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DETERMINATION OF SELECTED TERPENIC SUBSTANCES IN GRAPES AND WINE OF THE CULTIVAR PÁLAVA

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ABSTRACT

The presented study is focused on the determination of the content of terpenic substances in grapes and subsequently produced a wine of Czech variety Pálava (the Czech Republic, wine region Morava), which is a protected landscape with long-term tradition and culture. The aim of this study was the analysis of the aromatic profile of the cultivar Pálava, which was fermented by the original yeast strains from the Moravian wine region. Larger amounts of flavoring substances occur in grapes as bound flavoring substances, most often in the form of glycosides. One of the basic groups of aromatic substances is monoterpenes. The content of twelve free and bound terpenic substances was measured by the GC-MS method, namely linalool, ho-trineol, α-terpeniol, β-citronellol, nerol, geraniol, furan linalool oxide 1, furan linalool oxide 2, nerol oxide, epoxylinalol 1, epoxylinalol 2 and 2,6-dimethyl-3,7-octadiene-2,6-diol. The results were statistically compared by using a simple descriptive statistical method and ANOVA method. We noted a difference between the content of free and bound terpenes was very significant ($p \leq 0.05$). We found that using uncommercial yeasts could have an effect on the content of the volatile and terpenic compounds in wines. An important finding was that in fresh berry extracts there was a higher proportion of bound terpenes than free terpenes. The results have shown that the production technology of wine and the fermentation process has a clear impact on the content of the substances.

Keywords: terpens; grape; wine; GC-MS

INTRODUCTION

Pálava is a Moravian wine grape cultivar, named after the Protected Landscape Area of Pálava, which was created by crossing the cultivars Traminer Red and Müller Thurgau. The cultivar was bred by Ing. Josef Veverka, who started this breeding in 1953 at the Breeding Station in Velké Pavlovice and later continued in Perná. The cultivar has been entered in the State Variety Book in 1977 **(Kuneš et al., 2015)**.

The Pálava Protected Landscape Area (PLA) means a rich natural and cultural heritage. The vineyards cover approximately 16% of the total size of PLA **(Miklín and Smolková, 2011)**.

Despite the busy tourism in the area, wine and viticulture are an important phenomenon and the most important wine-growing area in the Czech Republic **(Miklín, 2012)**. Shrubs are moderately lush with dense leaves. The leaves are medium-sized, usually shallow-lobed. There is dense hair on the back, the blade surface is indefinitely wavy and leathery. The flowers are two-sexed, self-dusting, and five-flowered. The fringes are medium-sized, 100 – 160 mm long, conical, and sometimes winged **(Hakl et al., 2007)**.

The berries are light red with a gray tinge and have an oval shape with an average length of 14 mm. The skin is firm and the flesh is juicy, aromatic, with a spicy flavor. The average weight of the bunch is 169 g, the average weight of the berries is 1.1 g. It is moderately resistant to winter frosts and less resistant to spring frosts due to early budding. It is prone to attack by insects because it has very thick shrubs due to pecks. Resistance to other fungal diseases is average **(Kraus et al., 2005)**.

By **Butnariu and Butu (2019)** wine is one of the best selling agricultural products and by **Kong et al. (2019)** grapes (*Vitis vinifera* L.) are economically important fruit crops worldwide. The most important factors affecting the aroma in Muscat and non-Muscat cultivars are terpenic and aliphatic alcohols **(Matujašević et al., 2019)**.

Among the attributes that affect the marketability of wine, there are the production method and technological processes, such as the fermentation of fresh grapes, the application of the quantity and type of yeast, etc.

The study is focused on the analysis of the aromatic profile of the cultivar Pálava, which was fermented by the original yeast strains from the Moravian wine region. Using gas chromatography with mass detection (GC-MS) 12 terpenic substances were determined, which are important for shaping the character of the wine.

Scientific hypothesis

The difference between the results of the determination of free and bound terpenes in berries will be significant.

The content of the individual terpenes will be higher for the bound terpenes than for the free terpenes.

The content of terpenic substances is higher in fresh berries than in samples of the wine.

MATERIAL AND METHODOLOGY

The measured contents in grapes and wine were determined by extraction with methyl t-butyl ether. The weight of one sample was 100 grams of the cultivar Pálava. Samples of berries and wines were taken from the wine region of Moravia. Wine sampling was carried out one month before the end of the fermentation. At the same, we used a control sample which contained the only methyl t-butyl ether.

To fulfill the objectives of this study, there were used technology Shimadzu GC-17A, Autosampler: AOC – 5000, Detector: QP-5050A.

The results have been processed by using the software GC solution. Program LabSolutions, GC MS. Version 1.20. The separation conditions have been set as follow: Column: DB-WAX 30 m x 0.25 mm; 0.25 µm stationary phase (polyethylene glycol).

An injection volume of sample: 1 µL split ratio 1:5.

Carrier gas flow rate He: 1 $mL.min^{-1}$ (linear gas velocity 36 $cm.sec^{-1}$).

The injection chamber temperature was set at 200 °C.

The initial temperature of the 45 °C column was maintained for 3.5 minutes, followed by a temperature gradient: up to 90 °C by 15 $°C.min^{-1}$, up to 135 °C by 6 $°C.min^{-1}$, up to 207 °C by 9 $°C.min^{-1}$, up to 252 °C by 15 $°C.min^{-1}$.

The final temperature was held for 5 min. The total analysis time was 30 minutes. The detector operated in SCAN mode with an interval of 0.25 s in the range of 14 – 264. Detector voltage at 1.5 kV.

Substances were identified based on mass spectrum and retention time. Quantification was performed by comparing the sample peak area and the external standard with the correction for the internal standard.

The used analytical method, gas chromatography-mass spectrometry (GC/MS) was similar to the scientific study by **Pedersen et al. (2003)** and **Dziadas and Jeleń (2010)**.

Basic measured parameters of analyzed grapes were: weight 50 berries (76 g); red. sugar (22.8 °NM); pH (3.27); titric acids (7.6 $g.L^{-1}$).

The basic measured parameters of the analyzed wine were: red. sugar (0.1 $g.L^{-1}$), content of alcohol (11.7%); pH (3.5), titr. acids (7.94 $g.L^{-1}$).

Statistical analysis

After measuring terpene content in the samples, the results were statistically evaluated using simple descriptive statistics and the ANOVA method- one-factor method (without repetition). For statistical analysis, we used statistical software MS Excel (v.16.0, Microsoft-Windows).

RESULTS AND DISCUSSION

In the study, **González-Rodríguez et al. (2011)** three new commercial fungicides have been applied to wines according to GAP, which due to the aromatic structure of white wines. In the presence of fungicidal residues, the scent manifestation of geraniol was significantly reduced, thereby significantly reducing the floral nuance. However, it has not been shown that these fungicides have affected the formation of 1-hexanol and cis-3-hexen-1-ol formed during the fermentation processes, including harvesting, transport, crushing, and pressing.

Aim of the study **Zeleňáková et al. (2011)** was to examine of factors (manufacturer, temperature and storage time) influencing the variability of yeast amount and pH changes in bottled white wines. They discovered that storage time did not significantly impact the amount of yeast in wine ($p = 0.5507$).

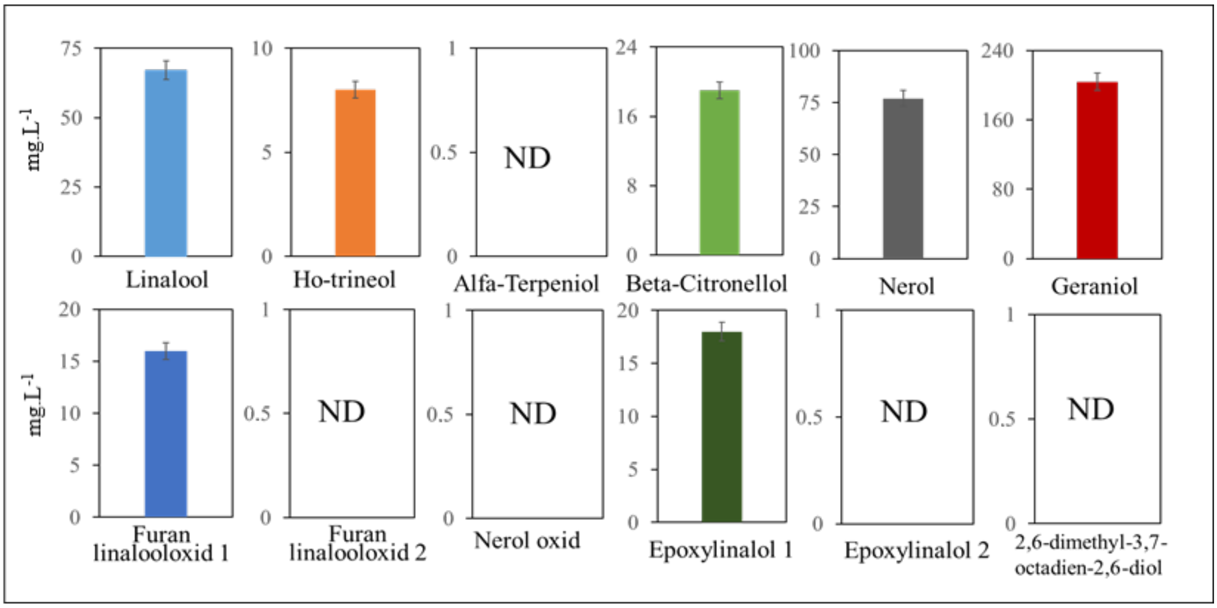

Figure 1 Values of the content of free terpenic substances in berries of the cultivar Pálava.

According to **Synos, Reynolds, and Bowen (2015)**, the use of various yeasts affects the content of ethyl isobutyrate, isoamyl acetate, 3-octanone, hexanoic acid, nerol, phenylethanol, and vitispiran. This claim was based in particular on the formation of various volatile substances found in wine. Using commercial yeast, the type of wine, berry processing, and fermentation is a factor for increasing the incidence of terpenes. **Lengyel and Panaitescu (2017)** studied the accumulation of free volatile terpene flavors (FVT) and bound precursor terpene flavors (BPT) under the action of 12 selected wine yeast strains. The 12 strains used in the alcoholic fermentation resulted in the determination of considerable amounts of terpene flavor compounds, between 370 $\mu g.L^{-1}$ and 1100 $\mu g.L^{-1}$, which recommends their use in making quality wines. By **Kačániová et al. (2019)** the wine grape berries share a complex microbial ecology including filamentous fungi, yeasts and bacteria. The study **Holešinský et al. (2020)** focused on the isolation of a consortium of microorganisms from spontaneously fermenting that naturally contain lactic acid bacteria, non-saccharomyces yeasts, and saccharomyces yeasts. The smallest amount of ethanol was formed from the isolates containing *Hanseniaspora uvarum*, while *Candida sake* isolate produced the lowest amount of hydrogen sulfide and *Zygosaccharomyces bailii* produced the highest. The killer proteins of *Pichia* spp. on the wine spoilage yeasts, studied by **Błaszczyk, Satora and Sroka (2015)**, are known for their broad spectrum of antifungal activity including pathogens such as *Candida albicans*. In this study, there was stated that the killer toxins could be used in the food industry as selective tools to control infections during the fermentation of wine. We agreed with this opinion. There are a lot of studies that determined the presence of microorganisms in wine and grape berries, namely e. g. **Drożdż et al. (2015)**; **Snopek et al. (2019)**; **Felšöciová, Kačániová and Vrábel (2020)**; **Kunová et al. (2020)**.

Yang et al. (2019) demonstrated that seventeen terpene glycosides were quantified in grapes and wines as pentosyl-glucopyranoside, the content of which ranged from 804 to 836 $\mu g.kg^{-1}$, and from 155 to 192 $\mu g.L^{-1}$. Eight free terpenes were present in wines with their content ranging from 40.1 to 59.7 $\mu g.L^{-1}$.

Compared to the above studies, it was noted that linalool (it has a floral aroma with a touch of lemon and spices) content was significantly higher for bound terpenic substances compared to free terpenic substances in berries ($p \leq 0.05$). Similar results were also observed in the determination of ho-trienol, α-terpeniol, geraniol, furan linalooloxid 1 and 2, nerol oxide, epoxylinalol 1, and 2, and ultimately 2,6-dimethyl-3,7-octadien-2,6-diol were not detected (Figure 1 and 2). To compare and obtain more accurate data, we measured each sample in three repetitions.

A statistically significant relationship between the individual measured attributes in the samples was demonstrated ($p \leq 0.05$).

Kostrz and Satora (2018) stated that the most common terpenes in wines are nerol (associated with citrus, magnolia aroma), citronellol (rose aroma), geraniol (with pleasant rose, geranium aroma), limonene (orange, citrus aroma), linalool (associated with floral, herbal, lavender), citral (with the smell of citrus) and β-ionone (with pleasant seaweed, violet, flower, raspberry).

By **Somkuwar et al. (2019)** among the studied varieties, Nielluccio wine recorded the highest concentration of total volatile compounds (191.53 $mg.L^{-1}$) while, it was least in Caladoc wines (15.45 $mg.L^{-1}$).

Comparing the measured terpene contents with the study **Matujašević et al. (2019)** the total relative content of terpenes in the control sample from 4.80% (Banat Muscat) to 24.78% (Radmilovac Muscat), in the sample with a lower dose of the enzyme (0.3 $g.kg^{-1}$) from 8.05% (Italia Muscat) up to 50.85% (Early Muscat) and in the sample with the higher dose of the enzyme (0.7 $g.kg^{-1}$) from 11.07% (Italia Muscat) to 34.78% (Radmilovac Muscat). The content of terpenic substances increased by an application of enzymes.

From this aspect, it can be deduced that in this way an increase in volatile and terpenic substances can be

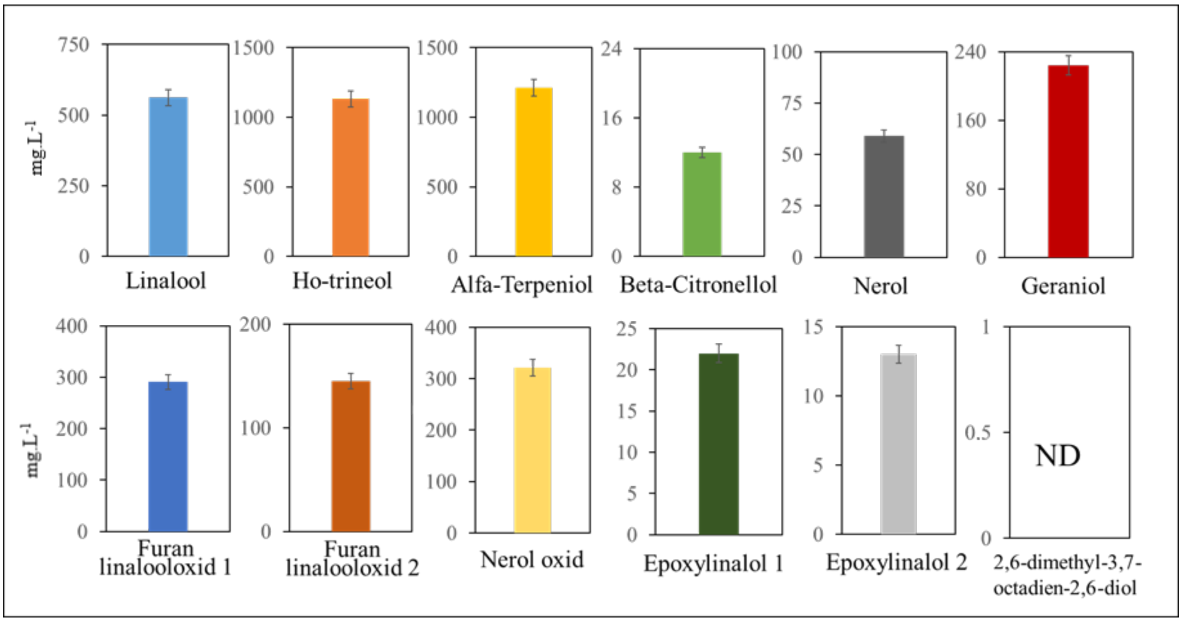

Figure 2 Values of content of bound terpenic substances in berries of cultivar Pálava.

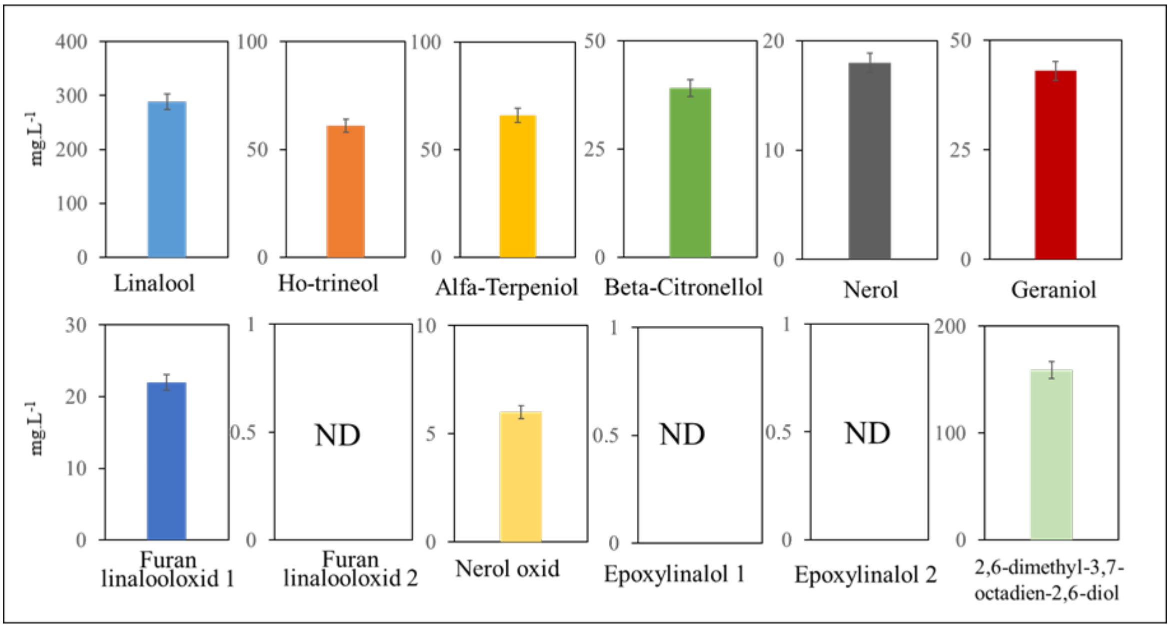

Figure 3 Values of total terpenic substances in wine of the cultivar ´Pálava´.

achieved.

By comparing results which are shown in Figure 3, we noted that despite β-citronellol, the content of terpenic substances was very low. 2-6-dimethyl-3,7-octadien-2,6-diol were measured only in wine samples wherein the samples of fresh berries were not detected.

We demonstrated a statistically significant meaning between the measured values of terpene compounds in the wine ($p \leq 0.05$).

Baron et al. (2017) deal with the determination of the content of both free and bound terpenes in berries and wine. They declared that the terpene content in wine increased gradually with the period of maceration. The highest and the lowest amounts of terpenes were recorded after 24 hours of maceration and no maceration, respectively. We agreed with this opinion because the length of maceration affects the content of terpens in wine. Otherwise, in the study **Blagoeva et al. (2020)** there was noted it is necessary to regulate the temperature of alcoholic fermentation to values of 14 – 16 °C to obtain a high-quality organoleptic profile.

Wang et al. (2020a) detected free terpenes by using solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC–MS). Their findings indicate that the matrix effect of phenolic acids can effectively control the release and modulate the global feature of wine aromas. In another similar study **Song et al. (2020)** the determination of the total concentrations of terpene glycosides were 7.32, 3.50, and 81.27 $mg.kg^{-1}$ in Ecolly, Cabernet Gernischet (CG), and Muscat Hamburg (MH) grapes, respectively. **Haygarov, Yoncheva and Dimitrov (2016)** determined the content of terpenes in selected grape varieties Rubin and Storgozia. The presence of terpenes was less marked and there was no significant difference in the quantity and quality of aromatic components. **Cehula et al. (2020)** presented in their study that wines with superior sensory properties did not contain higher levels of antioxidants or higher antioxidant activity. The formation of 13 volatile compounds was studied by **Lakatošová et al. (2013)**. Authentication of particular aromatic substances in typical Slovak wines and its saving in newly founded database can help to prevent wine falsification. They registered significant differences in the production of isoamyl acetate, 1-hexanol and 1-heptanol ($p \leq 0.05$).

Liu et al. (2017) noted that the major aroma components in grapes and wine include free volatile compounds and glycosidic nonvolatile compounds. Glycosidic aroma precursors are important reserves of grape and wine aroma components. Investigations concerning the chemical structures. The most used sulfur dioxide in winemaking owing to its antioxidant and antimicrobial properties. **Wang et al. (2020b)** studied to reveal the varietal characteristics of terpene glycosides (TGs) in ripe Meili grapes. They found that 49 of TGs were detected by UPLC–Q-TOF-MS. Monoterpenol pentosyl-glucosides were the main TGs, especially $C_{10}H_{18}O_2$ pentosyl-glucoside, which constituted 51.94 – 52.72% of the total concentration. **Shih et al. (2019)** found a novel application of terpene compound α-pinene for the alternative use of sulfur dioxide. They showed that α-pinene possessed an excellent antibacterial ability and could be a viable alternative for SO_2 in winemaking.

CONCLUSION

In this study, we demonstrated huge differences between measured results of content of terpenic substances in extracts of fresh berries of grape (*Vitis vinifera*) and extracts of wine. In both types of samples, we selected the typical Czech cultivar Pálava. We found significant relationships between total terpenic content ($p \leq 0.05$). Using uncommercial yeast could have a possible effect on the content of bioactive substances. We increased in wine volatile and terpenic substances by the used method and the results can be achieved. Also, we have once again justified the importance of wine production technology regarding fermentation.

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