Assessment of free and potentially volatile monoterpenes in Muscat Ottonel grapes variety

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Abstract. In this study the total content of free and potentially volatile monoterpenes present in the Muscat Ottonel grape variety from Murfatlar vineyard was quantified by using a rapid distillation-colorimetric method. The assays were performed on samples of mature and ripped grapes collected from the three different harvests. The level of free monoterpenes in the skin, pulp, and juice from the Muscat Ottonel grapes was lower than the level of bound monoterpenes found in the same structures. The majority of the free monoterpenes have been located in the grape skins. In the same time, the contents of free and potentially volatile monoterpenes in grape pulp obtained after pressing have been quantified. The evaluation of the quantity of potentially volatile monoterpene components is important for the possibility to enhance the free monoterpene contents in wines during the wine-making process.

Keywords: terpenes, grapes, wine aroma, Muscat Ottonel

1. Introduction

In wine are present more than 800 aromatic compounds, which are influenced by synergistic effect in their odour threshold concentration and quality.

Different monoterpene compounds were identified in *Vitis vinifera* L. grapes and wine, the most abundant of which are linalool, geraniol, nerol, citronellol and terpeniol [1]. These compounds contribute significantly to the characteristic flavour of grapes and are generally present only at low levels in the floral grape varieties. In grapes, aromatic compounds are present in free-odour form and more abundantly as non-volatile glycosides [2].

Glycosidically bound monoterpenes contribute significantly to aroma by hydrolysis [3]. To obtain the maximum intensity of such characteristic floral aromas in grapes and related aromatic varieties, several factors can be observed: period of grapes harvesting when the total terpene levels are at highest content [4]; obtaining the maximum available aroma components by using extended skin contact [5] and the hydrolysis of bound terpenes by using exogenous enzymatic preparation [6].

Different researchers advised that aromatic white grapes should be harvested based upon their potentially volatile monoterpene (PVT) accumulation during grapes ripening [1]. The concentration of monoterpenes (free and bound) may increase during maturation, while sugar concentration changes only slightly. The PVT concentration in the berry may continue to increase, even no increase in sugar concentration in the berry is observed.

Investigations related to the aromatic components in grapes and wines have grown rapidly as a result of the availability of more sophisticate analytical methods for their isolation and identification.

In this study, the level of free (FVT) and potentially volatile terpenes (PVT) in Muscat Ottonel grape variety from Murfatlar vineyard has been investigated.

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2. Experimental

2.1. Grape samples

Muscat Ottonel grapes were obtained from private vineyards in Murfatlar region during the period 2008-2010.

2.2.Sample preparation

Fresh grapes were picked at random to the total sample mass of 1000 g. Grape samples were frozen immediately after being picked. Prior to analysis, the grapes were thawed. To analyse the distribution of free monoterpenes in the skin, pulp and juice, berries from the variety Muscat
Ottonel were hand peeled and the seeds removed from the pulp. The pulp was homogenised and filtrated through cheesecloth to obtain clear juice. The weighed pulp and the skins were homogenised separately in about 200 mL phosphate buffer (pH 7.0) saturated with NaCl and filtrated. Then, the filtrates were adjusted to pH 6.6–6.8 with 20 % w/v solution of NaOH.

2.3.Isolation of monoterpenes

The rapid distillation analytical method described by Dimitriadis and Williams [5] and Šapceska *et al.,* [7] was used to determine the free volatile terpenes, as well as those released from their glycosidically bound forms by acid hydrolysis in the grape juice. A sample of 100 mL grape juice was steam-distilled until 25 mL distillate was recovered. This distillate was used for determination of the content of free volatile terpenes (FVT). Without interrupting the steam flow, the juice was acidified with 5 mL of 20% (v/v) H_3PO_4. The distillation continued until the next 40 mL distillate was collected. This distillate contained the potentially volatile monoterpenes (PVT), derived from the polyols and glycosidically bound forms.

2.4. Colorimetric determination of monoterpenes

A volume of 10 mL of each distillate (FVT or PVT) were individually shaken and pipetted into Eppendorf tubes. A blank sample was prepared with 10 mL of water. A volume of 5 mL of 2 % vanillin-H_2SO_4 reagent was added to each precooled tube. The contents were agitated with further cooling in an ice bath.

The colour was developed by heating the tubes in water bath at 60 ^oC for 30 minutes. The tubes were than cooled at 25 ^oC for 10 minutes and the optical densities were read at 608 nm using 1 cm plastic cuvettes.

The sample distillates, in reaction with the vanillin sulphuric reagents, form a complex with a blue-green colour, with a intensity proportional to the content of monoterpenes. The contents of monoterpenes in the distillates were calculated from the standard curve prepared with linalool standard solutions containing 20 – 100 mg/L linalool.

By using appropriate volumes of collected distillate, juice distilled, and aliquots taken for the colorimetric determination, the content of FVT and PVT was calculated as mg/L juice.

Each experiment was done in triplicate.

3. Results and Discussions

3.1. The monoterpenes content in grape juice

By using the rapid distillation-colorimetric method, free and glycosidically bound monoterpenes in Muscat Ottonel grapes variety were determined. The data for free terpenes (FVT) and potentially volatile terpenes (PVT) in the grape juice from the three grapes cultivars are shown in **Table 1**.

Table 1. Free (FVT) and potentially volatile monoterpene (PVT) in grape juice from different grape cultivars

Muscat Ottonel cultivars	**FVT (mg/L)**	**PVT (mg/L)**	**PVT/FVT**
2008	0.69	2.15	3.11
2009	0.78	2.47	3.16
2010	0.51	1.61	1.19

By analysing the data from **Table 1** it is clear that there are significant differences among the FVT and PVT contents of Muscat Ottonel grape variety during the period 2008-2010.

The content of free monoterpenes and potentially volatiles terpenes was much higher in

Muscat Ottonel variety 2009. Wine grape Muscat Ottonel variety 2008 and 2010 had lower content of both forms of monoterpenes. However, their PVT content was 1.61 to 2.47 times higher than FVT content. Muscat Ottonel harvest 2009 and 2008 have higher ratio of PVT/FVT than the Muscat Ottonel harvest 2010, suggesting a greater flavour potential. These results are in line with the ones about distribution of free monoterpenes and potentially volatiles terpenes in various grape varieties from Glen Osmond, South Australia, obtained by Dimitriadis and Williams [5]. The authors, also have investigated the recovery of monoterpenes with distillation colorimetric method, by using a mixture of linalool, α-terpineol, geraniol and furan linalool oxide (1:1:1:0.6). They have obtained 100 % recovery of the total free monoterpenes. In our study the linalool has been used as a referent monoterpene, and the recovery varied from 85 % to 98. The recovery of PVT during distillation of water mixture of standard linalool may result from linalool left in the condenser during the collection of the PVT distillate, or from transformation of this monoterpenol during acidification of the samples into other monoterpenols [8].

Other authors such Versini *et al.*, [9] have suggested that different contents of free and bound monoterpene is in correlation with the different geoclimatic vineyard regions in France. A difference in individual profiles of some terpenes such as linalool, geraniol, nerol, terpineol can be found. Further investigations of these monoterpene profiles are needed to explain the differences of individual content of monoterpenes in grape variety.

This initial evaluation for content of potentially volatile terpenes (glycosidically bound mono-terpenes) in grape varieties presents an interest for production of more aromatic wines in the Murfatlar vineyard.

By hydrolysing the glycosidically bound monoterpenes and releasing the free floral terpenes, it is possible to enhance the variety aroma in the flavoured wines during the wine making.

3.2. Free and glycosidically bound monoterpenes distribution in grape skins, juice and pulp

The interest has focused on the distribution and metabolism of these flavourings and their precursors in berries. The study of free and glycosidically bound monoterpenes in developing Muscat grapes showed clearly dynamic changes in content of these compounds during berry ontogeny [7, 10].

In grapes for winemaking, the knowledge of distribution as well as contents of free monoterpenes, flavourless polyols, and glycosides in skin and juice, is a valuable guide for applying skin contact and press condition to optimise flavourings in juice.

In this study, the distribution of free and glycosidically bound monoterpenes in the fractions of grape variety Muscat Ottonel during the 2008-2010 periods has been investigated. The distribution of FVT and PVT in the skins is shown in **Fig. 1.**

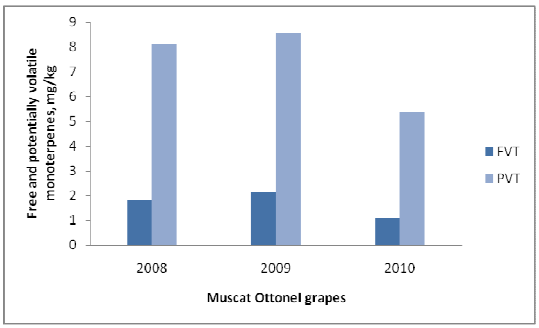

Fig. 1. The free and potentially volatile monoterpenes of grape skin

In figure 1 it can be seen that one kilogram grape skins of Muscat Ottonel grape harvest 2009 had the highest content of glycosidically bound monoterpenes (8.57 mg/kg). The levels of free volatile monoterpenes in all fractions were lower than the levels of bound monoterpenes.

In **Fig. 2** the free and potentially volatile monoterpenes in pulp of Muscat Ottonel grape variety are depicted. The distribution of free and potentially volatile monoterpenes in pulp of Muscat Ottonel grape variety has the same shape with one in skins. The highest content of glycosidically bound monoterpenes (2.82 mg/kg) was achieved in the case of Muscat Ottonel grapes harvest 2009.

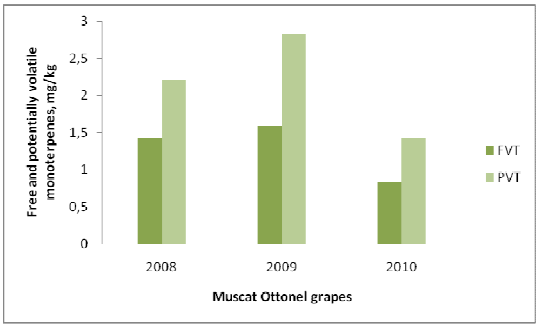

Fig. 2. The free and potentially volatile monoterpenes of grape pulp

The free and potentially volatile monoterpenes in juice of Muscat Ottonel grape variety are shown in **Fig. 3**.

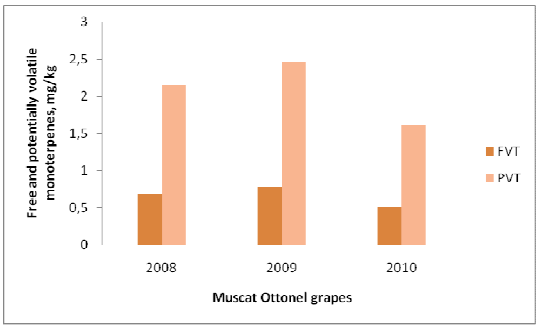

Fig. 3. The free and potentially volatile monoterpenes of grape juice

It was observed that monoterpene contents in grape berries would have been higher if distillation of grape juice had been done with the skins.

Others also found differences in monoterpene concentration between berries and must samples that might be due to the method of analysis [7].

Investigation about localisation of monoterpenes in Muscat of Alexandria (20.6 °Brix), White Frontignan (22.1 °Brix), and Gewürztraminer (21.0 °Brix) grapes were done by different authors [7, 11]. They quantified the major monoterpenes (free and bound) found in the skin, pulp, and juice. The Gewürztraminer, a Muscat-related variety, had the lower total amount of monoterpenes, while the other two Muscat varieties had higher levels. Distribution of the linalool in the berry fractions of Muscat of Alexandria showed that the skin had the majority of the linalool, with lower quantities in the juice and pulp.

Monoterpenes have a cycle of development in the grape. In general, the levels of free and bound monoterpene fractions increase with the maturation of the berry [12]. The bound fraction usually is abundant in the green stage, while the free fraction it was present in smaller quantities. The bound fraction was always larger than the free fraction throughout maturation. The total monoterpene content continues to increase even after ripeness has been reached [7].

Other authors indicated to implement greater press force and extended skin contact during winemaking in order to increase extraction of free and bound monoterpenes from grapes and to optimise the flavoured aroma content in must [13].

3.3. Free and potentially bound monoterpenes in pulp fraction after separation of juice

After the separation of juice (**Fig. 4**), the juice and the pulp fraction (pulp, seeds and skins) were analyzed for FVT and PVT (**Fig. 5**).

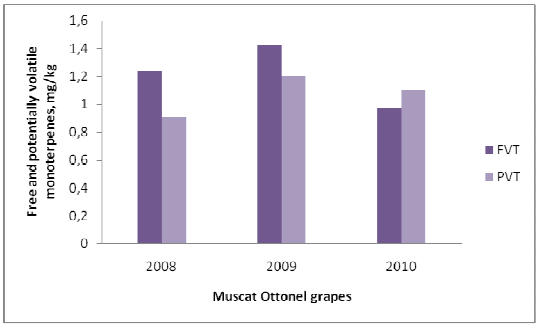

Fig. 4. The free and potentially volatile monoterpenes of grape juice after separation

The results indicate that the content of free and bound monoterpenes in grape juice was higher than before separation.

The presence of higher level of bound monoterpenes in juice and pulp fraction is characteristic for different grape varieties. Due to the hydrophylicity of the bound monoterpenes, they do not contribute to the wine aroma [7].

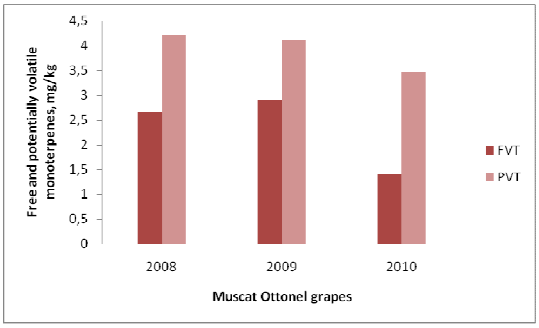

Fig. 5. The free and potentially volatile monoterpenes of grape pulp after separation

In this situation, winemakers are greatly interested in hydrolysing these potential aroma precursors to release the free floral terpenes to enhance the varietal aroma [11].

According to the level of free monoterpenes in pulp fraction, every step of their extraction in winemaking can be useful in obtaining better aroma in juice and wine.

4. Conclusions

In this study the level of free monoterpenes in the skin, the pulp, and the juice from the Muscat Ottonel grape during the period 2008-2010 was quantified. The level of free monoterpenes in the skin, the pulp, and the juice from the Muscat Ottonel was lower than the level of bound monoterpenes. The majority of the free monoterpenes were located in the skin.

The content of free and bound monoterpenes in grape juice was higher than before separation. The process of extended skin contact in which free and bound monoterpenes can pass in juice at higher content can be beneficial for production of higher quality of wine.

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6. References

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