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"Portugizac Mlado vino"; is the aroma of younger red wine more attractive?

Abstract

"Portugizac Mlado vino" is a local red wine with Protected designation of origin and Traditional term, and it is usually consumed very soon after alcoholic fermentation as a young wine. The maturation and aging of the wine affect the aroma composition, which is generally not pronounced and specific in the case of most red wines, but, as a sensory property, it is important for the perception of quality and consumer choice. The objective of this work was to analyze the most important aroma compounds of esters and higher alcohols, with the unpleasant volatile phenols too, in "Portugizac" wines, related to aging. The 9 young wines "Portugizac", PDO "Plešivica" were analyzed after 3 and after 15 months of bottle storage at 16 ºC. Ethyl esters of butanoic and hexanoic acid along with isoamyl acetate are considered to be the most important esters in the fruity aroma of wine, and their concentrations in all analyzed "Portugizac" young wine samples were higher than their perception thresholds. The concentration of ethyl acetate was in the range 30-123 mg/L. The concentrations of ethyl esters of octanoic and decanoic acid, as well as 2-phenyl ethyl acetate and diethyl succinate in all analyzed wines, were lower than their perception thresholds. The concentrations of higher alcohols were much higher than their perception threshold. Bottle storage significantly affected the concentrations of analyzed compounds; after 15 months, the concentrations of acetate and ethyl esters (except ethyl acetate and diethyl succinate), as well as terpene (linalool), decreased, while higher alcohols and ethyl phenols slightly increased if compared to three months. In general, the compounds responsible for the desired fresh, fruity aroma tones were altered in an undesirable manner, while, undesirable compounds of 4-ethyl-phenol and 4-ethyl-quaiacol, which were not present in young wines, were detected in aged wines.

It can be concluded that the bottle aging affects the aroma profile, undesirable changes were more pronounced than positive ones and therefore the consumption of young wine "Portugizac" might be more sensory attractive.

Keywords: *„Portugizac Mlado vino", aroma, bottle aging*

Introduction

Wine aroma is very important in determining overall wine quality and is therefore important in wine purchasing (Rimkute et al., 2015). It was shown that consumers with a good level of knowledge tend to look for specific sensory quality; aroma, taste, and complexity (Corduas et al, 2013). The composition of wine aroma or „bouquet" is the result of hundreds of volatiles that can be associated with few sources; origin, grape variety, and technology of grape and wine production (Robinson et al., 2014). Generally, the volatile compounds derived from fermentation are the most important contributors to the overall aroma of the wine (Belda et al, 2016). Maturation and/or bottling is one of the technological factors that significantly influence wine quality (Tao et al, 2014). Depending on the conditions and time of maturation, changes in a composition may be more or less pronounced, affecting sensory properties and perception of quality (Echave et al., 2021). The changes relate to the composition of individual compounds and their interrelationship and equilibrium. Namely, ethanol and other important fermentation compounds form an „aromatic buffer" with different levels of strength, and that

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cannot be easily broken (Ferreira, 2010). For example, if the wine concentrations in acetic acid are low, fusel alcohol acetates are hydrolyzed and can be found at appreciable concentrations only in young wines. In contrast, as the wine concentrations in ethanol are relatively high, the concentrations of fatty acid ethyl esters are relatively constant and the concentrations of the ethyl esters of isoacids steadily increase during aging, which causes a softening in the aroma of some red wines. Changes in the composition of volatiles, primarily fermentation ones, can significantly affect the sensory quality and attractiveness of wine, especially in varieties that do not have a pronounced varietal aroma.

Currently, the sources of a wine's aging potential remain speculative. The importance of varietal origin is especially emphasized in the case of varieties that depend on terpenes or yeast-generated ethyl and acetate esters because both tend to degrade comparatively rapidly during bottle aging (Jackson, 2009). The use of different analytical approaches has revealed that the volatiles of young red wines can be presented through several tens of odorants, but there are no remarkable qualitative differences between wines from different grape varieties (Lopez et al, 1999). This is confirmed throw different studies, on the example of fermentative volatiles, primarily esters and higher alcohols (Ugliano and Henschke, 2009). A good example is the influence of ethyl esters on the perception of fruity aromas in some red wines, such as Cabernet sauvignon and Merlot, in which the content of compounds of this group is significantly higher, compared to some other varieties, in which the concentrations of these esters are typically much lower, like in Pinot noir, and therefore are not considered to be important factors contributing to its aroma (Ferreira, 2010).

Some other studies also show that the fruity aroma in wine arises from a collective contribution, rather than individual contributions by esters (Escudero et al., 2007).

In the case of red varieties and wines, we can say that this is a general phenomenon. Portugizac (*Vitis vinifera* L.)is one such red variety that is used to produce young wines. It is widespread in most Central and Southeastern European countries, but its representation is symbolic in terms of total viticulture area. Portugizac is an early variety and has the characteristic of a high yield in good fertilization conditions. By appropriate technology, Portugizac grape results with a fresh, good color, soft, velvety wines, with a gentle fruity aroma and low astringency, especially drinkable as a young wine. Portugizac has special importance in the limited Croatian area, which is protected by the PDO (Protected designation of origin) label "Portugizac Plešivica", where it is traditionally consumed as a young wine ("Mlado vino").

"Mlado vino" represents a Traditional term with a description of product characteristic, 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.). The PDO label means obligatory certification of wine to Regulation (EU) No 606/2009, and the Traditional term „Mlado vino" is authorized for wines that must be placed on the market before the 31st of December of the calendar year in which grapes were harvested.

Young wine branding is an excellent example of the promotion of a particular region/country, and although it is not an important economic factor in the global wine market, it can be very important for the local community. For example, Japan is the number one export market for French Beaujolais Nouveau. In 2019, around five million bottles were exported to Japan, which is roughly 50% of the total exported volume, while the total quantity that came on the market was about 21 million bottles (Burgundy Report, 2020). Furthermore, the popularity of younger red wines increased tremendously in the last ten years (Indian Wine Academy, 2020), mostly due to a generational change and millennials. Younger consumers are more in tune with the natural wine philosophy, which includes young wines too, and producers can communicate it successfully with potential new consumers (Lembke and Cartier, 2020).

As already explained, in the case of neutral grape varieties, such as Portugizac, it is not possible to describe the grape/wine aroma with specific descriptors, but it is possible and ne-

cessary to monitor the aroma profile of wine to describe the variety, to understand changes during wine maturation and to find the way of influencing aroma quality with specific technologies. Furthermore, changes in aroma and quality during aging can be a signal in which direction the product should be branded.

In the case of Portugizac wine, there are no available references about varietal aroma and aroma profile concerning the maturation or aging.

The study aimed to determine some important aroma compounds in young wine Portugizac 3 and 15 months after bottling. The results can be a useful support key in the description and presentation of the wine aroma of Portugizac variety, with special emphasis on young wine. Indirectly, the results can help promote and position young wine as a local tourism product.

Materials and Methods

Wine samples

Nine red Portugizac bottled and labeled original wines with PDO "Plešivica" (Protected designation of origin) were obtained from the market. The wines included eight "Mlado vino" samples (young wine), and one young wine without the Traditional term "Mlado vino". All samples were declared on the market in accordance with the specified standard. Samples were analyzed 3 months and 15 months after bottling. The samples were stored under controlled conditions at a temperature of 16 °C during 12 months after the first analysis.

GC/MS analysis of aroma compounds

The determination of aroma compounds was performed by gas chromatograph 6890 coupled to a mass spectrometer 5973 (Agilent Technologies, Santa Clara, CA, USA). Prior to GC/MS analysis, aroma compounds were extracted from wine samples by solid phase microextraction (SPME). A detailed description of operating conditions is given by Tomašević et al. (2017). The identified and quantified aroma compounds included acetate esters (ethyl acetate, isoamyl acetate, 2-phenyl ethyl acetate), ethyl esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate), higher alcohols (isobutanol, isoamyl alcohol, 1-hexanol, phenyl ethyl alcohol), terpene linalool and ethyl phenols (4-ethyl guaiacol, 4-ethyl phenol).

Statistical analysis

Statistical analysis was done using Statistica V.10 software (StatSoft Inc., Tulsa, USA). Analysis of variance (ANOVA) was performed on all independent variables of analyzed aroma compounds. Tukey's HSD test was applied to the data to determine significant differences ($p < 0.05$) among wine samples aged 3 and 15 months.

Results and discussion

Tables 1 and 2 presented the aroma compounds of the "Portugizac" young wines after 3 and 15 months of bottling, grouped according to the chemical class (acetate and ethyl esters, higher alcohols, terpenes, and ethyl phenols). The SPME/GC-MS analysis allowed the identification of a total of 15 most important aroma compounds in the samples. The aroma description of analyzed compounds is presented in Table 1.

Among the quantified esters (Table 1), ethyl acetate presents the most important ester in wines, and its concentrations were in the range from 30 mg/L that can be described as an excellent fermentation result, to 123 mg/L, which can have an unpleasant effect on sensory quality. If the concentration exceeds a threshold, the aroma of acetone is present in wine. According to different authors (Francis and Newton, 2005; Ribéreau-Gayon et al.; 2006, Jackson, 2009) the perception threshold of ethyl acetate is between 75 and 180 mg/L, and if the concentration exceeds a threshold, it carries negative, sharp notes and aromas of nail polish and glue. Also, it is known that at lower concentrations (60-70 mg/L) this compound contribu-

tes to the complexity of the aroma (Ribéreau-Gayon et al., 2006). Furthermore, ethyl esters of butanoic and hexanoic acid along with isoamyl acetate are considered to be the most important esters in wine, and their concentrations in all analyzed "Portugizac" young wine samples were higher than their perception thresholds, which amounted 0.02, 0.08 and 0.26 mg/L, respectively (Lambrechts and Pretorius, 2000). In the case of isoamyl acetate, below 0.02 mg/L this compound is just one of the many sweet-fruity wine compounds with very weak sensory effect. Between 0.2 and 1.4 mg/L, the importance of isoamyl acetate grows to the point that it becomes a quite important contributor to the fruity note of the wine (Ferreira, 2010). Isoamyl acetate is the only ester capable of imparting its characteristic aroma nuance to wines; in wines made with Pinotage or Tempranillo varieties, it is a characteristic aroma compound (van Wyck et al., 1979; Ferreira et al., 2000). It is clear that these compounds affect the aroma of analyzed wines. Generally, ethyl butyrate gives the wine a note of strawberry, apple, and banana (Song et al., 2013), ethyl hexanoate is a carrier of apple and banana fruit flavors, and isoamyl acetate carries the preferred notes of banana and pear (Lambrechts and Pretorius, 2000).

On the other hand, the concentrations of ethyl esters of octanoic and decanoic acid, as well as 2-phenyl ethyl acetate in all analyzed wines, were lower than their perception thresholds (Table 1), which amounted 0.58, 0.2 and 0.25 mg/L, respectively (Lambrechts and Pretorius, 2000; Swiegers et al., 2005; Tao and Li, 2009). Given that it can be concluded that these compounds are not important for the aroma of presented wines. The same observation can be made for diethyl succinate, which is known as one of the main compounds responsible for the fruity aroma of red wines and is formed during alcoholic fermentation (Ivanova et al., 2013), with the perception threshold of 200 mg/L (Makhotkina, 2011).

Table 1. Concentrations of acetate and ethyl esters in "Portugizac" young wines after 3 and 15 months of bottle aging

Tablica 1. Koncentracije acetatnih i etilnih estera u mladim vinima "Portugizac" nakon 3 i 15 mjeseci odležavanja u bocama.

Compound/ Sample		**EA** (mg/L)		**AC** (mg/L)		**2-PA** (mg/L)		**EB** (mg/L)		**EH** (mg/L)		**EO** (mg/L)		**ED** (mg/L)		**DS** (mg/L)		**TE** (mg/L)	
Months		**3**	**15**	**3**	**15**	**3**	**15**	**3**	**15**	**3**	**15**	**3**	**15**	**3**	**15**	**3**	**15**	**3**	**15**
1	av	67.69^{a}	68.17^{a}	0.87^{a}	0.49^{b}	0.11^{a}	0.06^{b}	0.11^{a}	0.08^{b}	0.24^{a}	0.15^{a}	0.45^{a}	0.26^{b}	0.15^{a}	0.04^{b}	0.46^{b}	2.94^{a}	70.06^{a}	72.18^{a}
	SD	*3.42*	*0.30*	*0.03*	*0.03*	*0.00*	*0.00*	*0.01*	*0.00*	*0.04*	*0.01*	*0.01*	*0.01*	*0.04*	*0.01*	*0.01*	*0.07*	*3.53*	*0.32*
2	av	68.08^{b}	109.54^{a}	0.58^{a}	0.22^{b}	0.05^{a}	0.03^{a}	0.10^{a}	0.08^{a}	0.15^{a}	0.13^{b}	0.12^{a}	0.09^{a}	0.03^{a}	0.04^{a}	0.21^{b}	2.55^{a}	69.29^{b}	112.65^{a}
	SD	*3.02*	*0.50*	*0.02*	*0.00*	*0.00*	*0.00*	*0.01*	*0.01*	*0.00*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.05*	*3.07*	*0.44*
3	av	28.09^{a}	30.33^{a}	0.91^{a}	0.51^{b}	0.17^{a}	0.08^{b}	0.08^{a}	0.07^{a}	0.17^{a}	0.13^{b}	0.30^{a}	0.25^{a}	0.07^{a}	0.04^{b}	0.65^{b}	6.49^{a}	30.42^{b}	37.89^{a}
	SD	*1.96*	*0.59*	*0.05*	*0.01*	*0.01*	*0.01*	*0.01*	*0.00*	*0.01*	*0.01*	*0.08*	*0.01*	*0.01*	*0.00*	*0.06*	*0.07*	*2.19*	*0.70*
4	av	41.76^{b}	75.42^{a}	0.78^{a}	0.39^{b}	0.09^{a}	0.02^{b}	0.10^{a}	0.07^{a}	0.15^{a}	0.14^{a}	0.25^{a}	0.19^{a}	0.07^{a}	0.04^{b}	5.30^{b}	14.51^{a}	48.48^{b}	90.77^{a}
	SD	*4.04*	*0.68*	*0.03*	*0.01*	*0.01*	*0.00*	*0.00*	*0.01*	*0.01*	*0.01*	*0.04*	*0.01*	*0.01*	*0.00*	*0.97*	*0.31*	*5.04*	*1.03*
5	av	41.45^{b}	62.57^{a}	1.10^{a}	0.72^{b}	0.16^{a}	0.08^{b}	0.10^{a}	0.08^{a}	0.20^{a}	0.16^{b}	0.28^{a}	0.27^{a}	0.08^{a}	0.06^{a}	0.73^{b}	9.73^{a}	44.07^{b}	73.64^{a}
	SD	*1.75*	*1.50*	*0.05*	*0.02*	*0.00*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.02*	*0.02*	*0.01*	*0.01*	*0.01*	*0.06*	*1.85*	*1.58*
6	av	27.48^{b}	85.34^{a}	0.20^{a}	0.15^{a}	0.06^{a}	0.03^{b}	0.05^{a}	0.03^{a}	0.14^{a}	0.12^{a}	0.14^{a}	0.09^{a}	0.03^{a}	0.03^{a}	0.39^{b}	8.37^{a}	28.46^{b}	94.15^{a}
	SD	*3.63*	*2.32*	*0.04*	*0.01*	*0.01*	*0.00*	*0.01*	*0.00*	*0.03*	*0.01*	*0.02*	*0.00*	*0.02*	*0.00*	*0.04*	*0.03*	*3.79*	*2.35*
7	av	31.99^{b}	65.42^{a}	0.92^{a}	0.81^{a}	0.06^{a}	0.04^{a}	0.12^{a}	0.11^{a}	0.33^{a}	0.28^{a}	0.28^{a}	0.26^{a}	0.05^{a}	0.03^{a}	0.38^{b}	8.02^{a}	34.09^{b}	74.96^{a}
	SD	*2.38*	*0.96*	*0.06*	*0.02*	*0.01*	*0.00*	*0.01*	*0.01*	*0.02*	*0.01*	*0.05*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*2.55*	*0.98*
8	av	34.43^{b}	64.96^{a}	1.03^{a}	0.67^{b}	0.10^{a}	0.05^{a}	0.10^{a}	0.09^{a}	0.20^{a}	0.15^{a}	0.30^{a}	0.25^{a}	0.07^{a}	0.04^{a}	0.72^{b}	8.45^{a}	36.94^{b}	74.64^{a}
	SD	*0.45*	*0.01*	*0.07*	*0.02*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.01*	*0.03*	*0.01*	*0.01*	*0.01*	*0.00*	*0.03*	*0.40*	*0.01*
9	av	20.99^{b}	123.21^{a}	0.47^{a}	0.38^{a}	0.24^{a}	0.22^{a}	0.06^{a}	0.04^{a}	0.26^{a}	0.22^{a}	0.30^{a}	0.25^{a}	0.10^{a}	0.01^{b}	0.34^{b}	5.48^{a}	22.74^{b}	129.80^{a}
	SD	*2.38*	*2.24*	*0.22*	*0.01*	*0.00*	*0.01*	*0.01*	*0.01*	*0.02*	*0.00*	*0.04*	*0.01*	*0.02*	*0.00*	*0.01*	*0.10*	*2.55*	*2.17*

Legend: EA: ethyl acetate; AC: isoamyl acetate; 2-PA: 2-phenylethyl acetate; EB: ethyl butyrate; EH: ethyl hexanoate; EO: ethyl octanoate; ED: ethyl decanoate; DS: diethyl succinate; TE: total esters. Different letters within the same row represent the significant difference (Tukey's HSD test, $p < 0.05$) among the samples aged 3 and 15 months. / Legenda: EA: etil acetat; AC: *i*-amil acetat; 2-PA: 2-feniletil acetat; EB: etil butirat; EH: etil heksanoat; EO: etil oktanoat; ED: etil dekanoat; DS: dietil sukcinat; TE: ukupni esteri. Različita slova unutar istog reda predstavljaju značajnu razliku (Tukey's HSD test, $p < 0.05$) između uzoraka koji su dozrijevali 3 i 15 mjeseci.

Table 2. Concentrations of higher alcohols, terpene, and ethyl phenols in "Portugizac" young wines after 3 and 15 months of bottle aging

Tablica 2. Koncentracije viših alkohola, terpena i etilnih fenola u mladim vinima "Portugizac" nakon 3 i 15 mjeseci odležavanja u bocama

Compound/ Sample		B (mg/L)		AA (mg/L)		1-H (mg/L)		PEA (mg/L)		THA (mg/L)		L (µg/L)		4-EG (mg/L)		4-EP (mg/L)	
Months		3	15	3	15	3	15	3	15	3	15	3	15	3	15	3	15
1	av	57.10^{a}	61.66^{a}	248.71^{b}	265.11^{a}	0.54^{a}	0.60^{a}	40.41^{b}	48.84^{a}	346.75^{b}	376.20^{a}	5.34^{a}	1.91^{b}	0.24^{b}	0.34^{a}	0.42^{b}	1.14^{a}
	SD	*1.99*	*0.74*	*4.00*	*0.35*	*0.01*	*0.04*	*1.15*	*2.50*	*3.16*	*2.15*	*0.05*	*0.25*	*0.00*	*0.03*	*0.01*	*0.04*
2	av	68.80^{a}	56.21^{b}	261.48^{b}	282.03^{a}	0.91^{b}	1.16^{a}	41.34^{b}	53.10^{a}	372.52^{a}	392.49^{a}	5.61^{a}	2.21^{b}	0.00^{b}	0.23^{a}	0.00^{b}	0.85^{a}
	SD	*3.33*	*1.05*	*3.79*	*4.78*	*0.02*	*0.04*	*3.64*	*0.93*	*10.74*	*4.86*	*0.10*	*0.14*	*0.00*	*0.01*	*0.00*	*0.01*
3	av	39.89^{b}	59.91^{a}	270.01^{b}	291.75^{a}	1.38^{b}	1.81^{a}	113.22^{a}	71.32^{b}	424.49^{a}	424.79^{a}	10.03^{a}	4.26^{b}	0.00^{b}	0.06^{a}	0.00^{b}	0.34^{a}
	SD	*0.37*	*0.73*	*1.67*	*0.89*	*0.01*	*0.04*	*0.84*	*2.73*	*2.12*	*1.15*	*0.19*	*0.11*	*0.00*	*0.00*	*0.00*	*0.01*
4	av	46.40^{b}	71.81^{a}	189.32^{b}	201.79^{a}	1.31^{b}	1.63^{a}	50.94^{a}	35.16^{b}	287.96^{b}	310.37^{a}	6.87^{a}	3.81^{b}	0.00^{b}	0.41^{a}	0.00^{b}	2.56^{a}
	SD	*2.88*	*3.36*	*5.03*	*1.89*	*0.03*	*0.01*	*4.29*	*0.62*	*6.41*	*0.86*	*0.13*	0.08	*0.00*	*0.01*	*0.00*	*0.03*
5	av	30.52^{b}	56.51^{a}	197.36^{b}	208.47^{a}	1.12^{b}	1.30^{a}	63.17^{a}	37.99^{b}	292.17^{b}	304.26^{a}	12.13^{a}	6.41^{b}	0.00^{b}	0.16^{a}	0.00^{b}	0.94^{a}
	SD	*3.35*	*1.50*	*0.71*	*0.08*	*0.04*	*0.01*	*0.88*	*1.60*	*1.80*	*3.03*	*0.39*	*0.37*	*0.00*	*0.02*	*0.00*	*0.06*
6	av	33.04^{b}	51.95^{a}	190.78^{b}	218.31^{a}	1.56^{b}	2.06^{a}	47.57^{a}	36.73^{b}	272.94^{b}	309.05^{a}	11.58^{a}	5.23^{b}	0.00^{b}	0.16^{a}	0.00^{b}	1.01^{a}
	SD	*2.55*	*1.12*	*0.32*	*0.17*	*0.09*	*0.08*	*1.91*	*1.10*	*0.23*	*2.31*	*0.45*	*0.08*	*0.00*	*0.01*	*0.00*	*0.00*
7	av	41.76^{b}	57.16^{a}	258.49^{a}	284.95^{a}	1.34^{a}	1.42^{a}	59.29^{a}	69.26^{a}	360.88^{b}	412.78^{a}	14.99^{a}	5.78^{b}	0.00^{b}	0.11^{a}	0.00^{b}	0.39^{a}
	SD	*2.53*	*4.08*	*7.70*	*5.82*	*0.00*	*0.16*	*2.36*	*2.98*	*12.59*	*4.56*	*1.28*	*0.00*	*0.00*	*0.01*	*0.00*	*0.04*
8	av	98.69^{a}	72.30^{b}	259.74^{b}	290.02^{a}	0.75^{a}	0.88^{a}	50.49^{b}	66.70^{a}	409.66^{a}	429.90^{a}	9.02^{a}	3.93^{b}	0.10^{b}	0.21^{a}	0.26^{b}	1.05^{a}
	SD	*3.24*	2.78	*8.75*	*3.21*	*0.06*	*0.18*	*3.95*	*1.06*	*9.40*	*7.23*	*0.64*	*0.08*	*0.01*	*0.04*	*0.00*	*0.13*
9	av	43.09^{b}	87.52^{a}	327.70^{b}	359.73^{a}	2.03^{a}	2.48^{a}	127.61^{a}	152.25^{a}	500.42^{b}	601.97^{a}	19.99^{a}	10.08^{b}	0.00^{b}	0.14^{a}	0.00^{b}	0.33^{a}
	SD	*1.68*	*4.62*	*5.69*	*2.40*	*0.16*	*0.03*	*8.82*	*0.52*	*16.04*	*7.51*	*1.96*	*0.32*	*0.00*	*0.01*	*0.00*	*0.01*

Legend: B: isobutanol; AA: isoamyl alcohol; 1-H: 1-hexanol; PEA: phenyl ethyl alcohol; THA: total higher alcohols; L: linalool; 4-EG: 4-ethyl guaiacol; 4-EP: 4-ethyl phenol. Different letters within the same row represent the significant difference (Tukey's HSD test, $p < 0.05$) among the samples aged 3 and 15 months. / Legenda: B: izobutanol; AA: izoamil alkohol; 1-H: 1-heksanol; PEA: fenil etil alkohol; THA: ukupni viši alkoholi; L: linalol; 4-EG: 4-etil gvajakol; 4-EP: 4-etil fenol. Različita slova unutar istog reda predstavljaju značajnu razliku (Tukey's HSD test, $p < 0.05$) između uzoraka koji su dozrijevali 3 i 15 mjeseci.

In general, among the aroma compounds, the higher alcohols were the most abundant in all analyzed samples, constituting about 76-96% of the total for presented wines, respectively. The second most abundant group was that of esters (acetate and ethyl), which accounted for about 4-23% of the total for the wine samples, respectively. The other groups (terpenes and ethyl phenols) were detected at very low concentrations (< 1% of the total).

Among the quantified higher alcohols (Table 2), isoamyl alcohol was the most abundant in all analyzed "Portugizac" young red wines. It is one of the four most commonly present higher alcohols in wines, along with 1-propanol, isobutanol and phenylethyl alcohol, and it is most often present in concentrations 10-500 mg/L (Peinado et al., 2004). This compound impacts an aroma reminiscent of marzipan, It is also reported to give aromas of whiskey and nail polish to

wine (Song et al., 2013), and the perception threshold is 30 mg/L (Swiegers et al., 2005). The observed concentrations in wine samples were much higher than the perception threshold (Table 2) and it can be concluded that this compound is of great importance for the aroma of "Portugizac" young red wines. Moreover, one of the four higher alcohols determined in this study was also isobutanol, which is produced from amino acids, valine and methionine, via the catabolic pathway during fermentation (Ivanova et al., 2013). The perception threshold of isobutanol is 40 mg/L (Francis and Newton, 2005), and it gives the wine an alcoholic odor (Song et al., 2013). The aroma analysis revealed that isobutanol is present in all samples at a concentration higher than the perception threshold (Table 3). Hence, it contributes to the complexity of "Portugizac" wine aroma. Further, with a perception threshold of 1-hexanol of 8 mg/L (Culleré et al., 2004), it can be concluded that 1-hexanol does not contribute to the aroma of analyzed "Portugizac" young red wines, considering that the concentrations detected in all wines did not exceed even 2.5 mg/L (Table 3). Otherwise, 1-hexanol contributes to the wine with a green grassy aroma (Tao and Li, 2009). The last quantified higher alcohol in presented wines was phenyl ethyl alcohol. This compound is the most important phenol-derived higher alcohol present in wines, and it is produced during fermentation by the action of yeast from sugars and amino acids (Ivanova et al., 2013). The perception threshold of phenyl ethyl alcohol is 14 mg/L (Culleré et al., 2004), and it gives the wine a floral rose aroma (Swiegers et al., 2005). Given the concentrations of phenylethyl alcohol in analyzed samples (Table 2), it can be seen that this compound is significant for the aroma of "Portugizac" young red wines. In general, higher alcohols are thought to contribute to the complexity of wine aroma at concentrations below 300 mg/L, while concentrations above 400 mg/L harm the aroma (Swiegers et al., 2005).

Aroma compound belonging to the group of terpenes, such as linalool, was also detected and quantified in the wines (Table 2). This family of aroma compounds derives from grapes and represents so-called varietal aroma or primary aroma. The perception threshold of linalool is 25.2 µg/L (Francis and Newton, 2005), and in the concentrations around 20-50 µg/L, it contributes to the wine with a generic sweet-floral note (Ferreira et al., 2007). Given that it can be concluded that linalool has no important role in the overall aroma of Portugizac variety and analyzed wines.

Bottle storage significantly affected the concentrations of analyzed compounds (Table 1 and Table 2). When comparing the particular aroma composition after 3 and 15 months, it can be seen that concentrations of acetate and ethyl esters (except ethyl acetate and diethyl succinate), as well as terpene (linalool), slightly decreased, while higher alcohols and ethyl phenols slightly increased during 12 months' period (Tables 1 and 2). In the case of ethyl acetate, the concentrations have significantly and undesirable increased in some samples, which may affect the perception of the desired fresh, fruity aroma to which ethyl acetate contributes in the already mentioned acceptable concentrations. Concentrations of another important ester, isoamyl acetate, were reduced by bottle aging, in most samples significantly. The well-known contribution of these two compounds to the desirable fruity aroma in the case of Portugizac wine has therefore changed in an undesirable direction.

According to Ferreira (2010), 16 compounds conform 16 "primary colors" of wine aroma, and isoamyl acetate is one of them. These compounds can be explained as major contributors; they can transmit a characteristic (not specific) aroma nuance to some wines. Its omission will cause a strong quantitative effect and even a slight qualitative change. In the case of young Portugizac wine, bottle aging has affected a significant reduction of isoamyl acetate, which is definitely not desirable, and given the varietal potential which cannot replace the omission of any crucial aroma compound.

In case of ethyl esters of butanoic and hexanoic acid, that are also important in fruity aroma

expression, bottle aging cause reduction of its concentration, but not significantly. As previously explained, the concentrations of diethyl succinate, that is known as one of the main compounds responsible for the fruity aroma of red wines, were below the perception threshold in all samples. Although the content increased during bottle aging, the actual concentrations of diethyl succinate were still irrelevant to the aroma of wines. It needs to be noted that not all the compounds were detected in all the Portugizac samples on a significant level, however, the main findings regarding the effect of the bottle aging on the concentration of volatiles were observed for each sample, although some exceptions to this general observation existed.

Additionally, the total esters (TE) content was also calculated as a sum of the contributions of individual compounds (Table 1). From the given results, it can be observed that the content of TE was in the range from 23 – 70 mg/L after the first analysis, and in the range from 38 – 130 mg/L after 12 months period of aging. The observed increase was primarily due to an increase of ethyl acetate, the compound that is already explained as risky for sensory quality.

The concentrations of higher alcohols slightly changed and increased during 12 months of aging. This was also evident from the calculated sum of individual higher alcohols, namely THA content (Table 2). After 3 months, the content of THA ranged from 347 – 500 mg/L, while after 15 months it accounted for between 376 - 602 mg/L, respectively. As already mentioned, concentrations of higher alcohols above 400 mg / L may harm aroma and sensory quality (Swiegers et al. 2005), and increases in their content, as in the case of Portugizac samples, are not desirable. Moreover, analysis of individual compounds suggests that 1-hexanol definitely does not contribute to the aroma of Portugizac wines, and the other three compounds remain within the limits of a positive effect on aroma quality individually in most of the bottled young wines after 3 months. However, their concentrations in some samples after 15 months of bottle aging were in a range undesirable for aroma quality.

Generally, the wine undergoes different changes during aging in which are involved both volatile and non-volatile components. A variety of chemical reactions, such as hydrolysis, oxidation, and esterification, results in the decay or formation of various aroma compounds (Yang et al., 2020). During this stage, the fresh and fruity aroma of the wines is lost due to the decrease of esters (Pérez-Prieto et al., 2003), and that was also confirmed by our results (Table 1). Moreover, an increase in the concentration of diethyl succinate during wine aging was also previously reported (Verzeletti et al., 2016). Also, a well-known consequence of wine aging is a loss of varietal aroma due to decrease of monoterpenic alcohols (linalool, geraniol and citronellol) in wines (Pereira et al., 2020). On the other hand, higher alcohols mostly remain unchanged during aging, although an increase has been observed in some studies due to hydrolysis of the corresponding esters (Pérez-Prieto et al., 2003) or increased microbial activity in wines (Tomašević et al., 2017).

Another class of aroma compounds is microbial-derived volatile ethyl phenols, such as 4-ethyl guaiacol and 4-ethyl phenol, which were found in two samples of young wines (Table 2). The potential sensory impact of these compounds is dramatic due to their low sensory thresholds. The 4- ethyl phenol and 4- ethyl quajacol, require only 770 µg/L and 436 µg/L, respectively, to be recognized (Chatonnet et al. 1992).

During bottle aging the average value of 4-ethyl guaiacol and 4-ethyl phenol in wines was, mainly aged samples, above the values from which these compounds can negatively affect the wine aroma (0.14 mg/L and 0.62 mg/L, respectively) (Chatonnet et al., 1992), since these are responsible for the undesirable medicinal and horsey off-odors found in some red wines (Garde-Cerdán et al., 2010). Curtin et al (2008) found the aroma threshold of 4-ethyl phenol to be 368 µg/L, and for 4-ethylguaiacol to be 158 µg/L in Australian red wine.

According to Aznar et al (2003), at a concentration high enough (well above 1000 µg/L), the sensory effect of this molecule is the straightforward apparition of the off-odor note but, at smaller concentration, certainly below the recognition threshold, what the addition of this molecule to a fruity wine causes is the decrement of the fruity note. Some other authors ((Suarez et al, 2007). reported that concentrations above 1.74 mg/L 4-ethyl phenol present a negative effect on the wine aroma. Furthermore, it has been reported that most red wines contain very low concentrations of ethyl phenols or none at all after the alcoholic and malolactic fermentation, but during aging their concentrations can increase due to a certain yeast activity (Chatonnet et al., 1992), which was found in this study (Table 2).

Conclusion

The results of major aroma compounds analysis of original red young Portugizac wines conducted 3 and 15 months after bottling, showed that undesirable aroma changes were more pronounced than positive ones. In addition to the unwanted changes in volatile esters and higher alcohols that are important for aroma quality and intensity during the 12 month aging period, volatile phenols compounds with negative effects were detected. Following the obtained results, it is possible to conclude that the aroma of young Portugizac wines is more desirable, in terms of quality and expected consumer preferences. The results of this study require further research that should include sensory analyzes too, both expert and consumer, to better understand the relationship between aroma profile - sensory quality – sensory preferences.

Literature

Aznar, M., Lopez, R., Cacho, J., Ferreira, V. (2003) Prediction of aged red wine aroma properties from aroma chemical composition, Partial least squares regression models. *Journal of Agriculture and Food Chemistry*, 51(9), 2700-2707. DOI: 10.1021/jf026115z

Belda I, Ruiz J, Alastruey-Izquierdo A, Navascués E, Marquina D, Santos A (2016) Unraveling the -functional study of wine related yeast species. *Front Microbiology.* 7:12

Burgundy Report (2020) 2020 Beaujolais Nouveau. Retrieved from https://www.burgundy-report.com/2020/11/2020-beaujolais-nouveau/ (5. 3. 2021.)

Chatonnet, P., Dubourdie, D., Boidron, J. N., Pons, M. (1992) The origin of ethylphenols in wines. *Journal of the Science of Food and Agriculture*, 60(2), 165-178. DOI: 10.1002/jsfa.2740600205

Corduas, M., Cinquanta, L., Ievoli, C. (2013) The importance of wine attributes for purchase decisions: A study of Italian consumers' perception. *Food Quality and Preference*. 28: 407-418. DOI: 10.1016/j.foodqual.2012.11.007

Culleré, L., Escudero, A., Cacho, J., Ferreira, V. (2004) Gas chromatography– olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry*, 52(6), 1653-1660. DOI: 10.1021/jf0350820

Curtin, C., Bramley, B., Cowey, G., Holdstock, M., Kennedy, E., Lattey, K., Coulter, A., Henschke, P., Francis, L., Godden, P. (2008) Sensory perceptions of 'Brett' and relationship to consumer preference. Blair, R.J., Williams, P.J., Pretorius, I.S. (eds) *Proceedings of the thirteenth Australian wine industry technical conference*, 29 July – 2 August 2007, Adelaide, SA: Australian Wine Industry Technical Conference Inc.: Adelaide, SA: 207-211.

Echave, J., Barral, M., Fraga-Corral, M., Prieto, M. A., Simal-Gandara, J. (2021) Bottle Aging and Storage of Wines: A Review. *Molecules*, 26(3), 713. DOI: 10.3390/molecules26030713

Escudero, A., Campo, E., Farina, L., Cacho, J. & Ferreira, V., (2007) Analytical characterization of the aroma of five premium red wines. Insights into the role of odour families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry*. 55, 4501-4510.

Ferreira, V. (2010) Volatile aroma compounds and wine sensory attributes. In: *Managing Wine Quality: Viticulture and Wine Quality*. Reynolds, A.G., Ed.; Woodhead Publishing Limited: Cambridge, UK, pp. 3–28. DOI: 10.1533/9781845699284.1.3

Ferreira, V., Escudero, A., Campo, E., Cacho, J. (2007) The chemical foundations of wine aroma–A role game aiming at wine quality, personality and varietal expression. In: Williams, P. J., Blair R. J., Pretorius, I. S., ed. *Proceedings of the Thirteenth Australian Wine Industry Technical Conference,* Adelaide, South Australia: Australian Wine Industry Technical Conference, Inc (p. 142).

Ferreira, V., Lopez, R., Cacho, J. F (2000) Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*, 80(11), 1659-1667. DOI: 10.1002/1097-0010(20000901)80:11<1659::aid-jsfa693>3.0.co;2-6

Francis, I. L., Newton, J. L. (2005) Determining wine aroma from compositional data. *Australian Journal of Grape and Wine Research*, 11(2), 114-126. DOI: 10.1111/j.1755-0238.2005.tb00283.x

Garde-Cerdán, T., Lorenzo, C., Alonso, G. L., Salinas, M. R. (2010) Employment of near infrared spectroscopy to determine oak volatile compounds and ethylphenols in aged red wines. *Food Chemistry*, 119(2), 823-828. DOI: 10.1016/j.foodchem.2009.07.026

Indian Wine Academy (2020). Younger Wines healthier than Cellared Wines. Retrieved from https://www.indianwineacademy.com/item_2_856.aspx (5. 3. 2021.)

Ivanova, V., Stefova, M., Vojnoski, B., Stafilov, T., Bíró, I., Bufa, A., Felinger, A., Kilár, F. (2013) Volatile composition of Macedonian and Hungarian wines assessed by GC/MS. *Food and Bioprocess Technology*, 6(6), 1609-1617. DOI: 10.1007/s11947-011-0760-y

Lambrechts, M. G., Pretorius, I. S. (2000) Yeast and its importance to wine aroma-a review. *South African Journal of Enology and Viticulture*, 21(1), 97-129. DOI: 10.21548/21-1-3560

Lembke, S. and Cartier, L. (2020), "Wine consumers in British Columbia, Canada: Towards understanding how Millennials differ from previous generations", *International Journal of Wine Business Research*, 32(3), 441-455. DOI:10.1108/IJWBR-07-2019-0043

López, R., Hernández, P., Cacho, J.F. (1999) Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. *Journal of Food and Agriculture.* 79(11), 1461-1467. DOI: 10.1002/(SICI)1097-0010(199908)79:11<1461::AID-JSFA388>3.0.CO;2-K

Makhotkina, O. (2011) *Stability of New Zealand Sauvignon blanc aroma compounds: oxidation versus hydrolysis.* Doctoral dissertation. Auckland: The University of Auckland.

Peinado, R. A., Moreno, J. A., Muñoz, D., Medina, M., Moreno, J. (2004) Gas chromatographic quantification of major volatile compounds and polyols in wine by direct injection. *Journal of Agricultural and Food Chemistry*, 52(21), 6389-6393. DOI:10.1021/jf049369o

Pereira, A. G., Fraga, M., Garcia-Oliveira, P., Carpena, M., Jimenez-Lopez, C., Lourenço-Lopes, C., Barros, L., C.F.R. Ferreira, I., Prieto, M. A., Simal-Gandara, J. (2020) *Chapter Management of Wine Aroma Compounds: Principal Basis and Future Perspectives*. IntechOpen. https://library.oapen.org/handle/20.500.12657/49377 (13. 8. 2021.)

Pérez-Prieto, L. J., López-Roca, J. M., Gómez-Plaza, E. (2003) Differences in major volatile compounds of red wines according to storage length and storage conditions. *Journal of Food Composition and Analysis*, 16(6), 697-705. DOI: 10.1016/S0889-1575(03)00080-2

Ribéreau-Gayon P., Glories Y., Maujean A., Dubourdieu D. (2006) *Handbook of Enology, Volume 2: The Chemistry of Wine Stabilization and Treatments*, 2nd Edition., John Wiley & Sons, Inc.

Rimkute, J, Moraes, Ferreira, C. (2015) 'The effects of scent on consumer behaviour' *International Journal of Consumer Studies*, 40 (1), 24-34. DOI: 10.1111/ijcs.12206. T

Robinson, A.L.; Boss, P.K.; Solomon, P.S.; Trengove, R.D.; Heymann, H.; Ebeler, S.E. (2014) Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *Am. J. Enol. Vitic*. 65, 1–24.

Song, J. Q., Li, H., Liang, Y. Y., Tao, Y. S., Mi, C. Q., Qian, M. C., Wang, H. (2013) Characterisation of volatile components of red and sparkling wines from a new wine grape cultivar'Meili'(*Vitis vinifera* L.). *Vitis*, *52*(1), 41-48.

Suárez, R., Suárez-Lepe, J.A., Morata, A., Calderón, F. (2007) The production of ethylphenols in wine by yeasts of the genera Brettanomyces and Dekkera: A review. *Food Chemistry*. 102 (1), 10-21. DOI: 10.1016%2Fj.foodchem.2006.03.030

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., Pretorius, I. (2005) Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of grape and wine research*, 11(2), 139-173. DOI: 10.1111/j.1755-0238.2005.tb00285.x

Tao, Y. S., Li, H. (2009) Active volatiles of Cabernet sauvignon wine from Changli County. *Health*, 1(03), 176. DOI: 10.4236/health.2009.13028

Tao, Y., Garcia, J. F., Sun, D-W. (2014) Advances in Wine Aging Technologies for Enhancing Wine Quality and Accelerating Wine Aging Process. *Critical Reviews in Food Science and Nutrition,* 54(6), 817-835. DOI:10.1080/10408398.2011.609949

Tomašević, M., Gracin, L., Ćurko, N., Kovačević Ganić, K. (2017) Impact of pre-fermentative maceration and yeast strain along with glutathione and SO_2 additions on the aroma of *Vitis vinifera* L. Pošip wine and its evaluation during bottle aging. *LWT-Food Science and Technology*, 81, 67-76. DOI: 10.1016/j.lwt.2017.03.035

Ugliano, M., Henschke, P. A. (2009) *Yeasts and wine flavour*. In: Moreno-Arribas M.V., Polo M.C., eds. *Wine Chemistry and Biochemistry.* Springer, New York, NY.

Van Wyk, C. J., Augustyn, O. P. H., De Wet, P., Joubert, W. A. (1979) Isoamyl acetate - A key fermentation volatile of wines of Vitis vinifera cv Pinotage. *Am. J. Enol. Vitic*. 30, 167-173

Verzeletti, A., Echeverrigaray, S., Cardoso, A., Vanderlinde, R., Delamare, A. P. L. (2016) Evolution of aromatic compounds during the second fermentation and aging of Brazilian sparkling wine. In *BIO Web of Conferences.* Vol. 7, p. 02019. EDP Sciences. DOI: 10.1051/bioconf/20160702019

Yang, Y., Hu, W., Xia, Y., Mu, Z., Tao, L., Song, X., Zhang, H., Ni, B., Ai, L. (2020) Flavor Formation in Chinese Rice Wine (Huangjiu): Impacts of the Flavor-Active Microorganisms, Raw Materials, and Fermentation Technology. *Frontiers in Microbiology*, 11, 2541. DOI: 10.3389/fmicb.2020.580247

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Izvorni znanstveni rad

"Portugizac Mlado vino"; je li aroma mlađeg crvenog vina senzorno privlačnija?

Sažetak

"Portugizac Mlado vino" je lokalno vino sa zaštićenom oznakom izvornosti i tradicionalnim izrazom, te se obično konzumira kao mlado vino, odmah nakon alkoholne fermentacije. Dozrijevanje i odležavanje vina utječe na sastav i aromu koja u slučaju crnih vina uobičajeno nema posebnu prepoznatljvost i specifičnost, ali je kao senzorno svojstvo jako važna za percepciju kakvoće i potrošački izbor. Cilj ovog rada bio je analizirati najvažnije hlapljive spojeve estera i viših alkohola, te nepoželjnih hlapljivih fenola u mladim vinima Portugisca, u odnosu na vrijeme dozrijevanja vina u boci. Devet mladih vina "Portugizac", zaštićene oznake izvornosti "Portugizac Plešivica", s tržišta, analizirano je dva puta; 3 mjeseca i 15 mjeseci nakon punjenja u boce. Sadržaj etil estera butanske (maslačne) i heksanske (kapronske) kiseline zajedno sa izoamil acetatom, kao najvažnijih spojeva nositelja voćne arome bio je veći od praga senzorne prepoznatljivost u svim analiziranim vinima. Koncentracije etil acetata bile su u rasponu 30-123 mg/L. Koncentracije etil estera oktanske (kaprilne) i dekanske (kaprinske) kiseline, kao i 2-feniletil acetata i dietil sukcinata bile su ispod praga prepoznatljivosti u svim uzorcima. Koncentracije viših alkohola bile su značajno veće od pragova senzorne prepoznatljivosti. Čuvanje vina u boci značajno je utjecalo na promjene koncentracija analiziranih spojeva; 12 mjeseci nakon prve analize, koncentracije acetata i etil estera (osim etil acetata i dietil sukcinata), kao i terpena linalola su bile manje, dok su koncentracije viših alkohola i etil fenola bile veće nego u mladim vinima nakon prve analize. Sadržaj spojeva odgovornih za svježu, poželjnu voćnu aromu dozrijevanjem je promijenjen u neželjenom smjeru, te su utvrđeni i nepoželjni spojevi etil fenoli koji nisu bili prisutni u mladim vinima. Može se zaključiti kako je čuvanje vina u boci utjecalo na aromatski profil, uz izraženije nepoželjne promjene, te bi stoga, konzumacija mladog vina "Portugizac" mogla biti senzorno privlačnija.

Ključne riječi: *"Portugizac Mlado vino", aroma, dozrijevanje u boci*