

Secondary Aroma Compounds in Fresh Grape Marc Distillates as a Result of Variety and Corresponding Production Technology

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Summary

In order to investigate the composition of secondary aroma compounds of fresh grape marc distillates as a result of variety and production technology, 30 samples (6 varieties×5 samples) were analysed. White grape marc samples from Malvazija istarska, Chardonnay and Muscat Blanc were obtained as by-products in standard white wine production, while red grape marc samples from Teran and Cabernet Sauvignon were obtained after standard red wine production procedures. Marc from red grape variety Muškat ruža porečki was obtained during the production of rosé wines. All fermented marc samples were distilled using a traditional copper alembic. The obtained distillates were subjected to GC/MS and GC/FID analyses. Malvazija istarska distillates exhibited exceptionally high methanol content. Distillates from white grape varieties were found to be characterized by higher C₆ alcohol and 1-propanol concentrations, while red grape distillates contained higher amounts of the majority of alcohols, acids, and esters. In Muškat ruža distillates intermediate concentrations of many important aroma compounds were found. It was concluded that differences in the production technology parameters, depending on the variety, resulted in differences in secondary aroma profiles, most evident between distillates from white and red varieties. These findings were confirmed applying stepwise linear discriminant analysis (SLDA), which resulted in 100 % correct classification of distillates according to the variety and corresponding production technology.

Key words: secondary aroma compounds, grape marc distillates, variety, production technology, fermentation temperature

Introduction

Grape marc, from which distillates are produced, is obtained as a by-product after separation of grape juice in the production of wine (1–3). Therefore, characteris-

tics of the obtained marc depend on varietal potential and grape quality, but also on technological procedures applied in vinification, which are completely adapted and inseparably related to the characteristics of the variety (2–4). Unlike in industrial facilities for continuous

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distillation with a number of modern rectification columns, by the simple traditional marc distillation it is not possible to significantly influence the composition of a distillate, so its final quality depends mostly on the variety, grape processing and fermentation parameters.

Generally, grape marc is produced from both white and red grapes (5,6). In the production of white grape marc, *i.e.* white wine, pressing of the mash is performed immediately after crushing and mashing of the grapes and, in some cases, after short-time maceration. Such marc contains significant amounts of grape sugars and is generally subjected to alcoholic fermentation after appropriate ensilage and inoculation with yeasts (5,6). In traditional production, ensilage is often performed in plastic tanks because of their availability, lower price and practicality, while stainless steel tanks are mostly reserved for fermentation and stabilization of wine (2,7). As in the case of wine, for the fermentation of white grape marc temperatures between 15 and 20 °C are advisable in order to preserve the amounts of fermentation esters and limit the formation of excessive levels of fusel alcohols (4,6).

Red grape mashes are commonly pressed after longer period of fermentative maceration in stainless steel tanks. Grape juice or must is in contact with grape berry skins through the largest part or the whole fermentation period at higher temperatures, in order to increase the extraction of compounds responsible for colour and taste of red wines (4). Marc obtained in that way is distilled immediately after pressing, or ensiled and distilled as soon as possible (6).

Sensory quality of grape marc distillates significantly depends on volatile aroma compound composition (8). Except in some cases in which the contribution of varietal aroma compounds originating from the grapes is also important (9), the fundamental aroma of fresh distillates consists of secondary aroma compounds formed in pre-fermentative production steps and in fermentation, such as fusel alcohols, volatile esters, acids and aldehydes (2,8,10). Fusel alcohols were reported to be responsible for recognizable, fundamental features and character of strong alcoholic beverages (11). Numerous volatile esters contribute to fruity and flowery odours (4,12), while higher levels of ethyl acetate and ethyl lactate may cause undesirable smells (12–14). Acetic and other fatty acids are responsible for sharp smells described as sour, rancid, fatty and cheesy, which are generally considered negative (2,15), while the main aldehyde, acetaldehyde, can contribute positively or negatively, depending on its concentration (16).

The production of grape marc distillates in the region of Istria in Croatia has a long tradition, and today it is an inseparable part of local customs and gastronomy. It is mostly related to small family farms, where it is carried out using traditional copper alembics. Unlike in the case of other countries of the Mediterranean basin, where products such as Italian grappa, Spanish orujo, Portuguese bagaceira, French eau de vie, and Greek tsipouro are recognized and appreciated worldwide, the potential of monovarietal grape marc distillate production in the region of Istria is not adequately exploited. Marc obtained from different varieties is usually first blended and then ensiled and fermented because the suitability

of a particular variety for the production of distillates has not yet been established. Due to the lack of precise knowledge on the relationship between variety and corresponding production technology and the composition of distillates, distillers are obliged to make choices based on non-scientific assumptions, which often leads to unwanted results. As a result, distillates present on the regional market largely lack a characteristic aroma.

The aim of this work is to investigate the influence of variety and corresponding production technology on the secondary aroma compound composition in fresh grape marc distillates, produced by traditional distillation from the six most important varieties in the region of Istria in Croatia. White grape varieties which were included in the investigation were Malvazija istarska, autochthonous and the most important variety in Istria, and the world-known Chardonnay and Muscat Blanc. Red grape varieties chosen were Teran, an important autochthonous variety, Cabernet Sauvignon, which is globally important, and Muškat ruža porečki, autochthonous red variety commonly used for the production of rosé wines.

Materials and Methods

Grape marc samples

Five grape marc samples of each of the six investigated grape varieties (*Vitis vinifera* L.) were obtained in the region of Istria in Croatia, during the 2006 harvest, which was characterized as very favourable, yielding grapes with high sugars and high acid concentration. Marc samples were consigned voluntarily by local producers, and samples of the same variety were obtained by different producers in order to comprise the influence of the intravarietal variability due to different growth and production conditions.

All grape mashes were obtained after standard wine-making procedures (crushing, mashing, destemming of the grapes, and sulphiting) and were further processed using technology that is characteristic for the variety. White grape mashes from Malvazija istarska, Chardonnay and Muscat Blanc were pressed shortly after crushing and mashing and, in some cases, after short maceration, as usual in standard white wine production. Prior to pressing, mashes from red grape variety Muškat ruža porečki were subjected to mid-term maceration characteristic for the production of rosé wines. Red grape mashes from Teran and Cabernet Sauvignon were inoculated with selected *Saccharomyces cerevisiae* wine yeasts, subjected to lengthy fermentative maceration in stainless steel tanks, and then pressed. All mash samples were pressed with pneumatic presses. Summarized grape quality and production parameters are presented in Table 1. Data on pressing programmes were not available.

Fermentation of grape marc samples

Grape marc samples were collected immediately after mashes were pressed. Samples (70 kg) were transferred into appropriate 100-litre plastic containers previously thoroughly cleaned. Marc samples from white grape varieties and Muškat ruža porečki were subjected to fermentation by inoculation with 20 g per 100 kg of selected (Fermol Blanc, Pascal Biotech, AEB Group, Brescia,

Table 1. Grape quality and processing parameters in the production of wine and grape marc from six grape varieties

Grape quality and production parameter	Grape variety					
	Malvazija istarska	Chardonnay	Muscat Blanc	Muškat ruža porečki	Teran*	Cabernet Sauvignon*
Reducing sugars in grapes/(g/L)	214.8±19.4 (188–236)	209.0±18.4 (188–234)	205.6±20.2 (178–220)	232.0±21.4 (202–256)	208.2±11.6 (191–220)	210.2±3.4 (207–215)
Total acidity in grapes/(g/L)	7.7±1.3 (6.0–9.3)	7.6±2.2 (5.6–8.7)	7.8±1.1 (6.5–9.5)	8.3±0.7 (7.2–9.0)	9.0±0.5 (8.5–9.5)	7.9±0.4 (7.4–8.5)
SO ₂ added to mash ^a /(g/100 kg)	8.0±5.7 (0–15)	9.0±5.5 (0–15)	11.0±3.8 (7–15)	8.0±3.1 (3–10)	5.4±2.7 (3–10)	8.4±3.8 (4–13)
Pectolytic enzymes added to mash ^b /(g/100 kg)	2.6±1.8 (0–5)	0.4±0.9 (0–2)	2.6±1.8 (1–5)	1.9±1.4 (0–4)	1.8±1.1 (0–3)	2.0±0.7 (1–3)
Yeast added to mash/(g/hL)	–	–	–	–	19.8±0.44 (19–20)	23.4±9.4 (17–40)
Duration of mash maceration ^c /h	2.0±3.5 (0–8)	–	17.4±30.9 (0–72)	30.4±17.6 (12–56)	206.4±36.4 (168–240)	168.0±56.3 (120–240)
Temperature of mash maceration ^c /°C	19.0±1.4 (18–20)	–	17.7±2.5 (15–20)	20.6±5.6 (13–27)	26.6±2.2 (23–28)	27.8±1.1 (26–29)
pH of mash*/marc	(3.78±0.22) ^A (3.52–4.10)	(3.57±0.17) ^A (3.39–3.78)	(3.69±0.17) ^A (3.55–3.94)	(3.59±0.19) ^A (3.33–3.85)	(3.20±0.08) ^B (3.07–3.29)	(3.29±0.05) ^B (3.20–3.33)

Values expressed as mean±standard deviation, ranges in parentheses (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 one-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

*Simultaneous mash maceration and fermentation; ^a4 Malvazija istarska, 4 Chardonnay, and all other samples were sulphited; ^b4 Malvazija istarska, 1 Chardonnay, 5 Muscat Blanc, 4 Muškat ruža, 4 Teran and 5 Cabernet Sauvignon samples were treated; ^c2 Malvazija istarska, 3 Muscat Blanc, and all Muškat ruža, Teran and Cabernet Sauvignon samples were macerated; maceration temperature data were reported for these samples only

Italy) *Saccharomyces cerevisiae* wine yeast, at temperatures between 17 and 19 °C. Already fermented Teran and Cabernet Sauvignon marc samples were ensiled at 17–19 °C in order to keep the time interval from grape mashing to distillation the same for all samples. The surface of marc samples was sprayed with 5 g of dipotassium disulphite, and covered with plastic foil. The containers were duly sealed by pouring 25 L of water onto the plastic foil in order to ensure anaerobic fermentation conditions. Fermentation was carried out over a period of 22 to 25 days.

Distillation of fermented grape marc samples

Fermented grape marc samples were distilled in a 120-litre traditional copper alembic. The alembic consisted of an onion-shaped boiler in the stainless steel casing, topped by a still-head in the shape of a turban, and extended by a swan neck tube that turns into a coil and circulates through a cooling tank. Marc was placed up on a copper grate placed at the bottom of the boiler, under which 20 L of water had previously been added to prevent the marc from burning. Before the beginning of distillation, the alembic was hermetically sealed in order to prevent any vapour leakage. Heating of the boiler was conducted by direct fire, with natural gas as a heating source. Distillation flow rate was kept at approx. 20 mL/min by carefully regulating the flame, and water in the cooling tank was kept between 20 and 22 °C. The volume fraction of ethanol was measured in the collected 100-mL fractions by an areometer. Three main distillation fractions were obtained. The forerun part of the distillate (known as the 'head' fraction), comprising approx.

5 % of the distilled volume, and the after-run part of the distillate (known as the 'tail' fraction), collected after the alcohol content of a running distillate dropped under 50 % (by volume), were discarded. The middle-run part (the 'heart' fraction) was saved as a fresh distillate. Distillates were collected in dark bottles, stoppered, stored at 20 °C for three months, and then adjusted to 43 % (by volume) of ethanol with deionized water. Distillates were stored at 20 °C for an additional month, and then analyzed.

Chemicals

Pure standards of methanol, 1-propanol, 1-butanol, 2-butanol, isobutanol, 1-pentanol, isoamyl alcohol, 1-hexanol, *trans*-2-hexen-1-ol, *cis*-2-hexen-1-ol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, 1-heptanol, 3-heptanol, 1-octanol, 3-octanol, 1-nonanol, 1-decanol, benzyl alcohol, 2-phenylethanol, acetic acid, isobutyric acid, butyric acid, pentanoic acid, hexanoic acid, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid, methyl nonanoate, ethyl isobutyrate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl acetate, isobutyl acetate, isoamyl acetate, hexyl acetate, 2-phenethyl acetate, ethyl lactate, diethyl succinate, acetaldehyde, benzaldehyde and furfural were purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Dichloromethane, 99.8 %, and sodium sulphate, 99 % were supplied by Kemika (Zagreb, Croatia). Pure deionized water was obtained from an Elix 3 purification system (Millipore, Bedford, MA, USA).

Extraction and analysis of secondary aroma compounds

Secondary aroma compounds were extracted from grape marc distillates by liquid-liquid extraction with dichloromethane. A volume of 12 mL of the distillate sample was diluted with 150 mL of deionized water, and 75 g of ammonium sulphate were added in order to improve extraction efficiency. An aliquot of 250 μ L of internal standard solution (3-octanol, nonanoic acid, and methyl nonanoate in ethanol, 50 mg/L each) was added to control the extraction. Aroma compounds were extracted with 5 mL of dichloromethane in a 250-mL parafilm-sealed separatory funnel vigorously agitated for 90 min using an electronic shaker. Closed funnel was then kept in a freezer at -20 °C for 30 min. After the organic phase was separated, transferred into a screw-cap vial and kept cold, the sample was re-extracted with two 5-mL portions of dichloromethane, following the same procedure. Dichloromethane extracts were combined, dried over anhydrous sodium sulphate, and concentrated under a gentle stream of nitrogen until the extract volume was reduced to 0.5 mL. To control injection, 10 μ L of 3-heptanol ethanolic solution (1000 mg/L) were added as the second internal standard.

Identification of secondary aroma compounds was performed by GC/MS analysis using a Varian 3900 gas chromatograph coupled with a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Harbour City, CA, USA). The fused silica column used was a 60 m \times 0.25 mm i.d., 0.25 μ m film thickness Rtx-WAX (Restek, Bellefonte, PA, USA). A volume of 2 μ L of dichloromethane extract was injected in splitless mode. The GC oven parameters were as follows: initial temperature was 40 °C, then increased at 2 °C/min to 240 °C, and then kept at 240 °C for 10 min. Injector, transfer line and ion trap temperatures were 240, 80 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 *m/z*. 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 with those of pure standards when available, and with mass spectra from NIST05 library. Identification by comparison with mass spectra was considered satisfactory only for compounds with the obtained reverse match factor higher than 800. Additional identification was achieved by comparing linear retention indices, calculated for the identified compounds (relative to *n*-alkanes), to those from literature.

Quantitative determination of secondary aroma compounds was performed using a Varian 3350 gas chromatograph equipped with a flame-ionization detector (FID). The column and GC oven parameters were the same as previously described. Injector and detector temperatures were 240 and 245 °C, respectively. A volume of 2 μ L of dichloromethane extract was injected in splitless mode, and carrier gas was helium with a flow rate of 1 mL/min. Detector gases were hydrogen (with a flow rate of 30 mL/min), air (300 mL/min), and helium as make-up gas (30 mL/min). Calibration curves (relative peak area *vs.* ratio of aroma compound/internal standard 3-heptanol concentrations) were constructed when standards were

available, and quantification was performed by the internal standard (3-heptanol) method using Varian Star v. 4.51 software (Varian Inc.). For other compounds semi-quantitative analysis was carried out, and concentrations were calculated as mg/L of internal standard, assuming a response factor equal to one (for fusel alcohols and aldehydes as mg/L of 3-octanol, for fatty acids as mg/L of nonanoic acid, and for esters as mg/L of methyl nonanoate).

The efficacy of the extraction procedure was checked by performing five extractions of each of the five standard model solutions. Concentration ranges of chemical standards of targeted secondary aroma compounds in model solutions were chosen in order to encompass the concentration ranges found in literature (2,3,7,8,10,15). Concentrations of chemical standards of 1-hexanol, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, isoamyl acetate, ethyl lactate, diethyl succinate, and furfural ranged from 1 to 120 mg/L, while concentrations of other standards ranged from 0.1 to 12 mg/L. Model solutions contained 43 % (by volume) of ethanol, 360 mg/L of acetic acid, 225 mg/L of acetaldehyde, 1000 mg/L of ethyl acetate, 1000 mg/L of methanol, 275 mg/L of 1-propanol, 350 mg/L of isobutanol, and 1250 mg/L of isoamyl alcohols in order to simulate more accurately a real distillate sample matrix.

Extraction recoveries for the majority of investigated compounds and internal standards, 3-octanol, nonanoic acid and methyl nonanoate were higher than 90 % ($N=25$), while relative standard deviations did not surpass 10 %. For more polar compounds, such as butyric, isobutyric and pentanoic acid recoveries ranged from 71 to 84 %, while for ethyl lactate and diethyl succinate were 83 and 89 %, respectively. Relative standard deviations for more polar compounds were lower than 17.56 %. FID response was linear in the investigated ranges, with R^2 higher than 0.998 for all compounds. Constructed calibration curves were used in further quantification.

Major volatile aroma compounds acetaldehyde, ethyl acetate, methanol, 1-propanol, 1-butanol, 2-butanol, isobutanol, isoamyl alcohol and 2-phenylethanol were analyzed by direct injection of double diluted distillate sample on Varian 3350 gas chromatograph and Rtx-WAX column with the following parameters: initial oven temperature was 40 °C, increased 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. Injector and detector temperatures were 160 and 240 °C, respectively. The carrier gas was helium with a flow rate of 1.1 mL/min, and detector gases were air, and helium as make-up gas. Prior to injection, 500 μ L of internal standard (1-pentanol) solution were added to the diluted distillate sample. A volume of 2 μ L of the sample was injected in split mode (1:20). Major volatile compounds were identified by comparing their retention times to those of the pure standards. Calibration curves were constructed, and quantification was performed by the internal standard (1-pentanol) method using Varian Star v. 4.51 software.

Statistical analyses

All analyses were performed in duplicates, and average values were used in further data elaboration. Mean values of concentrations and their standard deviations

were calculated from five replicates, *i.e.* five samples for each investigated variety. One-way analysis of variance (ANOVA) was carried out using Microsoft Excel (Microsoft, Seattle, WA, USA), and least significant difference test was used to compare the means at the level of significance of $p < 0.05$. In order to differentiate grape marc distillates according to varietal origin, stepwise linear discriminant analysis (SLDA) was carried out using Statistica v. 8.0 software (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Secondary aroma compounds identified and quantified in monovarietal grape marc distillates (6 varieties \times 5 samples) are listed in Tables 2–5. A total of 87 compounds were identified: 20 alcohols, 10 acids, 51 esters, and 6 aldehydes.

Relatively high standard deviations of concentration mean values observed for a number of compounds (Tables 2–5) imply a certain intravarietal variability in composition, derived from the differences in grape quality and winemaking practices applied in the production of marc samples of the same variety. Mean values and rather wide ranges of various grape, mash, and marc quality and production parameters presented in Table 1 corroborate this thesis.

Apart from intravarietal differences, the results of one-way ANOVA analysis shown in Tables 2–5 suggest that marc distillates produced from different varieties differed significantly in secondary aroma compound composition. The differences in average sugar content as a measure of the degree of grape maturity among varieties were not significant (Table 1), and were therefore not considered to have a significant contribution. The influence of other parameters is discussed in further detail in the following sections.

Alcohols

During grape processing and fermentation, methanol is formed by the hydrolytic demethoxylation of esterified methoxyl groups of the pectin polymer by pectic enzymes (17). Although endogenous enzyme activity depends on many parameters, such as variety, type of crushing and mashing, and duration and temperature of maceration (4), higher pressure and larger number of cycles during pressing of the mash are probably the main factors that strongly contribute to the increase of methanol content in grape marc distillates (7,10). In fact, Versini *et al.* (18) denominated methanol and 1-hexanol as rough indicators of the degree of pressing in distillates.

The highest concentration of methanol was found in Malvazija istarska distillates (Table 2). Malvazija istarska grapes are characterized by a thick skin and a dense pulp, both rich in pectins, and often require the application of pectolytic enzymes and higher degree of pressing during processing (19). Although data on pressing programmes were not available, high methanol content indicates that higher pressures were applied. When compared to Teran and Cabernet Sauvignon, higher concentrations of methanol were also found in other white grape distillates, although in some cases without statistical significance. It is known that pH values of unfermented white grape

marc are generally higher in relation to those of red grape mashes due to removal of a significant part of acid content by pressing, as shown in Table 1. Higher pH values promote the activity of enzymes and liberation of methanol (6), so it was assumed that this parameter had a significant influence. Moreover, larger quantities of energy-producing compounds and the presence of air pockets in unfermented white grape marc were reported to enhance the effect (6). On the other hand, fermentation of red grape mashes involved a rather lengthy maceration, during which a significant amount of released methanol is extracted to must (20), which presumably resulted in lower amounts of methanol in the corresponding marc distillates. Excessive amounts of methanol can be harmful to humans, meaning that white grape marc distillates, especially from Malvazija istarska, might require an additional refinement by marc acidification and re-distillation.

During the production of wine, C_6 alcohols are formed mainly during prefermentative production steps, such as harvesting, transport, crushing, mashing and pressing of the grapes, by enzymatic degradation and subsequent reduction of long-chain fatty acids (21). Production procedures such as higher pressures and larger number of pressing cycles were often reported as determining factors which can significantly increase their amounts in distillates (10,15).

Concentrations of C_6 alcohols found in this investigation are reported in Table 2. Since 1-hexanol is denominated as a rough indicator of the degree of pressing (18), wide concentration ranges in distillates made from the same variety imply a possible influence of pressing. Significantly lower concentrations of 1-hexanol and hexenols in relation to other varieties were found in distillates from Teran and Cabernet Sauvignon. The observed phenomenon is possibly in a larger part due to lighter pressing, combined with lower pH (Table 1) that limited the activity of enzymes to a certain extent. Secondly, during fermentative maceration a significant amount of C_6 alcohols from grape skins is extracted to the must. The bonds between C_6 alcohols and skin structures after fermentative maceration become weak, and pressing permits an easy release to the liquid medium (20,22). It is probable that this phenomenon also contributed to lower amounts of these compounds in red grape marc samples.

It was assumed that higher concentrations of 1-hexanol and *cis*-3-hexen-1-ol contribute significantly to the aroma of Malvazija istarska, Chardonnay, Muscat Blanc and Muškata ruža porečki distillates, because they were notably higher than the corresponding odour perception thresholds of 20 and 3.5 mg/L, respectively, determined in an earlier investigation (23). Guichard *et al.* (24) reported the mentioned amounts as the highest in which these compounds can positively contribute to the aroma of distillates. The odours of C_6 alcohols were described as reminiscent of green grass and leaves (10). Excessive amounts of 1-hexanol may cause harsh and repulsive odours, as well as odours reminiscent of cocoa, licorice and even toothpaste, and seriously diminish the sensory quality (10,14,25). In light of the above discussion, it was concluded that in the production of distillates from marc obtained after white and rosé winemaking, special attention should be addressed to the regulation of C_6 alcohol content, especially in terms of pressing.

Table 2. Concentration of alcohols in monovarietal grape marc distillates produced in the region of Istria, Croatia

Aroma compound	RI	γ (monovarietal grape marc distillate)/(mg/L)					
		Malvazija istarska	Chardonnay	Muscat Blanc	Muškat ruža porečki	Teran	Cabernet Sauvignon
Methanol	–	(4543.9±1241.1) ^A	(2764.9±781.0) ^B	(1617.6±584.3) ^C	(1296.83±306.7) ^C	(910.43±235.80) ^C	(850.53±189.77) ^C
<i>C₆ alcohols</i>							
1-hexanol ^a	1356	59.75±43.02	62.41±48.42	65.32±22.89	54.03±45.51	12.66±4.01	19.57±4.88
<i>trans</i> -3-hexen-1-ol ^a	1361	1.00±0.55	0.96±0.44	0.63±0.23	0.97±1.46	0.14±0.05	0.42±0.12
<i>cis</i> -3-hexen-1-ol ^a	1379	1.72±1.20	1.44±0.15	3.31±3.78	4.56±4.03	0.60±0.51	0.40±0.18
<i>trans</i> -2-hexen-1-ol ^a	1399	(0.62±0.21) ^B	(1.16±0.55) ^A	(0.91±0.35) ^{AB}	(0.57±0.56) ^{BC}	(0.10±0.02) ^C	(0.14±0.03) ^C
<i>cis</i> -2-hexen-1-ol ^b	1408	(0.23±0.14) ^A	(0.20±0.07) ^{AB}	(0.17±0.10) ^{AB}	(0.05±0.05) ^C	(0.04±0.02) ^C	(0.11±0.04) ^{BC}
Total		63.32±44.43	66.17±48.57	70.33±25.28	60.17±51.44	13.55±4.12	20.64±5.14
<i>Fusel alcohols</i>							
1-propanol ^a	1025	(343.26±72.94) ^A	(332.16±56.56) ^A	(406.74±100.54) ^A	(202.94± 4.79) ^B	(150.13±75.45) ^{BC}	(91.54±28.09) ^C
2-butanol ^a	1019	–	(0.67±0.71) ^B	(2.77±1.64) ^A	(0.06±0.13) ^B	–	(0.00±0.00) ^B
isobutanol ^a	1100	316.05±50.05	415.64±77.44	339.20±124.50	427.76±160.67	397.86±72.01	326.75±68.82
1-butanol ^a	1137	6.95±0.73	6.43±1.13	8.04±2.15	7.47±0.19	6.14±2.05	5.25±0.94
isoamyl alcohol ^a	1206	(796.7±143.3) ^C	(836.0±117.0) ^C	(974.5±85.9) ^{BC}	(1438.7±443.3) ^A	(1302.9±297.8) ^{AB}	(1594.4±414.3) ^A
1-pentanol ^a	1246	0.77±0.37	0.82±0.33	1.08±0.25	1.14±0.81	1.18±0.63	1.67±0.48
1-octen-3-ol ^c	1446	(0.46±0.19) ^A	(0.32±0.12) ^{AB}	(0.37±0.18) ^{AB}	(0.46±0.38) ^A	(0.20±0.10) ^B	(0.12±0.03) ^B
1-heptanol ^a	1451	(1.63±1.01) ^A	(1.53±0.80) ^A	(1.07±0.45) ^{AB}	(0.97±0.41) ^{AB}	(0.62±0.26) ^B	(0.51±0.09) ^B
1-octen-4-ol ^d	1535	0.35±0.32	0.36±0.31	0.17±0.09	0.23±0.08	0.32±0.16	0.34±0.12
1-octanol ^a	1552	0.74±0.20	0.74±0.09	0.96±0.27	1.11±0.78	0.57±0.21	0.68±0.17
1-nonanol ^a	1652	0.82±0.37	1.71±0.57	1.47±0.63	1.61±1.00	1.03±0.50	0.84±0.12
1-decanol ^a	1755	0.35±0.09	0.55±0.11	0.71±0.14	1.35±1.16	0.62±0.42	0.58±0.10
benzyl alcohol ^c	1857	(1.30±0.48) ^A	(1.34±0.62) ^A	(0.72±0.22) ^B	(0.57±0.16) ^B	(0.33±0.12) ^B	(0.31±0.13) ^B
2-phenylethanol ^a	1893	(5.54±1.40) ^D	(5.93±0.99) ^{CD}	(5.92±1.18) ^{CD}	(11.77±3.03) ^{BC}	(17.43±7.85) ^{AB}	(20.13±7.15) ^A
Total		(1474.9±120.0) ^C	(1604.2±117.6) ^{BC}	(1743.7±96.3) ^{ABC}	(2096.2±576.7) ^A	(1879±287.6) ^{ABC}	(2043.2±476.0) ^{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 $p \leq 0.05$ determined by one-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

^aRetention time consistent with that of the pure standards, mass spectra consistent with pure standards and those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature; ^bretention time consistent with that of the pure standards, and mass spectra consistent with pure standards and those from the NIST05 electronic library; ^cmass spectra consistent with those from the NIST05 electronic library, and RI consistent with those found in literature; ^dmass spectra consistent with those from the NIST05 electronic library (tentative identification)

The concentrations of fusel alcohols determined in this investigation are presented in Table 2. Higher amounts of isoamyl alcohol and 2-phenylethanol, as well as total concentrations, were found in distillates from red varieties Teran and Cabernet Sauvignon, while higher amounts of 1-propanol and benzyl alcohol were found in white grape distillates. Several authors reported higher concentrations of isoamyl alcohol and total fusel alcohols, and lower concentrations of 1-propanol in wines obtained by fermentation at higher temperatures (4,26,27), which corroborates the assumption that up to 10 °C higher temperatures in the fermentation of Teran and Cabernet mashes influenced the differences in fusel alcohol content. In a recent investigation it has been shown that temperature of white grape marc ensiled in plastic containers also increases significantly during fermentation due to the metabolic activity of yeast (6). The same authors also found that it rather quickly equals the external temperature, which ranged from 17 to 19 °C in this experiment. It

was reported that the production of fusel alcohols is stimulated by higher pH values (20,26). Since this was not the case in this investigation, it was assumed that the influence of other factors prevailed. Besides fermentation temperature, other processing parameters which are known to affect fusel alcohol content, such as grape quality, yeast strain, maceration, and aeration (4,20,26) might have a certain influence. It is worth emphasizing that the concentrations of isoamyl alcohol, 2-phenylethanol and 1-propanol in Muškat ruža porečki distillates were between the amounts determined in white and red grape distillates (Table 2). This fact is especially interesting because it implicates that the reason for that is precisely the grape processing technology of this variety, which is characterized by some similarities in both white and red winemaking.

Contributing to medicinal odours reminiscent of alcohol and solvent (3,12), isoamyl alcohol, isobutanol and

1-propanol are very important for the complexity of aroma and fullness of distillate body (28). Determined concentrations of isoamyl alcohol in all samples were significantly higher than its threshold of 24 mg/L (29). Due to higher concentrations, it was concluded that isoamyl alcohol contributes more strongly to the aroma of red grape marc distillates from Teran and Cabernet Sauvignon ensuring a more pronounced alcoholic character.

The majority of authors described the impact of 2-phenylethanol with its odour of roses as positive (20). Relatively low concentrations found in this work in relation to previously determined odour perception threshold in spirits of 125 mg/L (29) suggest that the influence of 2-phenylethanol is only indirect.

Higher concentrations of 1-octen-3-ol were found in distillates from white grape varieties, in some cases with statistically significant difference. This compound has miscellaneous origin, but it is known that its presence in wine is mainly due to the activity of *Botrytis cinerea* on grapes. It contributes negatively to the aroma with the odour of mushrooms (20).

Fusel alcohols may positively contribute to the aroma of spirits when present in total concentrations below 4000 mg/L of absolute alcohol, while in higher amounts they may cause strong and heavy smells that can significantly suppress desirable odours and characteristic varietal aroma (30). In some samples studied in this work,

the sum of fusel alcohol concentrations was higher than 4000 mg/L of absolute alcohol (1720 mg/L), which suggests that in the production of marc distillates by standard red wine technology, the content of fusel alcohols should be additionally monitored.

Volatile acidity (acetic acid) and fatty acids

No statistically significant differences among mean values of acetic acid concentration in monovarietal grape marc distillates were found (Table 3). Determined values mostly depended on the level of oxygen that promotes the activity of acetic acid bacteria (4,15), which could have varied in the production of both white grape marc and red grape mashes. The contact with oxygen in different production phases is inevitable, but in the case of adequate ensilage and opportune distillation, acetic acid amount should remain at acceptable level, which was the case in this investigation.

The concentrations of other fatty acids found in this investigation are presented in Table 3. Short-chain fatty acids are commonly found in low concentrations in alcoholic drinks, but due to very high volatility they may exhibit a significant impact on aroma (2,14), which is confirmed by rather low odour perception thresholds of butyric, isobutyric and isopentanoic acid (11). These compounds are usually considered negative contributors (2,15,23,29) with sharp smells described as rancid, fatty and cheesy (13,31).

Table 3. Concentration of acids in monovarietal grape marc distillates produced in the region of Istria, Croatia

Aroma compound	RI	γ (monovarietal grape marc distillate)/(mg/L)					
		Malvazija istarska	Chardonnay	Muscat Blanc	Muškat ruža porečki	Teran	Cabernet Sauvignon
<i>Volatile acidity (acetic acid)</i>	–	121.93±30.06	143.12±78.68	88.20±13.19	104.20±33.61	144.41±24.04	116.31±24.19
<i>Short-chain fatty acids</i>							
isobutyric acid ^a	1554	0.15±0.11	0.18±0.05	0.17±0.07	0.18±0.11	0.25±0.07	0.26±0.08
butyric acid ^a	1612	0.07±0.02	0.08±0.02	0.10±0.04	0.05±0.03	0.07±0.02	0.07±0.01
pentanoic acid ^a	1720	0.06±0.01	0.09±0.03	0.06±0.01	0.08±0.02	0.05±0.03	0.07±0.02
Total		0.28±0.14	0.35±0.03	0.32±0.09	0.31±0.11	0.33±0.10	0.40±0.10
<i>Middle-chain fatty acids</i>							
hexanoic acid ^a	1830	(0.82±0.28) ^B	(0.95±0.41) ^B	(1.87±0.78) ^A	(0.73±0.32) ^B	(0.87±0.37) ^B	(1.13±0.37) ^B
heptanoic acid ^c	1935	0.11±0.03	0.06±0.06	0.04±0.05	0.10±0.04	0.10±0.04	0.10±0.04
octanoic acid ^a	2043	(1.24±0.57) ^{CD}	(1.33±1.11) ^{BCD}	(2.47±1.19) ^{ABC}	(1.10±0.52) ^D	(2.53±1.30) ^{AB}	(2.63±0.87) ^A
decanoic acid ^a	2257	1.39±0.79	1.27±1.19	2.25±0.91	1.91±0.64	2.17±0.62	2.93±0.94
Total		3.55±1.53	3.62±2.68	6.63±2.82	3.84±1.40	5.67±2.27	6.79±2.12
<i>Long-chain fatty acids</i>							
dodecanoic acid ^a	2467	(0.48±0.25) ^B	(0.48±0.19) ^B	(0.53±0.21) ^B	(0.41±0.24) ^B	(1.32±0.79) ^A	(1.59±0.63) ^A
tetradecanoic acid ^c	2679	(0.08±0.06) ^{BC}	(0.08±0.03) ^{BC}	(0.08±0.02) ^{BC}	(0.06±0.02) ^C	(0.14±0.07) ^{AB}	(0.18±0.08) ^A
hexadecanoic acid ^c	>2800	0.50±0.19	0.56±0.36	0.38±0.15	0.47±0.36	0.59±0.37	0.75±0.32
Total		(1.06±0.44) ^B	(1.12±0.53) ^B	(0.99±0.30) ^B	(0.94±0.36) ^B	(2.05±1.17) ^A	(2.52±0.72) ^A

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 one-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

^aRetention time consistent with that of the pure standards, mass spectra consistent with pure standards and those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature; ^cmass spectra consistent with those from the NIST05 electronic library, and RI consistent with those found in literature

Higher concentrations of isobutyric acid in relation to other varieties were found in Teran and Cabernet Sauvignon distillates, although without statistically significant difference. This result is partially in accordance with the observation that isobutyric acid is more abundant and important for the aroma of red wines (32), and could be linked to the fact that higher fermentation temperature favours the formation of branched short-chain fatty acids (33,34).

Middle-chain fatty acids usually do not exhibit important effects on the aroma of distillates (11) due to relatively high odour perception thresholds of 15 and 8 mg/L determined for octanoic and decanoic acid, respectively (35). The impact of odours that these acids produce is described as restrained but unpleasant (12,13,31,35).

Concentration mean values of the majority of middle-chain fatty acids were higher in Muscat Blanc, as well as in Teran and Cabernet Sauvignon distillates (Table 3). Higher amounts determined in Teran and Cabernet distillates might have emerged from the previously assumed lighter pressing applied for red marc distillates. This assumption is corroborated by the fact that high negative correlation coefficients were determined between the concentrations of total middle-chain fatty acids and the indicators of the degree of pressing methanol and 1-hexanol: -0.911 and -0.753 for Malvazija, -0.708 and -0.698 for Chardonnay, -0.748 and -0.660 for Muscat Blanc, -0.608 and -0.163 for Muškat ruža, 0.105 and -0.352 for Teran, and -0.533 and -0.230 for Cabernet Sauvignon.

Higher amount of middle-chain fatty acids in Teran and Cabernet distillates could be linked to higher temperatures at which the corresponding marc samples fermented. Torija *et al.* (33) found higher levels of middle-chain acids in yeast cells after fermentation at elevated temperatures. Knowing that acids, among other compounds, are liberated from the yeast cells at high temperatures during distillation (while in the production of wine are discarded together with yeasts) (36), the effect of the above-mentioned phenomena could have resulted in higher amounts of acids in red grape distillates.

Esters

The concentrations of esters found in this investigation are presented in Table 4. In the case of ethyl esters of short-chain fatty acids, slightly higher concentrations of ethyl butyrate were found in white grape marc distillates. On the other hand, higher average amounts of branched short-chain ethyl esters were found in distillates from Teran and Cabernet Sauvignon, with statistically significant differences in the case of ethyl 2-methylbutyrate and ethyl 3-methylbutyrate (Table 4), esters which contribute to odours of forest and berry fruits (13,31,32). This results corroborate in a way earlier findings where it was shown that the mentioned esters contribute more strongly to red wine aroma (32,37), and that higher fermentation temperature favours the formation of ethyl 2-methylbutyrate (34). As in the case of fusel alcohols, it is worth emphasizing that Muškat ruža porečki distillates contained intermediate concentrations.

Table 4. Concentration of esters in monovarietal grape marc distillates produced in the region of Istria, Croatia

Aroma compound	RI	γ (monovarietal grape marc distillate)/(mg/L)					
		Malvazija istarska	Chardonnay	Muscat Blanc	Muškat ruža porečki	Teran	Cabernet Sauvignon
<i>Ethyl esters of short-chain fatty acids</i>							
ethyl propanoate ^c	<1000	0.55±0.10	0.57±0.13	0.72±0.07	0.69±0.32	0.68±0.12	0.63±0.08
ethyl isobutyrate ^a	<1000	0.30±0.14	0.41±0.14	0.25±0.12	0.44±0.28	0.47±0.13	0.52±0.16
ethyl butyrate ^a	1030	1.88±0.24	1.66±1.16	2.42±1.39	2.20±1.23	1.43±0.61	1.35±0.32
ethyl 2-methylbutyrate ^a	1049	(0.08±0.06) ^B	(0.14±0.04) ^B	(0.12±0.02) ^B	(0.29±0.14) ^A	(0.35±0.04) ^A	(0.38±0.10) ^A
ethyl 3-methylbutyrate ^a	1065	(0.08±0.06) ^B	(0.09±0.05) ^B	(0.07±0.01) ^B	(0.10±0.04) ^B	(0.13±0.08) ^B	(0.22±0.11) ^A
ethyl pentanoate ^c	1132	0.03±0.02	0.03±0.01	0.03±0.01	0.05±0.05	0.03±0.02	0.02±0.01
Total		2.92±0.31	2.90±1.00	3.60±1.29	3.78±1.19	3.09±0.70	3.12±0.23
<i>Ethyl esters of middle-chain fatty acids</i>							
ethyl hexanoate ^a	1236	(5.99±1.65) ^B	(7.15±2.09) ^B	(14.07±5.51) ^A	(8.02±3.82) ^B	(6.86±2.03) ^B	(7.76±1.39) ^B
ethyl heptanoate ^a	1332	(0.10±0.11) ^C	(0.22±0.24) ^{BC}	(0.51±0.43) ^{AB}	(0.70±0.32) ^A	(0.61±0.41) ^A	(0.14±0.13) ^{BC}
ethyl octanoate ^a	1435	(13.49±6.32) ^B	(17.79±12.29) ^B	(36.08±16.58) ^A	(17.19±9.99) ^B	(37.81±10.51) ^A	(44.44±15.84) ^A
ethyl nonanoate ^c	1534	0.27±0.18	0.69±0.44	0.55±0.14	0.77±0.47	0.93±0.40	0.63±0.27
ethyl decanoate ^a	1637	(14.59±8.96) ^B	(21.19±14.61) ^B	(27.20±12.18) ^B	(17.37±8.71) ^B	(55.03±17.01) ^A	(74.33±28.51) ^A
Total		(34.44±15.27) ^D	(47.04±28.63) ^{CD}	(78.41±33.57) ^{BC}	(44.03±21.59) ^{CD}	(101.24±29.40) ^{AB}	(127.32±44.97) ^A
<i>Ethyl esters of long-chain fatty acids</i>							
ethyl dodecanoate ^a	1842	(7.25±2.49) ^B	(10.11±3.86) ^B	(9.95±2.99) ^B	(6.77±2.09) ^B	(22.03±4.26) ^A	(27.67±8.61) ^A
ethyl tridecanoate ^d	1943	(0.02±0.04) ^C	(0.11±0.10) ^A	(0.04±0.02) ^{BC}	(0.02±0.01) ^C	(0.09±0.03) ^{AB}	(0.04±0.02) ^{BC}
ethyl tetradecanoate ^c	2045	(3.15±0.59) ^B	(3.88±0.71) ^B	(3.57±0.29) ^B	(2.75±0.63) ^B	(7.54±0.99) ^A	(8.14±4.23) ^A
ethyl hexadecanoate ^c	2255	(18.60±2.26) ^B	(18.96±4.08) ^B	(13.49±1.52) ^C	(12.82±3.84) ^C	(25.74±5.23) ^A	(21.24±4.78) ^{AB}
ethyl palmitoleate ^c	2276	(1.51±0.35) ^{AB}	(1.52±0.43) ^{AB}	(1.00±0.26) ^B	(0.75±0.40) ^B	(2.28±0.93) ^A	(1.37±0.87) ^B

Table 4. – continued

Aroma compound	RI	γ (monovarietal grape marc distillate)/(mg/L)					
		Malvazija istarska	Chardonnay	Muscat Blanc	Muškat ruža porečki	Teran	Cabernet Sauvignon
ethyl heptadecanoate ^d	2353	(0.12±0.02) ^B	(0.10±0.01) ^{BC}	(0.08±0.02) ^{BC}	(0.06±0.03) ^C	(0.19±0.09) ^A	(0.19±0.05) ^A
ethyl octadecanoate ^c	2454	(0.74±0.07) ^{BC}	(0.67±0.10) ^{BC}	(0.90±0.17) ^{BC}	(0.62±0.17) ^C	(1.32±0.50) ^A	(0.98±0.16) ^B
ethyl oleate ^c	2474	(1.80±0.25) ^B	(1.99±0.98) ^{AB}	(1.24±0.16) ^B	(1.58±0.48) ^B	(2.11±0.63) ^{AB}	(2.92±1.23) ^A
ethyl linoleate ^c	2524	(12.47±1.31) ^A	(10.28±2.95) ^{AB}	(8.26±1.34) ^{BC}	(7.98±1.71) ^{BC}	(10.83±3.26) ^{AB}	(7.15±1.92) ^C
ethyl linolenate ^d	2586	(5.48±0.40) ^A	(5.17±1.13) ^{AB}	(3.92±0.79) ^C	(2.79±0.44) ^C	(4.06±1.57) ^B	(2.71±0.84) ^C
Total		(51.15±1.39) ^{BC}	(52.79±6.67) ^B	(42.45±6.43) ^{CD}	(36.14±4.73) ^D	(76.19±8.88) ^A	(72.42±13.51) ^A
<i>Acetate esters</i>							
ethyl acetate ^e	–	373.91±128.92	497.72±350.39	263.80±33.09	410.36±133.52	517.66±149.08	365.94±103.77
propyl acetate ^c	<1000	(0.48±0.17) ^{AB}	(0.45±0.13) ^B	(0.32±0.09) ^B	(0.29±0.11) ^B	(0.69±0.27) ^A	(0.33±0.15) ^B
isobutyl acetate ^a	1009	0.57±0.23	0.72±0.41	0.41±0.21	0.62±0.23	0.85±0.26	0.58±0.20
isoamyl acetate ^a	1120	1.91±0.59	2.85±2.06	1.77±0.41	3.63±1.88	3.91±1.16	3.62±1.41
hexyl acetate ^a	1272	0.32±0.30	0.40±0.50	0.20±0.11	0.28±0.28	0.08±0.05	0.09±0.03
cis-3-hexenyl acetate ^c	1316	(0.38±0.21) ^{BC}	(0.35±0.12) ^C	(0.37±0.11) ^C	(0.23±0.07) ^C	(0.65±0.33) ^{AB}	(0.81±0.28) ^A
2-phenylethyl acetate ^a	1803	(0.05±0.02) ^C	(0.09±0.06) ^{BC}	(0.05±0.02) ^C	(0.10±0.05) ^{BC}	(0.17±0.03) ^{AB}	(0.20±0.13) ^A
Total		377.62±130.17	502.58±353.60	266.92±33.80	415.50±135.00	524.01±150.09	371.57±105.34
<i>Esters of hydroxy and dicarboxylic acids</i>							
ethyl lactate ^a	1341	29.17±27.31	29.45±37.33	17.76±14.63	3.80±3.72	18.07±9.97	17.51±22.67
isoamyl lactate ^d	1561	0.35±0.22	0.25±0.25	0.27±0.16	0.07±0.16	0.33±0.12	0.21±0.19
diethyl malonate ^c	1570	0.05±0.02	0.04±0.02	0.05±0.03	0.08±0.06	0.11±0.07	0.06±0.03
diethyl succinate ^a	1667	(1.71±0.87) ^B	(2.08±1.50) ^B	(1.36±1.67) ^B	(1.31±0.82) ^B	(8.68±5.52) ^A	(5.99±6.93) ^{AB}
Total		31.28±27.95	31.82±37.96	19.44±16.12	5.26±3.93	27.19±12.96	23.78±29.61
<i>Other esters</i>							
isoamyl butyrate ^c	1266	(0.02±0.00) ^B	(0.02±0.01) ^B	(0.04±0.02) ^A	(0.04±0.02) ^A	(0.03±0.02) ^{AB}	(0.04±0.01) ^A
isobutyl hexanoate ^d	1358	(0.03±0.02) ^B	(0.03±0.01) ^B	(0.07±0.04) ^A	(0.04±0.01) ^{AB}	(0.06±0.03) ^{AB}	(0.03±0.01) ^B
methyl octanoate ^c	1387	(0.35±0.04) ^A	(0.25±0.16) ^{ABC}	(0.29±0.13) ^{AB}	(0.12±0.06) ^D	(0.15±0.08) ^{CD}	(0.19±0.05) ^{BCD}
isoamyl hexanoate ^c	1457	(0.18±0.06) ^C	(0.21±0.12) ^C	(0.57±0.36) ^A	(0.27±0.19) ^C	(0.35±0.15) ^{BC}	(0.47±0.10) ^{AB}
propyl octanoate ^d	1517	(0.05±0.01) ^B	(0.07±0.05) ^B	(0.14±0.08) ^A	(0.03±0.02) ^B	(0.06±0.02) ^B	(0.04±0.01) ^B
isobutyl octanoate ^c	1550	(0.06±0.03) ^C	(0.09±0.05) ^{BC}	(0.19±0.07) ^A	(0.07±0.04) ^{BC}	(0.18±0.07) ^A	(0.13±0.05) ^{AB}
methyl decanoate ^c	1591	(0.67±0.08) ^A	(0.62±0.26) ^{AB}	(0.43±0.14) ^B	(0.21±0.11) ^C	(0.51±0.20) ^{AB}	(0.60±0.09) ^{AB}
isoamyl octanoate ^c	1655	(0.70±0.30) ^C	(0.77±0.49) ^C	(1.24±0.49) ^{BC}	(0.70±0.48) ^C	(1.77±0.77) ^{AB}	(2.41±0.75) ^A
isobutyl decanoate ^d	1753	(0.14±0.05) ^C	(0.24±0.10) ^B	–	(0.08±0.12) ^{CD}	–	(0.52±0.08) ^A
methyl dodecanoate ^c	1798	0.40±0.03	0.43±0.13	0.30±0.07	0.18±0.05	0.16±0.11	0.54±0.57
hexyl octanoate ^c	1807	(0.04±0.02) ^B	(0.06±0.03) ^B	(0.12±0.06) ^A	(0.05±0.04) ^B	(0.03±0.01) ^B	(0.04±0.01) ^B
isoamyl decanoate ^c	1862	(0.36±0.12) ^C	(0.54±0.66) ^C	(0.64±0.35) ^C	(0.50±0.33) ^C	(2.15±1.05) ^B	(3.59±1.51) ^A
isobutyl dodecanoate ^d	1955	(0.04±0.02) ^C	(0.09±0.03) ^B	(0.07±0.01) ^{BC}	(0.04±0.01) ^C	(0.17±0.04) ^A	(0.19±0.04) ^A
butyl decanoate ^d	2008	(0.06±0.03) ^{BC}	(0.07±0.03) ^{BC}	(0.12±0.05) ^{AB}	(0.07±0.05) ^{BC}	(0.05±0.02) ^C	(0.16±0.08) ^A
isoamyl dodecanoate ^d	2063	(0.22±0.11) ^C	(0.35±0.18) ^C	(0.36±0.14) ^C	(0.25±0.14) ^C	(0.81±0.33) ^B	(1.22±0.47) ^A
methyl hexadecanoate ^c	2209	(1.15±0.34) ^A	(0.81±0.27) ^B	(0.34±0.04) ^C	(0.24±0.10) ^C	(0.27±0.13) ^C	(0.25±0.12) ^C
isobutyl hexadecanoate ^d	2365	(0.06±0.02) ^{BC}	(0.09±0.02) ^{AB}	–	(0.05±0.01) ^C	(0.09±0.06) ^{AB}	(0.10±0.02) ^A
methyl linoleate ^c	2415	0.07±0.01	0.07±0.01	0.07±0.01	0.06±0.01	0.07±0.03	0.08±0.02
methyl octadecanoate ^c	2418	(0.04±0.01) ^A	(0.02±0.01) ^B	(0.01±0.00) ^C	(0.01±0.00) ^C	(0.01±0.00) ^C	(0.01±0.00) ^C
Total		(4.63±0.38) ^{BC}	(4.83±1.98) ^{BC}	(5.19±1.32) ^{BC}	(2.98±1.38) ^C	(6.93±2.51) ^B	(10.61±3.10) ^A

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 one-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

^aRetention time consistent with that of the pure standards, mass spectra consistent with pure standards and those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature; ^cmass spectra consistent with those from the NIST05 electronic library, and RI consistent with those found in literature; ^dmass spectra consistent with those from the NIST05 electronic library (tentative identification); ^eretention time consistent with those of the pure standards

Due to their high concentrations in alcoholic beverages and volatility, ethyl esters of middle-chain fatty acids are considered to be very important, contributing positively to the aroma (8,14,24) with pleasant odours reminiscent of fruit (green apple, pear and grapes), and soap (12,13).

Comparing mean values of concentration in monovarietal grape marc distillates, the highest ethyl hexanoate level was determined in Muscat Blanc distillates (Table 4). The concentration found was approx. twofold higher than in other white grape distillates, which were produced under the same conditions. The observed difference was assumed to be a characteristic of Muscat Blanc under the given experimental conditions. Higher average concentrations of ethyl octanoate, and especially ethyl decanoate, were found in red grape marc distillates from Teran and Cabernet Sauvignon, in most cases with statistically significant difference. Strong correlation was observed between total middle-chain acids and the corresponding ethyl esters ($R=0.726$, $N=30$), so it was presumed that the amounts of the latter were significantly predetermined by the content of acid precursors. Like in the case of acids, high negative correlation coefficients between the concentrations of total middle-chain ethyl esters and methanol and 1-hexanol were observed, implying the influence of pressing: -0.897 and -0.495 for Malvazija, -0.509 and -0.763 for Chardonnay, -0.667 and -0.467 for Muscat Blanc, -0.560 and -0.090 for Muškat ruža, -0.087 and -0.382 for Teran, and -0.719 and -0.891 for Cabernet Sauvignon. Presumably higher temperatures of fermentation of red grape mashes of Teran and Cabernet Sauvignon, together with the release (36) and esterification reactions induced by distillation (38), may also have affected the observed differences.

Higher average amounts of ethyl esters of long-chain fatty acids were determined in Teran and Cabernet Sauvignon distillates (Table 4). Again, it was suspected that the observed increase is the combined effect of lower pressure and smaller number of cycles during pressing of the mash, maceration, and higher temperatures of fermentation.

Ethyl esters of long-chain fatty acids usually do not exhibit significant influence on the aroma of alcoholic beverages. Only when present in exceptionally high amounts, these may contribute to odours reminiscent of candle wax and stearin (8,37,39). Esters from this group are also poorly soluble in water, so their elevated concentrations may cause turbidity and flocculation, and therefore be important factors of distillate instability (40). This feature is especially relevant and implies that problems with turbidity may occur in distillates produced by standard red wine vinification technology.

Important group of odorants in alcoholic beverages are acetate esters. Among them ethyl acetate, the ester of ethanol, is ordinarily the most abundant (39). In the majority of samples, the concentration of ethyl acetate (Table 4) was lower than 720 mg/L, which was pointed out by Ferreira *et al.* (14) as the highest at which this ester does not exhibit a negative influence, and does not cause odours that resemble solvent and nail polish remover (3,12,13). At lower concentrations in order of magnitude of those found in this work, the influence of ethyl acetate fruity odour is generally considered positive (12).

No significant differences were determined among concentration mean values found in monovarietal distillates. It is known that the degree of esterification of ethanol and fusel alcohols with acetic acid depends directly on their amounts in the medium (8). Higher acetate levels are usually indicators of microbial spoilage and increased levels of produced acetic acid, and not of varietal origin or standard production technology. This fact is corroborated by very strong correlation determined between total volatile acidity and ethyl acetate concentrations ($R=0.934$, $N=30$).

Among other acetate esters, Teran and Cabernet Sauvignon contained higher concentrations of isoamyl and 2-phenylethyl acetate, and lower concentrations of hexyl acetate, in relation to white grape marc distillates. It was assumed that the mentioned differences are the direct consequence of higher amounts of isoamyl alcohol and 2-phenylethanol, and lower amounts of 1-hexanol in these samples (Table 2). Relatively high positive correlation coefficients were determined among the corresponding acetates and alcohols in 30 distillate samples: for isobutyl acetate/isobutanol 0.603, for isoamyl 0.512, for 1-hexyl 0.900, and for 2-phenylethyl 0.665. Similarly, relatively high coefficients were found between acetate esters and acetic acid 0.881, 0.715, 0.691 and 0.505, respectively. On the other hand, in Teran and Cabernet distillates, higher concentrations of *cis*-3-hexenyl acetate were found despite the fact that these samples contained lower amounts of its precursor *cis*-3-hexen-1-ol.

It was assumed that isoamyl, 2-phenylethyl and *cis*-3-hexenyl acetate contribute more to the aroma of red grape distillates with odours reminiscent of banana, green fruit, and flowers (14,37), and that higher amount of hexyl acetate, carrier of fruity notes (14,37), is characteristic for white grape distillates.

Esters of hydroxy and dicarboxylic acids are formed in significant amounts during malolactic fermentation by lactic acid bacteria (41). In the investigated monovarietal distillates, no statistically significant differences were found among concentration mean values of the majority of esters from this group (Table 4). In the distillates of the same variety, wide ranges of concentration were determined as a result of variations in different parameters which are known to affect the activity of lactic acid bacteria: pH, temperature, ethanol, SO_2 concentration, and concentrations of nutrients such as malic acid (5,41).

The most important ester from this group, ethyl lactate, was often associated with negative characteristics of distillates, mostly due to its odour which resembles rancid butter (42), yeast extract and wet bread (14). The concentrations found in all samples in this work were below the odour perception threshold of 250 mg/L (39). However, the importance of such amounts is not to be neglected because of the indirect influence this ester may exhibit by stabilizing the aroma and mellowing the taste and smell (25,35).

Statistically significant differences were found only in the case of diethyl succinate; notably higher concentrations were found in Teran and Cabernet Sauvignon distillates.

Among other esters, it is worth mentioning higher amounts of esters of hexanoic acid in Muscat Blanc dis-

tillates, higher levels of esters of isobutanol and isoamyl alcohol, and the majority of fatty acids in distillates from Teran and Cabernet Sauvignon (Table 4). It was assumed that their higher amounts are the direct result of higher levels of corresponding alcohol and acid precursors (Tables 2 and 3). High positive correlation coefficients were determined between corresponding esters and isoamyl alcohol: for isoamyl butanoate 0.641, for octanoate 0.645, for decanoate 0.673, and for dodecanoate 0.594. Similarly, high coefficients were determined between acids and their corresponding esters: for hexanoic acid/isobutyl hexanoate 0.594, hexanoic acid/isoamyl hexanoate 0.517, octanoic acid/propyl octanoate 0.503, octanoic acid/isobutyl octanoate 0.769, octanoic acid/isoamyl octanoate 0.857, decanoic acid/isoamyl decanoate 0.675, dodecanoic acid/isobutyl dodecanoate 0.815, and dodecanoic acid/isoamyl dodecanoate 0.867.

Aldehydes

As for the majority of aldehydes, no significant differences were determined among the concentrations of acetaldehyde in distillates from different varieties (Table 5). Factors like fermentation temperature and the amount of SO₂ in marc are some of the possible causes for the observed variability in the concentration (17). Moreover, it is known that significant acetaldehyde concentration may be formed by oxidation of ethanol by acetic acid bacteria in the presence of oxygen (17). In this work, high correlation coefficients were determined between total volatile acidity and acetaldehyde concentration for the majority of varieties: for Malvazija istarska 0.837, for Chardonnay 0.717, for Muscat Blanc 0.982, for Muškati ruža porečki 0.917, and for Teran 0.790. However, acetaldehyde concentration in any of the samples did not surpass the limit of 1200 mg/L, which was reported as the indicator of significant oxidation of ethanol during fermentation (23).

Odours that originate from acetaldehyde resemble hazelnuts, cherry and overripe apples (16,24,25). Taking

into account relatively low odour perception thresholds of this compound of 25, 60 and 80 mg/L, as determined by different authors (42,43), it was concluded that acetaldehyde significantly contributes to the aroma of all investigated distillates, in which up to ten times higher concentrations were found.

The highest concentration of benzaldehyde was determined in Chardonnay distillates. It is directly related to the concentration of benzyl alcohol, which is corroborated by higher concentrations of both compounds in Chardonnay distillates (Tables 2 and 5).

Aldehyde furfural is formed during distillation by acid hydrolysis or by heating of unfermented pentoses and Maillard reactions (44), especially by heating the alembic during distillation by direct flame (45). The highest average concentration of furfural was found in Malvazija istarska distillates, with statistically significant difference. It was assumed that causes for that could be specific grape berry composition and consistency of this variety, as well as excessive pressing of the corresponding marc (19).

Differentiation of monovarietal grape marc distillates by stepwise linear discriminant analysis

For a valid statistical classification and differentiation of grape marc distillates as a result of variety and corresponding production technology, forward stepwise linear discriminant analysis (SLDA) was applied on the data set comprising 30 cases (distillates) and 32 variables (compounds). Included variables were those for which significant differences between mean values of concentration were determined, except methanol, which does not influence the aroma. Applying SLDA, a 100 % correct classification was achieved. Twenty-one variables were selected according to Wilks' lambda criterion (Table 6).

The projection of monovarietal grape marc distillate samples classified according to the variety in two-dimensional space defined by the first two discriminant functions is shown in Fig. 1.

Table 5. Concentration of aldehydes in monovarietal grape marc distillates produced in the region of Istria, Croatia

Aroma compound	RI	\bar{x} (monovarietal grape marc distillate)/(mg/L)					
		Malvazija istarska	Chardonnay	Muscat Blanc	Muškat ruža porečki	Teran	Cabernet Sauvignon
<i>Aldehydes</i>							
acetaldehyde ^e	–	222.90±48.73	214.41±47.78	231.82±51.62	219.18±91.70	299.49±149.60	261.87±120.02
octanal ^c	1286	0.09±0.03	0.07±0.02	0.07±0.02	0.06±0.04	0.06±0.02	0.07±0.02
nonanal ^c	1389	0.30±0.12	0.39±0.10	0.48±0.31	0.44±0.28	0.24±0.10	0.27±0.05
furfural ^a	1451	(3.74±1.66) ^A	(1.34±0.71) ^B	(1.41±0.24) ^B	(0.91±0.52) ^B	(0.81±0.64) ^B	(0.48±0.14) ^B
decanal ^c	1493	0.08±0.02	0.08±0.03	0.08±0.02	0.08±0.04	0.08±0.04	0.09±0.02
benzaldehyde ^a	1508	(0.49±0.14) ^{AB}	(0.64±0.16) ^A	(0.36±0.10) ^B	(0.46±0.13) ^{AB}	(0.55±0.17) ^{AB}	(0.36±0.18) ^B
Total		227.60±49.83	216.94±48.53	234.20±51.94	221.13±91.89	301.24±149.16	263.13±120.12

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 one-way analysis of variance (ANOVA) and least significant difference (LSD) comparison test

^aRetention time consistent with that of the pure standards, mass spectra consistent with pure standards and those from the NIST05 electronic library, and retention indices (RI) consistent with those found in literature; ^cmass spectra consistent with those from the NIST05 electronic library, and RI consistent with those found in literature; ^eretention time consistent with those of the pure standards

Table 6. Standardized coefficients of the variables selected for the differentiation of monovarietal grape marc distillates based on the first two discriminant functions obtained by SLDA

Variable	Root 1	Root 2
ethyl dodecanoate	20.568	0.061
ethyl octanoate	7.210	-1.509
ethyl 2-methylbutyrate	8.781	-0.051
ethyl decanoate	47.055	-1.624
isoamyl hexanoate	-32.618	-1.028
<i>trans</i> -2-hexen-1-ol	-9.328	0.892
ethyl hexadecanoate	13.304	0.884
1-propanol	-6.827	0.071
<i>cis</i> -3-hexen-1-ol	-52.674	2.281
<i>trans</i> -3-hexen-1-ol	-43.019	-2.817
<i>cis</i> -3-hexenyl acetate	-6.756	-1.770
ethyl 3-methylbutyrate	-20.528	-3.791
diethyl succinate	15.452	1.271
isoamyl octanoate	37.318	2.061
isobutyric acid	18.619	2.125
ethyl hexanoate	31.888	2.269
hexanoic acid	-20.440	-1.499
decanoic acid	8.377	0.671
isoamyl alcohol	-6.224	-0.416
isobutyl octanoate	4.057	1.564
2-phenylethyl acetate	2.327	1.120

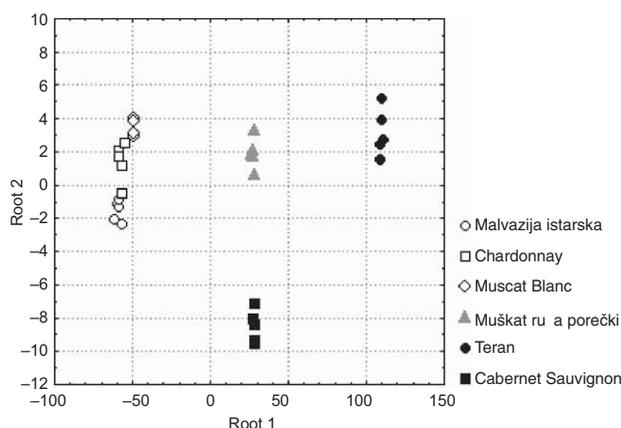


Fig. 1. Projection of monovarietal grape marc distillate samples, differentiated according to the varietal origin, in two-dimensional space defined by the first two discriminant functions

It is obvious that distillates made from white grape varieties were clearly differentiated from the red ones, which was corroborated by the highest Mahalanobis distances determined between them (data not shown). Judging by the obtained standardized coefficients (Table 6), separation of distillates made from red and white grape varieties based on the first function depended on the concentrations of the majority of esters (high positive coefficients) and hexenols (negative coefficients). Differentiation of distillates based on the second function seems to mainly be due to differences in the concentration of ethyl octanoate, ethyl decanoate, *cis*-3-hexen-1-ol, isoamyl octa-

noate, isobutyric acid, and ethyl hexanoate with positive, and *trans*-3-hexen-1-ol and ethyl 3-methylbutyrate with negative scores. White grape distillates were grouped rather close. Clear differentiation between Teran and Cabernet Sauvignon distillates, processed by a similar technology, implies a certain influence of their varietal potentials in terms of aroma precursors, although slight differences in grape quality, maceration conditions (Table 1), and other parameters which were not monitored precisely, could also have had an effect. Muškata ruža samples were grouped between white and red varieties, which again confirmed the influence of production technology as a characteristic inseparable from the variety used for production.

Conclusions

The present investigation, in contrast to laboratory-scale experiments, was set in real conditions of grape marc distillate production, so it was not possible to control and monitor the influence of each individual production parameter. Because of that, it was difficult to draw more precise conclusions about their impact on secondary aroma. However, it can be concluded with certainty that significant differences in the secondary aroma compound composition exist among the investigated monovarietal distillates as a result of variety and corresponding production technology. Marc distillates from red grape varieties Teran and Cabernet Sauvignon were found to contain higher amounts of the majority of fusel alcohols, fatty acids and esters, which ensures them a fuller body, more alcoholic character and more pronounced fruitiness of the aroma in relation to white grape distillates. On the other hand, in distillates produced from white grape varieties Malvazija istarska, Chardonnay, and Muscat Blanc higher and, in some cases, undesirable amounts of C_6 alcohols were found. Malvazija exhibited exceptionally high methanol content, indicating that serious problems may arise in the production of distillates from this variety. Especially interesting were the results for Muškata ruža porečki distillates, the variety ordinarily used for rosé wine production, in which the concentrations of many important volatiles were found to be between those determined in the distillates from white and red grape varieties. It must be kept in mind that the quality of distillates, especially those made from Muscat varieties, may significantly depend on the content of varietal aroma compounds, which were not investigated in this work. However, results of this study showed that characteristic aroma of monovarietal grape marc distillates obtained by traditional distillation does not necessarily originate only from the grapes, but that the applied production technology is also responsible for their specificity and recognizability.

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