“ LUCIAN BLAGA” UNIVERSITY OF SIBIU

FACULTY OF AGRICULTURAL SCIENCES, FOOD INDUSTRY AND ENVIRONMENTAL PROTECTION

Doctoral Dissertation

SUMMARY

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RESEARCH REGARDING THE PRIMARY AND SECONDARY AROMATIC POTENTIAL OF THE AROMATIC AND SEMIAROMATIC VARIETIES IN THE VINEYARD OF RECAS

Scientific Coordinators **Ph. D. Candidate**

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FROM THE AUTHOR

The present doctoral dissertation proposes to offer a series of information regarding one of the most important characteristics of wine, namely the aromatic complex , this being the characteristic leading the consumer to a choice when selecting the wine.

One of the regions which offers a big vineyard potential in the Banat region, which is very well known for its vineyards and wine centers, offering a series of highly appreciated wines for the market.

The doctoral dissertation follows two important directions: the physicochemical and aromatic evaluation of the aromatic and semiaromatic varieties in the Recas vineyard and on the other hand, the promotion of some improvement techniques for their aromatic profile.

The research took place during 3 years (2010 – 2013), the first of which being dedicated to bibliographical the studies. The experimental stage to place in the doctoral research laboratories of the "Faculty of Agricultural Sciences, Food Industry and Environmental Protection" of the "Lucian Blaga" University of Sibiu, the laboratories of the "Molecular Biology/ Microbiology Research Institute" of the "Nyíregyháza" University, Hungary and in the laboratories of the "National Research-Development Institute for Cryogenic and Isotopic Technologies - INC-DTCI ICSI" of Ramnicu Valcea.

The results obtained are synthesized in the 214 pages, 16 tables and 145 figures of the present doctoral dissertation.

The doctoral dissertation is structured in two distinctive parts, the first one being the theoretical one and the second one the experimental.

The theoretical part presents the present status of the research regarding the wine aromas and pedological and climate data, specific to the vineyard of Recas.

The experimental part focuses on the study regarding the aromatic potential of the aromatic and semi-aromatic varieties in the vineyard of Recas, but also on the techniques and methods which have the role of influencing this aspect.

Therefore, the influence of enzyme and yeasts selected in the formation of aromas are studied, by taking into consideration the conservation of the authenticity and type of the varieties through the isolation and conservation of autochthonous yeast with superior biotechnological qualities.

The dissertation contains the list of figures, of the graphs in the appendix, which syntheses the tables and notations which attend the information in the text.

Alongside me were Ph.D. eng. Ovidiu Tița and Ph.D. Letiţia Oprean, the scientific coordinators of the present doctoral dissertation, whom I wish to address special thanks for the material, moral and scientific support they have awarded me with during the entire doctoral dissertation.

The ideas, suggestions and result successful discussionss with my colleagues Ph.D. Angela Bănăduc, Ph.D. Dan Chicea, Ph.D. Mariana Păcală, Ph.D. Mihaela Tița, Ph.D. Ramona Iancu, Ph.D. Otto Ketney have also contributed to the finalization of the present essay, persons to whom I would hereby also like to address special thanks.

The experimental part wouldn't have been materialized without the help received from the professional collectivity at the vineyard Center of Recas, which had the kindness of allowing me to use raw material.

Also, the company "Begerow" from Germany and "Sodinal" of Bucharest, which offered me a series of wine products that were necessary during the research.

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Also, special thanks to the research collective of "Molecular Biology/ Microbiology Research Institute" of the "Nyíregyháza" University, Hungary, especially Ph.D. Laszlo Sikolya, where high accuracy research regarding the genetic identification of the yeasts from autochthonous strain could be conducted.

AIMS AND SCIENTIFIC OBJECTIVES OF THE DOCTORAL DISSERTATION

The present doctoral dissertation has the aim of capitalizing the primary and secondary aromatic potential of the aromatic and semiaromatic varieties coming from Recas, Romania, in view of obtaining wines with harmonious and balanced characteristics.

The following scientific objectives have been set:

- The evaluation of the physicochemical qualities of the aromatic and semi-aromatic yeasts of Recas;
- The identification and quantification of the phenolic and aroma compounds in the aromatic, semi-aromatic and red varieties, using modern methods in view of establishing the specific aromatic profile;

- The evaluation of the quantification and potentiating techniques of the aroma substances on the wine obtained through multiple biotechnological variants;
- The isolation and selection of indigene yeasts of the local varieties, in view of keeping the authenticity and typical potential of the wine of Recas;
- The identification and quantification of the aroma compounds in the aromatic, semiaromatic and red wines, obtained through the replacement of yeasts with autochthonous strain;
- The analysis and establishment of the isotopic profile of the region and the grape varieties in view of identifying the eventual fraud and supplementing the national database in this field of work;
- The influence of heavy metals on the quality of the wines of Recas.

CHAPTER IV

STUDIES REGARDING THE PHYSICAL-CHEMICAL AND AROMATIC EVALUATION OF THE AROMATIC AND SEMIAROMATIC MUSTS OF RECAS

4.1The physical-chemical characterization of the aromatic and semiaromatic musts of Recas

4.1.2. Materials and methods

The following must samples have been collected in view of accomplishing this study: Muscat Ottonel (MO), Chardonnay (CH), Sauvignon Blanc (SB), Cabernet Sauvignon (CS) and Pinot noir (PN), vineyard of Recas, harvest year 2012.

The physical chemical analysis of the selected musts have been carried out following the OIV accredited method, in conformity to the existing normative in our country and the recommendations of Hanna Instruments, www.hannainstr.com/manuals, and using the following kits:

- The sugar determination (SR 6182-25:2009 expressed in g/L)
- The pH determination (pH-meter)
- Total acidity determination (SR 6182-1:2008 expressed in g/L)
- The polyphenols determination (Folin-Ciocalteu method, expressed in mg/L)

And the glycerol has been determined using the -BIOPHARM(Cat NO10148270035) method, expressed in g/L.

4.1.3. Results and discussions

The following can be concluded based on the determinations: the musts contain a high quantity of sugars, varying between 205 and 235 g/L. Musts coming from aromatic varieties

present a sugar accumulation between 225 g/L and 228 g/L. Musts coming from aromatic varieties present a sugar accumulation between 205 g/L and 207 g/L (Sauvignon Blanc and 229g/L-235g/L (Chardonnay) and the red ones are situated between 205g/L-215g/L (Pinot noir) and 217g/L-224g/L (Cabernet Sauvignon). Due to the dry and sunny year, a substantial sugar accumulation took place in the selected varieties. Muscat Ottonel and Chardonnay present the highest sugar accumulation, situated between 225 g/L and 235 g/L.

The pH is situated between 2,95 for the Chardonnay and 3.23 in the Pinot noir, the obtained values reaching a maximum of 3.23.

The total acidity oscillates between 3.8 g/L in the case of the Sauvignon Blanc and Pinot noir, reaching 6.8 g/L for the Chardonnay. In the case of Cabernet Sauvignon, the values are situated between 5.2 g/L and 5.7 g/L, tartaric acid. The acidity of the aromatic Muscat Ottonel oscillates around 4 g/L, the results being between 4.1 g/L and 4.3 g/L, tartaric acid.

The lowest glycerol value is identified in the case of the Chardonnay and Sauvignon Blanc musts (31.2 mg/L – 43.9 mg/L).

4.1.4. Conclusions

- the glucose content exceeds 200 g/L, the pH evolves in balanced limits, so the result will be high quality wines
- the acidity is between the standard limits, no correction needed
- the glycerol varies depending on the variety, from 20 mg/L to 100 mg/L, these quantities conferring pleasant organoleptic nuances.

4.3 The evaluation of the volatile aromatic compounds in the aromatic and semiaromatic musts from the wine center of Recas

4.3.2.Materials and methods

- musts from the varieties: Muscat Ottonel (MO), Chardonnay (CH), Sauvignon blanc (SB), Cabernet Sauvignon (CS), Pinot noir (PN), from the wine center of Recas.
- the identification and quantification of the aroma substances using the GC-MS method – gas-chromatograph coupled to the mass spectrometer Varian GC 450/ 240 MS

4.3.3. Results and discussions

The above obtained results have been grouped and summed into categories of important compounds: terpenic compounds, aldehydes, superior alcohols, volatile fat acids, esters. Based on the determinations, it has been concluded that the musts from the wine center of Recas have accumulated terpenic compounds that presented different values depending on

the variety of origin. The sum of free and bonded terpenic compounds in the Muscat Ottonel reached 1.509 mg/L, and in the case of the two semiaromatic varieties, Chardonnay and Sauvignon blanc, the values vary between 0.190 mg/L and 0.186 mg/L, and the two red varieties Cabernet Sauvignon and Pinot noir between 0.384 mg/L and 0.322 mg/L. The sum of the aldehydes resulted from the gas-chromatograph analysis is situated between 0.923 for the Muscat Ottonel and 0.712 mg/L for the Chardonnay. It can be observed that the variation of the aldehydes quantum in the case of the five varieties taken into study does not present differences of more that 18-20%.

The superior alcohols are situated between 367.223 mg/L for the Chardonnay and a maximum of 526.325 mg/L for the Muscat Ottonel. It is observed that in the case of the aromatic varieties, this quantum is 20-30% higher than in the case of the semiaromatic varieties. Regarding the red varieties, Cabernet Sauvignon and Pinot noir, they present values that are 10-20% lower than the aromatic Muscat Ottonel. The fat volatile acids vary depending on the variety in percents between 5% and 22%, values which characterize these musts.

The minimum values of 198.867 mg/L have been found in Sauvignon Blanc, a semiaromatic variety, and maximum of 312.023 mg/L resulted in Pinot noir. In the case of the aromatic Muscat Ottonel 283.992 mg/L of fat volatile acids have been quantified.

The resulted ester sum leads to the conclusion that the aromatic and the red varieties have the highest content, varying between 272.843 mg/l for the Pinot noir, 278.224 mg/L for the Muscat Ottonel and 295.118 mg/L for the Cabernet Sauvignon.

For the semiaromatic varieties Chardonnay and Sauvingon Blanc, the ester sum oscillates between 156.892 mg/L and 167.778 mg/l, these values being 70-80% lower than the values determined in the case of the aromatic Muscat Ottonel and the red Cabernet Sauvignon and Pinot noir.

4.3.4. Conclusions

- the identification of the volatile aromatic and semiaromatic compounds from Recas leads to the establishment of some typical characteristics of these varieties, in their areal of cultivation, being the comparison base for ulterior studies.
- the accumulation of high quantities of terpenic compounds is favorable, effectively contributing to the realization of its own aromatic profile. The determined values lead to the conclusion that the aromatic and semiaromatic varieties in Recas have a well defined aromatic potential, which can be improved by applying alternative biotechnologies in view of growing the quantum of terpenic compounds.

- the aldehydes participate to the formation of aromas and aged bouquets of wines, so that a balance must be found regarding their quantity. A higher quantity implies an aroma oxide, but in moderate quantities, it presents caprylic compounds with nice, floral nuances.
- the aldehydes do not present spectacular values in musts related to different musts, these differences being visible as a result of the alcoholic fermentation.
- the sum of superior alcohols varies from variety to variety, the obtained values being similar during the must phase
- important differences can be observed between the aromatic and semiaromatic varieties, close values being only found between the aromatic Muscat Ottonel and Pinot noir.
- the fat volatile acids, responsible for the floral aromas, can be found in balanced quantum, the obtained values mirroring the aromatic specter of the aromatic and semiaromatic varieties from Recas
- significant differences can only be found between the red Pinot noir and the semiaromatic Sauvignon blanc, differences which are specific for the two varieties.
- esters are the components which confer a pleasant fruity, apples, pears, bananas, orange, grapefruit, berries. All these esters have been identified in the five varieties taken into study.
- the sum of esters, determined through the gas-chromatograph, leads to the conclusion that the aromatic varieties like Muscat and the red ones like Cabernet Sauvignon and Pinot noir, the values are close, superior to the semiaromatic ones, strengthening their fruity character.

CHAPTER V
TECHNOLOGICAL PROCEDURES REGARDING THE CAPITALIZATION OF THE AROMATIC POTENTIAL OF GRAPES

5.1. Studies regarding the influence of the maceration enzymes in the technology of aromatic, semiaromatic and red wine obtainment from the Recas vineyard

5.1.1. The extraction and potentiating of the aroma substances in the Muscat Ottonel grapes, using some enzymatic prepared

5.1.1.2. Materials and methods

- Muscat Ottonel grapes, harvested when fully mature in the vineyard of Recas, year 2012
- Extraction enzymes (5g/100 kg of grapes)
    - -Lafazyme CL, www.laffort.com
    - -Sihazym Extro, www.begerow.com
    - -Zymovarietal-aroma G, www.sodinal.com

-Enovin varietal, www.agrovin.com

- maceration temperature of 15°C,
- pelicular maceration
- maceration for 12 hours
- the identification and quantification of the aromatic substances using the GC-MS method has been carried out using the method described at point 4.3.2.
- Maceration variants for the Muscat Ottonel:

Variant I (V1) master sample

- Muscat Ottonel grapes, maceration without enzymes

Variant II (V2 / Lafazyme CL enzymes)

- Muscat Ottonel grapes, pelicular maceration with Lafazyme CL enzymes

Variant III (V3, Sihazym Extro enzymes)

- Muscat Ottonel grapes, pelicular maceration with Sihazym Extro enzymes

Variant IV (V4, Zymovarietal- Aroma G enzymes)

- Muscat Ottonel grapes, pelicular maceration with Zymovarietal- Aroma G enzymes

Variant V (V5, Enovin Varietal enzymes)

- Muscat Ottonel grapes, pelicular maceration with Enovin Varietal enzymes

5.1.1.3. Results and discussions

The determinations show that musts that have not been treated with enzymes present a 22-35% lower concentration of free terpene, compared to the musts that have been treated with extraction enzymes. The α terpine content has a 22% growth in variant V2 compared to the whiteness sample, while the percentage reaches 35% in variant V3, the linalool grows 23% in variant V3 compared to the whiteness sample, followed by variant V5 and variant V4, the lowest value being register for variant V2.

As far as the free terpene accumulation is concerned, they can be found in higher concentrations with linalool, exceeding 0.2 mg/L, while the lactones can be found in very small concentrations, under 0.0135 mg/L. The α terpineol, citroneol, polyols and the aldehydes are found in quantities between 0.01 and 0.05 mg/L, while the nerol, hotrienol and geraniol oscillate between 0.05 and 0.1 mg/L. As far as the linked glycoside terpene accumulation is concerned, the gas-chromatograph determinations show that they are present in the must with values exceeding by 30% the values of the free terpene. In the case of the pectolite enzymes usage, which also have a glycoside activity, the aroma precursors accumulate in significant values of up to 1-1.2 mg/L. When compared to the free terpene, an

accumulation of linked terpenic compounds can be seen, in higher quantities, exceeding 1.8 mg/L.

5.1.1.4. Conclusions

- the usage of enzymes prepared leads to a superior extraction of aroma compounds, while maintaining high values of β-glycoside.
- the values of the terpenic compounds grow in average by 20% through the usage of extraction enzymes.
- the usage of maceration enzymes in wine making leads to an optimization of the primary aroma potentiating process, having as a consequence wines with superior sensorial characters.
- the study demonstrated that the usage of enzymes prepared leads to important growth of the free and linked terpene in the must, the recommended enzymes being Sihazym Extro (Begerow, Germany).
- as far as the wine quality is concerned, it presents more accentuated fruity characters, through a more accentuated ester accumulation.

5.2 Studies regarding the influence of selected levures in the aromatic, semiaromatic and red wine processing technology at Recas

5.2.1. The extraction and potentiating of the aroma substances in the aromatic Muscat Ottonel varieties, using selected levures

5.2.1.1. Materials and methods

The must obtained from Muscat Ottonel grapes, macerated for 12 hours with the Sihazym Extro enzymes.

Technological fermentation parameters: linear fermentation for 3 days at 12°C, 20 days at 15°C, flotation cleaning, micro oxygenation, severe deburr, 100 mgSO_2/L.

Technological fermentation variants through microvinification:

V1 – spontaneous fermentation

V2 – fermentation through adding wine yeasts Saccharomyces cerevisiae, isolated from the resident epifita microflora – 20 g/hL.

V3 - fermentation through adding wine yeasts Saccharomyces cerevisiae Lalvin EC1118-20g/hL (Lallmande, France)

V4 - fermentation through adding wine yeasts Saccharomyces cerevisiae Fermactive muscat 20g/hL (AEB Spindal France)

V5 - fermentation through adding wine yeasts Saccharomyces cerevisiae Siha cryarome 20g/hL(Begerow, Germany)

- the determination of the physical-chemical characteristics of the resulted wines (methods described in www.hannainstr/manuals)
- the determination of the aroma compounds (method described at 4.3.2.)

5.2.1.2. Results and discussions

- *the physicochemical evaluation of the aromatic Muscat Ottonel wines, fermented with selected yeasts*

The aim of selecting the stem leading to a higher quality wine, with more pronounced aromatic characteristics, was the reason for the usage of multiple yeast stems for the fermentation process.

As a effect of the fermentations carried out in the microvinification system, the above mentioned technological parameters lead to the following physicochemical wine characteristics:

It can be concluded, as shown in figure 5.10, that the yeasts that were used in the present study lead to superior wines. Thus a 20% growth in alcoholic concentration can be observed in variant V5 and lower concentration in V3 and V4, the values being situated between 10.8%v/v and 12.5 %v/v. The glycerol value is doubled from 4.33 g/L to 8.11 g/L, which is benefic to the unctuous character of the wines. The residual sugar decreases, demonstrating the glucosidase activity of the used yeasts, the values oscillating between 3.2 g/L and 1.2 g/L.

The free SO_2 quantity grows between 11% and 40%, compared to the witness sample and the total SO_2 decreases with 1 to 9 points.

The volatile acidity decreases by 45% compared to the witness sample V1, the obtained values being situated between 0.27g acetic acid/L and 0.44 acetic acid/L. The total acidity increases with 28%, resulting in values oscillating between 4.2g tartaric acod/L and 5.8g tartaric acid/L. The values resulted from using the selected yeasts in the Lalvin EC1118 range, Fermactive muscat and Siha Cryarome do not present significant oscillations, the obtained results varying between 5.6g tartaric acod/L and 5.8g tartaric acid/L, which are balanced values for this wine assortment.

A growth of appreciatively 9-12% in the polyphenol quantum is observed, when using the selected yeasts in variants V3-V5, but with no alteration on the quality of the wine.

- *the aromatic evaluation of he Muscat Ottonel wines, fermented using selected levures*

The terpenic compounds are defining elements in the characterization of an aromatic wine, their quantification being extremely important. Observing the values in figure 5.14, it can be concluded that in the case of V1, the free and linked terpene compounds do not exceed

1800 mg/L, while variant V2 shows a 3% growth. The involvement of the selected yeasts in the release of terpenic compounds is visible in variants V3-V5, where a substantial growth can be observed.

In the Muscat Ottonel wines that have been obtained by using the selected Lalvin EC1118 (Lallmande, France) yeasts, the terpenic compounds accumulation reaches 2060 mg/L, 10% more than the witness sample. In the case of using the Fermactive muscat (AEB Spindal France) yeast, the values of the resulted terpenic compounds reach 2145 mg/L, 20% higher compared to the witness sample. In the case of variant V5, a 12% growth in the terpenic compounds can be observed, their value reaching 2099 mg/L. It can thus be concluded that the selected commercial yeasts lead to significant growth in terpenic compounds, the values for the last 3 variants being situated between 2060 mg/L and 2145 mg/L.

Regarding the evolution of superior alcohols, they are situated between 506.612 mg/L and 567.733 mg/L, the obtained values being decreased by an average of 8-10%. These values do not exceed the classical determined quantum in the special bibliography, the final results being appreciated for their odorous character of the aromatic Muscat Ottonel wines.

The fat volatile acids present values situated between 212.929 mg/L and 291.267 mg/L, values which significant for the aromatic characterization of the Muscat wines. The minimum values are found in variant V5, where the quantum of fat volatile acids are situated at around 212.929 mg/L. the variants V3 and V4 lead to the obtaining of values between 291.267 mg/L and 282.246 mg/L, values which are close to the witness sample, variant V1.

By using the selected yeasts, the ester quantum exceeds 360 mg/L, being representative for variants V3-V5. The most remarkable ester quantity is seen in variant V4, where they reach values as high as 395.008 mg/L, as much as 47% more as the witness sample.

Variant V5 leads to 32% growth in the esters that have been accumulated in the resulted wine, reaching values as high as 379.341 mg/L, while in the case of variant V1 the values were of 268.294 mg/L.

As far as the determinations in variant V3 are concerned, a 33% growth in esters can be seen, the values reaching 367.173 mg/L, compared to the variant V1, the witness sample.

The aldehydes are the carbon compounds which can lead to odorous perceptions in the aromatic wines, but during the same time, if consistent enough in components like acetaldehydes and butyraldehydes, they can affect the quality of the resulted wine. The aldehydes values that were obtained are situated between 171.529 mg/L and 189.378 mg/L,

the maximum values being highlighted in variant V2, where yeasts that were selected from the epifita micro flora of the Muscat Ottonel of Recas have been used. In the case of variant V3 these values are closer, reaching an quantum of 188.145 mg/L.

5.2.1.3. Conclusions

As a result of the physicochemical determinations of the wine, the five variants led to the following conclusions:

- by using the selected yeasts, the alcoholic concentration of the aromatic Muscat wines grows by cca. 20%
- the glycerol quantity is double, process which leads to obtaining some harmonious, onctuos, pleasant wines
- the residual sugar diminishes, fact which demonstrates the yeasts capacity to assimilate important quantities of carbohydrates
- the SO_2 quantity diminishes with about 13%, which is another benefit of the obtained wines
- the total acidity grows, conferring the wines with fresh and reviving characteristics
- the volatile acidity decreases, with an effect on the taste and the olfactive perception of the wine
- a growth of about 9-12% in the quantum of the total polyphenols is observed, but with no alteration on the quality of the final wine
- as far as the aromatic compounds are concerned, a growth of about 20% of the free and linked terpenic compounds can be observed, leading to the accentuation of the fruity character of the wines
- the superior alcohols and fat acids are decreasing their values in variants V3-V5, with about 10-12%, variants where the selected yeasts have been used. In exchange, a substantial growth can be found in the case of the esters, of around 47%, which are vital compounds in the formation of the aroma of the young wines
- the esters are actively participating at the aromatic characterization of the wines, conferring fruity aromas and firmly contributing to their sensorial definition
- the aldehydes, accumulated in moderate concentrations, lead to the obtaining of wines with a pleasant floral character, being also highly responsible for their organoleptic properties, the values obtained for five processing variants leading to the conclusion that the selected yeasts did not lead to spectacular modifications of this indicator
- the ratio between esters, superior alcohols and fat acids lead to an intensification of the bouquet and the decrease of the astringency of the wine

- the usage of selected yeasts with commercial provenience leads to the obtaining of harmonious, pleasant, with fruity odor notes, floral perfume aromatic wines, but also acidic with fresh notes.
- In view of maintaining the aromatic and authentic type of the Muscat Ottonel varieties of Recas, the improvement of the biotechnological characters of the isolated epifita micro flora is recommended.

6.4 The identification of the aromatic complex of the Muscat Ottonel wines that have been processed using autochthonous selected yeasts

6.4.2. Materials and methods

- cultures of *Saccharomyces cerevisiae* yeasts, isolated from local stems, having the following codes:

MOR 27 - *Saccharomyces cerevisiae* yeasts, isolated and multiplied in fermentative conditions, with an add of mineral nutritive substances of the Muscat Ottonel variety of Recas;

MOR 59 - *Saccharomyces cerevisiae* yeasts, isolated and multiplied in fermentative conditions, with an add of vitamin complex of the Muscat Ottonel variety of Recas;

MOR 234 - *Saccharomyces cerevisiae* yeasts, isolated and multiplied in fermentative conditions, with an add of yeast autolizat, mineral substances of the Muscat Ottonel variety of Recas;

MOR 298 – *Saccharomyces cerevisiae* yeasts, isolated and multiplied in fermentative conditions, with an add of activators of the Muscat Ottonel variety of Recas;

Used fermentative variants:

Variant I – Muscat Ottonel must, fermentation under the action of the epifita yeast existent in the must

Variant II – Muscat Ottonel must, fermentation under the action of the MOR 27 yeasts

Variant III – Muscat Ottonel must, fermentation under the action of the MOR 59 yeasts

Variant IV – Muscat Ottonel must, fermentation under the action of the MOR 234 yeasts

Variant V – Muscat Ottonel must, fermentation under the action of the MOR 298 yeasts

Technological fermentation parameters: linear fermentation for 5 days at 18°C and 14 days at 15°C; micro oxygenation; clearing through flotation with severe deburring and an add of 100 mg SO_2/L.

The quantitative determination of the aromatic compounds was done using the GC-MS methods, method described at point 4.3.2.

6.4.3. Results and discussions

Through the isolation of the yeast stems and the quality improvement of the biotechnological characteristics, the obtaining of the wine with superior sensorial qualities has been attempted.

Thus, it can be concluded that the total volatile compounds are situated between 730.878 mg/L (V1) and 866.729 mg/L (V3). By following their quantification, it can be concluded that a more profound accumulation took place in the case of variant V3, the variant for which the selected yeast MOR 59 has been used, followed by variant V5, where the selected yeast MOR 298 has been used. The differences between the two variants (variant V2 and variant V4) are not major and even those two can be recommended to the specialists.

The accumulation of the total volatile compounds varies between 16 and 34%, compared to the witness sample. Based on the chromatograph results, it can be concluded that the fruity aroma conferred by the isobutanol (1) grows up to the value of 27.794 mg/L and the vegetal one, conferred by 1-hexanol(2) up to 2,724794mg/L. The accumulation of alpha-terpineol (3) and linalool (4) are specific for the Muscat Ottonel variety, reaching values of 0.052794 mg/L.

Another pleasant aromatic characteristic of the Muscat Ottonel variety is the banana aroma conferred by the isoamyl acetate(8), aroma being quantified according to the chromatogram (Figure 6.44) to the maximum value of 54.333794 mg/L.

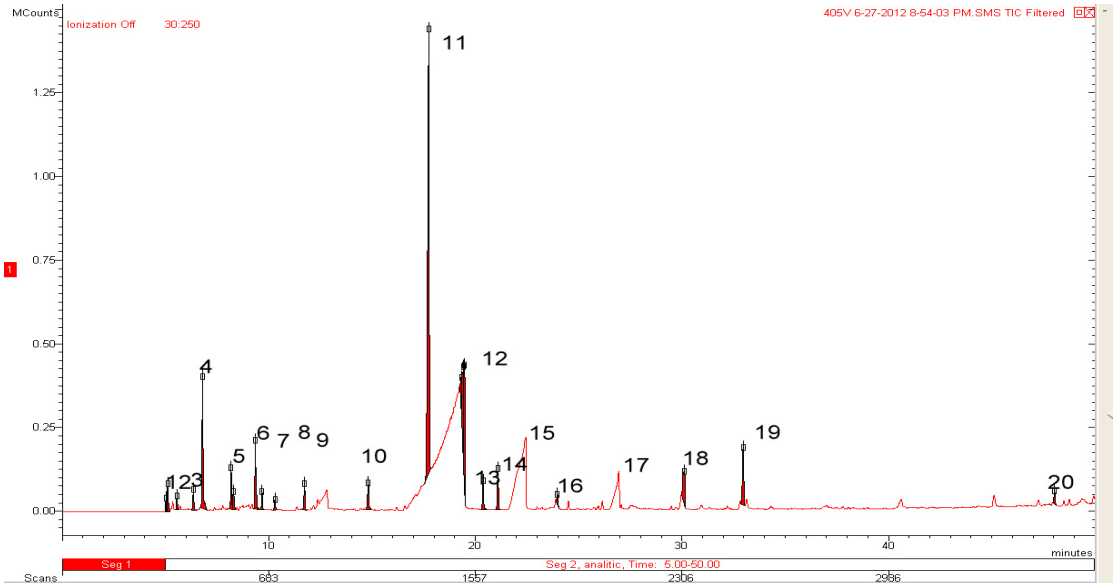

Figure 6.44. The chromatogram of the volatile compounds that have been identified in the Muscat Ottonel wine

6.4.4. Conclusions

The usage of the selected autochthonous yeasts led to the establishment of an authentic aromatic profile, specific to the area of Banat (Recas vineyard). The accumulation of terpenic compounds is specific to the Muscat Ottonel variety, in this case the recommendation being yeast MOR 234.

In view of insuring a balance regarding the superior alcohols in the wine, the usage of yeast MOR 298 and yeast MOR 59 is recommended.

The fat volatile acids lead to a positive influence on the aromatic profile of the Muscat Ottonel wine, in the case of the usage of yeast MOR 298.

The esters and aldehydes impose themselves through pleasant aromas, which effectively participate to the realization of a complex bouquet of the wine, especially when using the yeast MOR 298 and yeast MOR 234.

Practically, all the components identified through the GS-MS method led to the identification of the aromatic complex of the Muscat Ottonel wine, processed using the selected yeasts MOR 27, MOR 59, MOR 234 and MOR298. Thus, it can be observed that the selected yeasts have superior biotechnological properties and lead to the amplification of the aromatic characters, compared to the fermentations that have been realized under the action of the epifita yeasts existing in the must.

CHAPTER VIII

THE EVALUATION OF THE HEAVY METAL CONTENT IN THE WINES OF RECAS

8.2. Materials and methods

The present study has selected the samples coming from the wine center of Recas, from the following wine assortments: Chardonnay , Muscat Ottonel, Pinot noir, harvested during 2010, 2011, 2012, noted as follows:

-Muscat Ottonel- MOR,

-Pinot noir- PNR,

-Chardonnay- CHR,

The following heavy metals have been identified in the selected samples: Zinc, Nickel, Cadmium, Copper, Chrome and Led, using the atomic absorption spectrometer with graphite copper ICP-MS VARIAN 820. Pure water has been used, HNO_3 69% (g / v), concentrated with HF and HCl, Merck and purified water with a resistance of maximum 18,2 M Ω cm-1,

obtained by using a Millipore Milli-Q (Bedford, MA, USA) system. A small quantity of the sample, or standard solution is placed in the interior of the tubular graphite tube.

It is heated in a temperature program in order to eliminate the impurities by burning them. For the quantitative determinations a calibration curve has been obtained for each element.

8.3. Results and discussions

The zinc can be found in very small quantities in grapes, but they can reach the wine, where their quantum can exceed 5 mg/L. Milligram values resulted in the studied samples: the minimum value determined for the Muscat Ottonel of Recaș through the atomic spectrometry reaches 345.1μg/L in 2012 and the maximum value is of 1733μg/L, found in Recaș in 2010.

Nickel is one of the elements which can especially emanate from the stainless steel containers, but as shown in figure 8.2, the values are rarely exceeding 90μg/L.

Thus, minimum values of 20.4μg/L can be found in the case of the Muscat Ottonel of Recas in 2010 and maximum values of 105.9 μg/L, 126.9 μg/L in the case of the Pinot noir, respectively Muscat Ottonel from Recaș, 2012 and 2010.

A nickel accumulation in the human body is very harmful, close monitoring being the recommendation for the situation at hand.

Cadmium is one of the natural components of the wine emanating directly from the grapes, the maximum OVI admitted values being of 10 μg/L.

In large quantities, it is harmful for the human body, affecting the calcium in the bone structure and the kidneys. In general, values of under 1 μg/L or between 2 μg/L and 5 μg/L can be found.

Copper generally accumulates in the soil through the treatment process of the vineyard, finally reaching the wine. In large quantities, it can be toxic for the human body, but it is not assimilated into the organism through wine consumption.

It inhibits the activity of the levures in the must and as a result, quantities exceeding 2000 μg/L are not benefic for the wine. In general, the values do not reach alarming values, being in general found under the maximum admitted value of 1000 μg/L.

Chrome presents values situated between 0.14 μg/L and 5.12 μg/L, the accumulation of chrome being more accentuated in the Pinot noir of 2012.

Another one of the reference metals found in the characterization of a wine is the led. The values that have been determined in the wine of Recas are situated between 0.026 μg/L and 0.38 μg/L, the highest of the values being determined in the wines of 2012.

The lowest led quantity can be found in the wines of 2012, when this indicator does now exceed 0.103 μg/L.

8.4. Conclusions

- the metals that have been found in the samples subject to analysis are generally found in quantities under the maximum OIV admitted values, their influence on the aromatic qualities of the wine being minimum.
- the zinc presents very high oscillations and as a result there is no soil contamination in the studied areas.
- the nickel only exceeds the maximum admitted areas in one sample, the rest of the determination being under the admitted values.
- the cadmium can be found well under the maximum admitted limit and as a consequence it does not affect the health of the consumer.
- the copper is generally found under the value of 1000 μg/L, which is the maximum admitted value in the international regulations. It does however, in isolated cases, exceed this limit.
- the chrome is situated under the maximum OIV admitted limits, including the values determined in the case of the led.
- the metals that have been identified in the present study do not produce major changes in the aromatic profile of the wine due to the fact that the determined values present a low quantum, in several cases being even undetectable.

CHAPTER X

CONCLUSIONS AND PERSPECTIVES

10.1. Final conclusions

- The quality of the aromatic and semiaromatic wines of Recas is highly influenced by the approach of every one of the technological phases, a fact of major importance being the approach of the primary vinification.
- The usage of the enzymes prepared during the maceration and fermentation processes lead to the optimum extraction of the aroma compounds, conferring the aromatic, semiaromatic and red wines with superior quality characteristics.
- The optimum maceration leads to the accentuation of the floral characteristics, of the fruity properties of the wines thus developing their aromatic potential, especially through the superior terpenic compounds and ester extraction.
- The correlated physicochemical and enzymatic levels have been established, leading to the selection of the optimum grape processing variant, in view of obtaining wines with superior

sensorial qualities.

- The maceration operation leads to the selective optimization and extraction of the odorous fractions and of the anthocyanins, fact which leads to obtaining more corpulent, savory, full of color and aroma wines.
- The polyphenol and glycerol content grows with every maceration variant, which is benefic for the quality of the resulted wine.
- The usage of the commercial yeasts with known biotechnological properties leads to obtaining wines with the desired characteristics, fruity, with floral aromas, but during the same time isolated yeasts from the local epifita micro flora can be used.
- The combined usage of enzymes and selected yeasts influences the aromatic characteristics of the wine, onctuozity, corpulence.
- The isolation of the yeasts out of autochthonous stems is benefic for the conservation of the authenticity of the wine varieties in the Recas vineyard, but the co-inoculation of commercial yeasts in view of optimizing the fermentation process can also be recommended.
- A technological scheme for the production of superior aromatic, semiaromatic and red wine in the vineyard of Recas has been elaborated as a result of the obtained determination.

10.2. Personal contributions

- I have evaluated the physicochemical and aromatic characteristics of the aromatic and semiaromatic wines in the vineyard of Recas, wines which were obtained using different technological variants.
- I have studied the influence of the extraction enzymes on the physicochemical and aromatic characteristics of the aromatic and semiaromatic wines of Recas.
- I have isolated and identified the genetic yeasts from autochthonous varieties in view of maintaining the authenticity and type of the wines of Recas.
- I have established the quality parameters of the isolated yeasts from autochthonous stems, as well as the glycerol accumulation in view of selecting the stem with superior properties, the results being statistically interpreted.
- I have evaluated the aromatic configuration of the wines that have been processed with isolated yeasts from stems coming from the vineyard of Recas in view of improving their biotechnological properties.
- I have accomplished the isotopic printing of these wines, the obtained results being necessary for completion of the national data base.

- I have studied the effect of the accumulation of heavy metals in the wines and on their quality.
- I have suggested optimum processing methods for the aromatic and semiaromatic varieties in view of potentiating and capitalizing the aroma compounds.

10.3. Research continuation perspectives

- I recommend the continuation of the research regarding the obtaining of autochthonous yeasts with superior and specific biotechnological properties in view of keeping the typical characteristics of the varieties in this vineyard.
- Research can be continued regarding the accumulation of some phenol compounds like the rutin in certain varieties, which were less studied up to the present moment.
- Other technical specification can also be approached in view of making aromatic and semiaromatic wines in the vineyard of Recas more efficient by testing and establishing other technological parameters.
- Studies on the same theme can be carried out, also approaching other varieties specific for this vineyard, already knowing a series of aroma potentiating and inhibiting factors.
- A series of studies have been carried out regarding the aromatic compounds which are found in nanogram quantities using improved GC-MS procedures.
- Comparative tests can be carried out with other varieties in different vineyards in view of identifying the local specific characteristics.

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