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PHENOLIC COMPOUNDS AND ANTIRADICAL ACTIVITY IN TOKAJ WINES

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ABSTRACT

Eighteen wine samples (varieties - Lindenblaetrige, Yellow Muscat and Furmint), originated from Slovak and Hungarian part of Tokaj wine region, were analyzed for the content of total polyphenols, content of total flavonoids and antiradical activity by UV-VIS spectrometry. Our results showed that Slovak Tokaj wines had higher content of healthy useful phenolic and flavonoid compounds (all three varieties) and higher value of antiradical activity (two varieties) than Hungarian Tokaj wines. The highest total phenolic and total flavonoid contents we found out in wines variety Yellow Muscat. The highest value of antiradical activity was found out in wines variety Lindenblaetrige. Our results confirmed very strong linear correlations between all three analysed parameters (TPC, TFC and AA): TPC and TFC (r=0.867), AA and TPC (r=0.836), resp. TFC and AA (r= 0.766).

Keywords: antioxidants, antiradical activity, flavonoids, polyphenols, Tokaj, wine

INTRODUCTION

In recent years, much information has appeared about the role of oxidative stress in the development of a number of serious illnesses, such as certain cancers, cardiovascular diseases and age-related degenerative diseases, and about the possible therapeutic value of antioxidants against these illnesses. The terms antioxidant and free radical are popular expressions used by scientists, nutritionists and other healthcare professionals. Free radicals are chemical species, compounds and/or elements with one or two unpaired electrons in their outer layer, which can be created in a multiple ways. They can be exogenic (e.g. ultraviolet radiation, xenobiotics and infections) or endogenic. Antioxidants are molecules which can interact with free radicals and break the chain reaction before vital molecules are damaged. A lack of antioxidant or an overproduction in free radicals can lead to an imbalance between the oxidant and antioxidant system (**Barhé and Tchouya, 2016; Evans, 2007; Guerci *et al.*, 2001**).
Polyphenols are important constituents of plant cells, known for their multiple physiological roles including screening solar radiation and scavenging various free radicals (**Berli *et al.*, 2008; Pietta, 2000; Torel *et al.*, 1986**). Wine polyphenolic substances are usually subdivided into two groups: flavonoids and non-flavonoid compounds. The flavonoids have a common core, the flavane nucleus, consisting of two benzene rings (A and B) linked by an oxygen containing pyran ring (C) (C6C3C6). To date, over 3000 flavonoids have been identified. These can mainly be found in the pigments in flowers or in leaves (**Marfak, 2003; Timoracká, 2010**).
In the last few decades an intensive investigations underway to characterize the beneficial effects of moderate wine consumption. Wine is very rich source of polyphenols, such as catechins, epicatechins, quercetin, rutin, myricetin, anthocyanins, phenolic acids (gallic acid, caffeic acid, *p*-coumaric acid, etc.), *trans*-resveratrol and many others polyphenols and compounds. Many of these compounds have been reported to have multiple biological activities, including cardioprotective, anti-carcinogenic, antiviral, antidegenerative and antibacterial properties (**Bystrická *et al.*, 2014**; **King *et al.*, 2006; Santos-Buelga and Scalbert, 2000**).
In previous studies, we described some antioxidant properties, such as total polyphenol content, total flavonoid content, total anthocyanin content and antioxidant activity of the Slovak monovarietal red (**Bajčan *et al.*, 2012a,b; 2015, 2016**) and white (**Bajčan *et al.*, 2017**) wines originating in different vineyard areas, excluding Tokaj wine region.
Tokaj wine region lies in the Lower Zemplín in the south-eastern corner of Slovakia. It follows a wine region, most of which is located in Hungary. This area is also the smallest, but most attractive wine-growing region of Slovakia. It consists of the southern slopes of the Zemplín hills planted with vines after two millennia. Tokaj wine area is one of the few areas in the world where the grown grapes for the production of natural sweet wines. On the specific and unique taste of Tokaj wine has its share of both heavy sunlight, but also soil of volcanic origin. Very important is role of weather, long sunny autumn, which will enable aged berries attacked noble *Botrytis cinerea* mould, and dried berries that remain cibeba-raisins, without which the Tokaj wine quality can not be manufactured. Under these specific environmental conditions, pathogenesis is severely constrained, resulting in partial fruit dehydration and chemical transformation. These noble-rotted grapes produce some of the most sought-after, delicious, and expensive dessert wines (**Farkaš, 1983; Felšöciová *et al.*, 2015; Jackson, 2014**).
Numerous papers have been published on red and white wines but relatively few data are available on Tokay varietal non-botrytized wines. So we analyzed some of the abovementioned properties in the monovarietal wines typical for Tokaj wine region (Lindenblaetrige – Lipovina /Slovak nomenclature/ or Hárslevelű /Hungarian nomenclature/; Yellow Muscat – Muškát žltý /Slovak nom./ or Sárgamuskotály /Hungarian nom./; Furmint – Furmint/Slovak and Hungarian nom./) here, and compared them with those of selected white wine samples originating from various sources.

MATERIAL AND METHODS

Chemicals and instruments

All analysed parameters – total polyphenol content, total flavonoid content and antiradical activity in wines were analyzed by UV/VIS spectrophotometry (spectrophotometer Shimadzu UV/VIS – 1240, *Shimadzu, Japan*). The chemicals used for all analysis were: Folin-Ciocalteau reagent, monohydrate of gallic acid p.a., anhydrous natrium carbonate p.a., aluminum chloride p.a., sodium nitrite p.a., sodium hydroxide p.a., catechin hydrate 98%, methanol p.a., 2,2-diphenyl-1-

picrylhydrazyl (DPPH) radical p.a., Trolox (pure) /Sigma-Aldrich Chemie and Merck KGaA, Germany/.

Samples

Analysed, bottled, Tokaj wines – Lindenblaetrige (LB), Yellow Muscat (YM), resp. Furmint (FT) and their characteristics are mentioned in Table 1. Wine samples with origin in Tokaj vineyard area were purchased in retail network, to provide that analysed wine samples would have the same properties as wines that are consumed by common consumers (properties of wine affected by various factors, such as period and conditions of storage or distribution of wine).

Table 1 Characteristics of analysed Tokaj wine samples

Sample	Variety	Producer	Country	Vintage	Wine type
LB-S-1	Lindenblaetrige	Tokaj&CO, s.r.o.,Malá Tŕňa	Slovakia	2016	semisweet
LB-S-2		J&J Ostožovič, Veľká Tŕňa		2015	semidry
LB-S-3		Zlatý strapec - Anna Nagyová, Viničky		2013	semisweet
LB-H-1		BORANAL Kft., Sárospatak	Hungary	2016	semisweet
LB-H-2		Disznókő Szőlőbirtok és Pincészet, Mezőzombor		2015	dry
LB-H-3		Ungvár-Pince Kft., Sátoraljaújhely		2016	semisweet
YM-S-1	Yellow Muscat	Tokaj&CO, s.r.o.,Malá Tŕňa	Slovakia	2016	semisweet
YM -S-2		J&J Ostožovič, Veľká Tŕňa		2015	semidry
YM -S-3		Zlatý strapec - Anna Nagyová, Viničky		2013	semisweet
YM -H-1		BORANAL Kft., Sárospatak	Hungary	2015	semisweet
YM -H-2		Disznókő Szőlőbirtok és Pincészet, Mezőzombor		2015	semisweet
YM -H-3		Ungvár-Pince Kft., Sátoraljaújhely		2016	semisweet
FT-S-1	Furmint	Tokaj&CO, s.r.o.,Malá Tŕňa	Slovakia	2015	dry
FT-S-2		J&J Ostožovič, Veľká Tŕňa		2014	semidry
FT-S-3		Zlatý strapec - Anna Nagyová, Viničky		2013	dry
FT-H-1		BORANAL Kft., Sárospatak	Hungary	2016	semisweet
FT-H-2		Disznókő Szőlőbirtok és Pincészet, Mezőzombor		2015	dry
FT-H-3		Ungvár-Pince Kft., Sátoraljaújhely		2016	semisweet

Sample analysis

Total polyphenol content (TPC) was determined by modified method of **Singleton and Rossi (1965)**. 0.1 mL of wine sample was pipetted into 50 mL volumetric flask and diluted with 5 mL of distilled water. To diluted mixture 2.5 mL Folin-Ciocalteau reagent was added and after 3 minutes 7.5 mL of 20% solution of Na_2CO_3 was added. Then the sample was filled with distilled water to volume 50 mL and after mixing left at the laboratory temperature for 2 hours. By the same procedure the blank and calibration solutions of gallic acid were prepared. Absorbance of samples solutions was measured against blank at 765 nm. Total polyphenol (TP) content in wines was calculated as amount of gallic acid equivalent (GAE) in mg per 1 litre of wine.

Total flavonoid content (TFC) was assessed by aluminum chloride method (**Chang *et al*., 2002**). 1 ml of wine sample and 4 ml of deionized water were added to a 10 ml volumetric flask. 5 min after adding 0.3.ml of 5% sodium nitrite, 0.3. ml of 10% aluminum chloride was added. 2 ml of sodium hydroxide with concentration 1 mol/L was added to the reaction mixture after 6 min incubation. The final volume was immediately made up to 10 ml with deionized water. The absorbance of the solution was measured at 510 nm against blank solution. The content of total flavonoids (TF) in wine samples was calculated as amount of catechin equivalent (CE) in mg per 1 litre of wine.

Antiradical activity (AA) was assessed by method of **Brand-Williams *et al.* (1995)** using of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. Absorbance was read at 515.6 nm and antioxidant effectiveness was expressed as % inhibition of DPPH (quantitative ability of tested compound to remove in certain period a part of DPPH radical) and also as Trolox equivalent calculated from calibration curve. All chemical analyses were performed as four parallels.

Statistical analysis

The Shapiro-Wilk test showed that the data were not normally distributed. Therefore, nonparametric Kruskal-Wallis test was conducted in order to obtain statistically significant information about the differences between the tested samples and Spearman correlation was performed to investigate the relationship between the tested variables. All calculations were performed using MS Excel 2016 and XLSTAT **(Addinsoft, 2014)**.

RESULTS AND DISCUSSION

All studied parameters – the content of total polyphenols, the content of total flavonoids and antiradical activity of Tokaj wines are described in Table 2, 3 and 4.

Total polyphenol content in analysed wines variety Lindenblaetrige was in range from 287.6 to 540.3 mg GAE/L. Average content of TP in all wines variety LB was 411.3 mg GAE/L. Slovak wines variety LB had approximately a half higher values of TPC (average content 489.8 mg GAE/L) than Hungarian wines (average content 332.7 mg GAE/L) /Table 2/. It is very difficult to compare our results with the results of other authors because none of them have expressed the total content of polyphenols as the equivalent of gallic acid. **Eftimova (2016)** found that the content of total polyphenols in Tokaj wine – Lindenblaetrige originating in Slovakia was 182.6 mg of quercetin per liter.

Total flavonoid content in analysed wines variety Lindenblaetrige was in range from 31.0 to 101.2 mg CE/L. Average content of TF in all wines variety LB was 65.4 mg CE/L. Slovak wines variety LB had more than 2 times higher values of TFC (average content 89.3 mg CE/L) than Hungarian wines (average content 41.5 mg CE/L). Until now the content of total flavonoids in Tokaj wines has not been investigated.

Antiradical activity in analysed wines variety Lindenblaetrige was in range from 31.2 % inhibition of DPPH (0.354 mmol Trolox.L^{-1}) to 65.6 % inhib. of DPPH (0.768 mmol Trolox.L^{-1}). Average value of AA in all wines variety LB was 49.5 % inhib. of DPPH (0.568 mmol Trolox.L^{-1}). Slovak wines variety LB had much higher values of AA (average value 57.5 % inhib. of DPPH, resp. 0.664 mmol Trolox.L^{-1}) than Hungarian wines (average value 41.5 % inhib. of DPPH, resp. 0.471 mmol Trolox.L^{-1}). Our results are very similar to results reported by **Eftimova (2016)**, who found that antiradical activity in Tokaj wine – Lindenblaetrige originating in Slovakia was 53.5 % inhib. of DPPH.

Gained results exert statistically significant differences (at significance level $P<0.05$) between values of TPC, TFC and AA in wines variety LB made in Slovakia and Hungary.

Table 2 The content of total polyphenols, content of total flavonoids and antiradical activity in analysed wines variety Lindenblaetrige

Sample	TPC (mg GAE.L^{-1})	TFC (mg CE.L^{-1})	AA (% inhib. DPPH)	AA (mmol Trolox.L^{-1})
LB-S-1	491.8 ± 2.6	76.9 ± 1.3	51.6 ± 1.1	0.588 ± 0.014
LB-S-2	437.2 ± 5.3	101.2 ± 0.8	55.4 ± 1.1	0.635 ± 0.014
LB-S-3	540.3 ± 11.1	89.7 ± 0.7	65.6 ± 0.8	0.768 ± 0.010
Average LB-SVK	***489.8 ± 60.9^{a}***	***89.3 ± 14.4^{a}***	***57.5 ± 8.3^{a}***	***0.664 ± 0.106^{a}***
LB-H-1	420.8 ± 2.6	56.8 ± 0.8	50.6 ± 1.3	0.576 ± 0.016
LB-H-2	289.6 ± 2.6	36.6 ± 0.8	42.7 ± 0.6	0.484 ± 0.007
LB-H-3	287.6 ± 4.4	31.0 ± 1.0	31.2 ± 0.6	0.354 ± 0.007
Average LB-HUN	***332.7 ± 78.7^{b}***	***41.5 ± 15.2^{b}***	***41.5 ± 11.5^{b}***	***0.471 ± 0.131^{b}***
Total average LB	**411.3 ± 99.7**	**65.4 ± 27.7**	**49.5 ± 13.6**	**0.568 ± 0.163**

Legend: SVK – Slovakia, HUN – Hungary, LB - Lindenblaetrige, TPC – total polyphenol content, TFC – total flavonoid content, AA – antiradical activity.
Values of TPC, TFC and AA are expressed as arithmetic average ± standard deviation,
[a-b] Values with different letters denote significant differences (P<0.05) among countries.

Total polyphenol content in analysed wines variety Yellow Muscat ranged from 267.8 to 568.3 mg GAE/L. Average content of TP in all wines variety YM was 420.5 mg GAE/L. Slovak wines variety YM had much higher values of TPC (average content 525.6 mg GAE/L) than Hungarian wines (average content 315.4 mg GAE/L) /Table 3/. Other researchers reported diverse values of total polyphenol content in Tokaj wines variety Yellow Muscat: **Eftimova (2016)** – 252.3 mg of quercetin per liter and **Lugasi and Hovari (2003)** – 250 mg of catechin per liter.

Total flavonoid content in analysed wines variety Yellow Muscat was in range from 30.6 to 169.1 mg CE/L. Average content of TF in all wines variety YM was 83.0 mg CE/L. Slovak wines variety YM had more than 2 times higher values of TFC (average content 119.4 mg CE/L) than Hungarian wines (average content 46.7 mg CE/L).

Antiradical activity in analysed wines variety Yellow Muscat ranged from 26.6 % inhibition of DPPH (0.302 mmol Trolox.L^{-1}) to 67.4 % inhib. of DPPH (0.793 mmol Trolox.L^{-1}). Average value of AA in all wines variety YM was 47.2 % inhib. of DPPH (0.542 mmol Trolox.L^{-1}). Slovak wines variety YM had much higher values of AA (average value 58.8 % inhib. of DPPH, resp. 0.680 mmol Trolox.L^{-1}) than Hungarian wines (average value 35.7 % inhib. of DPPH, resp. 0.404 mmol Trolox.L^{-1}). Our results are quite similar to the results reported by **Eftimova (2016)**, who found that antiradical activity in Tokaj wine – Yellow Muscat originating in Slovakia was 63.8 % inhib. of DPPH.

Gained results confirmed statistically significant differences (at significance level P<0.05) between the TPC, TFC and AA values in wines variety YM produced in Slovakia and Hungary.

Table 3 The content of total polyphenols, content of total flavonoids and antiradical activity in analysed wines variety Yellow Muscat

Sample	TPC (mg GAE.L^{-1})	TFC (mg CE.L^{-1})	AA (% inhib. DPPH)	AA (mmol Trolox.L^{-1})
YM-S-1	513.7 ± 9.3	99.5 ± 1.3	57.6 ± 0.8	0.662 ± 0.010
YM -S-2	568.3 ± 13.3	169.1 ± 1.3	67.4 ± 0.6	0.793 ± 0.007
YM -S-3	494.8 ± 10.6	89.6 ± 1.5	51.4 ± 0.6	0.586 ± 0.007
Average YM-SVK	***525.6 ± 43.4^{a}***	***119.4 ± 47.0^{a}***	***58.8 ± 9.5^{a}***	***0.680 ± 0.122^{a}***
YM -H-1	345.0 ± 14.6	53.2 ± 0.8	38.4 ± 0.5	0.435 ± 0.006
YM -H-2	333.3 ± 12.0	56.4 ± 1.8	42.0 ± 0.6	0.476 ± 0.007
YM -H-3	267.8 ± 4.0	30.6 ± 1.0	26.6 ± 0.8	0.302 ± 0.010
Average YM-HUN	***315.4 ± 45.6^{b}***	***46.7 ± 15.2^{b}***	***35.7 ± 9.1^{b}***	***0.404 ± 0.103^{b}***
Total average YM	**420.5 ± 118.6**	**83.0 ± 54.7**	**47.2 ± 16.1**	**0.542 ± 0.194**

Legend: SVK – Slovakia, HUN – Hungary, YM – Yellow Muscat, TPC – total polyphenol content, TFC – total flavonoid content, AA – antiradical activity.
Values of TPC, TFC and AA are expressed as arithmetic average ± standard deviation,
[a-b] Values with different letters denote significant differences (P<0.05) among countries.

Total polyphenol content in analysed wines variety Furmint was in range from 241.8 to 457.7 mg GAE/L. Average content of TP in all wines variety FT was 359.3 mg GAE/L. Slovak wines variety FT had much higher values of TPC (average content 405.3 mg GAE/L) than Hungarian wines (average content 313.3 mg GAE/L) /Table 4/. Other researchers reported diverse values of total polyphenol content in Tokaj wines variety Furmint: **Eftimova (2016)** – 240.5 mg of quercetin per liter and **Sato *et al.* (1996)** from 312.5 to 373.2 ppm.

Total flavonoid content in analysed wines variety Furmint was in range from 28.3 to 117.6 mg CE/L. Average content of TF in all wines variety FT was 67.7 mg CE/L. Slovak wines variety FT had about 2 times higher values of TFC (average content 88.5 mg CE/L) than Hungarian wines (average content 46.9 mg CE/L).

Antiradical activity in analysed wines variety Furmint was in range from 27.1 % inhibition of DPPH (0.308 mmol Trolox.L^{-1}) to 63.2 % inhib. of DPPH (0.735 mmol Trolox.L^{-1}). Average value of AA in all wines variety FT was 47.0 % inhib. of DPPH (0.538 mmol Trolox.L^{-1}). Slovak wines variety FT had a slightly higher values of AA (average value 48.5 % inhib. of DPPH, resp. 0.552 mmol Trolox.L^{-1}) than Hungarian wines (average value 45.5 % inhib. of DPPH, resp. 0.523 mmol Trolox.L^{-1}). **Eftimova (2016)** found out similar value of antiradical activity in Tokaj wine – Furmint originating in Slovakia – 52.8 % inhib. of DPPH.

Gained results exert statistically significant differences (at significance level P<0.05) between values of TPC, resp. TFC (but not AA) in wines variety FT made in Slovakia and Hungary.

Table 4 The content of total polyphenols, content of total flavonoids and antiradical activity in analysed wines variety Furmint

Sample	TPC (mg GAE.L^{-1})	TFC (mg CE.L^{-1})	AA (% inhib. DPPH)	AA (mmol Trolox.L^{-1})
FT-S-1	457.7 ± 6.7	117.6 ± 1.0	52.6 ± 0.7	0.600 ± 0.009
FT-S-2	383.9 ± 13.3	64.7 ± 0.8	44.0 ± 1.1	0.499 ± 0.014
FT-S-3	374.3 ± 10.6	83.1 ± 1.0	49.0 ± 0.5	0.557 ± 0.006
Average FT-SVK	***405.3 ± 49.3^{a}***	***88.5 ± 31.3^{a}***	***48.5 ± 5.1^{a}***	***0.552 ± 0.060^{a}***
FT-H-1	356.6 ± 6.7	60.3 ± 0.5	46.2 ± 0.7	0.525 ± 0.009
FT-H-2	341.6 ± 10.6	52.1 ± 0.8	63.2 ± 0.9	0.735 ± 0.011
FT-H-3	241.8 ± 10.6	28.3 ± 0.5	27.1 ± 0.9	0.308 ± 0.011
Average FT-HUN	***313.3 ± 67.8^{b}***	***46.9 ± 18.9^{b}***	***45.5 ± 21.3^{a}***	***0.523 ± 0.252^{a}***
Total average FT	**359.3 ± 85.2**	**67.7 ± 35.2**	**47.0 ± 14.2**	**0.538 ± 0.168**
Total average Tokaj	**397.5 ± 89.3**	**72.0 ± 35.9**	**47.9 ± 12.1**	**0.549 ± 0.145**

Legend: SVK – Slovakia, HUN – Hungary, FT – Furmint, TPC – total polyphenol content, TFC – total flavonoid content, AA – antiradical activity.
Values of TPC, TFC and AA are expressed as arithmetic average ± standard deviation,
[a-b] Values with different letters denote significant differences (P<0.05) among countries.

Total polyphenol content in all analysed Tokaj wines ranged from 241.8 to 568.3 mg GAE/L. Average content of TP in all Tokaj wines was 397.5 mg GAE/L (Tab. 4). According to the average value of TPC an order for Tokaj varietal wines could be as following: wines Yellow Muscat (420.5 mg GAE.L^{-1}) > wines Lindenblaetrige (411.3 mg GAE.L^{-1}) > wines Furmint (359.3 mg GAE.L^{-1}). When comparing the average TPC of Tokaj wines with other white monovarietal wines, we can conclude that Tokaj wines have a significantly higher (about 20 – 50%) total content of phenolic substances.

Total flavonoid content in all analysed Tokaj wines ranged from 28.3 to 169.1 mg CE/L. Average content of TF in all Tokaj wines was 72.0 mg CE/L. According to the average value of TFC an order for Tokaj varietal wines could be as following: wines Yellow Muscat (83.0 mg CE.L^{-1}) > wines Furmint (67.7 mg CE.L^{-1}) > wines Lindenblaetrige (65.4 mg CE.L^{-1}). When comparing the average TFC of Tokaj wines with other white monovarietal wines, we can conclude that Tokaj wines have comparable value of the total flavonoid content.

Antiradical activity in all analysed Tokaj wines ranged from 26.6 to 67.4 % inhibition of DPPH (0.302 – 0.793 mmol Trolox.L^{-1}). Average value of AA in all Tokaj wines was 47.9 % inhibition of DPPH (0.549 mmol Trolox.L^{-1}). According to the average value of AA an order for Tokaj varietal wines could be as following: wines Lindenblaetrige (49.5 % inhib. DPPH) > wines Yellow Muscat (47.2 % inhib. DPPH) > wines Furmint (47.0 % inhib. DPPH). When comparing the average AA value of Tokaj wines with other white monovarietal wines, we can conclude that Tokaj wines have a significantly higher (about 10 – 50%) value of AA. **Staško *et al.* (2006), Fikselová et al. (2010)** also concluded in their works, that Tokay wines showed a very good scavenging ability, positioned between white and red wines.

The statistical evaluation of the obtained results confirmed strong positive relationship at significance level P<0.001 between all 3 studied parameters: TPC and TFC (r=0.867), AA and TPC (r=0.836), resp. TFC and AA (r= 0.766). This was consistent with many other articles in the literature, which also reported strong positive relationship between the total phenols, total flavonoids and antiradical properties of wines (**Chircu Brad *et al*., 2012; Li *et al*., 2009; Ma *et al*., 2014 and Minussi *et al*., 2003**).

CONCLUSION

The phenolic, resp. flavonoid content and antiradical properties of Tokaj wines (varieties: Lindenblaetrige, Yellow Muscat and Furmint) produced in Slovak and Hungarian parts of Tokaj vineyard area were evaluated in the present study. The highest total phenolic and total flavonoid contents were found out in wines variety Yellow Muscat. On the other hand, the highest value of antiradical activity was found out in wines variety Lindenblaetrige. Statistical analysis of the measured data showed that Slovak Tokaj wines had significantly higher content of healthy useful phenolic compounds and significantly higher value of antiradical activity than Hungarian Tokaj wines.

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