

Investigation of the Antioxidant Effect of Hungarian White Wines and Tokaj Wine Specialities

Kállay M.¹, Török Z.¹ & Korány K.²

¹University of Horticulture and Food Industry, Department of Enology
H-1118 Budapest, Ménesi út 45.

²University of Horticulture and Food Industry, Department of Food
Chemistry and Nutrition
H-1118 Budapest, Somlói út 14-16.

INTERNATIONAL
JOURNAL OF
HORTICULTURAL
SCIENCE

AGROINFORM
Publishing House, Hungary



Key words: Total Antioxidant Status (TAS), total polyphenol, leucoanthocianin, catechin, resveratrol determination

Summary: Authors investigated the antioxidant effect of wine-phenolics, expressed as total antioxidant status (TAS-value). This property may play an important role in elimination of the harmful effects of free radicals in human body. Independently of their polyphenol composition, special quality Tokaj wines did not show extremely high TAS-values. In Tokaj Aszu wines the total antioxidant status was independent of the "puttony" number but was influenced by the length of aging time.

Introduction

Free radicals are considered responsible for many illnesses of human body (Hallwell et al., 1989). Several scientific investigations deal with the inhibition of the disadvantageous reactions of these harmful substances (Riemerama et al., 1991; Stampfer et al., 1993; Rimm et al., 1993). Results promise the possibility of reducing the unhealthy effects of radicals by introducing antioxidant agents into the human organism. Kinsella et al. (1993) discussed in details the probable defensive role of antioxidants in obstructing the formation of cardiovascular and cancerogeneous illnesses. Due to their hypothesis flavonoids of plant origin possess significant antioxidant protecting characteristics. These compounds strongly connect with low density lipoproteins (LDLs) and behave like endogeneous antioxidant (Vitamin-E, Vitamin-C) analogs (Vinson et al., 1995). In other words they inhibit the oxidation of LDLs that might cause arteriosclerosis. An American research team has established, that polyphenols occurring in wines potentially have antioxidant activity as well (Esterbauer et al., 1992; Frankel et al., 1993; Frankel et al., 1995). Comparing the the antioxidant effect of red and white wines and Vitamin-C in vivo experiments it has been stated, that just after consumption ascorbic acid increased the blood serum antioxidant capacity by 22-29 %, red wine raised it by 18 % as an average, and in the case of white wine the value was 4-7 % (Whitehead et al., 1995).

A vast body of Hungarian enological research deals more and more with the world famous Tokaj Aszu wines. Tokaj wine-growing district extends in the north-eastern part of Hungary. Unique quality 3, 4, 5, 6 puttony (hod or back-basket) Tokaj Aszu wines became famous for the pourriture noble caused by *Botrytis cinerea*. Under proper weather conditions whole ripe grapes infected by *Botrytis* go dry on the vine. During this fermentation process unique bouquet substances (aromatic taste and flavour compounds) develop. Aszu wine making starts with the exposure of the aszu grapes. Then the aszu mash due to the puttony number in amount (e.g. in case of 3 puttony aszu wine: 60 kg aszu mash to 1 hl wine) is extracted with young wine or must for 24-48 hours and fermented afterwards (Eperjesi et al., 1998). Aszu wines have been supposed to possess curative power for a long time. At the beginning of the century they were indicated for anaemia and lack of appetite. Aszu wine is effective against sudden cardiac decompensation due to its high glucose content and has got relatively large amount of vitamins (Ambróczy, 1932), though wines are not considered remarkable vitamin sources. The Hungarian Medicine Administration book written by Issekutz suggests it for its roborant-curing effect (Issekutz, B., Issekutz, L., 1975).

As for the role of free radicals, our work aimed at the confirmation and proof of the medical power of Tokaj wines experimentally, furthermore the influence of polyphenolic content, vintage and puttony number on antioxidative status has been studied.

Materials and methods

The white wines derived from Eger, Tokaj wine growing district and Badacsony. Among Tokaj wine uniquenesses sweet szamorodni and 3, 4, 5 puttony aszu wines have been examined. The effect of vintages was followed by the proper selection of the samples. The total polyphenol, catechin and leucoanthocyanin contents of the wines were determined by the routine methods of the enological analytical practice.

The cis- and trans-resveratrol concentration was measured by direct HPLC injection (Kállay-Török, 1997).

The Total Antioxidant Status, called TAS afterwards, was determined by a special test produced by the RANDOX firm. The method is routinely applied in human medical practice for the determination of the antioxidant state of blood serum. The determination is based on measuring at 600 nm the absorbance of the stable blueish-green colour of a cation type free radical, formed by a peroxidase (methmyoglobin) enzyme coupled substance (2,2'-azino-di(3-ethylbenzthiazoline-sulphonate) in the presence of H_2O_2 . If the sample contains antioxidant components the decrease of the absorbance is proportional to their concentration. The antioxidant state of the samples have been expressed in $[mMol/dm^3]$ as the equivalent of the standard 6-hydroxi-2,5,7,8-tetramethylchromane-2-Carboxylic acid. In measuring white wines' TAS no special sample preparation was necessary.

The TAS values of the traditional antioxidant-like ascorbic and sulphurous acids have also been determined because they might have an influence on the results and might alter the effect of phenolic compounds.

In the evaluation of the result Microsoft Excel 5.0 and Statgraf 5.1 softwares have been used.

Results and discussion

In our investigations the TAS values of the traditional antioxidants applied in enology and beverage technology have been measured. The results are summarized in *Table 1*.

Table 1 – TAS values of the traditional antioxidants and catechin

Compound	Concentration $[mg/dm^3]$	TAS $[mMol/dm^3]$
SO ₂ (free)	100	0.04
Ascorbic acid	100	0.14
Catechin	100	1.18

Due to the results it can be stated, that at the concentration of application the antioxidant status of ascorbic and sulphurous acids is low (0.04-0.14 $mMol/dm^3$). Compared to them, that of the catechin is surprisingly high (1.18 $mMol/dm^3$). This fact suggests, that the role of sulphurous acid present in a concentration that matches the GMP of enology is insignificant, when determining the TAS of wines. The antioxidant state is decisively determined by the catechin content and other phenolic compounds possessing

antioxidant activity. The unusually low values measured for the traditional antioxidants must be connected to the specificity of the measurement.

The relationship between the TAS values and the major polyphenolic compounds were studied by linear regression. It has been established, that total polyphenolic content is in close connection with the antioxidative status ($r = 0.82$).

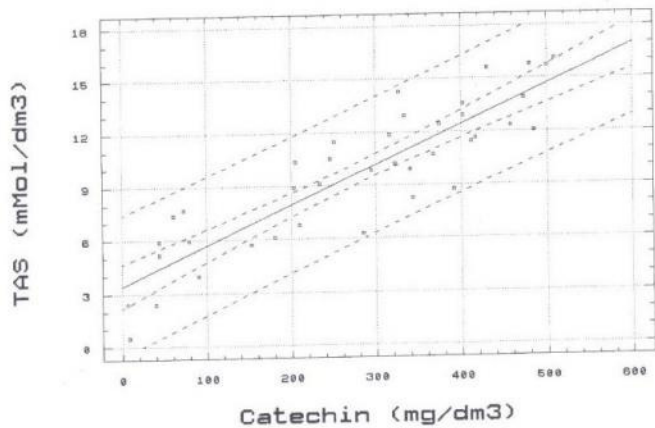


Figure 1 Linear regression analysis of the correlation between catechin contents and TAS-values

Figure 1 depicts the connection between catechin content and TAS. The correlation coefficient is 0.93 that means very close connection. The results of the investigations of the other compounds' role show, that the TAS-value is influenced the least by the resveratrole concentration, but leucoanthocyanins and anthocyanins present in red wines have a greater effect. The examination of the polyphenolic content and TAS-values of individual wines proves, that high TAS does not mean inevitably high polyphenolic or catechin content. Polyphenolic constituents presumably possess synergic features, furthermore other components might play role in forming the antioxidative status the investigation of that is of great importance.

The determination of the antioxidative status of white wines

The results of seven samples deriving from the three wine producing district mentioned are in the interval 0.38–0.89 $mMol/dm^3$, with an average of 0.57 $mMol/dm^3$ ($s = 0.03$).

Outstandingly high value, 1.81 $mMol/dm^3$ was measured for the Leányka from Eger, vintage 1996. Comparing its polyphenolic composition to that of the other Leányka from Eger vintage 1997, the conclusion can be drawn, that its total polyphenol, catechin and leucoanthocyanin content is lower as *Figure 3* shows. Consequently the extra-high antioxidant value is not necessarily accompanied with high polyphenolic content. Our research is intended to be conducted into this direction as well.

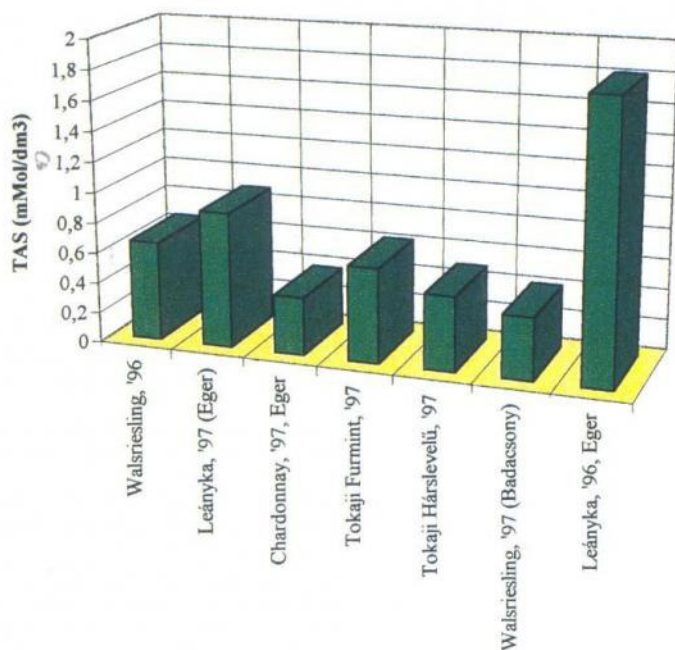
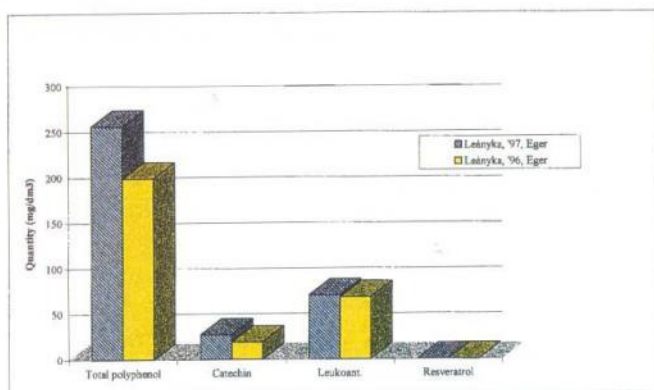


Figure 2 The TAS-values of the white wines examined



Figures 3 Polyphenol composition of Leányka wines deriving from Eger district

The determination of the antioxidative status of Tokaj wine unquities

The TAS-values of 16 aszu and 1 sweet szamorodni wines have been measured. The results can be found in Table 2.

Due to the results, the TAS of sweet szamorodni wine is 0.74 mMol/dm³ that approaches the antioxidant state of white wines. The probable cause of this phenomenon is the szamorodni production technology, where intact and aszu grapes are processed together in a large mass without any selection. The wine produced by this method contains less aszu grapes and its composition is more similar to special quality white wines made of over-ripe grapes than to aszu wines.

The TAS-value of aszu wines was 1.92 mMol/dm³ as an average ($s = 0.10$), three times higher than that of white wines. The explanation is hidden partly in the technology,

Table 2 – The TAS-values of Tokaj wine unquities

Sample	Vintage	TAS [mMol/dm ³]
6 puttony aszu	1995	1.45
5 puttony aszu	1992	2.68
5 puttony aszu	1993	1.93
5 puttony aszu	1993	2.10
5 puttony aszu	1995	1.79
5 puttony aszu	1996	1.80
5 puttony aszu	1997	1.80
4 puttony aszu	1988	0.44
4 puttony aszu	1992	2.60
4 puttony aszu	1993	1.95
4 puttony aszu	1995	1.80
4 puttony aszu	1996	1.79
4 puttony aszu	1997	1.78
3 puttony aszu	1995	1.79
3 puttony aszu	1996	1.81
3 puttony aszu	1997	1.79
Szamorodni (sweet)	1994	0.74

partly in the polyphenolic composition. In aszu wine making aszu grapes are extracted for 24–48 hours. The total polyphenolic content extracted during this time is threefold higher compared to the white wines. The difference is much larger considering the catechin and leucoanthocyanin content. The catechin concentration of aszu wines is five times higher and the leucoanthocyanin content is twentyfold larger than that of the white wines. The average total polyphenol, catechin and leucoanthocyanin contents of the examined aszu and white wine samples are summed up in Table 3.

Table 3 –The average polyphenol, catechin and leucoanthocyanin contents of the examined aszu and white wine samples.

Wine	Total polyphenol [mg/dm ³]	Catechin [mg/dm ³]	Leucoanthocyanin [mg/dm ³]
Aszu wines	679.5	94.4	1390.1
White wines	225.5	17.14	68.7

The antioxidant status of aszu wines is affected by several factors. In our experiments the influence of technology, puttony-number and the age of wine has been analysed. The effect of technology is well represented by a 4 puttony aszu wine of 1988 vintage. This vintage was outstandingly excellent. Technology was able to save and transfer all the treasures of the aszu grapes. It has to be noted that technology at that time contained and applied more oxidative operations. The antioxidative state of this wine was 0.44 mMol/dm³, much lower than in the other aszu wines. This big difference could partly be explained by the alterations of the polyphenol composition and maturation time only. The antioxidant agents of the wine were exposed to greater oxidative effect by the technology of that time.

The influence of puttony number has been studied by two variable variance-analysis. We wanted to answer the question, how the puttony number change alters the TAS-value, more exactly, the TAS-averages belonging to definite puttony numbers differ significantly or not. Figure 4 represents the effect of puttony number on TAS. In the case of six-

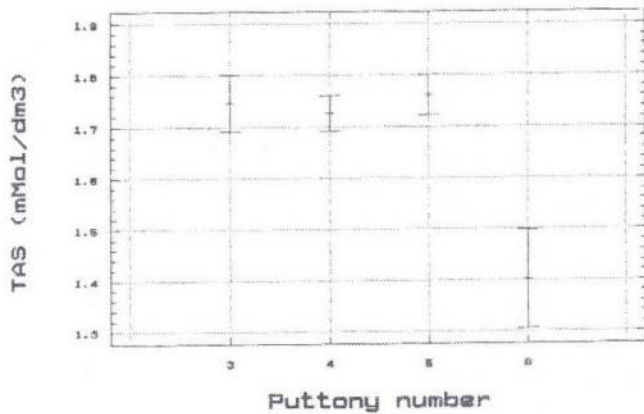


Figure 4 The effect of puttony number on TAS-values of Tokaj Aszu wines

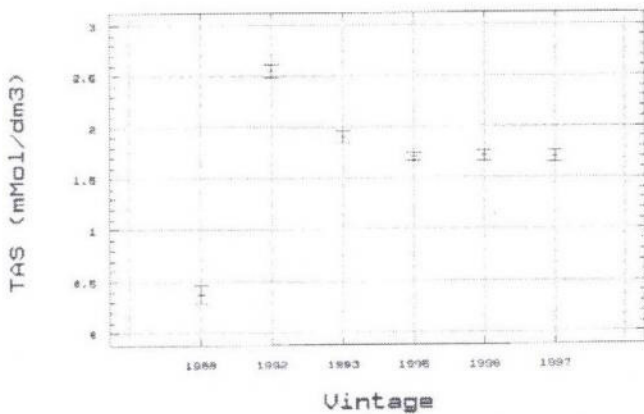


Figure 5 The effect of maturation time on Tokaj Wines' TAS-values

puttony aszu wine only one measurement could be carried out, therefore no strict conclusion was drawn.

The results of 3, 4 and 5 puttony aszu wines prove that no significant difference exists. Averages shown on the figure represent the TAS-values measured in the examined puttony number aszu wines and the limits mean the least significant differences at 95% probability level. Summing up the results of mathematical-statistical analysis it can be stated, that puttony number does not affect the antioxidant status of aszu wines.

The effect of maturation time on TAS-values has also been studied by two variable variance-analysis. The investigated samples derived from the vintages 1988, 1992, 1993, 1995, 1996 and 1997. In this case the question was, if the maturation time affected the TAS-values, in other words, do the TAS-averages of the aszu wines of the different vintages deviate significantly at the moment of the measurement, or not. The TAS-averages of the vintages 1993, 1992 and 1988 differ significantly at 95% probability level. It can be observed that the antioxidant status of young (1-3 year old) wines does not differ significantly, but in the case of 4 year old or older wines the difference is definite. The age (old vintage) is not accompanied with low TAS-values necessarily, for

instance, the antioxidative status of 4 and 5 puttony aszu wines from the 1992 vintage was the highest (2.6 and 2.68 mMol/dm³) among the samples investigated. Based on the mathematical-statistical analysis the conclusion can be drawn, that maturation time has got a significant effect on the antioxidative state of aszu wines. Our preliminary results suggest that 1-3 year old Tokaj Aszu wines possess identical TAS-values practically, while the antioxidative status of older wine unities shows a decrease. The shape of the curve indicates periodical, trend-like changes, the more exact determination of that is intended to be performed in the near future with a greater sample number.

Conclusion

The results of scientific investigations prove, that the polyphenolic compounds of wines have got antioxidant activity. For this characteristic, they might play a very important role in the inactivation of free radicals damaging human organism. Based on our work it has been stated that the unusually low values measured for the traditional antioxidants used in enology and beverage technology must be connected to the specificity of the measurement. The total antioxidant status of the examined Hungarian white wines was 0.573 mMol/dm³ as an average. It could be observed, that extreme high TAS-values are not inevitably accompanied with high total polyphenol content neither with large leucoanthocyanin and catechin amounts. It could be established that the antioxidant status of szamorodni is closer to the TAS of the white wines (< 1.0 mMol/dm³) among Tokaj wine unities, while that of aszu wines are much higher (average 1.92 mMol/dm³). This feature can be explained by the higher polyphenol content compared to white wines and the different technology. In forming the antioxidative status several factors play role. Beside the quality and quantity of phenolic compounds possessing antioxidant activity, TAS-values are also influenced by the wine making technology, because during grape processing and maturation different oxidative effects might occur in the wines. Among the polyphenolic fractions examined, TAS is affected by the catechin content to the highest extent, leucoanthocyanins have smaller influence and also the anthocyanins in the case of red wines. Resveratrol virtually has got no effect on this parameter. The quantity and composition of antioxidative polyphenols change during storage in the consequence of oxidation, polymerization and other reactions. Both the starting concentration of TAS forming substances and the maturation technology play role in change of antioxidative status during maturation. Besides, TAS is affected by maturation time as well. In case of Tokaj Aszu wines puttony number has got no influence on TAS.

References

- Ambróczy Á. (1932): Tokaj-hegyalja és néhány szellemi történeti vonatkozása. A központi sajtóvállalat kiadása, Budapest, 22-23.

- Eperjesi I., Kállay M., Magyar (1998):** Borászat, Mezőgazda, Bp.
- Frankel, E.N., Waterhouse, A.L., Teisseidre, P.L. (1995):** Principal Phenolic Phytochemicals in selected California Wines and Their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins. *J. Agric. Food. Chem.*, 43, 890–894.
- Hallwell, B., Gutteridge, J.M.C. (1989):** Free radicals in biology and medicine. Second ed. Oxford: Clarendon Press, 416–94.
- Issekutz, B., Issekutz, L. (1975):** Gyógyszerrendelés. Medicina Kiadó, Budapest
- Kállay M., Török Z. (1997):** Determination of resveratrol isomers in Hungarian wines. *Horticultural Science*, 29. (3–4), 78–83.
- Riemerama, R. A., Wood, D.A., MacIntyre, C.C.A., Elton, R.A., Gey, F.K., Oliver, M.F. (1991):** Risk of angina pectoris and plasma concentration of vitamins A, C and E and carotene. *Lancet* 337, 1–5.
- Rimm, E.B., Stampfer, M.J., Ascherio, A., Giovannucci, E., Colditz, E., Willett, W.C. (1993):** Vitamin E consumption and the risk of coronary artery disease in men. *N. Eng. J. Med.*, 328, 1450–1456.
- Stampfer, M.J., Hennekens, C.H., Manson, J.E., Colditz, G.A., Rosner, B., Willett, W.C. (1993):** Vitamin E consumption and the risk of coronary artery disease in women. *N. Engl. J. Med.*, 328, 1444–1449.
- Teisseidre, P.L., Waterhouse, A.L., Walzem, R.L., German, J.B., Frankel, E.N., Ebeler., Clifford, A.J. (1995):** Composés phénoliques du Raisin et du Vin et Santé. *Bulletin de l' O.I.V.*, 781-782., 251–275.
- Whitehead, T.P., Robinson, D., Allway, J., Syms, J., Hale, A. (1995):** Effect of Red Wine Ingestion on the Antioxidant Capacity of Serum. *Clin. Chem.* 41/1. 32–35.