

Bioactive Complexity of the Red Wine “Portugizac”; Is Younger More Beneficial?

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Summary

The young red wine “Portugizac Mlado vino” is a recognizable and successful local product with Protected Designation of Origin (PDO). This wine is produced of the variety Portugizac mostly, and it is expected to be consumed very soon after the fermentation. The aging of wine allows continuous chemical changes, and the polyphenolic properties are the basic indicator of quality and nutritional value during that process. The objective of this work was to analyze the parameters of polyphenolic quality; total phenols (TP), antioxidant capacity, and stilbene resveratrol and piceid in wine “Portugizac”, related to aging. Special importance is placed on bioactive *trans*-resveratrol and its corresponding compounds. The nine young wines “Portugizac”, PDO “Plešivica” were analyzed after bottling and after 12 months of bottle storage. An average concentration of TP in young wines Portugizac was 1429.8 mg L⁻¹ (gallic acid), with an average antioxidant capacity (FRAP) of 17.4 mmol L⁻¹ of Fe (II), and an average total stilbene concentration of 7.34 mg L⁻¹. After 12 months of bottle storage at optimal conditions, concentrations of all parameters decreased. An average concentration of TP was 1323.8 mg L⁻¹, an average antioxidant capacity (FRAP) of 16.0 mmol L⁻¹ of Fe (II), and an average total stilbene content of 7.15 mg L⁻¹. The most interesting bioactive stilbene compound is *trans*-resveratrol, and its concentration decreased by an average of 60%. It can be concluded that aging affects the content of polyphenols and nutritional complexity and that the consumption of young wine “Portugizac” might be more beneficial.

Key words

Portugizac, “mlado vino”, bottle aging, phenol content, stilbene, antioxidant capacity

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Introduction

The red grape variety Portugizac (*Vitis vinifera* L.) is grown in Croatia and some other countries of Central and Southeastern Europe. Fifty years ago, it was the most abundant red cultivar in continental regions of Croatia, but today its production is small; the total area of the Portugizac variety in Croatia is 36 ha, and the largest part of 71 % is in “Plešivica” subregion (APPRRR, 2020). However, this variety is a good example of importance at the local level; it is an important factor in the wine tourism of the region in central northwestern Croatia. Portugizac is a variety for the production of a local and popular red wine “Portugizac” with Traditional Term (TT) “Mlado vino”. This wine is traditionally consumed immediately after fermentation, and is part of the special offer of the City of Zagreb and open cellars from October to the end of the year. This tradition is similar to some other “young wine stories”, such as Beaujolais Nouveau in France, VINO Novello in Italy, and globally is not an economic factor in the wine industry, but is important and an added value to the wine sector of a particular area. According to available data, the interest in these wines is growing, and the new so-called millennial generations (Millennials) are prone to such seasonal products, which is in line with the “buy and taste now” trend (Indian Wine Academy, 2020).

Portugizac is officially called in EU countries by other names: Blauer Portugieser (Germany, Austria), Kékoportó (Hungary), Modry Portugal (Czech Republic, Slovakia), Portugais bleu (France), Oporto (Romania), Portoghese (Italy), Portugalka (Slovenia), and Português Azul (Portugal), and the origin is still an unanswered question, although the origin is assumed to be southern Styria (Maul et al., 2016). Portugizac is an early cultivar that ripen in the first epoch, has characteristic of a good fertilization and high yield. By application of appropriate ampelotechnical and technological procedure in cellar, Portugizac grape results in fresh, good color, soft, velvety wines, with fruity aroma and low astringency, especially drinkable as a young wine.

During the last decades, the additional investment in the application of new technological knowledge in the production of the Portugizac brand as a young wine with recognizable quality has been present. Young wine represents a well-defined category and Traditional Term with description of product characteristic in some EU countries, according to Regulation (EU) No 1308/2013 and Regulations (EU) 2019/33 and 2019/34, and is a part of EU eAmbrosia Register (European Commission, n.d.).

Although the healthful properties of wine were acknowledged in ancient cultures, considering that Hippocrates (460-370 BC) recommended specific wines to purge fever, disinfect and dress wounds, and as a diuretic, informing and promoting of moderate wine consumption is an ongoing process. Potential beneficial influence of wine is associated with different class of bioactive compounds, polyphenols mostly (German and Walzem, 2000), and epidemiologic studies supported the view that the positive and antioxidant effects were more specific to red wine because of greater complexity and concentrations of polyphenols, especially proanthocyanidins (Renaud et al., 1998; Guilford and Pezzuto, 2011; Golan et al., 2019). Additionally, the antioxidant abilities of red grapes, juices and wines have always been correlated with phenolics content (Simonetti et al., 1997; Paixao et al., 2007). Important antioxidant compounds include caffeic acid, catechin, chlorogenic acid, epicatechin, ferulic acid, myricetin,

protocatechuic acid, quercetin, and resveratrol (Weiskirchen and Weiskirchen, 2016).

The impact of young wines at global wine market is symbolic, and the quality of young wine, especially in relation to health benefits is not as interesting topic as in the case of common red wines, and research is modest (Pellegrini et al. 2000; Ma et al., 2014, Alpeza et al., 2006). The young wines are characterized by bright color, fundamentally due to monomeric anthocyanin pigments derived from the grape skin tissue (Somers and Verette, 1988). There is indication that the polyphenolic subgroup of monomeric anthocyanins, the conversion of which into polymeric forms increases with aging could be the main class of phenolics responsible for the antioxidant activity of young red wines (Ghiselli et al., 1998). Wine aging is the main factor influencing the antioxidant activity and polyphenolic spectra of red wines (Lachman et al., 2009). A recent study of red wines from Australia and New Zealand has found levels of healthy antioxidants resveratrol decreasing significantly over the 16-month period (Naiker et al., 2020). It was found that the concentration decreased in some wines by as much as 96 percent, so, it can be concluded that young red wine is more beneficial for human health. These results are definitely a window into a new dimension of young red wines, and will motivate and provoke further research. In this time when producers are faced with crisis caused by COVID 19 pandemic (Recarte, 2020), these results could be particularly emphasized and used to promote the consumption of local and young wines.

Our interest was to evaluate the beneficial phenolic composition and antioxidant capacity of branded young wine “Portugizac”, related to aging. Special importance is given to bioactive resveratrol and its relative compounds. We analyzed polyphenolic potential of “Portugizac” young wines from the Plešivica subregion in our previous research (Alpeza et al., 2006), but there is no evidence about bioactive stilbene compounds and quality changes during aging. The results will be useful to winegrowers of “Portugizac”, who, until now, lacked information on specific parameters of quality with potential health benefits. The results can be a great promoting tool for the consumption of local young red wine “Portugizac Mlado vino”.

Materials and Methods

Wines

Nine originally bottled Portugizac wines, labeled with PDO “Plešivica” were obtained from the local market. The wines included eight samples declared with Traditional Term „Mlado vino”, and one young wine (sample 8) without Traditional Term „Mlado vino. The PDO label means mandatory certification of wine in order to Regulation (EU) No 606/2009, and TT „Mlado vino” is authorized for wines with a PDO, and means the fermentation process which is either completed or partially completed. The wines declared with the TT “Mlado vino” must be placed on the market before 31st of December of the calendar year in which grapes were harvested. All samples were placed on the market in accordance with the specified standard. After the first analyses, the samples were stored under controlled conditions at a temperature of 16 °C for one year.

Analysis of Standard Quality Parameters

The determination of standard physicochemical wine parameters was performed by official methods (EC Regulation 2678/90).

Spectrophotometric Analysis

The analysis of total phenols was conducted using the Folin-Ciocalteu method (Singleton and Rossi, 1965), and the results were expressed as gallic acid equivalents. The antioxidant capacity (AC) of wine was measured using ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1996). The assay is based on the reducing power of a compound/s. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex that increases the absorption at 593 nm.

Stilbene Analysis

Stilbenes were analysed by 1100 Series HPLC- DAD (Agilent Technologies) using a modified method as described by Vrhovsek et al. (1995). The separation was performed on a Hypersil ODS C18 250 x 2.1 mm (5 μm) column connected to a Hypersil ODS C18 20x2.1 mm (5 μm) guard column (Thermo Scientific). The mobile phase A consisted of 1mM H_3PO_4 in water and B of acetonitrile. The linear gradient started at 0%B, to 60% B in 30 min, to 100 % B in 1 min B, to 0 % B in 1 min and 5 min post time at 0% B. The injection volume was 3 μL , the flow was 0.6 ml/min, and the column temperature was 40 °C. Compounds were identified by their UV-vis spectra and retention times. Quantifications based on peak areas at $\lambda = 310$ nm using a calibration curve of *trans*-resveratrol (trans 3,4,5-trihydroxystilbene) and concentrations of cis and *trans*-resveratrol and cis- and *trans*-piceid in wines were expressed as mg/L of *trans*-resveratrol equivalents. Internal standard trans-4-hydroxystilbene was used. Prior to the analysis a solid-phase extraction procedure for the isolation of stilbenes was used as described by Mattivi (1993).

Data Analysis

The statistical analysis of the data was performed by analysis of variance (ANOVA) using the Statistica V.10 software (StatSoft Inc., Tulsa, USA). Tukey's HSD test was used for comparison when samples differed significantly after ANOVA ($P < 0.05$).

Results and Discussion

The standard wine quality parameters of Portugizac young wines are presented in Table 1. The results are the means of two replicates. All results were in accordance with the specific proposition of PDO Plešivica, and good representatives of young wines. These wines tend to be produced without minimum of correction, and it can be a cause of larger range of some parameters value. It is known that Portugizac is not a variety with great potential, but in young wine, it is rich with a very high content of dry extract and minerals (Alpeza et. al., 2006). Some authors found wines from the same variety in Slovenia as dry, with an alcohol content of around 11 vol%, with a total acidity up to 7 g L^{-1} , a total extract of about 25 g L^{-1} , and a pH of 3.4 (Reščič et. al., 2015).

Table 1. The standard quality parameters of “Portugizac” young wines, vintage 2019

Parameter	Range
Relative density	0.9956 - 0.9982
Alcohol (vol%)	10.9 – 12.2
Reducing sugars (g L^{-1})	2.2 - 6.1
Total dry extract (g L^{-1})	27.8 - 32.9
Ash (g L^{-1})	3.1 - 4.3
Total acidity (g L^{-1} , tartaric)	6.1 - 8.0
pH	3.26 - 3.58
Volatile acidity (g L^{-1} , acetic)	0.2 - 0.5
Total SO_2 (g L^{-1})	21 - 96

In Tables 2 and 3 are presented the results of total phenols, antioxidant capacity, and stilbene composition of nine samples of Portugizac as a young red wine and wine stored for 12 months. It can be seen that the values obtained for total phenols (TP) in young wines were in the range from 941.82 to 2254.55 mg GAE L^{-1} (Table 2). Although there are many studies regarding phenolic compounds in wines, there is a lack of information about phenolic content in red wine from this variety. In a previous study of young Portugizac wine (18 samples from the 2005 harvest), values of total phenols up to 1754 mg L^{-1} , total anthocyanins up to 559 mg L^{-1} , and total flavanols up to 444 mg L^{-1} were found (Alpeza et al, 2006). Lachman and Šulc (2006) reported an average TP value of 1317.6 mg L^{-1} in red Czech wine made from the same variety, Blauer Portugieser, that coincides with our results. Average concentration of TP in young wines Portugizac was 1429,8 mg L^{-1} and 1323.7 mg L^{-1} in wines after 12 months.

Antioxidant capacity (AC) as a relative index of total phenols, and as a measure of free radical scavenging potential, is an applicable tool in estimating the health-protective properties of wine. As expected, the values of AC (FRAP) were accompanying those of TP in wine samples, ranging from 11.8 to 26.5 mmol/L of Fe (II). After 12 months of storage in controlled conditions, the concentrations of TP as well as the values of AC in “Portugizac Mlado vino” wines decreased, ranging from 863.2 to 2147.3 mg GAE L^{-1} and from 9.9 to 25.8 mmol/L of Fe (II) (Table 3). Similar values and relations were found in Italian young wines (“Vini novelli”), made of 16 different grape varieties in different combinations (Pellegrini et al, 2000). They analysed eight commercial Italian vini novelli from different geographical origins, prepared by carbonic maceration, and TP content range was from 1122 to 2143 mg L^{-1} (gallic acid equivalents). The average total antioxidant activity (16.8 ± 3.8 mmol L^{-1} Trolox equivalents, DPPH assay) of Vini novelli was higher than the corresponding values (12.3 ± 3.3 mmol L^{-1} Trolox equivalents) found for aged wines. Three couples of experimental wines were prepared from the same grapes by traditional or carbonic maceration.

Table 2. Total phenols and antioxidant capacity of “Portugizac” young wines related to bottle aging

Sample		Total phenols (mg L ⁻¹ gallic acid)		FRAP (mmol Fe ²⁺ L ⁻¹)	
		A	B	A	B
1	avg	1056.36 ^a	880.45 ^b	13.14 ^a	11.48 ^b
	stdev	7.71	8.36	0.00	0.02
2	avg	1416.82 ^a	1348.18 ^b	17.76 ^a	16.76 ^b
	stdev	1.93	10.29	0.06	0.03
3	avg	1223.64 ^a	1155.00 ^b	15.40 ^a	14.59 ^b
	stdev	9.00	3.21	0.04	0.06
4	avg	1492.73 ^a	1427.27 ^b	18.04 ^a	17.80 ^b
	stdev	2.57	6.43	0.02	0.04
5	avg	1714.55 ^a	1677.73 ^b	19.18 ^a	18.01 ^b
	stdev	6.43	7.07	0.02	0.03
6	avg	1407.73 ^a	1181.36 ^b	17.52 ^a	14.49 ^b
	stdev	8.36	5.79	0.05	0.02
7	avg	2254.55 ^a	2147.27 ^b	26.47 ^a	25.81 ^b
	stdev	15.43	5.14	0.02	0.05
8	avg	1357.73 ^a	1233.64 ^b	17.32 ^a	15.35 ^b
	stdev	7.07	1.29	0.03	0.02
9	avg	941.82 ^a	863.18 ^b	11.79 ^a	9.85 ^b
	stdev	1.29	1.93	0.08	0.03

Note: Data presented as average value of two analytical repetitions with standard deviation. The same letters in each row are not significantly different at the $P \leq 0.05$ level according to ANOVA. TP: total phenols; FRAP: Ferric ion Reducing Antioxidant Parameter. A: young bottled wines. B: wines after 12 months bottle storage

However, the average total antioxidant activities of the wines were similar, suggesting that aging (and not the wine-making technique) is the main factor influencing the antioxidant activity of red wines (Pellegrini et al., 2000).

The lower antioxidant capacity in aged wines seems to be related to different reactions, like polymerisation and aggregation, that result in more complex forms of polyphenolic compounds that are less bioavailable and, therefore with lower antioxidative properties. Namely, there are many factors that can influence chemical composition of wine, such as grape variety, climatic conditions, growing region, *viti*- and *vini*-practices, as well as storage and aging process (González-Domínguez et al., 2019). Every of these factors contribute to final physicochemical, nutritional, and sensory quality of wine. Particularly, during storage and aging of wine, phenolic compounds undergo various transformations. Unlike some other red wine varieties that have aging potential, Portugizac is known as red wine not suitable for long aging and it is mainly consumed as a young wine. In our study 7 samples (7/9) were closed with a screw cap. Screw caps are almost airtight and greatly limit the ingress of oxygen. In turn, the very

low oxygen ingress allowed by screw caps affects the wine chemical environment and yields more reductive characters (Coelho et al., 2018). Furthermore, the bottles are stored in optimal and constant temperature and humidity conditions that are important factors in wine aging. A temperature interval of 15–17 °C is acknowledged as optimum for wine preservation (Scrimgeour et al., 2015), and a $\approx 70\%$ relative humidity is accepted as optimal to store wine bottles (Arapitsas et al., 2014).

Furthermore, we can also see that a higher concentration of TP seems to be responsible for the higher antioxidant capacity in analyzed samples (Tables 2 and 3). It is known that phenolic compounds possess antioxidant properties which are mainly related to free radical scavenging and transition of metal chelating (Paixão et al., 2007). Additionally, most of them may exhibit biological properties of interest. Hence, special attention is drawn to stilbenes, particularly *trans*-resveratrol and other stilbene derivatives which are confirmed to have beneficial effects on cardiovascular and other diseases (Guerrero et al., 2009; Kiselev, 2011; Frombaum et al., 2012; Ruan et al., 2012; Ramírez-Garza et al., 2018).

Table 3. Stilbene composition of “Portugizac” young wines related to bottle aging

Sample		TS (mg L ⁻¹)		<i>trans</i> -R (mg L ⁻¹)		<i>cis</i> -R (mg L ⁻¹)		<i>trans</i> -P (mg L ⁻¹)		<i>cis</i> -P (mg L ⁻¹)	
		A	B	A	B	A	B	A	B	A	B
1	avg	7.34 ^a	5.67 ^b	2.90 ^a	0.95 ^b	0.95 ^b	1.95 ^a	2.33 ^a	2.15 ^b	1.16 ^a	0.62 ^b
	<i>stdev</i>	0.03	0.02	0.01	0.01	0.00	0.01	0.02	0.01	0.01	0.00
2	avg	10.07 ^b	11.11 ^a	3.39 ^a	1.97 ^b	1.31 ^b	3.67 ^a	3.48 ^b	4.09 ^a	1.89 ^a	1.38 ^b
	<i>stdev</i>	0.05	0.06	0.01	0.02	0.01	0.02	0.03	0.03	0.02	0.01
3	avg	7.95 ^a	7.39 ^b	2.46 ^a	1.34 ^b	0.80 ^b	1.73 ^a	3.33 ^b	3.70 ^a	1.35 ^a	0.62 ^b
	<i>stdev</i>	0.04	0.05	0.00	0.01	0.00	0.01	0.02	0.02	0.01	0.00
4	avg	7.43 ^b	7.76 ^a	2.49 ^a	1.23 ^b	1.08 ^b	2.47 ^a	2.61 ^a	2.37 ^b	1.25 ^b	1.69 ^a
	<i>stdev</i>	0.02	0.04	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01
5	avg	7.81 ^b	8.13 ^a	2.27 ^a	1.43 ^b	1.00 ^b	2.00 ^a	3.25 ^b	3.42 ^a	1.29 ^a	1.29 ^a
	<i>stdev</i>	0.05	0.06	0.03	0.01	0.00	0.01	0.04	0.02	0.01	0.01
6	avg	7.08 ^a	5.68 ^b	1.33 ^a	1.15 ^b	1.76 ^a	1.40 ^b	2.75 ^a	2.29 ^b	1.24 ^a	0.84 ^b
	<i>stdev</i>	0.04	0.03	0.01	0.00	0.01	0.01	0.03	0.01	0.01	0.00
7	avg	2.44 ^b	9.58 ^a	0.59 ^b	1.65 ^a	0.59 ^b	3.05 ^a	0.56 ^b	3.54 ^a	0.70 ^b	1.34 ^b
	<i>stdev</i>	0.01	0.05	0.00	0.01	0.00	0.02	0.00	0.03	0.00	0.01
8	avg	9.01 ^a	6.38 ^b	3.11 ^a	1.14 ^b	1.54 ^b	2.04 ^a	3.02 ^a	1.94 ^b	1.34 ^a	1.26 ^b
	<i>stdev</i>	0.06	0.04	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
9	avg	6.90 ^a	2.66 ^b	1.02 ^a	0.75 ^b	2.32 ^a	0.39 ^b	1.97 ^a	0.83 ^b	1.60 ^a	0.68 ^b
	<i>stdev</i>	0.05	0.02	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.00

Note: Data presented as average value of two analytical repetitions with standard deviation. The same letters in each row are not significantly different at the $P \leq 0.05$ level according to ANOVA. TP: total phenols; TS: total stilbenes; t-R: *trans*-resveratrol; cis-R: *cis*-resveratrol; t-P: *trans*-piceid; cis-P: *cis*-piceid. A: young bottled wines. B: wines after 12 months bottle storage

Therefore, studying the stilbene composition of wines is very important in view of wine production with high nutritional value. We quantified four stilbenes, precisely *trans*-resveratrol, *cis*-resveratrol, *trans*-piceid and *cis*-piceid. It can be observed that the concentration of total stilbenes (TS) was in the range from 2.44 to 10.07 mg L⁻¹ in young wines (Table 2). From all individual stilbene compounds, the highest portion falls on *trans*-resveratrol (0.59-3.39 mg L⁻¹) and *trans*-piceid (0.56-3.48 mg L⁻¹). Other stilbenes (*cis*-resveratrol and *cis*-piceid) were present in lower concentrations, reaching maximum values of 2.32 and 1.89 mg L⁻¹ (Table 2). This agrees with the observation from Adrian et al. (2000) who reported that the concentration of the *trans* isomers of both resveratrol and piceid are lower than those of the *cis* isomers in wines. Moreover, Pavloušek and Kumšta (2014) analyzed the content of *trans*-resveratrol, which is mostly found in red wines, and reported 2.67 mg L⁻¹ of *trans*-resveratrol in the same variety Blauer Portugieser. As shown in Table 3, the storage for 12 months led to a decrease in the concentration of TS in almost all samples with some exceptions. Although individual TS values ranged from

2.66 to 11.11 mg L⁻¹, on average total mean of TS of all samples was lower (7.15 mg L⁻¹) compared to the one observed before storage (7.34 mg L⁻¹). The same pattern was observed for individual stilbene compounds, the concentration of which in some samples decreased, while on the contrary, in some samples increased. The observed reduction in TS is primarily due to a decrease in the concentration of *trans*-resveratrol (0.75- 1.97 mg L⁻¹) and *cis*-piceid (0.62-1.69 mg L⁻¹) after 12 months of storage (Table 3). The concentration of free *trans*-resveratrol in wine is expected to increase with storage because of hydrolysis of piceid (a bound form of resveratrol). However, the results of this study show different pattern, which is in line with new research. For example, Naiker et al. (2020) measured resveratrol concentration in 16 red wines ranging from 1 to 6 years old from Australia, both initially and after a storage period of 16 months under ambient conditions. During the study period, *trans*-resveratrol concentration decreased by an average of 76%. The decay in *trans*-resveratrol was first order over the tested range of wine source and vintage, with a mean *trans*-resveratrol bottle-storage half-life of 8 (± 1 SD) months. Similarly,

the reduction in the concentration of bioactive compounds stilbene was found in the red Russian Fanagoria Merlot wine after 6 months of storage (Suprun et al., 2021). According to Naiker et al (2020), the data suggest isomerisation of *trans*- to *cis*-resveratrol via residual enzymatic activity, rather than being catalysed by light or acid. As already mentioned, bioactivity and health benefits are associated primarily with *trans*-resveratrol, and it is important to know the mechanisms if its activation is from inactive forms, such as the hydrolysis of piceid from the glycoside forms. Furthermore, as red wine ages, natural acid-catalyzed hydrolysis of piceid is expected to increase the levels of free *trans*-resveratrol in the wine, but these results suggest different life-mechanism of this compound. These results require further research and analysis of various factors in relation to red wine aging and *trans*-resveratrol. However, this study promotes young wines from the aspect of the polyphenolic structure.

Conclusion

To answer the question “is younger Portugizac more beneficial?”, this study analysed the Portugizac young wine samples before and after bottle storage for 12 months. An insight into the phenolic composition and antioxidant capacity has been shown, and the results demonstrate that young Portugizac wines have higher concentrations of total phenols and total stilbenes, as well as individual stilbene compounds, especially *trans*-resveratrol. At the same time, these wines also show a higher antioxidant capacity compared to wines stored for 12 months. Thanks to this research, the young Portugizac wine definitely gets a new nutritional value and a measurable dimension of quality. It can be concluded that aging changes the polyphenolic complexity and that the young wine “Portugizac Mlado vino” might be more nutritionally beneficial.

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