

## Technological characteristic, aromatic profile and biological potential of wines from the local white wine variety Kokorko

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### Abstract

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A study of the technological characteristic of Kokorko white wines was carried out. The object of the study were wines from two consecutive vintages (2019 and 2020), obtained from two growing regions. They were produced in the Experimental Wine Cellar of Agricultural University (AU) – Plovdiv (2019 and 2020), and the Yalovo Winery – Veliko Tarnovo (2019). The laboratory analyzes were performed at the Institute of Viticulture and Enology – Pleven. The results showed that the samples from AU – Plovdiv had higher alcohol content, total and sugar-free extract. The determined low titratable acidity was typical for the variety. A correlation was found between the content of the analyzed phenolic compounds and the antioxidant activity of the wines. Their amounts increased in the order: Kokorko, 2019 (Yalovo Winery) < Kokorko, 2020 (AU – Plovdiv) < Kokorko, 2019 (AU–Plovdiv).

A diverse volatile composition was identified in the studied wines, consisting of 17 compounds (1 aldehyde, 4 higher alcohols, 8 esters, 3 terpenes and methyl alcohol). The wine from the Yalovo Winery had the highest total volatile content. The highest total concentration of higher alcohols was found in the sample, 2020 vintage. The main identified representatives were 2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol and 1-butanol. The wine from the Yalovo Winery contained the most acetaldehyde and a total amount of esters. The least esters were found in Kokorko, 2020 vintage. The main representatives of the ester fraction identified in all wines were ethyl acetate and isopropyl acetate. Propyl acetate, isopropyl acetate, butyl acetate and isobutyl acetate were also identified. The terpenes  $\alpha$ -terpineol, nerol and geraniol were found, as the latter had the highest content. Methyl alcohol was present in all wines in quantities that meet the safety criteria of the drink. Kokorko wines, made in AU – Plovdiv, had better organoleptic features in terms of aromatic and taste properties.

**Keywords:** Kokorko variety; white wine; chemical composition; phenolic compounds; antioxidant activity; volatile components; organoleptic profile

### Introduction

The diverse climatic, soil and geographical conditions defined Bulgaria as a country with rich biodiversity of a number of local and introduced plant species. As a result of human activity and the economic conditions in the country in recent decades, the unique gene pool and genetic resources (local plant

varieties, wild ancestors and relatives of cultivated plants) had declined almost to the point of extinction. The accelerated global climate change had also implications for Bulgaria's biodiversity, given the country's transitional location between three major bioclimatic regions (Meine et al., 1994).

Due to the favorable ecological conditions, grapevines had been grown in our lands for thousands of years. The lo-

cal vine varieties were obtained through the cultivation of the wild vine and long-term selection, and later varieties were introduced from other parts of the world, therefore rich variety diversity was achieved in the country (Katerov et al., 1990).

Until the middle of the 20<sup>th</sup> century, wines were made in Bulgaria, mainly from local varieties. After the zoning of the viticulture, the training systems of the existing vineyards were changed and French varieties were widely planted, which together with other introduced ones found good conditions for development, cultivation and distribution. For these reasons, as well as under the influence of the economic factors, they had long been dominant in the sectors of viticulture and enology. As a result, over 20 old local Bulgarian varieties had almost completely disappeared ([https://www.bacchus.bg/vino/2017/05/17/2972972bulgarskite\\_sortove-nacionalna\\_cennost/](https://www.bacchus.bg/vino/2017/05/17/2972972bulgarskite_sortove-nacionalna_cennost/)).

In the last two decades, however, the interest of the Bulgarian grape-growers and wine-makers in a number of local varieties had increased. In their pursuit to find and impose their identity in the world wine markets, they rediscovered the qualities and features of the old varieties. The attitude of the producers towards these varieties was changing, their vine potential for obtaining quality white and red wines was revealed ([https://www.bacchus.bg/vino/2017/05/17/2972972bulgarskite\\_sortove-nacionalna\\_cennost/](https://www.bacchus.bg/vino/2017/05/17/2972972bulgarskite_sortove-nacionalna_cennost/)).

The Kokorko white wine variety had also been among the forgotten old local varieties. It might be found rarely, mainly as single vines in old plantations and ampelographic collections. Since 2014 the variety had been included in the list of classified wine grape varieties, allowed for cultivation in all wine regions of Bulgaria ([http://bulgarianwines.org/wp-content/uploads/2019/07/1714101413544264\\_Spissak-Vinini-Sortove.pdf](http://bulgarianwines.org/wp-content/uploads/2019/07/1714101413544264_Spissak-Vinini-Sortove.pdf)). The variety origin had not been established, but it had been grown in Bulgaria since ancient times. It was widespread throughout the country, but was also found in some parts of Romania and Serbia. It had been known under many synonyms in the different regions (Getov et al., 2014).

Kokorko is a medium-ripening wine variety and the grapes matured in the second half of September. It has high fertility and medium yield. The vines are strongly growing. It is sensitive to mildew and powdery mildew and relatively resistant to low winter temperatures. The grapes are practically resistant to gray rot. The variety grows and has good yield grafted to Shasla x Berlandieri 41 B, Berlandieri x Riparia Cober 5 BB and Rupestris du Lot rootstocks. Guyot training is the most suitable for ground cultivation, however it also develops well in stem training, which significantly increases the yield (Getov et al., 2014; <https://www.vinograd.info/sorta/vinnye/kokorko.html>).



**Fig. 1. Kokorko white wine variety (bunch and leaf)**

In its mechanical composition of the grapes, Kokorko is a typical wine variety. The cluster is medium-sized, conical and compact, with one, two or more wings. The berry is spherical, small. The skin is thick, elastic, greenish-yellow with abundant waxy deposits, with small and sparsely located dark brown spots. The berry texture is juicy and the taste – harmonious. The grapes do not accumulate enough sugars (15.1–17.3%) and have low titratable acidity (4.42–5.52 g/l). It is used for making white dry wines with good chemical composition, harmonious taste and specific aroma (Getov et al., 2014; <https://www.vinograd.info/sorta/vinnye/kokorko.html>).

White wines' composition and specifics had been determined by numerous factors, the major one being the characteristics and potential of the variety, the region of cultivation, the applied agricultural procedures in the vineyards and the technological practices used in grape processing and vinification. The terroir, with its geographical and soil-climatic conditions, was decisive in terms of the main biochemical indicators of must and wine – the ratio of sugars, acids, mineral, phenolic and aromatic composition (Lanaridis et al., 2002; Badamtestseg et al., 2012; Ciu et al., 2012; Yabaci Karaoglan et al., 2015; Bora et al., 2016; Urcan et al., 2016; Drava and Minganti, 2019). The conditions of the alcoholic fermentation, the maceration before, or during fermentation, with, or without the use of enzymes, subsequent clarifying and stabilizing treatments, as well as the duration of storage formed the specific profile of white wines (Jagartić–Korenika et al., 2014; Stoica et al., 2015; Giriboni et al., 2016; Blagoeva et al., 2020; Perez-Navarro et al., 2020; Aragon-Garcia et al., 2021).

The wines' chemical composition had been determinant of their characteristics and authenticity. The balance between the content of the various components had specified their

style and quality. The composition of white wines differed significantly from that of rosé and red wines, mainly in terms of acidity, aromatic profile, polyphenols and the related antioxidant activity.

The acid content of wines was of particular significance for their preservation and sensory characteristics. The titratable acidity of white wines varied widely from 4 to 8 g/l, as the acid composition depended on the variety, the degree of grape ripeness, its phytosanitary status, etc. Over 40 organic acids had been found in white wines, in free and bound state and of various origins. Some of them originated from grapes (tartaric, malic, citric acid), while others were formed as intermediate or final products of the alcoholic fermentation (Rajkovic et al., 2007; Slegers et al., 2017).

Typical of white wines was the lower amount of phenolic components compared to red ones. The applied wine-making technology had determined their content. The total phenolic compounds in white wines ranged from 50 to 350 mg/l, with the predominant groups being hydroxycinnamic and hydroxybenzoic acids, flavonols and flavan-3-ols. They had been crucial for the organoleptic properties of wines in terms of properties and stability of color, bitterness and tartness (Gawel et al., 2014; Jagartić–Korenika et al., 2014; Yabaci Karaođlan et al., 2015; Badamtestseg et al., 2012; Perez-Navarro et al., 2020). Flavan-3-ols in white wines were mainly represented by catechin and epicatechin. Their higher rates were usually associated with more intensive extraction of grape tannins with prolonged maceration (Banc et al., 2020). Flavonols and their glycosides were also extracted during the maceration. Their main representative in white wines was quercetin, decisive for the color (Jagartić–Korenika et al., 2014; Urcan et al., 2016).

The lower content of polyphenols in white wines had also determined their lower antioxidant activity compared to the red ones. Upon contact with the solid parts during the maceration, their extraction from the grape skins was enhanced therefore the antioxidant properties of the obtained wines were increased (Fuhrman et al., 2001; Jagartić–Korenika et al., 2014). The antioxidant activity had been determined more by the ratio of the individual phenolic components than by the total content of phenolic compounds. The higher antioxidant activity in Romanian white wines was associated with higher rates of flavan-3-ols (Banc et al., 2020), while in Slovak white wines – with a higher concentration of hydroxycinnamic acids – caffeine, gallic, vanilla acid (Laštincova, 2019).

The volatile components had been especially important for the formation of the aromatic profile of white wines. Their representatives belonged to different groups – esters, aldehydes, higher alcohols, terpenes, norisoprenoids, fat-

ty acids, etc. They originated from grapes or were formed during the alcoholic fermentation and storage. The must maceration significantly increased their concentration, especially of terpenes. The ongoing biochemical reactions during the fermentation caused significant changes in the aromatic profile of wines due to the synthesis of a large number of new compounds as a result of yeast metabolism. Their content in different ratios had a positive or negative effect on the wine aroma (Lanaridis et al., 2002; Stoica et al., 2015; Urcan et al., 2016; Slegers et al., 2017; Marcon et al., 2019; Perez-Navarro et al., 2020).

White wines had specific organoleptic features determined by their chemical composition. The typical varietal aroma, with fruity notes and harmony in the taste components, had been highly appreciated.

The increasing competition on the wine market in recent years had encouraged the production of wines from local varieties with specific characteristics and strong regional authenticity. The preservation of these varieties would protect them from potential extinction. Studies on the composition and characteristics of white wines from the local Kokorko white wine variety had been scarce and outdated due to its limited distribution. The objective of this study was to make technological characteristic of white dry wines from the Kokorko variety, in terms of basic indicators of chemical composition, phenolic, volatile composition and aromatic profile, antioxidant activity and tasting evaluation.

## Material and Methods

The study was carried out at the Institute of Viticulture and Enology (IVE) – Pleven, Agricultural University (AU) – Plovdiv and the Yalovo Winery – Yalovo village, district of Veliko Tarnovo. The object of the study were white dry wines from two consecutive vintages (2019 and 2020), obtained from two growing regions.

### *Grapes and winemaking*

#### **AU – Plovdiv**

The experimental vineyards of AU – Plovdiv are located in the South Central Vine and Wine Growing Region (Thracian Lowland), in the area of the village of Brestnik, district of Plovdiv. The Kokorko variety belongs to the ampelographic collection of AU. The vines were planted at 2.80 m distance between the rows and 1.10 m inside the rows. They were grafted to SO4 rootstock and grown on bilateral cordon training. The grapes, from both vintages in the amount of 30 kg, were harvested at the appropriate technological maturity with the chemical composition presented in Table 1. It was processed under the classical technology for dry white

wine making under the conditions of micro-vinification (Yankov, 1992) – crushing, straining off, pressing, sulfitation (60 mg/l SO<sub>2</sub>), must clarification with enzyme Novocclair speed (1 g/hl) of Novozymes, adding pure culture dry wine yeast Exellance FTH of Lamote-Abiet in the amount of 20 g/hl, fermentation temperature 17°C, decanting, further sulfitation, storage.

### The Yalovo Winery

The vineyard, from which the grapes were picked, is located in the South Vine and Wine Growing Region (Thracian Lowland), on the land of the village of Kamenovo, municipality of Nova Zagora. The main variety in the vineyard is Misket Cherven (Muscat Red), with some other varieties grown there. The planting distance of the vines is 3.00 m between the rows and 1.20 m inside the rows. The vines were grafted to SO4 rootstock and grown on the Umbrella training. In the vineyard, vines from the studied Kokorko variety were found as impurities and they were identified by classical ampelographic observation. Their grape was picked separately. The harvest was carried out in technological maturity, in the amount of the sample 30 kg. The chemical composition of the must was presented in Table 1. The grapes were processed in the Yalovo Winery according to the classical technology for dry white wine making under the conditions of micro-vinification (Yankov, 1992) – crushing, straining off, pressing, sulfitation (35 mg/l SO<sub>2</sub>), must clarification with enzyme Novocclair speed (1 g/hl) of Novozymes, adding pure culture dry wine yeast Melody of Ch. Hansen in the amount of 15 g/hl, fermentation temperature 16–20°C, decanting, further sulfitation, storage.

The grape chemical composition was determined according to the following methods (Ivanov et al., 1979): sugars, % – hydrometer of Dujardin; titratable acids (TA), g/l – titration with NaOH; pH – pH-meter; glucoacidimetric index (GAI) – calculation method as the ratio of sugars (%) and TA (g/l).

### • Laboratory analyses

The laboratory experimental work was performed in the laboratories of “Wine Technology” and “Gas Chromatography” of IVE – Pleven. The chemical and phenolic composition of wines, their antioxidant activity, their volatile and aromatic profile had been analyzed.

### ➤ Chemical composition

The main indicators of the wine chemical composition were analyzed by conventional methods in the wine-making practice (Ivanov et al., 1979): alcohol, vol. % – distillation method, Gibertini apparatus with densitometry, by the distillate density; sugars, g/l – Schoorl’s method; total extract (TE) g/l - Gibertini apparatus with densitometry, by the alcohol-free sample density; sugar-free extract (SFE), g/l – calculation method (the difference between TE and sugars); titratable acids, g/l – titration with NaOH; volatile acids, g/l – distillation method with subsequent titration with NaOH; pH – pH-meter.

### ➤ Phenolic composition and color characteristics

The content of total phenolic compounds (TPC), flavonoid phenolic compounds (FPC), non-flavonoid phenolic compounds (NPC) and the color characteristics were determined in the studied wines by the following methods (Chobanova, 2007): TPC, g/l – Singleton et Rossi method with Folin – Chicalteu reagent, FPC, mg/l catechin equivalent – Somers method, NPC, mg/l coffee equivalent – Somers method, color intensity I [abs. units] – Somers method.

### • Antioxidant activity

The antioxidant activity was determined according to the method of Wang et al. (1996) as antiradical activity against a stable product DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Germany). The wine samples were diluted to a total extract of 600.00 mg/l and the analysis was carried out on the samples thus diluted, respectively marked as TE = 600.00 mg/l.

### • Volatile composition

Gas chromatographic determination of the volatile components in wines (by the direct injection of wine distillate) was done. The content of major volatile compounds was determined on the basis of stock standard solution prepared in accordance with the IS method 3752:2005. The method describes the preparation of standard solution with one congener, but the step of preparation was followed for the preparation of a solution with more compounds. The standard solution in this study included the compounds with purity > 99.0%. The 2 µl of prepared standard solution was injected

**Table 1. Chemical composition of the grapes of Kokorko variety (data of the winemakers)**

| Sample  | Winemaker     | Vintage | Date of harvest | Sugars, % | Titratable acids, g/l | pH   | GAI  |
|---------|---------------|---------|-----------------|-----------|-----------------------|------|------|
| Kokorko | Yalovo Winery | 2019    | 20.09.2019      | 20.40     | 4.70                  | 3.45 | 4.34 |
| Kokorko | AU – Plovdiv  | 2019    | 03.10.2019      | 21.20     | 4.90                  | 3.50 | 4.32 |
| Kokorko | AU – Plovdiv  | 2020    | 15.10.2020      | 23.90     | 4.30                  | 3.68 | 5.56 |

in a gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF = 0.25  $\mu$ m), equipped with a flame ionization detector (FID). The used carrier gas was He. The hydrogen to support combustion was supplied to the chromatograph via a hydrogen bottle. The injection was manually by microsyringe.

The parameters of the gas chromatographic determination were: injector temperature – 220°C; detector temperature – 250°C, initial oven temperature – 35°C/retention 1 min, rise to 55°C with step of 2°C/min for 11 min, rise to 230°C with step of 15°C/min for 3 min. Total time of chromatography analysis – 25.67 min. The identified retention times of the compounds in the standard solution were: acetaldehyde (3.141), ethyl acetate (3.758), methanol (3.871), 2-propanol (5.170), isopropyl acetate (5.975), 1-propanol (6.568), 2-butanol (7.731), propyl acetate (9.403), 2-methyl-propanol (10.970), 1-butanol (11.509), isobutyl acetate (11.662), ethyl butyrate (12.710), butyl acetate (12.752), 2-methyl-1-butanol (13.054), 4-methyl-2-pentanol (13.629), 3-methyl-1-butanol (13.840), 1-pentanol (15.180), isopentyl acetate (15.965), pentyl acetate (16.033), 1-hexanol (16.276), ethyl hexanoate (16.376), hexyl acetate (16.510), 1-heptanol (16.596), linalool oxide (16.684), phenyl acetate (18.055), ethyl caprylate (18.625),  $\alpha$ -terpineol (19.066), 2-phenyl ethanol (19.369), nerol (19.694),  $\beta$ -citronellol (19.743), geraniol (19.831), ethyl decanoate (19.904). As an internal standard octanol was used.

After determination of the retention times of the compounds in the standard solution the identification and quantification of the volatile substances in the wine distillates was done. The volatile composition was determined based on the direct injection. The prepared samples were injected in an amount of 2  $\mu$ l in a gas chromatograph and was carried out an identification and quantification of the volatile substances in each of them.

#### • Organoleptic profile

The tasting characteristics of the studied wines were determined by a 5-member tasting commission by the method of the main features (Prodanova, 2008) for the indicators color (intensity, tint), aroma (intensity, harmony), taste (harmony, body, freshness) and aftertaste. A scale from 0 to 10 was used to quantify the indicators, corresponding to weak (0) – medium (5) – strong (10) manifestation.

#### • Statistical processing

Statistical data processing was performed, including determination of standard deviation ( $\pm$  SD), with three repetitions for each analysis. The determination of the indicator

was implemented by Excel 2007 from the Microsoft Office package.

## Results and Discussion

### *General chemical composition of the studied wines*

For the objective of the study, a comparative analysis of the chemical composition main indicators of the Kokorko variety white wines was made (Table 2).

The alcohol content of the analyzed wines was in correlation with the sugars found in the grape must (Table 1). They were mainly due to the impact of the weather conditions during the year and the terroir, with its soil and climatic characteristics, in the growing area (Bora et al., 2016; Slegers et al., 2017). The wine from the Yalovo Winery had the lowest sugar accumulation and the lowest alcohol ratio, respectively (12.22 $\pm$ 0.06 vol. %). In the rest of the samples from Plovdiv region, the sugar content in the grapes and respectively the alcohol concentration in the wines was significantly higher – 12.77 $\pm$ 0.04 vol.% (2019) and 13.81 $\pm$ 0.05 vol.% (2020).

The proper and complete course of the alcoholic fermentation had been decisive for the wines' composition and characteristics (Stoica et al., 2015; Perez-Navarro et al., 2020). The ratio of residual sugars in the wine was a proof for the occurrence of the process without deviations from the normal course. Their content in the experimental samples varied from 1.17 $\pm$ 0.14 to 1.97 $\pm$ 0.19 g/l, determining them as dry (Chobanova, 2012). Sugars ratio in wines within the range of 2-5 g/l defined them as smoother in taste (Bora et al., 2016).

An important indicator of the white wines composition was their extract content, being a set of their non-volatile components and depended on the variety specifics, the growing region, the method of vinification (Bora et al., 2016). The amount of total and sugar-free extract of the samples increased in the order Kokorko, 2019 (Yalovo Winery) < Kokorko, 2020 (AU-Plovdiv) < Kokorko, 2019 (AU-Plovdiv). The results showed that the wines made in AU-Plovdiv were more extractive compared to the sample from the Yalovo Winery. The reason was due to the impact of the terroir. The extract content of the experimental wines was within the specific limits indicated for the studied variety by Getov et al. (2014), and were respectively from 18.60 $\pm$ 0.20 to 19.20 $\pm$ 0.17 g/l (TE) and from 16.63 $\pm$ 0.32 to 17.72 $\pm$ 0.18 g/l (SFE).

The titratable acidity of white wines varied widely and was determined by a number of factors, the main of which being the variety and the degree of the grapes ripeness (Slegers et al., 2017). Generally, the wines' acidity was lower than that of must (Rajkovic et al., 2007), and that was the trend also in the present study (Table 1 and Table 2). Bora et

**Table 2. Chemical composition of the studied Kokorko wines**

| Indicators \ Wine       | Kokorko<br>Yalovo Winery | Kokorko<br>AU – Plovdiv | Kokorko<br>AU – Plovdiv |
|-------------------------|--------------------------|-------------------------|-------------------------|
| Vintage                 | 2019                     | 2019                    | 2020                    |
| Alcohol, vol. %         | 12.22±0.06               | 12.77±0.04              | 13.81±0.05              |
| Sugars, g/l             | 1.97±0.19                | 1.48±0.06               | 1.17±0.14               |
| Total extract, g/l      | 18.60±0.20               | 19.20±0.17              | 18.80±0.20              |
| Sugar-free extract, g/l | 16.63±0.32               | 17.72±0.18              | 17.63±0.21              |
| Titrateable acids, g/l  | 4.45±0.18                | 4.63±0.15               | 4.10±0.10               |
| Volatile acids, g/l     | 0.44±0.03                | 0.46±0.03               | 0.32±0.03               |
| pH                      | 3.39±0                   | 3.24±0                  | 3.13±0.01               |

al. (2016) found the lowest total acidity in Romanian white wines of Muscat Ottonel and Feteasca Alba (4.00±0.10 g/l), which they associated with the flat taste.

Typical for the Kokorko variety was also the low rate of titrateable acids in the grapes and respectively in the wines (Getov et al., 2014). That affected the taste indicators in their organoleptic analysis and defined them as not enough fresh. The data presented in Table 2 had confirmed these findings and did not show a significant difference in the titrateable acidity of the three samples. There was no clear distinction regarding the growing region. The indicator varied in the range from 4.10±0.10 (2020) to 4.63±0.15 g/l (2019), as the samples from the Plovdiv region had the lowest and the highest rate. Probably in this case the influence of the meteorological conditions of the year was more significant.

The experimental wines had normal volatile acidity (Chobanova, 2012; Perez-Navarro et al., 2020) and pH values (Table 2). The wine titrateable acidity and the related pH indicator were of particular importance in the wine-making process and ensuring its stability – microbiological, protein, oxidase (Rajkovic et al., 2007).

#### **Phenolic composition and antioxidant activity of the studied wines**

The data on the phenolic composition of the studied wines of the Kokorko variety were presented in Table 3. The total phenolic compounds, the flavonoid and non-flavonoid phenolic compounds, as well as the color intensity in the three analyzed samples were determined.

The TPC rates in the experimental wines were close. Their content was higher in the samples from AU – Plovdiv – 2019 vintage (0.42±0.03 g/l), followed by 2020 vintage (0.40±0.02 g/l), and the difference between them was very small. The amount of TPC in the wine from the Yalovo Winery was the smallest (0.37±0.02 g/l).

The results demonstrated that the differences in the TPC rates between the studied wines were not significant. The data obtained in this study correlated with the studies of Shadidi & Nazck (1995) and Li et al. (2009), who had found the total content of phenolic compounds in white wines in quantities from 50.00 – 2000.00 mg/l and from 189.00 – 495.00 mg/l.

Regarding the concentration of FPC in the studied wines, significant differences were observed compared to TPC. However, the tendency of higher presence of FPC in the wines of AU – Plovdiv had been preserved. The sample from the 2019 harvest contained 186.82±10.49 mg/l, and from the 2020 harvest – 172.91±11.64 mg/l. The lowest was the amount of FPC in the wine from the Yalovo Winery (150.48±11.34 mg/l).

The trend in the amount of NPC was absolutely identical to that for TPC and FPC, namely: Kokorko, 2019 (AU – Plovdiv) > Kokorko, 2020 (AU – Plovdiv) > Kokorko, 2019 (Yalovo Winery). The differences in the content of the studied component were clear, as the sample from AU – Plovdiv, 2019 harvest dominated quantitatively over the other variants with a rate of NPC of 71.17±3.58 mg/l. The wine from the 2020 harvest contained more NPC (57.67±10.56 mg/l).

**Table 3. Phenolic composition and color intensity of the studied Kokorko wines**

| Indicators \ Wine                            | Kokorko<br>Yalovo Winery | Kokorko<br>AU – Plovdiv | Kokorko<br>AU – Plovdiv |
|--|--------------------------|-------------------------|-------------------------|
| Vintage                                      | 2019                     | 2019                    | 2020                    |
| TPC, g/l gallic acid                         | 0.37±0.02                | 0.42±0.03               | 0.40±0.02               |
| NPC, mg/l caffeic equivalent                 | 41.84±5.43               | 71.17±3.58              | 57.67±10.56             |
| FPC, mg/l catechin equivalent                | 150.48±11.34             | 186.82±10.49            | 172.91±11.64            |
| Colour intensity IC <sup>a</sup> [abs. unit] | 0.183±0.02               | 0.165±0.02              | 0.147±0.01              |

than the wine from Yalovo Winery ( $41.84 \pm 5.43$  mg/l), where they were in the lowest content.

Data on the antioxidant activity of the studied wines were presented in Figure 2.

The antioxidant activity was in direct correlation with the phenolic composition of the wines. The results revealed that both samples from AU – Plovdiv (2019 and 2020) had almost similar antioxidant activity, eliminating almost identical amounts of DPPH radicals. At 5 minutes from the reaction in the wine from the 2019 harvest,  $51.61 \pm 0.04\%$  elimination of free radicals was registered, while in the sample from the 2020 harvest, the elimination was  $51.52 \pm 0.01\%$ . The 15 min reaction situation was similar. The wine from the 2019 harvest showed  $52.00 \pm 0.30\%$  elimination of DPPH, while from the 2020 harvest it had antioxidant activity of  $52.48 \pm 0.01\%$ .

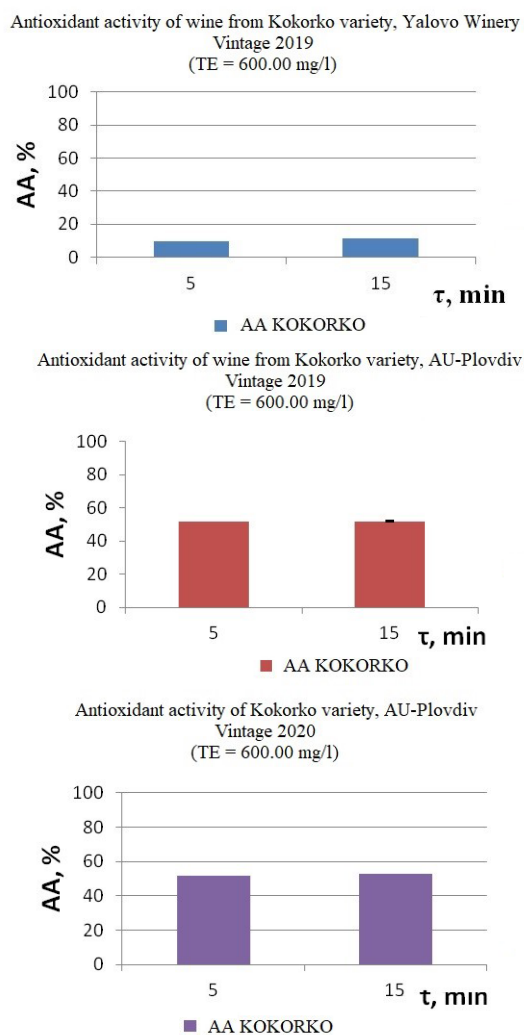


Fig. 2. Antioxidant activity of the studied Kokorko wines

The lowest antioxidant activity, significantly lower compared to the other two samples, was found in the sample from the Yalovo Winery. At 5 min of the reaction it was only  $9.79 \pm 0.10\%$ , at 15 min a slight increase was observed reaching  $11.60 \pm 0.01\%$  elimination of DPPH radicals.

There was a clear correlation between the content of the groups of phenolic compounds in wines and their antioxidant activity, namely – the samples from AU – Plovdiv, 2019 and 2020 harvests, showed higher rate of TPC, FPC, NPC, compared to the wine from Yalovo Winery, 2019 harvest. That directly reflected the higher antioxidant activity found in them.

The data obtained in the present study had been in full correlation with the findings in other studies (Katalinić et al., 2004; Marković et al., 2015), who established the antioxidant activity of white wines in the range from 10.30% to 70.20%.

#### Content of the volatile components in the studied wines

The data on the volatile compounds in the analyzed white wines were presented in Table 4.

Seventeen volatile compounds from different groups – esters, higher alcohols, aldehydes and terpenes had been identified (by GC-FID). One aldehyde, 4 higher alcohols, 8 esters, 3 terpenes and methyl alcohol were found as species composition.

Regarding the total amount of volatile compounds, significant differences were observed between the three analyzed samples. The highest rate ( $813.89 \pm 130.28$  mg/l) was found in the white wine from the Yalovo Winery. The difference between the other two samples for the indicator “total volatile composition” was smaller, but the one from the 2019 harvest prevailed, where a total amount of volatile compounds of  $662.62 \pm 105.75$  mg/l was found. The total content of volatile compounds in wine, 2020 vintage was the lowest –  $591.14 \pm 115.82$  mg/l.

The total content of higher alcohols was the highest ( $331.55 \pm 67.68$  mg/l) also in the sample from AU – Plovdiv, 2020 harvest. The other two wines showed a small difference for this indicator. The higher alcohols content ( $248.57 \pm 29.92$  mg/l) in the sample from AU – Plovdiv, 2019 harvest, was slightly higher than that from the Yalovo Winery, 2019 harvest ( $234.48 \pm 57.70$  mg/l). According to Chobanova (2012), the content of higher alcohols in white wines varied from 150.00 – 400.00 mg/l. The data in the present study fully correlated with this range.

Of the four higher alcohols identified, two were found in all analyzed samples – 2-methyl-1-butanol (active amyl alcohol) and 3-methyl-1-butanol (isoamyl alcohol).

3-methyl-1-butanol had been a higher alcohol identified in the highest concentration from the higher alcohols group

**Table 4. Volatile compounds in the studied Kokorko wines**

| Compounds , mg/l      | Wine | Kokorko<br>Yalovo Winery | Kokorko<br>AU – Plovdiv | Kokorko<br>AU – Plovdiv |
|-----------------------|------|--------------------------|-------------------------|-------------------------|
| Vintage               |      | 2019                     | 2019                    | 2020                    |
| Acetaldehyde          |      | 57.84±36.49              | 25.87±5.46              | 42.76±5.04              |
| Methanol              |      | 99.67±19.61              | 97.16±8.96              | 111.02±0.76             |
| 2-methyl-1-butanol    |      | 20.23±8.24               | 46.03±11.07             | 64.69±37.21             |
| 3-methyl-1-butanol    |      | 75.57±26.34              | 166.46±10.74            | 259.35±30.47            |
| 1-propanol            |      | 138.68±23.12             | ND                      | 7.51±0.00               |
| 1-butanol             |      | ND                       | 36.08±8.11              | ND                      |
| Total higher alcohols |      | 234.48±57.70             | 248.57±29.92            | 331.55±67.68            |
| Ethyl acetate         |      | 41.06±14.11              | 14.31±4.50              | 30.93±17.56             |
| Pentyl acetate        |      | ND                       | ND                      | 13.51±1.50              |
| Isopentyl acetate     |      | 379.52±2.12              | 74.99±3.72              | 8.18±2.34               |
| Propyl acetate        |      | ND                       | 22.73±13.08             | 23.44±11.34             |
| Isopropyl acetate     |      | ND                       | 8.25±2.75               | ND                      |
| Butyl acetate         |      | ND                       | ND                      | 12.83±6.08              |
| Isobutyl acetate      |      | ND                       | ND                      | 16.06±3.21              |
| Phenylacetate         |      | ND                       | 170.32±37.28            | ND                      |
| Total esters          |      | 420.58±16.23             | 290.60±61.33            | 104.95±42.03            |
| $\alpha$ -terpineol   |      | ND                       | ND                      | 0.37±0.16               |
| Nerol                 |      | ND                       | 0.42±0.08               | ND                      |
| Geraniol              |      | 0.62±0.25                | ND                      | 0.49±0.15               |
| Total terpenes        |      | 0.62±0.25                | 0.42±0.08               | 0.86±0.31               |
| Total quantity        |      | 813.19±130.28            | 662.62±105.75           | 591.14±115.82           |

\*ND – not detected

in two of the the studied wines (AU – Plovdiv, 2020 and AU – Plovdiv, 2019). Simultaneously, there were significant differences in its content in the three analyzed wine samples. Its quantity (259.35±30.47 mg/l) was dominant in the sample from AU – Plovdiv, 2020 harvest. Kokorko wine from AU – Plovdiv, 2019 harvest, showed smaller quantity of this higher alcohol (166.46±10.47 mg/l). The smallest content of this higher alcohol (75.57±26.34 mg/l) was identified in Yalovo Winery, 2019 sample – only in this sample it was not quantitative dominated higher alcohol. According to Chobanova (2012), this alcohol was present in wines in an average amount of about 200.00 – 500.00 mg/l. The data in the present study were in correlation with this range. The researchers (Šehović et al., 2007) defined 3-methyl-1-butanol as a quantitatively dominant alcohol, accounting for 40-70% of the total amount of higher alcohols. Our data for 3-methyl-1-butanol were also in line with the results of Gil et al. (2006), who found it in the rate of 200.00 mg/l in Spanish young white wines. It was supposed that the aroma given to wine by this compound was of malt, whisky and fruit (Francis & Newton, 2005; Gil et al., 2006; Selli et al., 2004).

In the content of 2-methyl-1-butanol, an identical trend was observed with regard to the found amounts of 3-meth-

yl-1-butanol. Respectively, its rate was the highest in the wines from AU – Plovdiv – 2020 harvest (64.69±37.21 mg/l), followed by 2019 harvest (46.03±11.07 mg/l), and the lowest identified rate was in the sample from the Yalovo Winery (20.23±8.24 mg/l). According to Chobanova (2012), the amount of this alcohol in wines was about 36.00 mg/l on the average. In the present study, the average ratio of 2-methyl-1-butanol for the three white wines was 43.65 mg/l, which was slightly higher than the findings of the above author. 2-methyl-1-butanol had been identified as a major representative of the higher alcohols in sparkling wines from Croatia and Brazil (Jagartić-Korenika et al., 2020; Verzeletti et al., 2016).

1-propanol was identified in two of the experimental samples. It was absent in the Kokorko white wine, 2019 vintage from AU – Plovdiv. It was found in higher content in the wine from Yalovo Winery (138.68±23.12 mg/l). It is noteworthy that in this sample its amount was higher than that of 3-methyl-1-butanol, which usually dominates. 1-propanol is also a yeast metabolite. The higher concentration of 1-propanol compared to 3-methyl-1-butanol found in the present study (at the sample Yalovo Winery) correlated with the data of Lorenzo et al. (2015) who investigated the vola-



tile composition of wines obtained from conventionally and organically grown grapes of the Monastrel variety. In wine obtained from organically grown grapes they found a higher amount of 1-propanol ( $110.88 \text{ mg/dm}^3$ ) compared to 3-methyl-1-butanol ( $92.79 \text{ mg/dm}^3$ ) – a trend identical to our study. In the sample from AU – Plovdiv, 2020 vintage, its amount was lower ( $7.51 \pm 0.00 \text{ mg/l}$ ). Lee et al. (2022) found a concentration of 1-propanol in wild grape wines in the range  $23.61 - 84.23 \text{ mg/l}$ . Muñoz-Gonzalez et al. (2011) had defined this higher alcohol as the main representative of the faction in the study of 25 mono-variety white wines (Xarel.lo variety) from different vintages in the region of Catalonia, Spain. Its content in the studied wines was within the range of  $22.78 - 57.93 \text{ mg/l}$ . The quantitative variation of 1-propanol, estimated in wines from different countries, ranged from  $11.00$  to  $125.00 \text{ mg/l}$  (IARC, 1987). The content of 1-propanol found in the present study had a normal range of quantitative availability, according to data from other studies, too.

The higher alcohols (2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol, 1-butanol) were yeast metabolites and their content in the wine was mainly determined by the fermentation conditions and the yeast strain used. A precursor for the formation of higher alcohols in wines are the amino acids of the grapes (Chobanova, 2012). This can be perceived as a specific varietal characteristic also having an impact on the final level of higher alcohols in the wine.

The aldehyde fraction was represented by acetaldehyde. It was found in the highest amount ( $57.84 \pm 36.49 \text{ mg/l}$ ) in the Kokorko wine, 2019 vintage, from the Yalovo Winery. The sample from AU – Plovdiv, 2020 harvest, had a higher content ( $42.76 \pm 5.04 \text{ mg/l}$ ) compared to that from 2019 harvest ( $25.87 \pm 5.46 \text{ mg/l}$ ). The acetaldehyde had been the main representative of this fraction, with concentrations in wines varied from  $10.00 - 200.00 \text{ mg/l}$ , which was 90% of the total aldehyde content (Chobanova, 2012).

The total ester content also revealed differences between the studied wines. The highest total content of esters ( $420.58 \pm 16.23 \text{ mg/l}$ ) was found in the sample from the Yalovo Winery. There was also a difference between the other two wines, with a higher concentration of esters ( $290.60 \pm 61.33 \text{ mg/l}$ ) in the sample, 2019 harvest, compared to that of 2020 ( $104.95 \pm 42.03 \text{ mg/l}$ ).

The esters identified actually in all studied wines were ethyl acetate and isopentyl acetate. Ethyl acetate was found in the highest concentration ( $41.06 \pm 14.11 \text{ mg/l}$ ) in the Kokorko wine from the Yalovo Winery, 2019 vintage, followed by the sample from AU – Plovdiv, 2020 vintage ( $30.93 \pm 17.56 \text{ mg/l}$ ), and its content ( $14.31 \pm 4.50 \text{ mg/l}$ ) was the lowest in the wine from AU – Plovdiv, 2019 harvest. According to Chobanova (2012) the ester rarely exceeded

$50.00 - 80.00 \text{ mg/l}$  in young wines. In these ratios it had a positive effect on the aroma. In the study of young red wines from four traditional varieties for the region of northwestern Spain, this ester was found in a concentration ranging from  $23.48 - 78.36 \text{ mg/l}$  (Cortes-Dieguez et al., 2015). The data in our research correlated with the results discussed in other studies regarding the normal quantitative presence of ethyl acetate.

Isopentyl acetate revealed the highest content ( $379.52 \pm 2.12 \text{ mg/l}$ ) in the wine from the Yalovo Winery. It was significantly higher compared to the other two wines. From them, isopentyl acetate was higher in the sample from 2019 harvest ( $74.99 \pm 3.72 \text{ mg/l}$ ) than from 2020 harvest ( $8.18 \pm 2.34 \text{ mg/l}$ ). The ester was essential for wines and imparts a banana aroma (Carpena et al., 2020). The higher levels of isopentyl acetate found in the Yalovo Winery wine were probably due to the mixed yeast culture used for vinification.

Propyl acetate was identified in the experimental wines from the two vintages from AU – Plovdiv. This compound concentration found in these samples were very close,  $22.73 \pm 13.08 \text{ mg/l}$  and  $23.44 \pm 11.34 \text{ mg/l}$ , respectively.

Other identified esters were isopropyl acetate, found only in the Kokorko wine, 2019 vintage from AU – Plovdiv ( $8.25 \pm 2.75 \text{ mg/l}$ ), butyl acetate and isobutyl acetate, found only in the 2020 sample, at concentrations of  $12.83 \pm 6.08 \text{ mg/l}$  and  $16.06 \pm 3.21 \text{ mg/l}$  respectively, phenylacetate, found only in the Kokorko wine, 2019 harvest from AU – Plovdiv ( $170.32 \pm 37.28 \text{ mg/l}$ ). The higher levels of phenylacetate found could be explained by the yeast culture used.

Terpenes, as metabolites of the vine plant with a very low threshold of aromatic perception and passing into the wine during the fermentation, had been also a significant component of the volatile aromatic composition, especially in Muscat varieties (Velkov, 1996). In wines from these varieties, the total amount of terpenes had been about  $2.00 \text{ mg/l}$  on the average (Chobanova, 2012). In our study, the highest total content of terpenes ( $0.86 \pm 0.31 \text{ mg/l}$ ) was found in the sample, 2020 vintage. The other two wines (2019 harvest) revealed lower amount of total terpenes, but the sample from the Yalovo Winery ( $0.62 \pm 0.25 \text{ mg/l}$ ) dominated over the sample from AU – Plovdiv ( $0.42 \pm 0.08 \text{ mg/l}$ ).

Three terpene alcohols were identified in the studied experimental wines.  $\alpha$ -terpineol was identified only in Kokorko, 2020 harvest, at a concentration of  $0.37 \pm 0.16 \text{ mg/l}$ . This terpene imparted a floral aroma to wines (Baron et al., 2017). Nerol was identified only in the sample from AU – Plovdiv, 2019 harvest, as its amount was  $0.42 \pm 0.08 \text{ mg/l}$ . The third terpene – geraniol was found in two of the experimental wines – Kokorko, 2019 vintage, from the Yalovo

Winery and Kokorko, 2020 vintage, from AU – Plovdiv, in content of  $0.62 \pm 0.25$  mg/l and  $0.49 \pm 0.15$  mg/l, respectively. That terpene was available in the largest amount compared to the other two. Geraniol and nerol (giving rose aroma) were normally present in wines in concentrations of  $0.323 - 1.060$  mg/l and  $0.014 - 0.45$  mg/l, respectively (Chobanova, 2012). The data in our study were in full correlation with this range.

Methyl alcohol, a normal component of the wine volatile fraction, had also been identified in wines. Its concentrations in Kokorko wines, 2019 vintage, from Yalovo Winery and AU – Plovdiv, were very close,  $99.67 \pm 19.61$  mg/l and  $97.16 \pm 8.96$  mg/l, respectively. At a higher concentration ( $111.02 \pm 0.76$  mg/l) this compound was found in the sample, 2020 vintage. According to OIV, the ratio of methanol in white wines should not exceed 250.00 mg/l (OIV, 2015). The normal methyl alcohol rate of Australian white wines had been found to be from 40.00 to 120.00 mg/l (Hodson et al., 2017). That component had also been found in Turkish white wines in the range from 30.50 to 121.40 mg/l (Cabaroğlu, 2005).

The data in our study had been in absolute correlation with the cited researches as well as the OIV-defined requirements for the normal safe presence of methyl alcohol in white wines.

### Organoleptic profile of the studied wines

The wines' chemical composition determined their organoleptic features. The content of extract, titratable acids, phenolic and aromatic compounds had been decisive for their aromatic and taste perceptions (Yabaci Karaoğlu et al., 2015; Marcon et al., 2019; Perez-Navaro et al., 2020; Aragon-Garcia et al., 2021).

The specified chemical composition of the studied Kokorko white wines, comprising the main indicators, the rates of phenolic and aromatic components, had been directly reflected in their tasting qualities. The organoleptic analysis results of the three samples were presented in Figure 3.

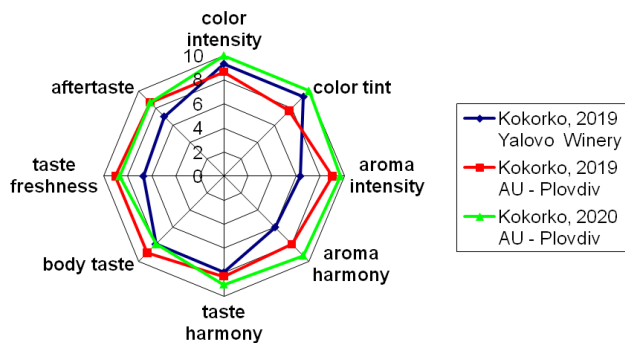


Fig. 3. Organoleptic profile of the studied Kokorko wines

In terms of colour indicators, Kokorko wine, vintage 2020 was determined to have the best intensity and tint. The color was characterized as straw-yellow with greenish hues. That was confirmed by the value of the colour intensity, which was the lowest of the three tested samples –  $0.147 \pm 0.01$  [abs. units] (Table 3). Despite the analyzed higher value of intensity ( $0.183 \pm 0.02$  [abs. units]), the wine from the Yalovo Winery showed better color characteristics compared to the wine from the same vintage (2019), but made in AU – Plovdiv (Table 3, Figure 3).

The aroma of the studied wines was dominated by floral notes with a subtle Muscat nuance. In terms of intensity and harmony in the aroma, the two samples from AU – Plovdiv were superior to the one from Yalovo Winery (Figure 3). The indicators in the wine from the 2020 harvest were more pronounced, despite the lowest rates found of esters and common aromatic components, but the highest one of higher alcohols and terpenes (Table 4).

The samples were evaluated in taste for their harmony, density, freshness and aftertaste. According to these indicators, the wines made in AU – Plovdiv were again superior compared to those from the Yalovo Winery. That was due to the higher rate of total and sugar-free extract found in them (Table 2), TPC, FPC and NPC (Table 3). The sample from the 2020 harvest was distinguished for its better harmony and balance in taste, but was inferior in density and freshness compared to that from the 2019 harvest. These wines also had significantly fuller and longer lasting aftertaste than the wine from the Yalovo Winery (Figure 3).

### Conclusion

From the obtained results of the presented study, it could be concluded the next.

Kokorko wines (2019 and 2020), from AU – Plovdiv, had higher alcohol rate than Kokorko wines (2019), from Yalovo Winery, due to the higher sugar content in the grapes.

The results showed that the wines made in AU – Plovdiv were more extractive. The amount of total and sugar-free extract of the samples increased in the order Kokorko, 2019 (Yalovo Winery) < Kokorko, 2020 (AU – Plovdiv) < Kokorko, 2019 (AU-Plovdiv).

The specific feature of the Kokorko variety with regard to the low content of titratable acids in grapes and wines, established by other researches, had been confirmed. No significant difference was found in the titratable acidity of the three samples. This indicator varied within the range from  $4.10 \pm 0.10$  to  $4.63 \pm 0.15$  g/l, as the samples from the Plovdiv region revealed the lowest and the highest value.

The defined phenolic composition had been typical for white wines. A correlation was found between the groups of analyzed phenolic compounds (TPC, FPC, NPC). Their content increased in the order: Kokorko, 2019 (Yalovo Winery) < Kokorko, 2020 (AU – Plovdiv) < Kokorko, 2019 (AU – Plovdiv).

There had been a clear correlation between the ratio of the groups of phenolic compounds in wines and their antioxidant activity. The samples from AU – Plovdiv, 2019 and 2020 vintage, showed higher antioxidant activity compared to that of the Yalovo Winery.

A diverse volatile composition had been identified in the studied wines, consisting of 17 volatile compounds (1 aldehyde, 4 higher alcohols, 8 esters, 3 terpenes and methyl alcohol).

The wine from the Yalovo Winery was distinguished with the highest total volatile content (813.89±130.28 mg/l), and the sample, 2020 harvest, with the lowest (591.14±115.82 mg/l).

The highest total content of higher alcohols was found in Kokorko, 2020 vintage (331.55±67.68 mg/l), and the lowest in the wine from the Yalovo Winery (234.48±57.70 mg/l).

The main identified representatives of higher alcohols were 2-methyl-1-butanol, 3-methyl-1-butanol and 1-propanol.

Acetaldehyde was found from the aldehyde fraction. Its concentrations varied from 25.87±5.46 mg/l (Kokorko, 2019, AU – Plovdiv) to 57.84±36.49 mg/l (Kokorko, 2019, Yalovo Winery) and were with the normal range for white wines.

The highest total amount of esters (420.58±16.23 mg/l) was found in the wine from the Yalovo Winery. The lowest rate (104.95±42.03 mg/l) was established in Kokorko, 2020 vintage.

The main representatives of esters (identified in all examined wines) were isopentyl acetate and ethyl acetate. Other esters identified were pentyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, and phenyl acetate.

Three terpenes were identified in the studied wines -  $\alpha$ -terpineol, nerol and geraniol, the latter having the highest concentrations. The total terpene content in the studied wines ranged from 0.42±0.08 mg/l (Kokorko, 2019, AU – Plovdiv) to 0.86±0.31 mg/l (Kokorko, 2020, AU – Plovdiv).

Methyl alcohol was found in all studied wines, ranging from 97.16±8.96 mg/l (Kokorko, 2019, AU – Plovdiv) to 111.02±0.76 mg/l (Kokorko, 2020, AU – Plovdiv). Its presence was in normal concentrations meeting the drink safety criteria.

Kokorko wines, made in AU – Plovdiv, had better organ-

oleptic qualities in terms of aromatic and taste characteristics compared to the wine from the Yalovo Winery.

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