Effects of maceration temperature on the anthocyanins composition of cv. Teran wine

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Summary

The evolution of free anthocyanins during the five different fermentation temperatures (control treatment at 22 °C, 5 days pre-fermentative cryomaceration at 5 °C followed by maceration at 25 °C, maceration at 25 °C, maceration at 30 °C and maceration at 25 °C + heated post-fermentation maceration to 35-40 °C after finished alcoholic fermentation) of Teran wine was studied. Sampling was made at day 5, 10, 15 and three months after the end of maceration during storage time. Anthocyanins were determined by high performance liquid chromatography method coupled with photodiode array detection (HPLC-PDA). The results showed that the modification of the temperature conditions during maceration increased the anthocyanin concentration for pre-fermentative cryomaceration and maceration at 25 °C, while the maceration at higher temperatures showed a decrease in the anthocyanin concentration in young Teran wine. Quantitatively, malvidin derivatives were the most abundant anthocyanin compounds in all studied wines. Malvidin-3-glucoside was the major anthocyanin in Teran wine, with concentrations up to 138.59±1.22 mg/L and 76.93±0.36 mg/L for C treatment in 2008 and 2009 vintage, respectively. Results of this study indicate that five days pre-fermentative cryomaceration at 5 °C followed by maceration at 25 °C is most appropriate in order to achieve maximum anthocyanin concentration in Teran wines.

Keywords: maceration, temperature, cryomaceration, anthocyanins, Teran

Introduction

Phenolic compounds greatly influence the organoleptic attributes of wine related to the extraction from grape skins during maceration. The extraction of anthocyanins from the grapes into the wine is therefore the main diffusion process affecting the colour of red wine (Gómez-Plaza et al., 2001; Moreno-Arribas et al., 2009). The rate and extent of extraction are influenced by concentration of anthocyanins, its localization in the berry, and processing methods where temperature and duration of maceration are determining factors (Romero-Cascales et al., 2005; Sacchi et al., 2005). Changing the temperature during processing is an effective method that influences extraction because temperature affects the permeability of the cells and membranes in grape berries (Koyama et al., 2007).
Several studies have studied the effect of the maceration temperature on red wine style and anthocyanin composition (Gil-Muñoz et al., 1999; Gómez-Míguez et al., 2007; Budić-Leto et al., 2008). Amerine (1955) found that among a range of fermentation temperatures, colour and flavour were best in both 21 °C and 27 °C treatments. More recently (Girard et al., 1997) showed that fermentations at 30 °C produced wines with a higher colour density and flavour than fermentations at 20 °C and increased anthocyanin content (Gao et al., 1997). Elevating fermentation temperature has frequently been used in varieties (such as Pinot noir) for which colour extraction is not appropriate (Reynolds et al., 2001). In recent years, low maceration temperatures (5-15 °C) prior to fermentation are also being tested in order to improve the extraction of pigments, tannins and aromas from the grape skins to the wine resulting more coloured and less brown wines (Goumy, 1996). The extraction of these compounds takes place in the absence of ethanol because these low maceration temperatures prevent yeasts from starting the fermentation process. Heatherbell et al. (1997) demonstrated that the use of cold soak to elaborate Pinot Noir wines, increased phenolic and anthocyanin content but reduced berry aromas and increased tobacco aroma and bitterness. Changing fermentation temperature has also been used to optimize the quality of red wines, for instance, increasing fermentation temperature from 12 °C to either 20 °C or 30 °C in Cabernet Sauvignon increased both colour and tannin extraction (Ough et al., 1960).

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Pre-fermentation pomace contact ("cold soak") and extending the maceration period past the end of fermentation are techniques traditionally used in France to improve colour in red wine. Reynolds et al. (2001) combined pre-fermentation cold soak and post-fermentation maceration with increasing fermentation temperature to produce Syrah wines. Several studies have shown that maximum anthocyanin extraction of red *Vitis vinifera* wines occurred early in fermentation (between 3 and 8 days of skin maceration) and then show a decrease (Sacchi et al., 2005). Gao et al. (1997) published on Pinot Noir the decline of anthocyanins after early fermentation, with roughly a 75 % decrease by bottling. He also reported that at the same time anthocyanins were decreasing, polymeric pigment was increasing, and that the formation of polymeric pigment increased greatly when the fermentation temperature was increased from 20 to 30 °C.

Teran is native red grapevine variety grown in Istria region, Croatia. Nowadays, the enhancement of Teran's wine anthocyanin concentration and more intense fruit and floral aromas has become the aim of many producers who wish to achieve high quality Teran wine with greater colour and flavour intensity. The influence of different maceration temperatures on anthocyanin concentration of wines produced in several wine regions has been extensively studied by many researchers (Romero-Cascales et al., 2005; Sacchi et al., 2005), but few data are available on Croatian red wines (Budić-Leto et al., 2008; Maletić et. al., 2009). However, there are no data on the influence of maceration temperature on the content and anthocyanin profile of Teran wine. The aim of the present work was to study the influence of different maceration temperatures on anthocyanin extraction and sensory properties of Croatian native red grape variety Teran. Maceration temperatures were the following: control treatment at 22 °C, 5 days pre-fermentative cryomaceration at 5 °C followed by maceration at 25 °C, maceration at 25 °C, maceration at 30 °C and maceration at 25 °C with heated post-fermentation maceration to 35-40 °C after finished alcoholic fermentation.

Materials and methods

Grapes and winemaking procedure

Experiments were carried out with *cv.* Teran (*Vitis vinifera*, L.) grapes harvested in two consecutive years (2008 and 2009) in good sanitary conditions from a seven years old vineyard at "Roxanich" estate located seven kilometres east from Poreč (Western Istrian vineyard, Croatia). The degree of grape ripeness was monitored by standard chemical analyses. Average harvest data were 86°Oe, 9.7 g/L titratable acidity (as tartaric), pH 3.09 and 84°Oe, 9.3 g/L titratable acidity, pH 3.12 for 2008 and 2009 vintage, respectively. The soil was the typical red Mediterranean soil (*Terra rossa*). Row and vine spacing were 2.8 x 1 m. Standard viticultural practices for the cultivar and region were performed during vineyard management. The experiments were set up in the experimental micro-vinification cellar of Agricultural department, Professional Study of Enology, Poreč. All the vinifications were done in duplicate using 130 L stainless steel jacketed fermentors with 5 cases of grapes (corresponding to 100 L of whole must). Grape harvesting was done manually in plastic cases (22 kg capacity). A random distribution of harvested grape cases among the different experiments was done to avoid any initial uncontrolled difference in grape composition. The temperature was controlled by chillers and heaters under five different treatments during the 25 day maceration, as shown in Table 1. Five skin maceration temperatures were assayed: control treatment at 22 °C (K), 5 days pre-fermentative cryomaceration at 5 °C followed by maceration at 25 °C (C), maceration at 25 °C (M25), maceration at 30 ˚C (M30) and maceration at 25 °C + heated post-fermentation maceration to 35-40 °C after finished alcoholic fermentation (MG25).

Table 1. Maceration protocol scheme for *cv.* Teran

Vinification*	Maceration duration / day	Maceration temperature / °C
K	25	average 22
C	25	5 (5 days) + 25
M25	25	25
M30	25	30
MG25	25	25 (fermentation) + heating 35-40

*Control (K), cryomaceration (C), maceration at 25 °C (M25), maceration at 30 °C (M30) and maceration at 25 °C and post-maceration heating between 35 - 40 °C (MG25)

The control wines (K) were vinified with standard red vinification treatment without any further treatment (see below). After the initial cooling to 5 °C for 5 days with dry ice the pre-fermentative cryomaceration (C) temperature was brought to 25 °C by means of heat exchangers and a standard red winemaking protocol was followed. After finished alcoholic fermentation (9th and 11th day for 2008 and 2009 respectively) at a moderate temperature MG25 wines were heated between 35-40 °C by means of heat exchangers until the 25th day. The grapes were destemmed, 25 mg/L SO_2 was added at crush and the pomace was transferred into ten stainless steel tanks for 25 day maceration. Each tank was inoculated with *S. cerevisiae* strain Uvaferm 299 (Lallemand Corp., Montreal, Canada). The tanks (K, M25, M30 and MG25) were supplemented with inactivated yeast just after sulfitation, while for the tanks subjected to cold maceration (C) the addition of inactivated yeasts was done after the cryomaceration, when the temperature reached 16 °C. During a 25 day skin contact the cup was punched down and mixed twice a day to encourage the extraction of phenolic compounds. Each wine received an additional 25 mg/L SO_2 at the end of maceration and prior to pressing. Each lot was pressed separately. After 10 days the wine was first racked and SO_2 (25 mg/L) was added. Finally, after three months period of maturation the wine was stored in 750 ml glass bottles with cork closures at cellar temperature and the remainders of the lots were racked to 225 L French oak barrels (2 usage years) for standard commercial aging. Any other operation not described above was done likewise during all experiments to avoid possible biases.

Must and wine samples

Must and wine samples (300 ml) were collected eight times from each replicate, at the beginning of the alcoholic fermentation, every 5th day during the maceration period, after pressing and three months after the end of maceration during storage time. All the samples were frozen until the moment of the analysis.

Standard grape analysis

The analytical methods recommended by O.I.V. (2005) were used to determine sugar content, titratable acidity and the pH of the grapes.

HPLC-PDA detection of free anthocyanins

The free anthocyanins content was determined with HPLC-PDA according to method of Berente et al. (2000). The wine samples were filtered through a 0.45 μm filter (Nylon Membranes, Supelco, Bellefonte, USA) before the HPLC analysis. Twenty microliters of each sample were injected for HPLC analysis using a Varian Pro Star Solvent Delivery System 230 (Varian, Walnut Creek, USA) and a Photodiode Array detector Varian Pro Star 330 (Varian, Walnut Creek, USA) using a reversed-phase column Pinnacle II C-18 column (Restek, USA) (250x4.6mm, 5μm i.d.). The following mobile phases were used: buffer: 10mM KH_2PO_4+H_3PO_4 to pH 1.6, solvent A: acetonitrile-buffer (5:95), solvent B acetonitrile-buffer (50:50). The oven temperature was 50 °C. Gradient elution was applied at 1 ml/min flow-rate according to the program which described by Berente et al. (2000). Chromatograms were recorded at 518 nm. Detection was performed with a Photodiode Array Detector by scanning between 200-600 nm, with a resolution of 1.2 nm. Individual anthocyanins were identified by comparing their retention times and visible spectra with those of authentic standards. Quantitative determinations were performed using standard curves of malvidin-3-O-glucoside (Polyphenols, Sandnes, Norway). The data acquisition and treatment were conducted using the Star Chromatography Workstation Version 5 software. All analyses were repeated three times, and the results were expressed as mean values in milligrams per litre of wine ± SD.

Sensory evaluation

The wines were subjected to sensory evaluation 4 months after the maceration, in February 2009 and February 2010 using five wine experts according to the 100-point O.I.V. / U.I.O.E method. In order to study the effect of different temperature maceration on sensory properties, tentative sensory attributes were selected by roundtable discussion.

Statistical analysis

All measurements were carried out in triplicate and the results were statistically analysed using the Statistica 6.0 program to determine the average value and standard error. ANOVA (two-ways, $p \leq 0.05$) and Duncan's multiple range test were performed to determine the influence of different maceration temperatures on anthocyanin extraction of red grape variety Teran.

Results and discussion

Changes of the concentration of total anthocyanins in control (K), cryomaceration (C), maceration at 25 °C (M25), maceration at 30 °C and post-maceration heating between 35-40 °C (MG25) during winemaking and maturation process for 2008 and 2009 vintage respectively are shown in Fig. 1 and 2. The concentration of total anthocyanins increased rapidly to a maximum at day 10 for K and MG25 treatment, and at day 15 for C treatment in both consecutive years. For M25 and M30 maximum was in 2008 at day 10 and in 2009 at day 5. The concentration of anthocyanins then decreased gradually thereafter throughout the remainder of fermentations, which is in agreement with other studies (Sacchi et al., 2005; Koyama et al., 2007). The decrease in the levels of anthocyanins after reaching the maximum could be due to fixation of the compounds on yeast cell walls or precipitation in the form of colloidal material together with tartaric salts, hydrolysis reactions (enzymatic deglycosilation), formation of more stable polymers by copigmentation as well as condensation reactions with other phenols (Moreno-Arribas et al., 2009).

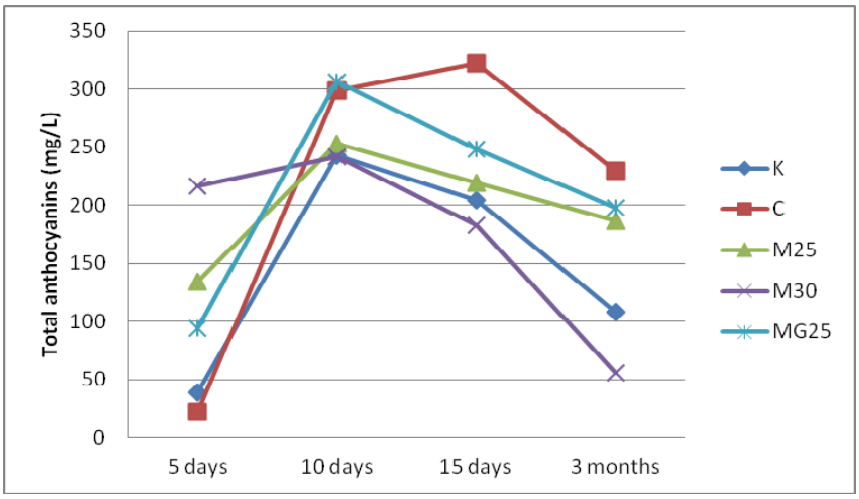

Fig. 1. Changes of the content of total anthocyanins in control (K), cryomaceration (C), maceration at 25 °C (M25), maceration at 30 °C and post-maceration heating between 35-40 °C (MG25) during winemaking and maturation process for 2008 vintage

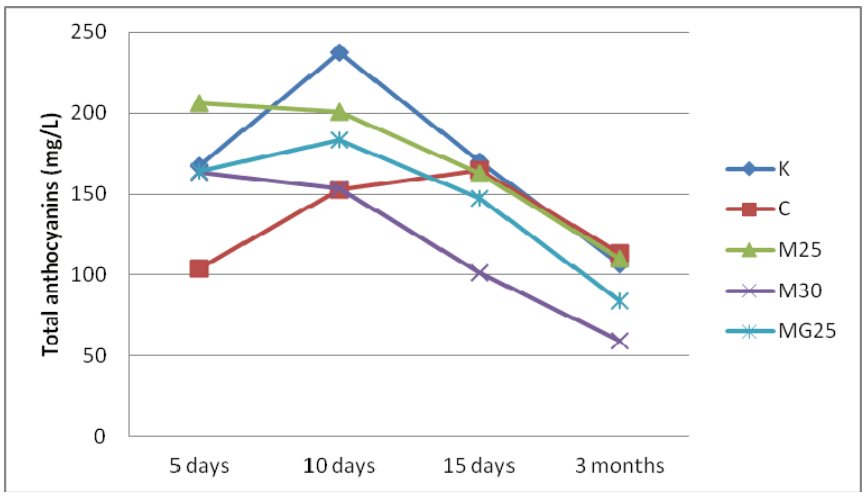

Fig. 2. Changes of the content of total anthocyanins in control (K), cryomaceration (C), maceration at 25 °C (M25), maceration at 30 °C and post-maceration heating between 35-40 °C (MG25) during winemaking and maturation process for 2009 vintage

The concentration of the main individual anthocyanins after 5, 10 and 15 days of maceration, for 2008 and 2009 vintage respectively is shown in Tables 2-7.

Table 2. Anthocyanin concentration in Teran wine after 5 days of maceration in 2008 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	0,48±0,09	0,22±0,05	0,40±0,10	4,99±0,36	0,56±0,16
Cy-3-Gl	0,50±0,11	0,03±0,00	0,11±0,02	0,56±0,12	0,15±0,03
Pt-3-Gl	0,63±0,09	0,25±0,05	0,30±0,09	11,47±0,19	0,67±0,10
Pn-3-Gl	2,26±0,10	0,47±0,10	6,15±0,13	8,92±0,23	4,87±0,10
Mv-3-Gl	20,90±1,22	14,04±0,32	87,13±0,30	128,84±1,15	59,29±0,35
Df-3-Gl-Ac	0,39±0,11	0,03±0,01	0,29±0,06	2,85±0,26	0,96±0,05
Cy-3-Gl-Ac	0,16±0,05	0,03±0,01	0,10±0,02	1,25±0,36	0,13±0,01
Pn-3-Gl-Ac	1,21±0,10	0,92±0,16	2,51±0,14	2,28±0,32	1,81±0,20
Mv-3-Gl-Ac	5,61±0,23	3,98±0,35	20,04±0,31	26,89±0,24	13,95±0,30
Pn-3-Gl-Cm	0,47±0,11	0,37±0,11	1,26±0,10	1,78±0,36	1,12±0,14
Mv-3-Gl-Cm	2,23±0,16	1,50±0,41	15,64±0,50	26,80±0,37	10,69±0,41
Sum of glucosides	24,77±0,11	15,00±0,22	94,09±0,25	154,77±0,30	65,53±0,20
Sum of acetates	7,37±0,20	4,96±0,13	22,94±0,10	33,27±0,16	16,84±0,15
Sum of coumarates	2,70±0,13	1,88±0,26	16,90±0,31	28,58±37	11,81±24
Total	34,84±0,22 d	21,84±0,18 e	133,93±0,23 b	216,62±0,33 a	94,18±0,26 c

The values followed by the same letter (a, b, c, d, e) indicate that they are not significantly different ($p \leq 0,05$).

Df, Cy, Pt, Pn and Mv-3-Gl, respectively: delphinidin, cyanidin, petunidin, peonidin and malvidin-3-monoglucoside, respectively.

Df, Cy, Pn and Mv-3-Gl-Ac, respectively: delphinidin, cyanidin, peonidin and malvidin-3-monoglucoside-acetate, respectively.

Pn and Mv-3-Gl-Cm, respectively: peonidin and malvidin-3-monoglucoside-*p* -coumarate, respectively.

Table 3. Anthocyanin concentration in Teran wine after 10 days of maceration in 2008 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	7,43±0,20	9,18±0,22	8,31±0,26	10,25±0,22	11,22±0,20
Cy-3-Gl	0,92±0,11	1,21±0,10	1,07±0,15	1,05±0,19	1,79±0,15
Pt-3-Gl	14,39±0,36	17,17±0,30	15,24±0,32	17,02±0,35	18,26±0,30
Pn-3-Gl	9,04±0,25	13,02±0,21	11,26±0,15	10,80±0,11	12,30±0,09
Mv-3-Gl	142,21±1,23	170,74±1,26	141,74±1,10	140,01±1,23	170,43±1,29
Df-3-Gl-Ac	3,58±0,15	3,97±0,10	3,30±0,16	3,77±0,10	3,80±0,16
Cy-3-Gl-Ac	1,29±0,32	1,72±0,30	1,59±0,32	1,32±0,30	2,02±0,36
Pn-3-Gl-Ac	5,15±0,37	5,62±0,31	5,63±0,39	2,29±0,35	7,73±0,32
Mv-3-Gl-Ac	31,87±1,01	38,12±1,11	32,73±1,01	27,78±0,99	41,45±1,23
Pn-3-Gl-Cm	0,58±0,14	0,75±0,10	3,08±0,16	1,39±0,21	2,28±0,25
Mv-3-Gl-Cm	26,29±0,45	37,73±0,40	29,20±0,45	26,07±0,40	34,51±0,42
Sum of glucosides	174,00±0,82	211,31±0,73	177,61±0,67	179,14±0,81	214,00±0,85
Sum of acetates	41,89±0,24	49,43±0,31	43,25±0,33	35,16±0,31	55,00±0.30
Sum of coumarates	26,88±33	38,47±0,27	32,28±29	27,45±0,32	36,79±0,32
Total	242,76±0,49 b	299,21±0,55 a	253,15±0,33 b	241,76±0,46 b	305,79±0,45 a

The values followed by the same letter (a, b) indicate that they are not significantly different ($p \leq 0,05$).

Table 4. Anthocyanin concentration in Teran wine after 15 days of maceration in 2008 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	5,98±0,26	11,80±0,21	6,89±0,26	7,23±0,28	7,02±0,29
Cy-3-Gl	0,79±0,16	1,65±0,19	1,18±0,22	0,84±0,20	1,00±0,19
Pt-3-Gl	11,80±0,32	21,02±0,30	12,88±0,31	12,63±0,31	14,00±0,35
Pn-3-Gl	8,13±0,20	16,09±0,22	10,75±0,20	8,47±0,25	10,43±0,20
Mv-3-Gl	138,16±2,14	184,01±2,01	129,58±2,12	109,22±2,01	151,21±2,11
Df-3-Gl-Ac	2,77±0,28	4,64±0,25	3,01±0,20	3,00±0,26	2,92±0,25
Cy-3-Gl-Ac	1,50±0,39	2,42±0,30	1,62±0,32	0,98±0,30	1,42±0,32
Pn-3-Gl-Ac	4,42±0,12	3,19±0,10	4,52±0,13	2,10±0,15	1,86±0,10
Mv-3-Gl-Ac	29,17±0,36	41,20±0,31	27,38±0,28	20,58±0,21	31,00±0,25
Pn-3-Gl-Cm	1,26±0,52	1,79±0,50	1,29±0,45	1,10±0,40	2,63±0,43
Mv-3-Gl-Cm	0,81±0,09	34,38±0,11	20,21±0,16	17,01±0,19	24,59±0,25
Sum of glucosides	164,86±0,43	234,58±0,47	161,29±0,53	138,40±0,55	183,67±0,60
Sum of acetates	37,86±0,11	51,45±0,15	36,53±0,17	26,66±0,20	37,21±0,21
Sum of coumarates	2,07±0,25	36,17±27	21,50±0,26	18,11±0,28	27,22±0,25
Total	204,78±0,30 c	322,20±0,33 a	219,32±0,32 c	183,17±0,39 d	248,10±0,46 b

The values followed by the same letter (a, b, c, d) indicate that they are not significantly different ($p \leq 0,05$).

Table 5. Anthocyanin concentration in Teran wine after 5 days of maceration in 2009 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	8,26±0,26	9,04±0,20	9,32±0,25	7,66±0,20	8,54±0,27
Cy-3-Gl	1,41±0,31	2,07±0,39	1,69±0,35	1,36±0,30	1,00±0,34
Pt-3-Gl	13,29±0,28	15,34±0,20	18,85±0,25	13,27±0,24	14,77±0,20
Pn-3-Gl	17,39±0,29	12,08±0,21	18,85±0,29	13,94±0,21	13,57±0,29
Mv-3-Gl	100,00±0,15	48,99±0,19	125,98±0,56	98,01±0,58	99,96±0,50
Df-3-Gl-Ac	1,06±0,09	0,66±0,11	0,29±0,10	1,05±0,16	0,23±0,19
Cy-3-Gl-Ac	2,13±0,52	1,68±0,50	1,49±0,51	2,70±0,45	1,02±0,40
Pn-3-Gl-Ac	3,24±0,32	6,61±0,30	4,80±0,39	3,28±0,30	2,86±0,36
Mv-3-Gl-Ac	19,83±0,19	6,61±0,19	22,28±0,21	19,73±0,29	20,74±0,24
Pn-3-Gl-Cm	0,58±0,12	0,29±0,16	0,90±0,17	0,28±0,10	0,24±0,16
Mv-3-Gl-Cm	0,34±0,09	0,29±0,10	1,53±0,19	1,97±0,10	1,34±0,19
Sum of glucosides	140,35±0,55	87,52±0,62	174,69±0,71	134,25±0,68	137,84±0,59
Sum of acetates	26,27±0,21	15,55±0,25	28,87±0,22	26,76±0,20	24,85±0,25
Sum of coumarates	0,92±10	0,58±0,11	2,43±0,25	2,26±0,11	1,58±0,18
Total	167,54±0,31 b	103,65±0,35 c	205,99±0,31 a	163,27±0,38 b	164,26±0,37 b

The values followed by the same letter (a, b, c) indicate that they are not significantly different ($p \leq 0,05$).

Table 6. Anthocyanin concentration in Teran wine after 10 days of maceration in 2009 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	10,90±0,28	6,37±0,20	10,29±0,25	7,04±0,22	8,60±0,26
Cy-3-Gl	2,04±0,12	0,92±0,19	1,54±0,24	1,43±0,07	1,24±0,16
Pt-3-Gl	20,36±0,35	12,86±0,30	18,60±0,36	12,39±0,31	15,26±0,36
Pn-3-Gl	23,28±0,10	13,78±0,19	19,63±0,27	13,50±0,26	15,11±0,21
Mv-3-Gl	141,85±0,89	94,56±0,80	121,10±0,89	93,39±0,89	113,27±0,79
Df-3-Gl-Ac	1,32±0,23	0,31±0,20	0,26±0,25	1,02±0,20	1,17±0,26
Cy-3-Gl-Ac	3,44±0,45	0,82±0,40	1,11±0,41	2,30±,43	2,59±0,40
Pn-3-Gl-Ac	4,69±0,78	2,27±0,72	4,69±0,70	2,99±0,72	3,72±0,70
Mv-3-Gl-Ac	26,84±0,36	18,47±0,30	21,14±0,36	17,77±0,30	20,51±0,36
Pn-3-Gl-Cm	1,25±0,58	0,29±0,01	1,07±0,56	0,39±0,15	1,00±0,50
Mv-3-Gl-Cm	1,09±0,23	1,76±0,23	1,14±0,20	0,93±0,26	0,59±0,20
Sum of glucosides	198,44±0,71	128,48±0,56	171,16±0,52	127,76±0,59	153,49±0,46
Sum of acetates	36,29±0,25	21,86±0,25	27,20±0,29	24,07±0,33	28,00±0,30
Sum of coumarates	2,35±0,33	2,06±0,16	2,21±0,31	1,32±0,25	1,58±0,35
Total	237,08±0,38 a	152,40±0,40 d	200,56±0,41 b	153,15±0,41d	183,07±0,46 c

The values followed by the same letter (a, b, c, d) indicate that they are not significantly different ($p \leq 0,05$).

Table 7. Anthocyanin concentration in Teran wine after 15 days of maceration in 2009 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	6,08±0,11	8,21±0,15	6,99±0,17	4,16±0,21	6,44±0,24
Cy-3-Gl	1,70±0,25	1,38±0,20	1,63±0,22	0,64±0,20	0,97±0,26
Pt-3-Gl	12,89±0,12	14,90±0,16	13,53±0,18	7,75±0,21	11,89±0,24
Pn-3-Gl	16,93±0,13	15,23±0,14	16,13±0,19	7,50±0,23	12,14±0,20
Mv-3-Gl	105,75±0,78	101,56±0,70	100,38±0,72	64,35±0,70	93,70±0,72
Df-3-Gl-Ac	0,87±0,36	0,43±0,35	0,30±0,29	0,96±0,25	0,86±0,20
Cy-3-Gl-Ac	1,92±0,14	1,09±0,10	1,04±0,15	0,58±0,20	1,42±0,25
Pn-3-Gl-Ac	2,87±0,25	1,79±0,23	3,53±0,20	2,23±0,24	2,80±0,20
Mv-3-Gl-Ac	18,76±0,13	18,41±0,10	17,10±0,19	12,14±0,24	16,06±0,21
Pn-3-Gl-Cm	1,44±0,36	0,63±0,32	1,23±0,38	0,86±042	0,92±0,35
Mv-3-Gl-Cm	0,62±0,01	1,07±0,05	0,52±0,09	0,44±0,15	0,30±0,10
Sum of glucosides	143,35±0,49	141,28±0,58	138,66±0,65	84,39±0,66	125,14±0,49
Sum of acetates	24,41±0,26	21,72±0,20	21,98±0,19	15,91±0,24	21,14±0,26
Sum of coumarates	2,06±0,11	1,70±0,17	1,75±0,21	1,30±0,24	1,22±0,11
Total	169,82±0,25 a	164,71±0,31a	162,39±0,32 a	101,61±0,41c	147,49±0,31b

The values followed by the same letter (a, b, c) indicate that they are not significantly different ($p \leq 0,05$).

Quantitatively, monoglucosides were predominant in all studied wines (70 – 72 % and 83 – 84 %), followed by acetates (15 – 18 % and 14 – 15 %), and *p*-cumarats (12 – 14 % and 1 – 2 %), for 2008 and 2009 vintage, respectively. The presence and relative abundance of anthocyanins acylated with acetic and *p*-coumaric acids seems to depend on the variety which is related to the grape's acetyl and cinnamoyl transferase activities. The grape variety determines the production of each of these enzymes since they are direct expressions of the genome (Moreno-Arribas et al., 2009). Acylated anthocyanins reached the maximum on the same day when the maximum anthocyanin concentration was reached, but they could be found throughout all the post-fermentation standing. Quantitatively, malvidin derivates were the most abundant anthocyanin compounds in all studied wines. Malvidin-3-glucoside was the major anthocyanin in Teran wine, with concentrations up to 184.01±2.01 mg/L and 141.85±0.89 mg/L for C treatment in 2008 and for K treatment in 2009 vintage, respectively. Malvidin-3-glucoside was the predominant constituent and its percentage to overall amount increased throughout the period studied, maximally accounting for 60 – 64 % of the overall amount in 2008 vintage and for 65 – 67 % in 2009 vintage in Teran wine, while cyanidin-3-glucoside occurred in the lowest proportions.

The cold soak limited the initial rise in the anthocyanin concentration, showing lower concentrations than those in other treatments. The retardation in the extraction was probably due to lower ethanol concentration during the early stage of maceration (Koyama et al., 2007). Canals et al. (2005) found a significant influence of the ethanol concentration on the extrability of anthocyanins. Extending the maceration period after the end of fermentation, the anthocyanin concentration was either similar to or rather higher from other treatments, like in 2008 vintage with concentrations up to 322.20±0.33 mg/L. In this study heating at the end of fermentation (13 and 14 days, in 2008 and 2009, respectively) increased anthocyanin extraction, especially in 2008, with concentrations up to 210.56±0.36 mg/L after 25 days of maceration. Heat treatment damages grape cell membranes, which results in an increased extraction of anthocyanins.

Anthocyanin content in Teran wine after 3 months for 2008 and 2009 vintage respectively, is shown in Tables 8 and 9.

Table 8. Anthocyanin concentration in Teran wine after 3 months of ageing for 2008 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	3,73±0,23	7,36±0,23	6,04±0,20	1,75±0,22	5,29±0,25
Cy-3-Gl	0,79±0,19	1,32±0,15	0,89±0,17	0,29±0,18	0,68±0,19
Pt-3-Gl	7,32±0,36	15,09±0,32	11,78±0,30	3,82±0,32	11,62±0,30
Pn-3-Gl	5,50±0,37	12,34±0,16	8,31±0,20	2,45±0,25	9,13±0,25
Mv-3-Gl	65,82±0,45	138,59±1,22	111,17±1,25	34,56±0,12	127,92±1,30
Df-3-Gl-Ac	2,21±0,12	1,65±0.24	1,05±0,20	0,54±0,16	1,04±0,21
Cy-3-Gl-Ac	0,69±0,11	0,14±0,05	0,18±0,04	0,09±0,03	0,12±0,05
Pn-3-Gl-Ac	2,15±0,10	5,01±0,14	4,70±0,20	1,21±0,19	3,09±0,22
Mv-3-Gl-Ac	10,88±0,30	29,12±0,31	23,20±0,30	5,88±0,28	18,36±0,33
Pn-3-Gl-Cm	0,45±0,22	1,62±0.24	0,75±0,20	0,41±0,16	0,51±0,19
Mv-3-Gl-Cm	8,68±0,31	17,68±0,33	18,98±0,30	4,52±0,25	20,52±0,31
Sum of glucosides	83,16±0,49	174,70±0,52	138,19±0,68	42,87±0,71	154,62±0,66
Sum of acetates	15,93±0,11	35,92±0,23	29,13±0,26	7,72±0,23	22,61±0,26
Sum of coumarates	9,13±0,17	19,30±0,25	19,73±0,21	4,93±0,23	21,03±0,22
Total	108,22±0,39 c	229,92±0,39 a	187,04±0,40 b	55,52±0,31 d	198,28±0,45 b

The values followed by the same letter (a, b, c, d) indicate that they are not significantly different ($p \leq 0,05$).

Table 9. Anthocyanin concentration in Teran wine 3 months of ageing for 2009 vintage (mg/L±standard deviation)

Anthocyanins	Control	Cryomaceration	Maceration at 25 °C	Maceration at 30 °C	Maceration at 25 °C + heating 35-40 °C
Df-3-Gl	2,92±0,25	3,68±0,20	5,34±0,21	1,79±0,19	2,84±0,24
Cy-3-Gl	0,65±0,12	0,54±0,15	1,14±0,10	0,15±0,06	0,63±0,06
Pt-3-Gl	7,45±0,32	9,40±0,30	9,70±0,32	4,52±0,30	6,43±0,31
Pn-3-Gl	11,07±0,55	10,26±0,50	11,08±0,51	5,10±0,45	7,29±0,40
Mv-3-Gl	70,83±0,30	76,93±0,36	72,84±0,32	40,12±0,30	54,53±0,39
Df-3-Gl-Ac	0,10±0,05	0,06±0,01	0,03±0,01	0,06±0,02	0,09±0,01
Cy-3-Gl-Ac	0,51±0,32	0,86±0,30	0,20±0,05	0,40±0,09	0,22±0,05
Pn-3-Gl-Ac	1,73±0,55	0,82±0,52	1,49±0,51	0,54±0,31	0,99±0,30
Mv-3-Gl-Ac	11,43±0,21	8,55±0,20	7,37±0,25	4,91±0,20	8,71±0,16
Pn-3-Gl-Cm	0,40±0,12	0,61±0,10	0,54±0,14	0,47±0,10	0,51±0,06
Mv-3-Gl-Cm	0,10±0,02	0,83±0,05	0,57±0,06	1,14±0,09	1,71±0,015
Sum of glucosides	94,70±0,45	99,02±0,52	100,10±0,55	51,67±0,12	71,72±0,35
Sum of acetates	10,30±0,25	13,76±0,20	9,08±0,26	5,91±0,25	10,01±0,26
Sum of coumarates	1,44±0,09	0,50±0,08	1,11±0,10	1,61±0,10	2,22±0,11
Total	106,44±0,39 a	113,29±0,36 a	110,29±0,25 a	59,19±0,22 c	83,95±0,29 b

The values followed by the same letter (a, b, c) indicate that they are not significantly different ($p \leq 0,05$).

Pre-fermentative cryomaceration increased the pigment extraction, and the wine produced by this technique contained higher content of total monoglucosides and total monoglucoside *p*-coumarats than the other wines. As it can be noted, pre-fermentative cryomaceration increased anthocyanin extraction in Teran wine when combined with lower fermentation temperatures (M25), but not high fermentation temperatures (M30) or post-fermentation heating (MG25). The maximum anthocyanin concentration 3 months after the end of maceration was found in C vinification technique in both consecutive years, with concentrations up to 229.90±0.39 mg/L and 113.29±0.36 mg/L in 2008 and 2009 vintage, respectively. The minimum anthocyanin concentration 3 months after the end of maceration was found in M30 vinification technique with concentrations up to 55.52±0.31 mg/L and 59.19±0.22 mg/L in 2008 and 2009 vintage, respectively. Specifically, the decline in total anthocyanin concentration in both years was roughly 70 % and 30 % for M30 and C treatment, respectively. According to Gómez-Míguez et al. (2007) the pre-fermentative cold maceration process not only increases the extraction of anthocyanins, but also their stability. Probably due to the higher fermentation temperature (30 °C) the anthocyanins were more decreasing while polymeric pigments

were increasing. The disappearance of anthocyanins and increase in polymeric pigment over time has been confirmed in other studies (Sacchi et al., 2005).

Fermentation rates

Fermentation rates were faster as vinification temperature increased what is in agreement with prior literature (Sacchi et al., 2005; Koyama et al., 2007). For Teran, fermentation rate for treatments K, C, M25, M30 and MG25 was complete on 11th, 11th, 9th, 8th and 9th day in 2008 vintage, whereas in 2009 vintage it was complete on 10th, 10th, 10th, 7th and 11th day, respectively. For pre-fermentative cryomaceration, fermentations were completed in 16 and 15 days of maceration, respectively.

Sensory analysis

Differences were found in terms of fruity flavour, colour intensity and body/finish. M25 wine was the best evaluated one showing increased dark red colour while conserving its fruit characteristics and good body/finish ready to be drunk young. Use of pre-fermentative cryomaceration (C) increased red colour, fruit and herbaceous aroma, good body/finish indicating a good potential for ageing. Increased fermentation temperatures (M30) also reduced red colour and fruity flavour, but extracted the tannins required to produce a premium wine capable for long aging. The heated post-fermentation maceration (MG25) wine had the lowest score, modifying the tannic structure and increasing the rustic character of wine but affected the colour intensity, which could be interpreted as an improvement.

Conclusions

The results of this research demonstrate the influence of different maceration temperatures on the anthocyanin content in the most important Istrian native red wine 'Teran'. Modification of the temperature conditions during maceration affected the progress of the anthocyanins concentrations, showing an increase in the concentration for pre-fermentative cryomaceration treatment, while the maceration at higher temperatures showed a decrease in the anthocyanin concentration in young Teran wine. Results of this study indicate that five days pre-fermentative cryomaceration at 5 ˚C followed by maceration at 25 ˚C is most appropriate in order to achieve maximum anthocyanin concentration in Teran wines, so this technique has shown to be very useful to improve the colour quality of young Teran wines.

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