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INFLUENCE OF ENZYMATIC TREATMENT AND STORAGE TEMPERATURE ON THE AROMATIC COMPOSITION OF VIORICA WINES

Natalia Vladei*, ORCID: 0000-0003-1094-6812,

Technical University of Moldova, 168, bd. Stefan cel Mare, Chisinau, Republic of Moldova
**natalia.furtuna@adm.utm.md*

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Abstract. The subjects of this paper are the grapes and wines obtained from local selection variety *Viorica* from Republic of Moldova. Following the spectrophotometric analysis of grape juice, significant differences between the content of free volatile terpenes and potentially volatile terpenes were noticed. In order to determine the influence of the enzymatic treatment on flavor compounds, the wines were subjected to GC-MS analysis. The analysis showed that musts that were not treated with enzymes have a concentration of about 40% lower terpenes compared to those macerated with pectolytic enzymes. Wine is constantly evolving and a particular interest is represented by the dynamics of odorous substances during wine storage. For this purpose wines produced by maceration with and without addition of pectolytic enzymes were analyzed. Young wines and wines aged for 6 months at different temperatures were studied. Experimental data showed that free and bound terpene compounds are significantly influenced by the storage temperature as well as by the origins of terpene compounds.

Keywords: *aroma, enzyme, flavor, free/bound terpenes, local selection variety, maceration, wine.*

Introduction

The aromatic profile is a fundamental element of the wine quality, and its handling is a challenge, first of all for the production of quality wines and also for producing products according to defined criteria [1]. The aromatic potential of grapes is characterized by the existence in berries of two types of flavors: free (odorous) and related to sugars (non-odorous), also known as "flavor precursors" because they have the ability to turn into volatile compounds, participating in the formation of the aromatic complex in wines after processing the grapes [2].

The quality of a wine is directly proportional to the chemical composition of the grapes, and the aromatic potential becomes a necessity for defining and controlling the overall aromatic quality of the wine [3]. This characteristic of the wine is essentially determined by the composition of the varietal aroma. The control of the quality and typicality of the wine aroma presupposes the knowledge of the aromatic precursors of the grapes, which are responsible for its aroma and the evaluation of the influence of some viticultural parameters on these precursors [4, 5].

The success of a wine on the world wine market is dictated by the originality and the typicality of it, these are due also to the terroir, including viticultural and oenological practices [1].

In Republic of Moldova, we cultivate mainly grape varieties of European origin, less than indigenous ones, that undermines the typicality of the wine. In this context, the aim of this work is to highlight the aromatic peculiarities of new selection grape variety – *Viorica*.

In Moldova there are very good climate and soil conditions for the cultivation of new selection interspecific hybrids, including the *Viorica* variety. The interest for *Viorica* variety has significantly increased in recent years. However, many vintners and winemakers are not sufficiently familiar with this variety.

This variety has a comprehensive resistance to biotic and abiotic environmental factors, being quite productive, with a particularly pronounced, harmonious aroma, with great technological potential for quality wines production, including organic ones [6]. Also, the planting of such varieties contributes both to the diversification of products and to the shaping of a new wine identity specific and typical for our country.

The new selection grape variety *Viorica* was created in 1969 within the Institute of Viticulture and Winemaking of the Republic of Moldova by crossing the interspecific hybrid *Seibel* 13-666 (France) and *Aleatico* (Italy), and in 1990 it was approved and included in the viticulture register of Republic of Moldova. The *Viorica* variety is classified as being an aromatic variety. Moreover, in the good harvest years, due to the high level of titratable acids and sugar, in addition to quiet dry and semi-dry wines, high quality sparkling wines can be produced [6].

Viorica dry white wine is described by a straw-yellow color, with greenish reflections. Intense aromas specific to the Muscat variety: white flowers (acacia, jasmine, linden flowers). The aroma of wine harmoniously interweaves a lot of flavors: muscat notes, spices with a predominance of bright, memorable flavor of basil, which define the typicality of this variety. A light aeration helps to open up the flower and fruit flavors: acacia, apricot and citrus fruits [7].

The analysis of flavor and odor composition can be challenging. When key components, responsible for specific aroma, are known and quantified, they can be utilized as a tool to optimize viticultural and oenological practices to obtain maximum grape and wine quality [8, 9, 10].

Experiment

In order to carry out this study, the grapes of local selection grape variety *Viorica* were manually harvested from the plantations of the Scientific-Practical Institute of Horticulture and Food Technologies from Chisinau and processed under micro-vinification conditions at the Technical University of Moldova.

Viorica is an interspecific grapes hybrid (*Seibel*13-666 x *Aleatico*) with medium or medium-late maturation period (depending on the climatic conditions of the year). The grapes are of medium size, cylindrical-conical in shape, sometimes winged, medium-compact berries. The berries are white, round, medium sized, when ripen they are amber-yellow covered with intense bloom, the part exposed to the sun often with a light bronze. The skin is thick, the pulp is juicy with muscat flavor [6]. *Viorica* grapes have a high sugar accumulation capacity, which depending on the climatic conditions of the year varies between 190–210 $g{\cdot}L^{-1}$. At the same time, one of the peculiarities of the variety is the increased content of titratable acids that varies between 8.5–9.5 g·L^{-1}, which favors it compared to other varieties. Due to these characteristics, *Viorica* can be used both for the production of dry white wines and for production of quality sparkling wines (as raw material) [7].

In order to obtain information regarding the evolution of aroma compounds from grapes to wine and afterwards with maturation, the free and glycozidic monoterpenes were determined by the spectro-photometric method in grapes and wines at different stages.

Fresh grapes were picked at random from whole bunches to give total sample mass of 500 grams. Grape samples were frozen immediately after being picked. Prior to analysis, the grapes were thawed, homogenised, and filtered through cheesecloth. The liquid must was then centrifuged at 4ºC (150 rpm) for 15 minutes.

Also, the influence of pectolytic enzymes addition during maceration was studied. The enzyme preparation Zymovarietal Aroma G (Sodinal, France) contains granulated pectinase with beta-glycosidase activity and was added to the must immediately after crushing. The maceration was carried out for 8 hours at 15 ^{o}C, in half of the total volume was added 2 g·hL^{-1} of enzyme. Both variants of wine were subject of the addition of sulfur dioxide in a concentration of 75-100 mg·L^{-1} applied on marc when macerating, gravitational racking and sulphitation after the completion of alcoholic fermentation.

The principle of the spectro-photometric determination method is based on separation of aroma compounds by steam distillation and subsequent colorimetric determination of free terpenes in neutral conditions and of bound terpenes (glycosylated precursors) in acid conditions by the vanillin/sulfuric coloring reaction. The analysis were carried out in the same experimental conditions as in previous studies of the author [11].

The samples were also analyzed using a Shimadzu GC system composed from a single quadrupole mass-spectrometer GC/MS QP2010SE coupled with a three-dimensional automated system for the injection of samples AOC-5000. The GC/MS was equipped with a Rtx-5MS (30 m x 0.25 mm; 0.25 μm film thickness) fused silica capillary column. Helium was used as the carrier gas adjusted to 0.8 mL·min^{-1}; with splitless injection of 1 μL of a hexane solution; injector and interface temperature were 200 ºC; oven temperature programmed was 40-240 ºC at 8 ºC min^{-1}; ion source temperature was 200 ºC. The mass spectrophotometer was operated in the selective ion mode under autotune conditions and the area of each peak was determined by ChemStation software (Agilent Technologies).

After headspace extraction and GC/MS analysis, mass spectral identifications of compounds was carried out by comparing their GC mass and retention data with those held in the NIST-08 mass spectral library and FFNSC 1.3, a library which was specially developed for flavours and fragrances. Total ion chromatogram and mass fragment of each component was compared with mass spectra from mentioned libraries and confirmed with retention data of compounds [12].

Results and discussion

a. Aroma compounds from grapes

The content of free (FVT) and bound (PVT) monoterpenes reveals obvious dynamic changes during the evolution of grape berries [13, 14]. In case of grape varieties for winemaking, the knowledge about the distribution in juice and skin, as well as the content of FVT and PVT, are very valuable for the application of contact treatments between the solid and liquid phase in order to optimize the aromatic quality of the wines [15].

Following the spectrophotometric analysis of Viorica grape juice, there were noticed significant differences between the content of free volatile terpenes (0.175 mg·L^{-1}) and potentially volatile terpenes (0.312 mg·L^{-1}), the PVT/FVT ratio being 1.783. The higher content of terpene glycosides means an increased potential of volatile terpenes, thus suggesting a higher aromatic potential. The distribution of FVT and PVT in juice, pulp and skin varies differently depending on the contact duration [11].

Volatile compounds were quantified as area percentage of total volatiles from GC-MS analyses. Estimation of the quantity of individual components in the samples (in ppm or mg·L^{-1}) has not been attempted. The peaks were identified by their mass spectra and retention data. The most abundant volatile compounds identified in *Viorica* grape juice, including terpenes, alcohols, esters, aldehyde and ketone compounds are listed in the order of their elution in Table 1.

Several studies have reported that the terpenoid compounds could be used analytically for varietal characterization [2 - 4, 9, 13]. It is known that terpene compounds are secondary plant constituents [14, 16 - 18]. In Viorica grape juice, the presence of a large range of monoterpenes ($C_{10}H_{16}$) was identified: eucalyptol, linalool oxide, linalool, p-menthanone, α-isomenthone, DL-menthol, α-terpineol, nerol, menthyl acetate. All these monoterpenes earlier were identified in other grapes of flavoured varieties [3, 4, 12]. The terpene monohydroxilic alcohols (terpenols) are the most important compounds with flavoring potential, because these represent volatile free flavors from aromatic type grapes. From the quantitative point of view, they represent about 40-50% of volatile odorant compounds with a very low perception threshold (0.1-0.5 mg·L^{-1}) [16].

In the present study, the Viorica grapes juice is distinguished by a huge amount of p-menthanone and DL-menthol (nearly 50 %), that gives it minty and camphoreous notes to the wines obtained from this variety.

Table 1

Main volatile compounds found in *Viorica* grapes juice: GC/MS retention data, compound identification, CAS number [19] and olfactory characteristics

Retention time	Compound	CAS no.	Relative peak area, %	Olfactory descriptors	Odor type
4.053	Isoamyl alcohol	123-51-3	1.50	Fusel, alcoholic, cognac, fruity, banana	fermented
6.550	Hexanol	111-27-3	15.64	Etherial, fusel oil, fruity sweet with a green top note	herbal
9.987	Eucalyptol	470-82-6	12.64	Eucalyptus, herbal, camphor	herbal
10.852	Linalool oxide <cis-> (furanoid)	5989-33-3	0.41	Citrus green	citrus
11.350	Linalool	78-70-6	1.37	Citrus, orange, floral, terpy, waxy and rose	floral
12.455	p-Menthanone	10458-14-7	23.49	Minty	mentholic
12.669	α-Isomenthone	491-07-6	2.37	Mentholic, cooling, minty and camphoreous	mentholic

Continuation Table 1

12.809	**DL-Menthol**	**89-78-1**	**24.14**	**Peppermint, cool woody**	**mentholic**
13.163	α-Terpineol	98-55-5	3.26	Lilac, citrus, woody floral, lily of the valley	floral
14.261	Nerol	106-25-2	3.09	Fresh, citrus, floral, green, sweet, lemon, magnolia	floral
14.990	Menthyl acetate	16409-45-3	4.73	Tea cooling, minty, fruity, berry	mentholic
16.461	β-Damascenone	23696-85-7	2.54	Woody, sweet, fruity, green floral nuances	floral
16.515	Ethyl 9-decenoate	67233-91-4	0.32	Fruity fatty	fruity
16.637	Ethyl caprate	110-38-3	0.85	Sweet, waxy, fruity, apple	waxy

b. The influence of enzymatic treatment on the aromatic composition of wines

In order to determine the influence of the enzymatic treatment on flavor compounds, the wines were subjected to GC-MS analysis. The overall results were recorded in Table 2. Analyzing the data from the table, it can be observed an insignificant increase of the total content of volatile compounds in the case of maceration enzymes addition (about 4%), instead it can be seen important differences for each class of compounds separately.

Thus, when pectolytic enzymes were added, in *Viorica* wine, were registered esters quantity over 50% higher than in the control sample. Similar results were observed for norisoprenoids (62% more), terpenes and thiols (about 40% more than the control sample).

Given that the *Viorica* variety is a potentially aromatic variety, i.e. most flavor compounds are found in the form of precursors, these results have a very important role for the choice of winemaking technology. Furthermore, in Table 2, in the case of wines with the enzymes addition at maceration, there is a content decrease of volatile phenolic compounds and aldehydes (especially acetic aldehyde), which are known for their phenolic and spicy aromas and, respectively, pungent odor.

Table 2

Content of volatile odorous compounds in *Viorica* dry white wine (control sample and sample with enzymes addition), mg·L^{-1}

Name of the volatile compound	control	enzymes	Δ, %
Higher alcohols	206.108	226.073	+9.69
Esters	61.682	93.850	+52.15
Acids	265.919	271.684	+2.17
Terpenes	0.926	1.286	+38.92
Norisoprenoids	0.011	0.017	+62.05
Thiols	0.155	0.212	+36.25

Continuation Table 2

Aldehydes	**58.897**	**42.736**	**-27.44**
Volatile phenolic compounds	0.291	0.258	-11.29
Lactones	0.568	0.725	+27.82
Totally volatile compounds	**604.491**	**626.905**	**+3.71**

Note:"+" increase of the value,"-" decrease of the value compared to the control sample

The percentage variation of the volatile odorous compounds content in wines macerated with enzymes addition compared to the control sample is represented graphically in Figure 1. The wine obtained by enzyme treatment showed a higher content in terpenes, this increase is due to their release from the bound forms during alcoholic fermentation, because of the residual enzymatic activity of the grapes or the enzymatic activity of the fermentation yeasts [8, 20, 21].

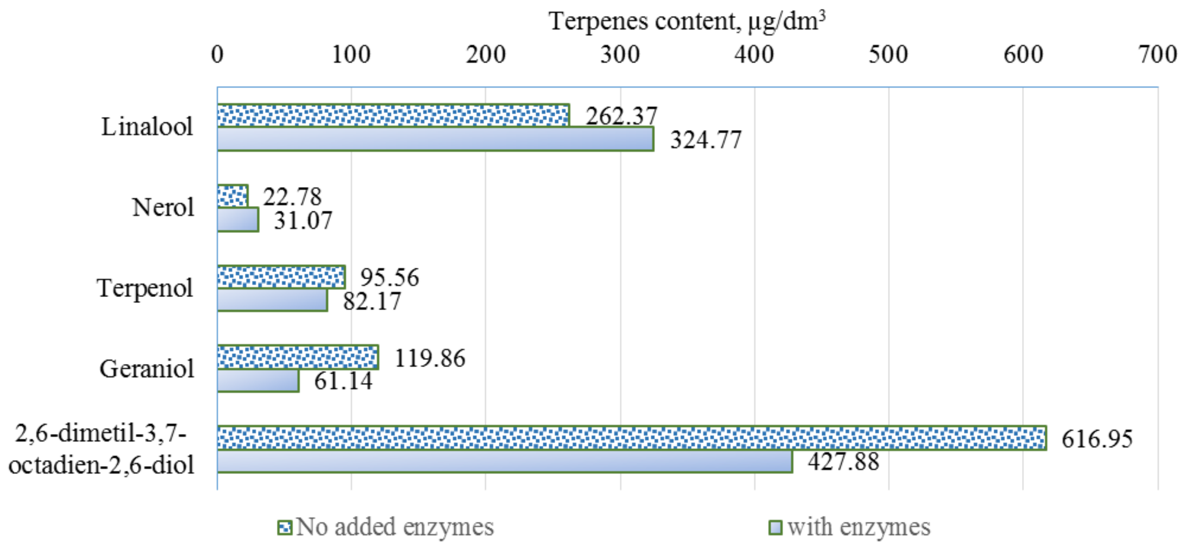

Figure 1. Influence of the enzyme addition on the content of the most important terpenes.

Cyclization of nerol, geraniol and linalool in an acid medium gives α-terpineol [22, 23], and cyclization in an acid medium with 2,6-dimethyl-3,7-octadien-2,6-diol leads to hotrienol [5, 9].

Figure 1 shows graphically the comparative concentration of the most important terpenes in terms of quantity and smell, respectively. The performed determinations showed that musts that were not treated with enzymes have a concentration of about 40% lower terpenes compared to those macerated with pectolytic enzymes. The linalool content increases by about 20% compared to the control sample.

The use of enzymes during maceration leads to the optimization of the varietal flavors increasing process [24, 25], resulting in wines with superior sensorial characteristics, the values of terpene compounds increasing by about 40%. Moreover, the study showed that the use of enzymatic preparations leads not only to significant increases in terpenes in wine, but they also are richer in esters, have very low values of acetaldehyde and volatile phenolic compounds. Thus, it can be concluded that the use of enzymes during maceration has led to increased efficiency due to increased content of compounds responsible for the profile and aromatic typicality of wines obtained from Viorica grapes, which is reflected through a value increase of the wine quality.

c. Influence of storage temperature on aromatic composition of wine

Wines are constantly evolving, so a special interest is the dynamics of terpenes during the wine storage [26]. For this purpose, the wines obtained with or without the addition of enzymes at the maceration and stored 6 months at 12 °C and 18 °C were analyzed by spectrophotometrical method. The variation of terpene compounds after six months of storage at different temperatures is shown in Figure 4.

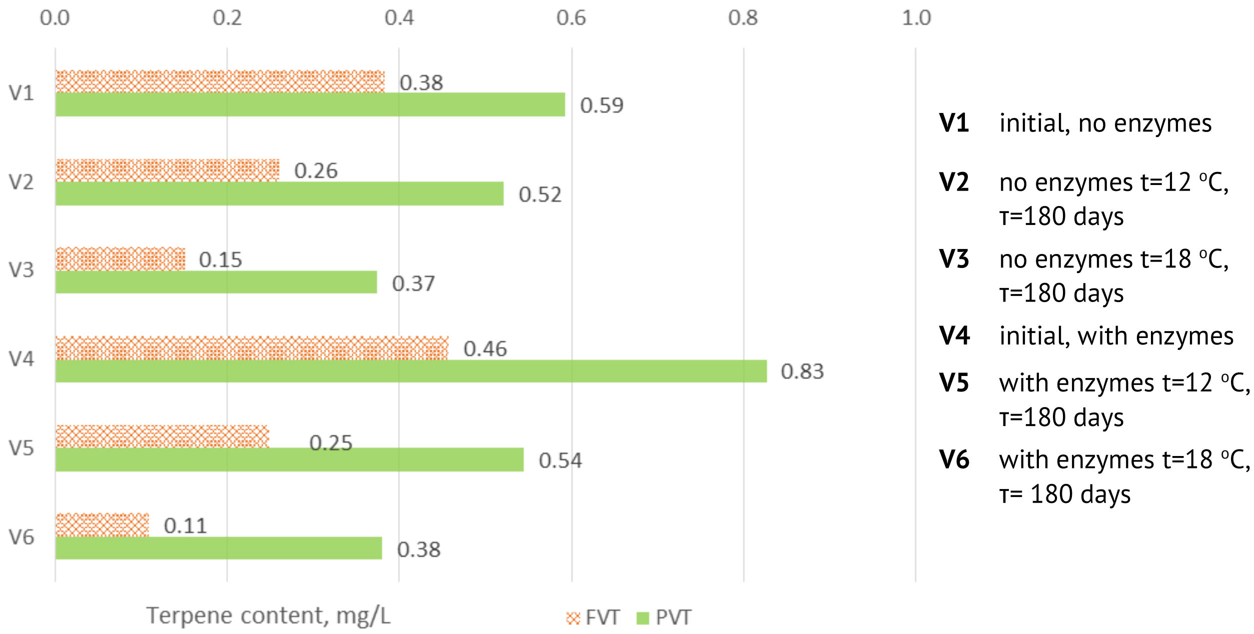

Figure 2. Variation of terpene compounds during storage at different temperatures.

After 6 months of storage at temperature of 12 °C it was noticed a decrease of free terpenes level with 30% and after storage at temperature of 18 °C with 60% in control samples. In wines obtained by addition of maceration enzymes free terpenes content decreased by 45% after 6 months of storage at temperature of 12 °C and by 75% after storage at temperature of 18 °C. Thus, it can be concluded that, increasing storage temperature from 12 °C to 18 °C leads to decrease of free terpenes content in wine samples about 30 %. Contrary to expectations, the use of exogenous enzymes at maceration presented a negative impact on wine aroma. After 6 months of storage at temperature of 12 °C, a decrease of free terpenes level about 12 % and at temperature of 18 °C about 15 % for the wine compared with the control was measured.

Regarding the content of bound terpenes, it also can be noticed the tendency to decrease along with increase of storage temperature. At the storage temperature of 12 °C slow processes of the bound terpenes content decreasing was measured. The decrease was about 10 % for the control wines and by 35% in wines produced with enzyme addition during maceration. At higher storage temperatures (18 °C), the enzymatic and acid hydrolysis of bound terpenes was enhanced, leading to a decrease of bound terpenes amount three times higher than at 12 °C, about 35 % for control samples and 55 % for samples obtained by enzyme maceration addition.

As a result of the research it can be resumed that, in order to diminish the degradation process of terpenic compounds, it is important to keep the wines at temperatures of about 12 °C.

At the same time, it was found that the use of enzymes in maceration on marcs leads to the production of wines with an increased content of terpene compounds, but which, after a 6 months storage, record terpenes amounts with 10-15% lower than in samples obtained by enzyme-free maceration.

Conclusions

As a result of the carried study, it can be mentioned that the choice of winemaking technology has a very important role for the aromatic characteristics of the wines obtained from local selection grapes *Viorica*.

Following the spectrophotometric analysis of grape juice, significant differences between the content of free and potentially volatile terpenes were noticed, the PVT/FVT ratio being 1.783. The higher content of terpene glycosides means an increased potential of volatile terpenes, thus suggesting a higher aromatic potential. The *Viorica* grapes juice is distinguished by a high amount of p-menthanone and DL-menthol (nearly 50 %), that gives it spicy and camphoreous notes to the wines obtained from this variety.

Moreover, the study showed that the use of enzymatic preparations leads not only to significant increases in terpenes in wine, but they also are richer in esters, have very low values of acetaldehyde and volatile phenolic compounds. Thus, it can be concluded that the use of enzymes during maceration has led to increased efficiency due to increased content of compounds responsible for the profile and aromatic typicality of wines obtained from *Viorica* grapes, which is reflected through a value increase of the wine quality.

Regarding the influence of the storage temperature on the terpene amount, it can be resumed that, in order to diminish the degradation process of terpenic compounds, it is important to keep the wines at temperatures of about 12 °C.

It can be resumed that the use of enzymes in maceration on marcs leads to the production of wines with an increased content of terpene compounds, but which, after a 6 months storage, record terpenes amounts with 10 - 15% lower than in samples obtained by enzyme-free maceration.

Consequently, experimental data showed that free and bound terpene compounds are significantly influenced by the storage temperature as well as by the origins of terpene compounds.

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