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Influence of enzyme maceration and alcoholic fermentation temperature on the terpenes concentration in Muscat wine distillates

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Abstract

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Various factors in grape processing are influencing terpene content in wines and base wine for distillation. The choice of an appropriate regime for Muscat wine distillate production is very important. Flavour perception, olfactory and gustatory, is most often given precedence when assessing the quality of Muscat wines and distillates, as the aroma of Muscat grape varieties is a legitimizing factor of primary importance in terms of quality. In the production of Muscat wines, it is necessary to regulate the temperature of alcoholic fermentation to values of 14-16°C in order to obtain a high quality organoleptic profile. In the present research, in the production of Muscat wine distillate, an alcoholic fermentation regime has been applied without temperature control and in combination with a pectolytic enzyme preparation. The aim is to assess the impact of the proposed regime on the quantity and quality of the terpenes in the obtained distillates compared to low-temperature fermentation regime. The following have been found: accelerated extraction of bound terpenes in base wine without regulating the fermentation temperature and in combination with a pectolytic enzyme preparation; increased concentration of monoterpenes in Muscat distillates by 40 and 79% under accelerated extraction conditions. Practical applications: this study contributes to choose an appropriate regime for production of high quality Muscat wine distillate.

Keywords: Muscat distillates; Muscat Ottonel; free and bound terpenes; enzyme maceration; fermentation temperature
Abbreviations: BW – base wine; CD – crude distillate; MD – Muscat distillate; OIV – International Organisation of Vine and Wine

Introduction

Characteristics and meaning of terpenes in grapes, wine and distillates. Flavour perception, olfactory and gustatory, is most often given precedence when assessing the quality of Muscat wines and distillates, as the aroma of Muscat grape varieties is a legitimizing factor of primary importance in terms of quality (Katerova et al., 2010; Yoncheva et al., 2012).

Approximately 550 representatives of the volatile components have been found in grapes and wines (Doneva-Šapceska et al., 2006). They may be provisionally grouped into representatives contained in grapes; obtained during the crushing of the raw material under the action of enzymes; formed during fermentation and distillation (Clarke & Bakker, 2004; Marais, 1983; Mateo & Jiménez, 2000).

It has been found that the floral, fruit, spice and herbal aroma nuances of grapes are mainly due to terpenic substances and their derivatives of the monoterpenes and sesquiterpenes groups (Clarke & Bakker, 2004; Marais, 1983). The dominant monoterpenes in Muscat varieties are linalool, geraniol, and nerol (Mateo & Jiménez, 2000). α-terpineol (α-alpha), hotrienol, nerol oxide and linalool oxides are also found (Blagoeva et al., 2016).

Terpenic substances are contained in all parts of the grapes – mainly in the skins, the flesh and the stems, and their concentrations change during the ripening of the grapes (Gomez et al., 1994; Lengyel, 2012). They are found in free form as aromatically active monoterpenes and aroma-free in the bound form (Doneva-Šapceska et al., 2006; Gomez et al., 1994; Lengyel, 2012).

Various factors in grape processing influencing terpene content in wines and base wine for distillation. A number of factors such as ambient pH and alcohol content, the concentration and activity of the enzymes, the regime of maceration, pressing and heat treatment of the grape must influence the content of terpenes in wines and base wine for distillation.

Applying low temperature maceration regimes 8-10°C slows the extraction of free and bound terpenes. The application of higher temperatures (20–25°C) reduces the extraction time to 6-8 hours, but together with the accelerated extraction of aromatic components, the extraction of polyphenols from the solids also intensifies. This is undesirable in winemaking and should be carefully compensated via regulating the duration of maceration (Stoica et al., 2008; Stoica et al., 2009).

Using enzyme preparations with β-glucosidase activity aids the degradation of the glucosidic bonds in the molecules of bound terpenes resulting in the release of volatile aromatically active monoterpenes. Essential for the activity of enzyme β-glucosidase is the concentration of sugars in the environment. The higher it is, the lower the enzyme activity is (Strömvall & Petersson, 1992).

Press-run contain 2 to 4 times higher concentrations of terpenes than free-run. The increase of geraniol, nerol and citronellol concentrations is the most substantial. Together with the increase of terpenic substances, the concentrations of phenolic compounds and fatty acids also increase, which restricts press pressure in winemaking (Reynolds & Wardle, 1997).

Ethanol content plays an important role in the extraction of terpenes from the grape. Ethanol is a good extract, even in low concentrations, and its action can be used in fermentation of grape must in distillate production technology.

Change of terpenes through the stages of production and storage. During the production and storage stages of wines and wine distillates, terpenic substances undergo transformations including auto transformations that alter the aroma characteristics. The increase in terpenic concentration results from the release of free forms from glycosidically bound ones, and losses are due to oxidation or other processes leading to the formation of new substances (Marais, 1983).

During alcoholic fermentation, yeast has the ability to convert some of the terpenes – geraniol forms citronellol and nerol. The concentration of citronellol in wine is significantly higher than that in grapes which have been vinified (Zoecklein et al., 1997).

During storage, oxidation of linalool to cis- and trans-linalool oxides takes place, which reduces the intensity of the aroma. In addition to the oxidation processes, other enzymatic and non-enzymatic reactions occur, accompanied by the formation of substances modifying the nature of the Muscat aroma. After 3 months of storage of wines and base wine, terpenic substances are reduced by 50–60%. There are also changes in the structure of terpenes with the formation of cyclic compounds, ketones, lactones (α- (alpha) and β-ionone (beta), vitispirane) (Lengyel, 2012).

Behaviour of terpenes during distillation. During distillation of fermented grapes, pomace and base wine, bound monoterpenes are gradually hydrolysed under the influence of high temperature and acidic reaction of the environment. Concentrations of the majority of the monoterpenes in the obtained distillates increase compared to those in the starting material (Lillo et al., 2005). A possible additional reason for increasing concentrations of monoterpenes in distillates during distillation with solids is their physical separation from the cells of the grapes, mainly from the skins, where they are contained in the highest concentrations.

Given the greater solubility of terpenes in ethanol and lower in water, higher concentrations are expected in the head. In practice, however, their relatively high boiling temperatures are critical in determining their behaviour during distillation. During periodic distillation, the tail with lower ethanol content distilled at higher boiling temperatures of the mixture contains higher total concentrations of monoterpenes.

During periodic distillation of Muscat Blanc fermented grape marc, the increase of terpenes from the head to the tail is more apparent for monoterpenic alcohols than for oxides, probably due to higher polarity, hydrophilicity and boiling temperature. Their disengagement is stimulated by the higher temperatures at the end of the distillation (Lukić et al., 2011).

According to Lukić et al. (2011), the concentration of linalool decreases in the tail. It is characterized by a relatively lower boiling temperature, higher sensitivity to acid hydrolysis (such as tertiary alcohol) than other basic monoterpenes. These are the possible causes for its reduction and, what is more, the hydrolysis of linalool glycoside precursors has probably largely ended during the first half of the distillation.

Experiments associated with the behaviour of varietal aroma compounds during periodic distillation indicate that monoterpenic concentrations increase during distillation and reach their peak in the tails. This secondary waste product is a valuable raw material for re-distillation and enhancing source materials with varietal flavours.

In the production of Muscat wines, it is necessary to regulate the temperature of alcoholic fermentation to values of 14–16°C in order to obtain a high quality organoleptic profile. In the present research, in the production of Muscat wine distillate, an alcoholic fermentation regime has been applied without temperature control and in combination with a pectolytic enzyme preparation. The aim is to compare the impact of the proposed regime on the quantity and quality of the terpenes in the distillates obtained to the low temperature fermentation regime.

Materials and Methods

Grape. In the experiments, grapes of the Muscat Ottonel variety from the vineyard in the region of Pravishte village, Saedinenie, Bulgaria were used. The grapes were hand-picked in trays with capacity of about 6 kg. Then, they were kept in refrigerator for one day and night at 10–12°C.

Enzyme and dry yeast. In addition to the grapes, a pectolytic enzyme preparation (Lafazyme Extract, Laffort), which facilitates the extraction of aromatic precursors, and dry yeast (Zymaflore VL1, Laffort) were used.

Obtaining base wine for distillation. Prior to processing, the total amount of grapes for the experiment was divided into three parts of an approximately equal quality of the material. The grapes were destemmed and crushed by hand and three experimental variants of 18-kilogram grape mash for fermentation were formed. The experimental variants were placed in 20 dm^3 containers and treated according to the information in Table 1 below.

After alcoholic fermentation, draining and pressing of the pulp of the three variants was carried out. The resulting BW, designated as I, II and III, were stored in a refrigerator for 10 days at 8–10°C and then decanted from the lees.

Distillation of base wine. Base wine was double-distilled. The first distillation was carried out in small Copper Pot Stills. The apparatus was equipped with a distillation vessel with a volume of 15 dm^3 and a cooler. The obtained variants of CD I, II and III had an alcohol content of 30-33% vol. The second distillation was carried out in a glass distillation apparatus with serpentine condenser. The volume of the boiling flask was 2.5 dm^3, the rectifier column had 10 real plate. During rectification, only heads with a volume of 1.5% of the volume of the crude distillate was separated. Tails were not separated due to the concentration of heavier desirable aromatic components. The alcohol content of the obtained MD I, II and III was between 66 and 69 % vol.

Determination of oenological parameters. Sugar content of grapes was 21.8% and was defined under the Schoorle method (Ivanov et al., 1979), titratable acids were 2.82 g/dm^3, and the pH value was 4.18, according to the OIV regulations. Alcohol content of obtained base wine was 12.6% vol. determined ebuliometrically with Dujardin – Salleron ebuliometer) (Ivanov et al., 1979), sugar content – 0.44 g/dm^3, titratable acids – 3.5 g/dm^3, pH – 4.10 (Ivanov et al., 1979).

Determination of concentration of total free and bound terpenes and individual representative of terpenes. BW I, II and III were analysed for total concentration of free and bound terpenes with colorimetric method of Dmitriadis & Williams (1984). CD and MD I, II and III were analysed for the content of individual representative of terpenes – linalool, cis- and trans-linalool oxides, geraniol, nerol and α-terpineol with sample preparation with solvent-solvent extraction method according to Vas et al. (1998). Apparatus GC-MS was Varian 431-GC gas chromatograph equipped with a 2200-MS Ion Trap Mass Spectrometer; manual sampling; Splitless injection of a 0.2 μL volume (split 1:20) was carried out at 250°C. The gas (helium) flow was 1 ml/min. Analytes were separated in a capillary column CP-WAX 52 CB-30×0.25×0.5 (Agilent). Oven temperature program was as follows: initial temperature 70°C, held for 10 min, increased to 220°C at 5°C/min. Temperature of the detector was 150°C. MSD conditions were ion source temperature, 220°C; electron energy, 70 eV; mass scan range, 41–250 m/z.

Statistical analysis. Statistical analyses were run in triplicate and results were reported as mean values ± standard deviation (SD). Data were subjected to analysis of variance (one-way ANOVA Excel 5.0).

Results and Discussion

Content of free and bound terpenes in base wines

Variations in the concentrations of free and bound terpenic forms were found in the variants of wine materials (Table

Table 1. Characteristics of experimental variants

	Variant I	Variant II	Variant III
Enzyme dose in mash	–	–	3 g/hl Lafaym Extract
Yeast	20 g/hl Zymaflore VL1 for all variants		
Fermentation temperature	In refrigerator at 12–15°C	No temperature regulation /ambient temperature/	

Table 2. Content of free and bound terpenes in base wine variants

	Free terpenes, mg/dm³	Bound terpenes, mg/dm³	Total terpenes, mg/dm³
BW I	4.61 ± 0.11	4.92 ± 0.16	9.53 ± 0.27
BW II	6.52 ± 0.14	5.58 ± 0.11	12.10 ± 0.25
BW III	6.19 ± 0.24	6.05 ± 0.12	12.24 ± 0.36

The results were expressed in mg/dm³ ± SD of three replicates

2). Applying a higher fermentation temperature to the solid phase (BW II) provided an increased concentration of both free and bound terpenes compared to those in BW I that had fermented at 12–15°C. The rise of free terpenes was by about 40% while bound terpenes rose by 13%.

Polygalacturonase activity enzyme dose in alcoholic fermentation without temperature regulation (BW III) aided further extraction of bound forms – precursors of the aromatically active free monoterpenes. An increase of the bound forms by about 13% was achieved compared to BW I and by about 8.5% compared to BW II.

Content of representatives of terpenes in crude distillates

Linalool was dominant in all crude distillates, followed by cis-linalool oxide, α-terpineol and geraniol, while the concentration of trans-linalool oxide and nerol was the lowest (Figure 1).

In the CD II variant obtained from wine fermented at a normal temperature, 30% higher concentrations of representatives of terpenes were found on average, compared to those in CD I obtained from wine fermented in a refrigerator at temperature of 12–15°C. This increase corresponds approximately to that observed between the different BW varieties.

Via distillation of BW III that fermented at ambient temperature with enzyme dose added to the grape mash, CD III was obtained, which demonstrated even higher concentrations of terpenes. The increase compared to CD I concentrations was as follows: 64% linalool, more than twofold α-terpineol, and between 42 and 49% for the other representatives, and compared to CD II by 26% linalool, 58% α-terpineol and by between 11 and 16% for the rest of the representatives.

Probable reasons for the increase in monoterpenes in CD II and III are the provided accelerated extraction conditions of bound forms during alcoholic fermentation of the grape mashes, namely the normal (higher) temperature and the dosing of a pectolytic enzyme preparation. During the distillation process, the bound forms degraded into free forms under the influence of ambient temperature and acidity.

Content of representatives of terpenes in Muscat wine distillates

Linalool was dominant in all Muscat distillates, followed by α-terpineol, cis-linalool oxide and geraniol, while the concentration of trans-linalool oxide and nerol was the lowest (Figure 2).

After the second fractional distillation of the CD variants, MD containing higher concentrations of the studied terpenes were obtained, as follows: 1.5–3 times for variant I and 1.5–2.5 times for variants II and III.

This increase corresponds approximately to the concentration of ethanol during distillation – from crude distillate with 30–33% vol., Muscat distillate with 66–69% vol. was obtained. On average, the concentrations were: 2.7-fold linalool, 2.5-fold α-terpineol, geraniol and nerol, and 1.8-fold linalool oxides.

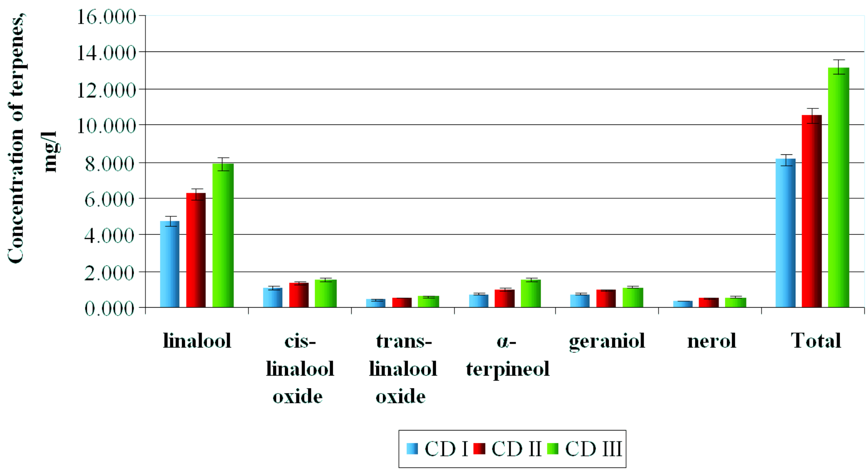

Fig. 1. Histogram of the content of terpenes in the three variants of Crude distillates

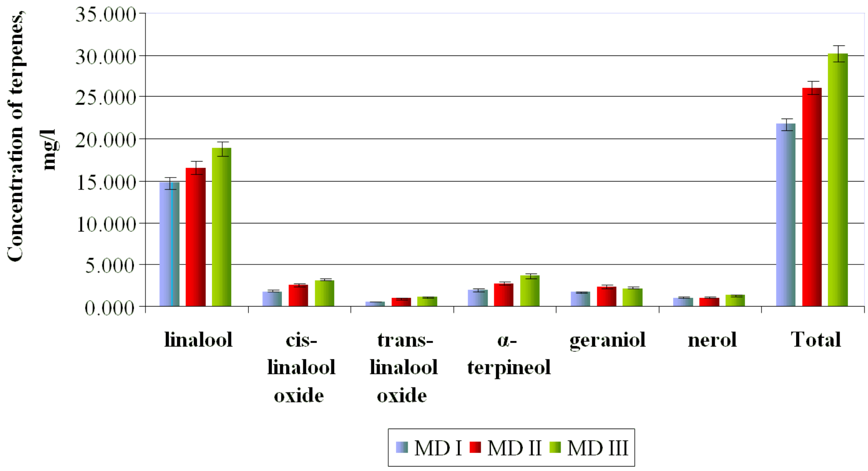

Fig. 2. Histogram of the content of terpenes in the three variants of Muscat distillates

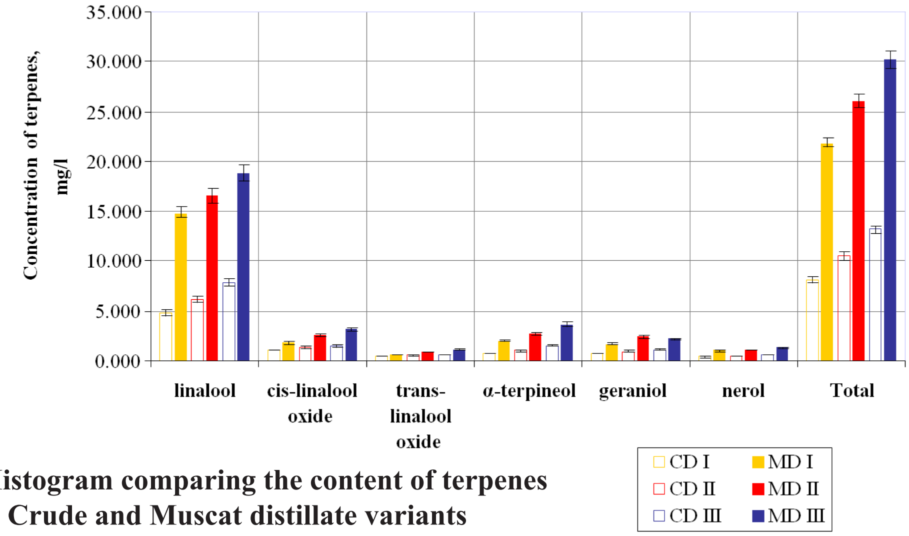

Fig. 3. Histogram comparing the content of terpenes in Crude and Muscat distillate variants

When comparing the concentrations of terpenes in the three MD variants, there were significant differences between some representatives as follows: an average of 40% higher concentration of linalool oxides, α-terpineol and geraniol in MD II, compared to MD I; an average of 79% higher concentration of linalool oxides and α-terpineol in MD III compared to MD I.

The behaviour of two representatives – geraniol and nerol – is excellent. Carrying out fermentation at normal temperature (MD II) compared to lower temperature (MD I) favours an increase in the content of cis- and trans-linalool oxide, α-terpinol and geraniol by about 1.4 times (40%), and the content of the nerol underwent almost no change. The application of fermentation at normal temperature with adding enzyme (MD III) compared to that without enzyme dosing (MD II) led to a smaller increase in trans-linalool oxide and nerol by about 1.2 times (20%) and geraniol content decreased. A probable cause for the behaviour of nerol and geraniol may be their being bound to a lesser extent.

In the three produced MD variants, starting from Ist to IIIrd, there was an increase in the concentrations of: linalool, cis-linalool oxide, α-terpineol and total terpenes. This means that the applied schemes without temperature regulation and especially with enzyme dosing favour the production of distillates by concentrating these representatives.

There was a trend towards a stronger concentration of linalool and nerol (by 3 and 2.6 times) in the MD at a lower concentration in the crude distillate (MD I) compared to the same in the other two variants (Figure 3).

Applying higher alcohol fermentation temperature and additional dosing of an enzyme with polygalacturonase ac-

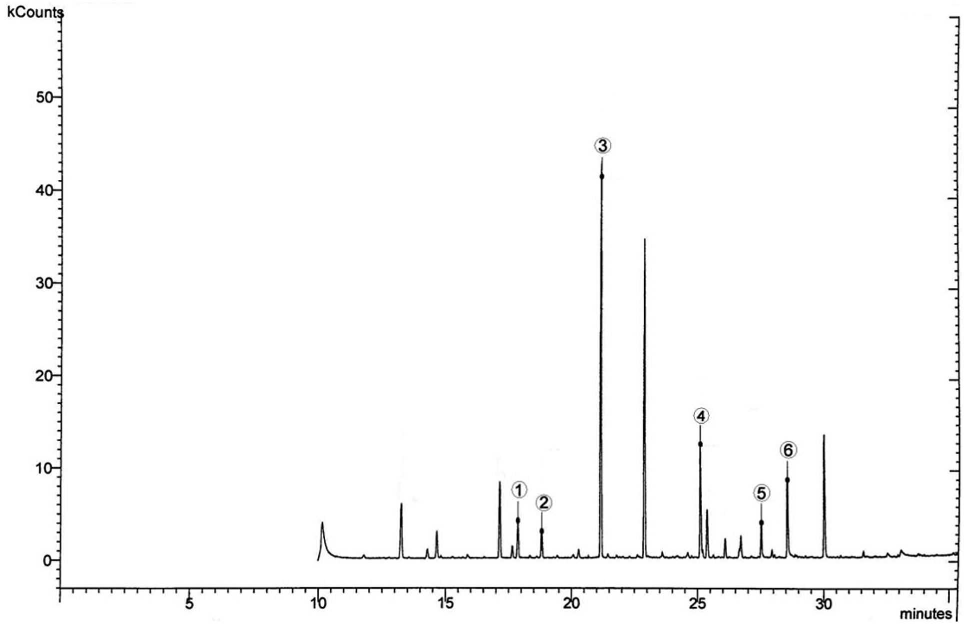

Fig. 4. Chromatogram of terpenes in crude distillate III:
1 – cis-linalool oxide; 2 – trans-linalool oxide; 3 – linalool; 4 – α-terpineol; 5 – nerol; 6 – geraniol

tivity (MD II and III) contribute to the production of MD with increased total concentrations of terpenes by 20 and 40%, respectively.

Figure 4 is a selected chromatogram of CD III after the analyses of distillate variants.

Conclusions

Under the conditions of the carried out experiment, the following conclusions can be drawn:

Accelerated extraction of bound terpenes in wine material for distillation is observed in alcoholic fermentation without temperature control and even more in combination with the use of a pectolytic enzyme preparation;

All crude and muscat wine distillates are dominated by linalool, followed by cis-linalool oxide, α-terpineol and geraniol, while the concentrations of trans-linalool oxide and nerol are the lowest;

Higher concentration of monoterpenes is found in two of the crude distillates. Possible reasons for this are the conditions created for the accelerated extraction of bound forms during alcoholic fermentation of the grape mash, namely higher temperature and dosing of a pectolytic enzyme preparation;

After a second fractional distillation of the crude distillate variants, Muscat distillates are obtained, which contain 1.5 to 3 times higher concentrations of representatives of the studied terpenes. This increase corresponds approximately to the concentration of ethanol during distillation;

Creating conditions for the accelerated extraction of related terpenes in the course of alcoholic fermentation favours the production of Muscat distillates with increased concentrations of representatives of monoterpenes – by 40 and 79 % on average.

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