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**STUDIES REGARDIGN TEH EVOLUTION OF THE
MICROBIAL ACTIVITIES DURIGN THE PROCESS OF
ELABORATION OF HIGH QUALITY WHITE WINES
(ABSTRACT)**

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CONTENTS

| | |
|--|-----------|
| INTRODUCTION..... | 1 |
| CHAPTER I: ASPECTS REGARDING STUDIES AND OBSERVATIONS REALIZED IN TIME, REGARDING THE PRESENCE OF THE MICROORGANISMS ON GRAPES, IN MUST AND WINE..... | 5 |
| I.1. Yeasts in the natural and technological circuit. Ecology of yeasts..... | 5 |
| I.1.1 Role of yeasts in formation and evolution of wine's flavor..... | 9 |
| I.2. Bacteria of wine | 36 |
| I.2.1 Lactic bacteria of wine in natural and technological circuit... .. | 37 |
| I.2.2 Role of bacteria in evolution and formation of wine's flavor..... | 39 |
| I.3 Aspects regarding the purification and stability of wines..... | 50 |
| I.3.1 General aspects regarding production of white and red wines..... | 51 |
| I.3.1.1 Chemical composition of wines..... | 52 |
| I.3.1.2 Main factors of wine's instability..... | 56 |
| I.3.1.3 Main techniques of purification and stability of wines..... | 58 |
| I.3.1.4 Filtration of wines | 59 |
| I.3.2 Application of tangential micro filtering by mineral membranes at purification and stabilization of wines..... | 61 |
| I.3.2.1 Semi – permeable membranes | 61 |
| I.3.2.2 Generalities on techniques of tangential micro filtering. | 62 |
| I.3.2.3 Polarization of concentration..... | 63 |
| I.3.2.4 Expression of the permeability flux..... | 64 |
| I.3.2.5 Application of tangential micro filtering in oenology | 66 |
| CHAPTER II: PURPOSE OF THE PAPER. RESEARCH OBJECTIVES. MATERIAL AND METHOD..... | 69 |
| II.1 Analytical methods | 70 |
| II.1.1 Chemical classical analyses of the must and wine..... | 70 |
| II.1.2 Measuring pH..... | 70 |
| II.1.3 Measuring of the total polysaccharides (PZ) | 70 |
| II.1.4 Determination and formation of phenol compounds | 70 |
| II.1.5 Measuring the total and protean nitrogen | 71 |
| II.1.6 Measuring the tartaric acid (Ta) | 71 |
| II.1.7 Measuring the main cations of the wine..... | 71 |
| II.2 Equipments | 71 |
| II.2.1 Laboratory installation..... | 71 |
| II.2.2 Membranes | 74 |

| | |
|---|----|
| II.3 Microbiologic control | 76 |
| III.3.1 Methods of numerical evaluation of microorganisms | 77 |
| II.3.1.1 Determination of the total number of cells with the aid of the counting chamber..... | 77 |
| II.3.1.2 Nephelometric method | 79 |
| II.3.1.3 Method of counting germs by microscopic examination on filtering membranes..... | 80 |
| III.3.2 Methods of determination of the number of viable germs..... | 81 |
| III. 3.2.1 Method of units forming colonies..... | 81 |
| III. 3.2.2 Method of filtering membrane for determination of the units forming colonies..... | 82 |
| III. 3.2.3 Method of titre..... | 83 |
| II.4 Control of wine's limpidity | 84 |
| II.4.1 Estimation of agitation..... | 84 |
| II.4.2 Determination of filtering index..... | 84 |
| II.4.3 Retention coefficient..... | 84 |

CHAPTER III: OBTAINED RESULTS AND THEIR INTERPRETATION 86

| | |
|---|-----|
| III.1 Establishing microbial content on grapes belonging to white types Fetească albă, Sauvignon and Italian Riesling, in vineyard Segarcea. | 86 |
| Conclusions of objective 1..... | 93 |
| III.2 Establishing microbial content of musts resulted from grapes gathered at different moments of harvest..... | 94 |
| Conclusions of objective 2 | 99 |
| III.3 Establishing microbial content of musts obtained at different moment of drainage and pressing must | 100 |
| Conclusions of objective 3 | 113 |
| III.4 Influence of must's purification operation, before development of the process of alcoholic fermentation, on microbial loading..... | 116 |
| Conclusions of objective 4 | 125 |
| III. 5 Influence of tangential filtering of wines on chemical composition and microbial content of wines..... | 128 |
| III.5.1 Arrangement of a cleaning sequence and of sterilization of membranes adapted for wine..... | 128 |
| III.5.2 Influence of the membranes on tangential filtration of wines.. | 133 |
| III.5.2.1 Average diameter of pores..... | 133 |
| III.5.2.2 Influence of the geometry of support..... | 140 |
| III.5.3 Study of the main operator parameters..... | 144 |
| III.5.3.1 Influence of tangential speed..... | 144 |
| III.5.3.2 Influence of trans membrane pressure | 149 |
| III.5.3.3 Influence of wine's temperature..... | 154 |
| III.5.3.4 Influence of filtering period | 156 |

| | |
|---|-----|
| III.5.4 Influence of tangential filtering on composition of wine. | 161 |
| III.5.5 Influence of characteristics of wines..... | 168 |
| III.5.5.1 Effect of the degree of purification of wine..... | 168 |
| III.5.5.2 Effect of the content of colouring substances and colloidal of wines..... | 172 |
| Conclusions of objective 5 | 178 |

| | |
|--|------------|
| CHAPTER IV: FINAL CONCLUSIONS AND RECOMMENDATION..... | 181 |
|--|------------|

| | |
|--------------------------|------------|
| BIBLIOGRAPHY..... | 188 |
|--------------------------|------------|

KEY WORDS:
microbial activity, high quality white wines, tangential microfiltration,
wine's chemical composition, clarification,
biological stability

Wine is a very complex mixture of compounds, which define to a great extent its aspect, flavour, bouquet and organoleptic property. Compounds that are responsible for these attributes came from three great sources: grapes, activity of the micro organisms, and when it is used, wood, most common oak wood.

After fermentation, substances that are responsible for agitation of the wine are mainly solid particles that come from must or from bunch without grapes and micro organisms. These can interfere also in numerous chemical soluble compounds, susceptible to be precipitated ulterior in crystalline form (calcium tartrate, bitartrate of potassium) or colloidal formless (colouring substance).

Despite appearances, wine is an alive products, in permanent evolution, very sensible to external attacks (oxidation, microbial attacks, etc).

During maturity process, certain constituents will flocculate, others will combine and the result is generally the apparition of agitation and / or deposits. We talk here about the natural normal evolution of wine and not an alternation phenomenon, but are absolutely indispensable to clear well the product before commercialization. The consumer, from this point of view, it is not forgiving.

In the case of a defective wine making process or the worst preservation of the products, chemical or microbiological accidents might lead to a degradation of the organoleptic degradation of the wine.

At the end of wine making process, wine is very loaded with all types of microorganisms, especially yeasts and lactic bacteria that are responsible with malolactic fermentation.

The purification of wines has as purpose the elimination of diverse unwanted constituents, to obtain a product of perfect purification and stable from the chemical point of view in the depositing period. It makes appeal to chemical methods (glue) and physical (centrifugation and filtering).

Selection of techniques of conditioning of wines before being commercialized depends on the products and its sensibility to external aggression, in the same measure with its composition.

Wines sold in bulk suffers generally a simple purification, meanwhile the ones sold in bottle must be conditioned with greatest care and perfect stability.

In this moment, the micro filtering techniques are used in a large scale in oenology, to ensure the biological stability of wines due to export. Their principle reveals the frontal (direct) filtration, which involves a rapid sealing of the filtering environment by retained particles. Used before putting into bottles, they allow an almost total elimination of the micro organisms, but can not be applied but to products that are already perfectly purified.

The research program that we proposed has as purpose the presentation of the existence of micro organisms, in a special manner of the yeasts and lactic bacteria, on grapes, in must and in wine, bringing this way scientific information, that are necessary to the oenologist, when he is in the situation of taking technical measures that are imposed for stimulation, of after case the inhibition of the activity of the micro organisms. It was also studies the effect realized by tangential filtration on the number of microorganisms and chemical composition, applied to the wine for its purification and stabilization.

Researches have been realized in the period 2005-2007, on grapes, in must and in wine at S.C. „Domeniul Coroanei” Segarcea.

The research thematic established the following objectives:

6. Establishing the microbial content on grapes that belong to white types Fetească albă, Sauvignon and Italian Riesling, in Segarcea vineyard;
7. Establishing the microbial content of must that resulted from grapes harvested at different moment of harvest ;
8. Surprising the microbial content of musts obtained at different moments of drainage and pressing must;
9. Influence of the purification operation on microbial content of musts, applied before development of the alcoholic fermentation process;
10. Influence of the tangential filtration of wines on chemical composition and the microbial content of wines.

For the present paper have been taken into study three types of white grapes, capable to obtain white wines of superior quality from Wine - growing Central Segarcea-Dolj, and namely Feteasca Albă, Sauvignon and Italian Riesling. Researches realized aims to establish the Total Number of Germs that are present in grapes and in musts obtained from their process of wine – making, as well as identification of the content in sugar, acidity, nitrate substances and non-reducing extract of musts obtained from grapes gathered in different harvest moments, to be established the correlation that exists between chemical composition of must and its microbial

loading, as well as the evolution of the main parameters of grapes' must depending on the applied technology and treatments.

Researches included the determination of the number of micro organisms and the main chemical compounds of the wine, after application of tangential filtration. For purification and stabilization of the wines, it was realized the tangential filtration of three wines belonging to the types: Sauvignon, Cabernet Sauvignon and rose wine, realized at S.C. „Domeniul Coroanei” Segarcea, in the year 2006.

The study was realized during three years: 2005- 2007.

All analytical methods involved in analyzes of wines into study are part of the category of the Analytical methods adopted by the International Organization of the Vineyard and the Wine (O.I.V.).

Gross results obtained as a result of the laboratory works, have been verified from statistic point of view and processed with two specialized programs: ”Statistics V.7” and „SPSS V.15”.

For the graphical representation of the dependence relations between certain factors variable that area taken into study, it was used a specialized program „Smart Draw 2008”.

As a result of the realized study we were able to mention the following conclusions:

Conclusions of objective 1: Establishing microbial content on grapes belonging to white types Fetească albă, Sauvignon and Italian Riesling, in vineyard Segarcea

1. Grapes that reached maturity, contain on their surface a heterogeneous micro biotic, which comes from external sources: soil of plantation, rains, insects.

The balance and nature of micro organisms depends on the degree of maturity, of the age of plantation, of conditions of cultivation and harvest.

2. Analyzing the microbial content of the grapes of the types taken into consideration (Fetească albă, Sauvignon and Italian Riesling) in the three years 2005, 2006 and 2007, we have observed that grapes situated in the superior third of the trunk, presents a microbial content much more reduced that the grapes situated in the inferior third, where the number of the yeasts is 4-5 times greater, and the one of the mould is 8-10 times higher.

3. Regarding the distribution of the microorganisms depending on the health status of the grapes, as a result of the analyzes resulted, we have observed that, cracked grapes have a content that is ten time higher, in comparison with grapes having intact peel, where the layer of pruin is like a barrier for microorganisms.

4. The number of microorganism does not registered great differences between the three types taken into study, but differences exist as part of the same type, in different years of harvest, as a result of the influence of climatic conditions.

Conclusions of objective 2: Establishing the microbial content of musts resulted from grapes harvested at different moment of maturation.

1. The chemical and biological composition of must made of grapes is very complex, being dependent of the type of grapes, of the degree of maturity, the climatic conditions in particular manner and the grape's harvest and processing modality.

From results obtained as a result of the three types– Fetească albă, Sauvignon and Italian Riesling, I have observed that, once with the evolution of maturity, takes place an increase of the content of sugar and nitrate substances, concomitant with diminishing of acidity.

The proportion, in which these modifications take place, is dependent mainly on climatic conditions.

2. The total number of germs, increases on extent in which we alienate on the moment of full maturity, on account of increasing concentration of sugar and nitrate substances.

Conclusions of objective 3: Establishing the microbial content of musts obtained at different moments of drainage and pressing must.

1. To obtain a quality products, a special attention must be granted to the use of the adequate technical means for each stage of the wine making process.

Squashing and taking out of the bunch are the first operations to which grapes are submitted in the process of processing and on the modality in which are produced, depends the quality of the final product- wine. Unwanted effects of some technological mistakes are manifested especially on the organoleptic quality of the products, by the presence of some increased proportions of tannin, oils, nitrate substances, which might appear from application of some great pressures at crushing grapes or from unjustified prolongation of the contact of the must with bunches.

Obtaining must of press, as technological operation, must be controlled and lead in conformity with the same principles of going easy on the parts of the grapes that mish enrich the must and wine in constituents with negative effect on quality (great content of tannin, nitrate substances, that come from exaggerate pressing of the peel and seeds).

2. Analyzing the microbial content and the chemical composition of musts resulted at different moments of pressing, obtained from grapes harvested at full maturity, in the case of the three types, we can observe that, as we advance in the technological flux, in the direction *must ravac* → *must from press I* → *must from II press*, take place modifications of the proportion of the chemical compounds, as well as the total number of micro organisms.

- The content of sugar will be diminished, meanwhile total acidity, nitrate substances and total number of germs, register increases of their value.

The differences between the sugar content between different must fractions, have values contained between 4 and 30 g/l, with an average difference of 15 g/l between two successive fractions, registering great diminishing between Must ravac and Must from press I, than between Must in press I and the one in the II press.

- Total acidity registers values that belong to normal limits, reported to sugar content, except year 2005, unfavourable to wine – growing in Romani, year characterized by low temperatures and abundant rains, which lead to an increased accumulation of acidity and a small content of sugar.

From analyzes realized on three types taken into study (Fetească albă, Sauvignon and Italian Riesling), we can observe that as we progress in the process of wine – making, the acidity registers an appreciation with 1,5-2,5 g/l in the first stage, after that it stops, observing either easy appreciation, either depreciations, with values under 0,3 g/l H₂SO₄.

The greatest appreciation of acidity as part of technological flux was observed in the year 2007, as part of Sauvignon type, of 3,45 g/l H₂SO₄, as well as part of Italian Riesling, of 2,38 g/l H₂SO₄, and the smallest increases have been met in the year 2005 (of 0,48 g/l H₂SO₄ at Sauvignon type and only 0,66 g/l H₂SO₄ at Italian Riesling type).

The Fetească albă type knew a linear evolution in which concerns the modification of the acidity during wine – growing process, registering constant values of the modification of acidity between the three fractions of must and during the three years.

This evolution of the acidity is based on the same explanation as in case of sugar, taking into account the fraction of must and the repartition of the chemical compounds in the interior of the grape. .

- Tannins registers a continuous increase of along technological flux, in the same direction *must ravac* → *must from press I* → *must from press II*, with greater values between ravac must and must from press I, and decreased between must in press I and the one in press II.

In this sense, increases registered are in average of 0,05 g/l between the two fractions and only 0,008-0,012 g/l between the last two fractions. Explanation in case of the other phenomenon is valid also in which concerns the increase of the content of tannins, with the specification that while we progress in the technological process, increases also the period of contact between tannin and the liquid phase of the must, these becoming more extractible.

- The Total number of Germs in the must, in the direction *must ravac* → *mu must from press I* → *must from press II*. This fact is due to the increase and multiplication of micro organisms, that are in the must, with a beneficent environment, rich in substances that are

necessary to their development. As we progress in the technological flux, increases the period of time that is necessary for the micro organisms to develop and reproduce, so that their number is higher and higher as we alienate from the moment of crushing grapes.

Conclusions of objective 4: Influence of must's purification operation, before development of the process of alcoholic fermentation, on microbial content.

The purification operations, applied before development of the alcoholic fermentation plays a very important role in the definition of the final product, as the quality of wine is connected to the quality of must from which comes.

Must resulted from grapes has a heterogeneous chemical composition formed from colloidal substances (gums, mucilage) together with land particles, rests of peels, seeds, bunches, that when are found in increased proportions, lead to the deterioration of the quality of wines and must be eliminated.

By application of purification treatments before starting alcoholic fermentation it is aimed the deposit of the substances situated in suspension and elimination of the formed deposit, operation named must's purification.

It was observed that wines resulted from musts to which have been applied these treatments present superior qualitative characteristics, having a fine and light taste, with flavour of a more pronounced type, more resistant at the action of the oxygen and with a smaller content of Fe, Pb, Zn.

To musts obtained from grapes of the types Fetească albă, Sauvignon and Italian Riesling, have been applied two purification treatments

- 1) bentonizat 1g/l + 60 mg SO₂ free;
- 2) cooling 7°C + 30 mg/l SO₂ free.

1. Analyzing the chemical composition and the microbial content of musts submitted to the operations of purification before development of the fermentation process, it can be observed a modification of the main parameters in the must, in case of application of the some treatments mentioned before.

- Sugar registers a slow fall, in the case of application of purification operation by gluing (bentonizat 1g/l + 60 mg SO₂ free, 24 hours), of 2-5 g/l, as well as in the case of purification operation by cooling, of 1-3 g/l, proportion in which this is diminished being easily increases in the case of the application of the treatment of purification with bentonite.

The diminishing of the content of sugar is due, mainly, to the action of SO₂-ului, which might combine between 2 and 6% from sugars. These diminishing are greater in the case of the

first treatment, due to the property of the bentonite to flocculate, so integrating a small part of sugar in formed floccules.

- The total acidity will be diminished to a small proportion, with values that are appreciatively equal in case of both applied treatments, at all types that are studies and during all years of the experiment. Diminishing the activity is smaller or equal with 0,1 g/l, the smallest value with which the acidity decreased, being registered in case of the Italian Riesling, in the year 2007, of only 0,06 g/l.

The small quantity of SO₂ which has been added in the case of both treatments (treatment I 1g/l + 60 mg SO₂ free and the treatment II - cooling 7°C + 30 mg/l SO₂ free) does not manifest an important role on the acidity of must, even if this can combine a part of acids that can be found in must.

- Nitrate substances face the most significant diminishing in rapport with the other parameters that are analyzed, these losses being almost double in case of application of the treatment of purification with bentonite (around 0,4 g/l) in comparison with cooling of must (around 0,2 g/l).

Great losses as part of the first treatment applied are due to the powerful character without proteins of bentonite, which might lead to diminishing between 30 and 45% in case of application of prevention treatments of copper cassation. SO₂ contributes also to the decrease of teh content f nitrous substances, by combination that they form with these compounds from must.

- The non- reducing extract also decreases, but in smaller proportions, of 0,5 g/l in the case of the first treatment and 0,2 g/l in the case of application of the second treatment, decreases provoked by the flocculating action of the bentonite and the combination action of SO₂. Greater diminishing appear in the case of the treatment with bentonite.

- NTG will be also diminished, based on the conjugated action of SO₂ which has a powerful antiseptic effect and the purification agents. The bentonite treatment determines double decreases of this parameter, as at flocculation of the proteins with bentonite, in floccules formes are gathered also in other particles, including micro organisms, bentonite can lead to elimination of micro organisms up to 80-100%.

Conclusions of the objective 5: Influence of tangential filtering of wines on chemical composition and microbial content of wines

1. Techniques of ultra filtration and tangential micro filtration proved to be performing and started to know a large spread in the food industry, including in the oenological one.

2. Taking into account the supplementary viability that it brings and the economies in which concerns the performances (automation, continual work), reduced consumption, it is considered that this technique is a future solution for the oenological industry.

3. In the great oenological production from Romania, using this technique almost begins, reason for which is considered that surprising some phenomenon and factors that might decide the success of this technique is absolutely necessary, especially that this type of studies have been a few in our country and sometimes we missed the shaping of all factors that influenced the success of this technique.

4. Our researches underlined as important the following aspects:

- the cycle of cleaning and sterilization of the membranes is sufficient and efficient when it is used the solution of NaOH in concentration of 2 % and at a temperature of 80 °C, allowing the perfect regeneration of the membrane, by destruction of the organic substance that was involved in sealing.

- the average diameter of the pores of the membrane does not interfere but very few on average permeability debits, but it characterizes phenomena of external and profound sealing. The ideal value of the diameters of the pores of the membrane, if this is due to purification and ensuring the stability of wines will be situated between 0,20 and 0,80 µm.

- the geometry of the support of multiple channel type, allows the diminishing of the hydraulic diameter of the channels, so the improvements of the filtration coefficients (easy increase of the debit of permeability of wine).

- the optimal tangential speed has a value contained between 4 and 5 m/s.

- the trans membrane pressure has a favourable influence on the performance of the technique, up to values of the pressure of 3-5 bars.

- the temperature of the wine that is submitted to tangential filtration must be between 20 and 25 °C.

- the technique offers the chance to put into practice very long filtration cycles, 20 hours per day as an average

- the rests of the tangential filtration of wine might not exceed 1,4 % from total volume, are viscous and from then can be recovered , with press filter, the liquid part.

- tangential micro filtration of wines that are very loaded with microorganisms and colloids, is as possible as can be, without excessive decrease of performances.

- the preliminary purification of wine, that will be filtered tangential, does not bring a quantitative improvement (of debits) or qualitative (degree of purification and sterilization)

- the coloring intensity of the wine and its content in colloids have an influence on the performance of the micro filtration. The deposit on the surface of the membrane is composed

from micro organisms and polysaccharides, sometimes from tannins (poly phenols), proteins, tartaric acid and potassium.

In exchange the internal sealing of the pores is due to phenomenon of physical – chemical interaction between very condensed alumina and tannins of the wine (yellow polymers).

5. Tangential micro filtration can operate with agitates musts and wines, and in comparison with classical techniques of purification and stabilization of the wines presents great advantages.

6. With all advantages that tangential filtration offers, in oenology, until present moment, it might be the perfect solution only for purification and stability of wines which in return can not be in the row of superior quality and with origin denomination. This technique can not be integrated in the technological scheme of obtaining great wines.

7. It is imperious necessary that in our country be continued researches in this field, to surprise many other aspects of tangential filtration, like: surface substances of alumina, which conditions the reactivity of membrane next to dissolved substances of must and wine, but also the main study of the structure and the physical – chemical reactivity of the main colloids of musts and wines (proteins, polyphenols, polysaccharide) and determination of the responsible fraction of the membranes.