

# Study of the aromatic profile of Traminer Rot (Gewürztraminer) by GC-MS

J. Sochor, M. Baron, L. Sochorova, and M. Kumsta

**Abstract**— This paper is focused on the study of the aromatic profile of Traminer rot must, cultivated in the Moravian wine-growing region in the Czech Republic. In our paper, we monitored selected terpenic substances during maceration after 0, 6, 12, 18, 24, 30, and 36 hours. The aromatic profile was studied by gas chromatography with mass detection (GC-MS). Our focus was on the determination of free, bound, and total terpenic substances, in addition to the determination of specific aromatic substances: linalool, geraniol, nerol, alpha-terpineol, and hotrienol. The study confirmed that increasing the maceration time also increases the content of free and total terpenic substances.

**Keywords**—aromatic profile, autochthonous yeast, gas chromatography, terpenes.

## I. INTRODUCTION

In grape vines, terpenic substances are mostly found in the berry skins [1]. These substances are characterised by floral, muscatel, or fruity scents. At present, we can identify approximately fifty monoterpenic compounds in grapes/wine grapes. The most commonly represented substances in this group are monoterpenic alcohols: linalool, geraniol, nerol, hotrienol, and also alpha-terpineol, which are most representative of “Muscat” varieties. Monoterpenes and their derivatives are therefore important precursors for varieties such as Muscat Ottonel-Traminer, but also for the Rhineland Riesling or Müller-Thurgau [2,3].

The main monoterpenes contained in wine grapes and their sensory manifestation are listed in Tab. 1.

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**Tab. 1** Sensory manifestation of the main monoterpenes contained in wine grapes

Terpenoid substance	Sensory manifestation
Hotrienol	Citrus, rose, linden
Linalool	Citrus, floral scent, lavender
Nerol	Rose, floral and fruit tones
Alpha-Terpineol	Floral tones
Geraniol	Rose, geranium

The content of these aromatic substances in grapes is largely dependent on a number of factors: the variety, physiological maturity, weather in a given year, grape damage, agro-technical works in the vineyard, and a set of factors known as terroir [4,5]. In addition, the content of terpenic substances in the wine depends on the technology used and, above all, the length and method of maceration and course of fermentation [6,7]. The concentration of terpenes increases during the development of the grapes, from the stage of engustment (softening of berries) up to full maturity. Ripe grapes may contain concentrations of terpenes that are five or six times higher than the concentrations found in unripe grapes. For this reason, the concentration of terpenes can serve as an indicator of the ripeness of grapes. The main monoterpenes and terpene polyols are therefore found in grapes in a glycosylated form, which is more frequent than the free form, while the proportions of free and bound forms are given by a particular variety. Due to the presence of  $\beta$ -glycosidases, some free aromatic terpenes are released from their non-aromatic glycosides [8,9].

This experiment monitors the effect of the maceration time on their content in the aromatic Traminer rot variety

## II. MATERIAL AND METHODS

### Design of the experiment

The Traminer rot variety harvested in the Moravian wine region, the Znojmo sub-region (Czech Republic), was used for the experiment. Specifically, it came from the “old vineyard” track in the wine village of *Havraníky*. In this region, the average annual precipitation and temperature are 570 mm and 9.8 °C respectively.

The collection was carried out on September 3, 2016, and the grapes were processed immediately after their import. They were crushed, pitted, and then macerated for a duration of 0, 6, 12, 18, 24, 30, and 36 hours. Subsequently, the

samples were taken, centrifuged, and prepared for further processing.

#### Used chemicals

10 M phosphoric acid, ethanol, Methyl t-butyl ether (MTBE), neohexane, 2-nonanol, cyclopentanone, terpenic standards: linalool, alpha-terpineol, nerol, hotrienol, geraniol (all Sigma Chemical Co., St. Louis, MO).

#### Used instruments

Instruments: Shimadzu GC-17A, Auto sampler: AOC – 5000, Detector: QP-5050A,

Software: GCsolution. Program: LabSolutions, GC MS solution. Version 1.20, Conditions of separation: column: DB-WAX 30 m x 0.25 mm; 0.25  $\mu$ m stationary phase (polyethylene glycol). Voltage of the detector: 1.5 kV. Individual compounds were identified on the basis of MS spectrum and retention time using NIST 107 library, which contains 107,886 spectra.

#### Conditions of separation

Column: DB-WAX 30 m x 0.25 mm; 0,25  $\mu$ m stationary phase (polyethylene glycol)

Sample injection volume: 1  $\mu$ l split ratio of 1:5

Carrier gas flow He: 1 ml/min (linear gas velocity 36 cm/s)

Spraying room temperature: 200 °C

The initial column temperature of 45 °C was maintained for 3.5 minutes, followed by a temperature gradient:

to 90 °C at 15 °C/min

to 135 °C at 6 °C/min

to 207 °C at 9 °C/min

to 252 °C at 15 °C/min.

The final temperature was held for 5 minutes. The total length of the analysis was 30 minutes.

The detector worked in a SCAN mode with a 0.25 second interval in the range of 14-264.

1.5 kV detector voltage.

Individual substances were identified based on MS spectrum and retention time.

The quantification was performed by comparing the peak area of the sample and the external standard with the correction for the internal standard.

#### Determination of aromatic substances by gas chromatography

Concentrations of individual volatile compounds in wine were determined according to an, until now, unpublished method of extraction with methyl tert-butyl ether. 20 ml of wine was pipetted into a 25-ml volumetric flask together with 50  $\mu$ l of 2-nonanol solution in ethanol; this compound was used as an internal standard (in concentration of 400 mg·L<sup>-1</sup>) and 5 ml of a saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution. The flask content was thoroughly stirred, and then 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 emulsion produced by centrifugation, and the clear organic phase was dried up with

anhydrous magnesium sulphate. Extract samples, adjusted in this way, were thereafter used for the GC-MS analysis.

The bound terpenic substances were determined after glycosidic linkage hydrolysis. 20 ml of must was pipetted into a 25 ml volumetric flask, with the pH adjusted to 3.3 with the help of 10M phosphoric acid or sodium hydroxide. The sample was heated at 70 °C for 3 hours. After cooling, the same procedure was followed for the determination of free terpenes.

### III. RESULTS AND DISCUSSION

Total free and bound terpenic substances were determined, as well as other selected terpenic compounds, such as linalool, hotrienol, alpha-terpineol, nerol, and geraniol. For the determination of bound terpenic substances, hydrolysis was performed prior to the analysis. The extraction of these terpenes depends on a number of factors: alcohol, glucose, acids, pH levels, etc.

Fig. 1 shows of chromatograph of terpenes in the must of the Traminer rot variety.

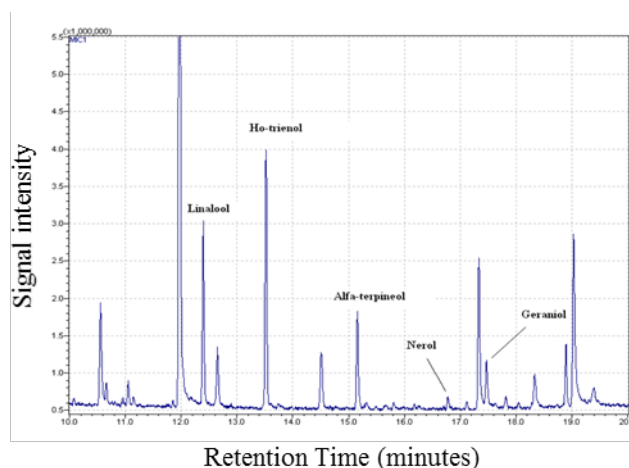


Fig 1. Chromatograph of terpenes in the must of the Traminer rot variety. Linalool (Retention time-RT 12.4), hotrienol (RT 13.5) alpha-terpineol (RT 15.1), nerol (RT 16.8), and geraniol (RT 17.5).

#### Determination of linalool

The Linalool terpene substance is described as a floral, musky to spicy fragrance. The perception threshold for humans is set at 50  $\mu$ g·l<sup>-1</sup>. We could not measure this value even at zero hour, but after 36 hours maceration, this threshold was exceeded almost 2x, and it rose up to 99  $\mu$ g·l<sup>-1</sup>. In the free form, this substance did not appear at all.

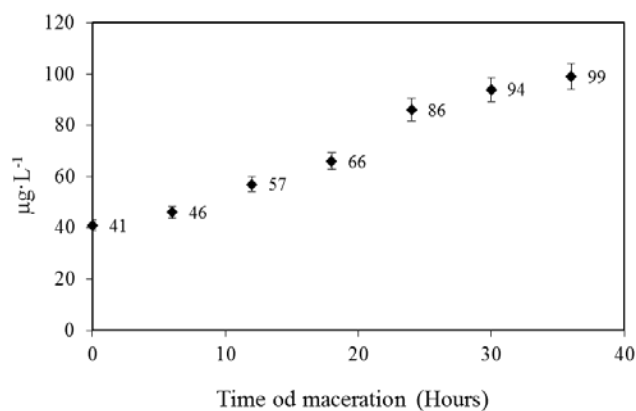


Fig 2. Values of linalool content after hydrolysis

#### Determination of Hotrienol

Hotrienol is perceived as a lime fragrance. Throughout the measurement, its values changed only minimally. Its value at time zero showed up as  $18 \mu\text{g}\cdot\text{l}^{-1}$  and increased to  $24 \mu\text{g}\cdot\text{l}^{-1}$  at 36 hours. The threshold for perception of this terpene is set at  $110 \mu\text{g}\cdot\text{l}^{-1}$ . Our values were only 1/4 of that threshold. No values for this substance were measured in free form.

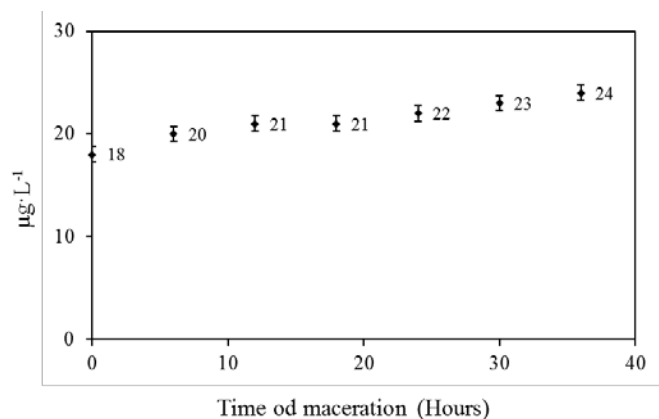


Fig 3. Hotrienol content after hydrolysis

#### Determination of alpha-terpineol

Alpha-terpineol manifests itself as a scent of lilac. Its perception threshold is set at  $400 \mu\text{g}\cdot\text{l}^{-1}$ . Our values were well below this limit. At time zero, it was only  $14 \mu\text{g}\cdot\text{l}^{-1}$ . Over the 18-24 hour period, there was a regular change in values. When reviewing the sampling process, no conclusion was arrived at as to what could have caused this. The final value rose to  $31 \mu\text{g}\cdot\text{l}^{-1}$ . This value is twice as high as the beginning at time zero.

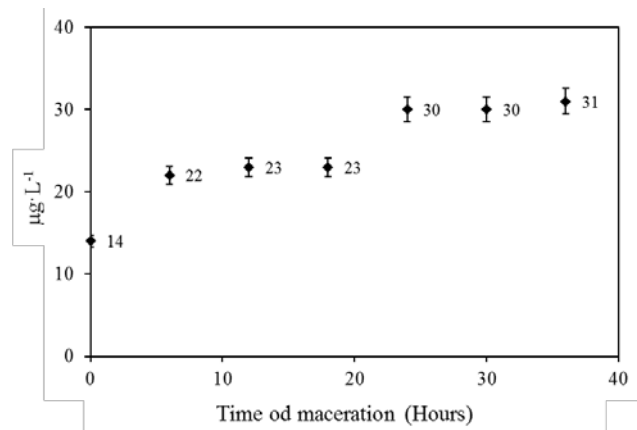


Fig 4. Alpha-terpineol content values after hydrolysis

#### Determination of nerol content

Nerol has a sweet rose scent. It was analysed in both free and bound forms. The free nerol displayed an almost linear line in the increase of values; at time zero, the measured value was  $0 \mu\text{g}\cdot\text{l}^{-1}$ , and at 36 hours the value was  $28 \mu\text{g}\cdot\text{l}^{-1}$ . The threshold of perception is recorded at  $400 \mu\text{g}\cdot\text{l}^{-1}$ . After 36 hours of maceration, these values were 14 times lower than we were able to capture organoleptically.

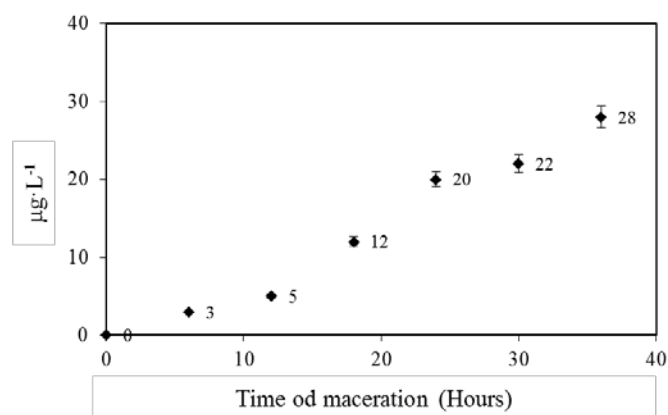


Fig 5. Values of free nerol content before hydrolysis

In the case of nerol released by hydrolysis, the values were paradoxically maintained at  $1 \mu\text{g}\cdot\text{l}^{-1}$  to  $8 \mu\text{g}\cdot\text{l}^{-1}$  at 36 hours. This figure does not correspond to the previous chart. Values are only at 1/3 of the values of the free nerol samples. The hydrolysis caused a substantial decrease.

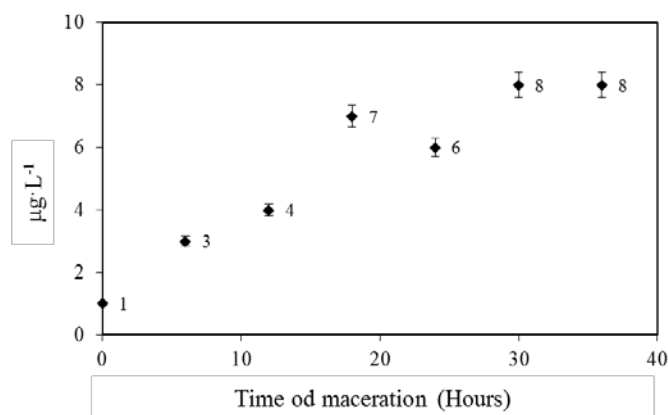


Fig. 6. Values of free nerol after hydrolysis

#### Determination of geraniol content

Monoterpenoid geraniol has a scent of roses. Interestingly, it has turned out to be suitable as a mosquito repellent. The geraniol perception threshold is set to  $130 \mu\text{g}\cdot\text{l}^{-1}$ . The free geraniol displayed an almost linear line. At time zero, the values were at  $12 \mu\text{g}\cdot\text{l}^{-1}$ , and at the end of the measured time axis they were at  $91 \mu\text{g}\cdot\text{l}^{-1}$ . Halfway through this period of time, there was a vacillation in the form of a double increase in values. The threshold of perception wasn't close to being reached. Geraniol in free form was the only one analysed against other measured terpenes.

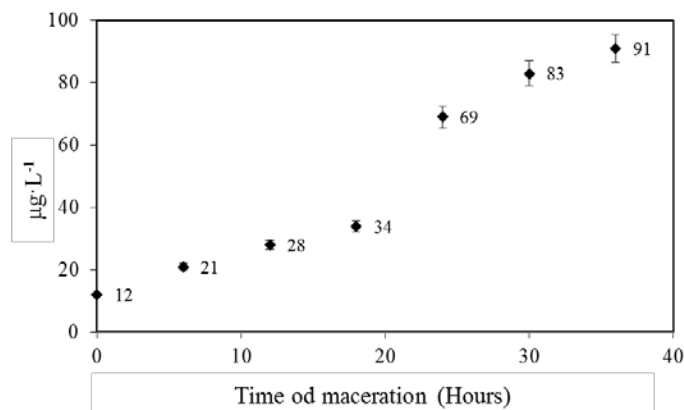


Fig 7. Values of free geraniol content before hydrolysis

The bound geraniol had a regular increase in values across the spectrum of samples taken. At the beginning, at time zero, it had a value of  $10 \mu\text{g}\cdot\text{l}^{-1}$ , and the last measured time had a value of  $39 \mu\text{g}\cdot\text{l}^{-1}$ . In comparing the free and bound geraniol, the same situation as in Nerol is repeated when the element is analysed in free form, with larger values as in bound form.

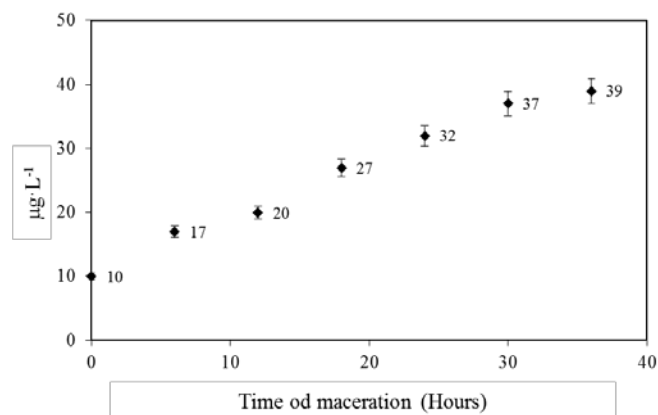


Fig. 8. Bound geraniol content after hydrolysis

#### Determination of free terpenes content

For all the samples taken, the terpenes were released into the must with the length of mash maceration. For the zero hour sample, the free terpenic content was  $12 \mu\text{g}\cdot\text{l}^{-1}$ , with the only value being geraniol. At 6 hours, the value rose to  $24 \mu\text{g}\cdot\text{l}^{-1}$ , and in that amount there was  $21 \mu\text{g}\cdot\text{l}^{-1}$  of geraniol and the nerol was present only at  $3 \mu\text{g}\cdot\text{l}^{-1}$ . These two substances gradually increased their values up to  $119 \mu\text{g}\cdot\text{l}^{-1}$ . Of this, geraniol was  $91 \mu\text{g}\cdot\text{l}^{-1}$  and nerol was only  $28 \mu\text{g}\cdot\text{l}^{-1}$ . The remaining three substances – linalool, hotrienol and alpha-terpineol – were not detected, and their value after 36 hours showed  $0 \mu\text{g}\cdot\text{l}^{-1}$ . Nerol has a perception threshold of  $400 \mu\text{g}\cdot\text{l}^{-1}$  and geraniol has a perception threshold of  $130 \mu\text{g}\cdot\text{l}^{-1}$ . None of these substances exceeded this limit.

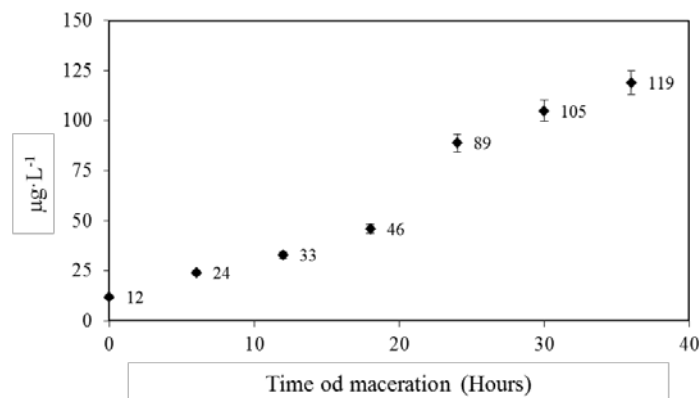


Fig 9. Free terpene content before hydrolysis

#### Determination of bound terpenes content

Bound terpenes released by hydrolysis (heating the must to  $70^\circ\text{C}$  for three hours) rose at time zero to  $72 \mu\text{g}\cdot\text{l}^{-1}$ . Over the course of 18 hours, they continued to rise to  $98 \mu\text{g}\cdot\text{l}^{-1}$ . At 24 hours, they decreased and maintained a value of  $82 \mu\text{g}\cdot\text{l}^{-1}$ . The largest change was measured for linalool, which was  $99 \mu\text{g}\cdot\text{l}^{-1}$ . The smallest change was found in nerol: only  $8 \mu\text{g}\cdot\text{l}^{-1}$ . Of all the perceived samples, the linalool was the only one to surpass the perception threshold.

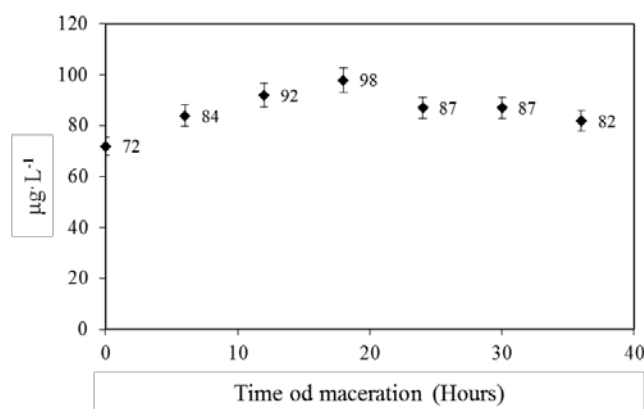


Fig 10. Content of bound terpenes after hydrolysis

#### Determination of total terpenes content

This linear graph shows an upward trend in the time axis for 0-36 hours. The hydrolysis caused a seven-fold increase in total terpenic substances. At time zero, the value was  $84 \mu\text{g}\cdot\text{L}^{-1}$ . After 36 hours, this value rose to  $201 \mu\text{g}\cdot\text{L}^{-1}$ , which is 2.3 times more.

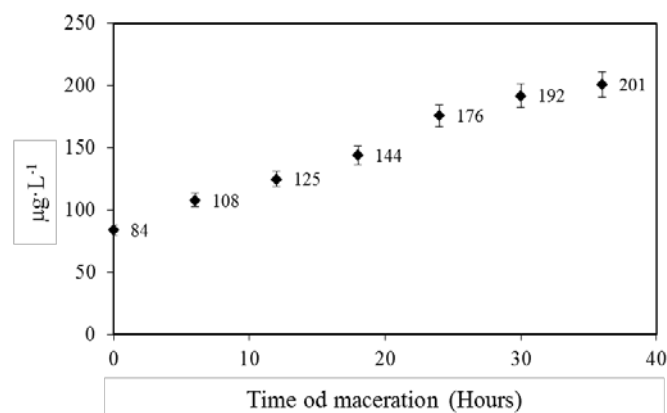


Fig 11. Total terpenes content after hydrolysis

Terpenic substances are a large group of aromatic substances that play a crucial role in the aroma and the character of wine. The most important representatives of terpenes are hotrienol, linalool, alpha-terpineol, geraniol, and nerol. In the grapes and must, we can sensorially find and name geraniol (rose) and nerol (lychees), which are present in grapes in free form. The others only manifested themselves in bound form, and as the time increased, the values also increased [10,11].

In the case of aromatic grape varieties, the terpene substance had mainly increased in the form of glycosidic binding rather than in free form. The results of this work coincide with this finding. Most of the terpenes appeared in intensive form only after hydrolysis. In free form, the values appeared only in nerol (lychees) and geraniol (rose) [12,13].

Mateo and Jiménez [14] divided the varieties of vines into three groups according to the content of terpenic substances. The first group included the intensively aromatic Muscat varieties, the terpene values of which are higher than 4-6 mg. The Traminer variety was also included in this group.

Our study has shown that the Traminer we were observing has more than 20 times lower values than those published in this study. Therefore, Traminers from our region cannot be included as intensively aromatic varieties.

Bureau et al. [15] and Calabretti et al. [16] found that, for aromatic varieties, most terpenes were present in their glycosylated forms. This claim also supports the results of this study, as the content of bound terpenes was higher than that of free terpenes.

The content values of terpenes in the study of Mateo and Jiménez [14] ranged from  $4000\text{--}6000 \mu\text{g}\cdot\text{L}^{-1}$ . The Traminer we were observing reached only  $201 \mu\text{g}\cdot\text{L}^{-1}$  after hydrolysis. The values of the terpenic substances mentioned in this study can also be determined in our country, but mostly in the case of Muscat varieties.

#### IV. CONCLUSION

The composition of these aromatic substances is crucial for wine quality. Their presence or increased concentration can lead to both positive and negative sensory properties of the wine. Moreover, the aromatic maturity is the most important parameter of the oenological potential of grapes. The lessons learned from our study can help wine producers to better understand the principles of volatile substance content in must and wine.

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