Effect of winemaking on phenolic profile, colour components and antioxidants in *Crljenak kaštelanski* (sin. *Zinfandel*, *Primitivo*, *Tribidrag*) wine

Ivana Generalić Mekinić¹ · Živko Skračić² · Ana Kokeza¹ · Barbara Soldo³ · Ivica Ljubenković³ · Mara Banović⁴ · Danijela Skroza¹

Revised: 11 January 2019 / Accepted: 3 February 2019 / Published online: 20 February 2019
© Association of Food Scientists & Technologists (India) 2019

Abstract *Crljenak kaštelanski* or *Tribidrag* is a Croatian autochthonous grape variety which is a parent of the America's most popular variety *Zinfandel* or Italian *Primitivo*. The aim of this study was to investigate the dynamics of extraction of phenolics and changes in antioxidant activity of the samples collected during maceration of *C. kaštelanski* grapes processed using two different macerating enzymes. According to the obtained results it can be concluded that wines from *C. kaštelanski* grapes are great source of bioactive phenolics although differences in phenolic profiles between control and enzyme-treated wines were detected. The highest content of total phenolics was detected in control wine (2691 mg GAE/L). Use of pectolytic enzyme Vinoxym Vintage improved anthocyanin extraction, while higher colour parameters were observed for wine samples produced using Sihazym Extro. The statistical analysis confirmed great influence of total phenolics and concentrations of some individual phenolic compounds (e.g. catechin, gallic acid,

epicatechin) on reducing and free radical scavenging activity of wine samples while the impact of anthocyanins was not detected. According to the obtained results it can be concluded that use of enzymes has slightly negative effect on total phenolics and wine antioxidant properties, but it increases the extraction yield of wine colour components what enables shorter maceration and prevents colour losses during the aging process.

Keywords Red wine · Vinification · Maceration enzymes · Phenolics · Anthocyanins · Antioxidants

Introduction

Croatia has historical tradition of grape vine cultivation and wine production. At the beginning of the last century, 400 different grape varieties were cultivated in Croatia, but since then the number of native varieties drastically decreased (Maletić et al. 2015). In the last few years considerable efforts have been taken to increase the areas under vines, and special attention was given to the native varieties and their revitalization.

Among those old cultivars, autochthonous Dalmatian red grape variety *Crljenak kaštelanski* is significantly outspread in Kaštela wine region (Central and South Dalmatia) (Maletić et al. 2015; Zdunić et al. 2013). It has attracted a lot of interest since American-Croatian team of scientists among more than 150 different autochthonous Dalmatian vines, discovered that *C. kaštelanski* (also called *Tribidrag* or *Pribidrag*) has an identical genetic profile as American most popular grape variety *Zinfandel* or Italian *Primitivo*. In the last few years, thanks to the *Zinfandel*'s reputation, this almost vanished grape cultivar has become economically important and recognized by Croatian winemakers

✉ Ivana Generalić Mekinić
gene@ktf-split.hr

✉ Živko Skračić
zivko.skracic@st.htnet.hr

¹ Department of Food Technology and Biotechnology, Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, 21000 Split, Croatia

² Secondary School "Braća Radić", Put poljoprivrednika bb, 21217 Kaštel Štafilić, Croatia

³ Department of Chemistry, Faculty of Science, University of Split, Ruđera Boškovića 33, 21000 Split, Croatia

⁴ Department of Food Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

who started to cultivate it increasingly. While 15 years ago, only a dozen vines were found in Dalmatian vineyards, *C. kaštelanski* is today planted on more than 75 ha (Žulj Mihaljević et al. 2015).

Due to proven health benefits of grape phenolics, and because of their importance to wine organoleptic properties and stability, the maceration of red grapes is found to be a crucial step in winemaking. Today, consumers are increasingly demanding highly coloured wines with the added biological value, meaning that extraction of anthocyanins from grape skin must be as complete as possible to ensure high colour intensity and improved stability during the aging process (Jackson 2000; Mattivi et al. 2006; Bautista-Ortín et al. 2007; Romero-Cascales et al. 2008; Moreno Arribas and Polo 2009; González-Neves et al. 2016; Generalić Mekinić et al. 2016). As traditional fermentation leads to a maximal extraction of anthocyanins up to 60%, the use of macerating enzymes to facilitate their extraction is a common practice. Therefore, these extraction processes modify not only colour of the red wine but also its stability, taste and structure (Bautista-Ortín et al. 2007; Romero-Cascales et al. 2008). The aim of this study was to compare the effects of two different winemaking enzymes on the evaluation and extraction of phenolics, colour components and antioxidants from *C. kaštelanski* during the vinification.

Materials and methods

Chemicals and instruments

All used reagents and solvents were of adequate analytical grade. Spectrophotometric measurements were performed on a Specord 200 (Analytik Jena GmbH, Germany) while phenolic compounds were analysed by high-performance liquid chromatography (HPLC) system (Perkin Elmer, Waltham, Massachusetts, USA) consisting of an autosampler, vacuum degasser, binary pump, UV/VIS detector and the Peltier column oven (all of Series 200).

Sample preparation

Grapes from *Vitis Vinifera* var. *C. kaštelanski* were hand-picked at the stage of technological maturity from the vineyard located in Kaštela (Dalmatia, Croatia). All vines were of the same age (approximately 7 years old) and their identity have been confirmed. Immediately after the harvest, grapes were transported to the winery and processed. For each experiment 100 kg of grapes was used.

Winemaking and sampling

In this study three different winemaking procedures were performed; the traditional procedure without use of enzymes (as a control) and two procedures with different enzymes, Vinoxym Vintage[®] FCE (Novozymes A/S, Bagsvaerd, Denmark) and Sihazym Extro (Eaton Begerow Product Line, Langenlonsheim, Germany) (3 g/100 L). The grapes were destemmed and crushed with a MGM-940 crusher (MIO, Osijek, Croatia) and distributed into different fermentation tanks. After the homogenisation, samples were treated with the enzymes, potassium metabisulphite (10 g/100 L) and inoculated by dry active yeasts SIHA[®], Aktiv Hefe 8 (Burgundy Yeast) (E. Begerow GmbH & Co., Langenlonsheim, Germany) (10 g/100 L). The cap of grape solid was kept soaked using the mechanical barrier. The fermentation temperature ranged from 25 to 27 °C. The maceration lasted for 5 days and, once the alcoholic fermentation has been finished, the must has been devatted and grape pomace was pressed by the manual screw press. Free-run juice and the pressed one were combined. The wine was then sealed by the tank's floating lid and by the paraffin oil. At the beginning, during the maceration (from day 1 to day 5) samples were taken every day and finally after the racks (approximately 40 and 160 days after the winemaking process started). The samples were kept at – 20 °C until the analysis.

Spectrophotometric analysis of total phenolics, anthocyanins and wine colour parameters

The content of total phenolics was determined by Folin-Ciocalteu method (Singleton and Rossi 1965) and the results are expressed as mg of gallic acid equivalents per litre (mg GAE/L).

The amount of monomeric anthocyanins was determined by bisulphite bleaching assay (Amerine and Ough 1980) and the results are expressed as mg of malvidin-3-glucoside equivalents per litre (mg M-3-gl/L).

The colour intensity (CI), hue (H) and chromatic structure [optical density (OD) at 420, 520 and 620 nm] were determined spectrophotometrically and calculated according to the equations described by Ribéreau-Gayon et al. (2006).

HPLC analysis of phenolic compounds

Separations, quantifications and identifications of the individual phenolics were performed by HPLC method. Prior to injection, wine samples were filtered through the syringe filters (0.45 µm membrane pore size) and adequately diluted.

The phenolic compounds were separated on an Ultra-Aqueous C₁₈ column (250 × 4.6 mm, 5 µm, Restek, Bellefonte, PA, USA) maintained at 25 °C. The elution solvents were 0.2% phosphoric acid in water (solvent A) and 50% acetonitrile in methanol (solvent B). A gradient was applied as follows: from 96% A at 0 min to 50% A at 40 min, to 40% A at 45 min, to 20% A at 50 min, to 20% A at 53 min, then from 20 to 96% A at 54 min, and maintaining 96% A for 11 min (65 min). The applied flow rate was 0.8 mL/min and the injection volume was 20 µL. The detection of phenolics was carried out at 280 nm and peaks were identified by comparing their retention times and absorption spectra with those acquired for corresponding standards.

The HPLC analysis of anthocyanins was performed according to the Fredotović et al. (2017) with some slight modifications. The separation was carried out on the Kinetex C18 core-shell column (150 × 4.60 mm, 5 µm, Phenomenex, Torrance, CA, USA). The temperature was set to 40 °C and the elution solvents were 0.3% perchloric acid in water (solvent A) and 0.3% perchloric acid in methanol (solvent B). The flow rate was 0.6 mL/min and the injection volume was 10 µL. The detection of anthocyanins was carried out at 520 nm. The separated peaks were identified by their retention times, while their concentrations were determined according to the standard curve obtained for malvidin 3-*O*-glucoside.

Antioxidant activity

The reducing activity was measured as ferric reducing antioxidant power (FRAP) as described by Benzie and Strain (1996). This method is based on the reduction of ferric to ferrous ions at low pH which causes the formation of a coloured ferrous-tripyridyltriazine complex. The absorbance of the samples was recorded at 593 nm and the final results are expressed in micromoles of Trolox equivalents per litre (µmol TE/L) (Katalinić et al. 2013).

The free radical-scavenging ability of the samples was determined using a stable 2,2-diphenyl-2-picrylhydrazyl radical (DPPH[•]) according to the procedure described by Katalinić et al. (2013) and Harlina et al. (2018). This method is based on the “quenching” reactions of antioxidants with DPPH resulting in discolouration of the purple coloured ethanol solution which is monitored at 517 nm, and the results are expressed as DPPH inhibition (in %).

Statistical analysis

Statistical analysis was performed using GraphPad InStat3 (GraphPad Software, San Diego, USA) and STATISTICA (Data Analysis Software System, v. 10, StatSoft Inc, Tulsa, OK, USA). Pearson's correlation coefficient was used for

determination of the relations between the variables and *p* value < 0.05 was considered statistically significant. Duncan's multiple comparison test was used to determine the significant differences between group means in an analysis of variance setting. All data are expressed as mean ± standard deviation (SD).

Results and discussion

Anthocyanins are the main pigments of red wines, and their content primary depends on phenolic composition of grapes and the applied winemaking procedure. Maceration is traditionally used in red winemaking and its duration is a critical step for obtaining stable wines with good colour that will withstand a maturation period. While short maceration results with wines that contain low amounts of anthocyanins and have weak colour, the prolonged periods give wines with poor and unstable colour characteristics (Jackson 2000; Bautista-Ortín et al. 2007). The use of macerating enzymes to facilitate the extraction of grape components is a common and well-known practice. Use of enzymes support the extraction, maximize juice yield, facilitate filtration and intensify the wine flavour and colour. The short vatting with the addition of pectolytic enzymes increases wine colour intensity, but the concentration of extracted tannins remains low what results with wines that taste less astringent (Zoecklein et al. 1995; Pardo et al. 1999; Bautista-Ortín et al. 2007; Romero-Cascales et al. 2008; Mojsov et al. 2010; Río Segade et al. 2015). According to the study of Romero-Cascales et al. (2008) enzymes accelerate the extraction of phenolic compounds from grape skin but, if maceration period is too long the control wine can reach similar values of colour intensity. Therefore, enzyme preparations are found to be more useful in short macerations. Numerous studies investigated the effects of different oenological practices and macerating enzyme additions on the yield of extracted phenolics (Bautista-Ortín et al. 2007; Mojsov et al. 2010; Soto Vázquez et al. 2010; Río Segade et al. 2015; González-Neves et al. 2016; Aguilar et al. 2016). Despite the initial expectations, contradictory results have been reported but they all improve knowledge about the importance of choosing the proper winemaking technique.

In our study, two commercial enzyme preparations were used; Vinozym Vintage and Sihazym Extro. According to the producer's product technical sheets the declared enzyme in both preparations was polygalacturonase with the activity of 7.500 and 7.600 polygalacturonase units/g in Vinozym Vintage and Sihazym Extro, respectively. Sihazym Extro enzyme also contained further defined secondary activities such as arabinosidase and hemicellulases.

The evolution of total phenolics and monomeric anthocyanins in *C. kaštelanski* wine during the vinification with and without enzymes is shown in Fig. 1. As expected, all samples had the maximum content of total phenolics at the end of vinification after the second rack and the highest value (2691 mg GAE/L) was detected for the control wine. While for the control sample the continuous growth of phenolics during vinification was recorded, a slight decrease of these compounds was detected for samples produced by other two vinification procedures. The results obtained for monomeric anthocyanins were completely different. The majority of monomeric anthocyanins were extracted during first few days of maceration, while in all cases the concentration of these compounds decreased from first to second rack. The extremely high values were

recorded for Vinoxym Vintage sample at second and for Sihazym Extro sample at fifth day of maceration but they were lower in samples collected after the second rack. Dimitrovska et al. (2015) also noted significant increase of total anthocyanins which occurred between the first and second day of the fermentation but soon after the maximum values were reached, the concentration continually dropped. This decrease of monomeric anthocyanins could be due to reactions of anthocyanins and formation of copigments by making weak linkages with other phenolic compounds. Furthermore, it is well known that the increase of alcohol content during vinification causes alterations in the unions responsible for copigmentation products, and the final result is usually a decrease of anthocyanins as a consequence of their fixation on the solid parts, adsorption

Fig. 1 Changes of total phenols (a) and total anthocyanins (b) in *Crljenak kaštelanski* wine during the vinification without enzyme addition and with the addition of enzyme A (Vinoxym Vintage) or enzyme B (Sihazym Extro). GAE- gallic acid equivalents, M-3-gl- malvidin 3-glucoside. Different letters (a–e/f) on a and (a–e/g) on b in superscripts denote statistically significant difference ($p < 0.05$) of total phenolics and total anthocyanins among samples of the same wine collected at different vinification stages

on yeast cell walls, precipitation in the form of colloidal material, degradation reactions and structural changes (Moreno Arribas and Polo 2009; Soto Vázquez et al. 2010). Finally, the highest content of monomeric anthocyanins was found in Vinozym Vintage wine (115 mg M 3-g/L). Mojsov et al. (2010) also reported that samples produced by the use of preparations Vinozym Vintage showed a more intensive extraction of anthocyanins in comparison to the control sample. As can be seen from the results presented in Fig. 1, used enzyme preparations had different effect on the yield of extracted phenolics and anthocyanins what supports the results of previous studies (Mojsov et al. 2010; Soto Vázquez et al. 2010; Ortega-Heras et al. 2012). Ortega-Heras et al. (2012) used also Vinozym Vintage enzyme preparation in vinification of Mencía grapes and the results are opposite to those obtained in our study where enzyme treated samples contained higher amounts of phenolics and anthocyanins. However, Parley et al. (2001) investigated the effects of pre-fermentation enzyme maceration on extraction and colour stability in Pinot Noir wine and also obtained lower content of phenolics, especially monomeric anthocyanins, in control sample than in enzyme treated wine. In the literature there are also numerous other reports showing that use of pectolytic enzymes do not improve anthocyanin extraction or that it may even cause decrease of their concentration (Soto Vázquez et al. 2010; Sun et al. 2011), probably due to polymerisation reactions or glycosidase side activities which may cause hydrolysis of these compounds.

Tables 1, 2, and 3 shows the results for individual phenolic compounds in samples detected by HPLC: three phenolic acids, two flavonoids from the group of flavan-3-ols, flavonol quercetin and stilbene resveratrol. Gallic acid was found to be the dominant phenolic acid and its highest concentration was detected in wine produced by the addition of Vinozym Vintage (about twofold higher concentrations in comparison to the control wine and wine produced with Sihazym Extro). Soto Vázquez et al. (2011) also reported the significant increase of this compound in wine produced using enzymes and tannins. Among flavonoids, catechin was the most abundant what is also in agreement with other reports (Bautista-Ortín et al. 2007; Soto Vázquez et al. 2010; Xia et al. 2010), followed by its epimer. Beside different concentrations of detected phenolic compounds among control wine and enzyme-prepared wines, the significant variations among samples at different stages of winemaking were also observed. Most of these individual phenolic compounds were detected in study by Soto Vázquez et al. (2010) who also obtained higher extraction yields of these phytochemicals in samples produced by the addition of enzymes and tannins. The presence of *trans*-resveratrol was confirmed in all samples with concentration range from 0.51 mg/L in wine produced

by the addition of Vinozym Vintage to 1.07 mg/L in wine produced by the addition of Sihazym Extro.

The qualitative and quantitative compositions of extracted anthocyanins are also shown in Tables 1, 2 and 3 where 16 different anthocyanin derivatives are presented. The dominant forms were those of 3-*O*-glucosides. Of these, malvidin is the most common pigment in *Vitis vinifera* varieties as well as the most stabile form of anthocyanins (Zoecklein et al. 1995; Río Segade et al. 2015; González-Neves et al. 2016). In wine produced without enzyme addition the share of 3-*O*-glucosides was 84% of total detected anthocyanins (95.60 mg/L), while in wines produced by the addition of enzymes it was 79% for Vinozym Vintage wine and 69% for Sihazym Extro, respectively. As expected, malvidin 3-*O*-glucoside was the dominant compound, followed by peonidin-3-glucoside, and its concentration in control wine was similar to those reported by Dimitrovska et al. (2015) for Merlot wine. Also, the concentrations of other monoglucosides in all samples were less abundant (lower than 10 mg/L). The concentration of other 3-*O*-glucosides was significantly higher in wine produced by the classic vinification in comparison to the wines produced with enzymes what is in accordance with results reported by Mattivi et al. (2006) for *Primitivo* (clone of *C. kaštelanski*) wine. Furthermore, changes in content of the individual anthocyanins during the maceration stages have been detected. The results for 3-*O*-glucosides in the control wine and wine produced with Vinozym Vintage showed concentration decrease of all detected 3-*O*-glucosides from second to fifth day of maceration what is in accordance with results obtained by Sun et al. (2011), while the application of Sihazym Extro caused increase of peonidin-3-*O*-glucoside (from 8.72 to 9.25 mg/L) and malvidin-3-*O*-glucoside (from 58.74 to 63.82 mg/L). Similar observations were obtained for *O*-coumaryoyl glucosides where increase of peonidins and petunidins was detected in wine produced using Sihazym Extro, while in all other cases decrease of these compounds was detected. In case of the control wine and Vinozym Vintage the content of 3-*O*-glucosides continued growth, the samples of wine produced using Sihazym Extro showed significantly lower concentrations. While Sun et al. (2011) in their study reported further decrease of all investigated anthocyanins at second rack (after 6 months), the results obtained in our study show similar trend for enzyme treated wines while the contents of all 3-*O*-glucosides except malvidin-3-*O*-glucoside in control wine were higher in samples from the second rack. An extremely significant correlations were found between the results for total monomeric anthocyanins and malvidin-3-*O*-glucoside ($n = 21$, $r = 0.8973$, $p < 0.0001$) and total malvidins ($n = 21$, $r = 0.9078$, $p < 0.0001$).

Table 1 Phenolic composition (mg/L) of *Crljenak kaštelanski* young wine produced by classic vinification without addition of enzymes

Group	Phenolic compound (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Rack 1	Rack 2
PA	Gallic acid	8.41 ± 0.04a	12.56 ± 0.18b	17.78 ± 0.33c	8.97 ± 0.05a	24.67 ± 6.12d	25.46 ± 3.90de	28.63 ± 0.04e
	Protocatechuic acid	1.29 ± 0.12a	0.95 ± 0.02bc	1.38 ± 0.13a	1.05 ± 0.02c	1.18 ± 0.12d	0.85 ± 0.00b	0.91 ± 0.05b
	<i>p</i> -hydroxybenzoic acid	n.d.	1.95 ± 0.02a	3.36 ± 0.67b	n.d.	4.38 ± 0.57c	4.53 ± 0.55c	4.74 ± 0.01c
F	Catechin	15.77 ± 0.05a	39.70 ± 0.24b	55.06 ± 0.51c	24.29 ± 7.22d	67.14 ± 7.34e	76.27 ± 8.06f	82.60 ± 0.18g
	Epicatechin	18.54 ± 2.67a	28.86 ± 0.28b	33.97 ± 0.24c	21.30 ± 0.41d	66.79 ± 2.65e	43.35 ± 3.49f	47.04 ± 0.53g
	Quercetin	0.17 ± 0.02a	1.37 ± 0.01b	0.98 ± 0.18c	n.d.	0.98 ± 0.07c	1.27 ± 0.02b	1.27 ± 0.03b
S	Resveratrol	0.10 ± 0.02a	0.44 ± 0.05b	0.50 ± 0.05c	0.30 ± 0.03b	0.50 ± 0.32c	0.68 ± 0.01d	0.70 ± 0.02d
A	Delphinidin-3- <i>O</i> -glucoside	1.80 ± 0.01a	5.35 ± 0.00b	4.62 ± 0.07c	4.60 ± 0.01c	3.84 ± 0.03d	5.88 ± 0.10e	7.49 ± 0.37f
	Cyanidin-3- <i>O</i> -glucoside	0.82 ± 0.07a	1.24 ± 0.07b	1.19 ± 0.05b	1.35 ± 0.07b	1.24 ± 0.13b	1.51 ± 0.15c	1.74 ± 0.34d
	Petunidin-3- <i>O</i> -glucoside	2.37 ± 0.04a	6.94 ± 0.01b	6.61 ± 0.23b	6.93 ± 0.18b	6.17 ± 0.36c	8.35 ± 0.52d	8.56 ± 0.40d
	Peonidin-3- <i>O</i> -glucoside	3.78 ± 0.11a	8.95 ± 0.46b	8.61 ± 0.56b	9.77 ± 0.38c	8.88 ± 0.39b	11.59 ± 0.46d	12.30 ± 0.65e
	Malvidin-3- <i>O</i> -glucoside	17.70 ± 0.15a	50.12 ± 0.38b	51.32 ± 1.05c	56.98 ± 0.36d	49.14 ± 1.15e	61.14 ± 0.36f	50.49 ± 0.15b
	Delphinidin-3- <i>O</i> -acetylglucoside	4.22 ± 0.27a	4.61 ± 0.04b	4.08 ± 0.19a	3.51 ± 0.13c	4.18 ± 0.44a	2.88 ± 0.14d	2.92 ± 0.17d
	Cyanidin-3- <i>O</i> -acetylglucoside	1.04 ± 0.00a	1.48 ± 0.03b	1.28 ± 0.02c	1.45 ± 0.02b	1.71 ± 0.10d	1.47 ± 0.02b	0.47 ± 0.02e
	Petunidin-3- <i>O</i> -acetylglucoside	1.78 ± 0.01a	2.58 ± 0.10b	2.32 ± 0.04c	2.55 ± 0.05b	2.76 ± 0.02d	2.60 ± 0.23b	0.91 ± 0.07e
	Peonidin-3- <i>O</i> -acetylglucoside	1.12 ± 0.00a	1.93 ± 0.00b	1.62 ± 0.01c	1.70 ± 0.00d	1.83 ± 0.02e	1.11 ± 0.01a	0.58 ± 0.05f
	Petunidin-(6- <i>O</i> -caffeoyl)glucoside	0.76 ± 0.00a	1.22 ± 0.01b	1.00 ± 0.01c	0.83 ± 0.03d	0.68 ± 0.01e	0.51 ± 0.03f	0.57 ± 0.01g
	Malvidin-3- <i>O</i> -acetylglucoside	1.15 ± 0.01a	3.17 ± 0.00b	3.22 ± 0.01b	3.63 ± 0.06c	3.09 ± 0.02d	3.49 ± 0.13e	2.53 ± 0.01f
	Malvidin-(6- <i>O</i> -caffeoyl)glucoside	0.67 ± 0.02a	2.09 ± 0.02b	2.08 ± 0.01b	1.96 ± 0.02c	1.58 ± 0.09d	1.85 ± 0.01e	1.43 ± 0.02f
	Cyanidin-(6- <i>O</i> -coumaroyl)glucoside	0.18 ± 0.00a	0.91 ± 0.01b	0.78 ± 0.08c	0.66 ± 0.00d	0.54 ± 0.01e	0.80 ± 0.01c	0.48 ± 0.01f
	Petunidin-(6- <i>O</i> -coumaroyl)glucoside	0.47 ± 0.00a	0.78 ± 0.01b	0.64 ± 0.00c	0.67 ± 0.01d	0.67 ± 0.00d	0.79 ± 0.00e	0.36 ± 0.01f
	Peonidin-3-(6- <i>O</i> -coumaroyl)glucoside	0.41 ± 0.01a	1.39 ± 0.01b	1.33 ± 0.05c	1.31 ± 0.01c	1.15 ± 0.01d	1.34 ± 0.01e	0.81 ± 0.02f
	Malvidin-3-(6- <i>O</i> -coumaroyl)glucoside	2.02 ± 0.01a	6.94 ± 0.01b	6.47 ± 0.10c	6.32 ± 0.02d	5.13 ± 0.03e	6.25 ± 0.04f	3.98 ± 0.01g

PA Phenolic acids, F flavonoids, S stilbenes, A anthocyanins, n.d. not detected

Table 2 Phenolic composition (mg/L) of *Crijenak kaštelanski* young wine produced with the addition of enzyme Vinozym Vintage

Group	Phenolic compound (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Rack 1	Rack 2
PA	Gallic acid	8.69 ± 0.12a	8.92 ± 0.03a	24.91 ± 0.89b	34.23 ± 0.14c	30.05 ± 0.18d	50.39 ± 0.30e	65.45 ± 0.11f
	Protocatechuic acid	0.92 ± 0.02a	0.93 ± 0.02ab	1.00 ± 0.03b	1.18 ± 0.04c	0.85 ± 0.11d	1.25 ± 0.08c	1.67 ± 0.02e
	<i>p</i> -hydroxybenzoic acid	1.82 ± 0.01a	1.10 ± 0.00b	4.65 ± 0.07c	5.35 ± 0.05d	5.34 ± 0.02d	6.59 ± 0.16e	6.20 ± 0.07f
F	Catechin	23.06 ± 2.80a	19.41 ± 0.29b	70.92 ± 0.52c	88.91 ± 0.15d	75.60 ± 0.52e	87.88 ± 0.98d	91.99 ± 0.10f
	Epicatechin	23.84 ± 1.20a	22.26 ± 2.13b	45.12 ± 0.37c	56.00 ± 1.30d	45.60 ± 0.64b	48.21 ± 0.89e	52.85 ± 0.38f
	Quercetin	0.34 ± 0.08a	0.12 ± 0.03b	0.52 ± 0.01c	0.65 ± 0.02d	0.43 ± 0.00e	1.41 ± 0.01f	2.18 ± 0.01g
S	Resveratrol	0.39 ± 0.04a	0.32 ± 0.02b	0.92 ± 0.00c	0.91 ± 0.05c	0.67 ± 0.04d	0.86 ± 0.01e	0.51 ± 0.00f
A	Delphinidin-3- <i>O</i> -glucoside	2.06 ± 0.03a	5.21 ± 0.25b	4.34 ± 0.22c	3.85 ± 0.04d	2.77 ± 0.01e	3.20 ± 0.05f	2.62 ± 0.01e
	Cyanidin-3- <i>O</i> -glucoside	0.79 ± 0.01a	2.45 ± 0.05b	1.52 ± 0.29c	1.33 ± 0.08d	0.93 ± 0.05a	0.95 ± 0.01d	0.56 ± 0.03e
	Petunidin-3- <i>O</i> -glucoside	2.83 ± 0.41a	8.69 ± 0.12b	6.78 ± 0.48c	6.47 ± 0.21c	4.67 ± 0.18d	5.68 ± 0.01e	4.03 ± 0.01f
	Peonidin-3- <i>O</i> -glucoside	5.91 ± 0.52a	13.05 ± 0.03b	12.07 ± 1.40c	9.96 ± 0.23d	7.61 ± 0.23e	8.54 ± 0.02f	5.37 ± 0.13a
	Malvidin-3- <i>O</i> -glucoside	20.89 ± 0.32a	63.48 ± 0.86b	63.12 ± 0.70b	57.82 ± 0.82c	46.12 ± 0.08d	51.82 ± 0.56e	36.29 ± 0.10f
	Delphinidin-3- <i>O</i> -acetylglucoside	7.71 ± 0.20a	11.79 ± 0.15b	6.94 ± 0.46c	4.54 ± 0.49d	3.00 ± 0.02e	2.05 ± 0.05f	1.53 ± 0.01g
	Cyanidin-3- <i>O</i> -acetylglucoside	1.25 ± 0.12a	1.22 ± 0.04a	1.02 ± 0.31b	1.09 ± 0.24ab	0.90 ± 0.01b	0.94 ± 0.02b	0.67 ± 0.00c
	Petunidin-3- <i>O</i> -acetylglucoside	2.28 ± 0.03a	1.99 ± 0.04b	1.78 ± 0.31cd	1.82 ± 0.13c	1.65 ± 0.01d	1.72 ± 0.02cd	1.47 ± 0.02e
	Peonidin-3- <i>O</i> -acetylglucoside	1.76 ± 0.22a	2.02 ± 0.02b	1.72 ± 0.12ac	1.64 ± 0.03c	1.48 ± 0.01d	0.99 ± 0.02e	0.58 ± 0.01f
	Petunidin-(6- <i>O</i> -caffeoyl)glucoside	1.27 ± 0.08a	2.67 ± 0.25b	1.59 ± 0.24c	0.95 ± 0.19d	0.62 ± 0.14e	0.49 ± 0.00ef	0.35 ± 0.01f
	Malvidin-3- <i>O</i> -acetylglucoside	1.28 ± 0.08a	4.25 ± 0.10b	4.39 ± 0.12b	3.59 ± 0.31c	2.81 ± 0.24d	3.37 ± 0.05e	2.17 ± 0.01f
	Malvidin-(6- <i>O</i> -caffeoyl)glucoside	0.71 ± 0.07a	2.28 ± 0.03b	2.18 ± 0.02c	1.70 ± 0.01d	1.17 ± 0.01e	1.50 ± 0.02f	0.83 ± 0.04g
	Cyanidin-(6- <i>O</i> -coumaroyl)glucoside	0.17 ± 0.00a	0.73 ± 0.01b	0.62 ± 0.01c	0.57 ± 0.01d	0.38 ± 0.00e	0.47 ± 0.04f	0.29 ± 0.00g
	Petunidin-(6- <i>O</i> -coumaroyl)glucoside	0.52 ± 0.00a	0.62 ± 0.01b	0.48 ± 0.03c	0.50 ± 0.01c	0.49 ± 0.00c	0.66 ± 0.00d	0.74 ± 0.01e
	Peonidin-3-(6- <i>O</i> -coumaroyl)glucoside	0.39 ± 0.06a	1.71 ± 0.05b	1.56 ± 0.01c	1.49 ± 0.10d	1.00 ± 0.01e	1.23 ± 0.01f	0.81 ± 0.01g
	Malvidin-3-(6- <i>O</i> -coumaroyl)glucoside	1.72 ± 0.18a	8.24 ± 0.16b	7.57 ± 0.03c	6.77 ± 0.07d	4.63 ± 0.00e	5.47 ± 0.06f	3.60 ± 0.01g

PA Phenolic acids, F flavonoids, S stilbenes, A anthocyanins, *n.d.* not detected

Table 3 Phenolic composition (mg/L) of *Crljenak kaštelanski* young wine produced with the addition of enzyme Sihazym Extro

Group	Phenolic compound (mg/L)	Day 1	Day 2	Day 3	Day 4	Day 5	Rack 1	Rack 2
PA	Gallic acid	5.38 ± 0.14a	7.19 ± 0.05a	9.21 ± 0.38a	14.45 ± 0.04b	23.75 ± 0.18c	44.58 ± 8.50d	45.04 ± 0.19d
	Protocatechuic acid	1.99 ± 0.02a	0.56 ± 0.03b	0.59 ± 0.02b	1.61 ± 0.02ce	1.40 ± 0.08d	1.56 ± 0.36cd	1.78 ± 0.02e
	<i>p</i> -hydroxybenzoic acid	1.83 ± 0.16a	1.63 ± 0.32ab	1.59 ± 0.05b	2.56 ± 0.02c	2.32 ± 0.20d	3.31 ± 0.01e	2.75 ± 0.01c
F	Catechin	14.72 ± 0.15a	24.75 ± 0.19b	30.16 ± 0.47c	47.54 ± 0.57d	72.86 ± 0.61e	87.53 ± 1.48f	55.49 ± 3.93g
	Epicatechin	10.82 ± 0.38a	19.60 ± 0.31b	18.93 ± 0.30c	24.99 ± 0.16d	34.75 ± 0.51e	48.65 ± 0.55f	22.03 ± 0.14g
	Quercetin	n.d.	0.71 ± 0.03a	0.43 ± 0.00b	0.67 ± 0.01a	1.48 ± 0.02c	1.34 ± 0.12d	5.19 ± 0.12e
S	Resveratrol	n.d.	0.35 ± 0.02a	0.44 ± 0.00b	0.68 ± 0.01c	0.89 ± 0.01d	0.90 ± 0.04d	1.07 ± 0.13e
A	Delphinidin-3- <i>O</i> -glucoside	3.24 ± 0.01a	6.91 ± 0.00b	5.16 ± 0.07c	5.27 ± 0.04c	5.19 ± 0.17c	3.81 ± 0.35d	2.32 ± 0.01e
	Cyanidin-3- <i>O</i> -glucoside	1.15 ± 0.04ac	1.30 ± 0.03b	1.01 ± 0.02a	1.23 ± 0.30bc	1.01 ± 0.05a	0.79 ± 0.08d	0.49 ± 0.00e
	Petunidin-3- <i>O</i> -glucoside	3.79 ± 0.00a	8.96 ± 0.06b	7.23 ± 0.10c	7.81 ± 0.14d	8.18 ± 0.21e	5.60 ± 0.51f	3.04 ± 0.03g
	Peonidin-3- <i>O</i> -glucoside	4.82 ± 0.02a	8.72 ± 0.11b	7.16 ± 0.13c	8.89 ± 0.13b	9.25 ± 0.04d	6.43 ± 0.46e	3.23 ± 0.03f
	Malvidin-3- <i>O</i> -glucoside	24.73 ± 0.04a	58.74 ± 0.31b	50.07 ± 0.59c	61.55 ± 0.87d	63.82 ± 1.05e	40.58 ± 4.12f	21.63 ± 0.09g
	Delphinidin-3- <i>O</i> -acetylglucoside	3.39 ± 0.00a	4.15 ± 0.25b	3.75 ± 0.06c	4.29 ± 0.15b	3.45 ± 0.17a	2.55 ± 0.19d	1.92 ± 0.05e
	Cyanidin-3- <i>O</i> -acetylglucoside	1.05 ± 0.01ac	0.92 ± 0.02ab	0.90 ± 0.02b	1.19 ± 0.00c	1.67 ± 0.09d	1.37 ± 0.14e	1.86 ± 0.27f
	Petunidin-3- <i>O</i> -acetylglucoside	2.05 ± 0.00a	1.76 ± 0.01b	1.68 ± 0.03b	2.35 ± 0.04c	2.73 ± 0.03d	2.48 ± 0.21e	2.96 ± 0.03f
	Peonidin-3- <i>O</i> -acetylglucoside	1.11 ± 0.00ac	1.17 ± 0.00a	1.15 ± 0.01a	1.54 ± 0.16b	1.57 ± 0.09b	1.01 ± 0.11c	0.80 ± 0.00d
	Petunidin-(6- <i>O</i> -caffeoyl)glucoside	0.67 ± 0.00a	1.26 ± 0.00b	0.87 ± 0.01c	1.01 ± 0.01d	0.99 ± 0.10d	0.60 ± 0.02e	0.48 ± 0.00f
	Malvidin-3- <i>O</i> -acetylglucoside	1.26 ± 0.01a	3.27 ± 0.00b	2.52 ± 0.03c	3.44 ± 0.03d	3.58 ± 0.35d	2.13 ± 0.07e	1.04 ± 0.01f
	Malvidin-(6- <i>O</i> -caffeoyl)glucoside	0.83 ± 0.01a	2.34 ± 0.09b	1.96 ± 0.03c	2.35 ± 0.01b	2.44 ± 0.18b	1.33 ± 0.14d	0.54 ± 0.00e
	Cyanidin-(6- <i>O</i> -coumaroyl)glucoside	0.34 ± 0.00a	1.15 ± 0.01b	0.84 ± 0.03c	0.93 ± 0.00d	0.89 ± 0.12cd	0.61 ± 0.08e	0.21 ± 0.00f
	Petunidin-(6- <i>O</i> -coumaroyl)glucoside	0.57 ± 0.00ac	0.54 ± 0.01a	0.45 ± 0.00b	0.61 ± 0.05c	0.75 ± 0.08d	0.80 ± 0.08d	1.16 ± 0.00e
	Peonidin-3-(6- <i>O</i> -coumaroyl)glucoside	0.57 ± 0.02a	1.43 ± 0.00b	1.16 ± 0.01c	1.42 ± 0.03b	1.57 ± 0.07d	0.93 ± 0.07e	0.50 ± 0.00f
	Malvidin-3-(6- <i>O</i> -coumaroyl)glucoside	3.24 ± 0.03a	8.92 ± 0.01b	6.90 ± 0.08c	7.91 ± 0.04d	7.95 ± 0.08d	4.92 ± 0.54e	2.24 ± 0.01f

PA Phenolic acids, F flavonoids, S stilbenes, A anthocyanins, n.d. not detected

Fig. 2 Distribution of anthocyanin derivatives in *Crljenak kaštelanski* wine during the vinification without enzyme addition (a) and with Vinoxym Vintage (b) and Sihazym Extro (c)

Figure 2 presents the distribution of anthocyanin derivatives in *C. kaštelanski* wine during the vinification with and without enzyme addition. As can be seen, the highest content of total delphinidins was detected in control wine what is in accordance with results reported by González-Neves et al. (2016). There were no significant differences among the detected concentrations of cyanidins in all samples, while the content of petunidins during the

vinification without enzymes increased almost 2-times. Peonidins increased in classic vinification (from 5.31 to 13.68 mg/L), while slight decrease was detected in enzyme-treated wines. In all cases, the most significant increase of malvidin derivatives was detected from first to second day of maceration and the highest concentrations were detected in the control wine (58.43 mg/L).

Table 4 Colour parameters of *Crljenak kaštelanski* wine produced by classic vinification and by the addition of enzymes

Sample	CI	T	Colour composition		
			OD 420 (%)	OD 520 (%)	OD 620 (%)
Classic vinification without enzyme addition					
Day 1	2.71	0.54	32.50	60.44	7.06
Day 2	4.93	0.48	30.38	62.76	6.86
Day 3	4.65	0.51	31.25	61.71	7.04
Day 4	5.08	0.51	31.38	61.79	6.83
Day 5	5.32	0.52	31.91	61.06	7.03
Rack 1	5.66	0.55	32.67	59.55	7.77
Rack 2	6.18	0.76	37.64	49.59	12.78
Vinification with the addition of Vinoxym Vintage enzyme					
Day 1	2.65	0.56	33.12	59.59	7.29
Day 2	5.30	0.52	31.91	61.30	6.79
Day 3	4.52	0.54	32.79	60.20	7.01
Day 4	4.22	0.56	33.22	59.58	7.20
Day 5	3.70	0.58	34.12	58.54	7.35
Rack 1	4.51	0.68	36.55	53.98	9.47
Rack 2	5.38	0.74	38.19	51.45	10.36
Vinification with the addition of Sihazym Extro enzyme					
Day 1	3.15	0.50	30.99	62.59	6.41
Day 2	4.01	0.46	29.77	64.42	5.81
Day 3	3.51	0.48	30.54	63.69	5.78
Day 4	4.86	0.48	30.63	63.48	5.89
Day 5	5.45	0.49	30.79	63.26	5.95
Rack 1	6.17	0.54	32.47	60.28	7.25
Rack 2	6.93	0.59	33.71	56.84	9.45

CI Colour intensity; T hue; OD optical density

Colour parameters for *C. kaštelanski* wines produced by classic vinification and by the addition of enzymes are shown in Table 4. CI represents the amount of colour while T indicates the development of a colour towards orange. These parameters vary a great deal from one wine and grape variety to another (Ribéreau-Gayon et al. 2006). The most intensive colour was detected for wine produced by the use of Sihazym Extro (final CI value was 6.93), and the lowest CI was detected for wine produced by the addition of Vinoxym Vintage. This is not in accordance with the results obtained by Mojsov et al. (2010) who, in their study on Vranec red wine produced with different pectolytic enzyme preparations (among which one of the used enzymes was Vinoxym Vintage), reported higher CI values for wines produced using enzymes. T value for young wines usually ranges from 0.5 to 0.7 and it increases throughout aging, reaching an upper limit around 1.2–1.3 (Pardo et al. 1999; Ribéreau-Gayon et al. 2006) what is in accordance with our results. Colour composition is the contribution of each of the three basic colour components

in the overall wine colour (Ribéreau-Gayon et al. 2006), and it can be defined as an optical density of the samples at 420 (yellow), 520 (red) and 620 (blue) nm, expressed in percentages. There were no significant differences between the OD 420 and OD 520 among samples produced without enzyme addition and with Vinoxym Vintage, while OD 420 was lower and OD 520 higher for wine produced with Sihazym Extro. The highest OD 620 was detected for the control sample (12.78%).

Although it is well-known that the phenolic composition of red wines varies significantly during vinification and storage, its effect on wine antioxidant activity has not been well elucidated (Sun et al. 2011). The antioxidant properties of investigated samples tested by two methods based on different mechanisms, FRAP and DPPH, are given in Fig. 3. All samples showed increase of reducing ability during the vinification. At the first day, the lowest FRAP value was detected for the control wine (3035 $\mu\text{mol TE/L}$), which at the end of winemaking (after second rack) showed the highest activity. Among other two samples, wine produced by the addition of Sihazym Extro had better antioxidant capacity. An extremely significant correlation has been confirmed between total phenolics and FRAP values ($n = 21$, $r = 0.9773$, $p < 0.0001$). Furthermore, the antioxidant capacity was determined by DPPH method. Again, extremely significant correlation has been confirmed between the results for total phenolic content and DPPH inhibition ($n = 21$, $r = 0.9404$, $p < 0.0001$). Other authors also observed a positive correlation between the phenolic content and wine antioxidant properties (López-Vélez et al. 2003; Katalinić et al. 2013; Paixão et al. 2007; Mitić et al. 2014). According to the obtained results it can be concluded that the antioxidant capacity of wine is largely influenced by the total phenolic content, while anthocyanins play a minor role (Fernández-Pachón et al. 2006; Cimino et al. 2007; Li et al. 2009; Mitić et al. 2014). In this study the antioxidant activity also didn't show correlation with anthocyanins what can be explained by their chemical structure which is not catechol-type what is one of the most characteristic structure antioxidant features (Sun et al. 2011; Skroza et al. 2016). On the other hand, the correlation between tested antioxidant activities and content of detected individual compounds was also tested. The reducing activity (FRAP values) showed extremely significant correlation with catechin content ($n = 21$, $r = 0.7204$, $p = 0.0002$) and very significant correlation with the concentration of gallic acid ($n = 21$, $r = 0.6591$, $p = 0.0012$) and epicatechin ($n = 21$, $r = 0.5483$, $p = 0.0101$). Also results for DPPH inhibition correlated very significant with catechin content ($n = 21$, $r = 0.5522$, $p = 0.0094$) and significant with gallic acid ($n = 21$, $r = 0.4946$, $p = 0.0227$). Red wine contains a wide variety of phenolic substances and we investigated only few of

Fig. 3 Comparison of the antioxidant properties of *Crljenak kaštelanski* wine samples collected during the vinification with and without enzymes obtained by FRAP (a) and DPPH (b) method. Enzyme A– Vinoxym Vintage, Enzyme B– Sihazym Extro. Different letters (a–g) on Fig 3a and (a–d/e) on b in superscripts denote statistically significant difference ($p < 0.05$) of FRAP and DPPH inhibition values among samples of the same wine collected at different vinification stages

them, so the influence of other compounds whose presence was not investigated should not be neglected. For example, Cimino et al. (2007) reported that the proanthocyanidin level has strong impact on the antioxidant efficiency of red wines, while according to the Fernández-Pachón et al. (2006) main contributors to a sherry and white wine radical scavenging activity are gallic, caffeic and caftaric acids and (–)-epigallocatechin gallate.

Conclusion

This study aimed to compare the classic vinification with those performed using macerating enzymes in order to investigate the phenolic potential of the grapes as well as the extraction of phenolics during the winemaking.

Although there were differences in the extraction kinetic of phenolics between control and enzyme-treated wines during maceration, at the end the highest yield of phenolics was detected in control wine produced without enzymes. According to the obtained results it can be concluded that use of enzymes increases extraction yield of anthocyanins and other wine colour components what enables shorter maceration and prevents their losses during wine aging. Generally, the obtained results for phenolic profile and antioxidant properties of *C. kaštelanski* grapes imply that the content of total phenolics and some individual phenolic compounds (especially those with catechol type structure) is primarily responsible for antioxidant activity of the samples, while anthocyanins play a minor role. Generally, the obtain results are conformation that *C. kaštelanski* grapes are a great raw material for the production of high-

quality, stabile and highly coloured wines with added biological value.

Acknowledgements This work has been partially supported by Croatian Science Foundation under the project IP-2013-11-8652. We thank Prof. Vida Šimat for assistance with statistical analysis and suggestions that greatly improved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

- Aguilar T, Loyola C, de Bruijn J, Bustamante L, Vergara C, von Baer D, Mardones C, Serra I (2016) Effect of thermomaceration and enzymatic maceration on phenolic compounds of grape must enriched by grape pomace, vine leaves and canes. *Eur Food Res Tech* 242:1149–1158. <https://doi.org/10.1007/s00217-015-2619-3>
- Amerine MA, Ough CS (1980) *Methods for analysis of musts and wines*. Wiley, New York
- Bautista-Ortín AB, Fernández-Fernández JI, López-Roca JM, Gómez-Plaza E (2007) The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. *J Food Comp Anal* 20:546–552. <https://doi.org/10.1016/j.jfca.2007.04.008>
- Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as measurement of “antioxidant power”: the FRAP assay. *Anal Biochem* 239:70–77. <https://doi.org/10.1006/abio.1996.0292>
- Cimino F, Sulfaro V, Trombetta D, Saija A, Tomaino A (2007) Radical-scavenging capacity of several Italian red wines. *Food Chem* 103(1):75–81. <https://doi.org/10.1016/j.foodchem.2006.07.042>
- Dimitrovska M, Bocevska M, Dimitrovski D, Doneva Sapceska D (2015) Evolution of anthocyanins during vinification of Merlot and Pinot Noir grapes to wines. *Acta Aliment* 44:259–267. <https://doi.org/10.1556/066.2015.44.0003>
- Fernández-Pachón MS, Villaño D, Troncoso AM, García-Parrilla MC (2006) Determination of the phenolic composition of sherry and table white wines by liquid chromatography and their relation with antioxidant activity. *Anal Chim Acta* 563(1–2):101–108. <https://doi.org/10.1016/j.aca.2005.09.057>
- Fredotović Ž, Šprung M, Soldo B, Ljubenkov I, Budić-Leto I, Bilušić T, Čikeš-Čulić V, Puizina J (2017) Chemical composition and biological activity of *Allium cepa* L. and *Allium × cornutum* (Clementi ex Visiani 1842) methanolic extracts. *Molecules* 22:1–13. <https://doi.org/10.3390/molecules22030448>
- Generalić Mekinić I, Skroza D, Ristovski B, Ljubenkov I, Bikić I, Svilović S, Banović M, Bocevska M, Katalinić V (2016) Anthocyanidins in Babica, Ljutun and Crljenak Kaštelanski grapes and kinetic of their degradation under different storage conditions. *J Food Nutr Res* 55(4):294–302
- González-Neves G, Favre G, Piccardo D, Gil G (2016) Anthocyanin profile of young red wines of Tannat, Syrah and Merlot made using maceration enzymes and cold soak. *Int J Food Sci Tech* 51:260–267. <https://doi.org/10.1111/ijfs.12958>
- Harlina PW, Ma M, Shahzad R, Gouda MM, Qiu N (2018) Effect of clove extract on lipid oxidation, antioxidant activity, volatile compounds and fatty acid composition of salted duck eggs. *J Food Sci Tech*. <https://doi.org/10.1007/s13197-018-3367-8>
- Jackson RS (2000) *Wine science, principles, practice, perception*. Elsevier Inc., London
- Katalinić V, Smole Možina S, Generalić I, Skroza D, Ljubenkov I, Klančnik A (2013) Phenolic profile, antioxidant capacity and antimicrobial activity of crude leaf extracts of six *Vitis vinifera* L. varieties. *Int J Food Prop* 16:45–60. <https://doi.org/10.1080/10942912.2010.526274>
- Li H, Wang X, Li Y, Li P, Wan H (2009) Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chem* 112:454–460. <https://doi.org/10.1016/j.foodchem.2008.05.111>
- López-Vélez M, Martínez-Martínez F, Del Valle-Ribes C (2003) The study of phenolic compounds as natural antioxidants in wine. *Crit Rev Food Sci Nutr* 43:233–244. <https://doi.org/10.1080/10408690390826509>
- Maletić E, Pejić I, Karoglan Kontić J, Zdunić G, Preiner D, Šimon S, Andabaka Ž, Žulj Mihaljević M, Bubola M, Marković Z, Stupić D, Mucalo A (2015) Ampelographic and genetic characterization of Croatian grapevine varieties. *Vitis* 54:93–98
- Mattivi F, Guzzon R, Vrhovsek U, Stefanini M, Velasco R (2006) Metabolite profiling of grape: flavonols and anthocyanins. *J Agric Food Chem* 54:7692–7702. <https://doi.org/10.1021/jf061538c>
- Mitić MN, Kostić DA, Pavlović AN (2014) The phenolic composition and the antioxidant capacity of Serbian red wines. *Adv Techn* 3(1):16–22
- Mojsov K, Ziberoski J, Bozinovic Z, Petreska M (2010) A comparison of effects of three commercial pectolytic enzyme preparations in red winemaking. *Int J Pure Appl Sci Technol* 1(2):127–136. <https://doi.org/10.15208/ati.2011.4>
- Moreno-Arribas MV, Polo C (2009) *Wine chemistry and biochemistry*. Springer, New York
- Ortega-Heras M, Pérez-Magariño S, González-Sanjosé ML (2012) Comparative study of the use of maceration enzymes and cold pre-fermentative maceration on phenolic and anthocyanic composition and colour of a Mencía red wine. *LWT Food Sci Technol* 48:1–8. <https://doi.org/10.1016/j.lwt.2012.03.012>
- Paixão N, Perestrelo R, Marques JC, Câmara JS (2007) Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. *Food Chem* 105:204–214. <https://doi.org/10.1016/j.foodchem.2007.04.017>
- Pardo F, Salinas MR, Alonso GL, Navarro G, Huerta MD (1999) Effect of diverse enzyme preparations on the extraction and evolution of phenolic compounds in red wines. *Food Chem* 67:135–142. [https://doi.org/10.1016/S0308-8146\(99\)00080-1](https://doi.org/10.1016/S0308-8146(99)00080-1)
- Parley A, Vanhanen L, Heatherbelle D (2001) Effects of pre-fermentation enzyme maceration on extraction and colour stability in Pinot Noir wine. *Aust J Grape Wine R* 7:146–152
- Ribéreau-Gayon P, Dubourdieu D, Donèche B (2006) *Handbook of enology, volume 2, the chemistry of wine, stabilization and treatments*, 2nd edn. Wiley, Chichester
- Río Segade S, Pace C, Torchio F, Giacosa S, Gerbi V, Rolle L (2015) Impact of maceration enzymes on skin softening and relationship with anthocyanin extraction in wine grapes with different anthocyanin profiles. *Food Res Int* 71:50–57. <https://doi.org/10.1016/j.foodres.2015.02.012>
- Romero-Cascales I, Fernández-Fernández JI, Ros-García JM, López-Roca JM, Gómez-Plaza E (2008) Characterisation of the main enzymatic activities present in six commercial macerating enzymes and their effects on extracting colour during winemaking of Monastrell grapes. *Int J Food Sci Technol* 43:1295–1305. <https://doi.org/10.1111/j.1365-2621.2007.01608.x>
- Singleton VL, Rossi J (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16:144–158
- Skroza D, Generalić Mekinić I, Svilović S, Šimat V, Katalinić V (2015) Investigation of the potential synergistic effect of

- resveratrol with other phenolic compounds: A case of binary phenolic mixtures. *J Food Compos Anal* 38:13–18
- Soto Vázquez E, Rfo Segade S, Orriols Fernández I (2010) Effect of the winemaking technique on phenolic composition and chromatic characteristics in young red wines. *Eur Food Res Technol* 231:789–802. <https://doi.org/10.1007/s00217-010-1332-5>
- Sun B, Neves AC, Fernandes TA, Fernandes AL, Mateus N, De Freitas V, Leandro C, Spranger MI (2011) Evolution of phenolic composition of red wine during vinification and storage and its contribution to wine sensory properties and antioxidant activity. *IJ Agric Food Chem* 59:6550–6557. <https://doi.org/10.1021/jf201383e>
- Xia EQ, Deng GF, Guo YJ, Li HB (2010) Biological activities of polyphenols from grapes. *Int J Mol Sci* 11:622–646. <https://doi.org/10.3390/ijms11020622>
- Zdunić G, Preece JE, Dangel GS, Koehmstedt A, Mucalo A, Maletić E, Pejić I (2013) Genetic characterization of grapevine cultivars collected throughout the Dalmatian region. *Am J Enol Vitic* 64:285–290. <https://doi.org/10.5344/ajev.2012.12085>
- Zoecklein B, Fugelsang KC, Gump B, Nury FS (1995) *Wine analysis and production*. Springer, New York
- Žulj Mihaljević M, Anhalt UCM, Rühl E, Tomić Mugoša M, Maraš V, Forneck A, Zdunić G, Preiner D, Pejić I (2015) Cultivar identity, intravarietal variation, and health status of native grapevine varieties in Croatia and Montenegro. *Am J Enol Vitic* 66:531–541. <https://doi.org/10.5344/ajev.2015.15023>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.