Based on the results of this study it is possible to conclude that the ‘Irsai Oliver’ variety of grape has a very high terpene content. To improve the aromatic profile of the wine, the grape must should be macerated for 24 hours.

Keywords: terpenes; grapevine; gas chromatography; basic parameters of wine, maceration

1 Introduction

Terpenes, norisoprenoids, methoxypyrazins, volatile phenols, and aromatic thiols are important compounds that occur in grapes and in wine made of grapevine berries. Flavour is the result of the interaction between certain chemical compounds in wine and the senses of smell and taste of the consumer. Approximately 550 volatile components have been identified in grapes and wine, and many of these components contribute to the aroma [1].

With respect to their origin, wine aroma components may be classified into four groups [1]: originating from the grapes, produced during the crushing of the grapes by the action of the enzymes, produced during fermentation, produced during maturation of wine.

Several studies have been undertaken regarding the chemistry of terpenes, their synthesis and their systematic classification [25]. Devon and Scott (1972) presented a classification of 4 000 terpenes and their derivatives. Of these, 400 are monoterpenes and 1 000 sesquiterpenes [6].

Monoterpenes and sesquiterpenes are characteristic components of the essential oils produced by plants. Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are the lower molecular weight representatives of the terpenoid components and comprise two and three isoprene units, respectively. A general representation of the biosynthesis of monoterpenes and sesquiterpenes, as well as some enzymatic transformations such as oxidation, reduction and dehydration, has been summarized by Croteau (1975) [7].
Terpenes are characterized by floral, muscatel or fruity aromas that are synthesized in berries and stored in their skin. Their concentrations in grapes and wine obviously depend on various factors, including cultivar, region and wine-making techniques. Although terpenes mainly give off a pleasant aroma, some may have a negative effect on the quality of wine. So, for example, yeast of the genus Streptomyces may synthesise sesquiterpenes either on the cork or in the barrel and their presence may jeopardize the sensory quality of wine [8]. Nearly 50 terpenes in grapes and wine are known at this stage, of which 46 were identified in grapes and 30 in wines. They include only monoterpenes and sesquiterpenes [9]. Aromatic properties of monoterpenes may be found mainly in the hydrated monoterpenes (limonene and myrcene), aldehydes (linalool, geranial), alcohols (linalool, geraniol), acids (linalool, geraniol) and their esters (linalyl acetate) [10]. In the cultivars ‘Muscatel’, ‘Traminer’ and ‘Riesling’ wine aroma is predominantly determined not only by monoterpenes; but also by C13 norisoprenoids and, to a lesser extent, by volatile thiols. Of the monoterpenes, the most important are the following: linalool, geraniol, nerol, α-terpineol, citronellol, and ho-trienol [11].

The experiment monitored the occurrence of terpenes in juice samples after different periods of maceration. The variety ‘Irsai Oliver’ was chosen due to the fact that it is one of the most frequently cultivated aromatic varieties in South Moravia (Czech Republic) so that the results of this experiment may be rather interesting. The aim of this experiment was to determine if (and to which extent) the length of maceration shows an effect on the increase in the content of terpenic and other compounds in produced wine.

Figure 1. Structural formulas of monoterpenes that occur most frequently in wine. A) linalool, B) geraniol, C) citronelool, E) nerol, D) α-terpineol, F) Ho-trienol G) epoxylinalool 1 (trans-linalool oxide (furanoid) cis-linalool oxide (furanoid)) and epoxylinalool 2 (trans-linalool oxide (pyranoid) cis-linalool oxide (pyranoid)) H) 2,6-dimetyl-3,7-octadiene-2,6-diol

2 Material and methods

2.1 Sampling site

Grapes originated from vineyards situated in the Velké Bílovice locality (wine-growing subregion of Velké Pavlovice). In this region, the average annual precipitations and temperature are 550 mm and 9.5°C, respectively.

2.2 Experimental variants of maceration and processing of samples

In this experiment, grapes of the variety ‘Irsai Oliver’ were used. These grapes were harvested on 11 September 2013 and their sugar content was assayed at 19 °NM (i.e. 19 kg of natural sugars in 100 liters of juice). Harvested grapes were crushed and destemmed in a stainless destemmer-crushing machine, and macerated for 0 hours; 5 hours; 12 hours and 24 hours at the temperature of 14 °C, and finally pressed. Wine was made from each juice sample and used for the determination of the following parameters: pH, alcohol content, total acidity and sugars. In individual wine samples, the terpenes were estimated as well.

2.3 Determination of total acidity (EEC No 2676/90)

The total acidity was estimated in an automatic titrator TITROLINE EASY (manufacturer SI Analytics GmbH, Germany). Titrations were performed with NaOH...
Heating process leads to hydrolysis of glycosidic bounds to determination free and bound terpenes [12]. The equilibrium microextraction was performed using 1 ml of methyl-t-butyl ether that contained 1% of cyclohexane. After the separation, the organic phase was dried with anhydrous magnesium sulphate and used for the GC-MS analysis.

2.6.2 Terpene analysis by gas chromatography

Concentrations of individual volatile compounds in wine was determined previously unpublished method of extraction with methyl-t-butylether (MTBE). Into a 25-ml volumetric flask, 20 ml of wine was pipetted together with 50 µl of 2-nonanol solution in ethanol; this compound was used as a 400 mg/L internal standard with the addition of 5 ml of a saturated (NH₄)₂SO₄ solution. The flask content was thoroughly stirred and 0.75 ml of the extraction solvent (MTBE with an addition of 1% cyclohexane) was added. After another thorough stirring and separation of individual phases, the upper organic layer was placed into a micro test tube together with the produced emulsion centrifuged and the clear organic phase was dried over anhydrous magnesium sulphate, prior to the GC-MS analysis. The extraction and subsequent GC analysis was performed three times. Average values and standard deviation were determined using Excel and Statistica 10.

Instruments: Shimadzu GC-17A, Autosampler: AOC – 5000, Detector: QP-5050A, Software: GCSolution. Program: LabSolutions, GC MS solution. Version 1.20, Conditions of separation: column: DB-WAX 30m x 0.25mm; 0.25μm stationary phase (polyethylene glycol). Voltage of the detector 1.5 kV.

Separation conditions:
Sample injection volume: 1 µl split ratio1:5
Carrier gas flow He: 1 ml/min (linear gas velocity 36 cm/s)
The temperature of the injection port: 180°C
Initial column temperature 45°C maintained for 3.5 minutes, followed by temperature gradient:
To 75°C gradient 6°C/min
To 126°C gradient 3°C/min
To 190°C gradient 4°C/min
To 250°C gradient 5°C/min, final temperature was maintained for the subsequent 6.5 min.
Total length of analyses was 60 minutes.
Detector worked in SCAN mode with interval 0.25 s in range 14-264.

Individual compounds were identified by comparison of the MS spectrum and the retention time with the NIST 107 library.
2.7 Statistical analysis of measured data

Statistical analyses and graphs were generated using Excel 2007 (Microsoft Office, USA) and Statistica 10 (Copyright © StatSoft). Results are expressed as mean ± standard deviation (SD). Differences with $P<0.05$ were considered significant. Author's statements: Informed consent has been obtained from all individuals included in this study. Ethical approval: The conducted research is not related to either human or animals use.

3 Results

In grape berries, organic compounds are present both in the pulp and in the skin. As far as the winemaking methods are concerned, making wine from aromatic varieties is not an easy and simple process. When growing these varieties, it is necessary to consider the vineyard, health of grapes, temperature and time (duration) of maceration, temperature of fermentation and other aspects of the vinification process [13].

Grapes of the aromatic grapevine variety 'Irsai Oliver' were macerated for different time intervals (0; 5; 12 and 24 hours). Wine was thereafter made from each sample and used for the determination of the following parameters: ethanol content; pH; total acidity; content of sugars and terpenes.

3.1 Determination of basic analytical parameters

The highest content of alcohol (10.87%) was found in wine produced after no maceration, while the lowest levels (8.20%) was found in wine after 12 hours of maceration (Figure 2A).

The pH values of wine increased depending on the length of the maceration period (Figure 2B). The pH values ranged from pH 3 after no maceration to 3.7 after 24 hours of maceration. In this case, the content of total acidity is

![Figure 2: Basic parameters of wine samples: A - content of ethanol; B – pH; C – content of total acids; D – content of sugars. n=3. Significantly different are values of alcohol between 0 and 12 hours, 5 and 12 hours of maceration, total acids and sugar.](image-url)
an illustrative example of the dependence of contents of these substances on the time of maceration: the longer the period of maceration, the lower the final acidity of the wine. In wine after no maceration, the highest content of total acids was 6.16 g/L, while the lowest value was 4.27 g/L in wine after 24 hours of maceration (Figure 2C).

Sugar content increased with time of maceration from 1.7 g/L with no maceration to 3.9 g/L after 12 or 24 hours of maceration. (Figure 2D). Table 1 shows correlations existing between contents of individual substances and the period of maceration. Significant correlations (p < 0.05000) are in red.

3.2 Determination of terpene content

The extraction of terpenes is dependent on many factors (content of ethanol and glucose, pH etc.). In this study, attention was paid to terpenes occurring in the berries of aromatic varieties. Terpene contents were estimated as bound and free forms.

3.2.1 Determination of terpene content in wine

Linalool has a characteristic floral aroma with spicy tones and lemon aroma, it is present in the pulp of berries of Muskat-like grapevine varieties. Its concentration increases after the stage of colouration of berries and its odour threshold is low so it can be easily detected in wine aroma by the drinker [14]. Samples of this variety indicate that the longer the period of maceration, the higher the carry-over of linalool into the wine. In wine after no maceration and 24 hours of maceration, levels of linalool were 600 and 1,014 μg/L, respectively. In all experimental variants, levels of linalool were higher than its odour threshold (i.e. 50 μg/L). Due to oxidation, linalool is transformed to α-terpineol. If the temperature of alcohol fermentation is not too high, its content changes only a little. In grapes, α-terpineol occurs only in small amounts. The identification of this compound is difficult and its effect on wine aroma is very low. A characteristic feature of α-terpineol is a floral aroma. Because the content of α-terpineol increases with the time of ripening of berries and of wine ageing, this compound may be used as an indicator for the ripeness of the grapes. Differences in its content after 0, 5 and 12 hours are not statistically significant. The content of α-terpineol increased only in wine after 24 hours of maceration, from 135 μg/L to 151 μg/L. The odour threshold of α-terpineol is 400 μg/L. This value was not exceeded in any experimental variant.

A characteristic feature of geraniol, found in grape skin [14], is its rosy and citrus-fruit aroma. In this case, the rosy odour contributes significantly to the aroma Muscat-like wine; in other varieties, however, this compound was not identified. As far as the Muscat-like varieties are concerned, the content of geraniol represented approximately 4% of all organic compounds. The highest levels of this compound may be found in fully ripened grapes; thereafter, its content slightly decreases. The content of geraniol increases in the course of the alcoholic fermentation with statistically significant differences. The odour threshold of geraniol is nearly the same as that of linalool. In the course of wine ageing, geraniol is transformed to α-terpineol [8].

Nerol is characterized by a sweet aroma that resembles roses and thyme; some people characterize its odour also as “fresh”. It occurs mostly in skins. Geraniol and nerol are cis-trans isomers with an identical structure. In the course of fermentation, the content of nerol decreases and in the course of wine ageing this compound is transformed to α-terpineol [15]. The length of maceration increased the levels of nerol and geraniol: as compared with zero maceration, their contents after 24 hours of maceration were two-times higher. The odour thresholds of nerol and geraniol are 400 and 130 μg/L, respectively. This limit was not exceeded in any of samples under study.

The length of maceration did not increase markedly the concentrations of hotrienol and β-citronellol. The odour threshold of ho-trienol is 110 μg/L; this limit was exceeded with no maceration when the content

### Table 1: Correlations existing between contents of individual substances and the period of maceration. Significant correlations (p < 0.05000) are in red.

<table>
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<tr>
<th>Time of maceration [h]</th>
<th>Content of sugar [g/L]</th>
<th>Content of alcohol [g/L]</th>
<th>pH</th>
<th>Content of total acids [g/L]</th>
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<td>Content of sugar [g/L]</td>
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<tr>
<td>Content of alcohol [g/L]</td>
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<td>-0.84</td>
<td>1.00</td>
<td>-0.72</td>
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<td>pH</td>
<td>0.89</td>
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<td>Content of total acids [g/L]</td>
<td>-0.95</td>
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of ho-trienol was only 114 μg/L. The odour threshold of β-citronellol is only 18 μg/L; this limit was exceeded only after 24 hours of maceration (19 μg/L).

The content of epoxylinalool 1 increased with the length of the period of maceration with no maceration, its content was 365 μg/L while after 24 hours of maceration it increased to 452 μg/L. The content of epoxylinalool 2 also increased with the time of maceration. With no maceration, its content was 134 μg/L and after 24 hours of maceration its level increased to 206 μg/L.

Also the content of 2,6-dimetyl-3,7-octadiene-2,6-diol increased gradually with the length of maceration. Although this compound is an important member of the group of polyhydroxyl monoterpenes, the intensity of its aroma is not high. With no maceration, the content of this compound in the sample was 591 μg/L while after 24 hours of maceration, the level of 2,6-dimetyl-3,7-octadiene-2,6-diol was 989 μg/L (Figure 3B). Table 2 shows correlations existing between contents of individual substances and the period of maceration. Significant correlations ($p < 0.05000$) are in red.

![Figure 3: Contents of total of free plus glycosylated terpenes under study in individual wine samples: A - linalool, B - 2,6-dimetyl-3,7-octadiene-2,6-diol, C - ho-trienol, D - α-terpineol, E - β-citronellol, F - nerol, G - geraniol, H - epoxylinalool 1, I - epoxylinalool 2. n=3. Significantly different are values of all monoterpenes, except Linalool between 5-12 hours, Ho-trienol, α-terpineol, epoxylinalool 1 between 0-12 hours and epoxylinalool 2 between 5-12 hours of maceration.](image-url)
Table 2: Correlations existing between contents of individual substances and the period of maceration. Significant correlations (p < 0.05000) are in red.

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3.3 Determination of terpenes in grape juice

The terpene contents of berries are presented in Fig. 4. Linalool was the most abundant terpene and levels of its two forms (i.e. epoxylinalool 1 and epoxylinalool 2) were increased. The levels of free and bound epoxylinalool 1 were 366 and 500 µg/L, respectively, while those of epoxylinalool 2 were 119 and 169 µg/L, respectively. As far as the contents of other terpenes were concerned, the next most abundant were hotrienol and α-terpineol. A nearly zero detection was recorded in case of β-citronellol. Contents of free and bound linalool, hotrienol and α-terpineol were 2,009 µg/L; 456 µg/L and 160 µg/L, respectively (Figure 4).

4 Discussion

Many studies show that terpenes are present not only in stalks and skins but also in the pulp of berries. They are the most frequent aromatic substances in such grapevine varieties as ‘Irsai Oliver’, ‘Moravian Muscat’, ‘Gewürz Traminer’, ‘Lena’ and some others. From the sensory point of view, terpenes determine the aromatic quality of these grapes which are usually described as flowery, fruity and/or muscatel taste [16].

Ribéreau-Gayon et al. (2006) determined that in aromatic varieties, most terpenes were present in their glycosylated forms a finding that is supported by the results of this study regarding the content of bound terpenes being higher than those of free terpenes. As the majority of monoterpenic compounds occur in skins, they are usually released by the maceration of the grape must[10]. As described by Radeka et al. (2008), the maceration temperatures of 20 - 25°C are used most commonly in making wine; but more recently wine makers are using cryomaceration at temperatures of 5 - 8°C. When macerating at temperatures of 20 - 25°C, the release of phenolic substances is usually associated with bitterness and acerbity of juice; phenolic compounds may also oxidize and cause an undesirable aroma as well as an oxidative browning of the processed grape juice[17]. In this case, the interval of maceration plays an important role.
Studies of terpenes in grapevine ‘Irsai Oliver’

In juices, a dissolution of cations takes place in the course of maceration and this can cause a decrease in total acidity either due to a neutralization or due to salinization of tartaric acid. This discovery is supported also by a decrease in the content of total acidity [10].

In an interesting study, Mencarelli and Tonutti (2013) monitored the presence of terpenes in berries of the varieties ‘Aleatico’ and ‘Moscato Bianco’. They found that monoterpenes predominated over all others. In these varieties, the content of monoterpenes is approximately 85% of the total content of aromatic substances present in ripe grapes; of this amount, about 10% and 90% are in a free or bound form, respectively. When harvest of these varieties was delayed for a period of two weeks, the weight of berries went down by 8 to 12% and the total content of organic compounds decreased by 8 to 10%. The results of this study also corroborated that in Muscat and Gewurztraminer-like varieties terpenic compound predominated over other organic compounds[19].

Hasalgrove et al. (2000) studied the localization of terpenic compounds in grapes of the variety ‘Muscat of Alexandria’. In this variety, altogether 94% and 96% of geraniol and nerol, respectively, were found in the skin while linalool was present nearly equally in pulp and skin. This means that the aromatic profiles of skin and pulp were different and that skin contact with juice made from pulp may improve both the quality and intensity of the aroma of produced wine. In the conventional processing of grapes, a rapid separation of skins from grape juice reduces the extraction of terpenes into the grape juice [20].

On the other hand, cryomaceration results in an increased content of terpenic compounds and in a suppression of the undesirable extraction of phenolic substances. Radeka et al. (2008) performed an experiment with the variety ‘Malvasia’, in which one batch of harvested grapes was macerated at 20°C for 10; 20 and 30 hours while the other one was processed at the temperature of 7°C also for 10; 20 and 30 hours. The aforementioned authors monitored the concentrations of free and bound monoterpenes and total phenols. In the variety under study, linalool and geraniol were the most common monoterpenes. As compared with the control (i.e. berries without maceration) the contents of all free and bound monoterpenes were increased. A sensory evaluation indicated that the best samples of wine were those that passed through the process of cryomaceration [17]. These wine samples showed a typical varietal bouquet. These authors observed that at the maceration temperature of 14°C, the highest levels of phenolic substances occurred in samples with a longer period of maceration. In the variety ‘Irsai Oliver’, linalool and 2,6-dimethyl-3,7 octadiene-2,6-diol were the most common terpenes.

Clarke et al. (2008) found that a lower temperature of maceration enabled the production of fresh and light wines with a marked fruity taste; they also observed that at higher temperatures and after longer periods of maceration the resulting wine was dark and with a less pronounced fruity character [18].

Ribéreau-Gayon et al. (2006) wrote that in juices, maceration could show a negative effect on the content of total acidity. In juices, a dissolution of cations takes place in the course of maceration and this can cause a decrease in total acidity either due to a neutralization or due to salinization of tartaric acid. This discovery is supported also by a decrease in the content of total acidity [10].
Espejo et al. (2010) found out that the release of terpenes from glycosidic bonds can be increased by pectolytic enzymes. The grape mush maceration time is another possibility to increase the release of those terpenic compounds that are present in skins. If must maceration is combined with the use of pectolytic enzymes then the extraction of terpenic substances may be increased. When macerating the must, it is also necessary to pay attention to the temperature because it can either accelerate or inhibit this process. When the grape mush is macerated at temperatures lower than 20°C, it is necessary to macerate the grape must for a longer time interval and to use higher doses of pectolytic enzymes [21].

5 Conclusions

The terpene content in wine increased in proportion to the length of the period of maceration. The highest and the lowest amounts of terpenes were recorded after 24 hours of maceration and zero maceration, respectively. The bound terpenes content was higher than that of free ones. Linalool and 2,6-dimethyl-3,7-octadiene-2,6-diol were the most common terpenes. Based on the results of this study, it is possible to conclude that ‘Irsai Oliver’ is a variety with a very high terpene content. To improve its aromatic profile and to make it more pronounced, it is recommended to macerate the grape must for 24 hours.

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