ISSN 1330-9862
(FTB-1873)

original scientific paper

Effect of Different Maceration Treatments on Free and Bound Varietal Aroma Compounds in Wine of *Vitis vinifera* L. cv. Malvazija istarska bijela

Sanja Radeka[1]*, *Stanka Herjavec*[2], *Đordano Peršurić*[1], *Igor Lukić*[1] *and Barbara Sladonja*[1]

[1]Institute of Agriculture and Tourism, Karla Huguesa 8, HR-52 440 Poreč, Croatia

[2]Faculty of Agronomy, University of Zagreb, Svetošimunska 25, HR-10 000 Zagreb, Croatia

Received: February 22, 2007
Accepted: July 13, 2007

Summary

During a two-year study, the effect of different grape mash maceration treatments (skin contact at 20 °C for 10, 20 and 30 h) and cryomaceration (skin contact at 7 °C for 10, 20 and 30 h) on the concentration of free and bound monoterpenes and total phenols in Malvazija istarska wine (the most spread white wine variety in Istria) was monitored. Monoterpenic alcohols linalool, α-terpineol, citronellol, nerol and geraniol were determined by gas chromatography after isolation on octadecylsilica (C_{18}) sorbent and elution with pentane/dichlorometane ratio of 2:1, while total phenols were determined spectrophotometrically with Folin-Ciocalteau reagent. Sensory evaluation of wines was performed using the Buxbaum method. The obtained results show that linalool and geraniol are the most abundant monoterpenes in Malvazija istarska, and that grape mash maceration treatments cause significant increase of free and bound monoterpene concentrations compared to those in control treatment wine obtained without maceration. The content of phenols, which are subject to oxidation, was found to be significantly lower in wines produced by cryomaceration treatments, while in those produced by maceration at 20 °C the concentration of total phenols increased proportionally with the duration of maceration, negatively influencing wine quality by causing higher colour intensity and bitter taste.

Key words: Malvazija istarska wines, maceration at 20 °C, cryomaceration, varietal aroma, monoterpenes, phenols

Introduction

Wine aroma is one of the most important parameters that determine wine character and quality, and it depends on the variety, grape maturity, prefermentative and vinification procedures, yeast activity, and wine ageing (*1–3*). *Vitis vinifera* cv. Malvazija istarska bijela is an autochthonous and the most spread cultivar in all vine growing areas of Istria, a viticultural region of Croatia (*4*), and up to now without any investigated varietal aromatic potential. Usually, dry wines with fruity-flowery aroma (descenent mostly from volatile esters) are produced from this grape variety, obtained with fast grape processing and must fermentation at low temperature (*5,6*). A deficiency in the production technology of this white wine is the must and, consequently, the wine is impoverished at the level of primary (varietal) aroma compounds originated from the grapes, prevalently contained in grape berry skin (*7,8*). Several authors underline that terpenic compounds play a significant role in varietal wine aroma because of their characteristic fruity-flowery odour (*1,9–11*). The main representatives of terpenes in grapes and wine are monoterpene alcohols linalool, geraniol, nerol, citronellol and α-terpineol (*10*). Since the majority of

*Corresponding author; Phone: ++385 52 408 339; Fax: ++385 52 431 659; E-mail: sanja@iptpo.hr

monoterpenes is contained in berry skin, type and duration of maceration can significantly influence their concentration in must and wine (*7,12–14*). In vinicultural practice, the most frequently applied are maceration treatments at temperatures from 20 to 25 °C, and cryomaceration with skin contact at 5–8 °C (*7,14,15*). Skin contact at temperatures from 20 to 25 °C leads to increased extraction of the phenolic compounds that increase wine astringency and bitterness (*16–18*), and with time oxidize, creating undesirable odours that suppress varietal aroma, as well as browning of must and wine (*14,15*). On the contrary, grape mash cryomaceration leads to increased extraction of aromatic compounds from berry skin cells, while the additional undesirable extraction of phenolic fraction is reduced to the highest possible degree (*7,12,14*). Namely, cryomaceration low temperatures favour must and wine enrichment with terpenic compounds, and inhibit the activity of oxidative enzymes, which is of crucial importance since cryomaceration can be performed without the addition of sulphur dioxide, which increases solubility, and as a consequence, extraction of phenols from the berry (*7,12, 14*). The aim of the present investigation is to establish whether there are differences in chemical parameters, especially in varietal aroma compound concentrations and total phenols, among Malvazija istarska wines produced without maceration, wines produced with maceration at 20 °C, and those produced with cryomaceration of grape mash. Besides that, the objective is to determine to what extent possible changes of these compounds influence sensory characteristics of the obtained wines.

Materials and Methods

Malvazija istarska bijela grape vinification

The experiment was performed during harvests of 2002 and 2003 with Malvazija istarska bijela grapes (originating from Western Istrian vine growing area) in a minivinification cellar of the Institute of Agriculture and Tourism in Poreč (Istria, Croatia). Maceration treatments were applied at temperature of 20 °C, and cryomaceration at 7 °C, both in durations of 10, 20 and 30 h. Must obtained by separation of liquid fraction from solid cluster parts right after grape crushing (without maceration) was used as a control treatment. Each of the mentioned treatments was performed in 3 repetitions in stainless steel vats of 100-litre volume (a total of 21 vats). Musts from control treatments were treated with 20 g/hL of potassium metabisulphite and sedimented for 24 h at 12 °C. Grape mash of the maceration at 20 °C was treated with 20 g/hL of potassium metabisulphite right after grape crushing, while in cryomaceration grape mash was treated with the same potassium metabisulphite concentrations only after the maceration (right before pressing). Alcohol fermentation of the control treatment must, as well as of musts obtained after maceration treatments, was performed under controlled temperature conditions at 17 °C in stainless steel vats of 100 L with the addition of selected wine yeast *Saccharomyces cerevisiae* VB1, Fermicru, produced by Gist Brocades Chile S.A. (Santiago, Chile). After alcohol fermentation, wines were decanted, subjected to detailed chemical analyses, and sensory evaluation was done 6 months after decantation.

Chemicals

Dichloromethane, pentane, ethanol 96 %, methanol and sodium sulphate, all of *p.a.*, were supplied by Kemika (Zagreb, Croatia). Pure deionized water was obtained from an Elix 3 purification system (Millipore, USA). Octadecylsilica sorbent prepacked in 500 mg/6 mL cartridges (BondElut C_{18}) was obtained from Varian (Harbor City, CA, USA). All monoterpene standards were of *p.a.* purity and were purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Polyvinylpolypyrrolidone (PVPP) was purchased from Merck. Pectolytic enzyme with specific β-glycosidase side activities, Everzym Arom, was purchased from EVER S.r.l. (Pramaggiore, VE, Italy). Folin-Ciocalteau reagent was purchased from Kemika, Zagreb.

Standard chemical wine analyses

Relative density, volume fraction of alcohol, concentration of total extract, total and volatile acidity, reducing sugars, ash, and pH were analyzed by OIV methods (*19*). Total phenols were determined spectrophotometrically with Folin-Ciocalteau reagent (*20*) following the method of Ough and Amerine (*21*) using UV/VIS spectrophotometer (Cary 50, Varian, USA) at wavelength of 760 nm.

Extraction of monoterpene alcohols

Monoterpenes were isolated from wine samples by solid-phase extraction (SPE) using octadecylsilica (C_{18}) sorbent prepacked in Varian Bond Elut cartridges and eluted with pentane/dichlorometane solvent ratio of 2:1 according to Di Stefano (*22*).

A volume of 50 mL of wine with the addition of 1 g of PVPP was stirred on electrical shaker for 10 min, cooled for 20 min, and then centrifuged at 4000 rpm for 15 min. A volume of 25 mL of limpid wine with the addition of 50 μL of internal standard solution (1-nonanol in 40 % ethanol) was passed through 6-mL SPE cartridges containing 500 mL of C_{18} sorbent previously activated with 5 mL of methanol and 5 mL of deionized water. Free monoterpenes were eluted from the sorbent with 7 mL of pentane/dichloromethane (2:1). The obtained extracts were dried over anhydrous sodium sulphate and then concentrated under the stream of nitrogen until the extract volume was reduced to 200 μL, of which 2 μL were directly injected in gas chromatograph. Bound monoterpenes were eluted from the sorbent with 7 mL of methanol, and methanol extracts were evaporated to dryness using rotary evaporator at 50 °C. For enzymatic release of aglycons, 5 mL of buffer solution containing Everzym Arom (5 g/L, pH=5) were added to the extract residue and 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, concentration and injection were performed in the same manner as for the free monoterpenes.

Gas chromatography analysis of monoterpenes

Gas chromatography analyses of linalool, α-terpineol, citronellol, nerol and geraniol were performed on a Varian 3350 gas chromatograph equipped with a split/splitless injector and a flame ionization detector (FID). Sepa-

ration of monoterpenes was done using a 30 m×0.25 mm i.d.×0.25 μm film thickness capillary column Rtx-Wax (Restek, USA).

A volume of 2 μL of pentane/dichloromethane extract was injected with the following parameters: initial oven temperature was 40 °C, then raised at 10 °C/min to 60 °C and kept for 2 min, then it was programmed at 2 °C/min to 176 °C, followed by the increase of 10 °C/min to 240 °C and then kept for 20 min. The injector and detector temperatures were 235 and 245 °C, respectively. The carrier gas was helium at velocity of 1.5 mL/s.

Data elaboration

Statistical analysis was performed using analysis of variance (ANOVA) and *t*-test on significance levels of 95 and 99 %. Aromatic index (expressed as odour unit value, OUV) was calculated as a quotient between individual monoterpenic alcohol concentration in a particular wine and its odour perception threshold found in literature (*10,23,24*).

Sensory evaluation of wine

Sensory evaluation of wine quality was performed by 5 trained judges, members of the Croatian Enological Society, all of them highly experienced in wine sensory testing, especially for Malvazija istarska wines. Evaluation took place at the Institute of Agriculture and Tourism in Poreč 6 months after the first decantation using the Buxbaum positive points method.

Results and Discussion

Basic chemical composition of wine

Results of Malvazija istarska wine basic chemical composition analyses are shown in Table 1, representing the average values of three repetitions.

The analyzed wines did not differ significantly according to the basic chemical composition, which is in accordance with results obtained by Peinado *et al.* (*25*) and Selli *et al.* (*26*). It is important to underline that in Malvazija istarska wines obtained with different maceration treatments, a significant decrease in total acidity was not recorded when compared to control treatment wine, contrary to the results from Gerbi *et al.* (*12*) and Test *et al.* (*27*), but in agreement with the results from Selli *et al.* (*26*) and Arnold and Noble (*28*). This fact is relevant considering the problems with low total acidity this grape variety shows in certain vintages.

Table 1. Basic chemical composition and the concentration of total phenols of Malvazija istarska wines*

Parameter	Year	Control	Maceration at 20 °C			Cryomaceration at 7 °C			LSD
			10 h	20 h	30 h	10 h	20 h	30 h	
Relative density (20/20 °C)	2002	0.9954	0.9958	0.9957	0.9960	0.9954	0.9957	0.9957	n.s.
	2003	0.9923	0.9930	0.9930	0.9933	0.9928	0.9932	0.9929	n.s.
φ(alcohol)/%	2002	12.01	11.76	11.71	11.66	11.70	12.14	11.95	n.s.
	2003	11.48	11.02	11.20	11.10	11.20	11.30	11.20	n.s.
γ(total extract)/(g/L)	2002	27.50	28.47	28.77	28.93	26.80	29.03	27.97	n.s.
	2003	19.73	19.60	20.43	20.07	18.60	21.47	19.20	n.s.
γ(reducing sugars)/(g/L)	2002	2.50	2.53	2.47	2.53	2.17	2.50	2.43	n.s.
	2003	1.80	2.03	1.50	1.70	1.15	2.03	1.50	n.s.
γ(total extract without reducing sugars)/(g/L)	2002	26.00	26.94	27.30	27.40	25.63	27.53	26.54	n.s.
	2003	18.93	18.57	19.93	19.37	18.45	20.44	18.70	n.s.
γ(total acidity as tartaric acid)/(g/L)	2002	7.03	7.33	7.20	7.45	7.27	7.33	7.23	n.s.
	2003	5.10	5.37	5.17	5.30	5.23	5.43	4.83	n.s.
γ(volatile acidity as acetic acid)/(g/L)	2002	0.46	0.50	0.45	0.42	0.52	0.51	0.51	n.s.
	2003	0.43	0.48	0.45	0.40	0.48	0.42	0.45	n.s.
γ(ash)/(g/L)	2002	4.30	4.50	4.53	4.37	4.30	4.45	4.37	n.s.
	2003	2.13	2.10	2.47	2.60	2.43	2.40	2.63	n.s.
pH	2002	3.66	3.56	3.68	3.58	3.60	3.59	3.58	n.s.
	2003	3.63	3.55	3.65	3.63	3.65	3.60	3.65	n.s.
γ(total phenols)/(mg/L)	2002	296.33^{Bc}	315.57^{ABb}	318.13^{ABb}	339.33^{Aa}	298.77^{Bbc}	317.47^{ABb}	331.27^{Aab}	5 %=17.32 1 %=24.29
	2003	284.23^{Bc}	317.08^{ABb}	325.95^{ABab}	353.95^{ABa}	296.70^{Bbc}	312.67^{ABbc}	317.74^{ABb}	5 %=30.49 1 %=42.75

*Capital letters represent significant differences at $p<0.01$ level, and lower case letters represent significant differences at $p<0.05$ level; n.s., not significant difference; LSD, least significant difference

Total phenols

According to the literature data, phenol content in wine is highly dependent on vinification technology, and the advantages of cryomaceration over maceration at higher temperatures have been widely reported (*7*,*12–14*). Namely, low temperature during cryomaceration has an inhibitory influence on oxidative enzymes, enabling maceration process to be performed without the addition of sulphur dioxide, which is responsible for increased solubility of polyphenols, and therefore, their extraction from berry skins. The majority of phenolic compounds in wine originates from the grapes (mostly from seed, skin and stems, while less from juice), and only a small part is produced as a yeast metabolism product (volatile phenols). Consequently, maceration duration and temperature can significantly influence the final total polyphenol concentration in the obtained wines. Results presented in Table 1 show that control treatment wines have the lowest total phenol concentrations and, moreover, that concentration in wines obtained by grape mash maceration increases proportionally to the duration of maceration and temperature, which is in agreement with literature data (*7*,*29*). It is important to emphasize that no increase in total phenols in wines obtained by cryomaceration for 10 h was observed, while maceration treatment at 20 °C of the same duration resulted in significant increase of total phenols.

Free monoterpenes

Free monoterpenes represent a monoterpenic volatile fraction and have a direct influence on wine aroma, as opposed to non-volatile glycosidically bound fraction, which indirectly contributes to varietal aroma profile, since these compounds are odour active only after enzymatic and acid hydrolyses (*30–35*). Concentrations of free monoterpenes in Malvazija istarska wines are presented in Table 2.

From a two-year investigation it can be concluded that mash maceration leads to an increase of total monoterpene concentration in analyzed wines, which is in accordance with literature data (*16*,*36*,*37*). Although a unique pattern of monoterpene increase as a result of maceration temperature and duration was not observed, it is obvious that control treatment wines contained significantly lower concentration of total free monoterpenes in both investigated years.

Results shown in Table 2 suggest that it is possible to obtain a significant increase in free monoterpene con-

Table 2. Concentrations and odour unit values (OUV) of free monoterpenes in Malvazija istarska wines, and the LSD between concentrations found in individual treatment wines*

Compound	Year	Control γ/(μg/L); OUV	Maceration at 20 °C γ/(μg/L); OUV			Cryomaceration at 7 °C γ/(μg/L); OUV			LSD
			10 h	20 h	30 h	10 h	20 h	30 h	
Linalool	2002	40.76^{Bc}	43.76^{ABbc}	46.08^{ABab}	42.60^{ABbc}	48.67^{Aa}	41.38^{Bc}	44.55^{ABabc}	5 %=4.50
		2.72	2.92	3.07	2.84	3.24	2.76	2.97	1 %=6.32
	2003	21.85^{D}	32.71^{Cc}	35.56^{BCc}	42.39^{ABab}	32.96^{Cc}	37.78^{ABCbc}	45.00^{Aa}	5 %=5.34
		1.46	2.18	2.37	2.83	2.20	2.52	3.00	1 %=7.48
α-Terpineol	2002	24.37^{Dd}	28.0^{Cc}	28.97^{BCc}	31.75^{Bb}	27.23^{CDc}	29.44^{BCbc}	36.58^{Aa}	5 %=2.44
		0.10	0.11	0.12	0.13	0.11	0.12	0.15	1 %=3.42
	2003	15.94^{BCb}	25.42^{ABa}	26.38^{Aa}	21.57^{ABCab}	14.96^{Cb}	15.76^{BCb}	20.15^{ABCab}	5 %=7.39
		0.06	0.10	0.10	0.08	0.06	0.06	0.08	1 %=10.36
Citronellol	2002	2.35^{ABbc}	3.40^{Aa}	2.67^{ABab}	2.59^{ABb}	1.73^{Bc}	1.75^{Bc}	2.46^{ABbc}	5 %=0.79
		0.13	0.19	0.15	0.14	0.10	0.10	0.14	1 %=1.10
	2003	6.17^{ab}	4.52^{b}	6.98^{a}	5.55^{ab}	4.42^{b}	5.18^{ab}	6.72^{ab}	5 %=2.31
		0.34	0.25	0.39	0.31	0.24	0.29	0.37	1 %=3.23
Nerol	2002	3.62^{Bc}	4.23^{Bb}	4.25^{Bb}	5.29^{Aa}	2.36^{Cd}	1.74^{Ce}	4.47^{ABb}	5 %=0.61
		0.009	0.01	0.01	0.01	0.006	0.004	0.01	1 %=0.86
	2003	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
Geraniol	2002	15.81^{Cc}	21.62^{BCb}	30.75^{Aa}	21.42^{BCb}	20.72^{BCb}	25.33^{ABb}	24.15^{ABb}	5 %=4.75
		0.53	0.72	1.02	0.71	0.69	0.84	0.80	1 %=6.65
	2003	17.54^{ABb}	23.85^{Aa}	25.53^{Aa}	24.20^{Aa}	17.43^{ABb}	14.97^{Bb}	23.87^{Aa}	5 %=5.84
		0.58	0.79	0.85	0.81	0.58	0.50	0.80	1 %=8.18
Total	2002	86.91^{C}	101.05^{Bb}	112.72^{Aa}	103.64^{ABb}	100.69^{Bb}	99.64^{Bb}	112.20^{Aa}	5 %=6.64
									1 %=9.31
	2003	61.50^{Cd}	86.50^{Ab}	94.46^{Aa}	93.87^{Aa}	69.76^{BCc}	73.64^{Bc}	96.26^{Aa}	5 %=7.52
									1 %=10.55

*Capital letters represent significant differences at $p<0.01$ level, and lower case letters represent significant differences at $p<0.05$ level; n.s., not significant difference; LSD, least significant difference

centrations by application of short-time cryomaceration treatment (10 hours), and to retain total phenol content at the level equal to that in control treatment wines, as indicated in previous section. On the other hand, application of maceration at 20 °C of the same duration also resulted in significant increase of monoterpene content, but simultaneously caused significant increase in total phenol content in relation to the control wines.

Among individual monoterpene alcohols, maceration had the highest influence on linalool, α-terpineol and geraniol content. Linalool had the highest influence on aromatic characteristics of Malvazija istarska, since its concentration in all wine samples exceeded corresponding odour perception threshold of 15 μg/L, as reported by Guth (*23*). Linalool OUV ranged from 1.46 to 3.24, depending on the treatment type, and was generally higher in macerated than in control wines, especially in the year 2003. Geraniol, the odour perception threshold of which is 30 μg/L according to Guth (*23*), was found to be another important monoterpene in Malvazija istarska wines with OUV ranging from 0.5 to 1.02. Odour unit values for other monoterpene alcohols were calculated as quotients between their concentrations in Malvazija istarska wines and corresponding odour perception thresholds found in literature, 250 μg/L for α-terpineol (*24*), 18 μg/L for citronellol (*10*), and 400 μg/L for nerol (*10*). Although concentrations of these monoterpenes in Malvazija istarska wines do not exceed their odour perception thresholds, they positively contribute to wine aroma because of their synergistic and cumulative influence (*10*).

Bound monoterpenes

In the year 2003 the influence of mash maceration on total concentration of bound monoterpenes was more evident than in the previous year of investigation (Table 3). Significantly lower concentration of bound monoterpenes was found in control treatment wine. Wines obtained with skin contact at 20 ºC contained 50–70 %, and wines obtained by cryomaceration had 75–90 % (proportional to the duration of maceration) higher concentrations of bound monoterpenes than control treatment wine.

Since glycosidically bound monoterpenes represent a non-volatile form of monoterpenes, which are considered to be the aromatic potential from which odour-active monoterpenic fraction is released by enzymatic or acid hydrolysis, the above mentioned increase in bound monoterpene concentrations turns out to be of crucial importance. This can explain the fact that wines obtained by grape mash maceration, particularly by

Table 3. Concentrations of bound monoterpenes in Malvazija istarska wines*

Compound	Year	Control γ/(μg/L); OUV	Maceration at 20 °C γ/(μg/L); OUV			Cryomaceration at 7 °C γ/(μg/L); OUV			LSD
			10 h	20 h	30 h	10 h	20 h	30 h	
Linalool	2002	9.36^{Bd}	9.51^{Bcd}	11.29^{ABabc}	10.51^{ABbcd}	12.62^{Aa}	12.17^{Aab}	12.18^{Aab}	5 %=1.79 1 %=2.51
	2003	6.78^{Bc}	9.23^{ABbc}	11.96^{ABabc}	10.27^{ABabc}	13.87^{ABab}	9.61^{ABbc}	15.43^{Aa}	5 %=5.46 1 %=7.66
α-Terpineol	2002	3.31^{B}	2.88^{B}	3.43^{B}	6.24^{A}	3.22^{B}	3.45^{B}	2.92^{B}	5 %=1.08 1 %=1.52
	2003	5.27^{Aa}	0.99^{Bc}	0.81^{Bc}	0.62^{Bc}	4.27^{Ab}	0.77^{Bc}	0.90^{Bc}	5 %=0.74 1 %=1.04
Citronellol	2002	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
	2003	0.76^{AB}	1.48^{A}	0.95^{AB}	0.36^{B}	0.75^{AB}	0.77^{AB}	0.72^{AB}	5 %=0.77 1 %=1.08
Nerol	2002	11.65^{ABCab}	12.70^{ABa}	8.77^{Cc}	13.58^{Aa}	11.91^{ABCab}	9.77^{BCbc}	12.40^{ABa}	5 %=2.33 1 %=3.27
	2003	5.09^{C}	13.31^{ABb}	11.89^{Bb}	13.88^{ABab}	12.41^{ABb}	16.69^{Aa}	14.25^{ABab}	5 %=3.22 1 %=4.51
Geraniol	2002	48.30^{ABbc}	55.80^{Aa}	43.83^{Bc}	50.88^{ABab}	52.21^{Aab}	49.86^{ABb}	52.72^{Aab}	5 %=5.74 1 %=8.05
	2003	28.52^{Cd}	45.87^{ABbc}	43.55^{Bc}	53.35^{ABab}	50.36^{ABabc}	56.04^{Aa}	56.66^{Aa}	5 %=8.49 1 %=11.91
Total	2002	72.62^{ABbc}	80.88^{Aab}	67.29^{Bc}	81.21^{Aa}	79.96^{Aab}	75.24^{ABabc}	80.27^{Aab}	5 %=8.26 1 %=11.58
	2003	46.42^{Bc}	70.88^{Ab}	69.16^{Ab}	78.48^{Aab}	81.67^{Aab}	83.88^{Aab}	87.97^{Aa}	5 %=15.22 1 %=21.34

*Capital letters represent significant differences at $p<0.01$ level, and lower case letters represent significant differences at $p<0.05$ level; n.s., not significant difference; LSD, least significant difference

cryomaceration, have prolonged expiry date, thus maintaining a specific varietal aroma for a longer period of time than control treatment wines. Increase in glycosidically bound monoterpenes, total concentration in wines obtained by Malvazija istarska mash maceration is in accordance with results of Nicolini *et al.* (*13*) for the Prosecco cultivar, and Tamborra (*14*) for the Moscatello selvatico cultivar.

Sensory evaluation of wine

In both years of investigation, wines obtained by mash cryomaceration at 7 °C, which were distinguished by typical varietal aroma and distinctive acacia flower odour in harmony with fruity fermentation odours, were graded with the highest scores by the judges (Table 4).

It was assumed that the complexity of the aroma of these wines is partially a result of increased concentrations of monoterpenes extracted from berry skins. Besides that, it is known that skin contact treatment may induce the increase in extraction of fermentation aroma precursors from berry skins, so the complexity of macerated wine aroma may be partially due to the increment in concentrations of volatile esters, higher alcohols and fatty acids, as reported by Selli *et al.* (*26*) and Cabaroglu and Canbas (*37*). Malvazija istarska wines obtained by cryomaceration were categorized by the judges as the top-quality wines.

Table 4. Results of sensory evaluation of Malvazija istarska wines using the Buxbaum positive points method*

Treatment	Year	
	2002	2003
Control	18.5	18.5
Maceration at 20 °C/10 h	18.2	18.1
Maceration at 20 °C/20 h	17.9	18.0
Maceration at 20 °C/30 h	17.7	17.5
Cryomaceration at 7 °C/10 h	18.8	18.7
Cryomaceration at 7 °C/20 h	18.5	18.7
Cryomaceration at 7 °C/30 h	18.5	18.6

*Reported sensory evaluation scores represent medians of five individual scores

Wines obtained by maceration treatments at 20 ºC were characterized by higher colour intensity and bitterness as a result of increased phenol extraction from berry skins, which is in accordance with literature data (*16–18*), and even though in some cases these wines contained higher amounts of free monoterpenes than those produced by cryomaceration, they were graded with lower sensory evaluation scores. Colour and bitterness intensity in these wines were proportional to mash maceration duration. Wine odour was not pleasant and harmonious as in wines obtained by cryomaceration. Wines produced by maceration treatments at 20 ºC were categorized by judges as quality wines.

Control treatment wines were characterized by a nice lighter yellow colour with pronounced nuance of green. The aroma of this wine was a typical fermentation aroma with accentuated green apple, banana and tropical fruit odour nuances, and light flowery note resembling acacia flower odour. Malvazija istarska wines obtained without maceration were also categorized by the judges as top-quality wines.

Conclusions

From the results of the present investigation, it can be concluded that mash maceration has a significant effect on the increase of free and bound monoterpene concentration, primarily on linalool and geraniol as the most abundant monoterpenes in Malvazija istarska wines. The advantage of cryomaceration treatments over maceration at 20 °C was in a significantly lower content of extracted phenols, which are subject to oxidation and negatively contribute to Malvazija istarska wine quality. It was also observed that it is possible to obtain a significant increase in monoterpene concentrations by application of a short-time cryomaceration treatment (10 h), and to retain total phenol content at the level equal to that in control treatment wines, which was not the case for the maceration at 20 °C of the same duration.

Results of sensory evaluation showed that in both investigation years the best rated wines were those obtained by grape mash cryomaceration. These wines were characterized by a typical varietal aroma with accentuated acacia flower odour, which was assumed to be partially a result of increased extraction of monoterpenes, varietal aroma compounds contained in grape berry skins. On the basis of the obtained results it can be concluded that Malvazija istarska belongs to a cultivar group with significant flowery varietal aromatic potential, which comes to full expression in wines obtained by cryomaceration treatments.

References

1. J.S. Câmara, P. Herbert, J.C. Marques, M.A. Alves, Varietal flavour compounds of four grape varieties producing Madeira wines, *Anal. Chim. Acta*, *513* (2004) 203–207.
2. S.E. Ebeler, Analytical chemistry: Unlocking the secrets of wine flavour, *Food Rev. Int. 17* (2001) 45–64.
3. P. Schreier, Flavor composition of wines: A review, *Crit. Rev. Food Sci. Nutr. 12* (1979) 59–111.
4. Đ. Peršurić, V. Kovačević, D. Gluhić, Influence of bud load and rootstock on characteristics of Istrian Malvasia on Cordon with shortcutting, *12th International GESCO (Groupe d'Etude des Systemes de Conduite de la Vigne) Viticulture Congress*, Montevideo, Uruguay (2003) pp. 183–187.
5. S. Radeka, The quality of Malvazija wine produced from late harvested and raisined grapes, *MSc Thesis*, Faculty of Agronomy, University of Zagreb, Croatia (2001).
6. K. Kovačević Ganić, B. Sladonja, S. Radeka, Đ. Peršurić, D. Gluhić, I. Lukić, G. Cargnello, Aromatical composition of must and wine for the cv. Istrian Malvasia and Chardonnay at different bud loads, rootstock and time of harvest on the spur cordon training system, *14th International GESCO (Groupe d'Etude des Systemes de Conduite de la Vigne) Viticulture Congress*, Geisenheim, Germany (2005) pp. 180–186.
7. T. De Rosa: *Technology of White Wine Production*, Brescia, Italy (1993) pp. 91–95 (in Italian).

8. S. Radeka, Grape mash maceration and varietal aroma of Malvazija istarska wine, *PhD Thesis*, Faculty of Agronomy, University of Zagreb, Croatia (2005).

9. A. Rapp, H. Mandery, Wine aroma, *Experientia, 42* (1986) 873–884.

10. P. Ribérau-Gayon, Y. Glories, A. Maujean, D. Dubourdieu: *The Chemistry of Wine: Stabilization and Treatments, Handbook of Enology, Vol. 2*, John Wiley & Sons Ltd., Chichester, UK (2000) pp. 187–206.

11. P.J. Williams, C.R. Strauss, B. Wilson, Hydroxylated linalool derivates as precursors of volatile monoterpenes of Muscat grapes, *J. Agric. Food Chem. 28* (1980) 766–771.

12. V. Gerbi, G. Zeppa, C. Manara, J.L. Minati, Experience in cold maceration with Muscat grapes, *L' Enotecnico*, *27* (1991) 57–66 (in Italian).

13. G. Nicolini, G. Versini, A. Dalla Sera, P. Barchetti, G. Menini, Experience in vinification with cold maceration in the production of Prosecco wine, *L' Enotecnico, 30* (1994) 75–82 (in Italian).

14. P. Tamborra, Influence of skin-contact on free and glycosidically-bound terpenes in wines cv. Moscatello selvatico, *Rivista di viticoltura e di enologia, 2* (1992) 35–45 (in Italian).

15. P. Berta, Skin maceration for the Muscat, *VigneVini, 17* (1990) 25–30 (in Italian).

16. T. Cabaroglu, A. Canbas, R. Baumes, C.L. Bayonove, J.P. Lepoutre, Y. Gunata, Aroma composition of a white wine of *Vitis vinifera* L. cv. Emir as affected by skin contact, *J. Food Sci. 62* (1997) 680–683.

17. B. Ferreira, C. Hary, M.H. Bard, C. Taisant, A. Olsson, Y. Lefur, Effect of skin contact and setting on the level of the C18:2, C18:3 fatty acids and C6 compounds in Burgundy Chardonnay musts and winnes, *Food Qual. Pref. 6* (1995) 35–41.

18. D. Ramey, A. Bertrand, C.S. Ough, V.L. Singleton, E. Sanders, Effect of skin contact temperature on Chardonnay musts and wine composition, *Am. J. Enol. Vitic. 37* (1986) 99–106.

19. Compendium of International Methods of Analysis of Wine and Musts, Office International de la Vigne et du Vin, Paris, France (1990) (in French).

20. O. Folin, W. Denis, On phosphotungstic–phosphomolybdic compounds as color reagents, *J. Biol. Chem. 12* (1912) 239–243.

21. C.S. Ough, M.A. Amerine: *Methods for Analysis of Musts and Wines*, John Wiley & Sons, New York, USA (1988).

22. R. Di Stefano, Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines, *Bull. OIV, 64* (1991) 219–223.

23. H. Guth, Quantitation and sensory studies of character impact odorants of different white wine varietes, *J. Agric. Food Chem. 45* (1997) 3027–3032.

24. V. Ferreira, R. López, J.F. Cacho, Quantitative determination of the odorants of young red wines from different grape varietes, *J. Sci. Food Agric. 80* (2000) 1659–1667.

25. R.A. Peinado, J. Moreno, J.E. Bueno, J.A. Moreno, J.C. Mauricio, Comparative study of aromatic compounds in two young white wines subjected to pre–fermentative cryomaceration, *Food Chem. 84* (2004) 585–590.

26. S. Selli, A. Canbas, T. Cabaroglu, H. Erten, J.P. Lepoutre, Z. Gunata, Effect of skin contact on the free and bound aroma compounds of the white wine of *Vitis vinifera* L. cv Narince, *Food Control, 17* (2006) 75–82.

27. S.L. Test, A.C. Noble, J.O. Schmidt, Effect of pomace contact on Chardonnay musts and wines, *Am. J. Enol. Vitic. 37* (1986) 133–136.

28. R.A. Arnold, A.C. Noble, Effect of pomace contact on the flavor of Chardonnay wine, *Am. J. Enol. Vitic. 30* (1979) 179–181.

29. S. Selli, A. Canbas, T. Cabaroglu, H. Erten, Z. Gunata, Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment, *Food Chem. 94* (2006) 319–326.

30. R. Di Stefano, Presence of precursors of linalool in Moscato bianco from Piemonte, *VigneVini, 9* (1982) 45–47 (in Italian).

31. Z. Günata, C. Bayonove, R. Baumes, R.E. Cordonnier, The aroma of grapes, I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components, *J. Chromatogr. A, 331* (1985) 83–90.

32. J.J. Mateo, M. Jimenez, Monoterpenes in grape juice and wines, *J. Chromatogr. A, 881* (2000) 557–567.

33. P.J. Williams, C.R. Strauss, B. Wilson, Classification of the monoterpenoid composition of Muscat grapes, *Am. J. Enol. Vitic. 32* (1981) 230–235.

34. P.J. Williams, C.R. Strauss, B. Wilson, R.A. Massy-Westropp, Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines, *Phytochemistry, 21* (1982) 2013–2020.

35. P.J. Williams, C.R. Strauss, B. Wilson, R.A. Massy-Westropp, Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D-glucosides rationalizing the monoterpene composition of grapes, *J. Agric. Food Chem. 30* (1982) 1219–1223.

36. J.E. Bueno, R. Peinado, J. Moreno, L. Moyano, L. Zea, Selection of volatile aroma compounds by statistical and enological criteria for analytical differentation of musts and wines of two grape varietes, *J. Food Sci. 68* (2003) 158–163.

37. T. Cabaroglu, A. Canbas, The effect of skin contact on aromatic composition of the white wine of *Vitis vinifera* L. cv. Muscat of Alexandria grown in Southern Anatolia, *Acta Aliment. 31* (2002) 45–55.