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Professional paper

FREE AND POTENTIALLY VOLATILE MONOTERPENES IN GRAPE VARIETIES FROM THE REPUBLIC OF MACEDONIA

Donka Doneva-Šapčeska[1*], Aco Dimitrovski[1], Trajko Bojadžiev[2], Goran Milanov[3], Borimir Vojnovski[3]

[1] *Faculty of Technology and Metallurgy, Ss Cyril and Methodius University, P. O. Box 580, MK-1001 Skopje, Republic of Macedonia*
[2]*AD Povardarie, Negotino, Republic of Macedonia*
[3]*Institute of Agriculture, Skopje, Republic of Macedonia*
*donka@tmf.ukim.edu.mk

The total content of free (FVT) and potentially volatile terpenes (PVT) present in the grape varieties Muscat Hamburg, Muscat Italia, Italian Riesling, Chardonnay, Vranec and Cabernet Sauvignon was determined by using a rapid distillation-colorimetric method. The assays were performed on samples of mature grapes. The FVT and PVT correlated with the recognized aroma characteristics of the cultivars examined. The level of free monoterpenes in the skin, the pulp, and the juice from the Muscat Italia grape was lower than the level of bound monoterpenes. Most of the free monoterpenes were located in the skin. However, the calculated contents of the FVT and the PVT present in the skin were much lower when expressed on the whole berry. Additionally, the contents of FVT and PVT in grape waste obtained after pressing was examined. The quantity of potentially volatile monoterpene components determined in the grape varieties investigated opens up the possibility of enhanceing the free monoterpene contents in the wine-making process.

Key words: grape; free volatile terpenes; potentially volatile terpenes

СЛОБОДНИ И ПОТЕНЦИЈАЛНО ИСПАРЛИВИ МОНОТЕРПЕНИ ВО НЕКОЛКУ СОРТИ ГРОЗЈЕ ОД РЕПУБЛИКА МАКЕДОНИЈА

Испитана е вкупната содржина на слободните (FVT) и потенцијално испарливите терпени (PVT) присутни во неколку сорти грозје: мускат хамбург, мускат италија, италијански ризлинг, шардоне, вранец и каберне совињон од тиквешкиот регион на Република Македонија. Примероците од зрело грозје беа испитани со брзиот дестилационо-колориметриски метод. Слободните и врзаните терпени корелираат со препознатливите ароматични карактеристики на испитуваните сорти. Нивото на слободните монотерпени во лушпата, пулпата и сокот кај грозјето мускат Италија беше помало од нивото на врзаните монотерпени. Слободните монотерпени ги имаше најмногу во лушпта. Но пресметаната содржина на слободните и потенцијално испарливите терпени во лушпата е многу помала во однос на сокот кога се изразува на цело зрно. Покрај тоа, испитана е содржината на FVT и PVT во гроздовиот остаток добиен по пресувањето. Определеното количество потенцијално испарливи монотерпени во испитуваните сорти грозје даваат можност овој потенцијал да се искористи за збогатување на содржината на слободните монотерпени при производството на вино.

Клучни зборови: грозје; слободни испарливи терпени; потенцијално испарливи терпени

INTRODUCTION

Various studies have been undertaken to investigate components, which have effect on the aroma – flavor of the grapes and wines. It was found that the most of the components that influence the wine aroma are monoterpenes. Approximately 50 monoterpene compounds, the most abundant of which are linalool, geraniol, nerol, citronellol and terpeniol, as well as a number of

other compounds of terpenic origin, have been identified in *Vitis vinifera* L. grapes and wine [1–3]. Monoterpene compounds contribute significantly to the characteristic flavour of grapes and are generally present only at low levels in the floral grape varieties. These aroma components, which are common constituents of many fruits, are present in free-odor form and more abundantly as non-volatile glycosides [4, 5].

Although the non-volatile glycosides (or glycosidically bound monoterpenes) have been shown to be tasteless at the levels at which they are found in grapes and wine, they can contribute significantly to aroma upon hydrolysis [6–8]. To obtain the maximum intensity of such characteristic floral aromas in grapes and related aromatic varieties, several factors can be manipulated: first, harvesting grapes when the total terpene levels are at highest content [9–11]; second, obtaining the maximum available aroma components by using extended skin contact or appropriate pressing system [12–14] and third, hydrolysing bound terpenes enzymatically [6, 7, 15].

Marais [1] advised that aromatic white grapes should be harvested based upon their potentially volatile terpene (PVT) concentration. The concentration of monoterpenes (free and bound) may increase dramatically during maturation, while sugar concentration changes only slightly. The PVT concentration in the berry may continue to increase, even if sugar concentration in the berry had ceased to grow.

In general, literature suggests that several berry composition factors must be considered when determining potential wine quality, specific to both the environmental conditions the grapes are grown under and the desired wine style. Investigations related to the aromatic components in grape and wine have grown rapidly as a result of the availability of more sophisticate analytical methods for their isolation and identification. In this paper, the level of free (FVT) and potentially volatile terpenes (PVT) in different grape varieties from Tikveš region in the Republic of Macedonia has been investigated.

EXPERIMENTAL

The rapid distillation analytical method described by Dimitriadis and Williams [16] 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 obtained from few vine cultivars.

Grape samples

Muscat Italia, Muscat Hamburg, Vranec and Cabernet Sauvignon were obtained from private vineyards in Tikveš region, and Italian Riesling and Chardonnay grapes were obtained from the Agriculture Institute, Skopje, Republic of Macedonia.

Sample preparation

Fresh grapes were picked at random from whole bunches to give total sample mass of 500 g. Grape samples were frozen immediately after being picked. Prior to analysis, the grapes were thawed, homogenised, and filtered through a cheesecloth. As a result, juice and pulp fraction (cellular debris of the flesh, pulp, seeds and skins) were obtained.

The pH of the juice was adjusted to 6.6–6.8 immediately prior to distillation by addition of 20 % w/v solution of NaOH**.** The weighed pulp fraction was rehomogenized in about 200 ml of citric phosphate buffer (pH, 7.0) saturated with NaCl, and allowed to stay for 72 hours at 4 ^{o}C in a refrigerator. The obtained extract was then filtrated, and, prior to analysis, adjusted to pH 6.6–6.8 with 20 % w/v solution of NaOH.

To determine the distribution of free monoterpenes in the skin, pulp (cellular debris of the flesh) and juice, berries from the variety Muscat Italia 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 were left for 3 and 16 days at 4 ^{o}C, respectively. Then, the skin and the pulp extracts were filtrated and filtrates were adjusted to pH 6.6–6.8 with 20 % w/v solution of NaOH.

Isolation of monoterpene

A sample of 100 ml neutral grape juice was steam-distilled until 25 ml distillate was produced. 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 solution of 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.

Colorimetric determination of monoterpenes

10 ml aliquots of each distillate (FVT or PVT) were individually shaken and pipetted into Pyrex test tubes fitted with silicone rubber seals. A blank sample was prepared with 10 ml of water. Five ml of 2 % vanilin-H_2SO_4 reagent was added to each precooled tube. The contents were thoroughly agitated with further cooling in an ice bath. The colour was developed by heating the tubes in water bath at 60 ^oC for 20 minutes. The tubes were than cooled at 25 ^oC for 5 minutes and within 20 minutes, the optical densities were read at 608 nm using 1 cm disposable plastic cuvettes. The examined distillates, in reaction with the vanillin-sulphuric reagents, gave blue-green colour, the intensity of which was 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 repeated three times.

RESULTS AND DISCUSSION

The content of monoterpene in grape juice

Grape varieties that are not Muscat varieties, such as Riesling, Gewürztraminer and Chardonnay, produce free terpene compounds in quantities about 10 times smaller than Muscat varieties. However, these varieties have components which are odourless, but capable of producing characteristic aromatic materials in the wine after being released upon hydrolysis [3].

By using the rapid distillation-colorimetric method, free and glycosidically bound monoterpenes in wine grape varieties (Italian Riesling, Chardonnay, Vranec, Cabernet Sauvignon) and Muscat varieties (Muscat Italia and Muscat Hamburg) were determined with the aim of obtaining information about the content of these terpenes in the Macedonian cultivars. The data for free terpenes (FVT) and potentially volatile terpenes (PVT) in the grape juice from the six grape varieties are shown in Table 1. It is clear that there are significant differences among the FVT and PVT contents of grape varieties investigated.

Table 1

Content of free (FVT) and potentially volatile monoterpene (PVT) in grape juice from different grape varieties

Grape variety	FVT (mg/l)	PVT (mg/l)	PVT/FVT
Italian Riesling	0.288	0.625	2.200
Chardonnay	0.181	0.307	1.700
Vranec	0.172	0.164	0.953
Cabernet Sauvignon	0.321	0.135	0.421
Muscat Italia	0.780	2.330	2.900
Muscat Hamburg	0.563	2.190	3.900

The content of free monoterpenes and potentially volatiles terpenes was much higher in Muscat varieties. Wine grape varieties Italian Riesling and Chardonnay had lower content of both forms of monoterpenes. However, their PVT content was 2.2 and 1.7 times higher than FVT content. Muscat Italia and Muscat Hamburg have higher ratio of PVT/FVT than the white wine varieties Italian Riesling and Chardonnay, suggesting greater flavour potential. Similar results about distribution of these terpenes in various grape varieties from Glen Osmond, South Australia, were obtained by Dimitriadis and Williams [16].

The same authors [16], investigating the recovery of monoterpenes with distillation colorimetric method, used mixture of referent monoterpenes (linalool, α-terpineol, geraniol and furan linalool oxide, 1:1:1:0.6), and obtained 100 % recovery of the total free monoterpenes. In our experiments with linalool as a referent monoterpene, the recovery varied from 84 % to 97.5 %, showing that this procedure can be efficiently used for calculating FVT [17]. However, the recovery of PVT during distillation of water mixture of standard linalool may result from linalool left in the con-

denser during the collection of the PVT distillate, or from transformation of this monoterpenol during acidification of the samples into other monoterpenols [16, 18].

In red grapes varieties Cabernet Sauvignon and Vranec, the ratio of PVT/FVT was lower than one, indicating that in these varieties glycosidically bound compounds couldn't significantly contribute to higher contents of free terpenes. For the grape varieties Vranec and Cabernet Sauvignon the data for the total contents of free and bound terpenes were not available. In these varieties, other components such as flavanols, norisoprenoides, benzoides, aliphatic compound or methoxypyrazines have much more influence on grape flavour and aroma [19, 20].

The content of PVT which Dimitriadis and Williams [16] had obtained in the juice from Riesling (0.88 mg/l) and Chardonnay (0.22 mg/l) were slightly higher than the PVT content in the varieties that we had investigated (Table 1). However, their FVT results for Riesling as a non-muscat aromatic variety (0.28 mg/l) and for Chardonnay as neutral variety (0.16 mg/l) were similar to the ones obtained in this study. Versini *et al*., [21] suggested that different profiles of the contents of free and bound monoterpene exist in different geoclimatic vineyard regions in France. It is possible that there is a difference in individual profiles of some terpenes such as linalool, geraniol, nerol, α-terpineol. A further investigation of these terpene profiles is needed to assess the differences of individual content of monoterpenes in grape varieties.

This initial screening for content of potentially volatile terpenes (glycosidically bound monoterpenes) in grape varieties is of a great interest for production of more aromatic wines in the Republic of Macedonia. By hydrolysing the glycosidically bound terpenes and releasing the free floral terpenes, it is possible to enhance the variety aroma in the wine.

Distribution of free and glycosidically bound monoterpenes among skin, juice and pulp of grape varieties

With growing understanding of the nature and behaviour of the various categories of monoterpenes in grape, the interest has focused on the distribution and metabolism of these flavorants and their precursors in berries. A study of free and glycosidically bound monoterpenes in developing Muscat grapes showed clearly dynamic changes in content of these compounds during berry ontogeny [18]. In grapes for winemaking, a knowledge of distribution as well as contents of free monoterpenes, flavourless polyols, and glycosides in skin and juice, would offer a valuable guide for applying skin contact and press condition to optimise flavorants in juice.

In this study, the distribution of free and glycosidically bound terpenes in the fractions of grape variety Muscat Italia has been investigated.

The distribution of FVT and PVT in the skins, pulp, and clear juice are shown in Fig. 1 (the basis is each berry part) and in Fig. 2 (the basis is whole berry). In Fig. 1 it can be seen that one kilogram grape skins had the highest content of glycosidically bound terpenes (7.73 mg/kg). The levels of free volatile monoterpenes in all fractions were lower than the levels of bound terpenes.

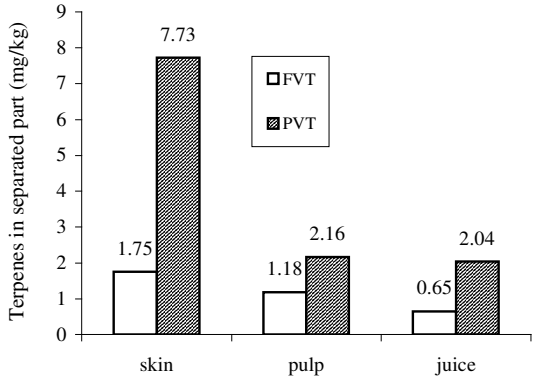

Fig. 1. Content of free and potentially volatile terpenes in grape variety Muscat Italia

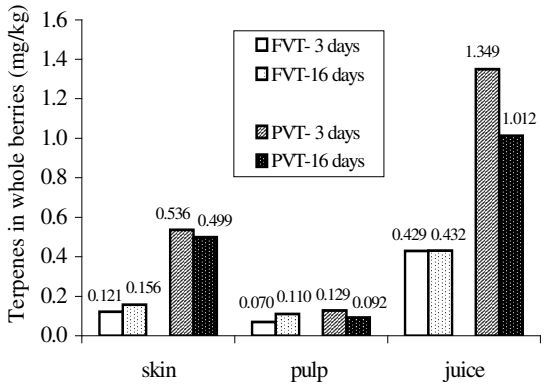

Fig. 2. Distribution of free and bound terpenes in different parts of grape berry Muscat Italia after 3 and 16 days of storage at 4 °C

The calculated values of 0.121 mg/kg free terpenes and 0.536 mg/kg bound terpenes (Fig. 2), indicate that the content of FVT and PVT present in the skin were much lower than those in the juice, when expressed on a whole berry basis. These results indicate that monoterpene contents in grape berries would have been higher if distillation of grape juice had been done with the skins. Reynolds *et al.* [13] also found differences in monoterpene concentration between berries and must samples that might be due to the method of analysis. They distilled the monoterpenes from entire berries.

The content of terpenes determined after keeping the fractions for 16 days in a refrigerator at 4 °C is also shown in Fig. 2. Higher content of free monoterpenes and lower content of PVT was observed in comparison with grape fractions stored for 3 days under the same conditions. These results suggested that longer storage enhanced liberation of free terpenes from their bound forms.

Similar investigation about localisation of terpenes in Muscat of Alexandria (20.6 °Brix), White Frontignan (22.1 °Brix), and Gewürztraminer (21.0 °Brix) grapes was made by Wilson *et al.* [22]. They quantified and tabulated the breakdown of the major monoterpenes (free and bound) found in the skin, pulp, and juice. The Gewürztraminer, a Muscat-related variety, had the least total amount of monoterpenes, while the other two Muscat varieties had higher levels. The linalool distribution in the berry fractions of Muscat of Alexandria showed that the skin had the majority of the linalool, with some present in the juice and very little in the pulp.

Similar to other major grape compounds, 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 [5]. The bound fraction was abundant (250–500 μg/kg) as early as the green stage, while as a free fraction it was present in smaller quantities (30–90 μg/kg). The bound fraction was always larger than the free fraction throughout maturation. The total monoterpene content continues to increase even after ripeness has been attained.

Gunata *et al.* [4] studied the development and distribution of monoterpenes in two Muscat varieties: Muscat of Alexandria and Muscat of Frontignan. Terpene levels were determined by extraction followed by identification and quantification with a GC/MS. Linalool and geraniol were the two most abundant terpenes in Muscat of Alexandria. Muscat of Frontignan had more nerol than Muscat of Alexandria.

Park and Noble [23] indicated that by implementing greater press force and extended skin contact during winemaking it is possible to increase extraction of free and bound monoterpenes from grapes which offers valuable guide in optimising the aroma content in juice.

Distribution of free and potentially bound terpenes in raw materials (pulp fraction) after separation of juice

After the separation of juice from 4 different grape varieties, both the juice and the pulp fraction (pulp, seeds and skins) were analyzed for FVT and PVT (Fig. 3). The results indicate that the content of free and bound terpineols in grape juice was higher than in the raw grape material (pulp fraction).

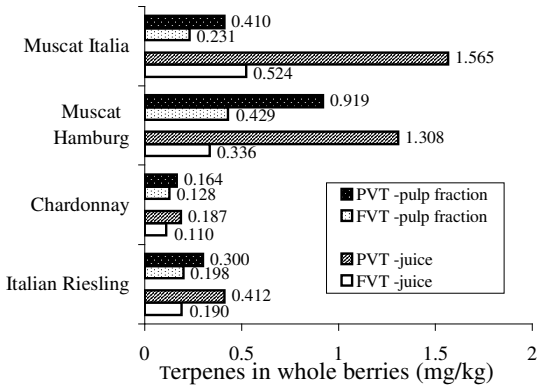

Fig. 3. Distribution of FVT and PVT in juice and raw grape material (pulp fraction) from different grape varieties

Higher level of bound terpenes in juice and pulp fraction (raw materials) is characteristic for various grape varieties. Due to the hydrophylicity of the bound monoterpenes, they do not contribute to the wine aroma. Therefore, winemakers are greatly interested in hydrolysing these potential aroma precursors to release the free floral terpenes to enhance the varietal aroma [24]. According to the level of free terpenes in pulp fraction, every step of their extraction in winemaking can be useful in obtaining better aroma in juice and wine. 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. With estimated optimal press conditions and development of winemaking technology that utilises these monoterpene glucosides as potential flavour compounds, an effect of increasing juice aroma could be obtained, which would result in an improved wine flavour.

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