

Effects of Maceration Duration on the Phenolic Composition and Antioxidant Capacity of ‘Teran’ (*Vitis vinifera* L.) Wine

Kristijan DAMIJANIĆ¹ (✉)

Mario STAVER¹

Karin KOVAČEVIĆ GANIĆ²

Marijan BUBOLA³

Ingrid PALMAN¹

Summary

Effects of maceration duration on the phenolic composition and antioxidant capacity in red grapevine variety ‘Teran’ (*Vitis vinifera* L.) was investigated in this study. Total phenolics, flavonoids, nonflavonoids, individual and total anthocyanins, vanillin index and antioxidant capacity measured by DPPH, ABST and FRAP methods were determined in ‘Teran’ wines during five different skin maceration periods (3, 7, 12, 17 and 21 days). The highest increase in the concentration of the most phenolic compounds and antioxidant capacity was obtained between the 3rd and 7th day of maceration. Prolonging the maceration from 7 to 21 days did not lead to significantly higher concentrations of total phenolics, flavonoids, nonflavonoids, total anthocyanins and antioxidant capacity measured with ABTS and FRAP methods. It is concluded that maceration duration of seven days is the most appropriate in order to obtain high concentrations of total phenolics and anthocyanins and high antioxidant capacity of ‘Teran’ wines.

Key words

maceration duration, phenolic composition, antioxidant capacity, ‘Teran’

¹ Polytechnic of Rijeka, Department of Agriculture, K. Huguesa 6, 52440 Poreč, Croatia

✉ e-mail: kristijan.damijanac@veleri.hr

² University of Zagreb, Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

³ Institute of Agriculture and Tourism, K. Huguesa 8, 52440 Poreč, Croatia

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Introduction

Phenolic compounds are important constituents in wines, since they contribute to wine organoleptic properties such as colour, astringency, bitterness and most of them may show biological properties related to their antioxidant capacity (Kelebek et al., 2009). Grapes contain non-flavonoid compounds mainly in the pulp, while flavonoid compounds are located mainly in the skins, seeds, and stems (Gómez-Plaza et al., 2001). The phenolic composition of wines depends on their concentrations in grapes, the winemaking technology used and their transformation during wine aging process (González-Neves et al., 2001; Sacchi et al., 2005).

Extraction of phenolic compounds during maceration, during which phenols turn into wine from solid parts of grapes, depends on vinification conditions (Budić-Leto et al., 2008), while the maceration duration has the greatest influence (Gómez-Plaza et al., 2001; Vrhovšek et al., 2002).

Among other factors, the optimum pomace contact time needed to achieve the proper level and composition of phenolics in wine depends on the desired wine style and cultivar. The colour of the young red wines mainly depends on the extraction of anthocyanins from grape skin during maceration process (Gómez-Plaza et al., 2001). Several studies demonstrated that the largest increase in anthocyanin extraction of red *Vitis vinifera* wines occurred early in fermentation (in the first 3 to 4 days of skin maceration), while little increase occurred after 10 days of skin contact and then show a decrease (Gómez-Plaza et al., 2001; Sacchi et al., 2005). Tannins and flavonoids continue to be extracted during skin maceration after anthocyanin extraction has reached a maximum, up to the end of alcoholic fermentation (Yokotsuka et al., 2000). High levels of tannins may stabilize the anthocyanins and wine color and may contribute to excessive astringency (Sacchi et al., 2005). The astringency and bitterness of the wine are mainly attributed to proanthocyanidins (Kovac et al., 1992). It was shown that the extraction of proanthocyanidins and catechins increased progressively with the length of maceration (Vrhovšek et al., 2002).

Another favorable aspect of phenolic compounds is their contribution to the antioxidant properties of food and so there is considerable interest in the possible health effects of these compounds (Budić-Leto et al., 2008). The antioxidant compounds present in wine are derived almost exclusively from grapes and have been identified as phenolic acids, flavonols, monomeric catechins and anthocyanidins (Katalinić et al., 2004). According to the same authors, high flavonoid content in red wines contributes to its increased antioxidant potential.

The influence of different maceration duration on phenolic composition of wines produced in several wine regions has been extensively studied (Kovac et al., 1992; Gómez-Plaza et al., 2001; Kelebek et al., 2009). However, there are no data on the influence of maceration duration on wine phenolic composition of 'Teran', a major autochthonous red grapevine variety in Istria (Croatia). Several studies have been published on the effect of enological practices on red wine antioxidant capacity (Netzel et al., 2003; Villaño et al., 2006), but few data are available on Croatian red wines (Katalinić et al., 2004; Maletić et al., 2009).

Furthermore, there is no available data concerning the variation of antioxidant capacity during different skin maceration periods of 'Teran' wine. Nowadays, the enhancement of 'Teran's' wine phenolic concentration has become the aim of many producers who wish to achieve high quality 'Teran' wine with optimized levels of natural antioxidants.

The aim of this research was to determine the influence of different maceration periods (3, 7, 12, 17 and 21 days of maceration) on the phenolic composition and antioxidant capacity of 'Teran' wines.

Materials and methods

The grapes of 'Teran' (*Vitis vinifera*, L.), cultivated near Poreč in West Istrian wine growing region were hand-harvested in the vintage 2006, when reducing sugars reached 21 Brix and 8.5 g/L of total acidity (expressed as g/L of tartaric acid) and pH 3.0. Standard viticultural practices for the cultivar and region were performed during vineyard management. Vinification was performed at the Experimental winery of the Department of Agriculture, Polytechnic of Rijeka, located in Poreč, Croatia. Experiment was carried out with 500 kg of grapes divided in four replicates. A random distribution of harvested grape among the different replicates was done to avoid any initial uncontrolled difference in grape composition. Grapes were crushed and destemmed immediately after harvest and then homogeneously transferred into four 130 L stainless steel tanks for maceration with the addition of sulphur dioxide (40 mg/L). Alcoholic fermentation was conducted by *Saccharomyces cerevisiae* "Premium Zinfandel" wine yeast (**Enologica Vason S.r.l.**, Verona, Italy). No pectolytic enzymes were added. Throughout the skin contact period, the cap was manually punched down twice a day to encourage the extraction of phenolic compounds. The initial fermentation temperature was 20°C and it was controlled to a maximum of 27°C. All vinifications were controlled daily by measuring the temperature and optical density. Wine samples (300 ml) were collected five times from each replicate (on days 3, 7, 12, 17 and 21 from the beginning of maceration). All samples were frozen until the moment of the analysis.

Total phenolics were evaluated as stated by Singleton and Rossi (1965) using Folin-Ciocalteu reagent. The quantification of total phenolics was carried out using a calibration curve prepared with known amounts of gallic acid and results are expressed as mg/L gallic acid equivalents (GAE). The flavonoid content was determined spectrophotometrically according to the method of Lee et al. (2003). Gallic acid was used as standard and results are expressed as mg/L GAE. The amount of non-flavonoid content was calculated as difference between total phenols and flavonoids in wine. Flavan-3-ols were determined using vanillin assay described by Di Stefano et al. (1989) and the results are expressed as mg/L (+)-catechin equivalents (CTE). The total anthocyanins content in wines was determined using bisulfite bleaching method (Ribéreau-Gayon and Stonestreet, 1965) and expressed in mg/L malvidin-3-glucoside equivalents.

The free anthocyanins content was determined with HPLC 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 was 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) (250x4.6 mm, 5 µm ID). The following mobile phases were used: buffer: 10 mM KH₂PO₄+H₃PO₄ to pH 1.6, solvent A: acetonitrile-buffer (5:95), and 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 that was 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 results were expressed in mg/L of wine sample.

Antioxidant capacity of wine samples was determined with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method according to the technique reported by Brand-Williams et al. (1995) and the results are expressed in mmol/L Trolox equivalents. Determination of antioxidant capacity was also estimated with 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS^{•+}) radical scavenging method using the procedure of Ivekovic et al. (1995) and the results are expressed as mmol/L Trolox equivalents. The ferric reducing antioxidant power (FRAP) assay was carried according to technique reported by Benzie and Strain (1996) and the results are expressed as mmol/L Fe SO₄. Data were analyzed by the analysis of variance (ANOVA) using Statistica software package (version 9. StatSoft, Tulsa, OK, USA). Means comparisons were performed by LSD test at $p < 0.05$.

Results and discussion

The concentration of total phenolic compounds, flavonoids and nonflavonoids increased with increasing skin contact period (Table 1) and are in accordance with other findings (Scudamore-Smith et al., 1990; Kovac et al., 1992; Auw et al., 1996). A significant increase in concentration of total phenolics, flavonoids and nonflavonoids was achieved between 3rd and 7th day of the maceration, while with longer maceration periods no significant increase in concentrations of these compounds occurred.

The highest concentration of total phenolics, flavonoids and nonflavonoids was achieved on the 17th day of maceration, but differences among 17th day and 7th, 12th and 21st day were not significant. Changes in the concentration of nonflavonoids after the 7th day of maceration were not significant, which agrees with previous findings that they are not influenced by extended pomace contact after the end of fermentation (Rossi et al., 1990; Scudamore-Smith et al., 1990). The decrease in total phenolics with longer maceration periods could be explained by the decrease of some fractions caused by precipitation, re-fixation on the solid parts and on yeast cell wall, degradation, anthocyanin-phenolics polymerization etc. (Kelebek et al., 2009). We found that the concentration of total phenolics decreased in 'Teran' wines after 21st day, although the increase slowed after 7th day. Yokotsuka et al. (2000) reported for 'Merlot' that total phenolics increased up to 36 days of pomace contact, though the increase slows around 10th day.

A significant increase in the concentration of total anthocyanins was achieved from 3rd to 7th day of the maceration. Increased maceration temperature (27°C), with concurrent action of alcohol, favourably influences the release of anthocyanins from grape skin cells (Sacchi et al., 2005), and this was the cause of a substantial increase in anthocyanin concentration after the third day of maceration. On the 12th day of maceration, the total amount of anthocyanins in 'Teran' wine reached a maximum of 762 mg/L. The decrease in anthocyanin concentration was observed from the 12th to 21st day of maceration (from 762 to 597 mg/L). The decrease in the level of anthocyanins in wines found from the 12th day of maceration could be due to the fixation of compounds on yeasts or solid parts and by reactions of degradation and condensation with tannins (Auw et al., 1996; Kelebek et al., 2009). Flavan-3-ols, evaluated by vanillin assay, in wines significantly increased with longer skin contact and reached the highest concentrations on the 21st day of skin maceration, which is in agreement with the research on 'Plavac mali' and 'Babić' wines (Budić-Leto et al., 2008).

The evaluation of individual wine anthocyanins is shown in Table 2. Eleven different anthocyanins were detected and determined quantitatively in 'Teran' wines. Delphinidin, cyanidin, petunidin, peonidin and malvidin were mainly found as 3-O-monoglucosides, and in a minor proportions, as esters of acetic and *p*-coumaric acids. The highest increase in the concentration of anthocyanins was found between days 3 and 7, and a further, but less pronounced increase was observed from 7th to 17th day of maceration. From 17th to 21st day of maceration

Table 1. Effects of maceration duration on the phenolic composition in 'Teran' wine

| Compounds | 3 days | 7 days | 12 days | 17 days | 21 days |
|---------------------------|--------|--------|---------|---------|---------|
| Total phenols (mg/L) | 615 b | 2003 a | 1815 a | 2425 a | 1863 a |
| Flavonoids (mg/L) | 508 b | 1578 a | 1485 a | 1993 a | 1448 a |
| Non-flavonoids (mg/L) | 108 b | 425 a | 330 ab | 433 a | 415 a |
| Total anthocyanins (mg/L) | 257 c | 653 ab | 762 a | 684 ab | 597 b |
| Vanillin assay (mg/L) | 403 c | 1250 b | 1401 ab | 1212 b | 284 ab |

Values within each row followed by the different letter are significantly different ($p \leq 0.05$)

Table 2. Effects of maceration duration on the anthocyanin content (mg/L) in Teran wine

| Compound | 3 days | 7 days | 12 days | 17 days | 21 days |
|------------------------------------------|--------|---------|----------|---------|----------|
| Delphinidin-3- <i>O</i> -monoglucoside | 10.7 b | 20.7 a | 26.0 a | 25.3 a | 18.5 ab |
| Cyanidin-3- <i>O</i> -monoglucoside | 1.6 b | 3.0 ab | 4.0 ab | 4.5 a | 3.1 ab |
| Petunidin-3- <i>O</i> -monoglucoside | 13.3 b | 22.9 ab | 26.5 a | 29.7 a | 25.4 a |
| Peonidin-3- <i>O</i> -monoglucoside | 13.5 | 21.6 | 24.4 | 24.7 | 20.5 |
| Malvidin-3- <i>O</i> -monoglucoside | 21.8 d | 76.8 c | 134.0 ab | 167.6 a | 109.8 bc |
| Delphinidin-3- <i>O</i> -acetylglucoside | 4.1 b | 4.9 ab | 6.3 ab | 7.8 a | 5.6 ab |
| Cyanidin-3- <i>O</i> -acetylglucoside | 0.4 c | 1.1 bc | 2.1 ab | 2.8 a | 2.6 a |
| Peonidin-3- <i>O</i> -acetylglucoside | 2.8 c | 3.9 bc | 4.7 ab | 6.4 a | 4.7 ab |
| Malvidin-3- <i>O</i> -acetylglucoside | 14.3 b | 18.1 b | 24.0 ab | 31.1 a | 19.7 ab |
| Peonidin-3- <i>O</i> -coumarylglucoside | 1.1 b | 2.1 ab | 2.7 ab | 3.3 a | 1.9 ab |
| Malvidin-3- <i>O</i> -coumarylglucoside | 8.6 | 11.0 | 13.0 | 16.4 | 12.0 |

Values within each row followed by the different letter are significantly different at ($p \leq 0.05$)

a slight decrease in the concentration of monomeric anthocyanins occurred. Almost all anthocyanins and their derivatives were present at the highest concentrations at day 17 of maceration, when alcoholic fermentation was finished. Several studies have shown that maximum pigmentation is reached between 3 and 4 days of skin maceration and further skin contact has no effect in increasing pigment concentration (Auw et al., 1996). Polymerisation, fixation of anthocyanins on yeasts or solid parts and reactions of degradation are important mechanisms occurring during vinification (Kelebek et al., 2009), and probably that are the reasons for the decrease in monomeric anthocyanins after the 17th day of skin maceration.

Anthocyanin monoglucosides were the major anthocyanins in 'Teran' wines (Table 2), as well as in most other *Vitis vinifera* cultivars (Mazza, 1995; Netzel et al., 2003; Kelebek et al., 2009). Malvidin-3-*O*-monoglucoside was the most dominant anthocyanin in all 'Teran' wines, with concentrations between 21.8 and 167.6 mg/L and it is the most dominant individual anthocyanin in most previous studies (Kelebek et al., 2009; Maletić et al., 2009). Petunidin-3-*O*-monoglucoside was the second most abundant pigment in 'Teran' wines. Cyanidin-3-*O*-monoglucoside was the anthocyanin present in the lowest concentrations in all 'Teran' wines, and it is the anthocyanin present in the lowest concentrations in most *Vitis vinifera* cultivars, with the exception of some cultivars (Yokotsuka et al., 2000). Kelebek et al. (2009) point out that cyanidin is considered by some authors to be the precursor of the other anthocyanidins and also as the most hydroxylated anthocyanin undergoes oxidation in the early hours after crushing.

The major acetyl and p-coumaryl derivatives present in 'Teran' wines were malvidin-3-*O*-acetylglucoside and malvidin-3-*O*-coumarylglucoside (Table 2). These findings are in accordance with previous studies of 'Tannat', 'Cabernet Sauvignon' and 'Merlot' cultivars (González-Neves et al., 2001). Acylated anthocyanins reached a maximum concentration on 17th day of skin maceration. As previously reported, malvidin-3-*O*-acetylglucoside along with malvidin-3-*O*-coumarylglucoside are considered to be the most important derivatives for the characterization of varieties (Mazza, 1995). We found that the proportion of total malvidin derivatives increased with increasing skin contact period, from 48.5% (3rd day) to 67.4% (17th day) and after that showed a slight decrease, but on average was close to 60% in 'Teran' wines. The

concentrations of malvidin derivatives decrease less during fermentation and in the post-fermentation period, because they are structurally more stable anthocyanic forms in comparison to other anthocyanins (González-Neves et al., 2001).

Antioxidant capacity was measured by radical cation decolourisation (ABTS), ferric reducing/antioxidant power (FRAP) and radical scavenging assay (DPPH). The *in vitro* antioxidant capacities of wines at day 3 were significantly lower than those at days 7 to 21 (Table 3). During prolonged maceration there is an enrichment of phenolic fraction (mainly flavan-3-ols and anthocyanins), which are proved to be the most potent in terms of antioxidant capacity (Villaño et al., 2006). This could explain the significant increase in the antioxidant capacity of 'Teran' wine during prolonged skin maceration.

Table 3. Effects of maceration duration on the antioxidant capacity in 'Teran' wine

| Antioxidant capacity | 3 days | 7 days | 12 days | 17 days | 21 days |
|-----------------------------------|--------|--------|---------|---------|---------|
| ABTS (mmol/L Trolox) | 23.1 b | 30.4 a | 30.2 a | 30.5 a | 30.6 a |
| FRAP (mmol/L Fe SO ₄) | 21.0 b | 28.1 a | 28.3 a | 28.3 a | 28.6 a |
| DPPH (mmol/L Trolox) | 2.85 c | 4.12 b | 4.27 b | 5.21 a | 5.52 a |

Values within each row followed by the different letter are significantly different at ($p \leq 0.05$).

However, the antioxidant capacity, measured by ABST and FRAP method was not modified by the variation of phenolic composition during prolonged skin maceration (from 7th to 21st day of maceration), indicating that the major reactions among polyphenols or reactions between phenols and other non-phenolics did not modify the *in vitro* antioxidant capacity, which agrees with previous findings (Villaño et al., 2006). On the other hand, the antioxidant capacity measured by DPPH method was significantly higher at 17th and 21st day of maceration in comparison to 7th and 12th day of maceration. According to Villaño et al. (2006) ABTS values greater than 11 are considered very high. 'Teran' wines showed high antioxidant capacity ranging from 23.1 (3th day) to 30.6 mmol/l (21st day), expressed as trolox

equivalents. Maletić et al. (2009) reported that ABTS values after 14 days of maceration were for Babić (18.1 mmol/L trolox) and for 'Plavac mali' wines (39.2 mmol/L trolox) showing that antioxidant capacity largely depends on variety. The high flavonoid content in red wine (80.6% for 'Teran' wine) contributes to its increased antioxidant potential (Katalinić et al., 2004). The antioxidant capacity of 'Teran' wines determined with FRAP test was similar to other monovarietal wines made from red grapevine cultivars in Croatia (Katalinić et al., 2004), which ranged from 20.6 to 32.3 mmol/L.

Conclusions

The phenolic composition of 'Teran' wine varied significantly during winemaking. There was a significant difference in total phenols, flavonoids, vanillin index, total anthocyanins and individual anthocyanins between three days of skin maceration and longer maceration duration. Three day maceration showed significantly lower antioxidant capacity than prolonged skin contact duration regardless of the antioxidant measurement method used. The concentrations of most phenolic compounds and antioxidant capacity did not vary significantly between the 7th and 21st day of maceration. Results of this study indicate that maceration duration of seven days is the most appropriate in order to achieve high concentrations of total phenolics and anthocyanins in 'Teran' wines with high levels of natural antioxidants.

References

- Auw J.M., Blanco V., O'Keefe S.F., Sims C.A. (1996). Effect of processing on the phenolics and color of Cabernet Sauvignon, Chambourcin, and Noble wines and juice. *Am J Enol Vitic* 47:279-286
- Berente B., García D.D.C., Reichenbacher M., Danzer K. (2000). Method development for the determination of anthocyanins in red wines by high-performance liquid chromatography and classification of German red wines by means of multivariate statistical methods. *J Chrom A* 871:95-103
- Benzi I.F.F., Strain, J.J. (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem* 239:70-76
- Brand-Williams W., Cuvelier M.E., Berset C. (1995). Use of free radical method to evaluate antioxidant activity. *Food Sci Tec* 28:25-30
- Budić-Leto I., Gracin L., Lovrić T., Vrhovšek U. (2008). Effects of maceration conditions on the polyphenolic composition of red wine 'Plavac mali'. *Vitis* 47(4):245-250
- Di Stefano R., Cravero M.C., Gentilini N. 1989. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico* 1:83-89
- Gómez-Plaza E., Gil-Muñoz R., López-Roca J.M., Martínez-Cutillas A., Fernandez-Fernandez J.I. (2001). Phenolic compounds and colour stability of red wines. Effect of skin maceration time. *Am J Enol Vitic* 52:271-275
- González-Neves G., Gómez-Cordéves C., Barreiro L. (2001). Anthocyanin composition of Tannat, Cabernet Sauvignon and Merlot young red wines from Uruguay. *J Wine Res* 12:125-133
- Iveković D., Milardović S., Roboz M., Grabarić B.S. (2005). Evaluation of the antioxidant activity by flow injection analysis method with electrochemically generated ABTS radical cation. *The Analyst* 130(5):708-714
- Katalinić V., Miloš M., Modun D., Musić I., Boban M. (2004). Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food Chem* 86:593-600
- Kelebek H., Canbas A., Selli S. (2009). Effects of different maceration times and pectolytic enzyme addition on the anthocyanin composition of *Vitis vinifera* cv. Kalecik karasi wines. *J Food Process Pres* 33:296-311
- Kovac V., Alonso E., Bourzeix M., Revilla E. (1992). Effect of several enological practices on the content of catechins and proanthocyanidins in red wines. *J Agric Food Chem* 40:1953-1957
- Lee K.W., Kim Y.J., Lee H.J., Lee C.Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *J Agric Food Chem* 51(25):7292-7295
- Maletić E., Karoglan Kontić J., Preiner D., Jeromel A., Patz C.D., Dietrich H. (2009). Anthocyanin profile and antioxidative capacity of some autochthonous Croatian red wines. *J Food Agri Environ* 7(1):48-51
- Mazza G. (1995). Anthocyanin in grape and grape products. *Crit Rev Food Sci Nutr* 35(4):341-371
- Netzel M., Strass G., Bitsch I., Konitz R., Christmann M., Bitcsh R. (2003). Effect of grape processing on selected antioxidant phenolics in red wines. *J Food Engin* 56:223-228
- Ribéreau-Gayon P., Stonestreet E. (1965). Le dosage des anthocianes dans le vin rouge et détermination de leur structure. *Bull Soc Chim Fr* 9(419):2649-2652
- Rossi I., Monteleone E., Dinardo E., Bertuccioli M. (1990). Fermentation and skin contact time effects on quality of Aglianico wine. *Dev Food Sci* 32:445-453
- Sacchi K.L., Bisson L.F., Adams D.O. (2005). A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am J Enol Vitic* 56:197-206
- Scudamore-Smith P., Hooper R., Mclaran E. (1990). Color and phenolics changes of Cabernet Sauvignon wine made by simultaneous yeast/bacterial fermentation and extended pomace contact. *Am J Enol Vitic* 41:57-67
- Singleton V.L., Rossi J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16:144-158
- Villaño D., Fernández-Pachón M.S., Troncoso A.M., García-Parrilla M.C. (2006). Influence of enological practices on the antioxidant activity of wines. *Food Chem* 95:394-404
- Vrhovšek U., Vanzo A., Nemanic J. (2002). Effect of red wine maceration techniques on oligomeric and polymeric proanthocyanidins in wine, cv. Blaufränkisch. *Vitis* 41:47-51
- Yokotsuka K., Sato M., Ueno N., Singleton V.L. (2000). Colour and sensory characteristics of Merlot red wines caused by prolonged pomace contact. *J Wine Res* 11(1):7-18