# PHENOLICS AND ANTIOXIDANT ACTIVITY OF WINES DURING THE WINEMAKING PROCESS

## OBSAH POLYFENOLŮ A ANTIOXIDAČNÍ AKTIVITA VÍN V PRŮBĚHU JEJICH VÝROBY

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## ABSTRACT

Content of polyphenols, composition of phenolic complex and antioxidative or antiradical capacity of wines could be affected by many extrinsic and intrinsic factors, as variety, wine growing area and climatic conditions, quality of wine, and not at least also technological procedures during wine-making. Changes in the content of total polyphenols (TP) and antioxidant antiradical activity (AARA) affected by grape variety, wine-growing area and winemaking process in five white (Müller Thurgau, Moravian Muscat MOPR, Pinot Blanc, White Riesling, Traminer) and four red (Saint Laurent, Blauer Portugieser, Pinot Noir, Zweigeltrebe) varieties from three wine areas (Žernoseky, Kutná Hora and Mělník) of Czech Republic (harvest 2004) were studied. 14-17 samples were taken during the wine processing. A spectrophotometric method using Foilin-Ciocalteau reagent was applied for determination of TP content and a spectrophotometric method using free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH·) was applied for determining AARA. Significant differences in TP among varieties were found. Analysis of variance showed statistically high differences among red and white wines and growing areas. Wines differed significantly in TP content and AARA increased significantly during wine making process. Statistically significant differences in AARA values were found among growing areas, wines and varieties. Significant positive correlations between TP and AARA were determined.

**Keywords:** wine; variety; growing region; winemaking; total polyphenols; antioxidant activity

## ABSTRAKT

Obsah a složení polyfenolických látek a jejich antioxidační aktivita ve vínech může být ovlivněna mnoha vnějšími a vnitřními faktory jako např. odrůdou, oblastí, klimatickými podmínkami, kvalitou vína a v neposlední řadě i technologickými postupy použitými při výrobě vína. Byly studovány změny v obsahu celkových polyfenolů (TP) a antioxidační aktivita (AARA) a prokázat vliv odrůdy, oblasti a procesu výroby vína. Pro studii bylo použito pět bílých odrůd (Müller-Thurgau, MOPR, Burgundské bílé, Ryzlink rýnský a Tramín červený) a čtyři modré odrůdy (Svatovavřinecké, Modrý portugal, Burgundské modré a

Zweigeltrebe). Vzorky pocházely z Žernosek, Kutné Hory a Mělníku, ze sklizně roku 2004. V procesu výroby vína bylo odebráno 14-17 vzorků k analýzám. Obsah celkových polyfenolů byl stanoven použitím spektrofotometrické metody s Foilin-Ciocalteauovým činidlem a antioxidační aktivita byla stanovena také použitím spektrofotometrické metody s volným radikálem 1,1-diphenylem-2-picrylhydrazylem (DPPH·). Byly nalezeny významné rozdíly mezi odrůdami. Analýza rozptylu prokázala značné rozdíly mezi červenými a bílými víny a pěstebními oblastmi. Vína se významně lišila v obsahu TP a AARA a jejich hodnoty stoupaly s průběhem procesu výroby vína. Statisticky významné rozdíly v AARA byly nalezeny mezi oblastmi a odrůdami. Nalezli jsme též pozitivní korelace mezi TP a AARA.

**Klíčová slova:** víno, odrůda, oblast, výroba vína, celkové polyfenoly, antioxidační aktivita.

## INTRODUCTION

The French have low coronary heart disease mortality with high fat consumption; this epidemiological anomaly is known as the "French Paradox" and is commonly attributed to the consumption of red wine (Vinson, *et al.*, 2001). Red wine is a complex fluid containing grape, yeast, and wood-derived phenolic compounds, the majority of which have been recognized as potent antioxidants (Burns, *et al.*, 2001). Antioxidative capacity of wine on human LDL oxidation is related to the content of polyphenols contained in wines (Hurtado, *et al.*, 1997), which improve aortic biomechanical properties (Mizutani, *et al.*, 1999). Content of polyphenols, composition of phenolic complex and antioxidative or antiradical capacity of wines could be affected by many extrinsic and intrinsic factors, as variety, wine growing area and climatic conditions, quality of wine, and not at least also technological procedures during wine-making (Faitová, *et al.*, 2004). Colour evolution during vinification and ageing has been attributed to the progressive changes of phenolic compounds extracted from grapes (Vivar-Quintana, *et al.*, 2002). In last years many studies focused on the dynamics of polyphenol extraction during maceration processes of grape varieties (Budic-Leto, *et al.*, 2003, 2005). Important is the evolution of grape polyphenol oxidase activity and phenolic content during wine maturation and vinification (Valero, *et al.*, 1989), changes and evolution of polyphenols in young red wines (Pellegrini, *et al.*, 2000), changes of the hydrophilic and lipophilic antioxidant activity of white and red wines during the wine-making process (Alcolea, *et al.*, 2003). Some authors put more emphasis on maceration process (Budic-Leto, *et al.*, 2005), the other on grape maturation (Jordao, *et al.*, 2001), when quantitative changes in oligomeric occur. Also changes in antioxidant capacity of Tannat red wines during early maturation were reported by Echeverry, *et al.* (2005). The aging of sparkling wines manufactured from red and white grape varieties (Pozo-Bayon, *et al.*, 2003) and different copigmentation models by interaction of anthocyanins and catechins play during the aging process of wine important role (Mirabel, *et al.*, 1999, Esparza, *et al.*, 2004, Monagas, *et al.*, 2005). Phenolic content and antioxidant activity could be also affected by storage conditions or conventional or ecological way of wine production (Zafrilla, *et al.,* 2003). To determine the importance and study factors influencing total polyphenol content (TP) and antioxidant antiradical activity (AARA) of red and white wines from different wine growing areas of Czech Republic, different viticulture, vinification and maturation processes of five white grape varieties and four red varieties were studied.

## MATERIAL AND METHODS

*Grape and wine samples*

Five grape varieties for white wines production (Müller Thurgau, Moravian Muscat MOPR, Pinot Blanc, White Riesling, Traminer) and four grape varieties for production of red wines (Saint Laurent, Blauer Portugieser, Pinot Noir, Zweigeltrebe) from three wine production areas of Czech Republic (Kutná Hora, Mělník, Žernoseky) from the harvest 2004 were investigated. Samples were analysed in dates given in Figs. I-IV in three parallel determinations. White varieties were immediately after harvest in October fermented, while red varieties were during October after harvest macerated and mashed and only after this procedure step fermented (both till the end of the year 2004). In the period January – March 2005 the wines maturated and in April they were bottled.

*Determination of total polyphenol content*

For the determination of total polyphenols (TP) adjusted method with Folin-Ciocalteau´s reagent was used. 1 mL of sample was pipetted into 50 mL volumetric flask and diluted with distilled water. Then 2.5 mL of Folin-Ciocalteau´s reagent was added and after agitation 7.5 mL of 20% sodium carbonate solution was added. After 2 hours standing at laboratory temperature absorbance of samples was measured on the spectrophotometer Heλios γ (Spectronic Unicam, GB) at wavelength λ=765 nm against blank. Extract of seeds was diluted before measuring at 1:50 ratio. Results were expressed as gallic acid (in mg/kg dry matter – DM and in the case of must mg/L fresh must, gallic acid Merck, D). Average results were obtained from three parallel determinations.

*Determination of antioxidant antiradical activity (AARA) by DPPH· method*

AARA was measured after the reaction with free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH·) according to Molyneux (2004). Fresh solution of DPPH in concentration of 25 mg DPPH in 1 L of methanol should be prepared before the determination. 3 mL of violet DPPH· solution is pipetted into plastic cuvettes of 10 mm length and absorbance at wavelength λ = 515 nm on the spectrophotometer Heλios γ (Spectronic Unicam, GB) is measured. Then 5 μL of sample extract is added and after stirring with the hand stirrer in cuvettes the reaction mixture is left to stand for 5 min. The absorbance is again measured and AARA is calculated from the decrease of absorbance in % according to relation:

$$\text{\% of inactivation} = 100-[(A_{t5}/A_{t0})\times 100] \qquad (1)$$

Calibration curve of ascorbic acid (Sigma), tannic acid (Fluka) and gallic acid (Merck) were made. Results were expressed as % of inactivation and calculated to the concentration of standard AA (mg/mL), which could provide the same inactivation as studied sample. Results were obtained from seven parallel determinations.

*Statistic evaluation*

Statistic evaluation of obtained results was made by Statistica programme by the analysis of variance with multiple grouping. More detail evaluation was performed by Scheffé test at $\alpha = 0.05$ and multiple regression analysis.

## RESULTS AND DISCUSSION

Significant differences among varieties were found after harvest (white varieties in av. 187.9 $mg.L^{-1}$, red var. 666.2 $mg.L^{-1}$. According to technological different winemaking procedures of white and red wines (in white wine vinification mashing is lacking) it was found, that at the end of wine making process red wines contained in av. 1425.6 $mg.L^{-1}$ TP, whereas white wines only 161.6 $mg.L^{-1}$. Thus red wines contained in av. 2 times more TP in comparison with grape must at the beginning of winemaking, whereas TP content decreased to 86 % of origin content in grape musts. The highest TP contents were found in Pinot Noir (1935 $mg.L^{-1}$), Zweigeltrebe (1522.2 $mg.L^{-1}$) and Blau Portugieser (1317.6 $mg.L^{-1}$). Analysis of variance (Scheffé test) at level of significance $P<0.05$ showed statistically high significance between red and white wines in TP content ($P=0.000$), Zweigeltrebe and all other varieties ($P=0.000 – 0.013$), Saint Laurent and Blauer Portugieser from all other varieties except Pinot Noir. Differences among white varieties were not significant. Variance analysis among wine growing areas confirmed highly significant differences (P ranged in interval 0.000 – 0.041, Tab. 3). Higher values were found in Mělník area (Zweigeltrebe 1257.7 $mg.L^{-1}$) as compared with Kutná Hora area (Zweigeltrebe 387 $mg.L^{-1}$). These results confirm the suggestions of Burns, *et al.* (2001) that the extraction of the phenolics was found to be influenced by vinification procedure, grape quality, and grape variety. We can confirm high influence of winemaking techniques on the polyphenolic composition as Budic-Leto, *et al.* (2003, 2005) referred in specific Croatian wines. White and red wines differed significantly in TP content course during vinification process. In all measured cases from the preparation of must and its fermentation in October could be seen moderate increase followed by almost constant content during further fermentation and maturation (November – December 2004, January – February 2005), followed finally by moderate decrease in March – April 2005 during and after bottling. TP content changes in white wines during their vinification were insignificant. On contrary, the procedures of wine making of red wines are characterised by dramatic changes in their TP content. Maceration and mashing in October was characterised by moderate or medium TP increase, while during fermentation in November - December intense increase of TP occurred followed by constant content or its moderate decrease during January – March 2005 period (maturation of wine) and then decrease in April (bottling and aging.). As a final result an increase of TP and AARA esp. in red wines during vinification was found (Graph 1 and 2). As Pellegrini, *et al.* (2000) suggested, aging is the main factor influencing the antioxidant activity and TP contents of wines. During maturation quantitative changes of catechins and oligomeric procyanidins were recorded (Jordao, *et al.*, 2001). Relative constant total polyphenol content in our results is in accordance with results of Echeverry, *et al.* (2005), suggesting the relevance of qualitative changes of phenolics. It could be concluded that red and white wines differ not only in final contents of phenolics, but also in their extreme increase in red wines during fermentation. Different evolution patterns during aging depending on the grape variety were also confirmed by Monagas, *et al.* (2005). Decrease is caused mainly by flavanols condensation reactions.

Another parameter investigated was antioxidant antiradical activity (AARA), which could be correlated with TP content. Statistically significant differences in AARA values were found in wine growing area, red and white wines and vine varieties (Tab. 2, 3). Analysis of variance (Scheffé test) at level of significance $P<0.05$ showed statistically high significance between red and white wines in AARA content ($P=0.000$). AARA increased during wine making process, esp. in Zweigeltrebe, St Laurent and Pinot Noir wines (Graphs 3, 4), suggesting thus better AARA of wines compared with grape juices at the beginning of wine making process (Alcolea, *et al.*, 2003). The highest increase was determined during fermentation and maturation of wine. Multiple correlation coefficients between TP and AARA revealed significant correlations in Kutná Hora ($R=0.4701$) and Mělník ($R=0.7379$) wine-growing regions.

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*Table 1. Variance analysis between varieties and TP content (Scheffé test)*

| Variety | {1} | {2} | {3} | {4} | {5} | {6} | {7} | {8} | {9} |
|---|---|---|---|---|---|---|---|---|---|
| | 390.15 | 1698.5 | 288.10 | 2315.5 | 116.41 | 436.79 | 1777.6 | 1211.1 | 263.52 |
| Moravian Muscat | | **0.0000** | 0.9997 | **0.0000** | 0.9137 | 1.0000 | **0.0000** | **0.0000** | 0.9994 |
| Saint Laurent | **0.0000** | | **0.0000** | 0.0052 | **0.0000** | **0.0000** | 0.9999 | **0.0048** | **0.0000** |
| Müller Thurgau | 0.9997 | **0.0000** | | **0.0000** | 0.9863 | 0.9948 | **0.0000** | **0.0000** | 1.0000 |
| Blauer Portugieser | **0.0000** | 0.0052 | **0.0000** | | **0.0000** | **0.0000** | 0.1254 | **0.0000** | **0.0000** |
| Pinot Blanc | 0.9137 | **0.0000** | 0.9863 | **0.0000** | | 0.7929 | **0.0000** | **0.0000** | 0.9980 |
| White Riesling | 1.0000 | **0.0000** | 0.9948 | **0.0000** | 0.7929 | | **0.0000** | **0.0000** | 0.9936 |
| Pinot Noir | **0.0000** | 0.9999 | **0.0000** | 0.1254 | **0.0000** | **0.0000** | | 0.0126 | **0.0000** |
| Zweigeltrebe | **0.0000** | **0.0048** | **0.0000** | **0.0000** | **0.0000** | **0.0000** | 0.0126 | | **0.0000** |
| Traminer | 0.9994 | **0.0000** | 1.0000 | **0.0000** | 0.9980 | 0.9936 | **0.0000** | **0.0000** | |

*Table 2. Variance analysis between varieties and AARA (Scheffé test)*

| Variety | {1} | {2} | {3} | {4} | {5} | {6} | {7} | {8} | {9} |
|---|---|---|---|---|---|---|---|---|---|
| | 3.385 | 29.070 | 7.073 | 16.573 | 2.108 | 2.348 | 12.373 | 21.634 | 11.183 |
| Moravian Muscat | | **0.0000** | **0.0000** | **0.0000** | 0.9984 | 0.9997 | **0.0000** | **0.0000** | **0.0000** |
| Saint Laurent | **0.0000** | | **0.0000** | **0.0000** | **0.0000** | **0.0000** | **0.0000** | **0.0000** | **0.0000** |
| Müller Thurgau | 0.2139 | **0.0000** | | **0.0000** | **0.0093** | **0.0184** | **0.0032** | **0.0000** | 0.0590 |
| Blauer Portugieser | **0.0000** | **0.0000** | **0.0000** | | **0.0000** | **0.0000** | 0.2315 | **0.0088** | **0.0199** |
| Pinot Blanc | 0.9984 | **0.0000** | **0.0093** | **0.0000** | | 1.0000 | **0.0000** | **0.0000** | **0.0000** |
| White Riesling | 0.9997 | **0.0000** | **0.0184** | **0.0000** | 1.0000 | | **0.0000** | **0.0000** | **0.0000** |
| Pinot Noir | **0.0000** | **0.0000** | **0.0032** | 0.2315 | **0.0000** | **0.0000** | | **0.0000** | 0.9987 |
| Zweigeltrebe | **0.0000** | **0.0000** | **0.0000** | **0.0088** | **0.0000** | **0.0000** | **0.0000** | | **0.0000** |
| Traminer | **0.0000** | **0.0000** | 0.0590 | **0.0199** | **0.0000** | **0.0000** | 0.9987 | **0.0000** | |

*Table 3. Variance test between area TP content and AARA (Scheffé test)*

| | TP | | | AARA | | |
|---|---|---|---|---|---|---|
| Area | {1} | {2} | {3} | {1} | {2} | {3} |
| | 1317.9 | 954.38 | 681.70 | 21.818 | 7.611 | 10.719 |
| Žernoseky | | **0.0056** | **0.0000** | | **0.0000** | **0.0000** |
| Mělník | **0.0056** | | **0.0411** | **0.0000** | | **0.0005** |
| Kutná Hora | **0.0000** | **0.0411** | | **0.0000** | **0.0005** | |

*Fig. I. Content of total polyphenols during winemaking in wines from area Kutná Hora*

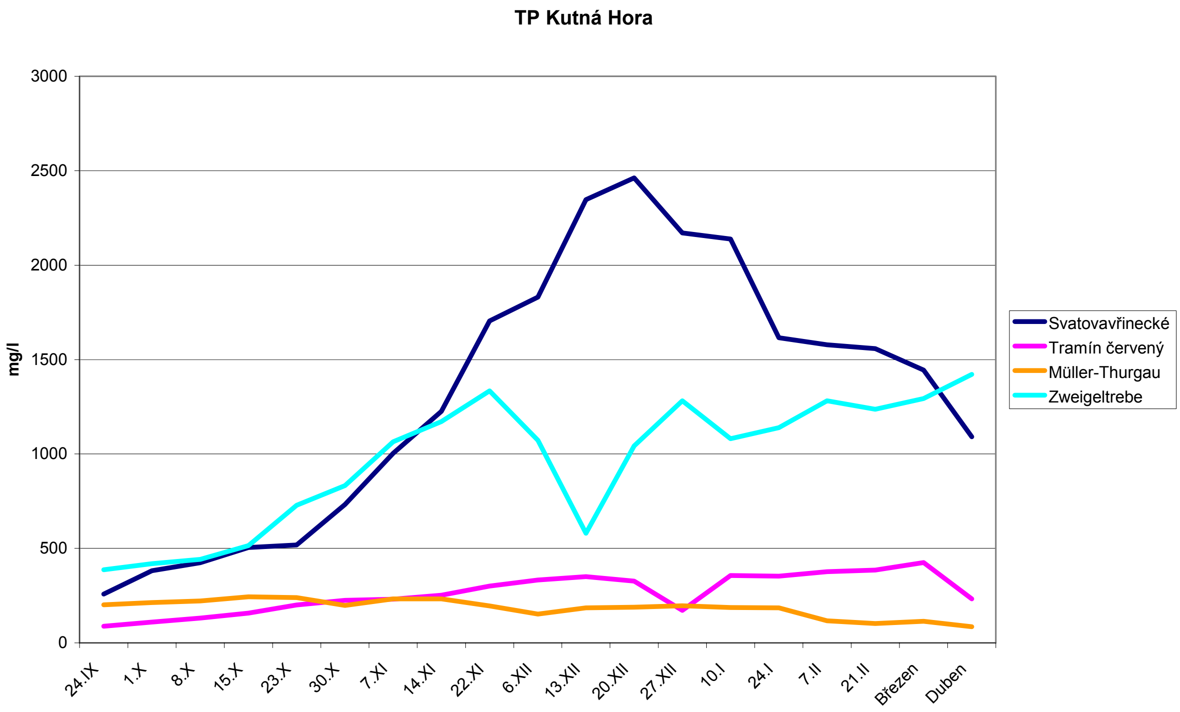



*Fig. II. Content of total polyphenols during winemaking in wines from area Mělník*

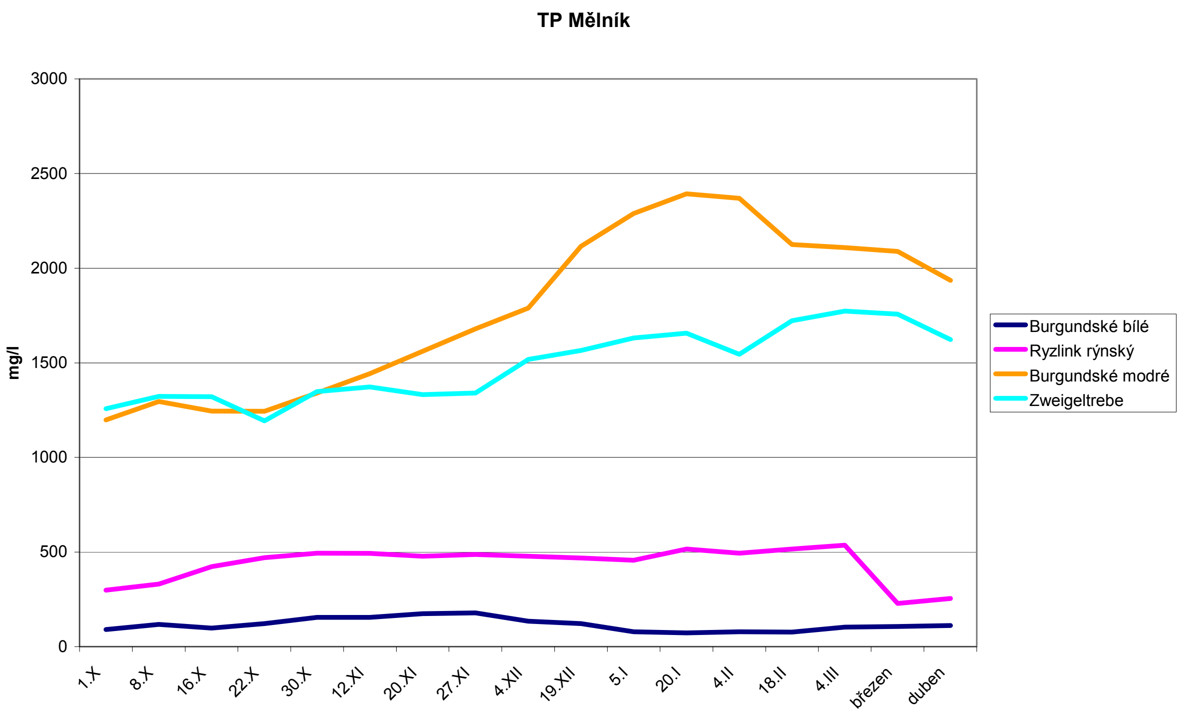


*Fig. III. Changes of antioxidant antiradical activity of wines during winemaking from area Kutná Hora*

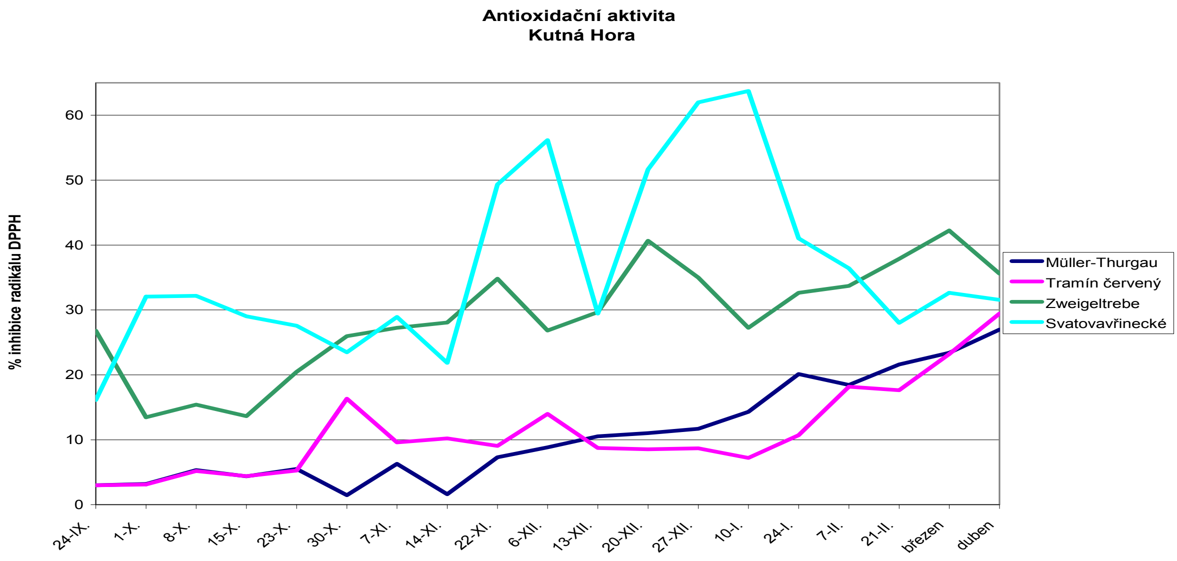
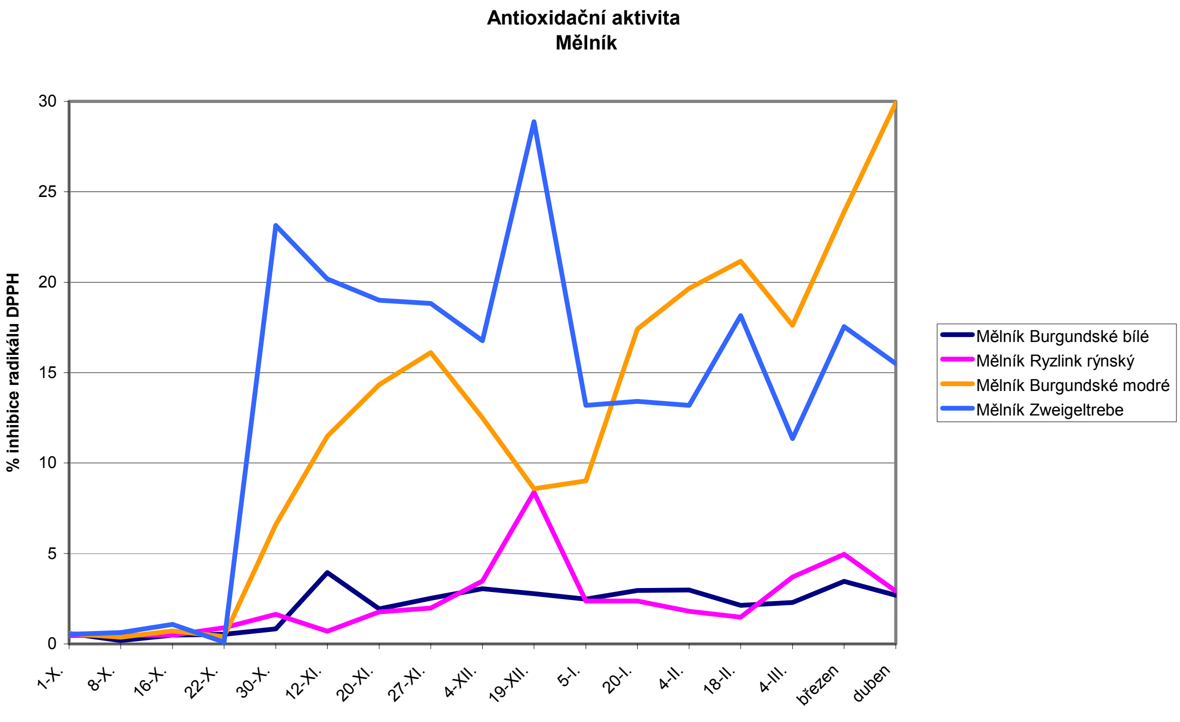



*Fig. IV. Changes of antiradical activity during wine making in wines from area Mělník*




*MT – Müller Thurgau; SL – Saint Laurent; PB – Pinot Blanc; WR – White Riesling; PN – Pinot Noir; ZW – Zweigeltrebe; TRA - Traminer