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E-mail: leeva@mail.bg

Competitive level and trends for development of the vine and wine sector in Bulgaria

DesislavaToteva

Institute of Agricultural Economics, 1113 Sofia, Bulgaria

SUMMARY

The aim of this report is to establish a competitive level and trends for the future development of the vine and wine sector in Bulgaria. After the accession of Bulgaria to the EU in 2007 Bulgaria's vine and wine sector develops in a highly competitive environment where exist mechanisms and tools of the CAP related to quality requirements and certain standard. Vine and wine sector is export oriented sector of agriculture which is highly dependent on trends in global wine trade. This requires market researches about the demand of wines, varietal structure of vineyards, cultivated area and production of grapes and wines.

Although Bulgaria has a competitive advantages in terms of favourable weather conditions for the development of wine viticulture over the past 10 years the production of wine grapes and wine decrease, which reveals reduced competitiveness of the sector. To establish a competitive level and a place of Bulgarian vine and wine sector in the structure of world trade is used Balassa index.

In the report is also made an analysis of the vine and wine sector after the accession of Bulgaria to the EU, conclusions and recommendations for development in the future are made.

Key words: competitiveness, wine viticulture, wine, trends, Bulgaria

INTRODUCTION

In the context of European integration, the wine sector in Bulgaria received both opportunities (access to the common European market, modernization, concentration and consolidation) and challenges (increased competitive pressure on the domestic market and loss of traditional markets in some European countries). The main factors affecting on the production of grapes and wine are many and interconnected. The wine sector is dependent on the natural conditions which determine the regional features of its specialization. The relief, soils, climate and the waters are most important. Other factors influencing the competitive level of viticulture and wine production are: demand, supply, prices, infrastructure, state policy, the Common Agricultural Policy of EU and others. In order to highlight the main problems in the sector and to provide specific recommendations for reform, it is necessary to conduct market studies, to analyze and to evaluate the results of the implementation of the EU CAP and the state policy for support of the sector.

MATERIAL AND METHODS

In economic literature, the most commonly used methodological approach for determining competitiveness is by analyzing production costs; market share and direct investment in production.

The vine and wine sector is an export-oriented agricultural sector that is heavily dependent on trends of wine demand in

RCA1, RCA 2 RCA 3.RCA1
, RCA2
, RCA3
(Balassa, 1989).

1
2000 . -
3143,7 . 2016 .
1207,7 .
376 903
2000 . 199500
2016 .

the national and world markets.

- This requires extensive market research on the demand of wines, the varietal structure of wine grape vines, the size of the areas and the applied production technologies. Wine and vine growing
- requires large capital investments, which are paid over an extended period of time, and therefore a market-oriented management approach based on the specificity of the industry is needed. In order to establish the competitive level and the place of the Bulgarian vine and wine sector in the structure of world trade is used Balassa index with its three varieties - RCA1, RCA2 and RCA3.RCA1 shows the share of Bulgarian exports of grapes and wines in world export with the same commodities.RCA2 shows what is the place of grapes and wines in Bulgarian agricultural export compared to the world export with the same goods. RCA3 shows whether the country is a net importer or net exporter of these goods (Balassa, 1989).

RESULTS AND DISCUSSION

Figure 1 presents the dynamics in the production of wine grapes and wine. Before Bulgaria's accession to the EU and before the start of reform in the vine and wine sector in our country are produced in 2000 – 3143.7 million liters wine. In 2016, the quantity of produced wine dropped to 1207.7 million liters. The production of wine grapes from 376 903 tones in 2000 decreased to 199 500 tones in 2016.

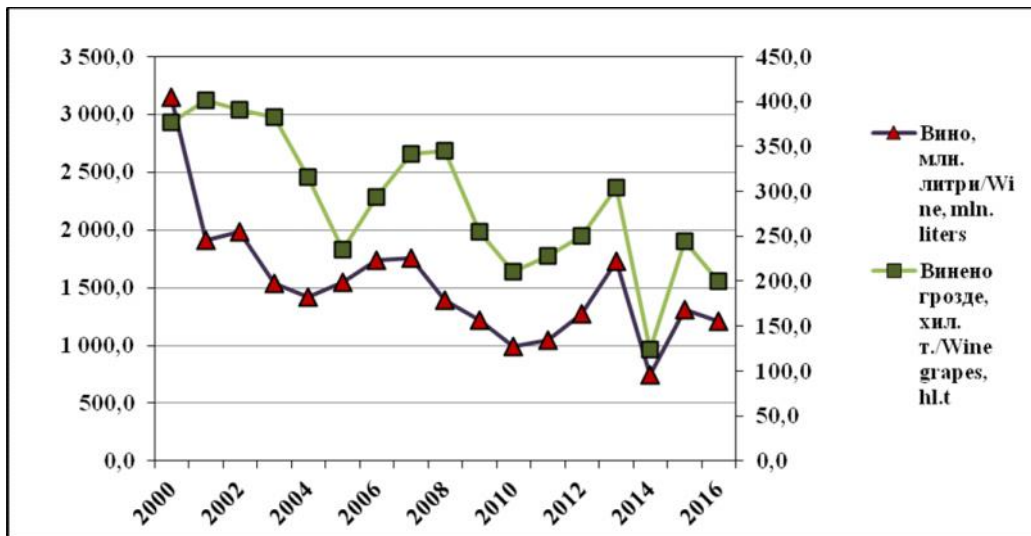


Fig. 1. Production of wine grapes and wine - Source: MAF, Directorate "Agrostatistics", 2001-2017

The reasons for the permanent reduction of wine vineyards in Bulgaria can be both the transition from a centrally planned to a market economy and the position of Bulgarian viticulture and wine production on the international markets after the accession of Bulgaria to the EU. In these years large areas of vines were abandoned, there was no investment to create new vineyards. The opening of the Bulgarian economy puts it in a situation of strong competition on the domestic market as well as on the international market.

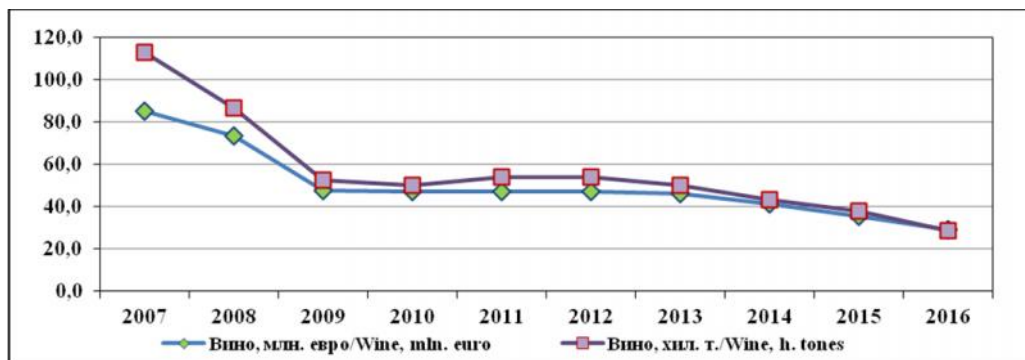
The problems of the Bulgarian wine production over the last seventeen years are the result of the reduced consumption of Bulgarian wines on the domestic market, the higher prices they are offered on domestic market and the great competition from the countries where the wine sector are strongly supported from the government of the state.

Trade with wine as a factor for the development of vine-growing in Bulgaria

ú after the accession to the EU.

- After the accession of Bulgaria to the EU, the export of Bulgarian wines is not stable. The increase of export in 2007 is the result of favourable structural changes in the vine and wine sector, greater opportunities for increase of production from high-quality wines due to available capacities and raw materials and a higher increase in wine export from a higher price category that provides greater volumes of currency earnings for our wine growers. After 2007 there is a collapse in the export of Bulgarian wines.

- The stagnation in the incomes of the population determines the stagnation in the consumption of wine and the decline in the export of wines. In 2016 the export of Bulgarian wines in quantity and value reduced twice compared to 2009 (Figure 2).

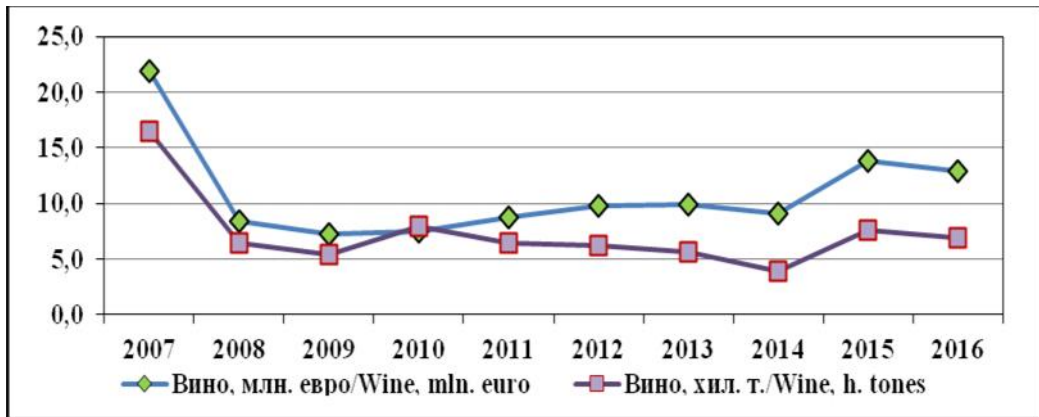


. 2.

:Eurostat, 2007-2016

Fig. 2. Dynamics of the export of Bulgarian wine in quantity and value - Source: Eurostat, 2007-2016

- After the accession of Bulgaria to the EU, there is a decrease in the import of wines in Bulgaria also. In 2016, the import of wines in the Bulgarian market in quantity and value decreased twice compared to 2007. In recent years Bulgaria imports wines mainly from Italy, France, Chile, New Zealand (Figure 3).



. 3.

:Eurostat, 2007-2016

Fig. 3. Dynamics of import of wines on the Bulgarian market in quantity and value -
Source: Eurostat, 2007-2016

RCA_{1, 2, 3} RCA₁

1. RCA₂

1. RCA₃

-1 1.

4

RCA₃

- The place of the Bulgarian vine
and winesector in world trade.

- In order to assess the place of the
Bulgarian vine and wine sector in the
structure of world trade with grapes and
wines, the Balassa index was used,
through its 3 varieties – RCA₁, RCA₂,
RCA₃. RCA₁ shows the share of
Bulgarian export of grapes and wine in
the world export with the same goods, as
the index cannot exceed 1. RCA₂ shows
what is the place of grapes and wine in
Bulgarian agricultural export compared to
the world export and thus the index may
exceed 1. RCA₃ shows whether the
country is a net importer or net exporter
of grapes and wine, thus the index values
ranging from -1 to 1.

5 Figure 4 and Figure 5 clearly show
the extremely low and non-increasing
values in the Balassa index, RCA₃ is
even negative. This shows that the
production of grapes is unsustainable and
our country is a net importer of grapes.

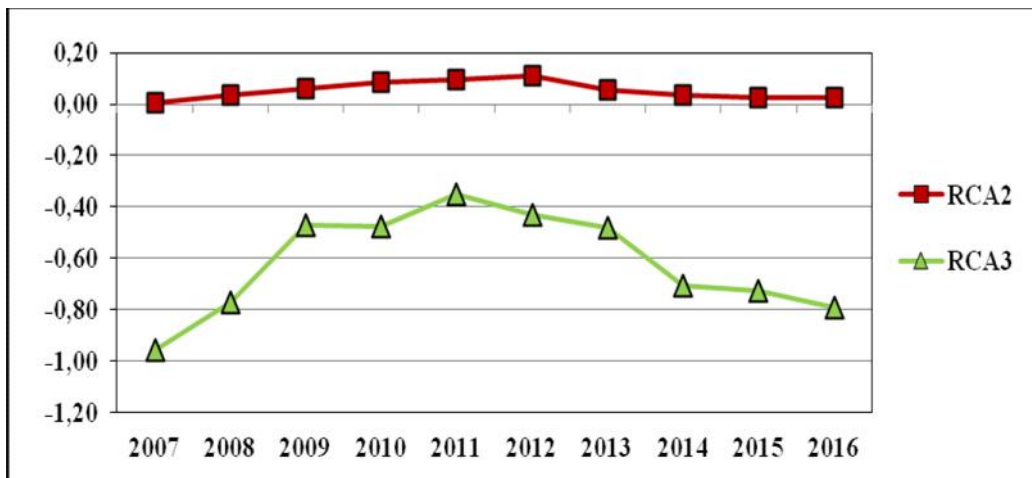


Fig. 4. Ballasa index for grapes - Source: ITC, International Trade Centre, International trade in goods (www.Intracen.org/), Grapes, fresh or dried – 0806

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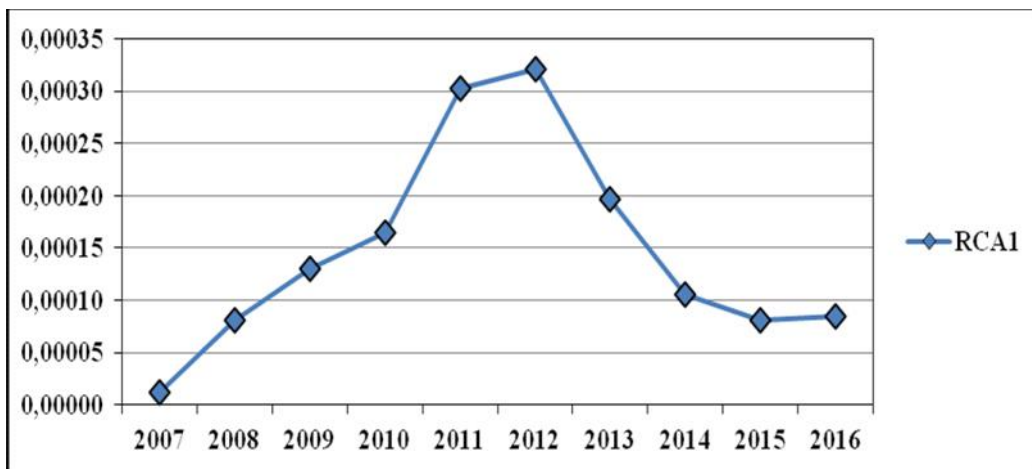


Fig. 5. Ballasa index for grapes - Source: ITC, International Trade Centre, International trade in goods (www.Intracen.org/), Grapes, fresh or dried – 0806

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RCA_1 ,
 RCA_2
 - 2,5 2007 . 0,3
 2016 .,
 2007 .

The Ballasa wine index shows low and declining RCA_1 values, which outlines the low share of Bulgarian wine export in world export. RCA_2 shows a reduction trend – from 2.5 in 2007 to 0.3 in 2016, indicating that in 2007 vine and wine had a good place in Bulgarian agricultural export compared to the world,

but in 2016 the sector lost its position (Figure 6 and Figure 7).

7).

2016
6

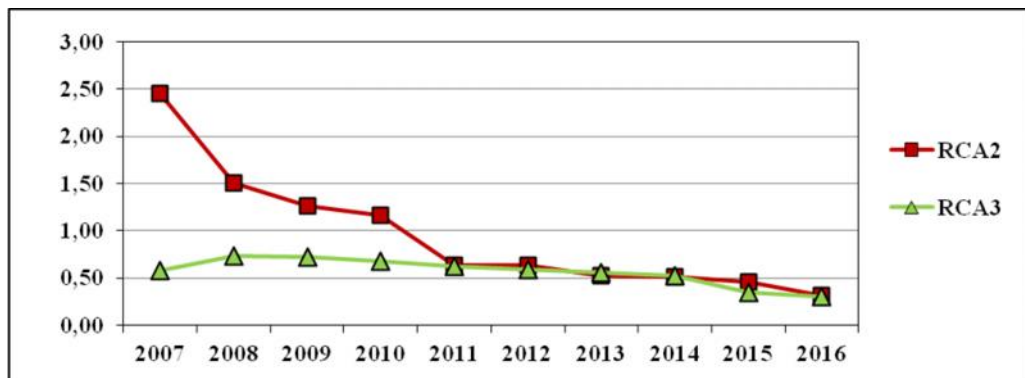


Fig. 6. Ballasa Index for wines - Source: ITC, International Trade Centre, International trade in goods (www.Intracen.org/), Wine of fresh grapes, incl. fortified wines; grape must, party fermented and of an actual – 2204

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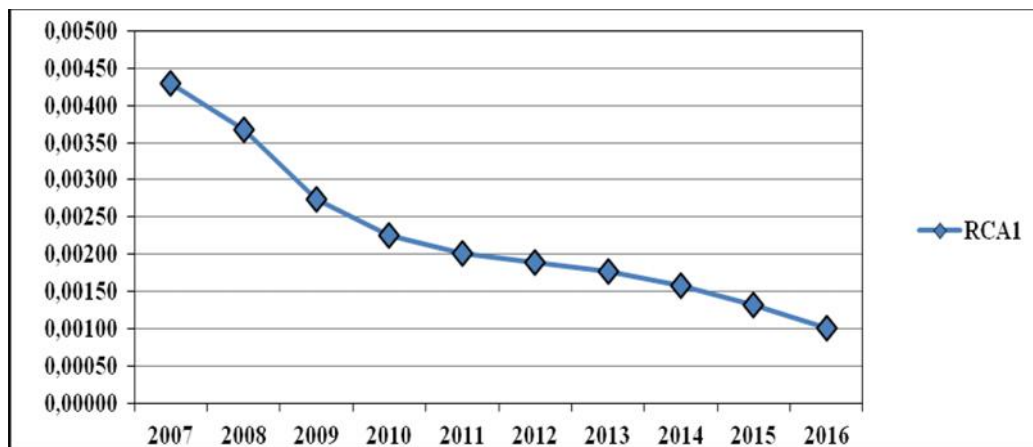


Fig. 7. Ballasa Index for wines - Source: ITC, International Trade Centre, International trade in goods (www.Intracen.org/), Wine of fresh grapes, incl. fortified wines; grape must, party fermented and of an actual ... – 2204

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CONCLUSIONS

The wine sector in the period of Bulgaria's EU membership and the implementation of the CAP is

4-5

(2014-2018) „
“.

- characterized as a descending
- development production in Bulgarian
- agriculture. This is confirmed by the main
- indicators characterizing the production of
- wine grapes and wine as well as the wine
- trade. Throughout the observed period,
- this sector reduces its indicators and
- values. This gives grounds for concluding
- that production is unsustainable and with
- declining competitiveness both on
- domestic market and foreign market.

- The reasons for the unstable
- positions of the vine and wine sector
- should be seek in the fact that, after the
- accession of Bulgaria to the EU, the main
- instrument influencing over the
- development of Bulgarian vine and wine
- sector was the National Support Program.
- The applied historical approach for the
- allocation of national financial packages
- accompanying the national vine and wine
- support programs in the EU, for Bulgaria
- did not reflect to the problems in the
- sector. Support to the Bulgarian viticulture
- places Bulgarian producers in a
- disadvantage compared to the producers
- from the other member states.

At the same time, over the past 4-5
years there is an increase in the area of
new vineyards, which is a good sign that
the competitive forces of the vine and
wine sector gradually start to strengthen,
but the united efforts are needed both of
the producers and from the state and
other institutions in order to look for
opportunities for expanding the supply of
Bulgarian wines in the foreign markets
and also for Bulgaria's participation in
programs for the promotion of Bulgarian
wines in the markets of third countries.

- In the future, the expectations and
- opportunities for development of vine and
- wine sector in our country are related to
- increasing the level of innovations in the
- sector. For this, the main measure of the
- new National Support Program
- (2014-2018) "Restructuring and
- conversion of vineyards" can contribute.
- Conversion and other activities affecting

- over the competitiveness of the sector lead to a higher market orientation of the varieties cultivated. Last but not least is the problem with the construction of meliorative equipment (drip irrigation systems). Global climate changes in recent years arise the issue of timely irrigation of vineyards particularly current.

- In order the sector to be successful in the coming years, Bulgaria has to take into account the taste preferences of consumers in the market, for whose attention the competition is extremely high. It is necessary for Bulgarian wines to participate in fairs and international exhibitions so that the potential consumers can see and experience them. In this respect, close cooperation between the National Viticulture and Wine Chamber and the Executive Agency for Vine and Wine is necessary.

- An important perspective for the future development of viticulture and wine production in Bulgaria is the active participation of our country in the international wine organizations such as the International Organization of Vine and Wine (OIV). It is also necessary to focus the administration's efforts for Bulgaria's membership of the Association of European Wine Regions (AREV).

(OIV).

(AREV).

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, 2500
E-mail: sd_krumov@abv.bg

Comparative assessment of the indicators actual fertility and yield of new introduced for the Kyustendil table grapevine cultivars

Simeon Krumov

Institute of Agriculture, 2500 Kyustendil, Bulgaria

SUMMARY

It was performed a comparative analysis of indicators characterizing the actual fertility and yield of newly introduced for the region of Kyustendil table grapevine cultivars – Misket russenski, Prista and Ryahovo. Super early Bolgar, the reference for the group of early ripening cultivars is accepted as a control variant. The research found that all investigated indicators of actual fertility and yields vary by year, but average values are fully within the requirements for table grapevine cultivars.

- There were no clear trends in the dynamics of change in the individual indications during the study.
- The investigated cultivars are characterized by approximately equal productive opportunities. The values of the indicators developed buds and fruit shoots with 1 cluster are higher in the control - Super early Bolgar. The percentage of fruit shoots by 2 clusters, the fertility coefficient of the leading shoot and fruit leading shoot, the average cluster mass and the

average yield per vine are higher in the cultivars Prista and Ryahovo. There are statistically proven differences between cultivars Prista, Ryahovo and control on some of the important ampelographic indicators – coefficient of fertility of fruit leading shoot, average cluster mass and the average yield per vine.

Key words: vine, table grapevine cultivars, introduction, actual fertility, yield, comparative analysis

INTRODUCTION

The vine (*Vitis vilifera* L.) is one of the most widespread cultures in Bulgaria, due to its high ecological plasticity and high quality of the fruits. Prerequisite for this is the excellent natural conditions that are very favorable for the production of grapes with different directions of use (Pandeliev et al., 2012).

Of all factors with greatest importance for the optimal use of the potential of environment in a concrete region has the choice of a suitable cultivar.

In the conditions of globalization, intensive development of viticulture and the constantly changing market situation of grapes, namely the choice of suitable cultivar is one of the most important for a high-quality and competitive production (Dimitrova et al., 2014; Dimitrova, 2015).

This requires the expansion of the assortment of table grapevine cultivars to satisfy the high requirements of customers (Simeonov et al., 2005). Most of the existing vine cultivars have a limited range of cultivation, others due to their greater ecological plasticity and their valuable economic qualities have been more widely distributed.

The introduction into the practice of new table grapevine cultivars with different agrobiological and technological

average yield per vine are higher in the cultivars Prista and Ryahovo. There are statistically proven differences between cultivars Prista, Ryahovo and control on some of the important ampelographic indicators – coefficient of fertility of fruit leading shoot, average cluster mass and the average yield per vine.

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The introduction into the practice of new table grapevine cultivars with different agrobiological and technological

(Stoev, 1964).

(Lazarov, 1998).

(Lazarov, 1998; Nakov et al., 2007; Simeonov et al., 2010; Pandeliev et al., 2012).

(Simeonov and Roychev, 2013).

(Roychev, 2012).

(Mokreva and Roychev, 2004; Mokreva, 2011). Katerov et al. (1990),

(Ivanov, 2011).

characteristics is associated with many studies and trials in order to select those, that show the best qualities and are better to cultivars distributed in the region (Stoev, 1964).

Many valuable cultivars of table grapevine have been created in Bulgaria, some of which are listed as examples of world selection, but the major problem of table grape production in our country is the cultivar structure of vineyards (Lazarov, 1998). Most of the newly selected table grapevine cultivars included in the National List of Varieties is poorly spread, although they exceed the standard cultivars to a range of biological and economic qualities (Lazarov, 1998; Nakov et al., 2007; Simeonov et al., 2010; Pandeliev et al., 2012).

One of the main criterions for the selection of each vine cultivar is its actual fertility, which is a highly accurate indicator of its productive capacity. It is a basic ampelographic criterion in assessing its economic significance and distribution opportunities in different regions and micro-regions (Simeonov and Roychev, 2013). Fruitfulness is the genetically determined ability of the cultivar to set a different amount of inflorescence in the winter eyes, to have a larger or smaller percentage of developed and fruit shoots, fertility coefficient and etc. (Roychev, 2012). It has a direct impact on the quantity of yield (Mokreva and Roychev 2004; Mokreva, 2011). According to Katerov et al. (1990), vine cultivars differ in their fertility, which can be high, medium or low. Depending on the direction of use of the grapes, the main requirement for the table grapevine cultivars is the high or medium actual fertility (Ivanov, 2011).

The aim of this investigation was to make a comparative analysis of the elements of the actual fertility and yield of three newly introduced in the region of

Kyustendil table grapevine cultivars.

MATERIAL AND METHODS

The research was conducted in experimental vine plantation at the Institute of Agriculture- Kyustendil during the period 2013-2015. The soil in the test area is highly leached, medium sandy-loam, weakly to moderately stony cinnamon forest soils (*Chromic Luvisols*) with a neutral pH.

The object of research was table grapevine cultivars – Misket russenski, Prista and Ryahovo, created in the IASS- "Obraztsov chiflik", Rousse. For comparative variant was used early repining cultivar (standard) Super early Bolgar, studied in the region of Kyustendil. The vines were planted in the spring of 2009 at 2,50 m distance between rows and 1,25 m inside the lines. They were grafted onto rootstock Berlandieri x Riparia SO4 and formed by the system ground Gyuyo, with annual cover with soil in the winter. The load during the research was the same in all cultivars – 18 buds (winter eyes) of the vine realized by 1 fruit-stick with 12 buds and 3 knots with 2 buds.

The study of vine cultivars was done in methodology described in Bulgarian ampelography, volume 1 (Katerov et al., 1990).

The experimental results were processed by the method of dispersion analysis, using the LSD test for proving statistical significance of the differences between the control and variants. The assessment was done at significance level $P < 0,05$, $P < 0,01$ and $P < 0,001$ (Maneva, 2007).

RESULTS AND DISCUSSION

The results of the indicators characterizing the actual fertility of the tested table grapevine cultivars show

2013-2015
(*Chromic Luvisols*)
2009
1,25 m
x
O4
- 18
1
3 2
(Katerov et al., 1990).
LSD-
 $P < 0,05$, $P < 0,01$
(Maneva, 2007).
 $P < 0,001$

considerable variation during the individual years of the experimental work (Table 1).

1.

2013-2015 .

Table 1. Parameters of actual fertility of table grapevine cultivars from the period 2013-2015

Cultivar	Year	Developed buds, %	Fruit shoots, %			Coefficient of fertility	
			Total	1 with 1 cluster	2 with 2 clusters	of developed shoot	of fruit shoot
Misket russenski	2013	74,7	68,6	66,7	33,3	0,88	1,29
	2014	80,5	76,3	81,8	18,2	0,92	1,21
	2015	80,5	64,7	67,6	32,4	0,83	1,28
	Average	78,6	69,9	72,0	28,0	0,88	1,26
Prista	2013	66,7	75,0	66,7	33,3	1,00	1,33
	2014	67,0	83,3	60,0	40,0	1,17	1,40
	2015	77,8	71,4	80,0	20,0	0,93	1,31
	Average	70,5	75,6	68,9	31,1	1,04	1,35
Ryahovo	2013	72,2	69,3	66,7	33,3	0,93	1,34
	2014	68,7	79,7	60,0	40,0	1,12	1,41
	2015	61,1	72,7	75,0	25,0	0,91	1,24
	Average	67,4	73,9	67,2	32,8	1,00	1,33
(control) Super early Bolgar	2013	77,9	71,4	80,0	20,0	0,86	1,20
	2014	78,7	82,5	72,7	27,3	1,00	1,22
	2015	79,8	64,3	77,8	22,2	0,81	1,26
	Average	78,8	72,7	76,8	23,2	0,89	1,23

“ ” . -
 -
 ()
 (78,8 %), -
 (67,4 %). -
 -
 77,9 % 79,8 %
 74,7 % 80,5 %
 66,7 % 77,8 %
 61,1 % 72,2 % .
 -
 2015 . (1).

According to the accepted classification, the share of developed shoots is "high" for all cultivars. With relatively higher percentage developed winter eyes (average for the study period) was the control cultivar Super early Bolgar (78,8%) and the lowest Ryahovo (67,4%). The dynamics of this indicator during the survey years varied in a relatively narrow range – from 77,9% to 79,8% in the control cultivar, from 74,7% to 80,5% in Misket russenski, from 66,7% to 77,8 % in Prista and from 61,1% to 72,2% in Ryahovo. For all cultivars except Ryahovo, the highest values of the indicator were established in 2015 (Table 1).

Average for the period of study, it was developed approximately the same amount of fruit shoots. Their share in all cultivars is defined as "high" under the conditions of the experiment. The values

69,9 % ()
 75,6 % (),
 72,7 % ()
 1).
 2015 .,
 64,7% () 72,7%
 (), - 64,3%.
 1 ,
 67,6 % ()
 80,0 % (), 77,8 %
 2014 .,
 2 ,
 2
 1,00 1,04
 (0,88),
 - 0,89.
 (1,33), - (1,35)
 - 1,23.
 (1,26),

- of this indicator ranged from 69,9% (Misket russenski) to 75,6% (Prista), compared to 72,7% in the control cultivar Super early Bolgar (Table 1).

- Less fruit shoots were recorded in 2015, probably due to the unfavorable climatic conditions during the formation of the inflorescences in the winter eyes the previous year. In investigated cultivars the percentage was from 64,7% (Misket russenski) to 72,7% (Ryahovo) and in the control – 64,3%.

In this year, the actual fertility is mainly determined by the shoot with one cluster, that was in the range from 67,6% (Misket russenski) to 80,0% (Prista) compared with 77,8% in cultivar Super early Bolgar.

- In other years, the percentage of fruiting shoots was higher, with the highest values was characterized 2014, when the share of the shoot with 2 clusters, except for cultivar Misket russenski was greatest.

- The less number of developed buds, most of which are fruiting, and the bigger number of fruit shoots with 2 clusters in cultivar Prista and Ryahovo, also determine their higher fertility coefficient of a developed and fruit shoots. The coefficient of fertility of developed shoot in both cultivars was 1,00 in Ryahovo and 1,04 in Prista.

- The lowest fertility coefficient of all investigated cultivars had Misket russenski (0,88), due to the lower percentage of fruit shoots. By this indicator, the control cultivar occupied a medial position – 0,89. Similar are the results for the fertility coefficient of fruit shoot, where again with higher values were cultivars Prista (1,35) and Ryahovo (1,33) and with the lowest Super early Bolgar – 1,23. The cultivar Misket russenski occupied an intermediate position on this indicator (1,26), which is due to the bigger number of fruit shoots

2 | with 2 clusters, compared to the control.

- The studied cultivars were harvested at the consumptive ripeness of the grapes, the yield elements were recorded (Table 2).

2.
2013-2015 .

Table 2. Parameters of the yield of table grapevine cultivars from the period 2013-2015

Cultivar	Year	Weight per cluster, g	Yield per vine, kg
Misket russenski	2013	304,0	3,70
	2014	259,1	3,50
	2015	292,0	3,40
	Average	285,0	3,53
Prista	2013	364,6	4,30
	2014	262,7	3,50
	2015	305,7	4,00
	Average	311,0	3,93
Ryahovo	2013	337,5	4,00
	2014	354,0	4,90
	2015	405,0	3,80
	Average	365,5	4,23
Super early Bolgar (control)	2013	302,1	3,60
	2014	268,5	3,80
	2015	263,3	3,00
	Average	278,0	3,46

(365,5 g), (311,0 g)
 (285,0 g). -
 1 (278,0 g).
 , " "

An average for the period with the highest average mass of cluster was cultivar Ryahovo (365,5 g), followed by Prista (311,0 g) and Misket russenski (285,0 g). With the lowest mass values of 1 cluster was the control (278.0 g). In regard to this indicator, all tested cultivars correspond to 'Extra' grape quality requirements.

-
 . C
 (4,23 kg),
 (3,93 kg).
 - -

The average yields per vine reflect to all other indicators in total and reveal exactly the productive potential of each cultivar. The newly introduced table grapevine cultivars are characterized by a higher yield than the control.
 - With the highest average yield per vine was cultivar Ryahovo (4,23 kg), followed by Prista (3,93 kg). In the cultivars with smaller mass of cluster the yield per vine is relatively lower - Misket

(3,53 kg)
(3,46 kg).

2015 .

3).

russenski (3,53 kg) and Super early Bolgar (3,46 kg).

The variation of the results by years and variants is slight, as 2015 is relatively lower values for this indicator, due to the fewer inflorescences formed in the previous year.

The comparative statistical analysis shows that the differences between the cultivars and the control in most indicators are insignificant (Table 3). There is no proven difference between the studied variants in the indicators – percentage of developed buds, fruit shoots and coefficient of fertility of a developed shoot. An exception is the indicator percentage of developed buds in the cultivar Ryahovo, where the value was lower than that to control variant. The differences between the Prista, Ryahovo and the control cultivar of the fertility coefficient of fruit shoot are proving positive.

3.

2013 – 2015 .

Table 3. Comparative analysis of the actual fertility and yield of table grapevine cultivars from the period 2013-2015

Cultivar	Developed buds, %	Fruit shoots, %	Coefficient of fertility of:		Weight per cluster, g	Yield per vine, kg
			developed shoot	fruit shoot		
Misket russenski	78,6 ^{n.s.}	69,9 ^{n.s.}	0,88 ^{n.s.}	1,26 ^{n.s.}	285,0 ^{n.s.}	3,53 ^{n.s.}
Prista	70,5 ^{n.s.}	75,6 ^{n.s.}	1,04 ^{n.s.}	1,35 ⁺	311,0 ⁺⁺	3,93 ⁺
Ryahovo	67,4 ⁻	73,9 ^{n.s.}	1,00 ^{n.s.}	1,33 ⁺	365,5 ⁺⁺⁺	4,23 ⁺⁺
() Super early Bolgar (control)	78,8	72,7	0,89	1,23	278,0	3,46
F	3,691	3,196	11,377	2,66	90,387	11,99
SD	4,258	2,196	3,18	4,934	5,905	0,147
LSD 0,05	10,43	5,381	7,782	0,12	14,47	0,361

* n.s. (/ non significant); +/- (P<0,05); ++/-- (P<0,01); +++/--- (P<0,001)

The average mass of clusters, as well as the generalizing indicator – yield per vine are proven to be bigger in cultivars Prista and Ryakovo than to the

(3).

control cultivar. From all biometric data it was found that there was no proven difference between cultivar Misket russenski and control. The values of most of the investigated indicators are higher in cultivar Super early Bolgar (Table 3).

CONCLUSIONS

All tested indicators of actual fertility and yield in the investigated cultivars, vary by years, but their average values are completely within the requirements of the table grapevine cultivars. There are no established clear trends in the dynamics of change of the different indicators during the study period. The studied cultivars are characterized by approximately equal productive capabilities.

The values of the indicators percentage of developed buds and fruit shoots with 1 cluster have a higher value in the control – Super early Bolgar, and fruit shoots with 2 cluster, coefficient of fertility of the developed shoot and fruit shoot, an average mass of cluster and average yield per vine – in cultivars Prista and Ryahovo.

There are mathematically proven differences between cultivars Prista, Ryahovo and control – Super early Bolgar in some of the important ampelographic indicators – coefficient of fertility of fruit shoot, average mass of cluster and average yield per vine. For all other indicators, the differences are insignificant.

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(*Vitis vinifera* L.)

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*E-mail: roytchev@yahoo.com

Comparative ampelographic study of table grape cultivars (*Vitis vinifera* L.)

Venelin Roychev*, Angel Ivanov, Todorka Mokreva

Agricultural University, 4000 Plovdiv, Bulgaria

SUMMARY

- A relative ampelographic study has been made of table grape cultivars and an elite hybrid form. It has been proven that there is a statistical diversity in phenotypic expression of many indicators of the mechanical analysis of the cluster and the berry. The elite form excels the known varieties in a number of economically valuable agro-biological and technological characteristics. Depending on their genetic proximity and relative weight, in the individual identity of varieties Brestovitsa-Palieri and Armira-Bolgar-Hybrid 10, significant similarity has been noticed. Super ran Bolgar has the lowest degree of similarity with the other varieties. The terms of the external environment least affect the productivity of the variety Bolgar and most strongly - Super early Bolgar.

Key words: table grape cultivars, relative ampelographic study, cluster analysis, environmental plasticity

INTRODUCTION

- The favourable climate and soil conditions of Bulgaria allow the cultivation of table grape cultivars for the production

(Zankov, 1984; Colapietra et al., 1995; Ivanov, 2009; Simeonov et al., 2012).

(Krumov, 2014; Simeonov et al., 2015).

et al., 2007).

(Nakov

of quality grapes for fresh consumption. A number of countries have produced new table grape cultivars with valuable business properties. Their agro-biological and technological features are always of interest for comparison with the old and famous varieties in this direction (Zankov 1984; Colapietra et al., 1995; Ivanov, 2009; Simeonov et al., 2012).

The relative share of foreign varieties in the structure of the vineyards in our country excels that of the local varieties. Over the last few decades, by introduction and the method of sexual hybridization, a large number of new early high-quality, middle and late ripening varieties of large-fruited table grape cultivars have been selected and tested (Krumov 2014; Simeonov et al., 2015). Depending on region, traditions and experience, the selection work in the field of viticulture is aimed at creating or introduction of table grape cultivars that ripen from the second half of July to late October (Nakov al., 2007). After studying the biological characteristics and the productive capacity of individual table grape cultivars, their most valuable ampelographic properties can be identified and the necessary information for their proper spatial distribution can be obtained.

The aim of this study is to compare important economic agro-biological characteristics of several seeded table grape cultivars and the identification of the most economically significant of them.

MATERIAL AND METHODS

The ampelographic study includes five table grape cultivars – Armira, Super ran Bolgar Brestovitsa, Bolgar, Palieri and a newly created elite hybrid form - Hybrid 10, grown in the Educational and experimental base of the Department of Viticulture at the Agricultural University in Plovdiv. This terroir is characterized by extremely favourable conditions for

SO4				growing table grape cultivars. They are grafted onto rootstock Berlandieri x Riparia SO4 and are grown on the formation Stem two-arm Gyuyo with aligned load in pruning with 2 fruit branches each with 11 winter eyes and 6-8 nubs each with 2 eyes and planting distances of 3,20/1,20 m. The experimental work includes no less than 25 vines of each variety. Their grapes are harvested in consumptive maturity. The vines of table grape cultivars are 18-25 years old, while the hybrid form is 12 years old.
	11	2	2	
3,20/1,20 m.		6-8	25	
18-25			-	
12				
2012).		(Roychev,		For five consecutive years the actual fertility of each variety, as well as the indicators of the mechanical analysis of the grapes and the berry have been identified (Roychev 2012). The experimental data of the individual analyses are presented as the average of all the years of the study and are statistically processed through a cross comparison test (Mokreva, 2007). Genetically close groups of varieties have been determined through cluster analysis (Everitt 1979; Philippeau 1990). The evaluation of environmental variability of the studied ampelographic indicators was carried out by the Wricke method (1962, 1965), which enables to determine the interconnectivity of the genotype with the environment. The low values of the estimated eco coefficients show greater ecological plasticity of the variety concerned (Mokreva al., 2001).
	(Mokreva, 2007).			
1990).	(Everitt, 1979; Philippeau,			
Wrick (1962, 1965),				
	(Mokreva et al., 2001).			

RESULTS AND DISCUSSION

Data from the comparative analysis of parameters of the mechanical analysis in the grape varieties and hybrid forms studied show that there is diversity in their phenotypic expression according to genotype and external conditions (Table 1). The productive potential of the studied varieties is best seen in reporting the indicators of performance of fruitfulness and yield. Regarding the *coefficient of fruitfulness*, there are no significant differences between the varieties and Hybrid 10. It varies from 0.78 (Brestovitsa

0,78 () 1,00
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 -
 - 18,22 %
 9,49 %.
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 :
 - 487,88 g 302,15 g
 ()/402,79 g ();
 - 15,16 cm - 11,19 cm
 ()/13,17 cm ().
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to 1.00 (Bolgar). The percentage of *millerandage berries* is proven to be the greatest only in Super ran Bolgar - 18.22% and Armira - 9.49%. The differences between all comparable varieties and Hybrid 10 have been proven for the indicators *mean weight of cluster*, *width of cluster*, *number of seeds in 100 berries*, *yield per vine*, *average weight of 100 berries*, *length of berries* and *sugar content*. The absolute value of some of them in Hybrid 10 is higher than those of all varieties: *average weight of cluster* – 487.88 g compared to 302.15 g (Super ran Bolgar) / 402.79 g (Armira); *width of cluster* – 15.16 cm – 11.19 cm (Palieri) / 13.17 cm (Bolgar). Unsubstantial are the differences between the hybrid form and varieties in the indicators *length of cluster* and *seed weight in 100 berries* (except Armira), *width of berry* (excluding Armira, Super ran Bolgar and Bolgar), *quantity of acids* (except Armina and Super ran Bolgar).

The multiway comparative analysis of the *coefficient of actual fruitfulness* and the *length of cluster* shows that the studied varieties and the hybrid form do not differ significantly among themselves, because only one group has been formed - a (Table 2). In the indicator *millerandage berries* three groups of proof are marked, as Armira and Super ran Bolgar differ from all other. The diversity of values at an *average weight of cluster* is even greater because each variety evidently differ from others, which means that the biology of the variety and the external conditions strongly influence this indicator. Regarding the *width of cluster* varieties also demonstrate sufficiently significant variety, with the meaning of shaping their table qualities and commercial appearance. Of the indicators related to seeds in berries, varieties differ less in their *weight in 100 berries*. The famous wine growing correlation between the size and number of seeds and the berry size is clearly demonstrated here.

1.

Table 1. Comparative analysis of parameters of fruitfulness and mechanical analysis in the grape varieties studied

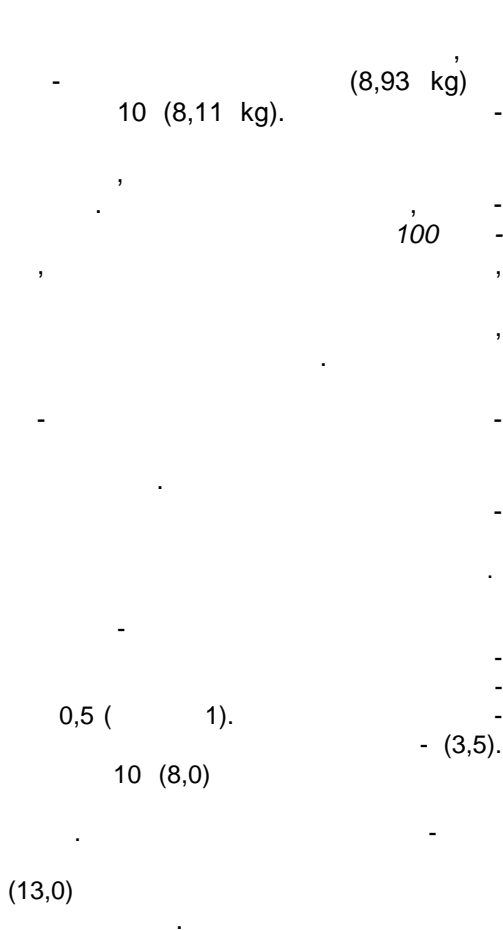
	Varieties	Fruitfulness coefficient of leading shoot	Millerandage berries %	Average weight of cluster g	Length of cluster cm	Width of cluster cm	100 Seeds in 100 grape berries /number	100 Seed weight in 100 grains g
1	Hybrid 10	0,86	3,99	487,88	22,41	15,16	192,14	13,47
2	Armira	0,86 n.s.	9,49 *	402,79 *	19,32 n.s.	12,02 *	242,79 *	21,83 *
3	Super ran Bolgar	0,84 n.s.	18,22 *	302,15 *	18,60 n.s.	11,38 *	176,38 *	10,09 n.s.
4	Brestovitsa	0,78 n.s.	3,31 n.s.	323,46 *	18,29 n.s.	11,68 *	201,54 *	11,44 n.s.
5	Bolgar	1,00 n.s.	4,26 n.s.	370,43 *	39,21 n.s.	13,17 *	146,47 *	11,03 n.s.
6	Palieri	0,86 n.s.	4,00 n.s.	351,55 *	19,51 n.s.	11,19 *	150,51 *	10,66 n.s.
	Varieties	Yield per grape vine kg	100 Average weight of 100 grape berries g	Length of a grape berry mm	Width of a grape berry mm	Sugars %	Acids g/dm ³	
1	Hybrid 10	8,11	602,66	25,81	22,01	16,96	5,05	
2	Armira	8,93 *	532,67 *	22,18 *	18,66 *	15,46 *	4,49	
3	Super ran Bolgar	5,63 *	464,22 *	24,65 *	19,59 *	14,85 *	4,24	
4	Brestovitsa	7,06 *	756,51 *	28,65 *	21,55 n.s.	17,52 *	4,99	
5	Bolgar	5,90 *	552,97 *	23,27 *	19,56 *	16,32 *	5,05	
6	Palieri	6,55 *	747,65 *	23,49 *	21,30 n.s.	15,90 *	5,21	

2.

Table 2. Multidirectional comparative analysis of the parameters of fruitfulness and mechanical analysis in the grape varieties studied

	Varieties	Fruitfulness coefficient of leading shoot	Millerandage berries %	Average weight of cluster g	Length of cluster cm	Width of cluster cm	100 Seeds in 100 grape berries /number	100 Seed weight in 100 grains g
1	<i>Hybrid 10</i>	0,86 ^a	3,99 ^c	487,88 ^a	22,41 ^a	15,16 ^a	192,14 ^c	13,47 ^b
2	Armira	0,86 ^a	9,49 ^b	402,79 ^b	19,32 ^a	12,02 ^c	242,79 ^a	21,83 ^a
3	Super ran Bolgar	0,84 ^a	18,22 ^a	302,15 ^f	18,60 ^a	11,38 ^{cd}	176,38 ^d	10,09 ^b
4	Brestovitsa	0,78 ^a	3,31 ^c	323,46 ^e	18,29 ^a	11,68 ^{cd}	201,54 ^b	11,44 ^b
5	Bolgar	1,00 ^a	4,26 ^c	370,43 ^c	39,21 ^a	13,17 ^b	146,47 ^e	11,03 ^b
6	Palieri	0,86 ^a	4,00 ^c	351,55 ^d	19,51 ^a	11,19 ^d	150,51 ^e	10,66 ^b

	Varieties	Yield per grape vine kg	100 Average weight of 100 grape berries g	Length of a grape berry mm	Width of a grape berry mm	Sugars %	Acids g/dm ³
1	<i>Hybrid 10</i>	8,11 ^b	602,66 ^b	25,81 ^b	22,01 ^a	16,96 ^b	5,05 ^a
2	Armira	8,93 ^a	532,67 ^c	22,18 ^e	18,66 ^c	15,46 ^d	4,49 ^b
3	Super ran Bolgar	5,63 ^d	464,22 ^d	24,65 ^c	19,59 ^{bc}	14,85 ^e	4,24 ^b
4	Brestovitsa	7,06 ^c	756,51 ^a	28,65 ^a	21,55 ^{ab}	17,52 ^a	4,99 ^a
5	Bolgar	5,90 ^d	552,97 ^c	23,27 ^d	19,56 ^{bc}	16,32 ^c	5,05 ^a
6	Palieri	6,55 ^c	747,65 ^a	23,49 ^d	21,30 ^{ab}	15,90 ^{cd}	5,21 ^a



All varieties are characterized by relatively *high yield per vine*, but the varieties with the greatest yields are Armira (8.93 kg) and Hybrid 10 (8.11 kg). With similar values of yields are Brestovitsa and Palieri, as well as Bolgar and Super ran Bolgar. The size of the berry, expressed through the *average weight of 100 berries*, also shows significant differences, as one group by this indicator includes the varieties Brestovitsa and Palieri, as well as Bolgar and Armina. In *length and width of berries* less statistical groups of proof have been formed and the observed diversity is not great. The amount of sugars and acids is within the requirements of table grape cultivars for their normal tasting characteristics.

The varieties Brestovitsa and Palieri have the highest degree of similarity in terms of the values of studied indicators with Euclidean distance of 0.5 (Figure 1). The second group includes the varieties Armira and Bolgar - (3.5). Hybrid 10 (8.0) joins the group formed by the previous two varieties. Super ran Bolgar has the lowest degree of similarity with the other varieties (13.0) and can be considered as a separate group.

Dendrogram using Average Linkage (Between Groups)

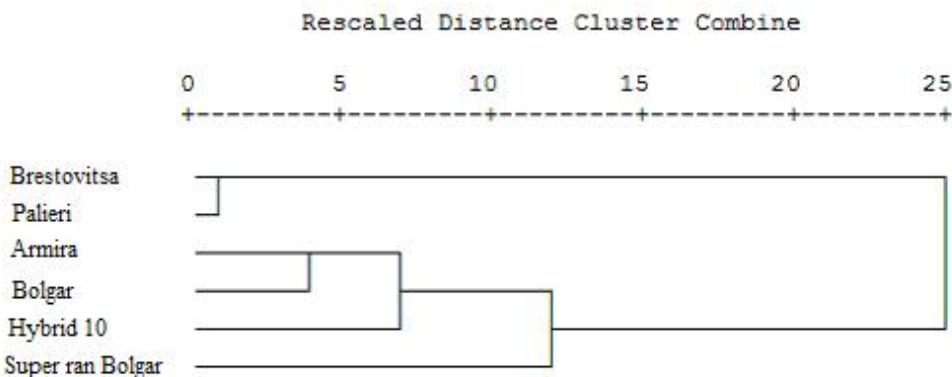
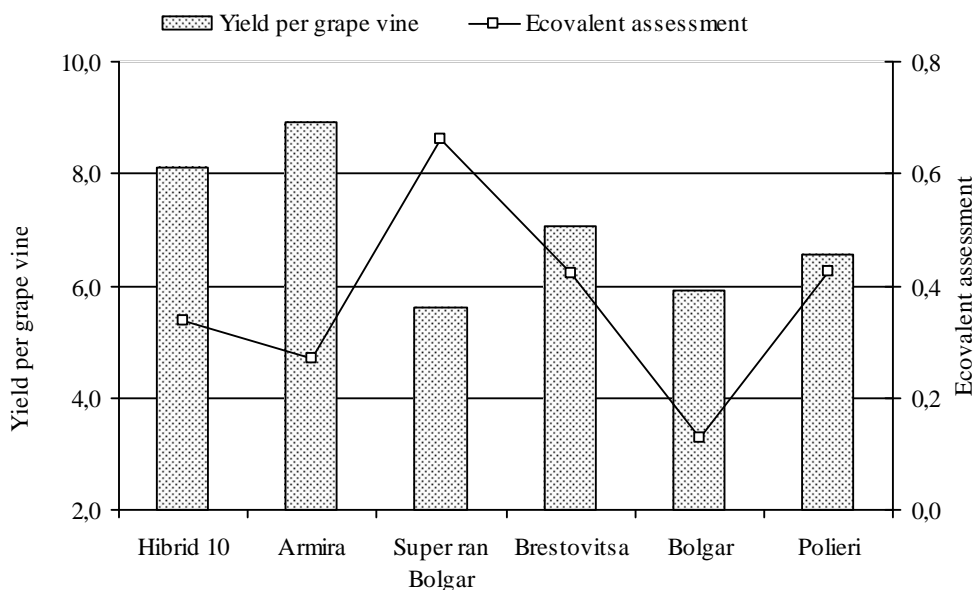


Fig. 1. Dendrogram - clustering of the table grape cultivars studied

(0,66) (2).

(0,13),

The variety Bolgar has the lowest values of eco coefficient in the indicator *yield per vine* - (0.13) which means that the conditions of the external environment least affect the change of its productivity and most unstable is Super ran Bolgar (0.66) (Figure 2). Other varieties occupy an intermediate position in terms of plasticity demonstrated compared to the impacts of the environment.



. 2.

Fig. 2. Graphic interpretation of the results from the environmental assessment of the table grape cultivars studied

1.

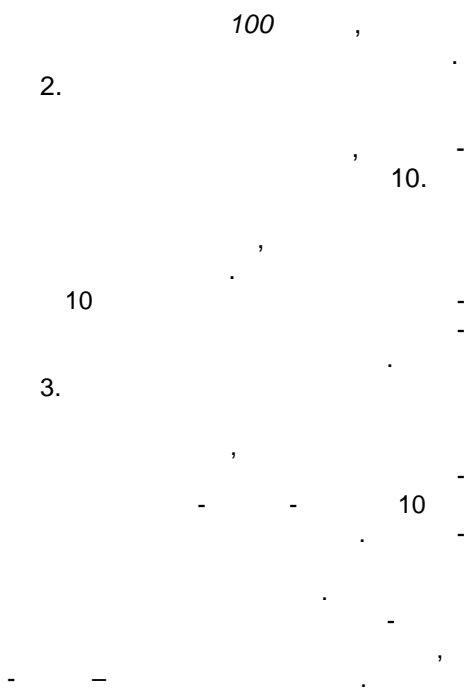
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100

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CONCLUSIONS

1. There is a proven statistical diversity in the phenotypic expression of the indicators of the mechanical analysis *mean weight of cluster, width of cluster, number of seeds in 100 berries, yield per vine, average weight of 100 berries, length of berry and sugar content* in the studied varieties and the hybrid form. With some exceptions, unessential are the differences between Hybrid 10 and the approved varieties in *length of cluster, seed weight in 100 berries, width of berry*



and quantity of acids.

2. All studied varieties are distinguished by a relatively high yield of grapes per vine, but the varieties with the highest yields are Armira and Hybrid 10. With similar values of yields are Brestovitsa and Palieri, as well as Bolgar and Super ran Bolgar. The elite form Hybrid 10 excels the known varieties in a number of economically valuable agrobiological and technological indicators.

3. Depending on their genetic proximity and relative weight of the various indicators, in the individual identity of the varieties Brestovita-Palieri and Armira-Bolgar-Hybrid 10, significant similarity has been noticed. Super ran Bolgar has the lowest degree of similarity with the other varieties. The terms of the external environment least affect the productivity of the variety Bolgar and most strongly - Super early Bolgar.

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*E-mail: pip.peeva@uni-sz.bg

**Preliminary study on content of *trans*-resveratrol
in table and wine varieties of grape**

Penka Peeva¹, Milena Tzanova², Miroslav Ivanov³, Anatoli Iliev³

¹Department of Plant production, Faculty of Agriculture, Trakia University,
Stara Zagora, Bulgaria

²Department of Biochemistry, microbiology and physic, Faculty of Agriculture, Trakia
University, Stara Zagora, Bulgaria

³Department of variety and variety maintenance, Institute of Viticulture and Enology,
Pleven, Bulgaria

SUMMARY

The dietary consumption of grape and its products is connected with a lower incidence of degenerative diseases such as cardiovascular disease and certain types of cancers. The focus of the recently studies has interpreted this fact with rich phenolic compounds in grape, including *trans*-resveratrol. The content of *trans*-resveratrol were analyzed in two varieties of introduced table grape (Muskat Hamburg and Moldova), one local (Mavrud), and four inter- and intra-species hybrids (Melnik 55, Rubin, Plevenski kolorit and Storgosia) and three introduced (Cabernet Sauvignon, Merlot and Syrah) wine varieties by RP-HPLC-PDA. Samples were taken at the moment when consumption/technological ripening was reached and from Northern and Southern wine region in Bulgaria. The

(RP-HPLC-PDA)

trans-
 15.03 ± 0.78 mg/kg,
 12.33 ± 0.62 mg/kg
 10.02 ± 0.84 mg/kg.

trans-
 0.13 mg/kg, - 1.24 ±
 - 1.46 ± 0.12 mg/kg
 - 1.71 ± 0.11 mg/kg.

: *trans*-

RP-HPLC-PDA,

- highest amount of *trans*-resveratrol was detected in variety Moldova - 15.03 ± 0.78 mg/kg, following of Syrah and Plevenski kolorit - 12.33 ± 0.62 mg/kg and 10.02 ± 0.84 mg/kg, respectively.

- The lowest was content in Muscat Hamburg – 1.24 ± 0.13 mg/kg, Cabernet Sauvignon – 1.71±0.11mg/kg and Merlot – 1.46 ±0.12 mg/kg.

- The impact of weather conditions during the growing season and the different regions of cultivation, growing technology and variety will be the factors that will be the subject of further research.

- **Key words:** *trans*-resveratrol, RP-HPLC-PDA, table grape, wine grape, Moldova, Syrah, Plevenski kolorit

INTRODUCTION

- Viticulture is a traditional branch of Bulgarian agriculture which products are present annual on our table.

- Nowadays, researching work on antioxidants has been greatly increased as their presence in foods and beverages determines their health potential.

- Polyphenol compounds belong to antioxidants, and table grapes and wines are products rich in flavonoids, stilbenes and phenolic acids (Nikov et al., 1990; Mandjukov, 2014). *Trans*-resveratrol is stilbene, naturally occurring in grapes and in its products (Bavaresco et al., 1999) and its biosynthesis is stimulated by the stress, including injury, infection or UV irradiation (LeBlanc, 2006).

(Nikov et al., 1990; Mandjukov, 2014). *Trans*-

(Bavaresco et al., 1999),

UV (LeBlanc, 2006).

- There are numerous studies in the literature which have been reported its beneficial effects on humans - cardiovascular protection, a powerful antioxidant, has anti-inflammatory action (Xia et al., 2010) and may even be used

(Xia et al., 2010)
 (Ndiaye et al., 2011).
 (Yoncheva et al., 2015)
 (Ivanov et al., 2007),
 2016) (Videnova, 2016)
 (Fartzov et al., 2012),
 trans-

as a prevention of skin diseases (Ndiaye et al., 2011).

In the Bulgarian literature there are studies on antioxidant capacity of wine (Yoncheva et al., 2015) and grapes (Ivanov et al., 2008), *trans*-resveratrol content in grape juice (Videnova, 2016) and wine (Fartzov, 2012), but there is no data on the amount of *trans*-resveratrol in grapes.

The aim of this study is to estimate the quantitative content of this antioxidant in some table and wine grape varieties.

MATERIAL AND METHODS

Plant material

2016
 (),
 (),
 (55,)
 ()
 55)
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 , 3-5 kg.
 ,
 -12°

Samples of ten varieties with red skin grains which were grown during vegetation season in 2016 are analyzed. Two varieties of introduced table grapes (Muskat Hamburg and Moldova), one local (Mavrud), four created by the methods of interdisciplinary and interspecific hybridization (Melnik 55, Rubin, Plevenski kolorit and Storgosia) and three introduced (Cabernet Sauvignon, Merlot and Syrah) wine grape varieties grown within Northern and Southern wine regions of Bulgaria were included in this study. Conventional farming is applied among the most of vineyards. The vineyard planted with Syrah variety during the investigated year was in transition period. Melnik 55 and Mavrud varieties are under biological technology of cultivation. The grapes are harvested at the moment of reached consumption ripeness and technological maturity by taking a representative sample of every vineyards consist 3-5 kg. At the laboratory, the grapes are flushed with distilled water and grains selected from the top, middle and the bottom part of the cluster are picked. Grain skins are pilled and then stored at -12 °C. Every sample is in triplicated. After all the samples were obtained, they have been analyzed.

trans-

(RM)

(min 99 %, HPLC),
CHROMASOLV®HPLC grade

Sigma-Aldrich.

RM

100 mg/L,

trans-
10 mg
100

RM
ml,
12 C.

trans-
0.05,

0.50, 1.0, 2.0 5.0 mg/L.

Thermo HPLC Surveyor LC
Pump Plus, Surveyor Auto sampler Plus
Surveyor photodiodearray detector PDA
Plus C18 column Hypersil Gold.

0.45 µm

1.0 mL/min;

= 306nm; Runtime: 10min

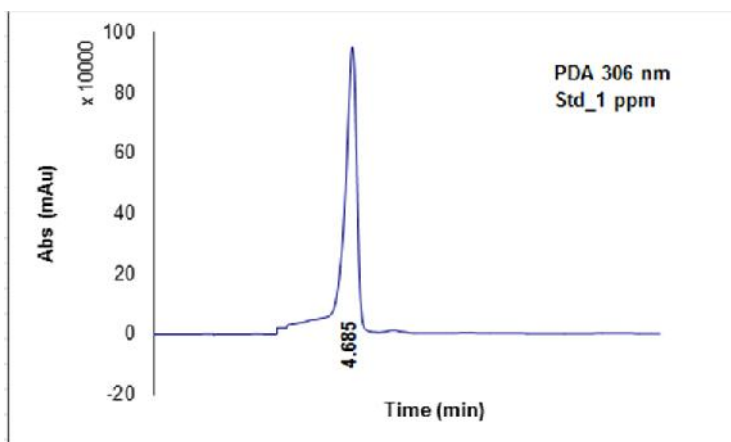
4.7

(1).

Used method

The chosen method for analyzing the quantitative content of *trans*-resveratrol is high performance liquid chromatography. For its application, reference material (RM) was used to construct a linearity plot. Reference material (RM), *trans*-resveratrol (min 99%, HPLC), methanol and acetonitrile CHROMASOLV®HPLC grade and glacial acetic acid are supplied by Sigma-Aldrich. From this RM, a 100 mg/L *trans*-resveratrol standard stock solution was prepared, such as 10 mg of RM in methanol to a final volume of 100 ml, which was stored in the dark at -12 °C. For the construction of the linearity plot, the standard stock solution was prepared by diluting working solutions of *trans*-resveratrol with a final concentration of 0.05, 0.50, 1.0, 2.0 and 5.0 mg/L. Thermo HPLC system was used with Surveyor LC Pump Plus, Survey or Autosampler Plus and Surveyor photodiode array detector PDA Plus and C18 column Hypersil Gold. Chromatographic conditions are: Mobile phase - water-acetonitrile-glacial acetic acid (70:29.9:0.1, v/v/v) was filtered through a 0.45 µm membrane and degassed before use; Elution – Isocratic at a flowrate of 1.0 ml.min⁻¹; Detection at = 306 nm; Runtime: 10min.

Under described chromatographic conditions, chromatograms were obtained – a primary record of the analysis, which showed that the retention time of the polyphenol sought was approximately 4.7 minutes (Figure 1).

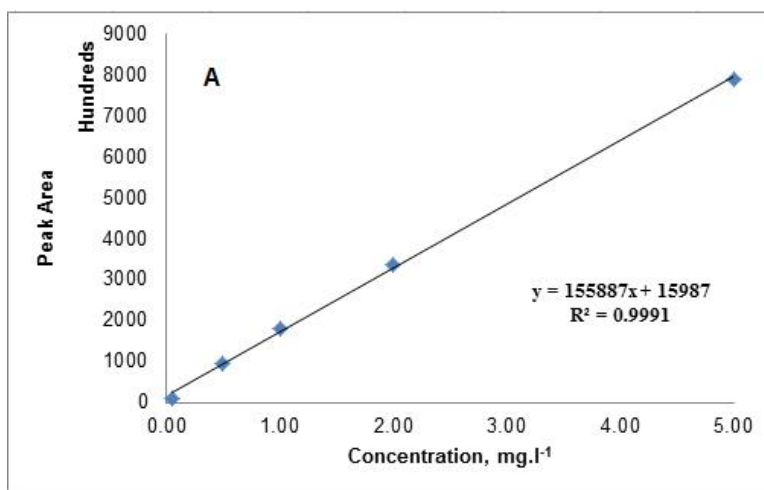


1. a *trans*-
1 mg/L

Fig. 1. Typical chromatogram of referent materials solution, containing 1 mg/L *trans*-resveratrol

mg/L e
(
, $R^2 = 0.998$),
(2).

Linearity plot has been done. An excellent linear dependence (regression coefficient, $R^2 = 0.998$) is achieved in the working range from 0.05 to 5.0 mg/L which is guarantee for accurate and reproducible results (Figure 2).

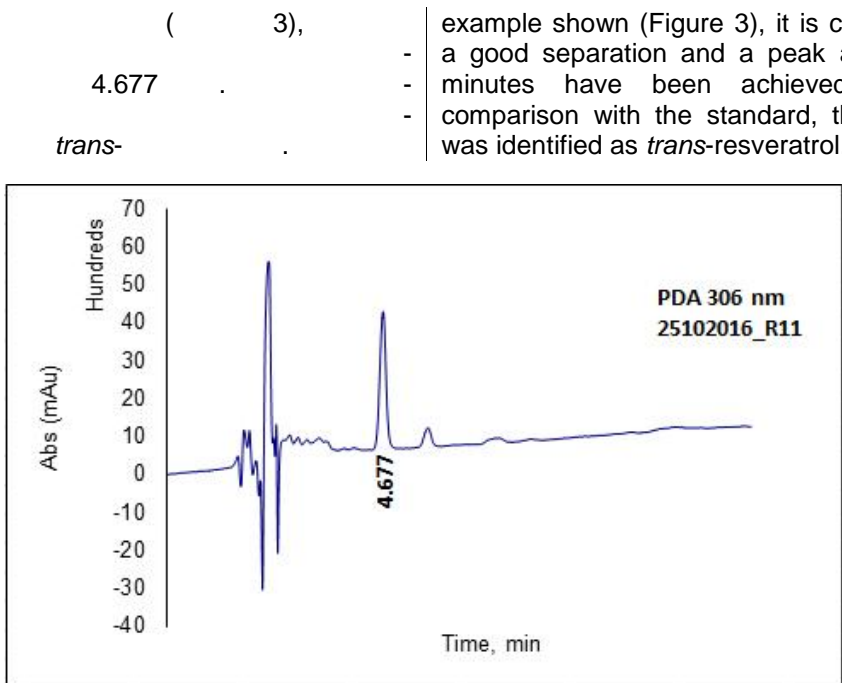


2. *trans*-

Fig. 2. Linearity plot of *trans*-resveratrol

RESULTS AND DISCUSSION

Chromatograms – primary analysis results were obtained under the described chromatographic conditions. From the



3. **Fig. 3. Typical chromatograms of sample solution**

trans-
 10
 15.03±0.78mg/kg
trans-
trans-
 (Cantos et al.,
 2000; Landfeld et al., 2015).
trans-
 - 1,24 ±0.13 mg/kg

example shown (Figure 3), it is clear that
 - a good separation and a peak at 4,677
 - minutes have been achieved. After
 - comparison with the standard, the peak
 was identified as *trans*-resveratrol.

The results of the assay for
 quantitative determination of *trans*-
 resveratrol in samples of studied varieties
 are presented in Table 1. From among all
 samples tested, the highest content of
trans-resveratrol - 15.03 ±0.78 mg/kg was
 found in the skins of berries from the
 Moldova variety. Moldova variety has a
 high resistance to stress factors, high
 fertility and yield.

Taking into account its rich content of
trans-resveratrol, it is justified to increase
 an area planted with it. The variety is
 suitable for storage, during which time the
 content of *trans*-resveratrol can be
 increased (Cantos et al., 2000; Landfeld
 et al., 2015).

The other table grape variety – Muskat
 Hamburg included in the present study
 has the lowest content of *trans*-
 resveratrol – 1.24 ± 0.13 mg/kg of all
 studied varieties.

Table 1. *Trans-resveratrol* content in the berry skins of the studied grape varieties

	/Region	/Variety	/ Year of production	<i>trans-resveratrol</i> in grape skins, mg/kg
1.	the village of Elenovo, Nova Zagora municipality	Mavrud	2016	1.81 ±0.14
2.	the village of Elenovo, Nova Zagora municipality	55 Melnik 55	2016	3.97 ±0.42
3.	Institute of viticulture and enology, the town of Pleven	Muscat Hamburg	2016	1.24 ±0.13
4.	Institute of viticulture and enology, the town of Pleven	Moldova	2016	15.03 ±0.78
5.	Institute of viticulture and enology, the town of Pleven	Plevenski kolorit	2016	10.02 ±0.84
6.	Institute of viticulture and enology, the town of Pleven	Storgosia	2016	1.78 ±0.84
7.	Institute of viticulture and enology, the town of Pleven	Rubin	2016	8.18 ±0.76
8.	the village of Mogilovo, Chirpan municipality	Merlot	2016	1.46 ±0.12
9.	the village of Mogilovo, Chirpan municipality	Cabernet Sauvignon	2016	1.71 ±0.11
10.	the village of Mogilovo, Chirpan municipality	Syrah	2016	12.33 ±0.62

- *trans-*
12.33 ±0.62
mg/kg.
8.18 ±0.76mg/kg,
10.02 ±0.84 mg/kg
trans-
(Laszlo et al.,
2005)
trans-

The highest content of *trans-resveratrol* in the berry skin among the wine varieties have been recorded for Syrah variety with 12.33 ± 0.62 mg/kg. Relevant value has been registered for Plevenski kolorit and Rubin varieties – 10.02 ± 0.84 mg/kg and 8.18 ±0.76 mg/kg, respectively.

There are numerous studies on the amount of *trans-resveratrol* in wine. In the study of wines from southern Hungary (Laszlo et al., 2005) it was found that the amount of *trans-resveratrol* varied over the years and between the varieties Syrah, Cabernet Sauvignon and Merlot, and the values among these varieties are

2002)	(<i>trans-</i>	1.71 ±0.11 mg/kg
1.46 ±0.12 mg/kg.	-		<i>trans-</i>	
			<i>trans-</i>	1.78 ±0.84 mg/kg.
			55	
			<i>trans-</i>	3.97 ±0,42
mg/kg	1,81 ±0.14 mg/kg.			
-				
			<i>trans-</i>	– 15,03 ±0.78 mg/k
			<i>trans-</i>	– 12.33 ±0.62 mg/kg.
mg/kg				– 10.02 ±0.84
				– 8.18 ±0.76mg/kg.

close (excluding 2002). In our study, the *trans-resveratrol* content in the grain skins of Cabernet Sauvignon and Merlot varieties is 1.71 ± 0.11 mg/kg and 1.46 ± 0.12 mg/kg, respectively.

The big difference in the higher content of *trans-resveratrol* in the Syrah variety may be due to the fact that the variety is cultivated according to the organic farming rules and the vineyard is in a transitional period. Subsequent analyses of grape samples in subsequent vegetations are required to confirm this assumption. The Storgozia variety is closely related to the Merlot and Cabernet sauvignon varieties in terms of the *trans-resveratrol* content and its present is 1.78 ± 0.84 mg/kg.

The biological method of cultivation is applied for Melnik55 and Mavrud varieties. The *trans-resveratrol* contents are 3.97 ± 0.42 mg/kg and 1.81 ± 0.14 mg/kg, respectively. The significantly lower content of the studied stilbene compared to its content in the Syrah variety can be interpreted with a different region in which these varieties have been planted and different influence of soil-climatic conditions, of a surely. This again demonstrated necessitates ongoing research and a reassessment of the climatic conditions during vegetation.

CONCLUSIONS

Among all table grape varieties included in this study, with the highest *trans-resveratrol* content of 15.03 ± 0.78 mg/kg is Moldova variety. This variety may be recommended as suitable for fresh consumption due to its high content of the stilbene.

The highest content of *trans-resveratrol* in the grain skin of the wine varieties included in the study is the Syrah variety – 12.33 ± 0.62 mg/kg. Plevenski kolorit variety (10.02 ± 0.84 mg/kg) and Rubin variety (8.18 ± 0.76 mg/kg) have relevant value.

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*E-mail: stoyanovnick@yahoo.com

Influence of the process of cold maceration and Non-saccharomyces yeast application in red winemaking

Nikolay Stoyanov^{1*}, Panko Mitev¹, Silvia Tagareva¹,
Stefcho Kemilev¹, Irina Melnik²

¹University of Food Technologies, Plovdiv, Bulgaria

²Odessa National Academy of Food Technologies, Odessa, Ukraine

SUMMARY

Red grape variety Merlot is vinified and two types of fermentation are applied – conventional winemaking process and other – cold maceration before alcoholic fermentation. Each of the samples is vinified with Non-saccharomyces - *Kluyveromyces thermotolerans* inoculation done 48 ours before Saccharomyces addition. For each trial the control sample is realized using only *Saccharomyces* yeasts. The resulting wines are analyzed for some basic compounds and also some specific aromatic compound is determined using GC-MS. High concentration of some ethyl esters are determined in Non-saccharomyces wine and also more neutral polysaccharides.

Key words: red wine, cold maceration, Non-saccharomyces yeast, GC-MS

INTRODUCTION

- The wine is the product of grape juice or crushed grape fermentation. Alcoholic fermentation is most commonly carried out by yeast *Saccharomyces cerevisiae*, which metabolizes grape sugars mainly in ethanol, CO₂ and glycerol as well as a number of other metabolites. Numerous studies of starter cultures are directed solely at *S. cerevisiae* for higher ethanol yield, better control of alcohol fermentation and inhibition of growth of non-*Saccharomyces* yeast in must, which may lead to stuck fermentation and other possible unwanted processes (Castelli, 1954; Spasov, 2012; Yoncheva, 2003). Due to the fact that non-*Saccharomyces* are often isolated from slow or stopped fermentations (Castelli, 1954, Ribereau and Peynaud, 1960), they have often been considered as a "harmful" microflora. Today, this group of microorganisms is becoming more and more interested. Non-*Saccharomyces* yeast is typically present in grape, grape must and on the winemaking equipment (Ciani et al., 2010). The most important strains are *Hanseniaspora / Kloeckera*, *Candida*, *Pichia*, *Zygosaccharomyces*, *Schizosaccharomyces*, *Torulaspota*, *Lachancea (Kluyveromyces)* and *Metschnikowia* (Fleet et al., 1984; Heard and Fleet, 1985; Pardo et al., 1989; Kurtzman, 2003).

- A number of studies investigate the influence of non-*Saccharomyces* yeast on the composition and sensory properties of the wine (Lema et al., 1996, and Egli et al., 1998, Henick-Kling et al., 1998). For these reasons over the past decade, the role of non-*Saccharomyces* in winemaking has been re-evaluated in terms of the possibility of the wine quality improving and increasing of the wine taste and aroma complexity (Rojas et al., 2001; Jolly et al., 2003, 2006; Swiegers et al., 2005; Domizio et al., 2007; Renouf et al., 2007; Renouf et al., 2007; Renouf et al., 2007; Anfang et al., 2009; Sadoudi et al., 2012).

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In some studies also are treated the improvement of "technological" parameters by revealing that co-inoculated fermentations with *Lach. thermotolerans* and *S. cerevisiae* also show reduced amounts of volatile acidity (Comitini et al., 2011). Increased concentrations of esters in mixed fermentations were also found. An increase concentration of ethyl-2-methylpropanoate and ethyl caprate (Gard-Cerdan and Ancin-Azpilicueta, 2006; Varela et al., 2009) was established in this fermentation. The study of Moreira et al (2008) confirms increased production of esters and reduction of ethyl acetate in mixed fermentations. Higher concentrations of 2-phenylethanol were also found when *T. delbrueckii* and *Lach. thermotolerans* participate in the fermentation (Comitini et al., 2011) as well as higher concentrations of glycerol (Ciani and Ferraro, 1998; Soden et al., 2000; Comitini et al., 2011). However, there are also contradictory data - the study by Gobbi et al. (2013) found a significant increase in ethylacetate concentrations, when *Lach. thermotolerans* and *S. cerevisiae* have been used as co-inoculants. Obviously, the applicability of non-*Saccharomyces* in wine fermentation is not unambiguous, and it is also necessary to take into account the influence of factors such as grape variety, process temperature, ethanol accumulation and a number of other factors that undoubtedly influence the growing of microorganisms in the grape must.

For a long time as a limiting factor for the non-*Saccharomyces* growing, it was mainly considered ethanol accumulation. More recent studies shown that *Kluyveromyces thermotolerans* (now classified as *Lachancea thermotolerans*) are less tolerant to the low oxygen levels and this affects to their growth more than their intolerance to the high ethanol level (Hansen et al., 2001).

Lachancea thermotolerans (*thermotolerans*),
 2-
thermotolerans/*S. cerevisiae*
 " " "
cerevisiae (Gobbi et al., 2013).
thermotolerans
S. Cerevisiae
 (Kapsopoulou et al., 2007; Gobbi et al., 2013).

Saccharomyces
Kluyveromyces thermotolerans,
 -
Kluyveromyces thermotolerans,

2014
 10 kg
 8÷10
 60 kg
 25 mg/kg.
 5
 70 dm³,

K. The main features of *Lachancea thermotolerans* (formerly *K. thermotolerans*) are the production of higher levels of lactic acid, glycerol and 2-phenylethanol during mixed fermentations of grape must. It was also reported for change in organoleptic properties of the wine co-inoculated with *K. thermotolerans* / *S. cerevisiae* in which wines "spicy flavors" and "freshness" are improved than in the wine produced only with *S. cerevisiae* (Gobbi et al., 2013). However, it is noted that the influence of the *L. thermotolerans* growing on the wine chemical composition also substantially depends on the time of inoculation with *S. cerevisiae* (Kapsopoulou et al., 2007; Gobbi et al., 2013).

- This relatively large number of data on the use of non-*Saccharomyces* and in particular *Kluyveromyces thermotolerans*, as well as somewhat unambiguous results, was also the starting point of the present study - to examine the chemical composition and organoleptic properties of wine produced by co-inoculation with *Kluyveromyces thermotolerans*, as well as the impact of a technological approach such as cold grape must maceration.

MATERIAL AND METHODS

- The experiment was conducted in the 2014 vintage using Merlot grapes variety. The grapes are picked in 10 kg cases and cooled in a refrigerated chamber up to 8 ÷ 10 °. During the processing, a uniform distribution was made, as the grapes from each case were distributed in five places and samples of 60 kg each were formed. The homogeneous samples are crushed and sulfur dioxide at 25 mg/kg is added to the fermentation tanks.

- For cold maceration and fermentation, 5 fermentation tanks with a volume of 70 dm³ are used, each fitted with a glycol cooling jacket and temperature

20÷22 °

Saccharomyces (*Viniflora Merit* –
Chr.Hansen) – (1) 20
g/HL, *Kluyveromyces thermotolerans*
(*Viniflora Concerto* –
Chr.Hansen) 25 g/HL –
.1(2) 50 g/HL – .2 (3).
.1 .2 48
Viniflora Merit
- 20 g/HL.

24÷26 ° .

25 % *Saccharomyces*
(.2 (4)),
Kluyveromyces
thermotolerans (*Viniflora*
Concerto – *Chr.Hansen*) 30 g/HL –
.3(5). .2 .3

11 ÷13 ° , .3 72
Viniflora Merit
(20 g/HL).
.2
Viniflora Merit (
75 %). .2 .3
11 ÷13 °
6 ,
24÷26 °

.3
Viniflora Concerto

12 ° .
1.000,
3

controllers. In the first three samples, the temperature of the crushed grape is increased to 20-22 °C and inoculated with *Saccharomyces* (product *Viniflora Merit* - Chr.Hansen) - control (K (1)) at a dose of 20 g/HL and *Kluyveromyces thermotolerans* (product *Viniflora Concerto* - Chr. Hansen) at doses of 25 g/HL - sample p.1 (2) and 50 g/HL - sample p.2 (3). For samples p.1 and p.2, after 48 hours, *Viniflora Merit* was added at a standard dose of 20 g/HL. In these three variants, the alcoholic fermentation process is carried out at a temperature of 24 ÷ 26 ° .

In the other two samples, the process of cold maceration was carried out, and the fourth variant was pre-inoculated with 25% of the dose of *Saccharomyces* (Control K.2 (4)) and the last sample was inoculated with *Kluyveromyces thermotolerans* (product *Viniflora Concerto* - Chr. Hansen) at a dose of 30 g/HL - sample p.3 (5). In samples K.2 and p.3 is maintained a temperature in the range of 11 ÷ 13 ° , and at p.3 after 72 hours a *Viniflora Merit* was added in a standard dose (20 g/HL). At the same time, in the K2 is added the remaining *Viniflora Merit* quantity (the remaining 75% of the dose). For samples K.2 and p.3, a temperature of 11 ÷ 13 °C is maintained for a total of 6 days, then increased to 24 ÷ 26 ° and maintained until the fermentation is complete. In the p.3 sample after rehydration, the *Viniflora Concerto* culture has gone through a period of two consecutive addition of must with low temperature and a gradual decrease in temperature to 12 °C.

After reaching a relative density of 1,000, each sample is stay for 3 days with the solids, after which the wine is drained and the marks are pressed. The press fraction is added to the bulk quantity.

After complete alcoholic fermentation, each wine is inoculated with lactic acid

Oenococcus oeni (Viniflora CH16 – Chr. Hansen).

25 mg/L
8÷10 °

(Method OIV-MA-AS311-01A).

AS313-02).

(2013)

FC (Méthode OIV-MA-AS2-10)

(GC-MS: 280nm)

(2011)

: 1.0 ml ()

(3 10 sec).
(10 min/22°C/13000 rpm).

500 µl
()

100 µl

7890

bacteria *Oenococcus oeni* (Viniflora CH16 - Chr. Hansen). After the malolactic fermentation, all samples were separated from sediments, sulfur dioxide is added to the free SO₂ content - 25 mg/L and stored at a temperature of 8 ÷ 10 °C until physicochemical analyzes were performed.

Methods of analysis:

Samples were analyzed by standard physicochemical analyzes:

- Determination of titratable acids expressed as tartaric acid (Method OIV-MA-AS313-01).

- Determination of the content of reducing sugars in wine (Method OIV-MA-AS311-01A).

- Determination of the volatile acids content expressed as acetic acid (Méthode OIV-MA-AS313-02).

- Determination of the alcohol content of the wine (by Dujardin-salleron ebulliometer)

- Determination of neutral polysaccharide content Delcheva (2013)

- Determination of the content of total phenolic compounds by the FC method (Méthode OIV-MA-AS2-10)

- A polyphenol index determined by absorbance at 280nm (from exercise manual).

- GC-MS: Preparation of samples and analysis are by Bav ar (2011) with some modification: Sample preparation: An equivalent amount of dichloromethane is added to 1.0 ml of wine (distillate). The resulting solution was vortexed (3 x 10 sec). Following is centrifugation (10 min/22 °C/13000 rpm). Gently pipette 500 µl of the organic phase (bottom layer) and evaporate to dryness under vacuum. To the dry residue was added 100 µl of dichloromethane.

Chromatographic conditions: The solution is injected into a system consisting of a gas chromatograph 7890A

(Agilent Technologies) -
 5975 (Agilent Technologies).
 HP-5ms -
 : 30 m, 0.32
 mm -
 0.25 μ m -
 : 40
 °C, 0 min,
 230 °C 5 °C/min, 10 min;
 250 °C; -
 1.0 ml/min; -
 m/z = 50 - 550;
 1 μ l
 (splitless).
 -
 (RI)
 -
 NIST'08 (National Institute of Standards and
 Technology, USA).

(Agilent Technologies) and a 5975C mass
 spectrometer (Agilent Technologies). The
 HP-5ms column was used with the
 following parameters: length 30 m,
 diameter 0.32 mm and film thickness 0.
 25 μ m with the following temperature
 program: start temperature 40 °C, hold 0
 min, increase to 230 °C with 5 °C/min,
 retention 10 min; Injector and detector
 temperatures 250 °C; Helium carrier gas
 at a flow rate of 1.0 ml/min; Range of
 scanning of the mass detector
 m/z = 50-550; Injected volume of sample
 1 μ l in splitless mode.

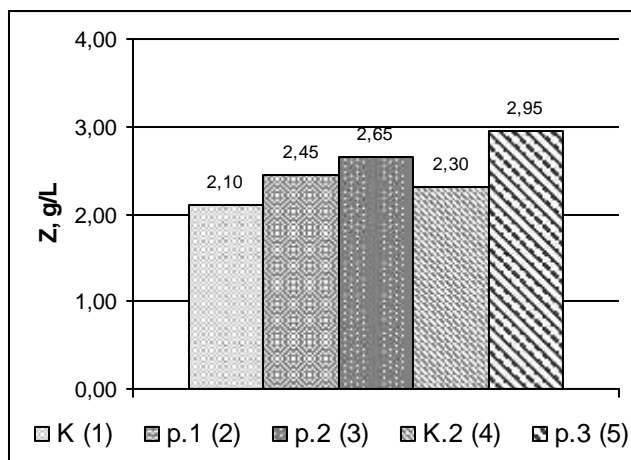
Compounds were identified by
 comparing retention times and relative
 indexes of Kovac (RI) to those of standard
 substances and mass spectral data from
 the NIST'08 library (National Institute of
 Standards and Technology, USA).

RESULTS AND DISCUSSION

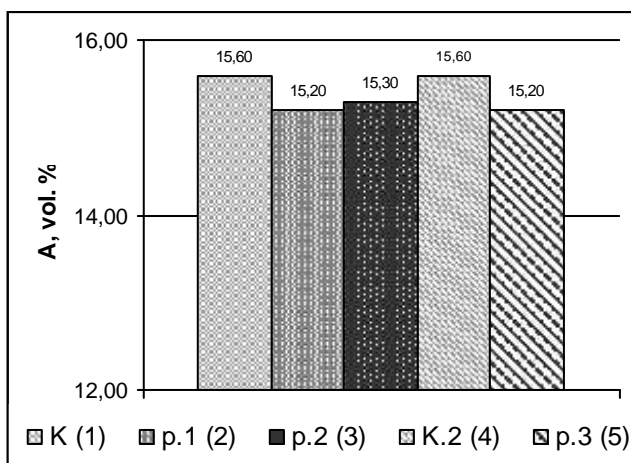
The content of residual sugars in
 the samples (Figure 1) shows that each
 samples are fermented to dry, moreover
 with a high sugar content of grape and
 obtained high alcohol content in the wine
 samples (Figure 2).

Indeed higher concentration of residual
 sugars was found in the samples obtained
 with *Kluyveromyces thermotolerans* but
 also a lower alcohol content of the wine
 which could be used as an opportunity to
 reduce the alcoholic strength of wines
 obtained In hot wine regions from grapes
 with high sugar content.

(1),
 ,
 (2). ,
 ,
Kluyveromyces thermotolerans
 -
 ,
 ,
 ,
 .



.1. (Z)
Fig. 1. Residual sugar content in wine samples

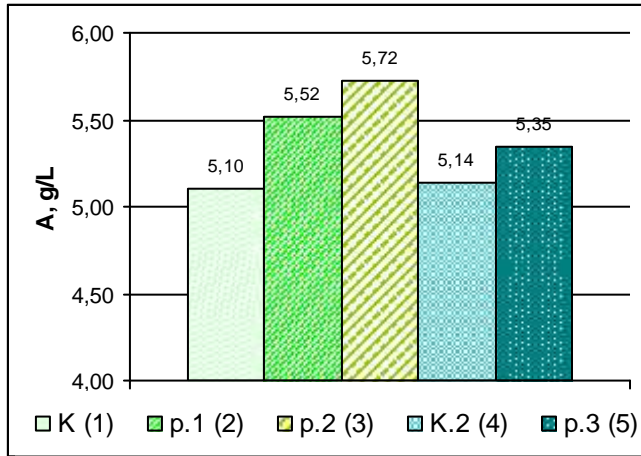


.2. ()
Fig. 2. Alcohol content in wine samples

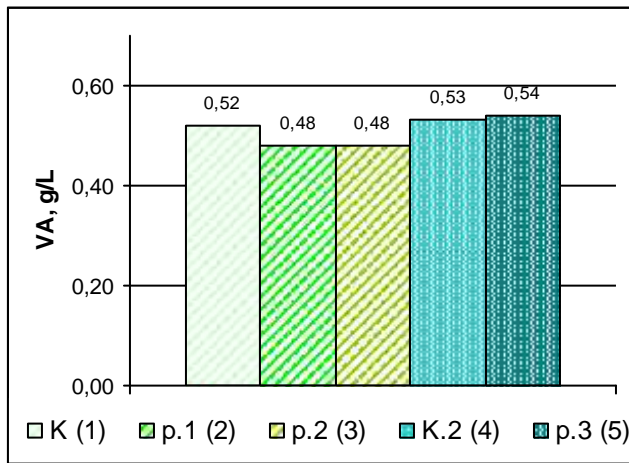
Viniflora Concerto ,
 -
 (3).
 (4) ,
Kluyveromyces thermotolerans ,

- In samples inoculated with *Viniflora Concerto* as expected a higher total acid content was established (Figure 3). At the same time, the content of volatile acids (Figure 4) in these samples does not increase, which supports the possibility inoculation with *Kluyveromyces thermotolerans* prior to alcoholic fermentation to be a technological possibility to obtain wines with lower alcoholic and improved acid content in wine regions characterized by high temperatures and grapes with high sugar

content.



3. ()
Fig. 3. Titratable acidity content in wine samples



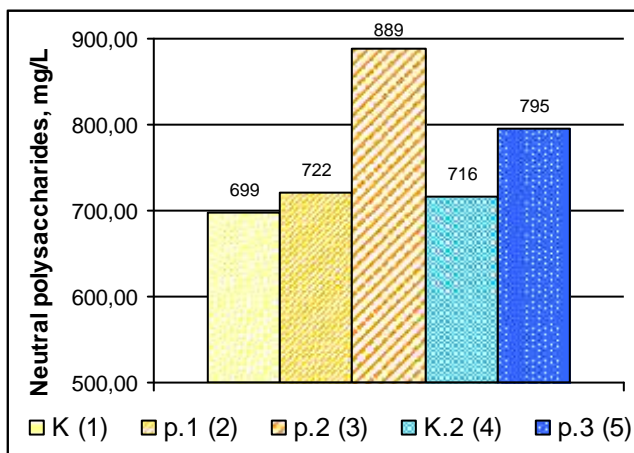
4. ()
Fig. 4. Volatile acidity content in wine samples

()
).
 ,
Viniflora Concerto –
 .2,
Viniflora Concerto
 – .3,
 -

The content of total phenolic compounds and index polyphenol does not differ significantly in the five samples (data from the analysis was not presented). At the same time, higher concentrations of neutral polysaccharides (Figure 5) and higher dry extracts (Figure 6) were found in the wine inoculated with a double dose of *Viniflora Concerto* - sample p.2, as well as in the sample

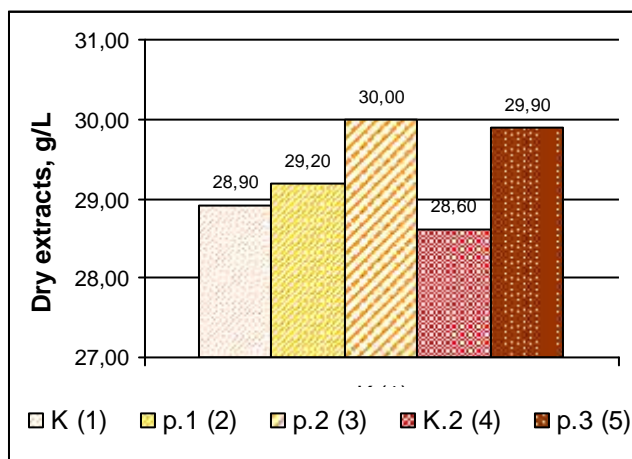
(5)
 (6).
Kluyveromyces thermotolerans
 -
 50 g/HL
 27 %.

inoculated with *Viniflora Concerto* and cold maceration applied - sample p.3. Obviously, the higher concentration of *Kluyveromyces thermotolerans* yeast cells has affected the content of that fraction of polysaccharides, which is considered to be the main source of the present yeasts in the wine and inoculation with 50 g/HL *Viniflora Concerto* is resulted to neutral polysaccharides content increase with 27 % compared with the control sample.



. 5.

Fig. 5. Neutral polysaccharides content in wine samples



. 6.

Fig. 6. Dry extracts content in wine samples

thermotolerans

7)

Kluyveromyces

-

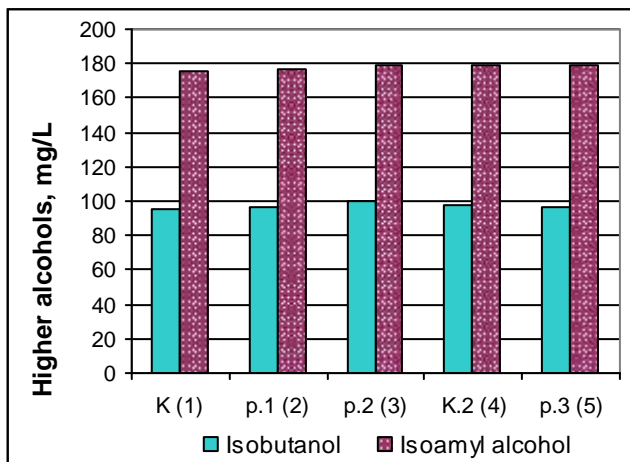
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The presence of *Kluyveromyces thermotolerans* during alcoholic fermentation did not result in the production of higher concentrations of higher alcohols (Figure 7), as the concentrations of the two abundant representatives - isoamyl and isobutyl alcohol remained relatively unchanged in the samples.



. 7.
Fig. 7. Higher alcohols content in wine samples

(8).

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(.1).

Viniflora Concerto,

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.3

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.2,

The using of cold maceration did not lead to an increase in the concentrations of some specific esters (Figure 8). The concentrations of ethylphenylacetate and ethyl octanoate, which aroma is associated with dried herbs, anise and sugared fruit syrup, are even lower in the control obtained with cold maceration (K.2) compared to the standard control samle (K.1).

Inoculation with *Viniflora Concerto*, however, resulted in "repairing" for these lower levels, and in sample p.3 were determined almost identical concentrations (ethylphenylacetate) or even higher (ethyloctanoate) than the standard control (K.1). The highest concentrations of the cited esters are found in sample p.2 obtained with a double level of *Viniflora Concerto* and a

Viniflora Concerto

(9),

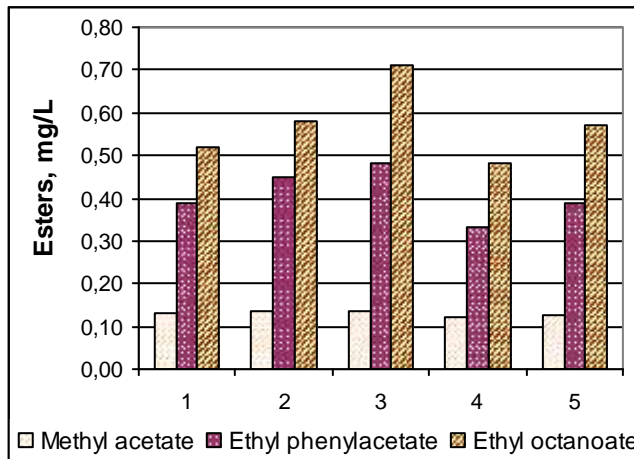
(10),

Viniflora Concerto – .3,

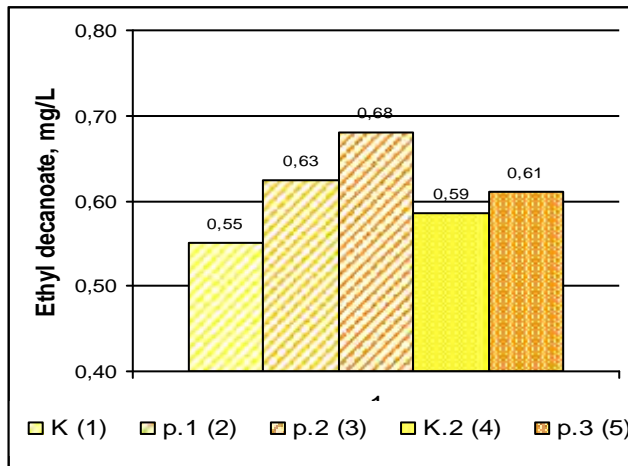
Kluyveromyces thermotolerans

standard fermentation temperature. In the same sample, the highest concentrations of ethyl decanoate (Figure 9) were found, the flavor of which is associated with floral and oily floral.

By comparing the winemaking at standard temperature of fermentation with cold maceration, in the second approach higher ethyl decanoate concentration is determined. Apparently this practice would encourage the appearance of floral and fruit aromas in wine to a higher degree, which is also supported by the established concentrations of ethylisovaleryate (Figure 10), whose aroma is associated with pineapple and sweet fruits. Highest concentrations of the quoted compound were found in the cold maceration sample and using *Viniflora Concerto* - p.3, which confirms the view that even at low temperatures of cold maceration, *Kluyveromyces thermotolerans* are able to grows and their specific metabolism contributes to enhancement the fruit and floral aromas in the wine.

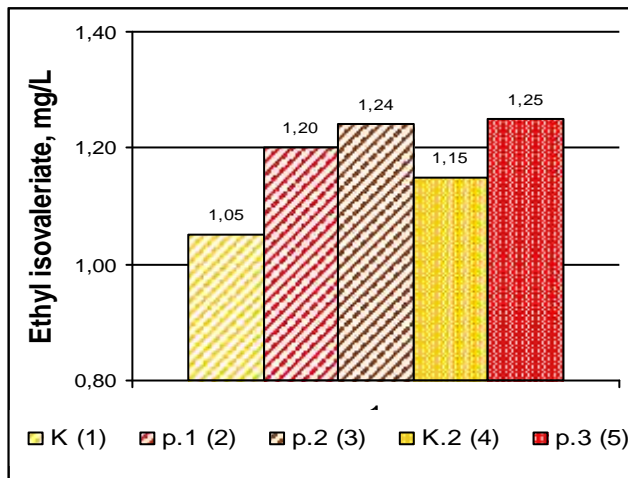


. 8.
Fig. 8. Esters content in wine samples



. 9.

Fig. 9. Ethyl decanoate content in wine samples



. 10.

Fig. 10. Ethyl isovalerate content in wine samples

Concerto	Viniflora
3-	(
11),	,
.	.2,
.	-
30 %	-

The use of *Viniflora Concerto* also resulted in higher levels of 3-mercaptohexanol (the aroma of tropical fruit) in the samples (Figure 11), higher concentrations of it are determined in the samples obtained under the standard temperature of fermentation. In sample p.2 obtained with double dose *Viniflora Concerto*, the highest values were found and a nearly 30 % increase compared with the control sample (K.1).

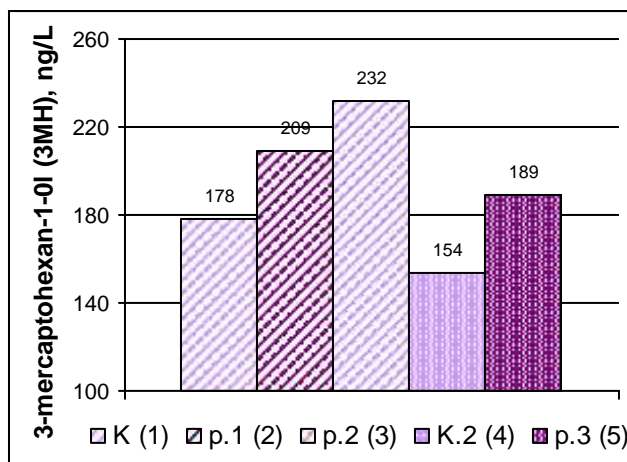


Fig. 11. 3-Mercaptohexan-1-ol content in wine samples

CONCLUSIONS

- In both used vinification methods of
- *Merlot* grape variety – conventional
- process and cold maceration before
- alcoholic fermentation, inoculation with
- non-*sacharomyces* yeast – *Kluyveromyces*
- *thermotolerans* (commercial product
- *Viniflora Concerto* - Chr. Hansen) resulted
- in higher amounts of total acids and
- neutral polysaccharides in trial wines
- compared to the controls. This is most
- definitely in the sample obtained under a
- standard vinification temperature and a
- double dose of *Kluyveromyces thermotolerans*
- inoculation.

- Using a relatively uniform equal quantity
- of *Viniflora Concerto*, higher
- concentrations of polysaccharides were
- found in the wine, in which making also
- cold maceration is applied. In trial
- samples wines also detected higher
- concentrations of ethyl esters as well as
- 3-mercaptohexanol (3MH), associated
- with floral and fruity wine aromas. The
- highest concentrations of which were
- found using inoculation with a double
- dose. Comparing the same starting
- quantities of *Kluyveromyces*
- *thermotolerans*, higher concentrations of
- 3MH was determined in the "standard"

Kluyveromyces thermotolerans (
Viniflora Concerto – Chr.Hansen)

Kluyveromyces thermotolerans.

Concerto -

3- (3),

Kluyveromyces thermotolerans,

„

- 3 , - temperature fermentation wine sample
- compared with this a cold maceration also
- is used.

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Viniflora
Concerto, Viniflora Merit Viniflora CH16

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