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Comparison of total polyphenols content and antioxidant potential of wines from 'Welschriesling' and 'Sauvignon Blanc' varieties during ageing on fine lees

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ABSTRACT

Phenolic compounds are key components of wine, since they contribute to wine characteristics such as colour, astringency and bitterness. They also act like antioxidants, with mechanisms involving free-radical scavenging that could prevent cardiovascular diseases and cancer. The aim of the present work was to compare the obtained results of total polyphenols content and antioxidant potential (AOP) of several white wines (welschriesling and sauvignon blanc) during ageing on fine lees. The total polyphenols content decreased in average for 16.1 % in welschriesling wines and for 18.7 % in sauvignon blanc wines in the period of three months of wine ageing on lees. In the same period AOP of wines decreased in average for 16.0 % in welschriesling wines and for 8.0 % in sauvignon blanc wines. Expectedly, the samples with added oak chips in grape must had higher antioxidant potential than others.

Key words: white wines, antioxidant potential, phenolic compounds, DPPH, yeast lees

IZVLEČEK

PRIMERJAVA VSEBNOSTI CELOKUPNIH FENOLOV IN ANTIOKSIDATIVNEGA POTENCIALA VIN IZ SORT 'Welschriesling' IN 'Sauvignon Blanc' MED STARANJEM NA FINIH DROŽEH

Fenolne spojine so ključne sestavine vina, ki prispevajo k značilnostim vina, kot so barva, trpkost in grenkoba. Delujejo kot antioksidanti z mehanizmi, ki vključujejo lovljenje prostih radikalov, kar lahko prepreči kardiovaskularne bolezni in raka. Namen dela je bil primerjati dobljene rezultate vsebnosti skupnih fenolov in antioksidativni potencial (AOP) belih sortnih vin laški rizling in sauvignon med zorenjem vina na finih drožeh. Trije meseci spremljanja so pokazali, da se je vsebnost skupnih fenolov zmanjšala v povprečju za 16,1 % v vinih sorte laški rizling in za 18,7 % v vinih sorte sauvignon. Vrednost AOP se je v tem času prav tako zmanjšala v povprečju za 16,0 % pri vinih sorte laški rizling in za 8,0 % pri vinih sorte sauvignon. Pričakovano so imeli vzorci z dodatkom trsk iz hrastovega lesa v mošt večji antioksidativni potencial kot ostali.

Ključne besede: bela vina, antioksidativni potencial, fenolne spojine, DPPH, droži

1 INTRODUCTION

Phenolic compounds are key components of wine that not only contribute to the organoleptic characteristics of wine but they also are the main cause of colour changes in wine. The light yellow colour as well as the brown colour in white wines is undesirable due to the higher content of phenolics and its oxidation. Nevertheless, phenolic compounds also act as antioxidants, with mechanisms involving free-radical scavenging that

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we are examining (Peréz-Serradilla and Luque de Castro, 2008).

White wines are characterised by lower concentrations of total polyphenols (typically 200-500 mg l^{-1}) than red wines because of a lack of red-coloured anthocyanins; white wines are additionally characterized by the predominance of hydroxycinnamic acids (Kilmartin, 2010). Phenolic compounds have received much attention in the prevention of human cardiovascular disorders and cancer due to their antioxidant properties (Paixão et al., 2007). Cells of aerobic organisms are constantly exposed to the effects of reactive oxygen species (ROS) – free radicals. Phenolic compounds have a functional role as they behave as antioxidants against the free radicals. We could say that they increase the antioxidant capacity in the human body after (especially red) wine consumption (Serafini et al., 1998).

We were monitoring the total polyphenols content and antioxidant potential of wine during ageing on fine lees. Yeast cells have been shown to exert a protective effect toward polyphenol oxidation during ageing on lees (Salmon et al., 2002). They have a role as a competing substrate for oxygen in wine. During alcoholic fermentation, yeast cells require oxygen for their metabolic activity and the reactive oxygen species that are produced can potentially oxidise wine polyphenols. At the end of fermentation, phenolic compounds take part in oxygen consumption. After completion of alcoholic fermentation, yeast lees can consume oxygen for up to 3 years in contact with ageing wine. The consumption of oxygen by yeast lees has been ascribed to a mild oxidation of the membrane lipids of the yeast lees that leads to lipid peroxides and further products that may add to the wine flavour (Kilmartin, 2010).

The aim of the present work was to compare the obtained results of total polyphenols content and AOP of several white wines (from 'Welschriesling' and 'Sauvignon Blanc' varieties) during wine ageing on fine lees.

2 MATERIALS AND METHODS

2.1 Samples

We collected 35 samples of Slovenian wines (18 samples of welschriesling and 17 samples of sauvignon blanc wines) and then examined the content of total polyphenols and AOP of these young wines. Investigated samples were taken one week after alcoholic fermentation was completed (at time t=0). Wine ageing was performed in the cooling room of Biotechnical Faculty at temperature 4 °C. No sulphite was added to welschriesling and sauvignon blanc wines. We were monitoring content of total polyphenols and AOP of wine at different times – at time t_1=0, t_2=14 days, t_3=28 days, t_4=48 days and t_5=76 days, during wine ageing on fine lees.

2.2 Folin-Ciocalteu assay

Total polyphenols were determined by Folin-Ciocalteu (FC) spectrofotometric method (Singleton and Rossi, 1965).

Undiluted samples of white wine and gallic acid (used as a standard) were incubated in sodium carbonate solution (20 %, w v^{-1}) and FC reagent for 2 hours at room temperature. The absorbance was measured at wavelength 765 nm. Measured absorbance is proportional to mass concentration of phenolic compounds and it is expressed as gallic acid equivalents (mg of gallic acid per litre of wine). The determination was performed in duplicate and the results are expressed as the mean value.

2.3 Determination of antioxidant potential (AOP) of wine with the 1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging method

Antioxidant potential of wine was determined by DPPH$^{\bullet}$ radical scavenging method (Brand-Williams et al., 1996). DPPH$^{\bullet}$ solution in 99 % methanol was added to 50 µl diluted sample (R=2). After 30 min, the absorbance was measured at 517 nm. For each sample there was a blank (methanol) and reference (DPPH$^{\bullet}$ solution). When antioxidant reacts with the DPPH$^{\bullet}$ radical and radical becomes a stable molecule, a decrease in

absorbance occurs. DPPH• scavenging ability is expressed as concentration of DPPH• in mmol l^{-1}. The determination was performed in triplicates and the results are expressed as the mean value.

3 RESULTS AND DISCUSSION

3.1 Antioxidant potential (AOP) of wines

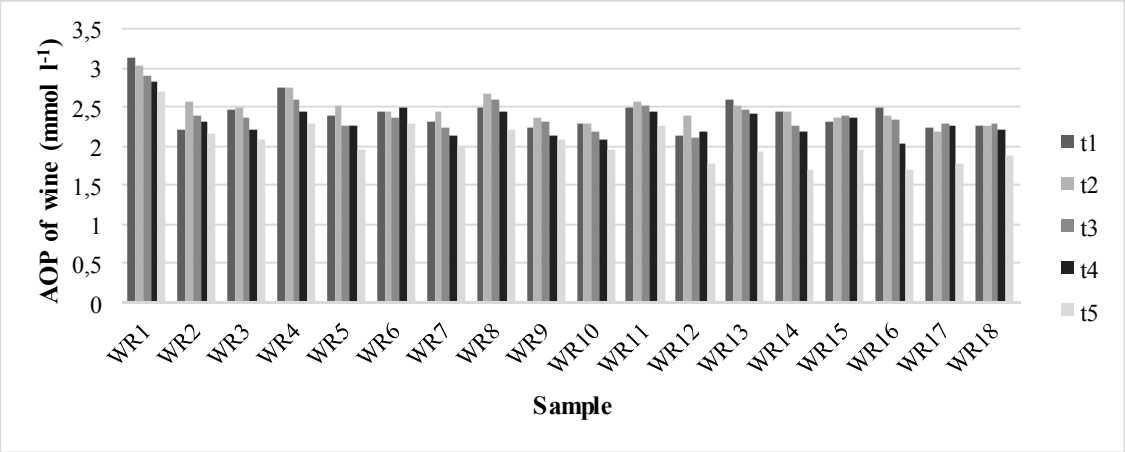

Figure 1: AOP of welschriesling wines at different sampling times ($t_1 = 0$, $t_2 = 14$ days, $t_3 = 28$ days, $t_4 = 48$ days and $t_5 = 76$ days) after completion of alcoholic fermentation

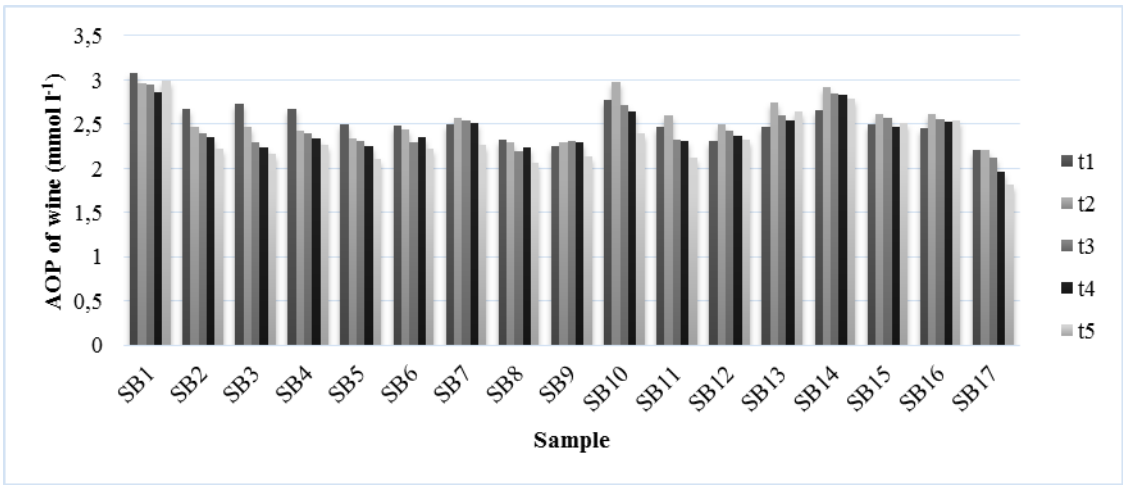

Figure 2: AOP of sauvignon blanc wines at different sampling times ($t_1 = 0$, $t_2 = 14$ days, $t_3 = 28$ days, $t_4 = 48$ days and $t_5 = 76$ days) after completion of alcoholic fermentation

The results of the AOP of welschriesling and sauvignon blanc wines are presented in Figure 1 and Figure 2, respectively. Both Figures show the gradually decrease of antioxidant potential of welschriesling and sauvignon blanc wines as a function of wine ageing on fine lees. We noticed the increase of AOP in some of the wine samples that decreased at the next sampling. The most obvious was this phenomenon of the AOP increasing at the second time of sampling in welschriesling wines WR2, WR8, WR11, and in sauvignon blanc wines SB7, SB10 and SB12-SB16. The increase of AOP of wine could be caused by the antioxidant defence system of yeast - the increase in antioxidant content and increase in the levels of antioxidant enzymes including superoxide dismutase and glutathione reductase on yeast entering into the stationary phase, may

constitute adaptive response to the enhanced oxidative stress (Jakubowski et al., 1999).

Table 1: Content of reducing sugars (RS), content of total polyphenols (PFT), FC index and antioxidant potential (AOP) after 76 days of wine ageing on fine lees.

Sample	RS ($g\ l^{-1}$)	PFT (mg GAE l^{-1})	FC index (/)	AOP (mmol l^{-1})
welschriesling wines				
WR1 (control)	117.2	444.6	36	2.70
WR2	3.1	327.6	6	2.17
WR3	1.1	322.1	6	2.08
WR4	1.5	331.1	10	2.28
WR5	3.6	299.5	9	1.96
WR6 (AM-HT[1])	1.9	353.1	9	2.28
WR7 (AM-HT[1])	1.4	331.1	11	2.01
WR8 (AM-HT[1])	1.4	325.8	10	2.22
WR9	1.7	324.1	7	2.07
WR10	1.2	305.4	8	1.95
WR11	7.7	334.1	7	2.27
WR12	3.4	297.6	7	1.76
WR13	2.4	320.1	10	1.93
WR14	31.4	328.6	3	1.70
WR15	1.9	328.6	10	1.95
WR16	1.5	297.1	10	1.70
WR17	1.4	309.0	11	1.77
WR18	1.7	322.1	10	1.87
sauvignon blanc wines				
SB1 (control)	121.7	451.1	38	2.99
SB2	2.3	320.6	7	2.22
SB3	1.3	310.1	6	2.16
SB4	2.1	315.6	9	2.27
SB5	3.4	298.6	9	2.11
SB6	5.0	322.2	9	2.23
SB7 (AM-MT[2])	5.2	328.6	9	2.26
SB8	1.0	284.1	4	2.06
SB9	1.2	252.6	6	2.14
SB10	1.3	340.6	8	2.39
SB11	1.3	311.7	7	2.12
SB12 (AM-MT[2])	1.1	294.9	4	2.33
SB13 (FR-MT[3])	1.2	356.6	10	2.65
SB14 (FR-MT[3])	1.7	371.6	9	2.79
SB15 (AM-MT[2])	1.5	342.6	9	2.51
SB16 (AM-MT[2])	1.7	342.6	9	2.54
SB17	9.1	233.1	7	1.81

[1] Addition of American oak chips (highly toasted), [2] Addition of American oak chips (medium toast), [3] Addition of French oak chips (medium toast)

The highest antioxidant potential was measured in a control sample SB1, where spontaneous fermentation was performed, and sample SB14 with addition of French oak chips (medium toast). As observed from Table 1, the control samples have the highest amount of reducing sugars ($\gamma_{RS(SB1)}$ = 121.7 g l^{-1} and $\gamma_{RS(WR1)}$ = 117.2 g l^{-1}; while the average amount of reducing sugars in

other samples is γ_{RS} = 3.3 g l^{-1}) and they interfere with free radical DPPH•. Because of that it shows greater antioxidant potential than it should.

The lowest antioxidant potential had sample WR16 with addition of commercial yeast strain EC-1118 (40 g hl^{-1}) and nutrient Naturferm (40 g hl^{-1}).

On Figure 1 and Figure 2 we can see that wines with added oak chips (samples WR6, WR7, WR8, SB7, SB10, SB12, SB13, SB14, SB15 and SB16) express stronger ability to scavenge DPPH• radical than other samples. AOP of samples SB12-SB16 was even higher at sampling time t_5 than at sampling t_0. This could be due to extracted polyphenols from oak chips to wine and therefore increasing AOP of investigated samples.

3.2 Content of total polyphenols

As observed from Figure 3 and Figure 4, content of total polyphenols is constantly decreasing during wine ageing on fine lees.

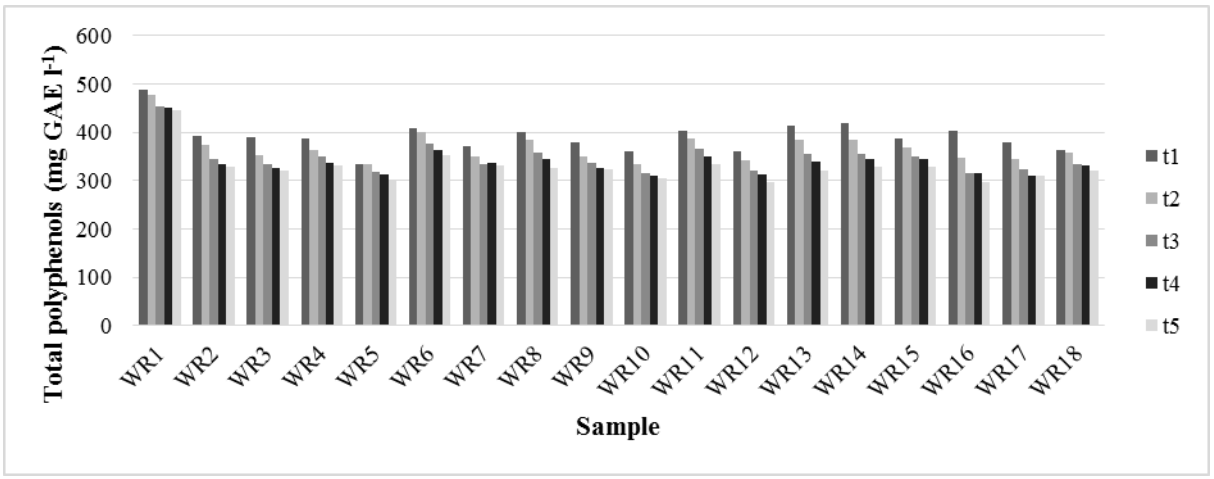

Figure 3: Content of total polyphenols in welschriesling wines at different sampling times (t_1 = 0, t_2 = 14 days, t_3 = 28 days, t_4 = 48 days and t_5 = 76 days) after completion of alcoholic fermentation

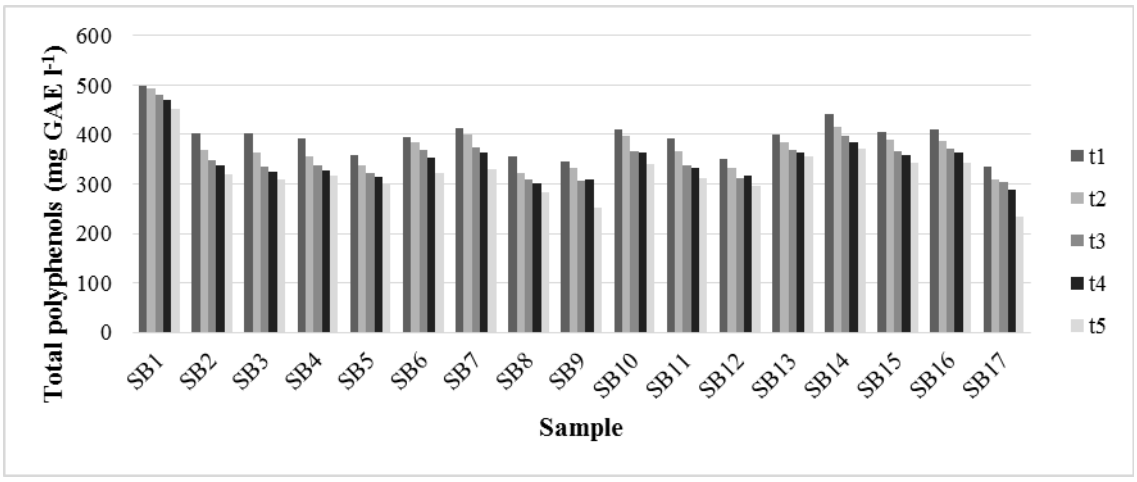

Figure 4: Content of total polyphenols in sauvignon blanc wines at different sampling times (t_1 = 0, t_2 = 14 days, t_3 = 28 days, t_4 = 48 days and t_5 = 76 days) after completion of alcoholic fermentation

The highest content of total polyphenols was determined in samples WR1 and SB1, produced by spontaneous fermentation.

Table 1 lists concentrations of total polyphenols and reducing sugars in both welschriesling and sauvignon blanc wines at sampling time t_5 = 76 days. It shows that samples with higher amount of

reducing sugars have considerably higher content of total polyphenols. The disadvantage of using Folin-Ciocalteu method for determination of total polyphenols is that the mentioned reagent reacts nonspecifically with all phenolic hydroxyl groups (-OH), including aromatic amino acids, ascorbic acid, reducing sugars and organic acids (Abramovič, 2011). We could clean up samples by a solid phase extraction (SPE) to remove other reducing compounds and to get more accurate results. Therefore our results indicate higher content of total polyphenols and the correction is needed.

Table 2: Correction of total polyphenol content (mg GAE l^{-1}) with FC reagent regarding the amount of reducing sugars (g l^{-1}) (Košmerl and Kač, 2010)

Reducing sugars (g l^{-1})	Division factor of total polyphenols concentration
0-10	/
10-25	1.03
25-100	1.06
100-200	1.10

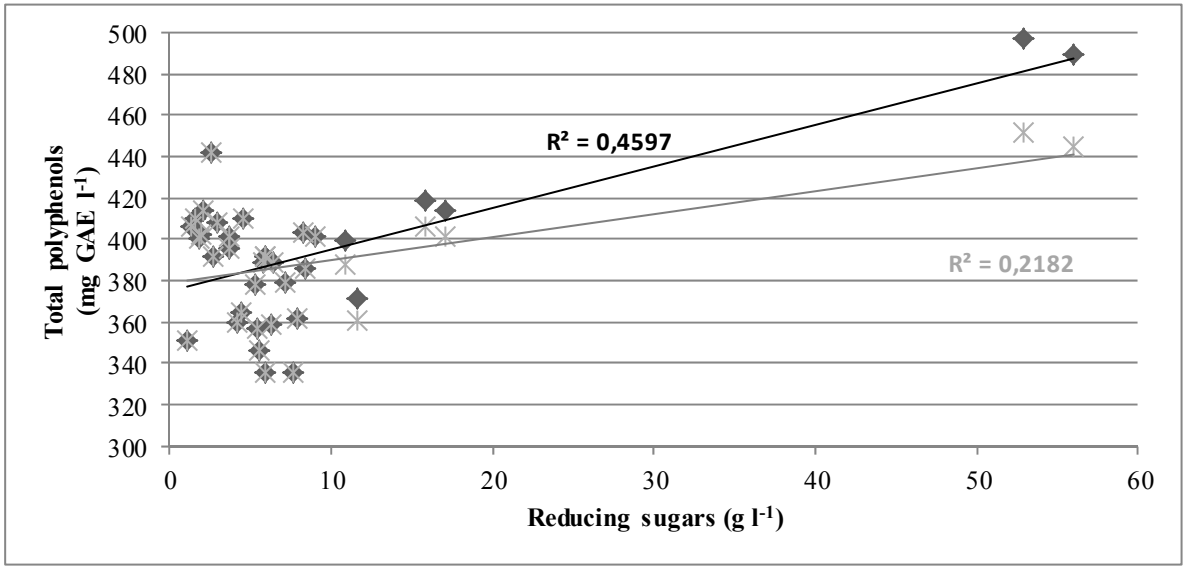

Figure 5: Relation between total polyphenols and reducing sugars with (*) and without correction (♦)

The correlation between reducing sugars and total polyphenols (PFT) was even worse after PFT-correction (R^2 = 0.2182) in comparison to non-correction (R^2 = 0.4597) for our investigated samples.

Expectedly, addition of oak chips in grape must of 'Welschriesling' and 'Sauvignon Blanc' varieties (samples WR6, WR7, WR8, SB7, SB10, SB12-16) resulted in much higher content of total polyphenols. We can assume that polyphenols were extracted from oak chips into wine during wine ageing on fine lees and therefore have increased content of total polyphenols.

3.3 Correlation between total polyphenols and AOP of wines

Figure 6 and Figure 7 presents the correlation between total polyphenols content and AOP of welschriesling and sauvignon blanc wines. It shows a strong positive dependence of AOP on the content of total polyphenols in all wines of both varieties.

The linear correlation between AOP of wine and total polyphenols concentration was better for sauvignon blanc wines than for welschriesling wines. From these obtained results we can

conclude that the correlation between AOP and total polyphenols is particularly varietal characteristic as also demonstrated Košmerl and Cigić (2008). Phenolic composition of individual variety has different influence on correlations of total polyphenols with AOP; polyphenols with higher numbers of hydroxyl groups and those having hydroxyl groups in ortho positions in the aromatic rings usually have higher antioxidant potential (Košmerl and Cigić, 2008).

It can be also summarized that the correlation between AOP of wine and total polyphenols concentration has increased during wine ageing on lees.

Table 3: Coefficient of determination (R^2) for welschriesling wines at different sampling times

Sampling	Coefficient of determination (R^2)
t_1	0.6538
t_2	0.5850
t_3	0.7224
t_4	0.7398
t_5	0.6530

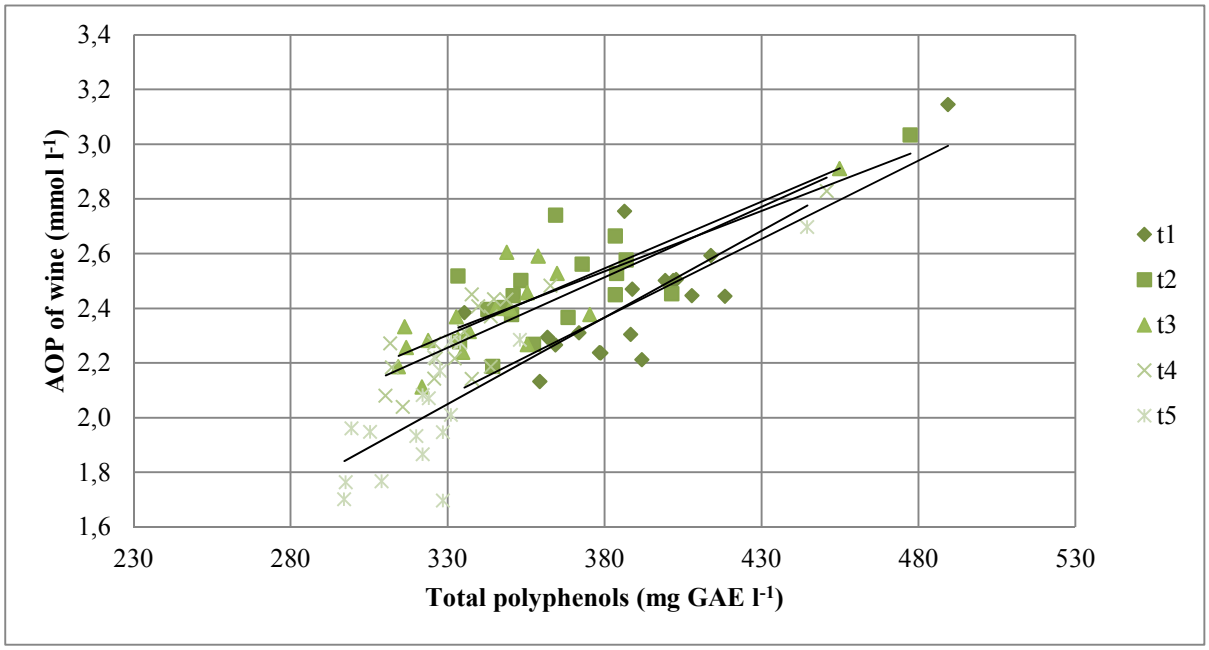

Figure 6: Correlation between total polyphenols and AOP of welschriesling wines at different sampling times (t_1=0, t_2=14 days, t_3=28 days, t_4=48 days and t_5=76 days) after completion of alcoholic fermentation

Table 4: Coefficient of determination (R^2) for sauvignon blanc wines at different sampling times

Sampling	Coefficient of determination (R^2)
t_1	0.7434
t_2	0.7141
t_3	0.7619
t_4	0.7868
t_5	0.8467

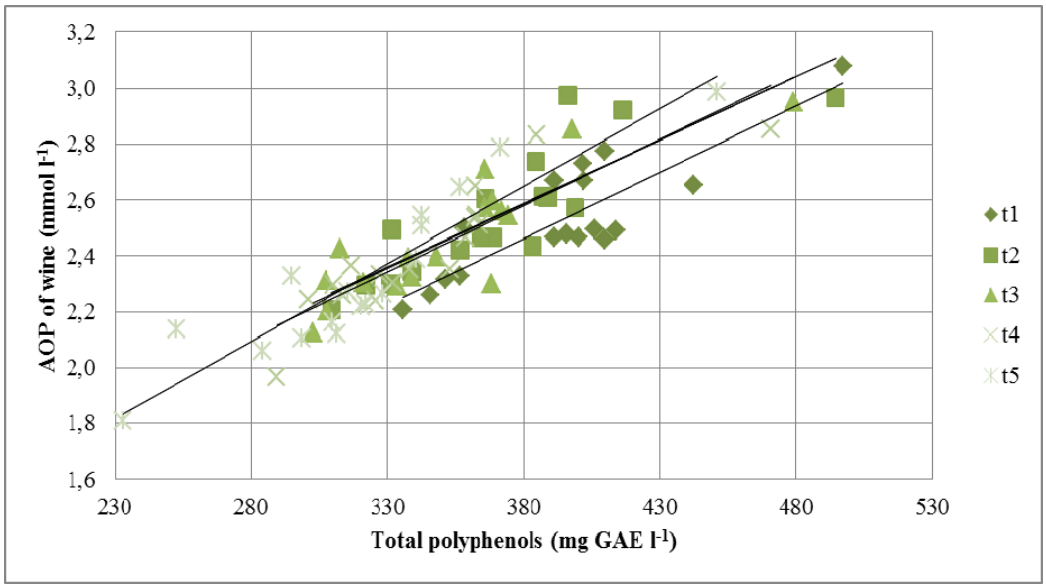

Figure 7: Correlation between total polyphenols and AOP of sauvignon blanc wines at different sampling times (t_1=0, t_2=14 days, t_3=28 days, t_4=48 days and t_5=76 days) after completion of alcoholic fermentation

3.3 Correlation between FC index and total polyphenols

In Table 1 are given informative values for FC index. In the first part we examined correlation between FC index and total polyphenols content and in the second part correlation between reducing sugars and FC index.

As shown in Figure 8, correlation between FC index and total polyphenols content was very weak in the case of sauvignon blanc wines and higher for welschriesling wines.

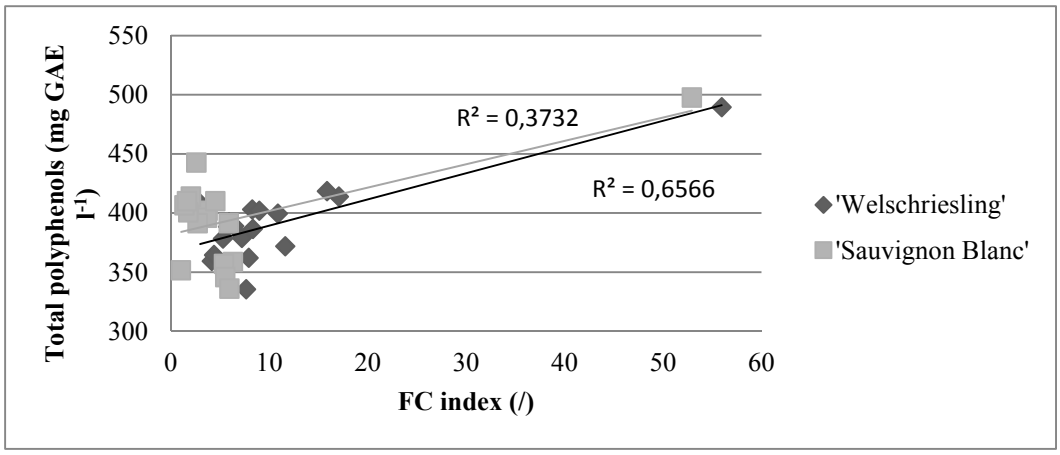

Figure 8: Correlation between FC index and total polyphenols content at sampling time $t_1 = 0$

On the other hand Figure 9 presents that correlation between reducing sugars and FC index at sampling time $t_1 = 0$ was strong ($R^2 = 0.6879$) of both welschriesling and sauvignon blanc wines. The correlation between reducing sugars and FC index was even stronger at sampling time $t_5 = 76$ days ($R^2 = 0.8449$). It means that the amount of reducing sugars influence on values of FC index.

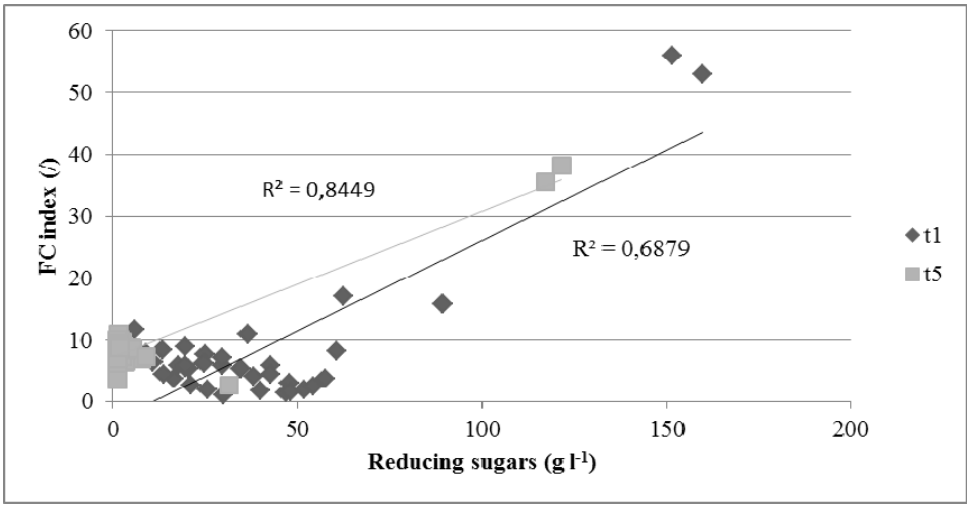

Figure 9: Correlation between reducing sugars and FC index at sampling times $t_1 = 0$ and $t_5 = 76$ days

4 CONCLUSIONS

Based on the obtained results, decrease of total polyphenols content and therefore decrease of antioxidant potential of wine during wine ageing on lees was observed. The total polyphenols content has decreased in average for 16.1 % (in the range from 36 to 105 mg GAE l^{-1}) in welschriesling wines and for 18.7 % (in the range from 44 to 103 mg GAE l^{-1}) in sauvignon blanc wines. Antioxidant potential of wines has decreased in average for 16.0 % (in the range from 0.04 to 0.80 mmol l^{-1}) in welschriesling wines and for 8.0 % (in the range from 0.09 to 0.57 mmol l^{-1}) in sauvignon blanc wines. We would like to emphasize that welschriesling and sauvignon blanc wines are not entirely comparable because oak chips are present in 3 samples of welschriesling wines, while oak chips were added to 7 samples of sauvignon blanc wines.

An increase of AOP was noticed in samples with added oak chips during wine ageing. We can assume that it is because of expected additional extraction of polyphenols from oak chips into wine. For comparison, in samples with added oak chips into grape must the total polyphenols decreased in average for 15.5 % in 76 days, while in the remaining samples they decreased for 18.4 %. AOP of wines with added oak chips has decreased in average for 3.7 %, while in other samples it decreased in average for 15.5 %. The correlation between total polyphenols content and antioxidant potential showed a strong positive dependence of AOP on the content of total polyphenols in all the wines of both varieties.

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