ASSAY OF THE PHENOLIC PROFILE OF MERLOT WINES FROM MACEDONIA: EFFECT OF MACERATION TIME, STORAGE, SO2 AND TEMPERATURE OF STORAGE

Violeta Ivanova1,2, Marina Stefanova2, Borimir Vojnoski1

1 “SS. Cyril and Methodius“ University of Skopje, Institute of Agriculture, Wine Department, Skopje, Republic of Macedonia
2 “SS. Cyril and Methodius“ University of Skopje, Faculty of Natural Sciences and Mathematics, Institute of Chemistry, Skopje, Republic of Macedonia

marinaiv@iunona.pmf.ukim.edu.mk

Spectrophotometric assays of total anthocyanins, total phenolics, total catechins, total flavonoids, color intensity and hue were performed on Merlot wines obtained with 3, 6 and 10 days of maceration, containing 30 and 70 ppm SO2. Changes of phenolic contents were observed during three stages of the wines: after maceration, after 6 and 16 months in order to check the effect of maceration time, SO2 and storage of the wines. Wines were stored at low and higher temperature to check also the influence of storage temperature on the studied parameters. It was found that maceration time influences the content of polyphenol compounds, observing increasing of their concentrations with increased maceration time, while lower contents were measured in the wines after 16 months of storage, respectively, in wine with 30 ppm SO2). SO2 had not a significant effect, whereas higher temperature caused slight changes of polyphenols contents.

Key words: total anthocyanins; phenolics; catechins; flavonoids; color intensity; hue; wine-making; SO2; spectrophotometry

ОДРЕДУВАЊЕ НА ФЕНОЛНИОТ ПРОФИЛ НА ВИНАТА МЕРЛО ОД МАКЕДОНИЈА: ЕФЕКТ НА ВРЕМЕТО НА МАЦЕРИРАЊЕ, ЗРЕЕЊЕТО, SO2 ИТЕМПЕРАТУРАТА НА ЧУВАЊЕ

Беа извршени спектрофотометрични испитувања на вкупни антацијани, вкупни феноли, вкупни флавоници, интензитет на боја и нијанса на вината мерло добиени со 3, 6 и 10 дена мацерација, кои содржат 30 и 70 ppm SO2. Промените на содржината на фенолните соединенија беа следени кај вината во различни фази на зреење: по мацерирање, по 6 и по 16 месеци, со цел да се провери влијанието на времето на мацерирање, SO2 и зреењето на вината. Вината беа чувани на ниска и повисока температура за да се провери ефектот на температурата на чувење на содржината на испитуваните параметри. Беше утврдено дека времето на мацерирање има влијане врз полиценолните компоненти, чија концентрација се зголемува со зголемување на времето на мацерирање, додека повисоки содржини беа измерени во вината по зреење од 16 месеци (3006, 1732 и 1602 mg/l вкупни феноли и 478, 188 и 98,5 mg/l вкупни антацијани, по мацерирање, по 6 и по 16 месеци зреење, соодветно, во вино со 30 ppm SO2). SO2 нема значително влијание, додека повисоки температури на чувење предизвикаа благи промени во фенолнот состав на вината.

Ключни зборови: вкупни антацијани; вкупни феноли; вкупни катехини; вкупни флавоници; интензитет на боја; нијанса; технологија за производство на вино; SO2; спектрофотометрија

1. INTRODUCTION

Polyphenolic compounds are very important constituents of red grapes and wines. They belong to two main groups, non-flavonoids (namely, hydroxybenzoic acid and hydroxycinnamic acid and their derivatives, stilbenes and phenolic alcohols) and flavonoids (namely, anthocyanins, flavan-3-ol
monomers and polymers, flavonols and dihydroflavonols) [1]. Free anthocyanins, extracted from grapes, are responsible for the color of young red wine and its color depends on the conditions in the medium (pH, SO₂). Tannins contribute to the mouth feel of wines, and they can form pigmented polymers with anthocyanins providing stable pigments which give longterm color stability of the wine [2].

The vinification process is an important operation in enology and results in the type of wine desired by the consumer. Several studies have been published on those wine-making technologies and conditions affecting greater extraction of phenolics, stable color and greater content of phenolic compounds in wine [3–9]. During storage and aging, wine color changes from a bright red to a reddish-brown hue. This is attributed to the formation of a new, more stable, polymeric pigments proceeding from reactions between anthocyanins and other phenolic compounds, including flavan-3-ol monomers, directly or mediated by acetaldehyde [10], vinyl phenols derivatives and pyruvic acid [11, 12].

Merlot is a variety of red grape cultivated in Republic of Macedonia used for production of high quality wines. In this study, the phenolic composition of Merlot wine is systematically assayed with regards to several factors. Merlot wines were prepared with different wine-making techniques, applying two doses of SO₂ (30 and 70 ppm SO₂) and three maceration times of 3, 6 and 10 days. Wines were stored at low (~15 °C) and higher temperatures (~25 °C). The total phenolic content, total anthocyanins, total flavonoids, total catechins, color intensity and hue of those wines have been determined with spectrophotometric assays in order to check the influence of maceration time, SO₂, temperature and bottle storage on the polyphenol content.

2. EXPERIMENTAL

Wine samples

Grapes from Vitis vinifera, the Merlot variety, cultivated in the Skopje region, were harvested at optimal maturity (2007 vintage) and transported to the experimental winery of the Wine Department, Institute of Agriculture in Skopje, Republic of Macedonia. Grapes were divided into 6 lots (11 kg for each lot) and using a mechanical crusher/destemmer, the grapes were processed separately in the same way, and crushed grapes were collected in 25 l plastic fermentation tanks.

Two different doses of aqueous solution of potassium metabisulfite were added to the grape mash and mixed, to give three tanks having 30 ppm total SO₂ (M30) and other three tanks, having 70 ppm total SO₂ (M70).

Vinalco yeast (Saccharomyces cerevisiae) was used for fermentation. Vinalco was selected by the Factory for yeast and alcohol manufacture (Bitola), from the Tikveš region and it was prepared by rehydrating (20 g/100 L) in water (30 °C for 15 min). The yeast was applied to the lots containing 30 ppm SO₂ (M30) and 70 ppm SO₂ (M70).

After addition of SO₂ and yeast, maceration time of 3, 6 and 10 days was applied in order to study the effect of maceration time on phenolics extraction, in the obtained six different variations.

All wines were mechanically “pumped over” twice a day during fermentation. After the period of maceration (3, 6 and 10 days), the wines were separated by mechanically pressing of the pomace. Pressed wines were stabilized at −4°C for a period of two weeks to induce tartaric stability and bottled. Bottled wines were stored in two different conditions: one set of wines was stored in a winery with temperature between 12 and 15 °C and other set of bottles was stored in a room with temperature between 24 and 27 °C. The labels for the obtained lots are given in Table 1.

| PC 1 | PC 2 | PC 3 | PC 4 |
|------|------|------|------|
| TP   | 0.9864 | −0.0052 | 0.0454 | 0.1076 |
| TA   | 0.7793 | 0.5966 | 0.0954 | 0.1206 |
| TF   | 0.7725 | −0.5799 | −0.1394 | −0.1881 |
| TC   | 0.8685 | −0.4251 | −0.1366 | 0.1738 |
| CI   | 0.7321 | 0.2410 | 0.5963 | 0.1738 |
| H    | −0.4191 | −0.4317 | 0.7859 | 0.1320 |

% of variance | 61 | 19 | 17 | 3 |

Labels: TP – total phenolics, TA – total anthocyanins, TF – total flavonoids, TC – total catechins; CI – color intensity, H – hue
Instrumentation and reagents

Analysis of polyphenolic components was carried out with a HP 8452 UV-Vis and Agilent 8453 UV-Vis spectrophotometers. All analyses were performed in duplicate.

The reagent p-(dimethylamino)cinnamaldehyde (p-DMACA), standards of gallic acid and (+)-catechin were from Fluka (Switzerland), and the Folin-Ciocalteu reagent was from Merck (Germany). All the other used reagents were of analytical purity grade.

Total anthocyanins assay

Determination of total anthocyanins was performed by the method proposed by Di Stefano et al. (1989) [13] by appropriate dilution of the samples with a solution consisted of ethanol/water/HCl = 70/30/1. The concentrations of anthocyanins was calculated using the equation:

$$\text{TA}_{540 \text{ nm}} (\text{mg/l}) = A_{540 \text{ nm}} \cdot 16.7 \cdot d$$

$A$ – absorbance at 540 nm, $d$ – dilution expressed as malvidin-3-glucoside equivalents.

Total catechins assay

The concentration of total catechins (procyanidins monomers) in wines was determined using the method of Di Stefano et al. (1989) [14] with the reagent p-DMACA and catechin as a standard for construction of the calibration curve. An appropriate diluted wine sample (1 ml) was added to a 10 ml volumetric flask, followed by adding of 3 drops of glycerol and 5 ml 1 % p-DMACA reagent, so that the total volume was made up to 10 ml with methanol. The absorbance was read after 7 min at 640 nm against the blank-methanol.

Total phenolics assay

The Folin-Ciocalteu method reported by Slinkar & Singleton (1977) [15] was slightly modified for the analysis of total phenolics in wine samples. In brief, an aliquot (1 ml) of appropriate diluted wine was added to a 10 ml volumetric flask, containing 5 ml of distilled water followed with addition of 0.5 ml of Folin-Ciocalteu’s reagent and 1.5 ml 20 % solution of Na₂CO₃ after 3 min, making up the total volume to 10 ml with distilled water. The measurements were performed after 16 min storage of the samples at 50 °C in sealed flasks, at 765 nm and expressed as gallic acid equivalent (GAE, mg/l) based on a calibration curve obtained with standard of gallic acid.

Total flavonoids assay

Total flavonoids were determined using the colorimetric assay with aluminium chloride and (+)-catechin as standard for calibration according to Zhishen et al. (1995) [16]. An aliquot of 1 ml of wine sample (appropriate diluted) was added to a 10 ml volumetric flask containing 4 ml of distilled water, followed with addition of 0.3 ml of 5 % NaNO₂ and 5 min later, 0.3 ml of 10 % AlCl₃ was added. After 6 min, 2 ml of 1 M NaOH was added to the mixture and the total volume was made up to 10 ml with distilled water, measuring the absorbance at 510 nm against the prepared water blank.

Color intensity and hue of wines

A direct measurement of wine absorbance at 420, 520 and 620 nm was carried out using a 2 mm optical path and the color intensity (CI) and hue (H) of wines were calculated [1]. The data were adjusted to 1 cm length path.

Statistical analysis

Statistical treatment of the data was performed, including calculations of mean and standard deviation. Principal component analysis was performed using the software TANAGRA 1.4.28 (Lyon, France).

3. RESULTS AND DISCUSSION

Spectrophotometric determinations of total anthocyanins (TA), total catechins (TC), total phenolics (TP), total flavonoids (TF), color intensity (CI) and hue (H) were performed for the Merlot wines obtained with different maceration times of 3, 6 and 10 days, containing two doses of SO₂ (30 and 70 ppm SO₂), during three stages: after period of maceration, after 6 and 16 months storage of the wines at low temperature in the winery. Measurements were performed in order to study the effect...
of maceration time and SO\textsubscript{2} on the extraction of polyphenols and, also, the influence of bottle storage on phenolic content. Concentrations of TA, TC, TP, TF, CI and H are presented in Table 2 and Figures 1, 2, 3, 4, 5a and 5b, respectively.

### Table 2

**Labels of Merlot wine samples prepared and stored under different vinifications**

| Vinification conditions | 30 mg/l SO\textsubscript{2} | 70 mg/l SO\textsubscript{2} |
|-------------------------|-----------------------------|-----------------------------|
|                         | Low temperature | Higher temperature | Low temperature | Higher temperature |
| 3 days of maceration    | V30-3d-L          | V30-3d-H               | V70-3d-L          | V70-3d-H               |
| 6 days of maceration    | V30-6d-L          | V30-6d-H               | V70-6d-L          | V70-6d-H               |
| 10 days of maceration   | V30-10d-L         | V30-10d-H              | V70-10d-L         | V70-10d-H              |

| Wines       | Storage | TP* | TA** | TF*** | TC*** | CI   | H    |
|-------------|---------|-----|------|-------|-------|------|------|
| M30-3d      | After macer. | 3006 ± 0.76 | 478 ± 2.12 | 413 ± 3.78 | 187 ± 3.87 | 4.03 ± 6.39 | 0.68 ± 6.29 |
|             | 6 m     | 1732 ± 2.28 | 188 ± 3.45 | 303 ± 3.07 | 74.0 ± 2.34 | 1.55 ± 5.03 | 0.57 ± 8.30 |
|             | 16 m    | 1602 ± 0.90 | 98.5 ± 2.43 | 331 ± 2.47 | 99.5 ± 1.49 | 1.45 ± 4.87 | 0.64 ± 8.23 |
| M30-6d      | After macer. | 2937 ± 3.56 | 429 ± 1.34 | 539 ± 2.69 | 250 ± 2.66 | 2.66 ± 5.67 | 0.57 ± 8.03 |
|             | 6 m     | 2689 ± 4.71 | 339 ± 2.33 | 432 ± 2.77 | 198 ± 2.34 | 1.98 ± 7.44 | 0.52 ± 7.10 |
|             | 16 m    | 2462 ± 3.56 | 156 ± 2.64 | 481 ± 3.09 | 209 ± 3.94 | 1.86 ± 6.06 | 0.66 ± 8.21 |
| M30-10d     | After macer. | 3467 ± 3.08 | 496 ± 3.21 | 566 ± 1.34 | 332 ± 2.29 | 2.65 ± 6.84 | 0.48 ± 5.49 |
|             | 6 m     | 3287 ± 2.82 | 402 ± 2.87 | 539 ± 1.73 | 327 ± 1.69 | 2.69 ± 5.21 | 0.5 ± 8.43 |
|             | 16 m    | 3022 ± 1.22 | 218 ± 2.43 | 719 ± 2.51 | 327 ± 3.04 | 2.37 ± 8.66 | 0.59 ± 8.84 |
| M70-3d      | After macer. | 3134 ± 3.22 | 431 ± 1.67 | 449 ± 0.78 | 220 ± 3.73 | 3.04 ± 7.72 | 0.66 ± 9.54 |
|             | 6 m     | 2138 ± 4.18 | 233 ± 2.41 | 363 ± 1.45 | 117 ± 2.68 | 1.54 ± 8.94 | 0.53 ± 7.59 |
|             | 16 m    | 2113 ± 0.67 | 118 ± 2.89 | 378 ± 2.06 | 187 ± 2.05 | 1.53 ± 8.27 | 0.67 ± 8.93 |
| M70-6d      | After macer. | 3133 ± 2.41 | 506 ± 2.06 | 539 ± 1.93 | 246 ± 3.67 | 2.70 ± 6.02 | 0.51 ± 9.53 |
|             | 6 m     | 2847 ± 3.42 | 404 ± 1.78 | 518 ± 2.48 | 191 ± 4.08 | 2.55 ± 5.35 | 0.49 ± 8.76 |
|             | 16 m    | 2764 ± 3.88 | 206 ± 3.22 | 481 ± 2.06 | 266 ± 3.74 | 2.25 ± 6.83 | 0.62 ± 7.59 |
| M70-10d     | After macer. | 3176 ± 2.32 | 445 ± 2.75 | 522 ± 0.78 | 295 ± 4.23 | 2.24 ± 6.74 | 0.49 ± 6.45 |
|             | 6 m     | 3031 ± 2.75 | 391 ± 2.03 | 482 ± 0.65 | 218 ± 3.45 | 2.39 ± 5.82 | 0.51 ± 7.21 |
|             | 16 m    | 2879 ± 1.85 | 221 ± 1.87 | 641 ± 1.47 | 230 ± 2.88 | 2.34 ± 5.08 | 0.61 ± 7.83 |

The values are mean ± RSD of two replicates.  
Labels: TP – total phenolics, TA – total anthocyanins, TF – total flavonoids, TC – total catechins, CI – color intensity, H – hue,  
after macer. – after maceration, 6 m – six months, 16 m – sixteen months, M – Merlot, 30 – 30 ppm SO\textsubscript{2}, 70 – 70 ppm SO\textsubscript{2}, 3d – three days of maceration, 6d – six days of maceration, 10d – ten days of maceration

*Concentrations expressed as gallic acid equivalents (mg/l)  
**Concentrations expressed as malvidin-3-glucoside equivalents (mg/l)  
***Concentrations expressed as catechin equivalents (mg/l)

Wines from the second lot, stored at higher temperature, were analyzed only after 6 months of storage and the results were compared with the corresponding wines stored at lower temperature.

Determination of total phenolics was performed with the commonly used colorimetric method, the Folin-Ciocalteu assay which is based on redox reactions in which polyphenols are oxidized. Determination of tannins using p-DMACA assay relies on the formation of a coloured product from the reaction between tannins and the aldehyde reagent [17]. And, the colorimetric method using AlCl\textsubscript{3} was applied for determination of total flavonoids, whereas anthocyanins, expressed as malvidin-3-glucoside equivalents, were determined by the method of Di Stefano et al. 1989 [13], using the already proposed equation because of the non-availability of an authentic standard.
Effect of maceration time

Fermentation and maceration time have a profound effect on the amount of anthocyanins, catechins and phenolics present in the final wine [2]. Anthocyanins are extracted at the early stage of vinification and at the beginning of the alcoholic fermentation when they reach the maximum and their content increases with maceration time. If we compare the results for anthocyanins (Fig. 1), obtained after maceration of 3, 6 and 10 days, it could be noticed that they reached the maximum in the wine macerated for 6 days, containing 70 ppm SO₂ and this value was close to the anthocyanin content of the wine macerated for 10 days with 30 ppm SO₂.

Longer maceration time means higher contents of extracted catechins [2], as it was observed for the analyzed wines. The concentration of catechins was lower for the wines macerated for 3 days and it increased during the maceration, reached the maximum in the wines macerated for 10 days (Fig. 2). This was expected because during 3 days of maceration, mainly anthocyanins and tannins from the skins are extracted and this period of contact with the grape mash is not enough for extraction of seeds tannins. In fact, they are protected by a lipid layer that needs alcohol to be disorganized. This is evident for the wines macerated for 6 and 10 days which contained higher concentrations of catechins due to the extraction of monomers in the later stages of vinification.

As it can be seen from the Figure 3, the content of total phenolics increased with the maceration time, as it was observed for total flavonoids (Fig. 4). The obtained data are in agreement with previously published data [6, 18]. The values for color intensity and hue were highest in the wines after 3 days of maceration, and lower values were noticed in the wines obtained after maceration time of 6 and 10 days (Fig. 5), which could be explained with the fact that anthocyanins could be adsorbed at the yeast cells or hard parts of the grapes.

Fig. 1. Changes in the concentration of total anthocyanins during storage: after maceration, after 6 and 16 months of storage, for the wines obtained with 3, 6 and 10 days of maceration, containing 30 and 70 ppm SO₂

Fig. 2. Changes in the concentration of total catechins during storage: after maceration, after 6 and 16 months of storage, for the wines obtained with 3, 6 and 10 days of maceration, containing 30 and 70 ppm SO₂

Fig. 3. Changes in the concentration of total phenolics during storage: after maceration, after 6 and 16 months of storage, for the wines obtained with 3, 6 and 10 days of maceration, containing 30 and 70 ppm SO₂

Fig. 4. Changes in the concentration of total flavonoids during storage: after maceration, after 6 and 16 months of storage, for the wines obtained with 3, 6 and 10 days of maceration, containing 30 and 70 ppm SO₂
Differences in the concentration of anthocyanins, catechins, phenolics, flavonoids, color intensity and hue were observed during the period of storage of the wines. Decreasing of anthocyanins and total phenolics was observed for all wines, as it was expected. Catechins decreased in the period of the first 6 months and then started to increase with aging (Fig. 2). The largest decrease of anthocyanin contents was in the wines macerated for 3 days, at the first 6 months of storage (Fig. 1). Short maceration time can lead to lower levels of tannin extraction and in this case, higher anthocyanin losses may occur since anthocyanins are not stabilized by polymerization with tannins. Wines macerated for 6 and 10 days contained higher concentrations of catechins which was expected because they are extracted at the end of fermentation. This way, forming of stable pigments decreases the anthocyanin and flavan-3-ol content, but the content of pigments increases in the wines.

Similar results were obtained for the total phenolics concentrations (Fig. 3), which were highest after the longest maceration period in all wines, observing decrease of their content during aging of the wines. The biggest decrease was observed for the wines macerated for 3 days, after 6 months of storage, as it was observed for the anthocyanins and color intensity. For the wines macerated for 6 and 10 days, slight decrease of the total phenolics was observed. Total phenolics decreased insignificantly in the wines obtained with 10 and 6 days of maceration during the aging, probably due to the precipitation. During the period of aging, total flavonoids behave similarly like catechins, their concentrations were lower at the first six months of storage of the wines. Wine macerated for 10 days with 30 ppm SO2, contained highest total catechin content after maceration, decreased after 6 and 16 months of storage. It was noticed that, during the six months of storage, catechins decreased in all wines and then their concentration increased after sixteenth month of aging.

Color intensity, which represents the amount of color in the wines, slightly decreased, with exception of 3 days macerated wines, where large decreasing of this value was observed after 6 months of storage, similarly like the decreasing of the concentration of anthocyanins. The hue indicates the development of a color towards orange, which increases throughout aging reaching upper limit, as it was observed for the analyzed Merlot wines which had the highest hue values after 16 months of storage.

**Effect of storage**

**Effect of SO2**

SO2, is an antioxidant and antimicrobial agent, which is commonly used in wine-making, before the fermentation starts. SO2 inactivates grape enzymes, such as polyphenoloxidases, and its early incorporation in churched grapes is very important because it protects polyphenols from oxidation and precipitation during the fermentation. Comparing the obtained results for total phenolics, anthocyanins, catechins, flavonoids, color intensity and hue values, for the wines containing two different doses of SO2 (30 and 70 ppm SO2), it was noticed that no significant differences on the extraction of those compounds were observed after finishing the fermentation; only slightly higher contents of the polyphenols from the different groups were observed in the wines with higher amount of SO2. This was in agreement with previ-
Assay of the phenolic profile of Merlot wines from Macedonia: Effect of maceration time, storage, SO\textsubscript{2} and temperature of storage

Assumedly published results, which showed that SO\textsubscript{2} content does not dramatically affect the extraction of phenolics [21]. Ough and Amerine, 1961 [22] found that increasing of the SO\textsubscript{2} levels affected Pinot noir phenolic extraction in fermentations at 53 °F (11.8 °C), but not at all at 70 °F (21.3 °C) or 80 °F (26.9 °C).

**Effect of storage temperature**

The differences found between both storage conditions (~15°C and ~25°C) of the wines, according to the obtained data were slight, perhaps because the range in temperature was not too different to give significant differences. However, lower concentrations were measured for anthocyanins in the wines stored at higher temperature, and slightly lower contents for total phenolics. The concentrations of total flavonoids and total catechins were higher, which could be explained that higher temperatures promoted rapid changes in wine composition and rapid reactions in which these components are involved. Results for total anthocyanins, total flavonoids and total catechins are presented in Fig 6.

![Fig 6. Changes of the concentrations of total anthocyanins (TA), total flavonoids (TF) and total catechins (TC) at both storage conditions (low temperature – L and higher temperature – H)](image)

**Principal Component Analysis**

Principal Component Analysis (PCA) was performed in order to check if the studied wines obtained with different maceration times, SO\textsubscript{2} and bottle storage, can be distinguished according to the obtained results for total phenolics, total anthocyanins, total flavonoids, total catechins, color intensity and hue.

The score plot of the first two PCs which account for 79.34 % of the variation of data for wines accounting PC 1 of 60.72 % and PC 2 of 18.62 % are given in Figure 7 A. Grouping was observed according to the storage period, and wines stored for 16 months were separated from the other wines. They were located in the negative part of PC 1 and PC 2. Within this group, further separation was observed between days of maceration, and wines macerated for 10 days (M70-10d-16, M30-10d-16) were located in the negative part of PC 1, wines with six days of maceration (M70-6d-16, M30-6d-16) around zero and –1, whereas the other two wines macerated for 3 days (M30-3d-16, M70-3d-16) were located in the more negative part of the principal component 2.

On the other hand, all wines analyzed in the first phase, after maceration, together with the ones analyzed after 6 months of storage were located in the positive part of PC 1, except wines M30-3d-6, M70-3d-6 and M30-6d-6 which were located in the positive part of PC 2. There is a significant grouping of the wines analyzed right after maceration, according to the maceration time, whereas the samples analyzed after 6 months of storage are spread in the wide range of values of the PC 1 with a slight grouping according to the days of maceration.
As it can be seen from the correlation scatter-plot (Fig 7B), all variables, with exception the hue value, were located in PC 1. The separation of the hue value implies that the studied wine samples can be distinguished by the hue value, which is actually a parameter combining the values of several individually measured parameters (anthocyanins, phenolics) in correlation with the maceration time and storage.

4. CONCLUSION

As it follows from the studied experimental samples of Merlot wines: the main factors affecting the contents of total phenolics, total anthocyanins, total flavonoids, total catechins, color intensity and hue are the maceration time and storage conditions. Higher concentrations of total anthocyanins, polyphenols, catechins and flavonoids were observed with increasing the time of maceration and lower contents during the bottle storage. SO2 levels in the wines do not have a significant effect on phenolic extraction, while temperature of the stogare has a little influence causing slightly higher concentrations of phenolic compounds.

REFERENCES

[1] P. Ribéreau-Gayon, Y. Glories, A. Maujean, D. Dubourdieu, *Handbook of Enology, Volume 2: The Chemistry of Wine and Stabilization and Treatments*, John Wiley & Sons Ltd., 2000.

[2] J. A. Kennedy, Grape and wine phenolics: Observations and recent findings, *Cien. Inv. Agr.*, 35 (2), 107–120 (2008).

[3] G. Mazza, L. Fukumoto, P. Delaquís, B. Girard, B. Ewert, Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem.*, 47, 4009–4017 (1999).

[4] K. Yokotsuka, M. Sato, N. Ueno, V. Singleton, Colour and sensory characteristics of Merlot red wines caused by prolonged pomace contact. *J. Wine Res.*, 11, 7–18 (2000).

[5] B. Sun, I. Spranger, F. Roque-do-Vale, C. Leandro, P. Belchior, Effect of different winemaking technologies on phenolic composition in tinta miúda red wines. *J. Agric. Food Chem.*, 49, 5809–5816 (2001).

[6] A. B. Bautista-Ortín, J. I. Fernández-Fernández, J. M. López-Roca, E. Gómez-Plaza, Wine-making of high coloured wines: extended pomace contact and run-off of juice prior to fermentation. *Food Sci. Tech. Int.*, 10, 287–295 (2004).

[7] B. Sun, M. I. Spranger, Changes in phenolic composition of tinta miúda red wines after 2 years of ageing in bottle: Effect of winemaking technologies. *Eur. Food Res. Tech.*, 221, 1165–1169 (2005).

[8] A. B. Bautista-Ortín, J. I. Fernández-Fernández, J. M. López-Roca, E. Gómez-Plaza, The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. *J. Food Comp. Anal.*, 20 (7), 546–552 (2007).

[9] M. S. García-Falcó, C. Pérez-Lamela, E. Martinez-Carballo, J. Simal-Gándara, Determination of phenolic compounds in wines: Influence of bottle storage of young wines on their evolution. *Food Chem.*, 105 (1), 248–259 (2007).
[10] H. Fulcrand, T. Doxo, N.E. Es-Safi, V. Cheynier, M. Moutounet, Study of the acetaldehyde induced polymerisation of flavan-3-ols by liquid chromatography-ion spray mass spectrometry, J. Chromatogr., A752, 85–91 (1996).

[11] J. Bakker, C. F. Timberlake, Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines, J. Agric. Food Chem., 45, 35-43 (1997).

[12] H. Fulcrand, C. Benabdeljalil, J. Rigaud, V. Cheynier, M. Mountounet, A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochemistry, 47, 1401–1407 (1998).

[13] R. Di Stefano, M. C. Cravero, I composti fenolici e la natura del colore dei vini rossi. L’enotecnico Ottobre, 81–87 (1989).

[14] R. Di Stefano, M. C. Cravero, N. Gentilini, Metodi per lo studio dei polifenoli dei vini. L’Enotecnico I. Maggio, 83–89 (1989).

[15] K. Slinkar, V. L. Singleton, Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic., 28, 9–55 (1977).

[16] J. Zhishen, T. Mengchneg, W. Jianming, The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals, Food Chem., 64, 555–559 (1999).

[17] M. J. Herderic, P. A. Smith, Analysis of grape and wine tannins, Aus. J. Graped&Wine Res., 11, 205–214 (2005).

[18] B. Sun, C. Leandro, J. M. Ricardo de Silva, I. Spranger, Separation of grape and wine proanthocyanidins according to their degree of polymerization. J. Agric. Food Chem., 46, 1390–1396 (1998).

[19] M. Schwarz, G. Hofmann, P. Winterhalter, Investigations on Anthocyanins in Wines from Vitis vinifera cv. pinotage: Factors influencing the formation of pinotin A and its correlation with wine age, J. Agric. Food Chem., 52, 498–504 (2004).

[20] I. Dugelay, Z. Gunata, J. C. Sapis, R. Baumes, C. Bayonove, Role of cinnamoyl esterase activities from enzyme preparations on the formation of volatile phenols during winemaking, J. Agric. Food Chem., 41, 2092–2096 (1993).

[21] K. L. Sacchi, L. F. Bisson, D. O. Adams, A Review of the effect of winemaking techniques on phenolic extraction in red wines, Am. J. Enol. Vitic., 56 (3), 197–206, (2005).

[22] C. S. Ough, M. A. Amerine, Studies on controlled fermentation. V. Effects on color, composition, and quality of red wines, Am. J. Enol. Vitic., 12 (1), 9–19 (1961).