

“ LUCIAN BLAGA” UNIVERSITY OF SIBIU

***FACULTY OF AGRICULTURAL SCIENCES, FOOD INDUSTRY
AND ENVIRONMENTAL PROTECTION***

Doctoral Dissertation

SUMMARY

Scientific Coordinator

Prof. Univ.Dr. Ing. Ovidiu Tița

Ph. D. Candidate

Ing. Mihaela Virginia Balteș

SIBIU 2016

“ LUCIAN BLAGA ” UNIVERSITY OF SIBIU
FACULTY OF AGRICULTURAL SCIENCES, FOOD INDUSTRY
AND ENVIRONMENTAL PROTECTION

**Capitalization of the by-products of
vinification with obtaining valuable
products for industry and food**

Scientific Coordinator

Prof. Univ.Dr. Ing. Ovidiu Tița

Ph. D. Candidate

Ing. Mihaela Virginia Balteș

SIBIU 2016

| | |
|--|-----------|
| TABLE OF CONTENTS | a |
| THE LIST OF THE USED NOTATIONS AND SYMBOLS | b |
| FIGURES LIST | c |
| TABLES LIST | d |
| FROM THE AUTHOR | e |
| THE AIMS AND SCIENTIFIC OBJECTIVES OF THE DOCTORAL DISSERTATION | e |
| PERSONAL CONTRIBUTIONS | |
| CHAPTER I | 1 |
| GENERAL INFORMATION ABOUT GRAPES | |
| CHAPTER II | 8 |
| CHEMICAL COMPOUND OF GRAPES | |
| 2.1. Nutrients and minerals that accumulate in the grapes | |
| 2.2. Watter in the grapes | 9 |
| 2.3. Sugar | 9 |
| CHAPTER III | 11 |
| CHARACTERIZATION OF BYPRODUCTS IN WINE INDUSTRY | |
| 3.1. Marc recovery processes | 14 |
| 3.1.1. The chemical composition of grape marc | 14 |
| 3.1.2. Extraction of soluble substances from pomace | 19 |
| 3.2. Grape seed | 21 |
| 3.2.1. Chemical characterization of grape seeds | |
| 3.2.2. Grape seed oil | 25 |
| 3.3. Wine yeast | 27 |
| 3.3.1. Capitalization of wine yeasts | |
| 3.3.2. Description of yeast sediment and our physicochemical characterization | 28 |
| 3.3.3. The morphology of yeast | 30 |
| 3.3.4. Description of the existing preparations based on yeast and their applications | 31 |
| 3.3.5. Effects of yeasts feed on milk production | 33 |
| 3.3.6. Effects of yeasts feed on rumen activity | 33 |
| 3.3.7. Alcohol recovery | 34 |
| CHAPTER IV | 34 |
| TECHNOLOGICAL INDICATORS DETERMINING THE QUALITY OF WINE SUB-PRODUCTS | |
| 4.1. Equipment and operations used in the production of wine by-products | |
| 4.1.1. Introduction | |
| 4.1.2. Types of presses | 35 |
| 4.2. TECHNOLOGICAL INDICATORS DETERMINING THE QUALITY OF WINE SUB-PRODUCTS | 39 |
| 4.3. THE INFLUENCE OF PRESSING PARAMETERS ON THE CONCENTRATION OF TANNIN IN MARC | 42 |

| | |
|--|-----------|
| 4.3.1. Introduction | |
| 4.3.2. Materials and methods | 44 |
| 4.3.3. Results and discussion | 45 |
| 4.3.4. Conclusions | 45 |
| CHAPTER V | 46 |
| INFLUENCE grape variety on the accumulation of polyphenolic compounds IN pomace | |
| 5.1. Introduction | |
| 5.2. Materials and methods | 46 |
| 5.3. Results and discussion | 46 |
| 5.4. Conclusions | 47 |
| CHAPTER VI | 47 |
| QUANTITATIVE AND QUALITATIVE ANALYSIS OF POLYPHENOLS IN RED MARC | |
| 6.1. Introduction | |
| 6.2. Materials and methods | 48 |
| 6.3. Results and discussion | 49 |
| 6.4. Conclusions | 55 |
| CHAPTER VII | 56 |
| INFLUENCE ON STORAGE TIME yield of alcohol from bagasse | |
| 7.1. Introduction | |
| 7.2. Materials and methods | 56 |
| 7.3. Results and discussion | 56 |
| 7.4. Conclusions | 60 |
| CHAPTER VIII | 60 |
| PHYSICAL, CHEMICAL AND AROMATIC CHARACTERISATION OF MARC BRANDY | |
| 8.1. Introduction | |
| 8.2. Materials and methods | 60 |
| 8.3. Results and discussion | 61 |
| 8.4. Conclusions | 65 |
| CHAPTER IX | 66 |
| IDENTIFICATION AND QUANTIFICATION OF TANNING SUBSTANCES FROM GRAPE BUNCHES (SKELETON) | |
| 9.1. Introduction | |
| 9.2. Materials and methods | 66 |
| 9.3. Results and discussion | 67 |
| 9.4. Conclusions | 73 |
| CHAPTER X | 74 |
| STUDIES ON THE CHEMICAL COMPOSITION OF OILS EXTRACTED FROM GRAPE SEEDS | |
| 10.1. Introduction | |
| 10.2. Materials and methods | 74 |
| 10.3. Results and discussion | 75 |
| 10.3.1. Identification and quantification of fatty acids | |

| | |
|--|------------|
| <i>10.3.2. Identification and quantification of tocopherols and tocotrienols</i> | 78 |
| <i>10.3.3. Identification and quantification of essential amino acids</i> | 79 |
| <i>10.3.4. Identification and quantification of non-essential amino acids</i> | 80 |
| <i>10.3.5. Identification and quantification of metals</i> | 81 |
| 10.4. Conclusions | 82 |
| CHAPTER XI | 83 |
| QUALITY CHARACTERIZATION wine yeasts | |
| 11.1. Identification of nitrogen compounds in the wine yeasts | |
| 11.1.1. Introduction | |
| 11.1.2. Materials and methods | 83 |
| 11.1.3. Results and discussion | 84 |
| 11.1.4. Conclusions | 87 |
| 11.2. Qualitative and quantitative identification of vitamins in the wine yeasts | 87 |
| 11.2.1. Introduction | |
| 11.2.2. Materials and methods | 88 |
| 11.2.3. Results and discussion | 88 |
| 11.2.4. Conclusions | 93 |
| 11.3. Physico-chemical and microbiological characterisation of wine yeasts isolated from sediments | 93 |
| 11.3.1. Materials and methods | |
| 11.3.2. Results and discussion | 95 |
| <i>11.3.2.1. Physical and chemical determinations, statistical results</i> | |
| <i>11.3.2.2. The results of microbiological analysis</i> | 108 |
| <i>11.3.2.2.1. Determining the viability of yeast cells</i> | |
| <i>11.3.2.2.2. Determining the total number of germs</i> | 108 |
| 11.3.3. Conclusions | 109 |
| CHAPTER XII | 110 |
| VALORISING THE RESULTS OF THE RESEARCH TO OBTAIN A COMPLEX ANIMAL FEED FODDER RESULTING IN A RAW MILK HAVING SUPERIOR QUALITATIVE CHARACTERISTICS | |
| 12.1. Materials and methods | 110 |
| 12.2. Results and discussion | 111 |
| 12.3. Conclusions | 117 |
| CHAPTER XIII | 117 |
| FINAL CONCLUSIONS | |
| PERSONAL CONTRIBUTIONS | 118 |
| PERSPECTIVES TO CONTINUE THE RESEARCH | 118 |
| REFERENCES | 119 |
| APPENDIX | 132 |

KEYWORDS: BY-PRODUCTS, MARC, YEAST, BRANDY, POLYPHENOLS, FODDER

FROM THE AUTHOR

The PhD thesis entitled “Capitalization of the by-products of vinification with obtaining valuable products for industry and food” aims at analysing a series of waste resulting from wine-making processes, so as to reveal their qualities. The PhD thesis focuses on two important directions: a documentary one, synthesising the latest developments in the field, and an experimental one, with the purpose to evaluate valuable compounds in these wine sub-products, so as to recover them. The doctoral thesis is 131 pages long, comprising 5 tables and 118 figures. The documentary part reviews information about grapes and their chemical composition, as they make up the *de facto* raw material from which wine products are derived. Another chapter focuses on studies conducted by researchers on the subject of the PhD thesis, while the bibliography is topical and includes more than 200 titles. The experimental part studies the factors influencing the quality of wine sub-products, their physical and chemical analysis and the valorisation of the research results to obtain a complex animal feed fodder. The thesis also contains a series of lists, such as the list of figures, tables, annexes, abbreviations. This thesis would not have been possible without the priceless help of my scientific coordinator, professor engineer Ovidiu Țița, PhD., whom I would like to thank especially. I would also like to thank the advisory committee, whose advice helped me finalise the thesis. Regarding the experimental part, I would like to thank all my colleagues who were kind enough to support me, both at the Centre for Research in Biotechnology and Food Engineering within the Faculty of Agricultural Sciences, Food Industry and Environmental Protection, at the National R&D Institute for Cryogenics and Isotopic Technologies – ICSI Râmnicu Vâlcea, as well as the engineers and laboratory at the wine Centres, who never refused any of my requests.

THE PURPOSE AND OBJECTIVES OF THE PHD THESIS

The wine industry has grown remarkably over the last period, as technology and biotechnologies have developed like never before. More and more yeasts, bacteria or enzymes – vital products for modern winemaking technologies – emerge on the market. However, a more and more interesting sector nowadays is the study and recovery of wine sub-products.

Scientific research has determined that wine sub-products resulting from the recovery technology amount to 25% of the annual grape harvest. Each winemaking or wine conditioning technological operation results in by-products whose properties vary depending on numerous ecological, technological and biological factors (ripeness, grape variety). Marc, seeds, skin and waste resulting from winemaking contain a lot of bioactive compounds which can be valued on the market. To this end, the thesis aims at conducting a series of studies on the recovery of wine sub-products followed by obtaining valuable products for industry and food consumption. In order to achieve this purpose, the thesis aims to fulfil the following objectives:

- qualitative and quantitative analysis of valuable substances in winemaking sub-products
- physical and chemical analysis of valuable substances in winemaking sub-products
- optimising extraction procedures of valuable substances in winemaking sub-products
- obtaining a complex animal feed fodder resulting in a raw milk having superior qualitative characteristics.

ACKNOWLEDGEMENTS

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/133675.

GENERAL INFORMATION ABOUT GRAPES

People have known and processed grapes from ancient times, as they constituted the raw material from which the “nectar of the gods”, i.e. wine, was made. Rich in nutrients and antioxidants, the grapes are appreciated both as fruit, and processed (juice, must, wine). Rich in sugars, but also other compounds, they offer a multitude of elements, regardless of the method used to process them. Phenolic compounds are among the most valuable.

Phenolic compounds are a diverse group of secondary metabolites, found both in grapes and in wine. The phenolic content and the composition of processed grape products (wine) are heavily influenced by the technological practice to which grapes are exposed. During grape handling and ripening, several chemical changes can occur, leading to new compounds and/or to the disappearance of others, the subsequent modification of ratios of the total content of phenolic compounds, as well as their qualitative and quantitative profile (Garrido et al., 2013). Grapes produce organic compounds that can play a role in defending the plant from invading phytopathogens. The metabolites include numerous phenolic compounds which are also active against human pathogens. Grapes are used to produce a great variety of wines, grape juice and raisins.

TECHNOLOGICAL INDICATORS DETERMINING THE QUALITY OF WINE SUB-PRODUCTS

Depending on the type of press used, a series of characteristic technological indicators can be established, such as yield, productivity, loss, the amount of sediments. From the viewpoint of reusing wine sub-products, these indicators can represent information sources for the industry, making up a base of valuable components.

Yield is an indicator presenting the percentage ratio between the total mass used and the resulting amount of must. In the case of presses, the yield is 50%, while in the case of continuous screws, it is 90%. Hydraulic vertical presses or mechanical or pneumatic horizontal presses reach a yield of 75%, even 80%, according to Figure 1.

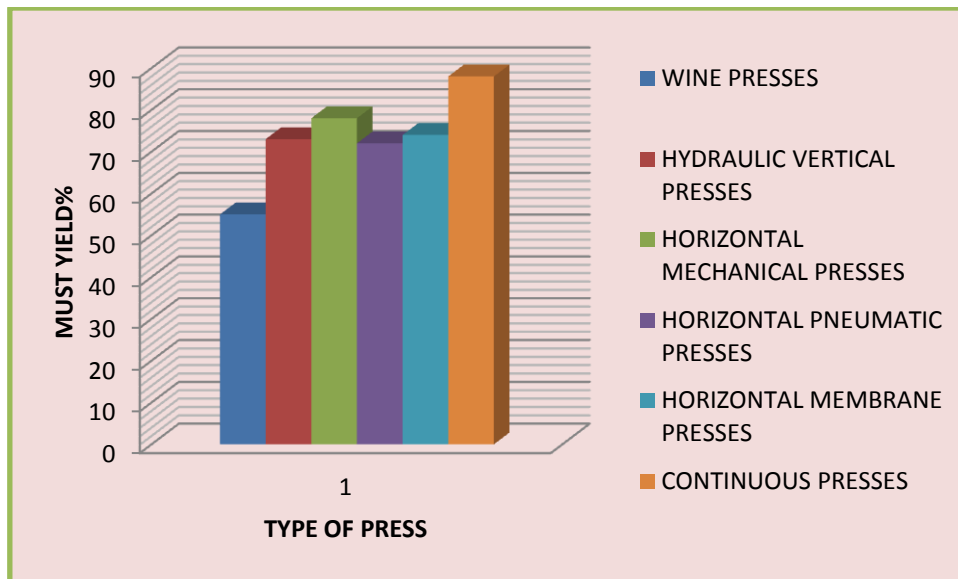


Figure 1. Must yield according to the type of press

The yield of marc can also be established depending on the type of press, which is important due to the ever-increasing interest to reuse this wine sub-product. As shown in Figure 2, marc yield is inversely proportional to that of must; the most significant values were recorded in the case of winepresses and hydraulic vertical presses, reaching percentages between 35% and 40%. The lowest values (only 12%) were recorded in the case of continuous screws, because a great part of marc elements get into the must as sediments.

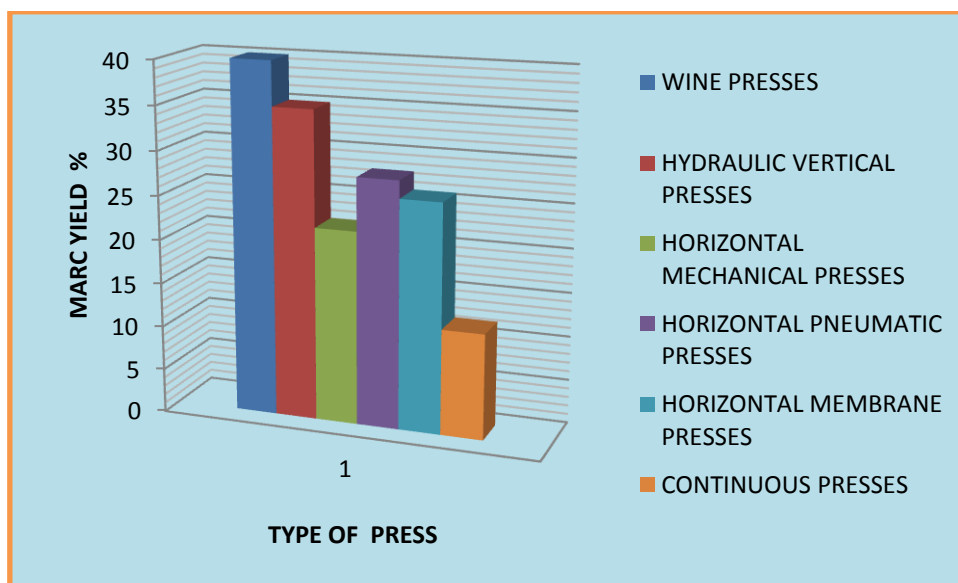


Figure 2. Marc yield according to the type of press

Productivity defines the wine centre, as results are in the interest of facilities recovering wine sub-products, and the volume of raw material establishing constant suppliers.

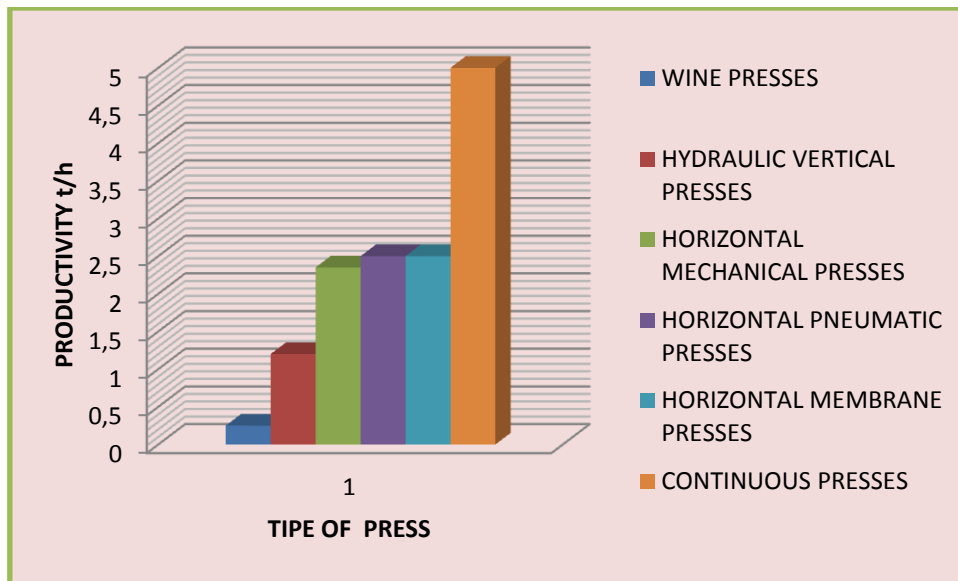


Figure 3. Productivity of presses (ton/hour)

Figure 3 shows that lowest productivity is recorded in the case of winepresses, although their marc yield is superior, while the highest productivity is recorded in the case of continuous screws, which present a very low marc yield.

The amount of must sediments is a wine quality indicator, as it confers a specific, astringent, herbaceous or unpleasant taste. A small amount of must sediments is beneficial, 3-4% at the most; it is recommended that these should remain in marc. As shown in Figure 4, the highest amount is recorded when using continuous screws (33%), and the lowest when using winepresses, hydraulic vertical presses and horizontal membrane presses (2%).

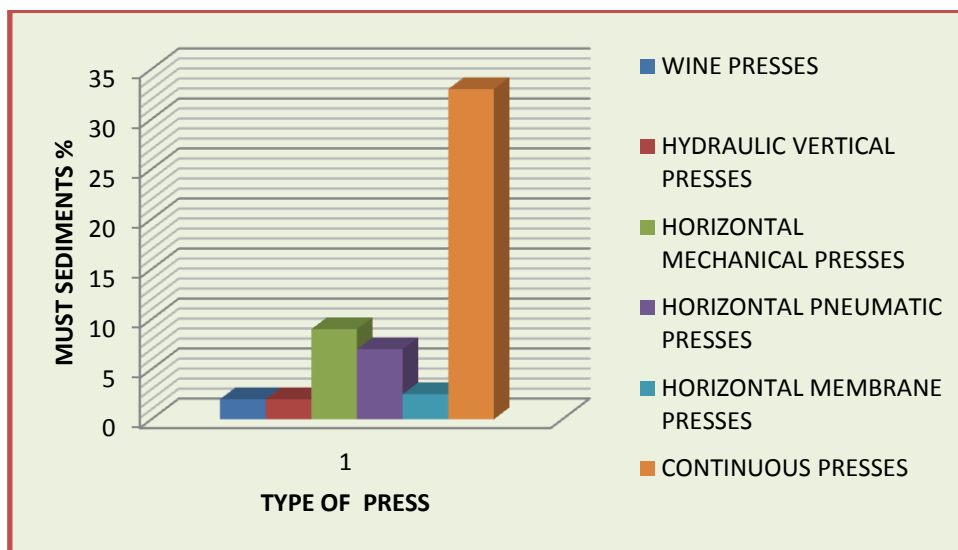


Figure 4. The amount of must sediments according to the type of press

The figures above show a balanced ratio between must and marc yield and that of must sediments and of productivity when using horizontal membrane presses, which are often employed in the winemaking industry. Winepresses, although recording high amounts of

marc, have low productivity and thus cannot become raw material suppliers for the wine sub-products processing industry.

THE INFLUENCE OF PRESSING PARAMETERS ON THE CONCENTRATION OF TANNIN IN MARC

Materials and methods

In order to establish the influence of pressing on the concentration of tannins in marc, a series of studies were conducted, based on a grape lot of the variety Fetească regală, one of Fetească albă and one of Welschriesling from the Sebeş Wine Centre. The grapes were pressed at pressures of 1, 2 and 3 bar, in mechanical-hydraulic horizontal presses. The tannins which remained in marc were determined through colorimetric spectroscopy.

This method consists of determining procyanidins in grapes, marc and other wine product, using colour reagents – p-dimetilamino cinamaldehyde (DMACA) and vanillin – in an acid environment (Țârdea, 2007).

Results and discussions

As shown in Figure 5, the concentration of tannins expressed in catechin varies according to the pressure exerted on marc. Thus, at a pressure of 1 bar, the concentration of tannin in marc reaches 0.5 g/kg, at 2 bar, 0.32 g/kg, and at 3 bar, 0.25 g/kg were determined in marc in the case of the variety Fetească regală. The variety Fetească albă reached a maximum of 0.48 g/kg, while Welschriesling 0.51 g/kg. At a pressure of 2 bar, the values recorded were between 0.31 g/kg and 0.33 g/kg. At a pressure of 3 bar, the value of tannins decreased by 50%, which indicated that they passed in the must. This is a favourable aspect for wine, but, on the other hand, lower amounts of tannin will be extracted from marc.

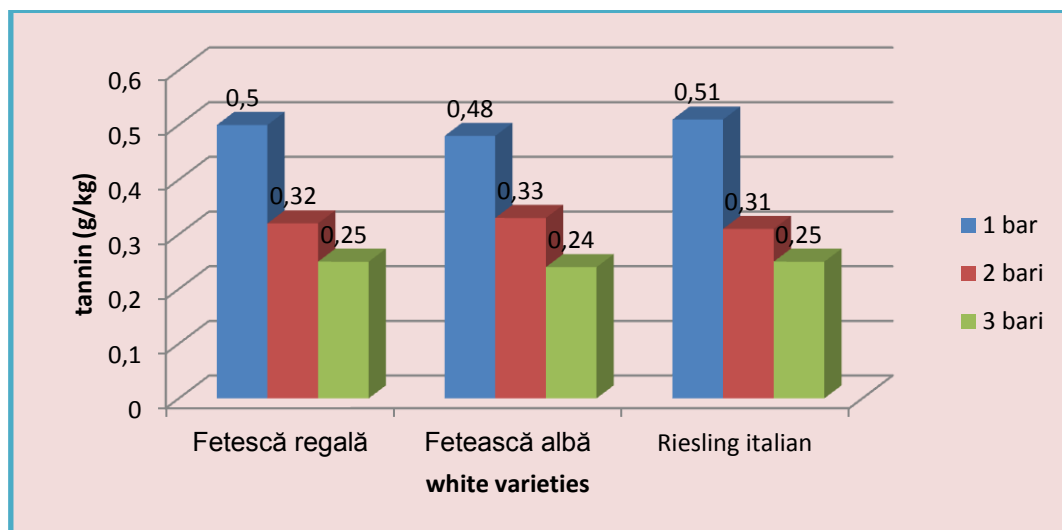


Figure 5. The amount of tannin in marc after pressing

Conclusions

This research showed the influence of pressing on the concentration of tannin in marc: we can state that superior pressing decreases the amount of tannin in marc. It is ascertained that the bigger the pressing pressure, the lower the amount of tannin in marc.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF POLYPHENOLS IN RED MARC

Materials and methods

Red marc from the following varieties: Cabernet Sauvignon, Merlot, Pinot Noir, Fetească neagră, from 2014 and 2015.

For the purpose of this study, dry, grinded marc underwent an extraction process with a solvent made of ethyl alcohol and distilled water at a 50% alcohol concentration in a ratio of 1:1.

We used the modified Folin-Ciocalteu method for the quantitative evaluation of polyphenols in red marc.

Results and discussions

In the samples under study, we determined amounts of polyphenols between 558.6 ml/L in the case of 2014 Cabernet Sauvignon and a maximum of 608.2 ml/L in the case of Pinot Noir. Intermediary values were recorded in the case of Merlot and Fetească Neagră, in which the amounts determined reached values between 569.7 ml/L and 574.9 mg/100g in the case of the year 2014, and 587.5 mg/100g and 566.3 mg/100g in 2015.

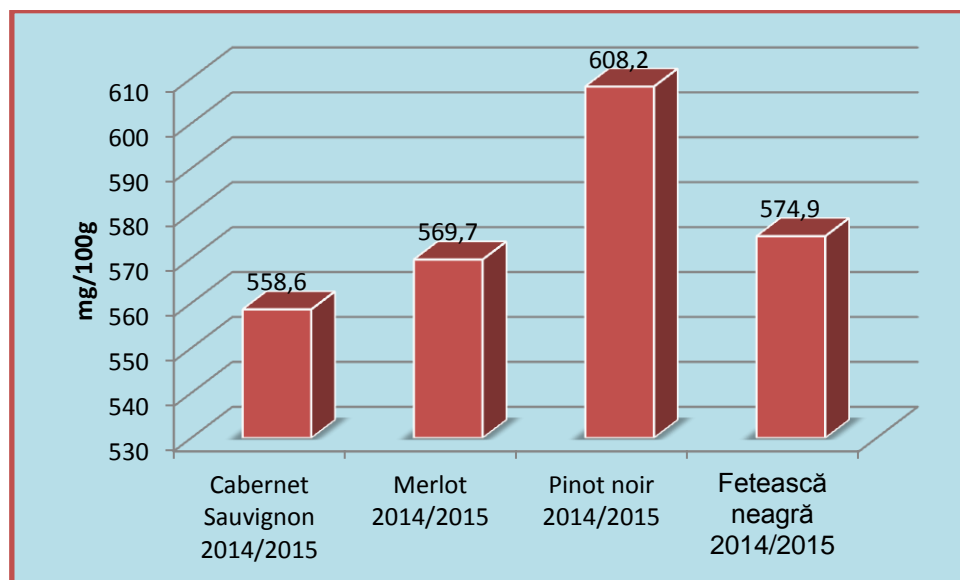


Figure 6. Evolution of the concentration of polyphenols in the red marc samples from 2014 and 2015 Cabernet Sauvignon, Merlot, Pinot Noir and Fetească Neagră

Figure 6 also shows that the most important amounts are characteristic of the Pinot Noir variety (between 598.8 mg/100g and 608.2 mg/100g). The lowest amounts were found in the variety Cabernet Sauvignon: between 558.6 mg/100g and 572.4 mg/100g. In the case of the

varieties Merlot and Fetească Neagră, the values recorded fell between 569.7 mg/100g and 587.5 mg/100g, respectively 566.3 mg/100g and 574.9 mg/100g.

Conclusions

Identifying the compositional structure of red marc is essential, as the identifiable elements constitute a valuable rich natural contribution.

Based on the results obtained, we can state that red marc is rich in phenolic compounds that remain after pressing.

PHYSICAL, CHEMICAL AND AROMATIC CHARACTERISATION OF MARC BRANDY

Materials and methods

Six marc brandy samples (P1, P2, P3, P4, P5, P6) were collected from six certified producers (boilermakers); the probes' authenticity was clear, they were produced in 2015, distilled in copper boilers, through rectification.

- pH was determined using a digital pH meter,
- acidity was determined in accordance with OIV-MA-AS313-01,
- the concentration of ethanol, methanol and aromas was determined using the method elaborated and optimised by Stegăruş (2015), GC/FID (gas chromatography coupled with flame ionization detector using the HeadSpace method in advance).

Results and discussions

- pH was between 4.2 (P5) and 5.8 (P1), equal values were visible in the case of samples P4 (4.5) and P2 (4.5).

Values close to 5 were recorded in the case of samples P3 (4.9) and P6 (5.2).

The total acidity recorded values between 0.11g acetic acid/100 ml alcohol anh. and 0.47 g acetic acid/100 ml alcohol anh.

Maximum values were determined in the case of samples P5 and P2, while minimum values in the case of P1 and P6. Samples P3 and P4 presented values that were 32% lower than the maximum values obtained.

Alcoholic concentration is an important characteristic of marc distillates; we noticed there were no significant differences between the six samples, producers achieving values between 41.5231% vol. (P3) and 43.3245% vol. (P5). Thus, intermediary values were identified in the case of samples P1 (42.8712% vol.), P4 (42.9987% vol.), P2 (43.0012% vol.) and P6 (43.0187% vol.).

Methanol recorded values between 235.6877 mg/L (P2) and a maximum of 1329.2153 mg/L in sample P5. Sample P4 contains 6.5% more methyl alcohol than sample P2, while sample P1, 29.7% more methyl alcohol than the minimum. Sample P3 reaches 871.9505 mg/L, and samples P5 and P6 exceed 1000 mg/L, reaching 1329.2153 mg/L (P5), respectively 1193.2098 mg/L (P6).

The determinations obtained using GC/FID show that acetaldehyde is detected in small amounts in sample P3 (0.0088 mg/L), reaching 0.0201 mg/L in P4 and 0.0204 mg/L in P1. No amount was determined in samples P2, P5 and P6.

Volatile higher alcohols are aromatic substances conferring drinks with flavour, a pleasant taste and a smooth feeling. In the case of marc brandy, through distillation, they pass from the grape skins to the top and the middle, contributing to the formation of the aromatic bouquet. These values range between 152.0257 mg/L (P4) and a maximum of 462.9826 mg/L in sample P3. Samples P1 and P2 contain 417.7767 mg/L, respectively 386.0628 mg/L higher alcohols. Lower values were recorded in samples P5 and P6: 300.9183 mg/L, respectively 337.6753 mg/L.

Terpene compounds are specific to aromatic grapes, but they can basically be found in all varieties of *Vitis vinifera*, passing into wine sub-products through pressing. Following determinations, they were found in all the six samples under study, especially in samples P4 and P5, in which the values reached 6.8401 mg/L, respectively 8.1635 mg/L terpene compounds. Sample P2 recorded 50% less terpene compounds than the maximum (4.0240 mg/L). Sample P3 recorded 1.2984 mg/L, three times lower than sample P2. In the case of samples P1 and P6, terpene compounds were determined in modest amounts: 0.1251 mg/L, respectively 0.0772 mg/L.

Benzyl alcohol, also called phenyl carbinol, bentanol or o-Hydroxytoluene, is a colourless liquid, with a sharp burning taste and a pleasant, light aromatic smell. It presents low toxicity, is partially soluble in water and fully miscible with alcohols and diethyl ether, ethanol, ether, benzene, methanol, chloroform and acetone. From the six samples under study, low amounts of benzyl alcohol were determined in three: sample P3, recording 1.4111 mg/L, sample P5 1.4740 mg/L and sample P6 1.6571 mg/L, 10.2% higher than in the case of sample P5. No benzyl alcohol was found in samples P1, P2 and P4.

Conclusions

Analysing the results obtained, we can state that the six samples of marc brandy are in accordance with the Regulation on the definition, description, presentation and labelling of Romanian traditional drinks of 13.06.2008, stipulating a minimum alcohol concentration of 37.5% vol. The boilers used for distillation were made of copper, fermentation took place in fermentation tanks, and wooden and glass recipients were used for storage.

The total acidity of samples P2, P3, P4 and P5 exceeds admissible values; only samples P1 and P6 fall within the legal limits of maximum 0.25 g acetic acid/100 mL alcohol anh.

PH records values between 4.2 (P5) and 5.8 (P1), which are characteristic of distillates made of grape marc.

The identified concentrations of methanol, although recording different values, do not exceed admissible maximum values of 1000 g/hL 100% alcohol in the case of samples P1, P2 and P4, in accordance with the Regulation on the definition, description, presentation and labelling of Romanian traditional drinks of 13.06.2008. In the case of samples P3, P5 and P6, the determined values exceed the maximum stipulated in the regulation in effect.

Acetaldehyde was identified in very low amounts in samples P1, P3 and P4, but not detected in samples P2, P5 and P6.

Higher alcohols were found in all samples; the lowest value, below the admissible limit was determined in sample P4, followed by sample P5.

Terpene compounds (nerol and geranial) were identified in important amounts in samples P2, P4 and P5, reaching values that confer these distillates with pleasant aromas and smoothness.

Benzyl alcohol was not found in samples P1, P2 and P4, but reached average amounts of 1.5 mg/L in samples P3, P5 and P6.

STUDIES ON THE CHEMICAL COMPOSITION OF OILS EXTRACTED FROM GRAPE SEEDS

Materials and methods

- 5 samples of cold-pressed grape seed oil, from five certified producers, noted P1, P2, P3, P4 and P5
- the physical-chemical characterization of the oil samples was made in accordance with standards SR EN ISO 10539, STAS 145/20-88, STAS 145/67 and SR EN ISO 662
- the identification and quantification of fatty acids was done using the method GC-MS, described by Dulf et al., 2013
- the identification and quantification of tocopherols and tocotrienols was done using the method HPLC, described by Oomah et al., 1998
- the identification and quantification of amino acids was done using the equipment Hitachi Amino Acid Analyzer L-8800, applying the method optimized by Kamel et al., 1985
- the identification and quantification of metals was done according to the standardized method, using an atomic absorption spectrophotometer NOVA A 300

Results and discussions

The identification and quantification of fatty acids

As shown in Figure 7, saturated fatty acids present amounts of compounds between 0.02% myristic acid and 7.84% palmitic acid. The amounts of myristic acid vary between 0.02% in sample P4 and 0.07% in sample P2. Intermediary values are recorded in samples P1 (0.06%), P3 (0.05%) and P5 (0.04%).

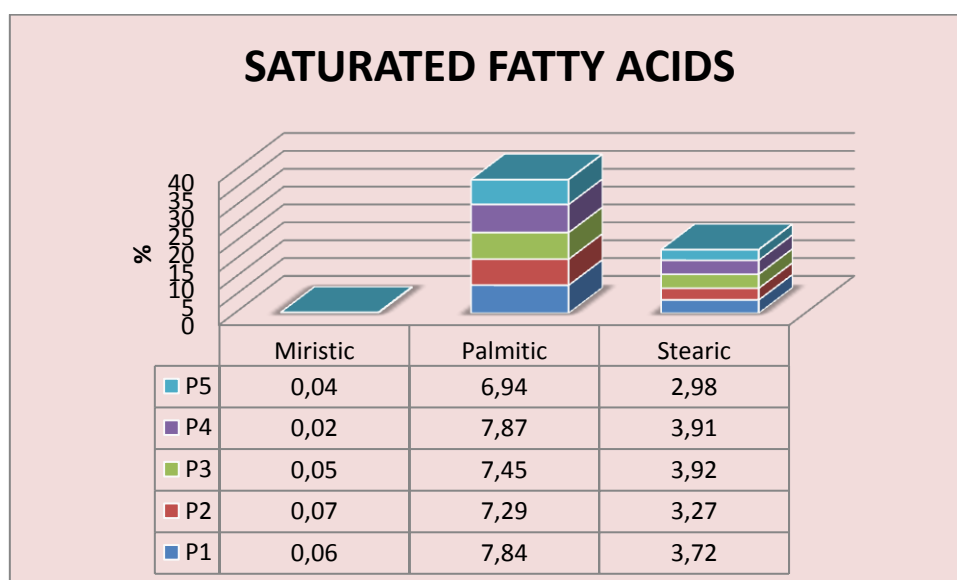


Figure 7. The identification and quantification of saturated fatty acids in the five grape seed oil samples

Palmitic acid reaches the highest amounts; thus, minimum values are recorded in sample P1 (6.94%), and the maximum in sample P4 (7.87%). The amount in sample P1 is close to the maximum (7.84%), while P3 reaches 7.45% and P2, 7.29%. Stearic acid is one of the significant elements identified in grape seed oil, recording values between 2.98% (P5) and 3.92% (P3). These values are specific to this type of oil; 1 unit variations depend on the grape variety from which the seed, and the oil respectively, comes. Samples P1 and P4 also contain significant amounts of stearic acid, the values determined reaching 3.72%, and 3.91% respectively. Sample P2 contains intermediary values – 3.27%.

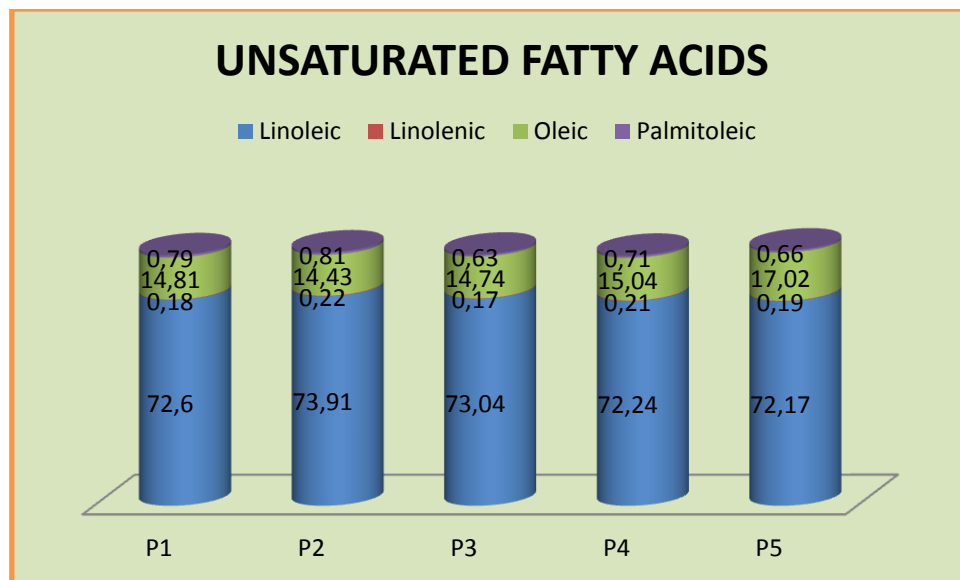


Figure 8. The identification and quantification of unsaturated fatty acids in the five grape seed oil samples

Figure 8 shows that unsaturated fatty acids record important amounts. Therefore, the amounts of linoleic acid range between 72.17% in sample P5 and a maximum of 73.91% in sample P2. In sample P4, the amount of linoleic acid reaches 72.24%, while in samples P1 and P3 the values recorded are 72.60%, respectively 73.04%. Another unsaturated fatty acid is oleic acid, ranging between 14.43% and 17.02%. Samples P2 and P5 presented these values. Samples P1 and P3 contained 14.81%, respectively 14.74% oleic acid, and sample P4, 15.04%. Linoleic and palmitoleic acid present subunit values, so that the amounts determined do not exceed 0.22%, respectively 0.81%. Linoleic acid records, on average, 0.194%, while palmitoleic acid reaches an average of 0.72%.

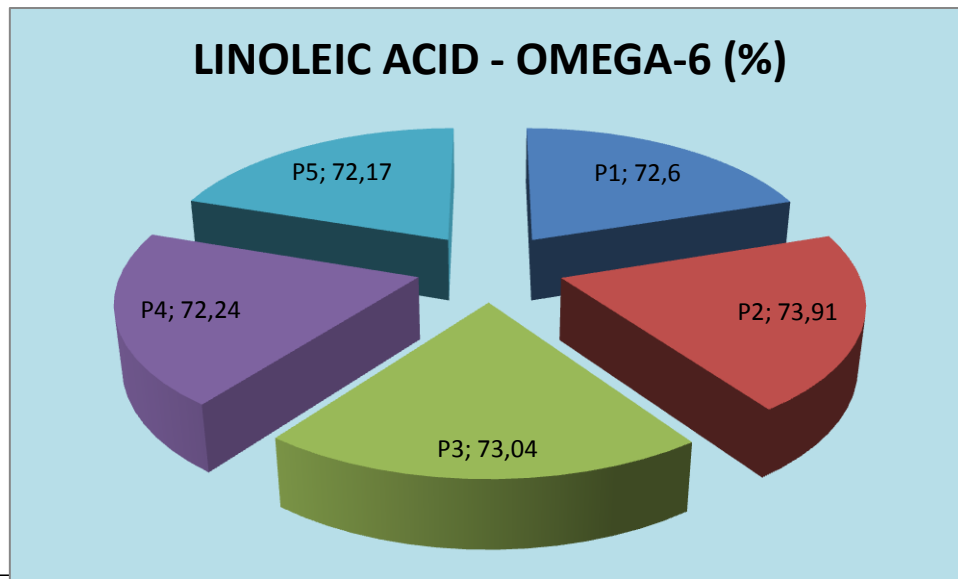


Figure 9. Evaluating the concentration of linoleic acid (omega-6) in the five samples of grape seed oil

Omega-6 is a family of polyunsaturated fatty acids, including linoleic acid, gamma-linolenic acid and arachidonic acid. Linoleic acid is the main omega-6 in food; it is found in the highest amounts in corn and sunflower oil, but grape seed oil should not be ignored either. Linoleic acid is considered a fatty acid, because it cannot be synthesized in the body. Inside the human body, omega-6 fatty acids, linoleic acid especially, are turned into arachidonic acid, which is incorporated in cell membranes.

Linoleic acid also generates anti-inflammatory molecules, so that, at the level of the vascular endothelium, omega-6 fatty acids have anti-inflammatory properties, suppressing the production of adhesion molecules, chemokines and interleukins, the key mediators of atherosclerosis.

As shown in Figure 10, the concentration of linoleic acid determined in the five samples of grape seed oil falls between 72.17% in sample P5 and 73.91%, in sample P2, significant values being found in virtually all the five samples under study.

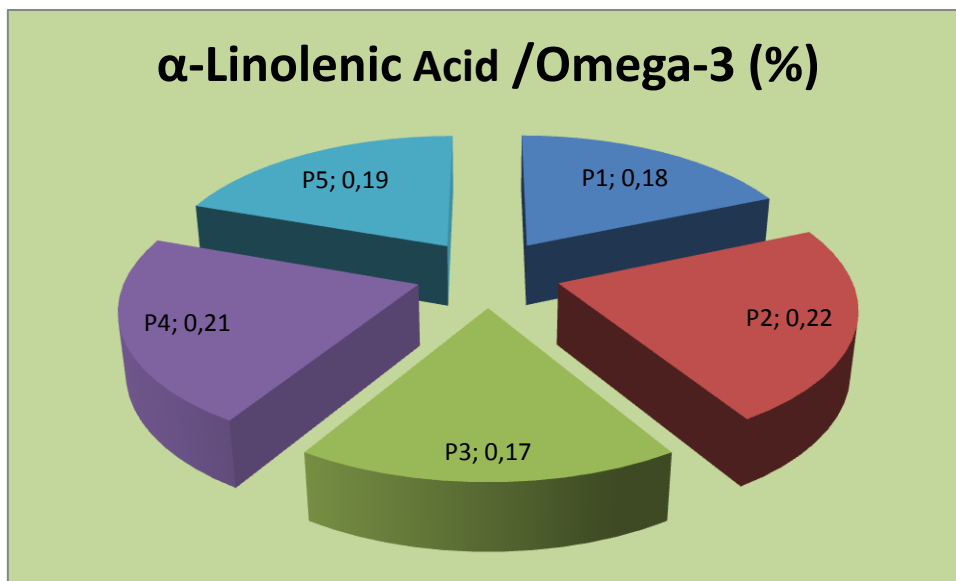


Figure 10. Evaluating the concentration of α -linoleic acid (omega-3) in the five samples of grape seed oil

Omega-3 fatty acids are considered essential fatty acids, necessary for human health, but which the body cannot produce naturally; they have to be taken from food. Omega-3 fatty acids can be found in certain types of fish, seafood, certain plants, seeds and nuts. Polyunsaturated fatty acids or omega-3 fatty acids play an important role in brain function and in the normal development of the body. They can reduce the risk of cardiovascular diseases. Research has shown that omega-3 fatty acids reduce inflammation and diminish the risk of cardiovascular diseases, cancer and arthritis. Omega-3 fatty acids are found in high concentrations in the brain and contribute to cognitive processes and the behaviour function. Consumption of food that is rich in these fatty acids leads to a healthy diet, with long term benefits. As shown in Figure 9, the amounts recorded fell between 0.17% and 0.22%, high values for this type of oil.

The identification and quantification of tocopherols and tocotrienols

Tocopherol is an essential vitamin for the human body. Vitamin E is made up of 8 tocopherols, α -tocopherol being the most efficient. A strong antioxidant, tocopherol plays an important role in protecting vitamin A, carotenes and vegetal oils. Moreover, vitamin E are involved in reproduction, facilitates glycogen storage in the liver and muscles. Tocopherols play a part in the metabolism of fats, calcium, phosphorus, but also in protein synthesis, limit the production of cholesterol, preventing the aging of cells, protecting the heart and arteries from atherosclerosis.

Tocopherols protect the blood vessels, the lungs and liver, strengthening the immune system. As shown in Figure 11, the most significant amount recorded is that of α -tocopherol, with values between 1.9987 mg/100 g (P4) and a maximum of 3.9815 mg/100 g (P3). Sample P1 records 2.6723 mg/100 g, while sample P2 contains 31.8% more. β -tocopherol was determined in amounts ranging between 0.9956 mg/100 g (P2) and 1.3428 mg/100 g (P4). Significant amounts were also recorded in samples P1 and P5, between 1.2134 mg/100 g,

respectively 1.2223 mg/100 g. Γ -tocopherol was found in similar amounts, reaching values between 0.9998 mg/100 g (P2) and a maximum of 1.7661 mg/100 g (P5).

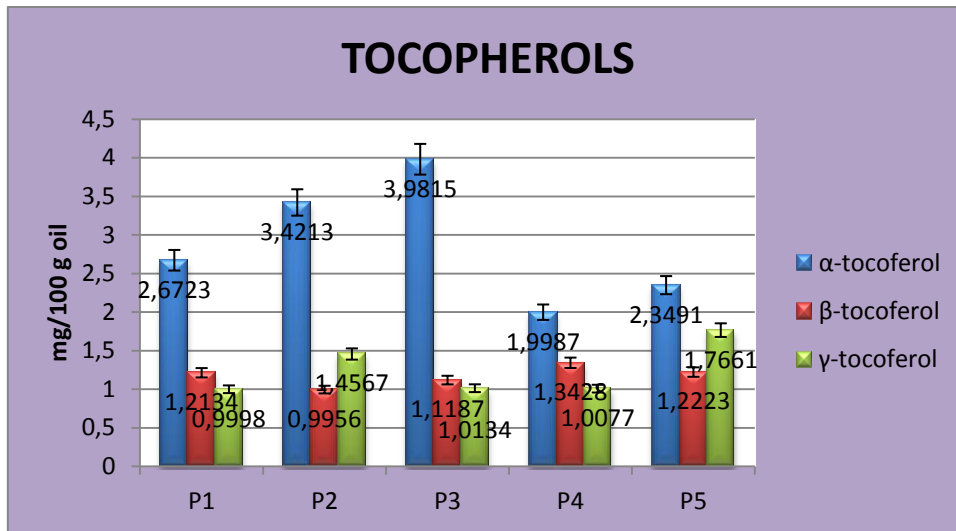


Figure 11. The identification and quantification of tocopherols in the five grape seed oil samples

Tocotrienols are part of vitamin E, and are extracted from plants in their natural, non-esterified form. Tocotrienols are antioxidants, the amounts found ensuring protection against the thickening of the walls of the arteries. Tocotrienols slow the liver enzyme which plays a part in cholesterol synthesis. As shown in Figure 12, two groups of tocotrienols were identified in grape seed oil, i.e. α -tocotrienol and γ -tocotrienol. The amounts of α -tocotrienol range between 7.2234 mg/100 g (P2) and 11.1121 mg/100 g in sample P4. In samples P1, P3 and P5, we determined values of 9.8891 mg/100 g, 10.9821 mg/100 g, respectively 10.0001 mg/100 g, with an average of 9.8413 mg/100 g.

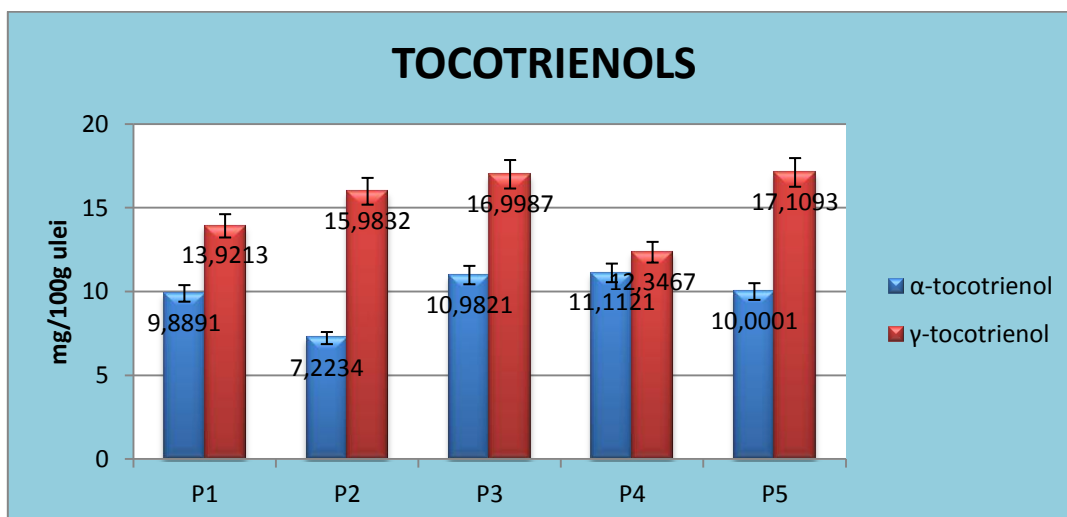


Figure 12. The identification and quantification of tocotrienols in the five grape seed oil samples

The amounts of γ -tocotrienol range between a minimum of 12.3467 mg/100 g in sample P4 and 17.1093 mg/100 g in sample P5. Sample P1 contained 13.9213 mg/100 g, sample P2

15.9832 mg/100 g, while sample P3 16.9987 mg/100 g, being the closest to the maximum. γ -tocotrienols reached an average of 15.2718 mg/100 g, 68% higher than that of α -tocotrienols.

Identification and quantification of essential amino acids (AA=amino acids, N=nitrogen)

Amino acids are essential for the health of the body. These are molecular structures that work together to form proteins in the body, which are vital for its correct functioning. Some of them cannot be synthesized by the body, and are taken from food; these are called essential amino acids. In the five samples under study, nine essential amino acids were identified and quantified, recording values ranging between 1 g AA/16 g N and 8 g AA/16 g N. Figure 13 shows that arginine was found in the highest amounts, between 6.99 g AA/16 g N (P4) and 7.48 g AA/16 g N (P2).

Similar values were determined in sample P5, where arginine reached 7.42 g AA/16 g N and in P1, with 7.39 g AA/16 g N. Phenylalanine recorded similar values to lysine and threonine. Thus, the concentration of phenylalanine ranged between 2.17 g AA/16 g N and 2.91 g AA/16 g N, while that of lysine ranged between 2.25 g AA/16 g N and 2.56 g AA/16 g N. Compared to the two essential amino acids, threonine ranged between 2.13 g AA/16 g N and 2.77 g AA/16 g N. Histidine and methionine recorded values under 2, ranging between 1.07 g AA/16 g N and 1.56 g AA/16 g N in the case of histidine, respectively 1.01 g AA/16 g N and 1.29 g AA/16 g N in the case of methionine. Leucine reached values between 5.21 g AA/16 g N and 6.01 g AA/16 g N, with an average of 5.72 g AA/16 g N. The essential amino acid isoleucine was found in amounts which are, on average, 62% lower than those of leucine, ranging between 3.21 g AA/16 g N and 3.64 g AA/16 g N. Valine reached values between 4.28 g AA/16 g N (P3) and 4.89 g AA/16 g N (P2), with an average of 4.43 g AA/16 g N.

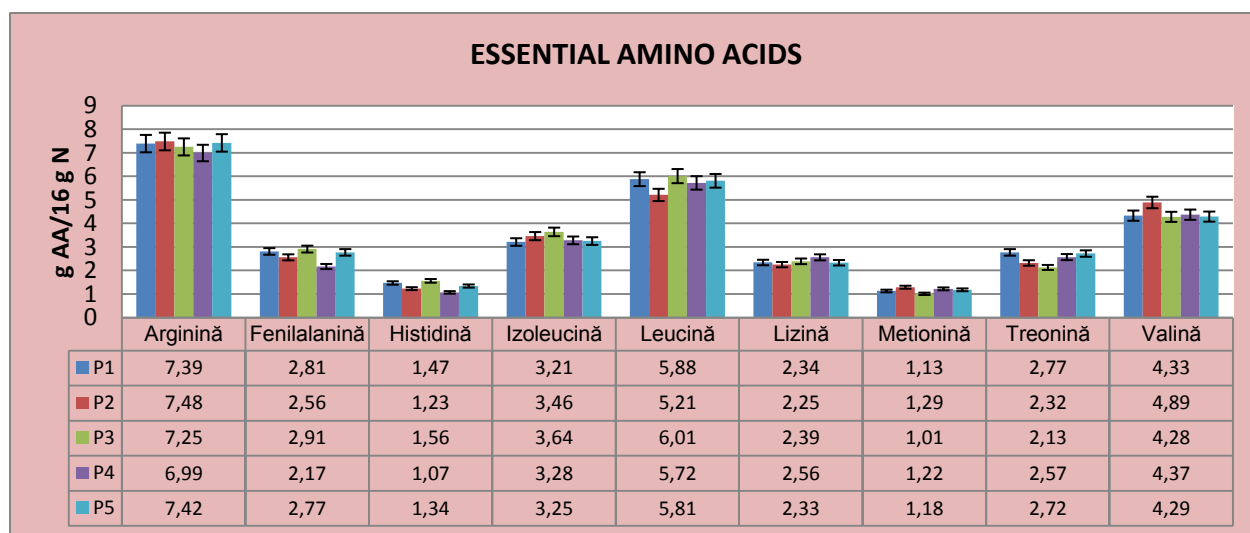


Figure 13 The identification and quantification of essential amino acids in the five grape seed oil samples

Identification and quantification of nonessential amino acids (AA=amino acids, N=nitrogen)

Nonessential amino acids are amino acids that can be synthesized by the body, playing a significant role in the synthesis of proteins, together with essential amino acids.

Figure 14 reveals that important values of 8 nonessential amino acids were determined, i.e. alanine, aspartic acid, cystine, glycine, glutamic acid, proline, serine and tyrosine. The highest

values were identified for glutamic acid, between 17.01 g AA/16 g N and 21.17 g AA/16 g N. The average of the five samples was 19.96 g AA/16 g N. Cystine and tyrosine recorded values between 0.55 g AA/16 g N and 1.21 g AA/16 g N, respectively 0.35 g AA/16 g N and 1.28 g AA/16 g N. Average values ranged between 0.94 g AA/16 g N, respectively 0.98 g AA/16 g N, with a difference of 0.5%.

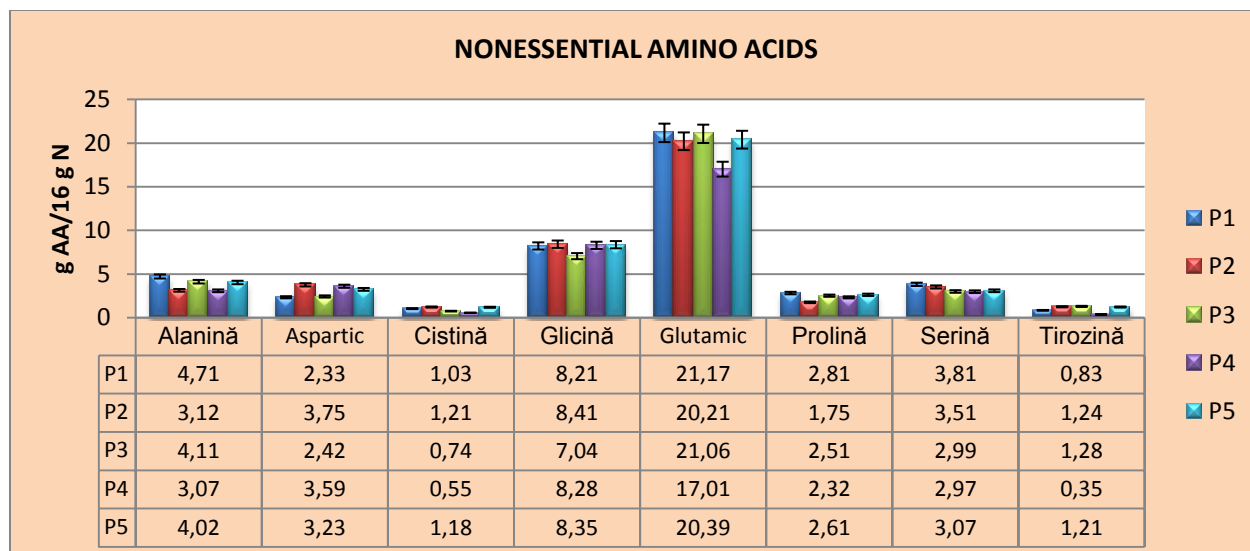


Figure 14. The identification and quantification of nonessential amino acids in the five grape seed oil samples

Alanine recorded a minimum value of 3.07 g AA/16 g N (P4), with an average of 3.8 g AA/16 g N and a maximum of 4.71 g AA/16 g N (P1), while aspartic acid reached values that were, on average, 54% lower. Glycine ranged between 7.04 g AA/16 g N and 8.41 g AA/16 g N, values that are, on average, double compared with those of alanine.

Proline and serine recorded average values of 2.40 g AA/16 g N, respectively 3.27 g AA/16 g N, remarkable for this type of oil.

The identification and quantification of metals in the five grape seed oil samples

Metals are generally a source of food contamination, but they are not a problem if found in very low amounts. These metals can pass in food from various sources, one of which can be the soil. Low amounts of metals can get in the grape seed oil from sources such as the plant (the vine), machines, storage areas, spraying substances. The present study looked at the concentrations of iron, zinc, copper and chromium found in the five samples of grape seed oil. Figure 15 shows that the amounts of iron were the highest, reaching 182.072 µg/L (P1), with an average of the five samples of 161.3694 µg/L. Zinc oscillated between 38.921 µg/L (P3) and 78.824 µg/L, samples P3 and P5 recording similar values, with a difference of 3.1%. The amounts determined in sample P2 were 53% lower than those found in sample P4, i.e. 41.545 µg/L. Copper was found in low amounts, ranging between 23.354 µg/L (P2) and 51.209 µg/L (P4), with an average of 41.356 µg/L.

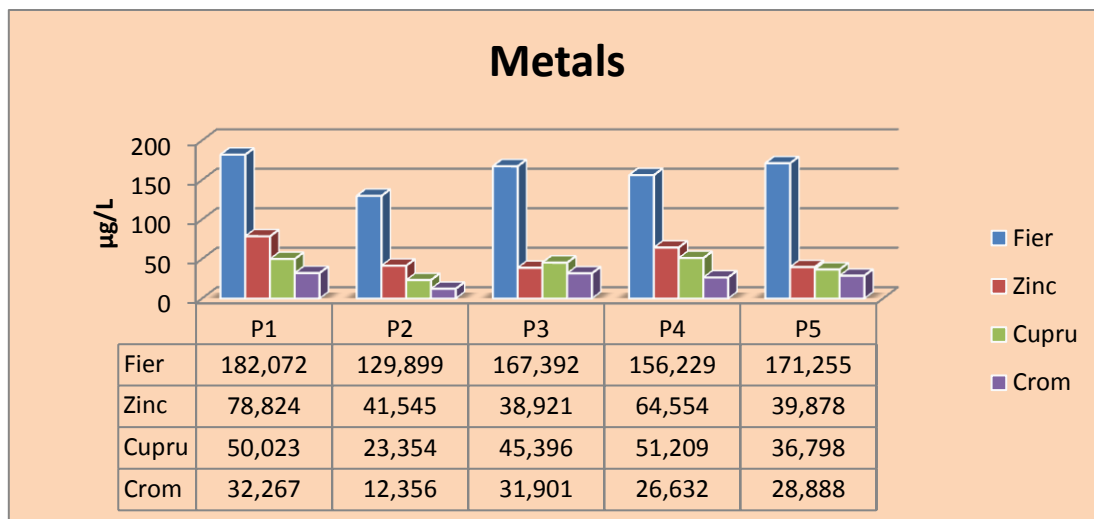


Figure 15. The identification and quantification of certain metals in the five grape seed oil samples

The lowest values identified were those of chromium, which did not exceed 32.267 µg/L. Sample P2 recorded the lowest value (12.356 µg/L), while samples P4 and P5 reached 26.632 µg/L, respectively 28.888 µg/L. Chromium values in sample P3 were 3.1% lower than the maximum.

Conclusions

Significant values of saturated fatty acids were identified and quantified, such as myristic acid, palmitic acid and stearic acid, which contribute positively to human diet.

Regarding unsaturated fatty acids, the most significant values were recorded in the case of linoleic acid, followed by oleic acid, palmitoleic acid, while the lowest values identified were those of linoleic acid.

Linoleic acid (omega-6) was found in grape seed oil in important amounts, lower than those found in sunflower or pumpkin oil.

Polyunsaturated fatty acids or omega-3 fatty acids were found in grape seed oil in reasonable amounts.

Tocopherols and tocotrienols recorded significant amounts in all the five samples under study; they are known to play a role in the metabolism of fats, in protein synthesis, limiting the production of cholesterol, protecting the heart and arteries from atherosclerosis.

Grape seed oil presented both essential and nonessential amino acids in amounts which recommend it to be consumed for an optimum functioning of the body.

The amounts of metals found in grape seed oil fell into the order of micrograms/L, thus posing no threat to human health.

VALORISING THE RESULTS OF THE RESEARCH TO OBTAIN A COMPLEX ANIMAL FEED FODDER RESULTING IN A RAW MILK HAVING SUPERIOR QUALITATIVE CHARACTERISTICS

The product is designed as a nutritional intake combined between marc and residual yeast added to animal feed.

Materials and methods

- dehydrated and grinded red marc
- dehydrated residual yeast
- 10:1 mix

In order to recover red marc from wine centres, it is recommended to use it in animal feed; the present study focuses on the possibility to increase the qualitative parameters of milk. Thus, taking into account the chemical properties of marc (especially polyphenols) and of yeast (vitamins, nitrogenous substances), an existing recipe of fodder was completed with a mix made up of the two components, as shown below:

- 50% silage, 15% marc and yeast mix (10:1 ratio), 25% sunflower, soybean, corn and barley meal, 8% lucerne (hay), 2% mineral premix (phosphorus, calcium).

The milk was harvested daily from a sample of 25 milk cows fed with the standard recipe and from a sample of 25 milk cows fed with the recipe completed with the suggested mix of marc and yeast. With a view to interpreting the results as objectively as possible, each milk lot was homogenized and evaluated weekly, resulting in an average of its quality. The monitoring was done over the course of 10 weeks at a private farm in the county of Alba. Milk analysis were also done using the equipment ECOMILK TOTAL, a device offering 10 automatic parameters: fat, protein, non-fat dry matter (NFDM), lactose, pH, freezing point, density, temperature, added water in milk, conductivity: (<http://www.analiticlaboratory.ro/analizor-de-lapte-ekomilk-total/>)

Results and discussions

According to the estimates of the cow farm owner, the average amount of milk in the ten weeks was 23.81 L/head/day of using classic fodder and 26.14 L/head/day of using fodder enriched with 10:1 mix of red marc and wine yeast /15%.

Table 1. Evaluation of the parameters of the milk harvested from the sample of cows fed with the standard recipe over the course of ten weeks

| Week | FAT % | NFDM % | PROT % | DEN g/cm ³ | AWM % | pH | Z mS/cm | T °C | LAC % |
|------|-------|--------|--------|-----------------------|-------|------|---------|------|-------|
| S1 | 3.28 | 9.2 | 3.65 | 34.9 | 0 | 6.55 | 3.77 | 19.2 | 5.52 |
| S2 | 3.19 | 9.8 | 3.77 | 34.7 | 0 | 6.56 | 3.89 | 21.4 | 5.63 |
| S3 | 3.23 | 10 | 3.78 | 35.1 | 0 | 6.62 | 3.64 | 21.8 | 5.88 |
| S4 | 3.30 | 10.2 | 3.69 | 34.6 | 0 | 6.53 | 3.91 | 19.4 | 5.48 |
| S5 | 3.27 | 10.1 | 3.58 | 34.8 | 0 | 6.61 | 4.06 | 19.8 | 5.67 |
| S6 | 3.25 | 9.9 | 3.68 | 35.5 | 0 | 6.62 | 4.07 | 19.4 | 5.71 |
| S7 | 3.22 | 10.3 | 3.56 | 34.6 | 0 | 6.59 | 3.95 | 20.1 | 5.66 |
| S8 | 3.18 | 10.6 | 3.63 | 35.2 | 0 | 6.64 | 4.1 | 20.3 | 5.59 |
| S9 | 3.24 | 9.7 | 3.74 | 35.9 | 0 | 6.55 | 4.13 | 20.5 | 5.81 |
| S10 | 2.26 | 10.1 | 3.75 | 36.1 | 0 | 6.53 | 4.11 | 20.6 | 5.83 |

Table 1 and Table 2 above systematize the results obtained over the course of the ten weeks when the parameters were monitored.

The two tables show that added water was not found in any milk sample (AWM %), so that the samples can be considered trustworthy and safe.

Table 2. Evaluation of the parameters of the milk harvested from the sample of cows fed with the suggested recipe [– 50% silage, 15% marc and yeast mix (10:1 ratio), 25% sunflower, soybean, corn and barley meal, 8% lucerne (hay), 2% mineral premix (phosphorus, calcium)] over the course of ten weeks

| Week | FAT % | NFDM % | PROT % | DEN g/cm ³ | AWM % | pH | Z mS/cm | T °C | LAC % |
|------|-------|--------|--------|-----------------------|-------|------|---------|------|-------|
| S1 | 3.58 | 10.2 | 3.94 | 34.8 | 0 | 6.45 | 3.79 | 19.2 | 5.84 |
| S2 | 3.29 | 10.5 | 3.89 | 34.7 | 0 | 6.56 | 3.90 | 21.4 | 5.73 |
| S3 | 3.47 | 10.5 | 3.98 | 35.2 | 0 | 6.61 | 3.61 | 21.8 | 5.82 |
| S4 | 3.39 | 10.9 | 3.79 | 34.5 | 0 | 6.56 | 3.89 | 19.4 | 5.73 |
| S5 | 3.52 | 10.8 | 3.82 | 34.9 | 0 | 6.60 | 4.01 | 19.8 | 5.65 |
| S6 | 3.5 | 10.9 | 3.8 | 35.5 | 0 | 6.61 | 4.02 | 19.4 | 5.7 |
| S7 | 3.42 | 10.9 | 3.76 | 34.7 | 0 | 6.57 | 3.94 | 20.1 | 5.62 |
| S8 | 3.18 | 10.7 | 3.83 | 35.4 | 0 | 6.62 | 4.1 | 20.3 | 5.79 |
| S9 | 3.41 | 10.7 | 3.85 | 36.0 | 0 | 6.55 | 4.11 | 20.5 | 5.82 |
| S10 | 2.48 | 10.9 | 3.81 | 36.2 | 0 | 6.50 | 4.04 | 20.6 | 5.81 |

Conclusions

The intake of red marc and residual yeast added to animal feed leads to:

- an increased percentage of fat in milk, on average by 6.1%
- an increased percentage of non-fat matter in milk, on average by 8.7%
- an increased percentage of proteins in milk, on average by 9.2%
- no added water found in milk
- milk density remaining constant
- pH showing a slight decrease, which reveals an increase of milk acidity
- conductivity recording values that oscillated insignificantly, not influencing the milk quality
- lactose reaching optimum values in both cases, with small exceptions which have to do with the cows' food source, especially the ones in sample 2, fed with intake of marc and residual yeast
- the intake of 10:1 red marc and wine yeast 15% in the fodder ratio of cows resulting in increased milk quality, but implicitly to its quantitative increase by an average of 9.8%.

FINAL CONCLUSIONS AND PERSPECTIVES TO CONTINUE THE RESEARCH

The studies and analysis conducted on the different wine sub-products led to the conclusions below:

- stronger pressing of grapes results in a lower amount of tannin in marc
- red grape varieties contain an average amount of tannin that is six times higher than that of white varieties, part of it passing into wine, but also into marc
- red marc is rich in phenolic compounds that remain after pressing
- brandy obtained from marc distillation depends on storage time and the chosen work version
- tannin extraction from grape bunch stems depends on the concentration of the solvent, on extraction time and temperature

- regarding unsaturated fatty acids, the most significant values were recorded in the case of linoleic acid, followed by oleic acid, palmitoleic acid, while the lowest values identified were those of linolenic acid
- important amounts of linoleic acid (omega-6) was found in grape seed oil, lower than those found in sunflower or pumpkin oil
- reasonable amounts of polyunsaturated fatty acids or omega-3 fatty acids were found in grape seed oil
- tocopherols and tocotrienols recorded significant amounts in all the five samples under study
- grape seed oil presented both essential and nonessential amino acids in amounts which recommend it to be consumed for an optimum functioning of the body
- the amounts of metals found in grape seed oil fell into the order of micrograms/L, thus posing no threat to human health
- the wine yeasts selected to be studied have presented different protein values and significant amounts of vitamins, depending on their origin, but also on the starter culture used in the fermentative process.

The intake of red marc and residual yeast added to animal feed leads to:

- an increased amount of milk, on average by 9.8%
- an increased percentage of fat in milk, on average by 6.1%
- an increased percentage of non-fat matter in milk, on average by 8.7%
- an increased percentage of proteins in milk, on average by 9.2%.

The results obtained are only a segment of the multiple possibilities to study wine sub-products and use them for various purposes. The scope of the study can be expanded, as there is a possibility to tackle a series of valuable compounds extraction systems, but also to expand applicability to related fields or to the cosmetic-pharmaceutical domain.

PERSONAL CONTRIBUTIONS

The present paper characterizes for the first time a series of wine sub-products from indigenous vineyards, identifying compounds by means of a state of the art methodology. Therefore, phenolic compounds in marc were identified and quantified, residual wine yeasts were characterized and a polyphenolic profile of red marc was done. Another first was the study of procedures to optimize extraction processes of valuable compounds from wine sub-products and the study of the milk from cows fed with a complex fodder, made after an original recipe.

SELECTIVE REFERENCES

1. Agustin-Salazar S., Medina-Juárez L.A., Soto-Valdez H., Manzanares-López F., Gámez-Meza N., 2014, Influence of the solvent system on the composition of phenolic substances and antioxidant capacity of extracts of grape (*Vitis vinifera* L.) marc, Australian Journal of Grape and Wine Research, vol. 20 (2), 208–213
2. Bail S., Stuebiger G, Krist S., Unterweger H., Buchbauer G., (2008), Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity, Food Chemistry, vol. 108, (3), 1122–1132

3. Balteș M, (2015) Studii privind caracterizarea unor drojdii de vin reziduale în scopul utilizării acestora în rațiile furajere ale animalelor, cap. VI, 165-185, coordonatori Tița O și Oprean C. (Perspective actuale privind dezvoltarea durabilă), ed Prouniversitaria București, 2015
4. Balteș M., (2015), The influence of storage time on the yield of alcohol extraction from marc, *Acta Universitatis Cibiniensis, Series E: Food technology*, 10.1515/auaft-2015-0008, vol IX 1:81-86
5. Balteș M., (2015), Identification and characterization of useful sub products of grape wine products, case study: wine yeast, *Proceeding of the International Conference Agri-Food Sciences, Processes and Technologies, Agri-Food, Sibiu, Romania, May, 2015*, cd
6. Cotea D.V., Zănoagă C.V., Cotea V.V., (2009), *Tratat de oenochimie, vol.I*, Ed. Academiei Române București
7. Cotea V. D., Zănoagă C.Z., Cotea Valeriu V., (2010b), *Tratat de oenochimie, vol.II*, Editura Academiei Române, București
8. Cotea V.V., Cotea V.D., (2006), *Tehnologii de producere a vinurilor*, Editura Academiei Române
9. Doshi P., Adsule P., Banerjee K., Oulkar D., (2015), Phenolic compounds, antioxidant activity and insulinotropic effect of extracts prepared from grape (*Vitis vinifera* L) byproducts, *Journal of Food Science and Technology*, vol. 52, (1), 181-190
10. Jayaprakasha G.K., Selvi T., Sakariah K.K., (2003), Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts, *Food Research International*, vol. 36, (2), 117–122
11. Jayaprakasha G.K., Singh R.P., Sakariah K.K., (2001) Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro, *Food Chemistry*, vol 73, (3), 285–290
12. Kaur M., Agarwal R., Agarwal C., Grape seed extract induces anoikis and caspase-mediated apoptosis in human prostate carcinoma LNCaP cells: possible role of ataxia telangiectasia mutated-p53 activation., *Mol Cancer Ther.*, vol. 5, (5), 1265-74.
13. Kaur M., Mandair R., Agarwal R., Agarwal C., (2008), Grape seed extract induces cell cycle arrest and apoptosis in human colon carcinoma cells., *Nutr Cancer.*, vol. 60 Suppl 1, 2-11.
14. Lengyel Ecaterina. *Aroma vinurilor bănățene*, Ed. Universității Lucian Blaga Sibiu, 2014.
15. Lengyel, E., Tita, O., Oprean, L., Gaspar, E., Sipos, A. 2011. "Practical considerations regarding the physiological active state and the autolized one of *Saccharomyces bayanus* cultures isolated from Tarnave and Sebes-Apold wineryard". *Annals of RSCB*, 16(1): 283-285.
16. Maier T., Schieber A., Kammerer D. R., Carle R., (2009) Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants, *Food Chemistry*, vol. 112, (3), 551–559
17. Mendel F., (2014), Antibacterial, Antiviral, and Antifungal Properties of Wines and Winery Byproducts in Relation to Their Flavonoid Content, *J. Agric. Food Chem.*, vol., 62 (26), 6025–6042, DOI: 10.1021/jf501266s,

18. Natella F., Belevi F., Gentili V., (2002), et al. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans., *J Agric Food Chem.*, vol. 50, (26), 7720-5.
19. Navarra, Tova (2004). *The Encyclopedia of Vitamins, Minerals, and Supplements*. Infobase Publishing. p. 155.
20. Nicula, A., Nicula, A.T., Socaciu, C., Dubreucq, P. 2009. "Application of Advanced Drying Technologies for Obtaining Bioactive Beer Yeast and Grape Seed Extract Powders". *USAMV Bulletin*, 66:345-355.
21. Oaknin-Bendahan S., Anis Y., Nir I., Zisapel N., (1995), Effects of long-term administration of melatonin and a putative antagonist on the ageing rat, *NeuroReport*, vol. 6, (5), 785–788
22. Oprean Letiția. *Drojii industriale*, Ed. Universității Lucian Blaga Sibiu, 2014.
23. Oprean Letitia, Iancu Ramona Maria and Ecaterina Lengyel, *Microbiologie generală : note de curs* (Ed. Universității Lucian Blaga Sibiu), 2014.
24. Oprean Letitia, Iancu Ramona Maria and Ecaterina Lengyel, *Microbiologie generală : îndrumar de laborator* (Ed. Universității Lucian Blaga Sibiu), 2014.
25. Oprean, L., Dezsai, C., Iancu, R., Lengyel, E. 2012 " Practical applications of yeast strains with superior biotechnological properties". *Management of Sustainable Development*, 4(1): 41-44.
26. Oprean, L., Lengyel, E., Gaspar, E., Vințean, A. Chicea, D., Tița, O., Tița, M., "Practical aspects regarding the physiological active state and the autolysis of the starter *Saccharomyces cerevisiae* culture" (paper presented at the Proceedings of the 5th International Conference, Integrated Systems for agri-food production SIPA 2007, Sibiu, Romania, november 22-24, 161-165, 2007).
27. Patti A. F., Issa G. J., Smernik R., Wilkinson K., (2009), Chemical composition of composted grape marc., *Water Sci Technol.*, vol. 60 (5), 1265-71
28. Pomohaci N. , Stoian V., Gheorghită M., Sîrghi C., Cotea V. V., Nămoșanu I., (2001), *Oenologie*, vol.II, Ed. Ceres, București;
29. Pomohaci N., Stoian V., Gheorghită M., Sîrghi C., Cotea V. V., Nămoșanu I., (2000) - *Oenologie* vol. I, *Prelucrarea strugurilor și producerea vinurilor*, Editura Ceres, București
30. Ramchandani A.G., Karibasappa G.S., Pakhale S.S., (2008), Antitumor-promoting effects of polyphenolic extracts from seedless and seeded Indian grapes., *J Environ Pathol Toxicol Oncol.*, vol. 27, (4), 321-31.
31. Robinson Philip, *Yeast products for growing and lactating ruminants: A literature summary of impacts on rumen fermentation and performance* (animalscience.ucdavis.edu, 2010).