Influence of Different Maceration Treatments on the Aroma Profile of Rosé and Red Wines from Croatian Aromatic cv. Muškat ruža porečki (*Vitis vinifera* L.)

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Summary

The influence of different grape mash maceration treatments on the varietal and secondary aroma profiles of wines produced from an aromatic red grape variety, Muškat ruža porečki, have been investigated. Two essentially different techniques were applied, fermentative maceration at room temperature and prefermentative cryomaceration, both in durations of one, three and five days. Generally, higher concentrations of free and bound varietal aroma compounds were found in wines obtained by maceration at room temperature in relation to cryomaceration. Regarding the effect of the duration of maceration, the highest concentrations were determined in wines obtained by three-day maceration treatments, in both fermentative and cryomaceration treatments. Secondary aroma compounds followed a less uniform pattern. The compounds with the highest odour unit values in all investigated wines were linalool, citronellol, geraniol, β-damascenone, β-ionone, isoamyl alcohol, 2-phenylethanol, ethyl hexanoate, ethyl octanoate, isoamyl acetate, ethyl acetate, and diethyl succinate. It has been shown that Muškat ruža porečki is an aromatic variety, producing wines with notable monoterpenol fraction, which are characterized by a typical varietal Muscat aroma with a dominant rose odour accompanied by red fruit nuances. Sensorially, longer maceration treatments improved odour and overall wine quality, together with the intensity and recognisability of varietal Muscat aroma, while short-term cryomaceration emerged as a preferable technique for the production of light rosé wines with pronounced Muscat aroma and low phenolic content. The presented maceration techniques were shown to be applicable for the production of different types of Muškat ruža porečki rosé and red wines.

Key words: red aromatic grape variety, Muškat ruža porečki wine, maceration at room temperature, cryomaceration, varietal aroma, secondary aroma, sensory evaluation

Introduction

Generally, in the production of red wines, grape pomace is macerated throughout the whole or the largest part of fermentation at relatively high temperatures, primarily in order to increase the extraction of phenols responsible for bitterness, astringency and colour of wine (*1,2*). Maceration of rosé wines is particularly delicate, especially in the case of aromatic red varieties, since an optimum phenolic content has to be extracted along with a high level of varietal aroma in order to obtain adequate gustative and chromatic features, as well as pronounced fruitiness and freshness of the aroma typical for a variety (*3*). In both cases, the achievement of a targeted balance is largely dependable on the regulation of two main maceration parameters, temperature and duration, which must be selected with care considering the variety and grape quality (*3–5*).

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Maceration at low temperature, *i.e.* cryomaceration (4–10 °C), when adapted to the variety and grape quality, may exhibit several advantages over a more common maceration at room temperature. In the case of ambient maceration, when conditions incorporate higher temperature, SO_2 and alcohol formed in the fermentation, the extraction of phenols is stimulated and it is unselective, and may result in excessive transition of some undesirable compounds from grapes to must, causing increased astringency and bitterness, inadequate colour, and herbaceous odour (*3,4,6–8*). On the other hand, cryomaceration favours the extraction of varietal aromas such as monoterpenes, and at the same time limits the extraction of phenols (*9,10*). The low temperature of cryomaceration inhibits the activity of oxidative enzymes, so it can be performed without the addition of SO_2 in the initial phase (*9,11*). It is generally conducted prior to fermentation so it passes without the formation of ethanol. Cryomaceration is frequently applied in the production of white wines (*9,10,12,13*), although its effects on the quality of rosé and red wines have been also investigated recently (*1,3*). However, there is a significant gap in the literature between the aroma research findings reported for white wine making referring entirely to the influence of short-term maceration (not applicable for red wine production), and the investigations dealing with prolonged maceration in rosé and red wine production almost exclusively oriented towards research on phenols. Information related to the influence of extended prefermentative and fermentative maceration on the concentrations of single and total volatile aroma compounds in red and rosé wines is limited, so there is a need to investigate it.

The variety chosen for this study was Muškat ruža porečki, a Croatian autochthonous aromatic red grape cultivar, synonymous to Moscato rosa (Rosenmuskateller) from South Tyrol (a region in North Italy), where it was introduced from Croatia (*14*). Nowadays, it is mainly grown in the vine-growing region of Istria, the largest peninsula in the Adriatic Sea, located on the very west part of Croatia. It is generally used for the production of dry to sweet rosé and red wines. Muškat ruža porečki belongs to a larger group of Muscats, and wines produced in an appropriate manner are characterized by a specific Muscat aroma with a predominant rose-like odour, accompanied by red fruit nuances. Because of its specific properties (red aromatic variety), maceration of Muškat ruža porečki requires a special approach, and many producers in Istria encounter problems in choosing an adequate winemaking technology.

The aim of this work is to investigate the response of varietal and secondary aroma compounds to different skin contact treatments in the production of Muškat ruža porečki wines, including fermentative maceration at room temperature and prefermentative cryomaceration, both in durations of one, three and five days. A preliminary screening of total phenols was also included, but a more in-depth discussion on the effect of the applied maceration treatments on the colour and phenolic composition of Muškat ruža wines shall constitute the aim of the forthcoming paper. Although an autochthonous cultivar, Muškat ruža porečki is considered a reliable representative of other red aromatic varieties worldwide. It is supposed

that the obtained result will significantly deepen the knowledge in this field, and contribute to a greater understanding of the existing problems in the achievement of a delicate balance of wines produced from red aromatic grape varieties. Finally, it is worth pointing out that the present investigation is the first report referring to the composition of aroma compounds of Muškat ruža porečki wines.

Materials and Methods

Muškat ruža porečki grape vinification

The experiment was performed during the harvest in 2007 of Muškat ruža porečki grapes, originating from Western Istrian vine-growing area, in the minivinification cellar of the Institute of Agriculture and Tourism in Poreč (Istria, Croatia). Maceration treatments were applied at room temperature ((20±1) °C), and cryomaceration at 5 °C, both in durations of 1, 3 and 5 days. Each of the mentioned treatments was performed in duplicate in 50-litre stainless steel vats (a total of 12 vats) equipped with a cooling system. Grape mash macerated at room temperature was treated with 10 g/hL of potassium metabisulphite immediately after grape crushing, while in cryomaceration, the grape mash was treated with the same concentration of potassium metabisulphite just after the maceration (before pressing). After crushing and mashing, selected wine yeast *Saccharomyces cerevisiae* Uvaferm 299 and fermentation activator Fermaid E (Lallemand, Montreal, Canada) were added to the mash. Alcoholic fermentation of the mash macerated at room temperature was running simultaneously with maceration, and continued in the musts obtained after pressing. Fermentation of cryomaceration treatments started after the completion of maceration (after pressing) and was conducted at room temperature ((20±1) °C), as in the case of maceration at room temperature. During maceration, mash samples were punched down three times a day. After alcoholic fermentation, the wines were decanted, and after 6 months subjected to physicochemical analyses and sensory evaluation.

Analysis of standard physicochemical parameters and total phenols

Relative density, volume fraction of alcohol, concentration of total extract, total and volatile acidity, reducing sugars, ash, and pH were analyzed according to Office International de la Vigne et du Vin (OIV) methods (*15*). Total phenols were determined spectrophotometrically with Folin-Ciocalteu reagent using a Cary 50 UV/VIS spectrophotometer (Varian Inc., Harbor City, CA, USA) at 760 nm after solid-phase extraction on Bond Elut C_{18} cartridges and elution with methanol and water (*16*). Gallic acid was used as a chemical standard for calibration. Total phenolic concentration was expressed in mg per L of gallic acid.

Extraction of varietal and minor secondary aroma compounds

Varietal aroma compounds were isolated from wine samples by solid-phase extraction (SPE) on octadecylsil-

ica (C$_{18}$) sorbent prepacked in Varian Bond Elut cartridges (6 mL, 500 mg; Varian Inc.) according to Di Stefano (*17*). Prior to SPE, wine samples were treated with polyvinylpolypyrrolidone (PVPP; 1 g per 25 mL) and centrifuged in order to remove high levels of phenols capable of competing with volatiles for the adsorption on the active surface of the sorbent during SPE, and inhibiting glycosidase activity. A volume of 25 mL of wine diluted fourfold with deionised water with the addition of 50 µL of internal standard solution (1-nonanol in 40 % ethanol) was passed through C$_{18}$ sorbent previously activated with 5 mL of methanol and 5 mL of deionised water. Free monoterpenes were eluted with 7 mL of pentane/dichloromethane (2:1). Extracts were dried over anhydrous sodium sulphate and then preconcentrated under the stream of nitrogen to 200 µL. Bound monoterpenes were eluted with 7 mL of methanol, and methanol extracts were evaporated to dryness using rotary evaporator. For the enzymatic release of aglycons, 5 mL of citrate-phosphate buffer solution (pH=5) containing a pectolytic enzyme with specific β-glycosidase side activities Everzym Arom (5 g/L, EVER S.r.l., Pramaggiore, Veneto, Italy) were added to the extract residue and the solutions were left at 37 °C for 16 h. After that, 50 µL of internal standard solution were added and solutions were passed through activated C$_{18}$ sorbent. Further elution, extract drying, and preconcentration were performed as for free monoterpenes.

Minor secondary aroma compounds were isolated from wine samples by SPE on Varian Bond Elut C$_{18}$ cartridges (6 mL, 500 mg) following the method developed by Lukić *et al.* (*18*). A volume of 25 mL of limpid wine diluted twice with deionized water, with the addition of 100 µL of internal standard solution (3-octanol in 40 % ethanol) was passed through the activated C$_{18}$ sorbent. Secondary aroma compounds were recovered by elution with 4 mL of dichloromethane. The extract was dried over anhydrous sodium sulphate and then preconcentrated with nitrogen to 0.5 mL.

Identification and quantification of varietal and secondary aroma compounds

Identification of varietal and minor secondary aroma compounds was performed by GC/MS analysis using a Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc.). The fused silica column used was a 60 m×0.25 mm i.d.×0.25 µm film thickness Rtx-WAX (Restek, Belafonte, PA, USA). A volume of 2 µL of the pentane/dichloromethane (2:1) or dichloromethane extract was injected in splitless mode. The GC oven parameters were as follows: initial temperature was 40 °C, then increased to 240 °C at 2 °C/min, and then kept at 240 °C for 10 min. Injector, transfer line and ion trap temperatures were 240, 180 and 120 °C, respectively. Mass spectra were acquired in the electron impact mode (70 eV) at 1 scan/s, using full scan with a mass acquisition range of 30–450 amu. Helium was used as a carrier gas with a flow rate of 1 mL/min. The identification of compounds was performed by comparing their retention times and mass spectra to those of pure standards when available, and to mass spectra from NIST05 library (National Institute of Standards and Tech-

nology, Gaithersburg, MD, USA). Additional identification was achieved by comparing calculated linear retention indices to those from literature.

Quantification of varietal aroma compounds was performed using Varian 3900/Saturn 2100T system under conditions described in the previous section. Calibration curves were constructed, and quantifications were performed by the internal standard method using Varian MS Workstation software v. 6.66 (Varian Inc.), on the basis of the peak area of corresponding quantification ions. When chemical standards for varietal aroma compounds were not available, semi-quantitative analysis was carried out, and concentrations were calculated as µg/L of similar compounds quantified using calibration curves, assuming a response factor equal to one.

Quantification of minor secondary aroma compounds was performed on a Varian 3350 gas chromatograph (GC) equipped with a split/splitless injector and a flame ionization detector (FID). GC/FID was used for quantification instead of GC/MS in order to avoid possible inaccuracies in quantitative determination due to ion fragment recombination in the GC/MS trap in the case of higher concentration compounds. The column and GC oven parameters were the same as described for the GC/MS system. The injector and detector temperatures were 235 and 245 °C, respectively. A volume of 2 µL of dichloromethane extract was injected in splitless mode. Carrier gas was helium with a flow rate of 1 mL/min. Calibration curves were constructed, and quantifications were performed by the internal standard method using Varian Star Workstation software v. 4.51 (Varian Inc.).

Quantification of the major secondary aroma compounds (acetaldehyde, ethyl acetate, methanol, 1-propanol, 1-butanol, isobutanol and isoamyl alcohol) was performed on the same GC/FID described above, following the method proposed by Peinado *et al.* (*19*). Prior to injection, a 500-µL volume of internal standard solution (1-pentanol in 40 % ethanol) was added to the doubly diluted wine. Samples were then deacidified with calcium carbonate. A volume of 2 µL of treated wine was injected (split ratio 1:20), with the following parameters: initial oven temperature was 40 °C, then raised after 4 min at 5 °C/min to 90 °C, then it was programmed at 15 °C/min to 235 °C and then kept for 10 min. The injector and detector temperatures were 160 and 240 °C, respectively. Carrier gas was helium with a flow rate of 1 mL/min. Major aroma compounds were identified by comparing their retention times to those of the pure standards. Calibration curves were constructed, and quantifications were performed by the internal standard method using Varian Star Workstation software v. 4.51. The accuracy and precision of the method were checked by standard addition and repeated measurements, and the results were very satisfactory (data not shown). No problems such as peak broadening or malformation, bad peak separation, *etc.* were observed.

Sensory evaluation

Sensory evaluation of wines took place at the Institute of Agriculture and Tourism in Poreč. It was performed by a panel of five trained certified tasters, all of them members of Croatian Enological Society and highly

experienced in Muškat ruža wine sensory evaluation. Tasters were seated in separate purpose-made booths, and the environment was free of interference in terms of noise, visual stimulation and ambient odour. Wine samples stored at 14 °C were served in coded standard wine tasting glasses (20) at room temperature (20 °C) under white light. Before sensory evaluation, criteria of the tasters were attuned by tasting representative samples of Muškat ruža porečki wine.

For descriptive sensory analysis, the tasters used a 10-point structured scale to rate the aroma intensity of each attribute (0=attribute not perceptible, 10=attribute strongly perceptible). Attributes were selected on the basis of high experience in Muškat ruža porečki wine tasting as those best describing its sensory characteristics. Wines were sniffed and tasted. They were also assessed on a regular basis as in commercial wine handling by the 100-point OIV method (21).

Wines were also evaluated by the ranking method according to Zoecklein et al. (22) on the basis of the following attributes: overall aroma intensity, varietal aroma recognisability, taste quality and overall impression. Wines were assessed in two sessions where each comprised six samples, each sample representing a replicate of a particular maceration treatment. For each attribute, wines were ranked in decreasing order with numbers from 1 to 6, where wine of the highest quality was assigned the score of 1, while of the lowest quality the score of 6.

Data elaboration

All analyses were performed in duplicates, and average values were used in further data elaboration. Concentration mean values and standard deviations were calculated from two replicates, *i.e.* two samples for each maceration treatment. Two-way analysis of variance (ANOVA) was carried out using Microsoft Excel (Microsoft, Seattle, WA, USA), and Fischer's least significant difference test was used to compare the means at the level of significance of $p \leq 0.05$.

Results and Discussion

Standard physicochemical parameters and total phenols

The results of standard physicochemical wine parameter analyses are presented in Table 1. Higher total acidity concentration was observed in wines obtained by one-day cryomaceration in relation to maceration at room temperature. A slight increase in dry extract and ash concentration, and a rather significant increase in total acidity as a function of the duration of maceration were determined for wines obtained by maceration at room temperature. Similar results were obtained for cryomacerated wines, except for three-day maceration. The case of total acidity is especially relevant, because it suggests that no significant degree of potassium bitartrate precipitation occurred, which is often the case during maceration (2,9), although results that corroborate this study have also been published (10,23).

As can be seen in Table 1, the content of total phenols increased after three days of maceration in relation to wines macerated for one day, and remained roughly the same in wines macerated for five days. This pattern was more evident in maceration at room temperature. The influence of maceration temperature emerged as an extremely important factor, since wines macerated at room temperature contained approximately double concentration when compared to cryomacerated wines. This was especially evident in three-day maceration where wines obtained by maceration at room temperature contained 112 % higher concentrations. Similar was observed by Budić-Leto et al. (24), who also observed cessation of the increase of total phenolic content at some point during maceration due to the decrease in the content of anthocyanins.

Varietal aroma compounds: monoterpenes

Free and bound monoterpenes identified in Muškat ruža porečki wines obtained by different maceration treatments are listed in Table 2.

Table 1. Standard physicochemical parameters and total phenols in Muškat ruža porečki wines obtained by different maceration treatments

Standard physicochemical parameter	Maceration at 20 °C			Maceration at 5 °C		
	t/day			*t*/day		
	1	3	5	1	3	5
φ(alcohol)/%	13.21±0.19	13.58±0.65	14.12±0.03	13.84±0.69	13.66±0.06	13.68±0.40
γ(dry extract)/(g/L)	(23.70±0.14)[B]	(25.70±0.85)[A]	(24.85±0.78)[AB]	(25.85±0.49)[A]	(23.60±0.14)[B]	(25.45±1.06)[AB]
γ(total acidity as tartaric acid)/(g/L)	(5.20±0.00)[C]	(5.95±0.35)[BC]	(6.10±0.14)[AB]	(6.05±0.35)[AB]	(5.30±0.28)[C]	(6.65±0.21)[A]
γ(volatile acidity as acetic acid)/(g/L)	(0.64±0.11)[A]	(0.43±0.01)[AB]	(0.37±0.01)[B]	(0.58±0.18)[AB]	(0.41±0.00)[AB]	(0.46±0.08)[AB]
γ(ash)/(g/L)	(2.11±0.01)[BC]	(2.37±0.09)[A]	(2.26±0.15)[AB]	(1.86±0.02)[C]	(2.22±0.08)[AB]	(2.28±0.17)[AB]
pH	(3.29±0.04)[A]	(3.27±0.04)[AB]	(3.27±0.01)[AB]	(3.22±0.03)[BC]	(3.24±0.00)[AB]	(3.18±0.04)[C]
γ(total phenols as gallic acid)/(mg/L)	(1037.50±17.68)[B]	(1265.00±56.57)[AB]	(1275.00±106.07)[A]	(532.50±10.61)[C]	(597.50±3.54)[C]	(625.00±0.00)[C]

Values expressed as mean±standard deviation (*N*=5); upper case superscripts indicate significant differences among mean values within rows at the level of significance of $p \leq 0.05$ determined by two-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

Table 2. Concentrations of free and bound varietal aroma compounds in Muškat ruža porečki wines obtained by different maceration treatments

Varietal aroma compounds	RI	Maceration at 20 °C			Maceration at 5 °C		
		t/day			*t*/day		
		1	3	5	1	3	5
		$\gamma/(\mu g/L)$					
Free varietal aroma compounds:							
monoterpenes							
trans-linalool furan oxide[a]	1436	11.93±0.71	12.97±0.57	12.84±0.25	11.46±0.52	11.90±0.87	11.96±0.10
cis-linalool furan oxide[a]	1464	(8.72±0.16)[AB]	(9.15±0.47)[A]	(9.01±0.05)[A]	(7.62±0.02)[C]	(8.29±0.35)[BC]	(8.00±0.16)[C]
linalool[a]	1542	(147.35±9.69)[BC]	(183.30±30.26)[A]	(171.40±11.74)[AB]	(112.70±14.14)[C]	(144.15±8.98)[BC]	(122.15±6.58)[C]
4-terpineol[b]	1593	1.89±0.16	2.17±0.02	1.88±0.12	1.62±0.42	1.74±0.05	1.77±0.02
α-terpineol[a]	1684	(81.97±4.43)[BC]	(100.54±12.39)[A]	(89.78±2.78)[AB]	(65.70±8.75)[C]	(81.79±7.38)[BC]	(75.27±6.19)[BC]
citronellol[a]	1758	(78.22±17.76)[BC]	(122.30±23.76)[A]	(105.32±12.57)[AB]	(61.57±5.46)[C]	(98.40±9.05)[AB]	(67.78±5.90)C
nerol[a]	1791	(113.25±7.57)[B]	(147.70±0.57)[A]	(165.55±7.28)[A]	(87.32±15.25)[B]	(89.43±24.56)[B]	(106.85±4.45)[B]
geraniol[a]	1838	(54.22±5.49)[A]	(57.43±6.22)[A]	(53.71±5.44)[AB]	(32.53±1.82)[C]	(44.06±0.70)[B]	(34.09±1.95)[C]
geranic acid[a]	2319	(40.84±7.64)[BC]	(80.28±16.13)[A]	(68.43±7.31)[A]	(28.70±4.12)[C]	(67.23±3.19)[A]	(46.14±0.31)[B]
farnesol[b]	2341	6.49±2.51	13.49±6.91	11.30±5.00	9.27±1.71	8.01±0.09	8.52±2.74
total monoterpenes		(544.87±24.12)[C]	(729.33±44.51)[A]	(689.21±21.56)[AB]	(418.48±23.72)[C]	(554.98±28.84)[BC]	(482.53±12.15)[C]
C13-norisoprenoids							
β-damascenone[a]	1809	(5.66±0.97)[B]	(9.47±0.77)[A]	(5.82±0.32)[B]	(6.94±0.50)[B]	(9.25±0.39)[A]	(6.47±0.83)[B]
β-ionone[a]	1923	(0.22±0.02)[A]	(0.14±0.02)[B]	(0.11±0.00)[C]	(0.11±0.02)[C]	(0.13±0.01)[BC]	(0.10±0.00)[C]
3-hydroxy-β-damascone[b]	2634	9.23±0.16	12.27±3.68	9.61±1.30	9.65±0.42	12.21±1.00	8.32±1.09
3-hydroxy-7,8-dihydro-β-ionol[b]	2762	6.63±0.43	7.03±2.51	7.45±4.23	3.49±0.22	8.01±0.09	8.52±2.74
total C13-norisoprenoids		21.74±1.07	28.91±4.52	22.99±4.44	20.19±0.69	29.60±1.08	23.41±3.06
miscellaneous							
benzaldehyde[a]	1508	(5.49±0.90)[BC]	(8.12±1.84)[AB]	(10.07±1.57)[A]	(4.96±1.47)[C]	(6.96±0.17)[BC]	(7.29±1.84)[BC]
benzyl alcohol[a]	1857	(8.60±0.40)[AB]	(9.89±1.83)[A]	(9.88±0.72)[A]	(3.38±0.27)[C]	(8.57±1.28)[AB]	(6.99±0.50)[B]
total miscellaneous		(14.09±0.98)[AB]	(18.01±2.60)[A]	(19.94±1.73)[A]	(8.34±1.49)[B]	(15.53±1.29)[AB]	(14.28±1.91)[AB]
Bound varietal aroma compounds:							
monoterpenes							
limonene[a]	1196	2.83±0.06	2.97±0.02	3.33±1.04	2.21±0.24	2.15±0.01	2.95±0.39
eucalyptol[a]	1214	(2.35±0.04)[A]	(1.67±0.05)[AB]	(2.01±0.73)[AB]	(1.29±0.14)[B]	(1.35±0.15)[B]	(1.29±0.10)[B]
trans-β-ocimene[b]	1253	(3.83±0.71)[BC]	(3.82±0.39)[BC]	(5.56±1.74)[A]	(2.44±0.01)[C]	(2.61±0.01)[C]	(4.27±0.45)[AB]
α-terpinolene[b]	1281	(2.16±0.26)[A]	(1.59±0.01)[AB]	(2.15±0.67)[A]	(1.31±0.10)[B]	(1.31±0.07)[B]	(1.75±0.15)[AB]
trans-linalool furan oxide[a]	1436	18.13±1.10	21.63±2.39	19.99±0.24	17.29±3.59	15.50±2.75	17.56±1.63
cis-linalool furan oxide[a]	1464	17.94±0.56	20.83±1.51	19.56±0.25	18.80±3.20	15.88±3.13	18.26±1.53
linalool[a]	1542	(77.95±14.64)[AB]	(101.26±22.83)[A]	(101.22±10.30)[A]	(55.31±1.85)[B]	(69.27±8.43)[B]	(57.05±8.83)[B]
α-terpineol[a]	1684	(30.58±1.47)[AB]	(37.28±5.93)[A]	(36.73±1.74)[A]	(28.08±3.29)[B]	(31.57±1.61)[AB]	(27.39±2.90)[B]
geranial[b]	1738	(14.69±0.16)[AB]	(17.63±3.85)[A]	(15.26±1.10)[AB]	(9.99±0.24)[C]	(12.93±0.01)[BC]	(10.24±0.18)[C]
trans-linalool pyran oxide[b]	1726	14.25±2.92	12.98±1.65	12.63±0.26	10.42±0.76	10.75±0.25	10.92±0.18
citronellol[a]	1758	(32.07±4.19)[B]	(40.24±6.14)[A]	(33.01±0.23)[B]	(23.98±0.70)[C]	(32.39±1.62)[B]	(27.51±4.10)[CB]
nerol[a]	1791	(481.35±34.29)[B]	(602.20±57.84)[A]	(547.50±8.49)[A]	(328.80±2.55)[C]	(471.90±10.47)[B]	(359.85±29.34)[C]
geraniol[a]	1838	(1674.0±84.85)[B]	(2073.0±207.89)[A]	(1871.0±79.20)[AB]	(1173.5±21.92)[C]	(1737.5±45.96)[B]	(1225.5±89.80)[C]
geranic acid[a]	2319	(538.19±44.11)[A]	(767.46±99.50)[A]	(410.17±93.91)[B]	(412.31±14.21)[B]	(652.02±9.84)[A]	(470.77±22.68)[B]
total monoterpenes		(2910.3±102.79)[B]	(3704.5±238.99)[A]	(3080.1±123.61)[B]	(2085.7±26.97)[C]	(3057.1±49.12)[B]	(2235.3±97.71)[C]
C13-norisoprenoids							
3-hydroxy-β-damascone[b]	2634	(92.93±22.57)[C]	(138.77±9.79)[A]	(54.10±10.57)[D]	(95.70±8.53)[C]	(125.45±2.56)[AB]	(100.61±8.27)[BC]
3-hydroxy-7,8-dihydro-β-ionol[b]	2762	(12.86±1.11)[C]	(22.08±3.29)[A]	(7.79±1.46)[D]	(11.92±0.31)[CD]	(19.66±0.73)[AB]	(14.87±2.72)[BC]
total C13-norisoprenoids		(105.79±22.60)[C]	(160.84±10.33)[A]	(61.89±10.67)[D]	(107.62±8.54)[C]	(145.11±2.66)[AB]	(115.47±8.71)[BC]

Table 2. – continued

Varietal aroma compounds	RI	Maceration at 20 °C			Maceration at 5 °C		
		t/day			*t*/day		
		1	3	5	1	3	5
		γ/(μg/L)					
miscellaneous							
benzaldehyde[a]	1508	2.56±0.01	2.84±0.21	2.42±0.02	2.84±0.54	2.35±0.31	2.65±0.46
benzyl alcohol[a]	1857	(51.23±0.79)[B]	(77.67±11.20)[A]	(57.87±3.04)[B]	(49.96±1.09)[B]	(48.56±2.02)[B]	(51.17±3.40)[B]
eugenol[a]	2152	(7.85±1.78)[AB]	(7.89±0.02)[AB]	(6.16±1.12)[B]	(7.80±0.11)[AB]	(8.31±0.02)[A]	(7.19±1.12)[AB]
total miscellaneous		(61.64±1.95)[B]	(88.40±11.20)[A]	(66.44±3.24)[B]	(60.59±1.22)[B]	(59.22±2.04)[B]	(61.00±3.61)[B]

Values expressed as mean±standard deviation (*N*=5); upper case superscripts indicate significant differences among mean values within rows at the level of significance of p≤0.05 determined by two-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

[a]retention time and mass spectra consistent with those of the pure standards, mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature; [b]mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature (semi-quantitative analysis)

Total monoterpene concentrations ranged from 2.5 to 4.4 mg/L, confirming that Muškat ruža porečki belongs to the group of aromatic varieties, according to the classification proposed by Mateo and Jiménez (*25*). Among free volatile monoterpenes, linalool was found at the highest concentration, while significant amounts of other major monoterpenols α-terpineol, citronellol, nerol and geraniol were determined. Exceptionally high amounts of bound geraniol were found. This result is in accordance with previous findings where geraniol was found to be the most abundant monoterpenol in wines made from aromatic red grape varieties such as Moscato Rosa and Moscato di Scanzo (*26*). Significant concentrations of oxygenated derivatives of geraniol were found, such as geranic acid in free and bound, and geranial in bound form. It is worth mentioning a high level of bound nerol, and a notable level of bound linalool. In contrast to linalool, α-terpineol and citronellol, lower levels of free in relation to bound forms of geraniol and nerol were found, which were probably mainly the result of monoterpene conversions by yeast during and/or after fermentation in which free geraniol and nerol are partially converted to free citronellol, linalool and α-terpineol (*26*, *27*). It has been shown in previous investigations that during fermentation the concentration of free linalool remains rather constant, concentrations of free nerol and geraniol significantly decrease, while the concentration of free α-terpineol increases for 300 to 400 % (*28–30*). High concentrations of bound nerol and geraniol that remained in finished Muškat ruža porečki wines support earlier findings where it was shown that from 77 to 89 % of terpenoid glycosides found in juice were not hydrolysed at the end of fermentation (*28*).

Higher concentrations of the majority of monoterpenes were found in wines macerated at room temperature in relation to cryomaceration of the same duration. It was assumed that higher temperature enhanced the solubility of free and bound monoterpenes, and increased their extractability from grape skin to must. The levels of total free and bound monoterpenes were from 30 to 43 %, and from 21 to 40 %, respectively, higher in wines macerated at room temperature. It is important to emphasize that such difference was much less pronounced than in the case of total phenols ranging from 95 to 112 %, which means that by cryomaceration phenolic content can be manipulated and decreased without losing a major part of varietal Muscat aroma. Considering that during maceration a major portion of monoterpenes is extracted in the form of glycosides, the observed difference between two treatments is compatible with that of McMahon *et al.* (*31*), who found that maceration at 20 °C increased the rate of extraction of total and phenol-free glycosides compared to cold maceration at 10 °C in the production of Cabernet Sauvignon wines. The authors indicated the possibility that the hydrolysis of complex precursors and subsequent liberation of glycosides was more expressed at higher temperature due to limited endogenous grape enzyme activity at 10 °C. Similar results were obtained by Salinas *et al.* (*3*), who found the highest total terpenol concentration in wines macerated at 15 °C in relation to 5 and 10 °C.

Regarding the influence of the duration of maceration, a similar pattern was observed for the majority of monoterpenes, as well as for total concentrations. Wines obtained by one-day maceration, both at room temperature and by cryomaceration, contained the lowest amounts, in free and in bound form. In wines macerated for three days a significant increase was observed for the majority of monoterpenes. Concentrations dropped again in wines analyzed after five days of maceration, although without statistically significant differences in some cases.

Rise and subsequent decline of glycosidically bound monoterpenes in Muškat ruža porečki wines macerated and fermented simultaneously at room temperature is comparable, by analogy, to previously published results. Zoecklein *et al.* (*29,32*) observed a slight increase, followed by a decrease, in total and phenol-free glycosides representing potentially volatile terpenes, during fermentation of Riesling must. Interestingly, similar to this study, the concentration of glycosides increased during the first three days of fermentation, and then decreased. A similar pattern was noted during fermentative maceration of Cabernet Sauvignon pomace (*31*). The observed initial increase could be an aftereffect of acid hydrolysis

of components from grapes contributing to glycoside concentration (*33*). On the other hand, McMahon *et al.* (*31*) and Zoecklein *et al.* (*29,32*) explained the decline in glycoside concentration after several days of fermentation as a result of the combination of factors including precipitation, absorption, and hydrolysis. A number of authors reported that glycoside concentration could be reduced due to adsorption and assimilation into yeast cells, which may proceed without the release of aroma-enhancing aglycones (*33,34*), but that its decline during fermentation is mostly due to hydrolysis (*34*). Although contradictory results have been reported concerning yeast β-glucosidase activity, the same authors confirmed that *S. cerevisiae* is able to hydrolyse glycosides. Alternatively or complementary, the decrease of glycosides could have been the result of hydrolysis by acids (*35*).

Most of the above-mentioned phenomena are not applicable for the elucidation of the decrease in monoterpene concentrations in Muškat ruža porečki wines obtained after five days of prefermentative cryomaceration at 5 °C. Fermentation was inhibited by low temperature, meaning that no yeast biomass was generated to allow adsorption and assimilation of monoterpene gylcosides into yeast cells or their hydrolysis by *S. cerevisiae* enzymes during maceration. Furthermore, endogenous enzyme activity was probably extremely limited by low temperature. It is possible that some other mechanism, involving precipitation or fixation of glycosides on different macromolecules and solids extracted from grapes at higher quantities after the third day of maceration, and subsequent removal by settling and pressing, was responsible for the observed decrease (*36*). Similar pattern of the initial increase and subsequent decrease of monoterpene concentrations was established, but not explained, in a number of previous studies for short-term prefermentative maceration in the production of white wine (*4,5,23,37*).

Varietal aroma compounds: C$_{13}$-norisoprenoids

Free and bound C$_{13}$-norisoprenoids found in Muškat ruža porečki wines obtained by different maceration treatments are listed in Table 2. The most important C$_{13}$-norisoprenoid identified was free volatile β-damascenone, a very potent odorant in wines, reported to be responsible for fruity-flowery, exotic fruit, rose-like, honey-like, dried plum and stewed apple odours (*2,38–40*).

Interestingly, β-damascenone was not identified in bound form. This compound can be generated *via* enzymatic and acid hydrolysis from multiple non-volatile precursors, involving different glycoconjugated moieties, as well as non-glycosidic compounds derived from transformations of carotenoids (*41*). It is possible that β-damascenone found in Muškat ruža porečki wines mainly derived from the degradation of carotenoids, and that grapes of this variety contain a limited portion of related glycosides. This hypothesis could be related to the findings of Baumes *et al.* (*42*) and Oliveira *et al.* (*43*), who established a negative correlation between the levels of carotenoids and bound β-damascenone in wine, and to those of Esti and Tamborra (*44*), who did not find β-damascenone after enzymatic and chemical hydrolysis of glycosides in wines from two varieties. Another possi-

bility is that β-damascenone glycosides were completely hydrolyzed during fermentation of Muškat ruža porečki musts. Furthermore, it was suggested that the formation of β-damascenone from glycosidic precursor must be a multi-step process, as it does not possess a hydroxyl group through which glycoconjugation could occur (*41*). It is possible that complex glycosidic precursors of β-damascenone were not hydrolyzed by the action of β-glucosidase in the analysis of bound fraction in this work, although Oliveira *et al.* (*43*) determined its presence by applying similar enzymatic hydrolysis. This assumption is supported by the fact that norisoprenoid derivatives with hydroxyl group which can form simple glycoconjugates, 3-hydroxy-β-damascone and 3-hydroxy-7,8-dihydro-β-ionol, were identified in bound form. Although rather odourless and negligible for wine aroma, 3-hydroxy-β-damascone and 3-hydroxy-7,8-dihydro-β-ionol can be transformed into odoriferous β-damascenone during wine ageing (*45*).

Another important C$_{13}$-norisoprenoid identified in this work was β-ionone, a compound which exhibits an odour reminiscent of violets (*2*). Similarly to β-damascenone, it can be formed by carotenoid degradation or by precursor hydrolysis (*43*). It was also identified only in free form.

As in the case of monoterpenes, an increase in the concentrations of free β-damascenone and the majority of norisoprenoids was observed between the first and third day of maceration, which was followed by a decrease. A rather sharp drop in the concentrations of bound 3-hydroxy-β-damascone and 3-hydroxy-7,8-dihydro-β-ionol was noted, but it was not accompanied by the increase in the concentrations of free forms. β-Ionone exhibited a peculiar behaviour in wines obtained by maceration at room temperature where its concentration decreased proportionally with the duration of maceration, which was rather unexpected and remained unexplained at this stage of investigation. The influence of maceration temperature on β-ionone was established in wines obtained by one-day maceration, where using maceration at room temperature significantly higher concentration was extracted. In contrast, the largest difference for 3-hydroxy-β-damascone and 3-hydroxy-7,8-dihydro-β-ionol was noted between wines macerated for five days, where wines obtained by cryomaceration contained significantly higher amounts.

Varietal aroma compounds: miscellaneous

Two benzenoids and a volatile phenol eugenol were identified (Table 2). In some cases higher concentrations were found in wines macerated at room temperature. Regarding the effect of the duration of maceration, similar behaviour as in the case of monoterpenes was observed. Zoecklein *et al.* (*29*) observed an analogous pattern in the case of benzyl alcohol glycosides during fermentation of White Riesling must.

Secondary aroma compounds

The concentrations of prefermentation and fermentation aroma compounds investigated in this study are presented in Table 3.

Table 3. Concentrations of secondary aroma compounds in Muškat ruža porečki wines obtained by different maceration treatments

Secondary aroma compounds	RI	Maceration at 20 °C			Maceration at 5 °C		
		t/day			*t*/day		
		1	3	5	1	3	5
		γ/(mg/L)					
Prefermentation aroma compounds:							
methanol[b]	–	$(77.82\pm7.76)^C$	$(95.80\pm0.85)^B$	$(113.17\pm2.40)^A$	$(74.72\pm9.04)^C$	$(96.08\pm2.52)^B$	$(101.52\pm1.15)^{AB}$
1-hexanol[a]	1356	$(0.64\pm0.07)^B$	$(0.43\pm0.06)^C$	$(0.40\pm0.04)^C$	$(0.43\pm0.07)^C$	$(1.02\pm0.08)^A$	$(0.49\pm0.05)^{BC}$
cis-3-hexen-1-ol[a]	1379	$(0.14\pm0.02)^{AB}$	$(0.12\pm0.01)^{BC}$	$(0.11\pm0.01)^{BC}$	$(0.09\pm0.00)^C$	$(0.17\pm0.01)^A$	$(0.10\pm0.00)^C$
total C$_6$-alcohols		$(0.78\pm0.07)^B$	$(0.55\pm0.06)^C$	$(0.51\pm0.04)^C$	$(0.52\pm0.07)^C$	$(1.19\pm0.08)^A$	$(0.59\pm0.05)^{BC}$
Fermentation aroma compounds:							
higher alcohols							
1-propanol[a]	1025	$(15.22\pm0.23)^B$	$(16.96\pm0.94)^B$	$(17.65\pm1.11)^{AB}$	$(15.92\pm0.96)^B$	$(19.78\pm1.13)^A$	$(20.99\pm1.15)^A$
isobutanol[a]	1100	$(53.23\pm1.39)^{BC}$	$(48.88\pm4.62)^C$	$(58.82\pm0.93)^B$	$(56.38\pm3.09)^B$	$(56.05\pm0.28)^B$	$(67.31\pm4.89)^A$
1-butanol[a]	1137	3.14 ± 0.65	4.13 ± 1.82	2.74 ± 0.86	1.77 ± 0.45	2.69 ± 0.57	2.04 ± 0.20
isoamyl alcohol[a]	1206	$(445.74\pm11.89)^A$	$(371.12\pm4.36)^B$	$(399.84\pm31.19)^{AB}$	$(364.81\pm24.35)^B$	$(390.13\pm9.69)^B$	$(363.01\pm19.86)^B$
2-phenylethanol[a]	1893	$(39.15\pm0.79)^A$	$(30.38\pm1.61)^C$	$(35.78\pm1.89)^B$	$(30.34\pm1.09)^C$	$(27.18\pm3.15)^{CD}$	$(26.49\pm0.82)^D$
total higher alcohols		$(556.48\pm12.02)^A$	$(471.46\pm6.87)^B$	$(514.82\pm31.29)^{AB}$	$(469.22\pm24.59)^B$	$(495.83\pm10.27)^B$	$(479.84\pm20.50)^B$
fatty acids							
hexanoic acid[a]	1830	$(1.74\pm0.00)^{AB}$	$(1.63\pm0.11)^B$	$(1.99\pm0.22)^A$	$(1.99\pm0.27)^A$	$(1.99\pm0.04)^A$	$(1.52\pm0.01)^B$
octanoic acid[a]	2043	$(2.00\pm0.12)^{AB}$	$(1.86\pm0.35)^B$	$(2.15\pm0.08)^{AB}$	$(2.54\pm0.57)^A$	$(2.14\pm0.06)^{AB}$	$(1.82\pm0.15)^B$
decanoic acid[a]	2257	$(0.19\pm0.03)^B$	$(0.30\pm0.04)^A$	$(0.30\pm0.01)^A$	$(0.21\pm0.02)^B$	$(0.31\pm0.03)^A$	$(0.31\pm0.03)^A$
total fatty acids		$(3.93\pm0.12)^{AB}$	$(3.78\pm0.37)^B$	$(4.44\pm0.23)^{AB}$	$(4.74\pm0.63)^A$	$(4.44\pm0.08)^{AB}$	$(3.65\pm0.15)^B$
ethyl esters							
ethyl hexanoate[a]	1236	$(0.37\pm0.02)^{AB}$	$(0.33\pm0.04)^{BC}$	$(0.42\pm0.01)^A$	$(0.30\pm0.03)^C$	$(0.42\pm0.01)^A$	$(0.35\pm0.04)^{BC}$
ethyl octanoate[a]	1435	0.40 ± 0.02	0.35 ± 0.08	0.45 ± 0.01	0.36 ± 0.03	0.41 ± 0.01	0.35 ± 0.03
ethyl decanoate[a]	1637	$(0.09\pm0.01)^{BC}$	$(0.12\pm0.01)^A$	$(0.11\pm0.00)^{AB}$	$(0.08\pm0.02)^C$	$(0.10\pm0.00)^{ABC}$	$(0.11\pm0.01)^{AB}$
total ethyl esters		$(0.86\pm0.03)^{AB}$	$(0.80\pm0.09)^{AB}$	$(0.98\pm0.01)^A$	$(0.74\pm0.05)^B$	$(0.93\pm0.01)^{AB}$	$(0.81\pm0.05)^{AB}$
acetates							
isoamyl acetate[a]	1120	$(0.35\pm0.01)^B$	$(0.50\pm0.00)^A$	$(0.54\pm0.07)^A$	$(0.39\pm0.05)^B$	$(0.54\pm0.06)^A$	$(0.53\pm0.09)^A$
2-phenethyl acetate[a]	1803	$(0.07\pm0.00)^B$	$(0.09\pm0.00)^A$	$(0.08\pm0.01)^{AB}$	$(0.07\pm0.01)^B$	$(0.09\pm0.01)^A$	$(0.08\pm0.01)^{AB}$
total acetate esters		$(0.42\pm0.01)^B$	$(0.59\pm0.00)^A$	$(0.62\pm0.07)^A$	$(0.46\pm0.05)^B$	$(0.63\pm0.06)^A$	$(0.61\pm0.09)^A$
other esters							
ethyl acetate[a]	–	$(63.66\pm6.15)^{AB}$	$(29.27\pm1.62)^C$	$(31.99\pm3.79)^C$	$(67.72\pm18.58)^A$	$(30.60\pm0.40)^C$	$(43.85\pm5.42)^{BC}$
diethyl succinate[a]	1667	$(5.35\pm1.23)^A$	$(2.02\pm0.04)^B$	$(1.92\pm0.14)^B$	$(2.61\pm1.08)^B$	$(2.21\pm0.09)^B$	$(1.66\pm0.06)^B$
acetaldehyde[b]	–	$(13.85\pm1.87)^{BC}$	$(25.25\pm5.20)^A$	$(15.75\pm0.93)^{ABC}$	$(12.90\pm5.95)^C$	$(16.56\pm4.04)^{ABC}$	$(24.40\pm5.15)^{AB}$

Values expressed as mean±standard deviation (*N*=5); upper case superscripts indicate significant differences among mean values within rows at the level of significance of p≤0.05 determined by two-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

[a]retention time and mass spectra consistent with those of the pure standards, mass spectra consistent with those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature; [b]retention time consistent with that of the pure standards

Regarding the influence of maceration temperature, no unique pattern was established. In general, slightly higher concentrations of several higher alcohols and ethyl esters were found in wines macerated for one and five days at 20 °C, and in wines cryomacerated for three days. As far as the duration of maceration was concerned, some tendencies were noted for several compounds.

The increase of methanol content with the duration of maceration was found to be completely linear since

very strong correlation with the number of days of maceration was determined: R=0.9999 for maceration at room temperature, and R=0.9459 for cryomaceration. It is generally known that methanol content increases during maceration due to enzymatic hydrolysis of pectins prevalently contained in grape berry skins (*2*).

There are results that show that macerated wines generally contain higher amounts of C$_6$-compounds in relation to control wines (*4,5,23,37*), but that after a sharp

initial increase their content tends to drop during maceration (*12,36*). Such behaviour was observed in this work in the case of cryomaceration (Table 3). In wines obtained by maceration at room temperature, C_6-alcohol concentrations decreased with maceration duration, possibly due to fixation to macromolecules, as reported by Ferreira *et al.* (*36*). The concentrations of the major C_6 compound 1-hexanol found in Muškat ruža porečki were low in comparison with other rosé and red wines (*3,46*).

Several authors observed a decrease in the concentration of higher alcohols as a function of maceration temperature (*3,12*), which was the case in this work for isobutanol (Table 3). The opposite results were found for isoamyl alcohol after one-day cryomaceration. A slight increase in the concentration of 1-propanol during cryomaceration was observed, which is in agreement with the results of several authors (*1,4,5,23,37*). Higher concentrations of 2-phenylethanol were found in wines macerated at room temperature, which corresponds to the findings of Salinas *et al.* (*3*), and is contrary to the results of Ramey *et al.* (*12*).

Concentrations of fatty acids exhibited a rather slight non-linear rise during maceration at room temperature (Table 3). A decrease of hexanoic and octanoic acids was observed in wines obtained by five-day cryomaceration, possibly as a result of the inhibition of fatty acid biosynthesis and consequent removal from must by assimilation into yeast cells (*47*).

The concentration of acetates, except for ethyl acetate, was found to increase during the first three days of both investigated maceration treatments, and then remained rather constant (Table 3), which coincides with a number of previous findings (*1,5,23,37*), although the opposite results have also been reported (*1,4*).

The impact of volatile compounds crucial for the typical aroma of Muškat ruža porečki wines

Odour unit values (OUV) of aroma compounds which were presumed to have a crucial impact on the typical aroma of Muškat ruža porečki wines (OUV>1) are presented in Table 4 (*2,10,13,37–40,48–50*). Among monoterpenes, citronellol and geraniol, and especially linalool, were found to significantly contribute to the aroma of all investigated wines, and are the most responsible for their recognizable varietal Muscat character.

Results presented in Table 4 suggest that the most pronounced Muscat character can be expected in wines obtained by three and five days of maceration at room temperature, followed by one day of the same treatment and a three-day cryomaceration. It must be kept in mind that high concentrations of bound monoterpenes (Table 2) may turn out to be a very important feature of Muškat ruža porečki wines, since these compounds can gradually hydrolyze in the bottle releasing compounds in free volatile form, which can impact the aroma. Judging on total concentrations (Table 2), it was concluded that wines macerated at room temperature for three days possess the highest varietal aroma reserve, which can prolong their expiry date in terms of aromaticity and typical Muscat aroma.

Apart from monoterpenes, key compounds probably responsible for the typical and recognisable rose-like component of the Muškat ruža aroma were 2-phenylethanol, and especially β-damascenone with the highest OUV. Considering the diversity of odour descriptors linked to the latter, such as fruity-flowery, exotic fruit, rose-like, honey-like, dried plum and stewed apple (*2,38–40*), β-damascenone possibly contributed to the complexity of other nuances of Muškat ruža wine aroma. Moreover, it was reported that β-damascenone may act as an enhancer of red fruit aroma in red wines by lowering the perception threshold of certain fruity ethyl esters (*39*), which may also be the case in rosé wines. β-Ionone had OUV>1 in all analyzed wines, which implied that it contributed to violet nuances and overall floral aroma. The most important contributors to the fruity aroma of Muškat ruža wines were fermentation esters such as ethyl octanoate and hexanoate, followed by isoamyl acetate.

Table 4. Odour perception thresholds, odour descriptions, and average odour unit values (OUV) of the key odorants in Muškat ruža porečki wines obtained by different maceration treatments

Volatile compound	Odour perception threshold	Odour description[f]	Maceration at 20 °C			Maceration at 5 °C		
			t/day			*t*/day		
	μg/L		1	3	5	1	3	5
linalool	15[a]	floral, rose, sweet	9.82	12.22	11.43	7.51	9.61	8.14
citronellol	18[b]	fruity/floral, citrus, citronella	4.35	6.79	5.85	3.42	5.47	3.77
geraniol	30[a]	floral, rose	1.81	1.91	1.79	1.08	1.47	1.14
β-damascenone	0.05[a]	fruity/floral, dried plum, stewed apple, rose, lilac, honey	113.20	189.40	116.40	138.80	185.00	129.40
β-ionone	0.09[e]	floral, violet	4.45	2.87	2.14	2.11	2.49	1.99
isoamyl alcohol	60 000[c]	solvent, fruity-winey	7.43	6.18	6.66	6.08	6.50	6.05
2-phenylethanol	7.5[d]	floral, rose	5.22	4.05	4.77	4.04	3.62	3.53
ethyl hexanoate	0.014[e]	fruity, red fruit, green apple	26.40	23.57	30.00	21.43	30.00	25.00
ethyl octanoate	0.005[e]	fruity/floral, banana, pear, sweet	80.00	70.00	90.00	72.00	82.00	70.00
isoamyl acetate	0.03[a]	fruity, banana, sweet	11.67	16.67	18.00	13.00	18.00	17.67
ethyl acetate	12 000[c]	fruity, pineapple, solvent, balsamic	5.31	2.44	2.66	5.64	2.55	3.65
diethyl succinate	1.2[c]	fruity, melon	4.46	1.68	1.60	2.17	1.84	1.38

OUV=concentration/odour perception threshold (*10*)
odour perception thresholds from references: [a](*48*), [b](*2*), [c](*13*), [d](*49*), [e](*50*); [f]odour descriptions from references *2,13,37–40*

Sensory evaluation

The results of descriptive sensory evaluation are shown in Fig. 1 as a spiderweb diagram for average wine aroma intensity scores. The aroma profile of the majority of assessed Muškat ruža porečki wines was dominated by the characteristic moderately intense flowery, rose-like Muscat aroma, probably as a result of the high OUVs of monoterpenes, 2-phenylethanol and β-damascenone. Notable intensities of stewed fruit and red stone fruit aromas were also perceived, which could be linked to the strong influence of β-damascenone and fruity esters (Table 4). Very low intensities observed for grassy odour correspond to low levels of 1-hexanol found (Table 3), and can be looked on as a positive general feature of wines from this variety. The OUV of isoamyl alcohol was relatively high (Table 4), and the concentration of total higher alcohols was higher than a critical limit of 400 mg/L (*37*) in all the investigated wines (Table 3), but the corresponding solvent-like odour (*13*) was not perceived during sensory evaluation. Interestingly, some authors underlined the positive contribution of the concentrations of higher alcohols comparable to those determined in this study to the overall fruity aroma of red wines (*1*).
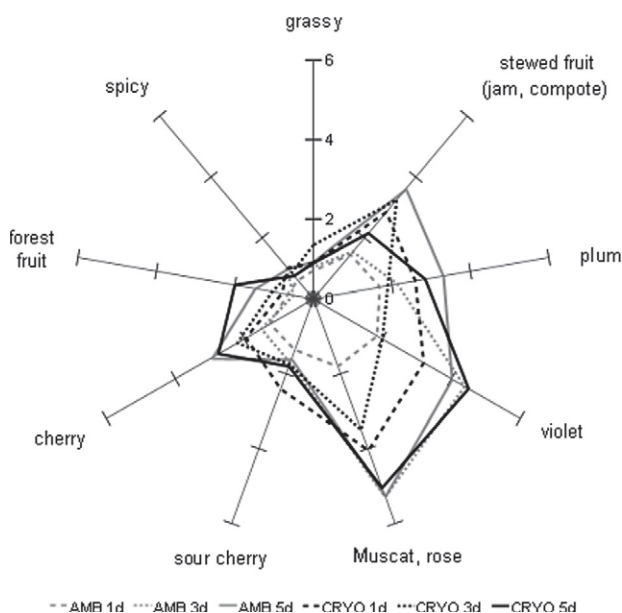

Fig. 1. Aroma profiles of Muškat ruža porečki wines obtained by different maceration treatments (mean scores of five tasters)
AMB 1d, AMB 3d, AMB 5d – maceration at 20 °C for one, three, and five days respectively
CRYO 1d, CRYO 3d, CRYO 5d – cryomaceration at 5 °C for one, three, and five days respectively

Considering the influence of maceration temperature, it is worth mentioning notably higher intensities of rose Muscat, violet, stewed fruit, plum, and cherry aromas perceived in wines obtained by cryomaceration for one day in relation to wines obtained by maceration for one day at room temperature. Apparently, such outcome could not be linked solely to the concentrations of monoterpenes since no correlation was observed. It is possible that notably higher OUV determined for β-damascenone

in one-day cryomacerated wine (Table 4) had a key impact, where this compound contributed directly to stewed fruit and plum odours, and indirectly as an enhancer of red fruit aroma originating from ethyl esters. Another possibility is that because of their higher content (Table 3) and OUV (Table 4), the odours of higher alcohols (especially isoamyl alcohol) partially masked monoterpene and other positive aromas, as suggested previously (*2*), in wines macerated for one day at room temperature. The observed fruitiness and varietal aroma, together with significantly reduced phenolic content (Table 1), suggest that short-term cryomaceration could be a promising technique for the production of fresh and light rosé wines. Maceration temperature also influenced several taste attributes, and wines macerated at room temperature exhibited stronger, but still only medium intensities of bitterness, astringency and body, which were considered by the tasters as very acceptable for this variety (data not shown). The same applies for the colour of these wines, which was described as ruby red. It has been shown that under controlled experimental conditions, maceration at 20 °C even for five days does not produce over-astringent Muškat ruža porečki wines.

Maceration at room temperature for three and five days significantly increased the intensity of rose Muscat aroma in relation to the wines macerated for one day. In this case, very strong correlation between the intensities of Muscat aroma and total monoterpene concentrations was determined (R^2=0.98). On the other hand, in wines cryomacerated for three days, the correlation was not observed due to lower Muscat aroma intensity. It is possible that higher intensities of stewed fruit and cherry odours suppressed the characteristic Muscat aroma to some extent. Increased stewed fruit and cherry intensities perceived in the wines obtained by cryomaceration for three days could be linked to higher β-damascenone and ethyl ester OUVs found in relation to wines cryomacerated for three and five days (Table 4). Besides Muscat aroma, longer skin contact generally resulted in increased intensities of the majority of aromas, as well as of taste attributes like bitterness, astringency, body, and colour intensity (data not shown). Violet nuance was perceived in all analyzed wines but its intensity could not be linked to the contents and OUVs of β-ionone since no correlation was noted. It is possible that the influence of β-ionone was modulated by interactions with other odorants, or that violet aroma originated from other odoriferous compounds.

The results of wine assessment by the ranking method are represented in Fig. 2. Wines produced by both maceration at room temperature and cryomaceration for five days were ranked as those of the highest quality for all four attributes, with no statistically significant differences established between them. The majority of tasters ranked wines macerated for one day at room temperature as the worst. This outcome was confirmed by the results of the 100-point OIV method, where wines obtained by five-day maceration at room temperature and cryomaceration were graded by the highest average scores of 79.1 and 78.1, respectively, followed by three-day maceration at room temperature with the score of 75.7. Wines macerated for one day at room temperature were rated with the lowest score of 62.8.
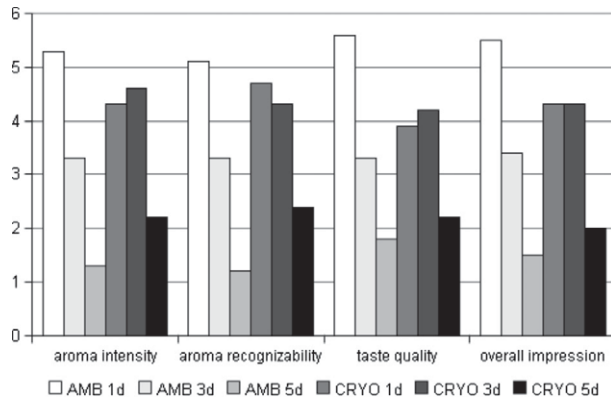
Fig. 2. The results of sensory evaluation by the ranking method of Muškat ruža porečki wines obtained by different maceration treatments (mean scores of five tasters; wine of the highest quality was assigned the score of 1, while of the lowest quality the score of 6)
AMB 1d, AMB 3d, AMB 5d – maceration at 20 °C for one, three, and five days respectively
CRYO 1d, CRYO 3d, CRYO 5d – cryomaceration at 5 °C for one, three, and five days respectively

Conclusions

The results of this investigation showed that by varying maceration temperature and duration, it is possible to significantly influence free and bound varietal aroma and, to a lesser extent, secondary aroma compound composition of wines produced from an aromatic red variety, Muškat ruža porečki. Generally, higher concentrations of the majority of varietal aroma compounds were found in wines obtained by maceration at room temperature in relation to cryomaceration. Regarding the effect of maceration duration, an increase in the concentration of the majority of varietal aromas was observed in wines obtained after three days of both maceration treatments, followed by a decrease in wines macerated for five days. Secondary aroma compounds followed a less uniform behaviour. It was shown that Muškat ruža porečki is an aromatic variety, producing wines with notable monoterpenol fraction, which are characterized by a typical varietal Muscat aroma with a dominant rose odour accompanied by red fruit nuances. Twelve key odorants for Muškat ruža porečki wine aroma were established: linalool, citronellol, geraniol, β-damascenone, β-ionone, isoamyl alcohol, 2-phenylethanol, ethyl hexanoate, ethyl octanoate, isoamyl acetate, ethyl acetate, and diethyl succinate. Cryomaceration proved to be a suitable technique for the regulation of phenolics without losing varietal aroma potential, since low temperature reduced phenolic content to a much higher extent than that of monoterpenes and the sensorial intensity of varietal aroma. Sensorially, longer skin contact treatments generally improved overall floral character and fruity notes, together with the intensity and recognisability of the typical varietal Muscat rose-like aroma and overall impression. Cryomaceration exhibited superior sensory evaluation results over maceration at room temperature in the case of short term one-day treatment, and emerged as a preferable technique for the production of light rosé wines with pronounced Muscat aroma and low content of phenols.

The current investigation addressed deficiencies in the available literature and provided new data as the basis for a greater understanding of the use of different maceration techniques in the production of wines from red aromatic varieties, from which future research in this oenological area would possibly benefit. It has practical relevance because it refers to the aspects of Muškat ruža porečki vinification that were identified by producers as problematic and were requiring investigation. The obtained results are likely directly applicable in winemaking practices and enable winemakers to make decisions based on more scientific information.

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