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CUPRINS

HPLC ANALYSIS FOR QUANTIFICATION OF BENZOIC ACIDS IN TÂRGU BUJOR AND COTNARI WINES

UTILIZAREA ANALIZEI HPLC, IN CUANTIFICAREA ACIZILOR BENZOICI DIN VINURI DE TÂRGU BUJOR ȘI COTNARI

COTEA V.V.[1], MUSTEA M.[1], NICULAUA M.[2], NECHITA C.B.[1], COLIBABA Lucia Cintia[1], LUCHIAN Camelia[1]
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Abstract. *This study determined and correlated the benzoic acids profile of four wines from Cotnari wine area (Frâncuşă, Fetească albă, Grasă de Cotnari şi Tămâioasă românească), and four wines from Tîrgu Bujor wine region (Băbească gri, Fetească regală, Fetească albă, Italian Riesling) with the geographical origins and vinification year, using high performance liquid chromatography. The wines produced are dry or semi-dry wines of good quality (DOC) with high alcoholic degree similar to the quality of the grapes that were used. All the wine samples from Cotnari vineyard from 2010 contain a higher quantity of gentisisc acid that the 2011 samples. At the same time, Cotnari samples register a higher total quantity of besoic acids (gentisic acid, galic acid, protocatechic acid) than those from Targu Bujor vineyard.*
Key words: *wine, HPLC, benzoic acids*

Rezumat. *Acest studiu a determinat și corelat profilul acizilor benzoici a patru vinuri din zona Cotnari (Frâncușă, Fetească albă, Grasă de Cotnari si Tămâioasă românească), și patru vinuri din regiunea viticolă Târgu Bujor (Băbească gri, Fetească regală, Fetească albă, Riesling italian), cu originea geografică și anul de vinificație, utilizând cromatografia de lichide de înaltă performanță. All the wine samples from Cotnari vineyard from 2010 contain a higher quantity of gentisisc acid that the 2011 samples. At the same time, Cotnari samples register a higher total quantity of besoic acids (gentisic acid, galic acid, protocatechic acid) than those from Targu Bujor vineyard.*
Cuvinte cheie: *vin, HPLC, acizi benzoici*

INTRODUCTION

Grapes and grape-derived products are an abundant source of polyphenols and represent an important dietary component. This versatile group of phytochemical compounds is classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. All of these compounds have a strong influence on the quality and character of the wine, and are therefore important not only for the wine characterization but also, reflects the history of the wine producing process,

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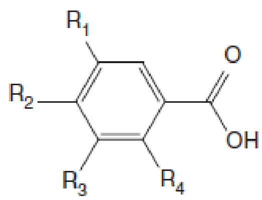
including the grape variety, the containers used for fermentation and storage, and the enological practices (Harris et al., 2007).

Non-flavonoid phenolic compounds can be: hydroxybenzoic and hydroxycinnamic acids, volatile phenols, stilbenes and miscellaneous compounds. Although non-colored, the non-flavonoid constituents are known to stabilize the color of wines by intra- and intermolecular reactions (Huang et al., 2009).

The most common derivatives found in wine are gallic acid, gentisic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid, salicylic acid, and vanillic acid (Moreno Arribas and Polo, 2009; Ribereau Gayon et al., 2006).



$R_1 = R_2 = R_3 = OH$; $R_4 = H$: Gallic acid
$R_1 = R_4 = OH$; $R_2 = R_3 = H$: Gentisic acid
$R_1 = R_2 = OH$; $R_3 = R_4 = H$: Protocatechuic acid
$R_1 = R_4 = H$; $R_2 = OH$; $R_3 = OCH_3$: Vanillic acid
$R_1 = R_3 = OCH_3$; $R_2 = OH$; $R_4 = H$: Syringic acid

Fig.1. Chemical structures of the hydroxybenzoic acids found in wines.

The aim of this research is to investigate the variation of benzoic acids content in wines obtained from grapes of Moldova different regions with year of winemaking. The method used for determining the benzoic acids content is a HPLC method (Castellari., 2002; OIV, 2012).

MATERIAL AND METHOD

In this study we have selected four wine grape varieties from Cotnari area: Frâncuşă (encoded Frş I 10 and Frș I 11), Fetească albă (Fa I 10 and Fa I 11), Grasă de Cotnari (Grs I 10 and Grs I 11) şi Tămâioasă românească (Tr I 10 and Tr I 11). Four wines from Tîrgu Bujor wine region were selected: Băbească gri (encoded Bg II 10 and Bg II 11, Fetească regală (encoded Fr II 10 and Fr II 11), Fetească alba (encoded Fa II 10 and Fa II 11), Italian Riesling (encoded IR II 10 and IR II 11). To simplify encoding was done for samples as following: variety - region - year (eg Frş I 10 - Frâncuşă Cotnari 2010).

The grapes were harvested at technological maturity from Iaşi and Cotnari vineyard and processed by classical technology for obtaining white wines. Before fermentation a fining procedure was made to remove all rough organic parts and afterword's a co-inoculation whit enzymes (2 g/hL) and yeasts (30 g/hL) was done. After its alcoholic fermentation, the wine was racked at room temperature. After 7-8 days the wine was filtered and bottled with the help of an Enomatic Tenco device. Immediately after taking a dose of sulphur dioxide by 40 mg/L per glass, they were closed semi with a Mini TS.

The experiments were done during September 2010 – March 2012, at the Oenological Research Centre of the Romanian Academy, Iaşi branch, and at the Oenology Laboratory of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iaşi. Each wine, after decarbonisation, was analysed: volatile acidity OIV-MA-AS313-02, total acidity OIV-MA-AS313-01, specific gravity at 20°C OIV-MA-AS2-01B, alcoholic strength by frequency oscillator OIV-MA-AS312-01A, reducing substances OIV-MA-AS311-01A, total dry matter and non-reducing substances OIV-MA-AS2-03B were done according to present standards (6) and specific literature (Ribereau Gayon et al., 2006). After the second decanting, at 6 months from fermentation, wines were analysed for their hydroxycinnamic acids content with HPLC methods.

Reagents and standard for HPLC: the standards solutions for HPLC analysis were supplied by Sigma and Alfa Aesar. By dissolving known amounts of the analytical-reagent grade standards in methanol the calibration solutions were prepared.

The wine hydroxycinnamic compounds were carried out with high-performance liquid chromatograph (HPLC) Shimadzu equipped with two chromatographic columns Merck Chromolith Performance RP-18.

RESULTS AND DISCUSSIONS

Table 1 registers the content of bensoic acids in wines obtained from Cotnari vineyar. The samples were processed in 2010 and 2011. In 2010, bensoic acids are in a higher quantity.

High values are registered for protocatehic acid (8.34 mg/L) for the sample Frş I 10, Cotnari vineyard, gallic acid (2.10) in Frş I 10 sample, in 2010. All wine samples from 2010 have a higher quantity of gentisic acid that the 2011 samples.

Table 1

Variation of benzoic acids content in wine samples from Cotnari area vinified in 2010 and 2011 (mg/L)

Wine sample	***protocatehic acid***	***p-hidroxybenzoic acid***	***vanilic acid***	***gallic acid***	***syringic acid***	***gentisic acid***
Frş I 10	**8.34**	0.24	0.30	**2.10**	3.66	**220.50**
Fa I 10	8.12	0.32	0.38	0.91	8.23	**33.35**
Grs I 10	8.34	0.24	0.30	2.10	3.66	**220.50**
Tr I 10	7.59	0.24	1.05	1.95	4.18	**292.35**
Frş I 11	7.87	0.22	**0.62**	1.44	11.30	103.36
Fa I 11	7.85	**0.40**	0.28	0.97	SLD	28.25
Grs I 11	8.00	SLD	0.31	1.61	**17.16**	125.51
Tr I 11	7.92	0.55	0.47	1.38	3.01	SLD

Table 2 contains the quantities of bensoic acids from wine samples obtained from grapes harvested in Târgu Bujor vineyard in 2010 and 2011.

The variation of the bensoic acids content is random according to harvest year and compound. High values for syringic acid were found in 2011 samples: Frâncuşă 17.90 mg/L, Fetească albă 21.52 mg/L, Băbească gri 16.62mg/L. In 2010 Frâncuşă wine sample, the concentration of gentisic acid is higher than in the 2010 sample (125.96 mg/L).

One can notice that the wine samples obtained from grapes harvested in Cotnari vineyard have a higher content of bensoic acids (gentisic acid, gallic acid, acid protocatehic).

Table 2

Variation of benzoic acids content in wine samples from Târgu Bujor area in 2010 and 2011 (mg/L)

Wine sample	*protocatehic acid*	*p-hidroxybenzoic acid*	*vanilic acid*	*gallic acid*	*syringic acid*	*gentisic acid*
Rl II 10	7.96	0.38	0.14	**1.35**	5.12	125.96
Fr II 10	7.96	0.38	0.14	1.35	5.12	**125.96**
Fa II 10	8.49	1.56	**0.51**	**1.48**	0.97	3.29
Bg II 10	8.94	0.92	**0.44**	1.54	14.28	32.10
Rl II 11	7.79	0.56	0.15	0.76	0.89	128.94
Fr II 11	7.96	1.15	0.30	1.74	**17.90**	35.01
Fa II 11	7.93	0.85	0.27	0.92	**21.52**	**58.54**

CONCLUSIONS

1. All the wine samples from Cotnari vineyard, obtained in 2010 have a much higher quantity of gentisic acid than the 2011 samples, also a higher quantity of bensoic acids (gentisic acid, galic acid, protocatehic acid) compared to the ones from Târgu Bujor vineyard.

2. All samples from 2010 from Cotnari vineyard have a much hiogher qunatity of gentisic acid than the 2011 samples.

3. In the case of syringic acid, higher values were identified in 2011 samples from Târgu Bujor: Frâncușă 17.90 mg/L, Fetească albă 21.52 mg/L, Băbească gri 16.62mg/L.

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STUDIES ON ORGANIC ACIDS CONCENTRATION OF TĂMÂIOASĂ ROMÂNEASCĂ WINE FROM COTNARI VINEYARD

STUDIUL ACIZILOR ORGANICI DIN VINURILE DE TĂMÂIOASĂ ROMÂNEASCĂ DIN PODGORIA IAŞI

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Abstract: *In this study, the must obtained in 2011 and 2012 from Tămâioasă românească variety was subjected to a number of 9 prefermentative treatments with oxalic acid, lactic acid, succinic acid, silicon dioxide, tannins, bentonite, graphen, chitosan and activated charcoal. The results showed that malic acid concentation increased due prefermetative treatments application in Tămâioasă românească wines obtained in 2011 from 1,24 g/L (M) to 1,61 g/L (V6). Acid succinic content in wine decreased after oxalic acid (V1) addition.*
Key words*: Tămâioasă românească, oxalic acid, chitosan, activated carbon*

Rezumat. *În acest studiu, mustul obţinut din soiul Tămâioasă românească în anii de recoltă 2011 şi 2012, a fost supus unui numar de 9 tratamente prefermentative cu acid oxalic, acid lactic, acid succinic, silicat de sodiu, tanin, bentonită, grafen, chitosan şi cărbune activ. Rezultatele au arătat că tratamentele prefermentative au condus la creşterea concentraţiei de acid malic din vinurile din 2011 de la1,24 g/L (M) la 1,61 g/L (V6). Tratamentul efectuat cu acid oxalic (V1) a condus la reducerea concentaţiei de acid succinic din vin.*
Cuvinte cheie*: Tămâioasă românească, acid oxalic, chitosan, cărbune activ*

INTRODUCTION

Besides the grape processing technology, the treatments applied to the must before fermentation also have an important role in deciding the wine's quality. (Ribéreau-Gayon and Dubourdieu, 2006). Several studies have been performed on the effect of enological practices on the wine's composition (Losada et al., 2010; Puig-Deu et al., 1996; Villanõ et al., 2006). The increasing of the titrable acidity and the total acidity of wine can be achieved by lactic acid addition.

Lactic acid can favour the biological evolution and maturation of wines and can also influence the obtaining of balanced wines from a gustatory point of view (***OIV, 2013). For adjustment of must acidity, oxalic or succinic acid can be used. Oxalic acid is also used to demonstrate the presence of calcium in a liquid as it causes turbidity and precipitation (Ribéreau-Gayon and Glories, 2006). As for succinic acid, its bitter- salty taste

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causes salivation and accentuates the wine's flavour and vinous character (Ribéreau-Gayon and Glories, 2006; Jackson, 2008). The exogenous tannins are frequently added to wines during the winemaking process for a number of reasons: to stabilize colour, to modify mouth-feel, to mask green characters, to increase polyphenolics and aromatic stability (Harbertson et al., 2011; Parker et al., 2007). Sodium silicate is used to clarify wines (***OIV, 2013).

The use of bentonite as a clarifying agent means to prevent the formation of protein haze in wines. Treating must with bentonite is recommended for wines which are to be clarified shortly after the completion of alcoholic fermentation (Ribéreau-Gayon and Dubourdieu, 2006). Recently characterized as "the thinnest material of the universe" (Geim and MacDonald, 2007), graphen is the two-dimensional version of graphite consisting of a two-dimensional arrangement of carbon atoms disposed in a hexagonal grid.

Graphene is the best known conductor of electricity and heat. Graphene presents a whole range of special properties, which gives it great potential for the practical production of new uses. Chitosan treatment can be an effective method to clarify the must and to prevent protein haze (Rao et al., 2010; Domingues et al., 2011, ***OIV, 2013).

It was observed that commercial preparations of β -glucosidase can be immobilized on chitosan and used in winemaking for the purpose of improving the aromatic potential of wines (Gallifuoco et al., 1998). Also, chitosan shown to effectively remove the polyphenols contained in white wines with a high polyphenol content and to have a stabilization capacity comparable to that of potassium caseinate (Spagna et al., 2000).

Another clarifying agent is activated charcoal, useful for correcting organoleptic issues of wine obtained from musts affected by fungi such as grey rot (*Botrytis cinerea*) or oidium (*Uncinula necator*), to eliminate, possible contaminants, to correct the colour from white musts derived from the white juice of red grapes, from very yellow musts derived from white grape varieties and from oxidized musts (Ribéreau-Gayon and Glories, 2006).

Knowledge of the organic acids content in wine present a great interests because these acids contributes to wine stability and affects the organoleptic qualities of wines, especially white wines (Ribereau-Gayon et al., 1982, Jackson, 1994). Their preservative properties also enhance wines' microbiological and physicochemical stability. The objective of the present study is to evaluate the influence of different enological treatments on the organic acids content of Tămâioasă românească wine.

MATERIAL AND METHOD

Grape samples and winemaking

Tămâioasă românească grapes from Cotnari vineyard were harvested in 2011 and 2012 at optimal maturity. The grapes were destemmed and crushed, and each must obtained was transferred in glass containers. Before fermentation, nine treatments were applied to the must :

- oxalic acid - 0,6 g/L (V1),
- lactic acid – 3 g/L (V2),
- succinic acid – 2 g/L (V3),
- silicon dioxide – 2,4 g/L (V4),
- tannins – 0,05 g/L (V5),
- bentonite – 1 g/L (V6),
- graphen – 1 g/L (V7), chitosan – 1 g/L (V8)
- activated charcoal – 1 g/L (V9).

The must were stirred to ensure a homogenous fermentation. After alcoholic fermentation, wines were filtered using a filtration-filling device-Tenco Enomatic® followed by sulfur dioxide addition (40 mg / L) to preserve wine from microbiological damage. Bottling was done with a semi-automatic device. After six months of storage the wines were analysed. Also, for each grape variety, a control sample (V) was obtained without prefermentative treatment.

Regents for pre-fermentative treatments: tannin (Taniblanc® - from AEB Spa, Italy), bentonite (Bentonita Clarit PLV 45 – Sodinal, France). Oxalic acid, lactic acid, succinic acid, sodium silicate, graphen, chitosan and activated charcoal were purchased from Sigma-Aldrich, Germany.

For analysing the organic acids from wines, the used methods were those recommended by the OIV (MA-E-AS313-04-ACIORG and MA-E-AS313-17-ACSHIK). The samples were processed by using a Shimadzu HPLC composed of: autoinjector Shimadzu series Prominence SIL-20AC (used injection volume: 10 μL, samples temperature 20 °C), quaternary pump Shimadzu series Prominence LC-20AD with five channels dagasing device Shimadzu series Prominence DGU-20A5, column oven Shimadzu series Prominence CTO-20AC, Photo Diode Array Shimadzu series Prominence SPD-M20A (used scanning segment: 200-440 nm), controler of cromatographic system Shimadzu series Prominence CBM-20A and PC conectivity through LAN.

RESULTS AND DISCUSSIONS

In Romanian wines, tartaric acid is found in concentrations between 0,3–4 g/L (Cotea et.al., 2009). Figure 1 registers the fact that the limits are not exceeded: 1,44 g/L (V4 - 2011) and 2,18 g/L (V1 – 2012). The wines from 2012 have a slightly higher concentration that the 2011 wines. Regarding the malic acid concentration, this has grown after using prefermentative treatments to 2011 wines.

The lactic acid in the new wines that did not undergo malolactic fermentation, is registered in a very low quantity, up to 0,5 g/L, while in wines that did undergo malolactic fermentation, it reaches up to 2–4 g/L (Cotea et.al., 2009). Except the variant where a treatment with lactic acid was applied and where a quantity of 6,62 g/L (V2 -2012) was registered, the other treatments did not influence the lactic acid concentration in wine, the values being quite similar among them (fig. 1).

In 2012 wines, the acetic acid values are very small in samples treated with sodium silicate (V4), tannin (V5) and chitosan (V8).

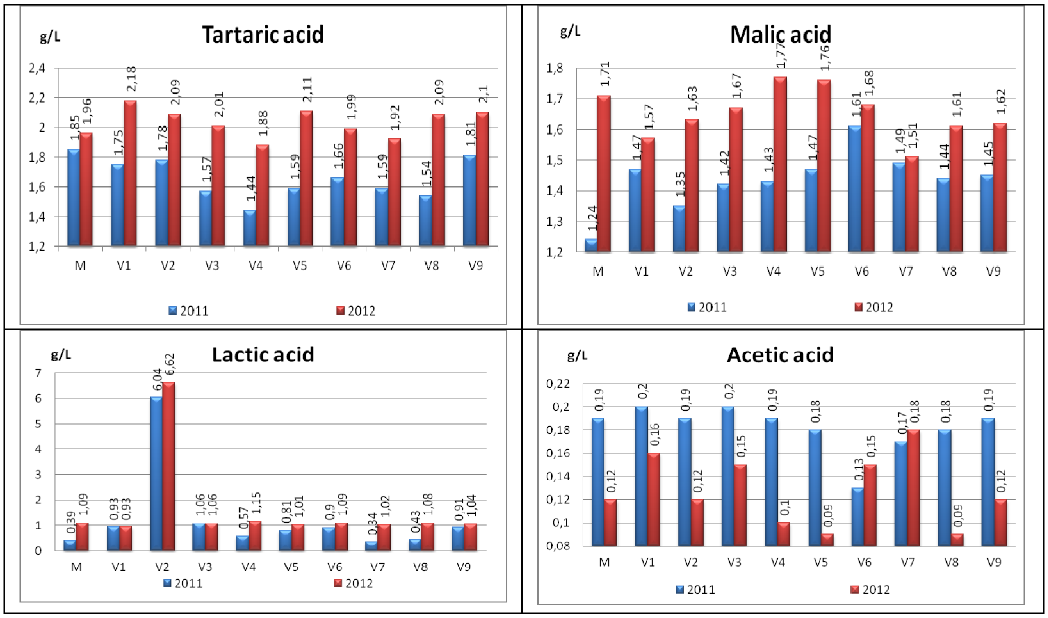

Fig.1. Tartaric acid, malic acid, lactic acid and acetic acid concentrations from Tămâioasă românească wines.

Due to the application of prefermentative treatments with oxalic acid and succinic acid (fig. 2), the concentration of these acids in wine registers values which are much higher than the other samples, such as the concentration of the succinic acid reaches a value of 1,47g/L (V3 – 2012), while oxalic acid is noted to be 80, 04 mg/L (V1 – 2011).

The succinic acid content in wine has decreased after applying the oxalic acid treatment (V1). 2012 Wines with pre-fermented treatments noted a decrease in concentrations of citric acid compared to the control.

The shikimic acid concentration (fig. 2) has much higher values in 2011 wines (V4 - 3,77 mg/L), compared to 2012 wines where the maximal values is of 1,59 mg/L (V4).

Fumaric acid content varies from 0,18 mg/L (V8 – 2011) to 0,99 mg/L (V4 – 2012) (fig. 3).

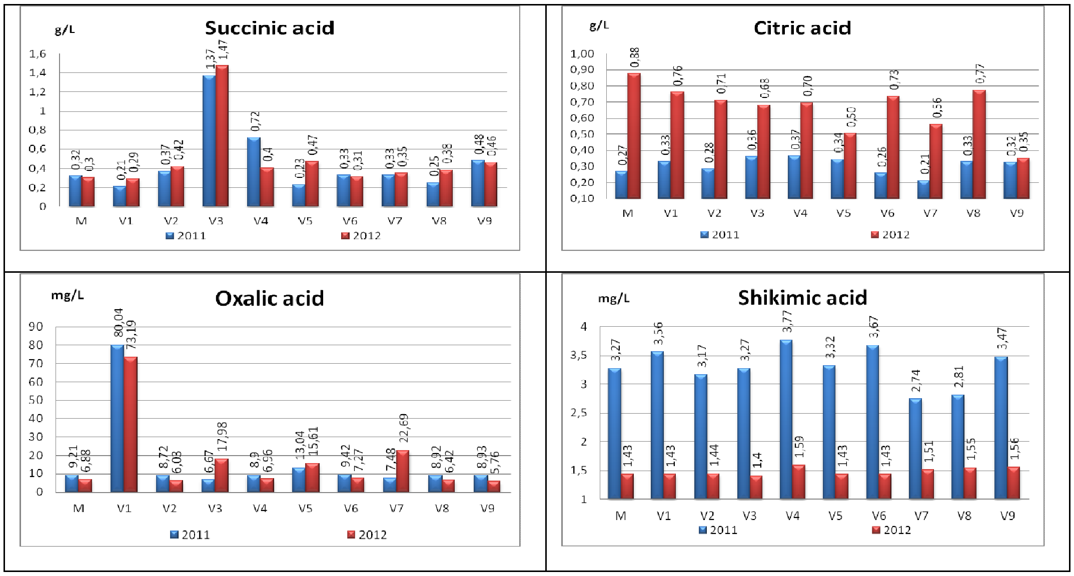

Fig.2. Succinic acid, citric acid, oxalic acid and shikimic acid concentrations from Tămâioasă românească wines.

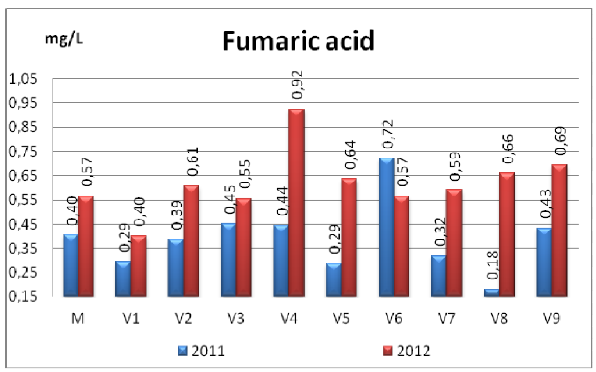

Fig.3. Fumaric acid concentration from Tămâioasă românească wines.

CONCLUSIONS

2012 wines registered higher values of tartaric acid than in 2011, while the malic acid concentration was raised after applying pre-fermentative treatments compared to the 2011 control sample.

The succinic acid content in wine has diminished after treating with oxalic acid (V1).

Treatment with sodium silicate (V4) led to a growth of the content of shikimic acid.

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STUDIES ON THE INFLUENCE OF VARIOUS OENOLOGICAL TREATMENTS ON SOME WINES FROM COTNARI VINEYARD

STUDII ASUPRA INFLUENȚEI DIVERSELOR TRATAMENTE OENOLOGICE ASUPRA UNOR VINURI DIN PODGORIA COTNARI

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Abstract. *The specific sensorial profile of each wine is determined by its aroma compounds . The different different oenological treatments applied to the wines can strongly influence its nose and character. The famous grape variety Grasa de Cotnari cv. was experimentally processed by using 4 commercial yeasts, enzymes and nutrients. A GC analysis revealed the sensorial profile of each sample. All the treated samples show a change in concentrations of aroma compounds compared to the control.*

Key words: *Grasa de Cotnari, wine, gaschormatography, oenological treatments*

Rezumat. *Profilul senzorial specific fiecărui vin este determinat de compuși de aromă. Diversele tratamente oenologice aplicate vinurilor pot influența puternic naul și caracterul său. Celebrul soi de struguri Grasa de Cotnari cv. din podgoria Cotnari a fost vinifcat în mod experimental prin utilizarea 4 drojdii comerciale, enzime și substanțe nutritive. O analiză gazcromatografică a identificat profilului senzorial al fiecărei probe. Toate probele tratate prezintă o modificare a concentrațiilor de compuși aromatici în comparație cu martorul.*

Cuvinte cheie: *vin, Grasa de Cotnari, gazcormatografie, tratamente oenologice*

INTRODUCTION

Aromatic bouquet formation depends on many factors related to the culture conditions of the vine, the technology of production, wine fermentation conditions and its aging (Cotea, 1985 Colibaba, 2010). Some classes of compounds such as the many alcohols, aldehydes, esters, acids, terpenic compounds and other minor form, in general, the volatile fraction present in grapes and it occurs during the process of fermentation and maturation (Bayonove, 1993; Baumes, 1989).

Cotnari aromatic compounds in wines are topics of endless debate. Aromas of dried apricot , honey and nuts specific to Grasă de Cotnari wines (Cotea, 1985), notes of newly mown hay of Frâncușă or those of dewy vine in flower of Fetească albă, ending with the unmistakable scent of pear and basil of Tămâioasă românească are the result of miraculous combining of hundreds of

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compounds, the concentration of which is often very small, i.e. part per billion (mg $L^{-}1$).

A study involving the use of oenological products (selected yeasts, nutrients, enzymes for fining or enzymes for extraction), so often used in winemaking practices is extremely necessary to detect their influence on the sensory profile of wines produced .

MATERIAL AND METHODS

Grapes of the Grasă de Cotnari grape variety were used, harvested from Cotnari vineyard, in September 2011. The experimental samples were obtained by using the specific processes of aromatic wines; during maceration, different yeasts, enzymes and nutrients were used:

Gr M - fermentation appeared spontaneously, no extraction enzymes were used, but a maceration-fermentation of 8-12 hours was used (control sample);

The extraction enzyme Vulcazyme arome® (3 g/hL) was used for the next 4 samples.

Gr V1 - selected yeasts (Cross Evolution® - 20 g/hL) were added to the must;

Gr V2 - selected yeasts (Cross Evolution® - 20 g/hL) and nutrients (Fermoplus integrateur®, 35 g/hL) were added to the must;

Gr V3 - selected yeasts (Cross Evolution® - 20 g/hL) and nutrients (Fermoplus integrateur® , 35 g/hL) and limpidity enzymes (Zymoclaire CG® 1,5g/100 kg grapes) were added;

Gr V4 - selected yeasts (Zymaflore X 16® - 20 g/hL), nutrients (Fermoplus integrateur® -35 g/hL and limpidity enzymes (Zymoclaire CG® - 1,5 g/hL) were added.

GC analysis methods

The samples obtained through the process described above were subjected to the SPE extraction by means of LiChrolut EN/RP-18 (40-120 µm) 100 mg and RP (40-63 µm) 200 mg, 6mL Standard PP și LiChrolut EN (40-120 µm) 500 mg, 6 mL Standard PP

20 mL wine samples were passed through a C18 bed SPE cartridge. The adsorbent bed was first conditioned with 10 mL dichloromethane, 10 mL methanol and 10 mL aqueous solution of ethanol 13% v/v. The adsorbent bed was dried up by means of a 20-minute forced air jet. The compounds retained in the adsorbent layer were then recovered by percolating the bed with 2 mL dichloromethane. The resulting extract was sealed hermetically and then injected into the Shimadzu 5 GC-2010 gas-chromatograph coupled with a QP2010 Plus mass spectrometer.

1000 µL extract were injected splitless into the chromatographic pipe. The aroma compounds were determined by means of the NIST 08, Wiley 08 and SZTERP spectrum library.

GC-MS parameters:

1. Gas chromatographer:

- oven temperature: 35 °C;
- injector temperature: 220 °C;
- injection mode: splitless;
- carrier gas: He;
- column flow: 1 mL/min;
- oven temperature programme: 35 °C for 5 mins, rising at a rate of 4 °C minute up to 250 °C, where itstays for 13,25 mins;

-temperature of the ion source: 250 °C;

-interface temperature between gas-chromatographer and mass spectrometer: 250 °C;
-mass domain: 50–200 m/z;
-detector sensitivity: 1,05 V

2. Injection parameters:
-syringe of: 10 μL;
-prewashing of syringe with solvent: 3 times
-syringe volume filling: 5 μL;
-prewashing of syringe with sample: 2 times;
-post-washing with solving of the syringe: 5 times

RESULTS AND DISCUSSIONS

The profile of volatile acids varies according to the used treatment. Octanoic acid is found in the highest quantity, its concentration decreasing as different products are used. Isobutiric acid is not identified in samples GC M and GC V4. Isobutiric acid is found in natural form in the Ceratonia siliqua plant, while isovaleric acid is identified in many essential oils, as well as plants. Isovaleric acid has an odor reminiscent of cheese and sweat, while its esters have a nice smell, being used in the perfume industry.

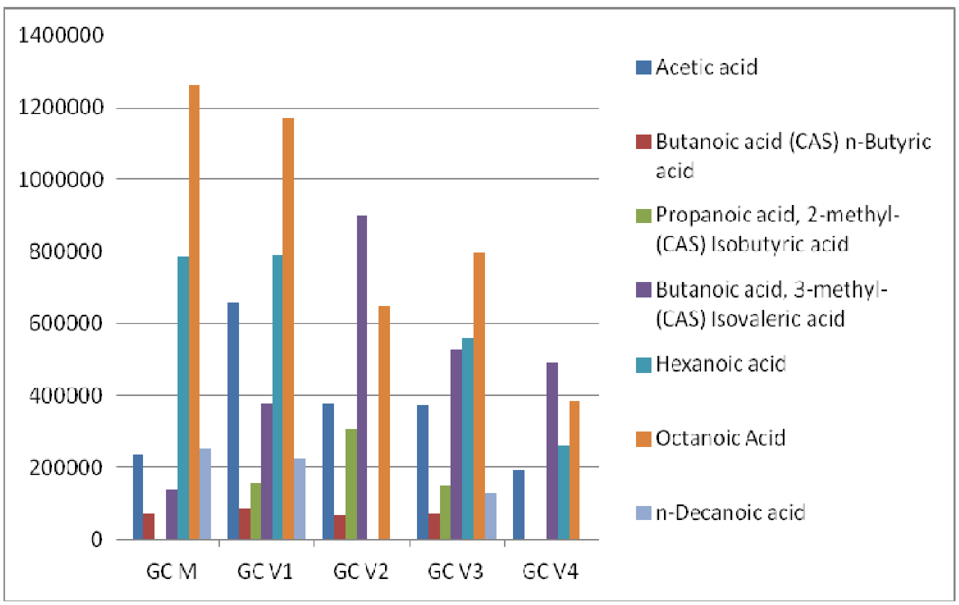

Fig. 1 - Graphical representation of acids identified in wines of Grasă de Cotnari obtained experimentally with different yeasts, nutrients and enzymes, in 2010

Wine esters are formed during fermentation and during wine maturation or aging. Killian E. and Ough C.S., 1979 accentuate the fact that a great influence on ester formation is the temperature during alcoholic fermentation. At temperatures lower than 10 °C, fruity esters are formed (isoamyl acetate, isobutyl acetate, ethylic butyrate and hexyl acetate). At temperatures between 15 and 20 °C, esters with a big molecule are formed (ethyl octanoate, ethyl decanoate etc) with waxy sweet smells.

Many esters, having a nice smell, are used as artificial essences, some with fruity aromas. For example, amyl acetate has a typical smell of bananas, ethyl butyrate has strong notes of pineapple, isoamyl butirate smells of pears. In general, all the esters of the isovaleric acid have similar odors similar to that of pears.

The number and the quantity for the identified esters in the Grasă de Cotnari samples (fig. 1) differ according to the products or the treatments used. Within the analysed samples, the maximum concentration is in the case of isoamyl acetate (banana oil), in all of the samples. Second in concentration, ethyl lactate, with creamy notes, similar to those of coconut, butter and fruits, is only identified in the samples GC M, GC V1, GC V2 and GC V3. In the variant GC V4, the commercial yeast strain Zymaflore X 16® was used, in comparison to the Cross Evolution® yeasts in the rest of the samples.

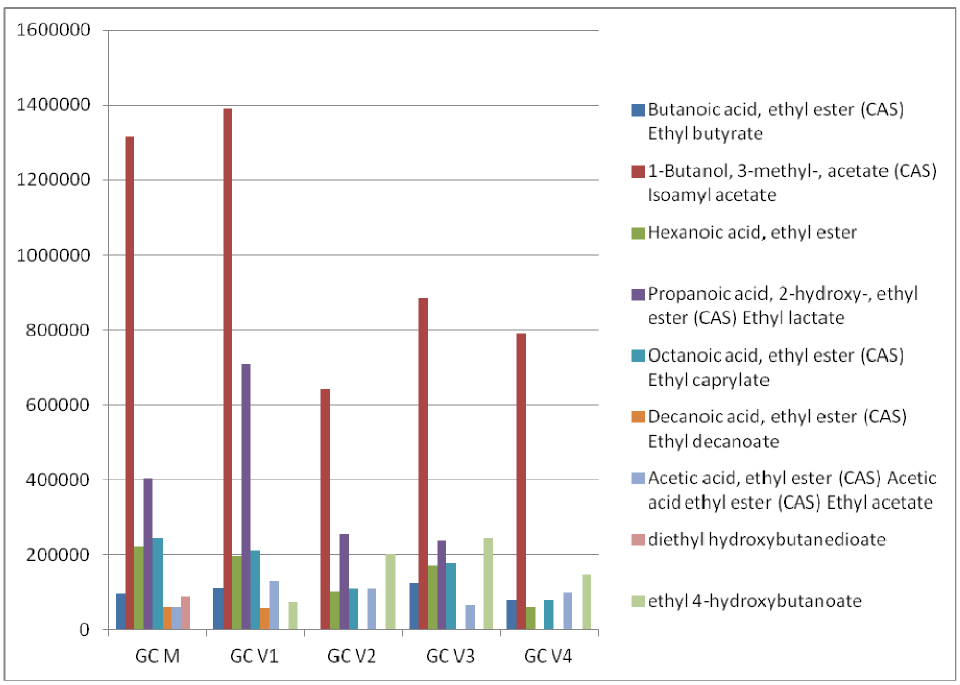

Fig. 2 - Graphical representation of esters identified in wines of Grasă de Cotnari obtained experimentally with different yeasts, nutrients and enzymes, in 2010

A large diversity of the esters is found in the analysed samples. Among the identified esters are: ethyl butirate, isoamyl acetate, hexanoic acid, ethyl ester, ethyl lactate, esters of fatty acids, ethyl acetate.

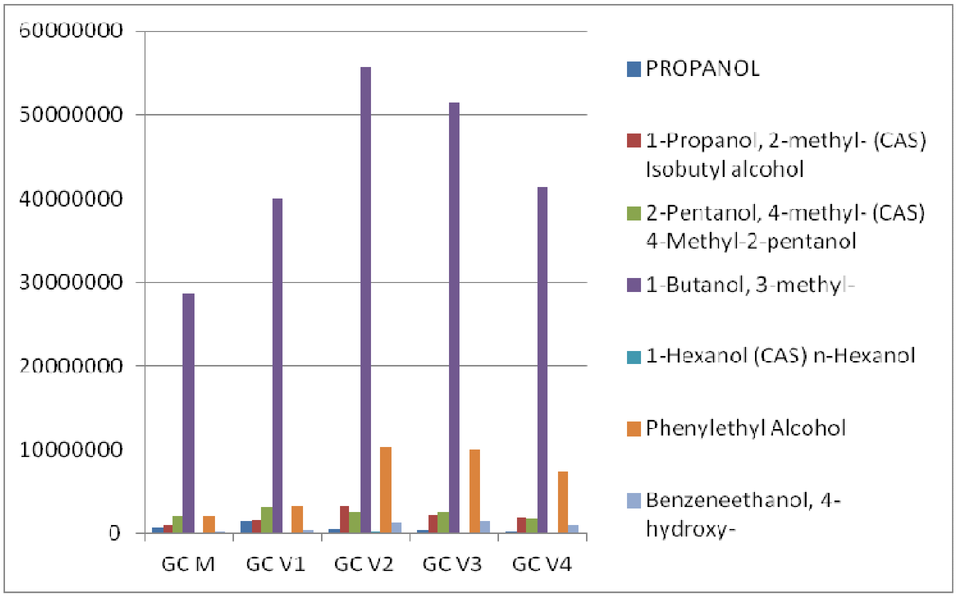

Fig. 3 - Graphical representation of alcohols identified in wines of Grasă de Cotnari obtained experimentally with different yeasts, nutrients and enzymes, in 2010

Superior monohydroxilic aliphatic alcohols are formed during desamination and decarboxilation of must's aminoacids. Superior alcohols belong to the aliphatic aromatic compounds, contributing directly to forming the aging bouquet of wine or indirectly, by forming esters.

The highest quantity of 1-butanol, 3-methyl is identified in CD V2, where the must was treated with selected yeasts (Cross Evolution® - 20 g/hL) and nutrients (Fermoplus integrateur®, 35 g/hL) were added to the must; phenylethylalcohol, with sweet rose notes, is found in its highest concentration, also in GC V2, similar to CG V3.

CONCLUSIONS

1. Esteres identified in Grasă de Cotnari differ according to the used oenological products and treatments.

2. Superior alcohols: 1-butanol,3-metil and phenylethylalcohol were identified in Grasă de Cotnari wines processed with selected yeasts (Cross Evolution® - 20 g/hL) and nutrients (Fermoplus integrateur®, 35 g/hL).

3. Isoamyl acetate, the ester found in its highest concentration in the analysed wine samples, is found at highest value in wines obtained from must where selected yeasts (Cross Evolution® - 20 g/hL) were added.

4. Taking into analysis the quantity and the diversity of the aroma compounds identified, the best results were obtained in GC V1 and GC V2, as well as the control sample.

***ACKNOWLEDGMENTS**: This study was realised and published within the research project POSCCE-A2-O2.1.2-2009-2 ID.653, code SMIS-CSNR 12596.*

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ASSESSMENT OF CINNAMIC ACIDS IN WINES FROM NATIVE GRAPES VARIETIES IN COPOU REGION

EVALUAREA UNOR ACIZI CINAMICI LA VINURILE UNOR SOIURI AUTOHTONE DIN CENTRUL VITICOL COPOU

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Abstract. *Evaluation of cinnamic acids is a crucial factor for evaluating the quality of wines from technological point of view and allows in most cases to characterize sensory characteristics felt by consumers at a fundamental level. In this case are presented some wines produced with grapes from the 2010 and 2011 harvest as following: Frâncuşă, Italian Riesling, Fetească regală, Fetească albă, Grasă de Cotnari, Tămâioasă românească and Băbească gri varieties. A optimization of the method of analysis was performed for p-coumaric, ferulic, caffeic and sinapic acids..*
Key words: *native grapes varieties, LC-DAD, cinnamic acids*

Rezumat. *Evaluarea acizilor fenolici este un factor tehnologic cu importanţă crucială pentru evaluarea calităţii vinurilor şi permite în cele mai multe cazuri caracterizarea caracteristicilor senzoriale resimţite de consumatori la nivel fundamental. În cazul de faţă sunt prezentate unele vinuri obţinute cu struguri din recolta anului 2010 și 2011 pentru varietăţile: Frâncuşă, Riesling italian, Fetească regală, Fetească albă, Grasă de Cotnari, Tămâioasă românească, Băbească gri. S-a realizat optimizarea metodei de analiză pentru acizii p-cumaric, ferulic, cafeic și sinapic.*
Cuvinte cheie: *soiuri autohtone, LC-DAD, acizi cinamici*

INTRODUCTION

Phenolic acids have great importance on reducing oxidation of the wine and indirectly on the biological properties of the wine which is recognized as a food supplement.

In the wine can be found about 300 organic acids (taking into account their conformers), some of them in traces, and of these only about 40 of them have known methods for rapid and clear determination which are useful to characterize at any time the evolution of the wine.

In the last half of the twentieth century, important discoveries in the field of chemical analysis were made. A number of methods have been improved, so called fast methods with shortened analysis time.

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Many of the methods were effective in separation, but the lack of structural information (conferred by IR, MS and NMR spectrometry, as mentioned - are economically inaccessible at the moment) may have serious consequences if missing. This shortcomings in assuring the information about compounds cannot determine the exact amount of substance (effect in an over -or under -evaluation of compound quantity).

MATERIAL AND METHOD

In this study we analysed the 2010 and 2011 wines of the varieties grown in the ampelographic collection didactical farm "V. Adamachi" belonging to the "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iaşi. Wines varieties: Frâncuşă, Italian Riesling, Fetească regală, Fetească albă, Grasă de Cotnari, Tămâioasă românească and Băbească gri were analyzed.

The harvesting was made on 18.09.2010 and 20.09.2011 when the grapes have reached technological maturity and were processed according to traditional technology of production of white and rosé/aromatic wines. A slight sulphitation with 30-40 mg/L was performed and after 2-3 hours must was decanted, then inoculated with enzymes Zymoclare HG® (2 g/hL) and selected yeasts Fermactiv AP® (30 g/hL) purchased from S.C. Sodinal S.R.L.

For the must Grasă de Cotnari, Tămâioasă românească and Băbească gri, 24 h maceration was performed prior to fermentation with Zymoclare HG® and Fermol Aromatic® followed by fermentation for 7-8 days. The wine produced was sterile filtered and bottled using a Enomatic Tenco device. To each bottle was added a dose of 180-200 mg/L of sulphur dioxide, before being sealed with polypropylene extruded corks in Mini TS closing machine.

Analyses were carried out from September 2010 to March 2012 at the Research Centre for Oenology of the Romanian Academy - Iasi Branch and at Laboratory of Oenology at the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iași.

After 1 year of bottling, wines were analysed to determine the concentration of specific phenolic acids. Samples were roughly and then sterile filtered through membrane filters 0.45 µm in vials, washed with 2 mL of the sample. At present there is no general method approved by the entire scientific community as a standard reference for phenolic acids. We have used as a starting point a method presented in Journal of Chromatography A by the team of Prof. Castellari (Castellari M. et al., 2008).

For the analysis of phenolic acids, samples were processed on a Shimadzu HPLC (Figure 1) consisting of: quaternary pump Shimadzu Prominence series LC-20AD with five-channel degasser DGU-20A5 Shimadzu Prominence series, autoinjector SIL-20AC Shimadzu Prominence series (injection volume: 10 µL, sample temperature 20 °C), column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M20A Shimadzu Prominence series (200-440 nm), fluorescence detector (Shimadzu FLD RF-10Axl) in order to achieve a double spectral certification for analyts, chromatographic system controller CBM-20A Shimadzu Prominence series PC connectivity via LAN.

Fig. 1 - HPLC system used in the determination of phenolic acids

RESULTS AND DISCUSSIONS

The method used involves the use of a monolithical type of column (or generically kwon as sponge columns) because the separation is not made on the silica particles with certain dimensions (as in conventional methods), but within a macrostructure like a block silane, having 2 μm micropors and mesopors of 13 μm. The advantage of this type of column is the performance of separation in linear flow at high speeds. Thus, for efficient separation in a short time (minutes) it has a high capacity for peaks, being able to separate about 80-200 compounds on column which can be analyzed with a lower flow rate at the expense of a relatively long analysis time (60-90 min separation). In the case of phenolic compounds in the literature almost all the methods recommended to use a gradient chromatographic separation by use of two or more eluents for separation whit relatively long time (Valle et al., 2004). From previous experiments we could observe that in some instances known separation columns and methods are not sufficiently effective in the separation of complex mixtures such as the case of wine (Maria del Alamo et al., 2004; Ortega et al., 2003, Silva et al., 2005).

Monolithic columns manufacturers declare in their presentations that these columns have currently the highest capacity of peaks per unit length, which can diminish the existing problems in the separation of wine compounds. Given that unlike conventional columns, coupling multiple columns does not lead to a sharp increase in backpressure on the system, we combined two such columns to increase the separation capacity of the method. The column system is composed of a pre-column Chromolith Guard Cartridge 5×4.6 mm and two Chromolith Performance RP-18 endcapped 100×4.6 mm columns manufactured by Merck.

Table 1 show the new optimized gradient reached using trifluoroacetic acid (TFA) as an eluent acidification of 1% MeOH (A channel) and 50% MeOH (B channel). Whit this method a much easier separation of tannins (420 nm) and anthocyanins (520 nm) in the same chromatogram was achieved. In this case and anthocyanin profile should be slightly better improved. Chromatographic eluents used in this case are acidified to a pH of 2.15-2.20 with TFA.

Table 1

The elution program for chromatographic separation method with TFA

time	module	action	value
0	Pump	%A	100 %
5	Pump	%A	100 %
15	Pump	%A	82 %
27	Pump	%A	75 %
30	Pump	%A	65 %
35	Pump	%A	65 %
60	Pump	%A	25 %
70	Pump	%A	0 %
80	Pump	%A	0 %
85	Pump	%A	100 %

In developing the method a fluorescent detector was used but it was abandoned because fluorescence detector was considered to be less useful because the separation condition are relative good and because the operating costs are much higher than for diode array detector (DAD). Peaks capacity (resolve capacity), with this method has come up to 120-180 in a period of 90 minutes as separation occurs (Fig. 2).

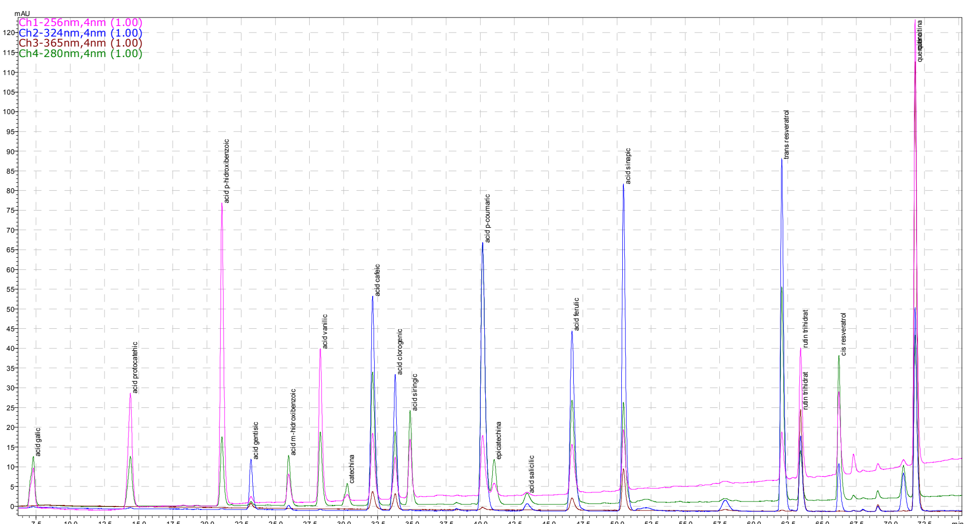

Fig. 2 - Chromatogram of standard mixture of phenolic acids and flavones with the TFA method

It was found in the evaluation of both identification methods DAD and FLD, the *m*-hydroxybenzoic acid and salicylic acid are found in all samples and an interfering substance is present in wines, that can give false quantitative results in most cases. This is why we can't present the values in the present study for these two compounds (they are reasons to discuss in total confidence these substances).

For the vast majority of the compounds linearity range is from 0.5-1 to about 200-300 mg/L, with the exception of gallic acid that has been standardized between 5 mg/L to 2 g/L. The limits of quantification (LQ) of the method is organized around a few tens of ppm (parts per millionth, 10^{-6}), and limits of detection (LOD) are even at 100 ppm or ppb (parts per billionth 10^{-9}) (in the case of sinapic acid).

Table 2

Values of cinnamic acids in wine samples from Copou viticultural centre, Iasi vineyard

Wine variety production year // mg/L	caffeic acid	*p*-coumaric acid	ferulic acid
Frâncuşă 2010	0.99	2.62	0.68
Italian Riesling 2010	1.18	2.99	0.59
Fetească regală 2010	1.06	2.28	0.94
Fetească albă 2010	0.91	2.61	0.95
Grasă de Cotnari 2010	5.18	3.52	0.87
Tămâioasă românească 2010	5.01	3.63	0.92
Băbească gri 2010	4.57	3.69	0.70
Frâncuşă 2011	0.29	SLD	SLD
Italian Riesling 2011	2.95	2.28	0.53
Fetească regală 2011	0.78	SLD	0.71
Fetească albă 2011	2.26	2.29	0.80
Grasă de Cotnari 2011	13.63	2.37	0.82
Tămâioasă românească 2011	46.84	4.27	0.94
Băbească gri 2011	1.43	2.80	0.72

Table 2 shows the values for some cinnamic acids in the analysed wines. In all analysed samples sinapic acid could not be identified (below 100 ppm). For this case an addition of acids were made, to standard values, but for the 200 ppb, it was not possible to identify in the samples. The samples were concentrated to a level of 10 ppb, but without any success.

In samples obtained by maceration fermentation, Grasă de Cotnari and Tămâioasă românească, have the highest values of caffeic acid, slightly higher in 2011 compared to 2010. For Băbească gri wine the 2011 values are lower than those in 2010.

p-coumaric acid can differentiate between the different grape varieties used for wines, more concretely in analysis the 2010 as technological options used. In 2011, *p*-coumaric acid in Fetească regală and Frâncuşă is below limits of identification. The Băbească gri wine has lower values for all three cinnamic acids, for the decreases in the concentrations of phenolic compounds of wine, a genetic or a climatic factor is responsible for.

A comparison between the two years, 2010 and 2011, ferulic acid values are very similar, with Frâncuşă and Riesling italian varieties among the lowest values. They do not have a high phenolic impact on the perception from these compounds.

CONCLUSIONS

1. Method for separation and analysis by liquid chromatography is optimized and allows analysis of five major cinnamic acids in wines with optimum resolution.
2. At wines taken in the analysis sinapic acid could not detect even in trace levels, not even at the theoretical limit of detection.
3. Wines made from Frâncuşă variety have the lowest values of phenolic acids among all wines analysed, so the wine does not has a potential for aromatic phenolics.
4. Varieties with premaceration before fermentation have elevated values compared to the classic method and for each year evolution of different acids is influenced by the nature of the raw material.

ACKNOWLEDGMENTS*: This study was realised and published within the research project POSCCE-A2-O2.1.2-2009-2 ID.653, code SMIS-CSNR 12596.*

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STUDIES ON THE INFLUENCE OF VARIOUS OENOLOGICAL TREATMENTS ON FETEASCĂ ALBĂ WINES FROM COTNARI VINEYARD

STUDII ASUPRA INFLUENȚEI DIVERSELOR TRATAMENTE OENOLOGICE ASUPRA VINURILOR DE FETEASCĂ ALBĂ DIN PODGORIA COTNARI

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Abstract. *The specific sensorial profile of each wine is determined by its aroma compounds. The different oenological treatments applied to the wines can strongly influence its nose and character. The very spread grape variety Fetească albă cv. was experimentally processed by using 4 commercial yeasts, enzymes and nutrients. A GC analysis revealed the sensorial profile of each sample. All the treated samples show a change in concentrations of aroma compounds compared to the control.*
Key words: *Fetească albă wine, gaschormatography, oenological treatments*

Rezumat. *Profilul senzorial specific fiecărui vin este determinat de compuși de aromă. Diversele tratamente oenologice aplicate vinurilor pot influența puternic naul și caracterul său. Mult răspânditul soi de struguri Fetesacă albă cv. din podgoria Cotnari a fost vinifcat în mod experimental prin utilizarea 4 drojdii comerciale, enzime și substanțe nutritive. O analiză gazcromatografică a identificat profilului senzorial al fiecărei probe. Toate probele tratate prezintă o modificare a concentrațiilor de compuși aromatici în comparație cu martorul.*
Cuvinte cheie: vin *Fetească albă, gazcormatografie, tratamente oenologice*

INTRODUCTION

The flavour profile of a wine is made up of three components: the primary aromas, characteristic of the variety of the grape used, secondary aromas, originating from yeasts during the fermentation process, and tertiary aromas (bouquet) developed during the maturing process of the wine in oak barrels or bottle (Etievant, 1991 and Piñeiro et al., 2006).

Cotnari aromatic compounds in wines are topics of endless debate. Aromas of dried apricot honey and nuts specific to Grasă de Cotnari wines (Cotea, 1985), notes of newly mown hay of Frâncușă or those of dewy vine in flower of Fetească albă, ending with the unmistakable scent of pear and basil of Tămâioasă românească are the result of miraculous combining of hundreds of compounds, the concentration of which is often very small, i.e. part per billion ($mg\ L^{-}1$).

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Aromatic bouquet formation depends on many factors related to the culture conditions of the vine, the technology of production, wine fermentation conditions and its aging (Cotea, 1985 Colibaba, 2010). Some classes of compounds such as the many alcohols, aldehydes, esters, acids, terpenic compounds and other minor form, in general, the volatile fraction present in grapes and it occurs during the process of fermentation and maturation (Bayonove, 1993; Baumes, 1989).

A study involving the use of oenological products (selected yeasts, nutrients, enzymes for fining or enzymes for extraction), so often used in winemaking practices is extremely necessary to detect their influence on the sensory profile of wines product.

MATERIAL AND METHOD

Grapes of the Fetească albă grape variety were used, harvested from Cotnari vineyard, in September 2011. The experimental samples were obtained by using the specific processes of aromatic wines; during maceration, different yeasts, enzymes and nutrients were used:

FA M - fermentation appeared spontaneously (control sample);

FA V1 - selected yeasts (Zymaflore X 16® - 20 g/hL) were added to the must;

FA V2 - selected yeasts (Zymaflore X 16® - 20 g/hL) and nutrients (Fermoplus integrateur®, 35 g/hL) were added to the must;

FA V3 - selected yeasts (Zymaflore X 16® - 20 g/hL), nutrients (Fermoplus integrateur®, 35 g/hL) and limpidity enzymes (Pecvine V® - 3 g/100 kg grapes) were added;

FA V4 - selected yeasts (IOC Expression® - 15 g/hL), nutrients (Fermoplus integrateur®, 35 g/hL) and limpidity enzymes (Pecvine V® - 3 g/100 kg grapes) were added.

GC analysis methods

The samples obtained through the process described above were subjected to the SPE extraction by means of LiChrolut EN/RP-18 (40-120 μm) 100 mg and RP (40-63 μm) 200 mg, 6mL Standard PP and LiChrolut EN (40-120 μm) 500 mg, 6 mL Standard PP

20 mL wine samples were passed through a C18 bed SPE cartridge. The adsorbent bed was first conditioned with 10 mL dichloromethane, 10 mL methanol and 10 mL aqueous solution of ethanol 13% v/v. The adsorbent bed was dried up by means of a 20-minute forced air jet. The compounds retained in the adsorbent layer were then recovered by percolating the bed with 2 mL dichloromethane. The resulting extract was sealed hermetically and then injected into the Shimadzu 5 GC-2010 gas-chromatograph coupled with a QP2010 Plus mass spectrometer.

1000 μL extract were injected splitless into the chromatographic pipe. The aroma compounds were determined by means of the NIST 08, Wiley 08 and SZTERP spectrum library.

GC-MS parameters:

1. Gas chromatographer:

- oven temperature: 35 °C;
- injector temperature: 220 °C;
- injection mode: splitless;
- carrier gas: He;
- column flow: 1 mL/min;

- oven temperature programme: 35 °C for 5 mins, rising at a rate of 4 °C minute up to 250 °C, where it stays for 13,25 mins;

-temperature of the ion source: 250 °C;

-interface temperature between gas-chromatographer and mass spectrometer: 250 °C;

-mass domain: 50–200 m/z;

-detector sensitivity: 1,05 V

2. Injection parameters:

-syringe of: 10 μL;

-prewashing of syringe with solvent: 3 times

-syringe volume filling: 5 μL;

-prewashing of syringe with sample: 2 times;

-post-washing with solving of the syringe: 5 times

RESULTS AND DISCUSSIONS

Fetească albă samples processed by adding different yeasts, nutrients and enzymes (Fig. 1), show a wide range of acids, which is influenced by the applied processing technology. The control sample, FA M, and FA V1, where selected yeasts were used (Zymaflore X 16 ®) at a dose of 20 g / hL and FA V2, where in the must selected yeasts were added (Zymaflore X 16 ®) at a dose of 20 g / hl as well as nutrients (Fermoplus integrateur ®) at a dose of 35 g / hL, show a small change in the acid number and the determined concentration in most of the fatty acids, which have the specific odor of manure, sweat, goats and livestock.

In samples FA V3, with selected yeasts (Zymaflore X-16 ®) at a dose of 20 g / hl, nutrient (Fermoplus integrateur ®) at a dose of 35 g / hL and fining enzyme (Pecvine V ®) and FA V4 (yeast selected (IOC Expression ®) at a dose of 15 g / hL, nutrient (Fermoplus integrateur ®) at a dose of 35 g / h and fining enzymes (Pecvine V ®), one can immediately observe a distinct change and expanded both the number of acids identified as well as their concentration. Thus, if the FA V3, acetic acid, butanoic acid and octanoic acid are no longer detected, while the amounts of isobutyric acid, isovaleric acid, hexanoic acid and decanoic acid decrease to almost half compared to the control sample. In FA V4, while butanoic acid is again identified, the amounts of acids do not change significantly.

Esters identified in samples of Fetească albă (fig. 2) have a pretty diverse palette, but varying concentrations depending on oenological products used. Isoamyl acetate, ester with a strong smell of bananas and found in highest concentration in all samples, has a maximum concentration in FA V2 variant produced by using selected yeast (Zymaflore X-16 ®) at a dose of 20 g / hL and nutrient (Fermoplus integrateur ®) at a dose of 35 g / hl.

Esters of fatty acids complement their flavors of tropical fruit and apple, pear and strawberry, to the identified sensory profile.

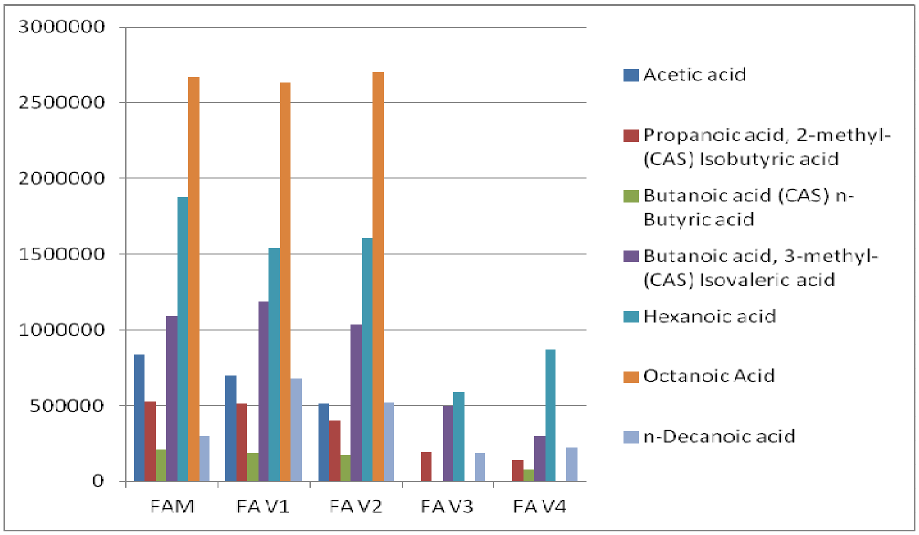

Fig. 1 - Graphical representation of acids identified in wines of Fetească albă obtained experimentally with different yeasts, nutrients and enzymes, in 2011

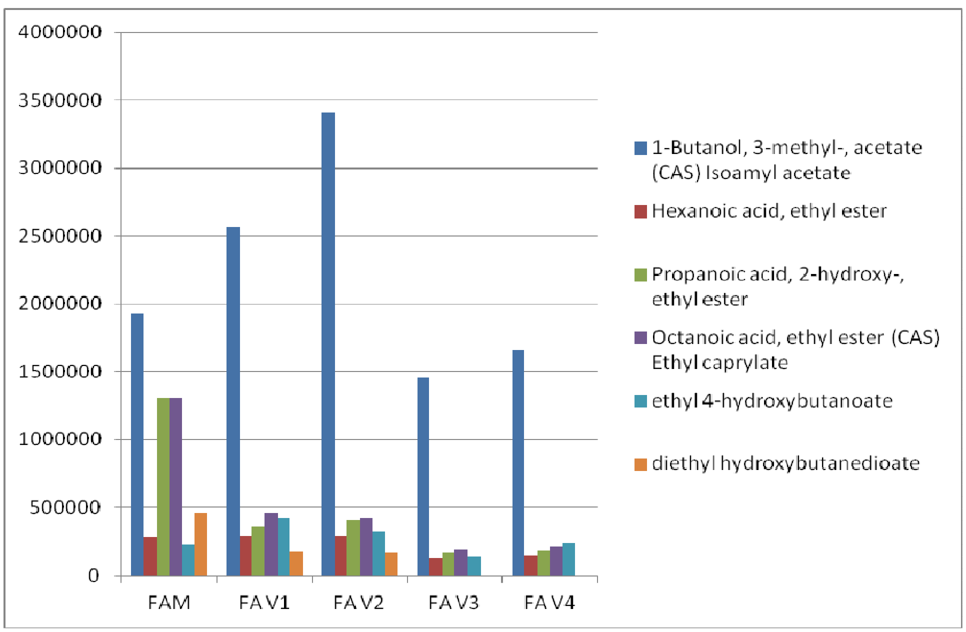

Fig. 2 - Graphical representation of esters identified in wines of Fetească albă obtained experimentally with different yeasts, nutrients and enzymes, in 2011

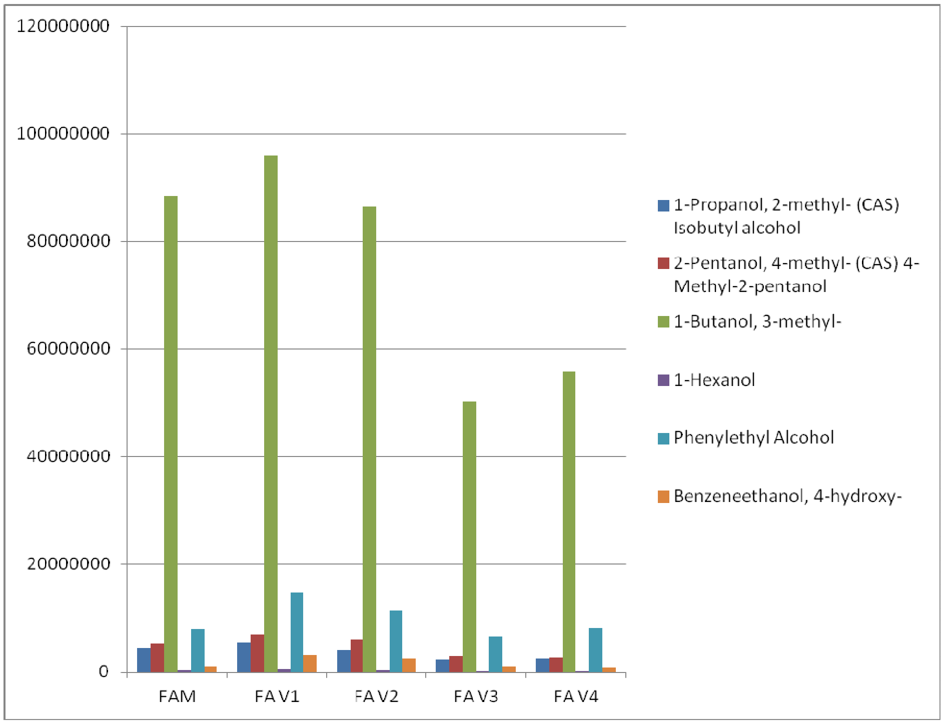

Fig. 3 - Graphical representation of superior alcohols identified in wines of Fetească albă obtained experimentally with different yeasts, nutrients and enzymes, in 2011

From the point of view of identified superior alcohols (fig. 3) 1-butanol 3-methyl occupies in the samples for the experimental Fetească albă a leading position in relation to the quantity, being about 9 times higher than the concentration of phenylethylalcohol, the following alcohol in quantitative terms. The range and diversity of higher alcohols differ depending on oenological products used. Phenylethylalcohol has a strong rose aroma and it is found to be in similar amounts in all samples, the highest , however, being recorded in FA V1.

CONCLUSIONS

1. Esters identified in Fetească albă wines obtained under different experimental conditions or treatments vary depending on the products used.

2. Superior alcohols 1-butanol 3-metil and phenylethylalcohol have been identified in the maximum amount of Feteasca alba FA V1 processed with selected yeasts (Zymaflore X 16®) at a dose of 20 g/hL.

3. Isoamyl acetate, found in the highest concentration from all the esters, is identified in the wines obtained from grape must where selected yeasts (Zymaflore X 16®) at a dose of 20 g / hl and nutrient (Fermoplus integrateur®) at a dose of 35 g / hL were added.

4. From the point of view of quantity and the number of the aroma compounds, the best results have been observed in samples FA M, FA V1 and FA V2.

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ANALYSIS OF HYDROXYCINNAMIC ACIDS CONTENT FROM WINES OF COTNARI AND TÂRGU BUJOR VINEYARDS

ANALIZA CONȚINUTULUI DE ACIZI HIDROXICINAMICI ALE VINURILOR DIN PODGORIILE COTNARI ȘI TÂRGU BUJOR

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Abstract. *This study determined and correlated the hydroxycinnamic acids profile of four wines from Cotnari wine area (Francuşă, Fetească albă, Grasă de Cotnari şi Tămâioasă românească), and four wines from Tîrgu Bujor wine region (Băbească gri, Fetească regală, Fetească albă, Italian Riesling) with the geographical origins and vinification year, using high performance liquid chromatography. Ferrulic acid was registered in high concentrations in wine samples from 2010 (grapes from Cotnari vineyard), especially in Grs I 10 (0.72 mg/L) and Tr I 10 (0.81 mg/L). In the same samples, harvested in 2011, the quantity of ferrulic acid was under the decetion limit. The highest chlorogenic acid concentration was registered in RI II 10 sample (4.70 mg/L) while the lowest, under the dection limit, was identified in Bg II 11 sample (Târgu Bujor).*

Key words: *wine, HPLC, hydroxycinnamic acids*

Rezumat. *Acest studiu a determinat și corelat profilul acizilor hidroxicinamici a patru vinuri din zona Cotnari (Frâncușă, Fetească albă, Grasă de Cotnari si Tămâioasă românească), și patru vinuri din regiunea viticolă Târgu Bujor (Băbească gri, Fetească regală, Fetească albă, Riesling italian), cu originea geografică și anul de vinificație, utilizând cromatografia de lichide de înaltă performanță. S-au identificat valori mari pentru acidul ferulic in probele vinificate in 2010 (struguri din zona Cotnari) mai ales Grs I 10 (0.72 mg/L) si Tr I 10 (0.81 mg/L) pe când în aceleași probe, dar vinificate în 2011 cantitatea de acid ferulic a fost sub limita de detecție. Cantitatea de acid clorogenic cea mai mare a fost determinata în proba RI II 10 (4.70 mg/L) iar cantitatea cea mai mica, sub limita de detecție în proba Bg II 11 (zona Târgu Bujor).*

Cuvinte cheie: *vin, HPLC, acizi hidroxicinamici*

INTRODUCTION

The composition of wines and associated organoleptic properties are greatly dependent on many factors such as: grape varieties, soil, climate (sunlight), ripening time, must – fermentation time, yeasts and enological microflora, wine – making technologies, wine ageing type and bottle stora (Harris et al., 2007; Huang et al., 2009).

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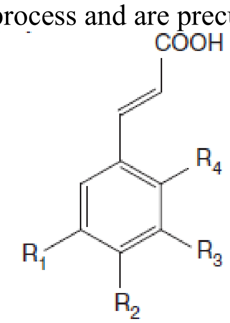
Wine polyphenolic compounds, on the basis of their carbon skeleton, are usually subdivided into two major classes: non-flavonoids and flavonoids (Moreno Arribas and Polo, 2009; Garrido et al., 2011).

The non-flavonoids include hydroxybenzoic and hydroxycinamic acids, stilbenes (mainly cis- and trans-resveratrol), phenolic alcohols, and aldehydes.

Characterized by a C6–C3 skeleton, hydroxycinamic acids are one of the most representative classes of phenolic acids found in both grapes and wine. Their derivatives are almost exclusively derived from p-coumaric acid, caffeic acid and ferulic acid, whereas sinapic acid is, in general, less encountered. They commonly occur esterified to sugars, various alcohols, or organic acids, mainly tartaric acid. Moreover, hydroxycinamic acids are also associated with the wine browning process and are precursors of volatile phenolic compounds (Castellari et al., 2002).



$R_1 = R_2 = R_3 = R_4 = H$: Cinnamic acid
$R_1 = R_2 = R_3 = H$; $R_4 = OH$: o-coumaric acid
$R_1 = OH$; $R_2 = R_3 = R_4 = H$: m-coumaric acid
$R_1 = R_3 = R_4 = H$; $R_2 = OH$: p-coumaric acid
$R_1 = R_4 = H$; $R_2 = OH$; $R_3 = OCH_3$: Ferulic acid

Fig.1- Chemical structures of the several hydroxycinnamic acids found in wines.

The aim of this research is to investigate the variation of hydroxycinnamic acids content in wines obtained from grapes of Moldova regions: Tîrgu Bujor and Cotnari with year of winemaking. The method used for determining the benzoic acids content is a HPLC method (OIV, 2012).

MATERIALS AND METHODS

In this study we have selected four wine grape varieties from Cotnari area: Frâncuşă (encoded Frş I 10 and Frș I 11), Fetească albă (Fa I 10 and Fa I 11), Grasă de Cotnari (Grs I 10 and Grs I 11) şi Tămâioasă românească (Tr I 10 and Tr I 11) It was selected four wines from Tîrgu Bujor wine region Băbească gri (encoded Bg II 10 and Bg II 11, Fetească regală (encoded Fr II 10 and Fr II 11), Fetească albă (encoded Fa II 10 and Fa II 11), Italian Riesling (encoded IR II 10 and IR II 11).

To simplify encoding was done for samples as following: variety - region - year (eg Frş I 10 - Frâncuşă Cotnari 2010).

The grapes were harvested at technological maturity from Târgu Bujor and Cotnari vineyard and processed by classical technology for obtaining white wines. Before fermentation a fining procedure was made to remove all rough organic parts and afterword's a co-inoculation whit enzymes (2 g/hL) and yeasts (30 g/hL) was done. After its alcoholic fermentation, the wine was racked at room temperature. After 7-8 days the wine was filtered and bottled. Immediately after taking a dose of sulphur dioxide by 40 mg/L per glass, they were closed semi with a Mini TS.

The experiments were done during September 2010 – March 2012, at the Oenology Laboratory of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iaşi. Each wine, after decarbonisation, was analysed: volatile acidity OIV-MA-AS313-02, total acidity OIV-MA-AS313-01, specific gravity at 20°C OIV-MA-AS2-01B, alcoholic strength by frequency oscillator OIV-MA-AS312-01A, reducing substances OIV-MA-AS311-01A, total dry matter and non-reducing substances OIV-MA-AS2-03B were done according to present standards (6) and specific literature (Ribereau Gayon et al., 2006).

Reagents and standard for HPLC: the standards solutions for HPLC analysis were supplied by Sigma and Alfa Aesar. By dissolving known amounts of the analytical-reagent grade standards in methanol the calibration solutions were prepared.

The wine phenolic compounds were carried out with high-performance liquid chromatograph (HPLC) Shimadzu equipped with two chromatographic columns Merck Chromolith Performance RP-18.

RESULTS AND DISCUSSIONS

Table 1 registers the content of hydroxycinnamic acids in wines obtained in Cotnari vinyard. The samples were processed in 2010 and 2011. In 2010, the hydroxycinnamic acids quantity is higher (the quantity of chlorogenic acid and p-coumaric acid). The quantity of sinapic acid was under the decetion limit in all wine samples analysed in 2010 and 2011. High values of ferullic acid in wine samples from 2010 are identified, especially in Grs I 10 (0.72 mg/L) and Tr I 10 (0.81 mg/L). In the samples from the same grape varieties but different harvest year, 2011, the ferullic acid was under the detection limit. The p-coumaric quantity was under the detection limit in the following samplese: Fa I 11, Grs I 11, Tr I 11, from 2011.

Table 1

Variation of hydroxycinnamic acids content in wine samples from Cotnari area vinified in 2010 and 2011 (mg/L)

Wine sample	*Clorogenic acid*	*Cafeic acid*	*p-cumaric acid*	*Ferulic acid*	*Sinapic acid*
Frş I 10	**5.44**	0.19	**3.12**	0.72	SLD
Fa I 10	**4.47**	5.66	**4.51**	1.07	SLD
Grs I 10	**5.44**	0.19	**3.12**	**0.72**	SLD
Tr I 10	1.84	2.09	**3.71**	**0.81**	SLD
Frş I 11	1.73	2.43	2.80	0.48	SLD
Fa I 11	1.66	0.20	SLD	0.82	SLD
Grs I 11	3.95	0.60	SLD	SLD	SLD
Tr I 11	4.11	**17.82**	SLD	SLD	SLD

Table 2 shows the hydroxycinnamic acids quantities in wine samples from Târgu Bujor in 2010 and 2011. The sinapic acid quantity was under the detection limit for all analysed samples.

The variation of the quantity of bensoic acids is can be randomly characterised by the harvest year and the compound. The chlorogenic acid was highest in RI II 10 sample (4.70 mg/L) while the lowest, under the detection limit, was in Bg II 11. Fa II 10 sample contains the the highest quantity of caffeic acid (11.40 mg/L).

Table 2

Variation of hydroxycinnamic acids content in wine samples from Târgu Bujor area vinified in 2010 and 2011

Wine sample	*acid clorogenic*	*acid cafeic*	*Acid p-cumaric*	*acid ferulic*	*acid sinapic*
RI II 10	**4.70**	3.94	**3.34**	0.48	SLD
Fr II 10	3.37	7.63	4.25	0.76	SLD
Fa II 10	2.48	**11.40**	2.29	1.72	SLD
Bg II 10	2.92	5.48	4.61	0.57	SLD
RI II 11	3.82	1.09	**SLD**	SLD	SLD
Fr II 11	3.42	8.76	4.45	1.14	SLD
Fa II 11	3.75	1.47	2.34	0.98	SLD
Bg II 11	**SLD**	**8.95**	4.77	**1.06**	SLD

CONCLUSIONS

1. The obtained wines are dry or demi-dry (DOC), whith an 11,5% alchoolic concentration.

2. High values for ferullic acid in wine samples from 2010 from Cotnari vineyard are registeres, especially Grs I 10 (0.72 mg/L) and Tr I 10 (0.81 mg/L). In the samples from 2011, the ferullic acid was under the detection limit.

3. The chlorogenic acid quantity was highest in RI II 10 sample (4.70 mg/L) and the lowest quantity, under the detection limit, was identified in Bg II 11 sample (from Târgu Bujor).

ACKNOWLEDGMENTS: *This study was realised and published within the research project POSCCE-A2-O2.1.2-2009-2 ID.653, code SMIS-CSNR 12596 and by the grant no. 5525 / 25.04.2013 of USAMV Iasi.*

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STUDIES ON AROMA COMPOUNDS OF TĂMÂIOASĂ ROMÂNEASCĂ WINE FROM COTNARI VINEYARD

STUDIUL COMPOZIŢIEI DE AROME DIN VINURILE DE TAMÂIOASĂ ROMÂNEASCĂ DIN PODGORIA IAŞI

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Abstract: *In this study, the must obtained in 2011 from Tămâioasă românească variety was subjected to a number of 9 prefermentative treatments with oxalic acid, lactic acid, succinic acid, sodium silicate, tannins, bentonite, graphen, chitosan and activated charcoal. The results showed that the acetic acid, hexyl ester has been identified only in the samples treated with succinic acid (V3), sodium silicate (V4) and graphen (V7). The amount of ethyl octanoate range between 18, 28 ppm (V8) and 236, 18 ppm (V2).The sensorial analysis of the wine samples demonstrated the influence the prefermentative treatments have on wines' aroma.*

Key words: *Tămâioasă românească, oxalic acid, chitosan, activated carbon, ester*

Rezumat*. În acest studiu, mustul obţinut din soiul Tămâioasă românească în anii de recoltă 2011, a fost supus unui numar de 9 tratamente prefermentative cu acid oxalic, acid lactic, acid succinic, silicat de sodiu, tanin, bentonită, grafen, chitosan şi cărbune activ. Rezultatele au arătat că acetic acid, hexyl ester a fost identificat numai în probele tratate cu acid succinic (V3), silicat de sodiu (V4) şi grafen (V7). Cantitatea de ethyl octanoate a variat de la 18, 28 ppm (V8) până la 236, 18 ppm (V2). Analiza senzorială a probelor demonstrează influența puternică a tratamentelor prefermentative aplicate.*

Cuvinte cheie: *Tămâioasă românească, acid oxalic, chitosan, cărbune activ, ester*

INTRODUCTION

Several studies have been performed on the effect of enological practices on the wine's composition (Losada et al., 2010; Puig-Deu et al., 1996; Villanõ et al., 2006). The increasing of the titrable acidity and the total acidity of wine can be achieved by lactic acid, oxalic or succinic acid addition. The exogenous tannins are frequently added to wines during the winemaking process to stabilize colour, to modify mouth-feel, to mask green characters, to increase polyphenolics and aromatic stability (Harbertson et al., 2011; Parker et al., 2007). Sodium silicate is used to clarify wines (***OIV, 2013).The use of bentonite as a clarifying agent means to prevent the formation of protein haze in wines. Treating must with bentonite is recommended for

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wines which are to be clarified shortly after the completion of alcoholic fermentation (Ribéreau-Gayon and Dubourdieu, 2006). Recently characterized as “the thinnest material of the universe” (Geim and MacDonald, 2007), graphen is the two-dimensional version of graphite consisting of a two-dimensional arrangement of carbon atoms disposed in a hexagonal grid. Graphene is the best known conductor of electricity and heat. Graphene presents a whole range of special properties, which gives it great potential for the practical production of new uses. Chitosan treatment can be an effective method to clarify the must and to prevent protein haze (Rao et al., 2010; Domingues et al., 2011, ***OIV, 2013). Another clarifying agent is activated charcoal, useful for correcting organoleptic issues of wine obtained from musts affected by fungi such as grey rot (*Botrytis cinerea*) or oidium (*Uncinula necator*), to eliminate, possible contaminants, to correct the colour from white musts derived from the white juice of red grapes, from very yellow musts derived from white grape varieties and from oxidized musts (Ribéreau-Gayon and Glories, 2006).

The objective of the present study is to evaluate the influence of different enological treatments on the esters content and on the aromatic and taste profile of of Tămâioasă românească wine.

MATERIAL AND METHOD

Grape samples and winemaking

Tămâioasă românească grapes from Cotnari vineyard were harvested in 2011 at optimal maturity. The grapes were destemmed and crushed, and each must obtained was transferred in glass containers. Before fermentation, nine treatments were applied to the must : oxalic acid - 0,6 g/L (V1), lactic acid – 3 g/L (V2), succinic acid – 2 g/L (V3), sodium silicate – 2,4 g/L (V4), tannins – 0,05 g/L (V5), bentonite – 1 g/L (V6), graphen – 1 g/L (V7), chitosan – 1 g/L (V8) and activated charcoal – 1 g/L (V9). The must were stirred to ensure a homogenous fermentation. After alcoholic fermentation, wines were filtered using a filtration-filling device-Tenco Enomatic® followed by sulfur dioxide addition (40 mg / L) to preserve wine from microbiological damage. Bottling was done with a semi-automatic device. After six months of storage the wines were analysed. Also, for each grape variety, a control sample (V) was obtained without prefermentative treatment.

Regents for pre-fermentative treatments: tannin (Taniblanc® - from AEB Spa, Italy), bentonite (Bentonita Clarit PLV 45 – Sodinal, France). Oxalic acid, lactic acid, succinic acid, sodium silicate, graphen, chitosan and activated charcoal were purchased from Sigma-Aldrich, Germany.

Analysis of esters

The obtained extract was injected into a Shimadzu GC coupled with a QP2010 Plus mass-spectrometer. 1000 μL extract are injected into a

Supelco SLB 5 ms GC column, of 15 m length, column oven temperature 30 ^{o}C, injection temperature 250 ^{o}C, in splitless mode, initial temperature 30 ^{o}C for 1 minute, then if grows at a rate of 8 ^{o}C until 240 ^{o}C where it stays for 2.75 minutes. The carrier gas was Helium, column flow 0.75 mL/min, ion source temperature 250 ^{o}C, interface temperature 250 ^{o}C, detector voltage 0.9 kV, The aroma compounds were determined by means of the NIST 08, Wiley 08 and SZTERP spectrum library. The program lasts for 30 minutes.

Sensory analysis of wines

The wines were tasted by a group of 12 specialised wine tasters. Tulip glasses of 30 mL, accordimg to ISO requirements, were used.

The wines were analysed taking into consideration the visual, olphactive, tactile and taste. Each descriptor was graded from 1 to 5. A tasting sheet was used, where a series of visual, ophactory and taste descriptors were underlined. The grades were centralised and the mean of each descriptor was calculated.

RESULTS AND DISCUSSIONS

The esters that have been identified in Tămâioasă românească wines obtained through different prefermentative treatments are represented in table 1. Results shows that 1-Butanol, 3-methyl-, acetate is present in small quantity in the sample that was not subjected to prefermentativ tratments (M). Higher quantities were found in wines treated with chitosan (V8) and actvated carbon (V9). The addition of oxalic acid (V1) and activated carbon (V9) in wine contributed at increasing the amount of hexanoic acid, ethyl ester. The acetic acid, hexyl ester has been identified only in the samples treated with succinic acid (V3), sodium silicate (V4) and graphen (V9). The amount of ethyl octanoate range between 18, 28 ppm (V8) and 236, 18 ppm (V2) and the smallest quantity of decanoic acid, ethyl ester was found in sample treated with activated carbon.

Table 1

Contents in esters (ppm)

Esters	M	V1	V2	V3	V4	V5	V6	V7	V8	V9
1-Butanol, 3-methyl-, acetate	3,15	32,54	13,81	45,19	39,67	15,10	35,69	51,96	49,58	48,76
Hexanoic acid, ethyl ester	18,24	40,24	33,16	28,19	19,63	20,37	34,00	28,58	24,61	43,35
Acetic acid, hexyl ester	-	-	-	0,29	0,33	-	-	0,76	-	-
Ethyl octanoate	160,47	225,25	236,18	127,64	105,90	111,39	218,18	164,62	18,28	118,12
Decanoic acid, ethyl ester	25,07	51,47	63,06	32,93	28,88	33,95	47,68	35,76	58,88	10,32

The results of the organoleptic analysis of Tămâioasă românească wines can be seen in figures 1, 2 and 3.

The 2011 wines have a more pronounced vegetal aroma in the control sample (M) and in the sample treated with graphen (V7), a more pronounced moneral hue in the wine that was clarified with activated charcoal (V9), while a citric note is stronger in the sample where bentonite was used as fining agent (V6). A ripe fruits note were accentueated by the oxalic acid treatment (V1), sodium sillicate (V4) and tannin (V5). In wines that were fined with bentonite and activated charcoal, a more intense whif of exotic fruits was perceived.

Dry fruits were present in oxalic acid treated wines (V1) as well as activated charcoal (V9). The strongest sensation of green fruits was registered in the control sample and the sample fined with activated charcoal. This treatment did not suppres the sensorial profile of 2011 Tămâioasă românească. Moreover, the flwery, fruity and dry grass notes were more powerful. The same intense flowery note was identified in wines treated with bentonite (V6), graphen (V7) and chitosan (V8).

The wines that were fined with charcoal have a hig hacidity and a persistent taste. A high acidity was found in samples treated with lactic acid (V2) and succinic acid (V3). A slightly phenolic character was noticed in samples treated with sodium silicate (V4) and bentonite (V6). Evaluating onctosity and texture, the wines that were obtained through prefermentative treatments with graphen (V7) and chitosan (V8) were graded as best.

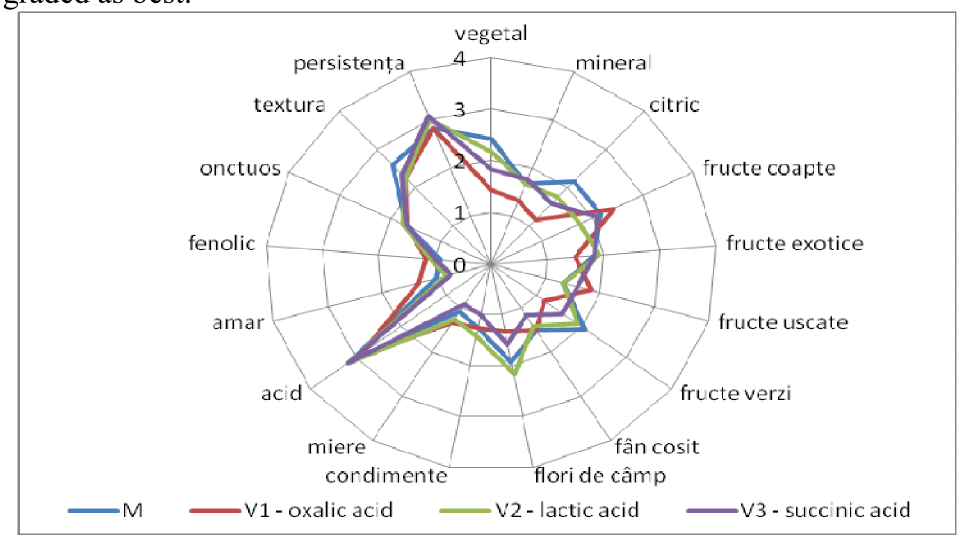

Fig. 1- *Aromatic profile of Tămâioasă românească wines obtained by oxalic acid, lactic acid and succinic acid addition*

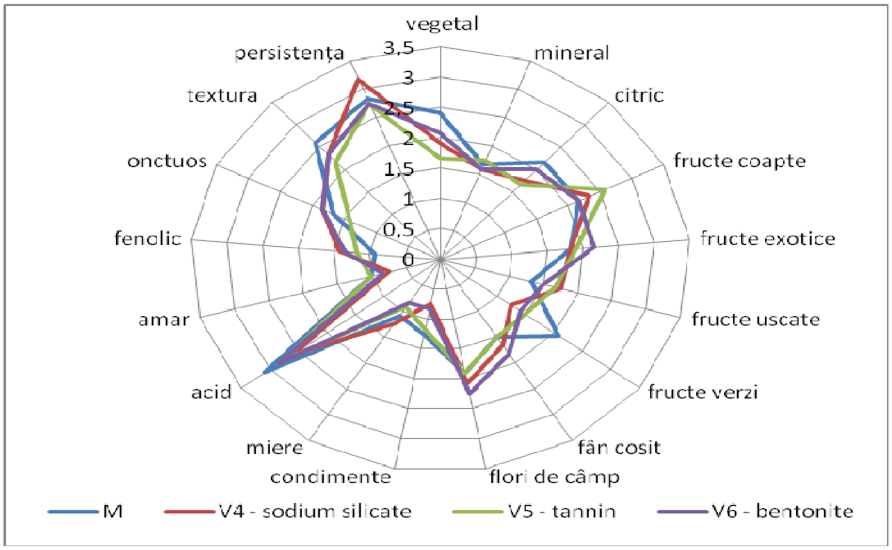

Fig. 2 - *Aromatic profile of Tămâioasă românească wines treated with sodium silicate, tannin and bentonite*

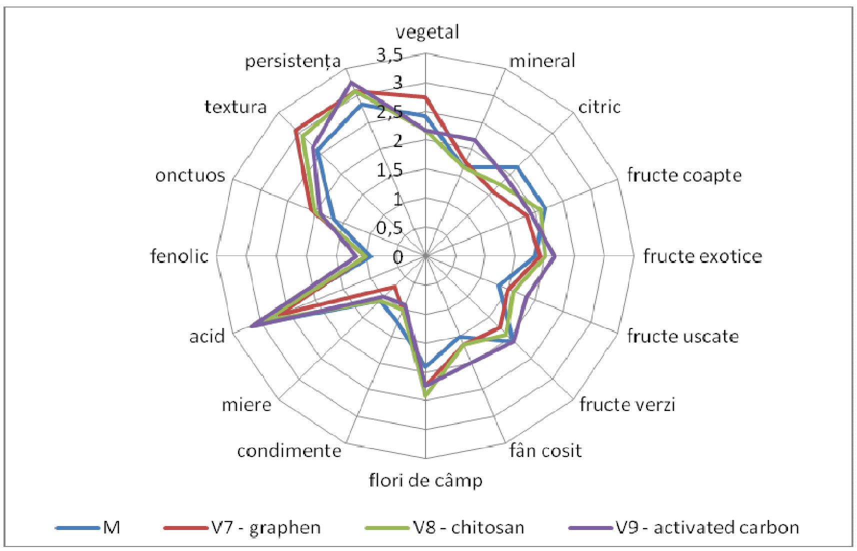

Fig. 3 - *Aromatic profile of Tămâioasă românească wines treated with graphen, chitosan, activated charcoal*

CONCLUSIONS

The aroma compounds identified in Tămâioasă românească wine samples are influenced by the applied treatments.

The acetic acid, hexyl ester has been identified only in the samples treated with succinic acid (V3), sodium silicate (V4) and graphen (V9).

The aromatic profile of Tămâioasă românească wines was influenced by the treatements that were appllied during the prefermentative period, resulting in intens notes of ripe fruits in the wine samples treated with oxalic acid, sodium sillicate and tannin.

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QUANTITATIVE STUDY OF BENZOIC ACIDS IN WINES FROM NATIVE VARIETIES FROM AMPELOGRAPHICAL COLLECTION OF USAMV IAȘI

STUDIU CANTITATIV AL ACIZILOR BENZOICI LA VINURILE UNOR SOIURI AUTOHTONE DIN COLECȚIA AMPELOGRAFICĂ USAMV IAȘI

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Abstract. *Evaluation of cinnamic acids is a crucial factor for evaluating the quality of wines from technological point of view and allows in most cases to characterize sensory characteristics felt by consumers at a fundamental level. In this case, some wines produced with grapes from the 2010 and 2011 harvest are presented as followes: Frâncușă, Riesling italian, Fetească regală, Fetească albă, Grasă de Cotnari, Tămâioasă românească and Băbească gri varieties. A optimization of the analysis method was performed for protocatechic, p-hydroxybenzoic, vanillic, gallic, syringic, gentisic, m-hydroxybenzoic and salicylic acids.*

Key words: *native grapes varieties, LC-DAD, benzoic acids*

Rezumat. *Evaluarea acizilor fenolici este un factor tehnologic cu importanţă crucială pentru evaluarea calităţii vinurilor şi permite în cele mai multe cazuri caracterizarea caracteristicilor senzoriale resimţite de consumatori la nivel fundamental. În cazul de faţă sunt prezentate unele vinuri obţinute cu struguri din recolta anului 2010 și 2011 pentru varietăţile: Frâncuşă, Riesling italian, Fetească regală, Fetească albă, Grasă de Cotnari, Tămâioasă românească, Băbească gri. S-a realizat optimizarea metodei de analiză pentru acid protocatehic, acid p-hidroxibenzoic, acid vanilic, acid galic, acid siringic, acid gentisic, acid m-hidroxibenzoic şi acid salicilic.*

Cuvinte cheie: *soiuri autohtone, LC-DAD, acizi benzoici*

INTRODUCTION

Phenolic acids manifeste some antiseptic proprieties. In the concentrations that are present naturally they may slightly change yeast and bacteria activity, whose enzymatic equipment is less complete than that of yeasts. For this reason, phenolic acids may be considered as an antiseptic in wine practice (Cotea D. V. et. al., 2009).

Grapes and wine contain benzoic and cinnamic acids. Concentrations are on the order of 100–200 mg/l in red wine and 10–20 mg/l in white wine. In grapes, they are mainly present as glycoside combinations, from which they are

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released by acid hydrolysis, and esters (gallic and ellagic tannins), from which they are released by alkaline hydrolysis. They are, however, precursors of the volatile phenols produced by the action of certain microorganisms (yeasts of the Brettanomyces genus and bacteria) (Ribereau-Gayon et. al., 2006).

MATERIAL AND METHOD

In this study we analysed wines from 2010 and 2011: Frâncuşă, Riesling italian, Fetească regală, Fetească albă, Grasă de Cotnari, Tămâioasă românească and Băbească gri varieties. The harvesting was made on 18.09.2010 and 20.09.2011 when the grapes have reached technological maturity and were processed according to traditional technology of production of white and rosé/aromatic wines. A slight sulphitation with 30-40 mg/L was performed and after 2-3 hours must was decanted, then inoculated with enzymes Zymoclare HG® (2 g/hL) and selected yeasts Fermactiv AP® (30 g/hL) purchased from S.C. Sodinal S.R.L. For the must Grasă de Cotnari, Tămâioasă românească and Băbească gri, 24 h maceration was performed prior to fermentation with Zymoclare HG® and Fermol Aromatic® followed by fermentation for 7-8 days. The wine produced was sterile filtered and bottled using a Enomatic Tenco device. To each bottle was added a dose of 180-200 mg/L of sulphur dioxide, before being sealed with polypropylene extruded corks in Mini TS closing machine.

Analyses were carried out from September 2010 to March 2012 at the Research Centre for Oenology of the Romanian Academy - Iasi Branch and at Laboratory of Oenology at the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iași. After 1 year of bottling, wines were analysed to determine the concentration of specific phenolic acids. Samples were roughly and then sterile filtered through membrane filters 0.45 µm in vials, washed with 2 mL of the sample. We have used as a platform we developed a method presented in Journal of Chromatography A (Castellari M. et al., 2008). For the analysis of phenolic acids, samples were processed on a Shimadzu HPLC consisting of: quaternary pump Shimadzu Prominence series LC-20AD with five-channel degasser DGU-20A5 Shimadzu Prominence series, autoinjector SIL-20AC Shimadzu Prominence series (injection volume: 10 µL, sample temperature 20 °C), column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M20A Shimadzu Prominence series (200-440 nm), fluorescence detector (Shimadzu FLD RF-10Axl) in order to achieve a double spectral certification for analyts, chromatographic system controller CBM-20A Shimadzu Prominence series PC connectivity via LAN. We optimized the gradient using trifluoroacetic acid (TFA) as an eluent acidification of 1% MeOH (A channel) and 50% MeOH (B channel). The column system is composed of a pre-column Chromolith Guard Cartridge 5×4.6 mm and two Chromolith Performance RP-18 endcapped 100×4.6 mm columns manufactured by Merck. It was found in the evaluation of both identification methods DAD and FLD, the *m*-hydroxybenzoic acid and salicylic acid are in samples and a interfering substance is present in wines, that can give false quantitative results in most cases. This is why we can't present the values in the present study for these two compounds (there are reason to discuss these substances in total confidence).

RESULTS AND DISCUSSIONS

Protocatechic acid shows high values for most wines. In 2010, the distribution of values is in a logical order with the lowest value in Frâncuşă wine and highest in Băbească gri, slightly colored variety which presents high values due to maceration. In 2011 we have low values in Fetească regală wine while aromatic wine Grasă de Cotnari şi Tâmâioasă românească have values somewhat lower than the rest (Table 1).

Table 1

Values of some bensoic acids in wine samples from Copou

Wine variety production year // mg/L	protocatechiuc acid	p-hydroxybensoic acid	vanillic acid
Frâncuşă 2010	7.95	0.96	0.56
Riesling Italian 2010	7.98	0.48	0.15
Fetească regală 2010	8.04	0.56	0.17
Fetească albă 2010	8.18	0.57	0.22
Grasă de Cotnari 2010	8.14	0.65	0.16
Tămâioasă românească 2010	8.12	0.71	0.21
Băbească gri 2010	8.25	0.95	0.44
Frâncuşă 2011	7.80	0.91	0.58
Riesling Italian 2011	7.82	0.36	0.90
Fetească regală 2011	7.42	0.34	0.23
Fetească albă 2011	8.28	0.51	0.31
Grasă de Cotnari 2011	7.91	0.97	0.32
Tămâioasă românească 2011	7.89	1.22	0.20
Băbească gri 2011	8.39	0.83	0.49

p-hydroxybensoic acid is present in all wines with values that are not much influenced by the year of production. In case of Fetească varieties in both years lower values were recorded while in aromatic and rose varieties they have higher values. It is interesting that the wine from Frâncuşă has 2-3 times higher values than Riesling italian.

In the case of vanillic acid again we see higher values in Frâncuşă comparable to those of Băbească gri. At aromatic wines in both years of production, vanillic acid is present in low quantities by contrast whit the other products.

Table 2

Values of some bensoic acids in wine samples from Copou

Wine variety production year // mg/L	gallic acid	syringic acid	gentisic acid
Frâncuşă 2010	2.62	1.78	145.51
Italian Riesling 2010	0.90	2.51	90.65
Fetească regală 2010	1.64	1.42	109.40
Fetească albă 2010	1.31	1.51	79.67
Grasă de Cotnari 2010	1.68	12.92	87.29
Tămâioasă românească 2010	1.07	12.51	70.51
Băbească gri 2010	2.00	13.50	38.72
Frâncuşă 2011	1.27	19.19	150.53
Riesling Italian 2011	0.71	12.61	99.83
Fetească regală 2011	1.32	13.90	91.73
Fetească albă 2011	1.77	2.88	30.73
Grasă de Cotnari 2011	1.01	12.75	0.38
Tămâioasă românească 2011	1.02	11.77	0.39
Băbească gri 2011	4.04	18.03	80.97

In table 2, gallic acid is present in Băbească gri and has normal high values for gray type grape variety. Surprisingly, and in this case, even if the Francuşă wine does not have high concentrations of alcohol (Niculaua M. et. al., 2013) the content of gallic acid are significant, in contrast again to smallest values for the Riesling italian.

For gentisic acid, as in case of syringic acids, we have unexpectedly high values comparable to red wines made from black grapes. There is however clear differences between the two years at flavoured wines varieties.

CONCLUSIONS

1. The method for separation and analysis in liquid chromatography is optimized and allows the analysis of 8 important bensoic acids from wines with optimum resolution at the level of chemical standards, but with some there were some problems in the case of *m*-hydroxybenzoic and salicylic acids.
2. The wines analyzed had relatively normal protocatechiuc, p-hydroxybensoic and gallic acids values whether it was before fermentation technology. It may be observed that the wines do not present phenol related acidity, aggressive or green tannins.
3. Despite the fact that we made a fining and prefermentation stage at some varieties the resulted wines had high amounts of syringic acid in those varieties where the prefermentation stage was not applied in 2011 and normally to the ones where maceration was done before fermentation in 2010. It may be observed that the year of harvesting is a factor that influenced the course of fermentation.
4. For gentisic acid, as in case to syringic acids, we have unexpectedly high values comparable to red wines made from black grapes.

***ACKNOWLEDGMENTS**: This study was realised and published within the research project POSCCE-A2-O2.1.2-2009-2 ID.653, code SMIS-CSNR 12596.*

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OBSERVATION ON SOME SOIL PHYSICAL PROPERTIES IN COTNARI VINEYARD

OBSERVAȚII ASUPPRA UNOR PROPRIETĂȚI FIZICE ALE SOLULUI DIN PODGORIA COTNARI

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Abstract. *Soil compaction is one of the major causes of soil degradation in modern agriculture and forestry. Neither runoff nor soil erosion were measured in the field, but the proportions of water-stable aggregates were determined as a relevant soil erodibility indicator. The highest percentage (80.10%) of* WSA_{1-2mm} *was recorded on 30-40 cm depth fallowed by 20-30 cm laye.*
Key words: *soil degradation, penetration resistance, water stable aggregates, bulk density*

Rezumat. *Compactarea solului este una dintre cauzele majore ale degradării solurilor în agricultura modernă. În câmp nu s-a determinat eroziunea propriu-zisă a solului dar s-a cuantificat stabilitatea hidrică a agregatelor de sol, un indicator foarte relevant al erodabilității. Cel mai mare procent la macroagregatelor hidrostabile s-a regăsit la 30-40 cm adâncime (80.10%) urmat de orizontul 20-30 cm*
Cuvinte cheie: *vin, degradare solului, rezistența la penetrare, stabilitatea hidrică, densitatea aparentă.*

INTRODUCTION

Viticulture is an important economic activity in Cotnari area. A large part of the territory is devoted to this crop. According to the latest data from the International Organisation of Vine and Wine (OIV, 2009), there were just over 7.8 million ha of vineyards worldwide in 2008.

Soil in vineyards is subject to frequent tractor traffic associated with soil tillage, the application of chemicals and grape harvesting. In highly mechanized viticulture, the number of tractor passes per year can be up to 22 in traditionally cultivated and 20% less in grass covered vineyards (Lisa et al., 1995).

Very little research has been done to investigate soil compaction effects in vineyards. Few papers showed (Ferrero et al., 2001; Van Dijck and van Asch, 2002) that long-term traffic in vineyards results in topsoil and subsoil compaction below the frequent tillage depth. Van Dijck and van Asch (2002) revealed that subsoil compaction in a vineyard is mostly attributed to wheel load.

Soil compaction is one of the major causes of soil degradation in modern agriculture and forestry. The overuse of machinery has been identified as the main reason contributing to soil compaction. Due to its persistence, subsoil compaction can be considered as a long-term degradation, although compaction also concerns

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surface layers. Compaction affects soil physical fertility adversely, in particular by impeding the storage and supply of water and nutrients. This leads to decreased porosity, increased soil strength and hence soil resistance to root penetration and plant emergence, and decreased soil water infiltration and holding capacity. These adverse effects also reduce fertilization efficiency and crop yields, increase waterlogging, runoff and soil erosion with undesirable environmental problems (Soane and van Ouwerkerk, 1994). Thus, knowing the changes in soil compaction with changes in water content and bulk density is essential when planning farm operations at appropriate water contents (Arvidsson et al., 2003), or when decreasing soil bulk density by increasing its organic matter content through the retention of crop and pasture residues or appropriate soil tillage (Hamza and Anderson, 2005).

Poor structure resulting from soil compaction by tractor traffic in vineyards has been shown to reduce root development and yields of grapes (Louw and Benni, 1991; Van Huyssteen, 1988). Soil compaction has been described as the most serious environmental problem caused by conventional agriculture because, it not only affect crop productivity, but also the workability and sustainability of the soil (McGarry, 2001).

MATERIAL AND METHOD

The plots from Cotnari vineyard are mostly located on slopes with gravelly soils, where surface crusts cause downslope rills and colluvial deposits and increased runoff leads to fertilizer and pesticide loss to surface waters.

The investigations were done in the Cotnari vineyard, situated in a climatic transition zone with cool climate elements in the Suceava Plateau and more continental in the Moldova Plain, on a Aric Haplic Chernozem (WRB-SR, 2006) with secondary calcium carbonate accumulation in the upper part of the soil, as a results of climatic changes (rising the temperatures and less precipitation), with a clay texture.

The following varieties are planted: *Grasa de Cotnari, Feteasca alba, Francusa and Tamaioasa romaneasca.* The quality of the grapes and wine depends on natural factors such as climate, soils, tillage techniques that attempt to control the hydrological functioning of soils and to maintain their fertility

The relief of Cotnari vineyard is very fragmented, with a general orientation to S-E, with some famous parts: Catalina Hill (395 m), Stanca Hill (360 m), Voda's Hill (347 m), Piciorul Racului (337 m), Liteanca (330 m). The average slope does not exceed 20°. The geological substrate is „Sarmatiene", within which are two different stories: one in the lower floor whose altutide is around 250-260 m, composed of marle and clay and an upper floor consisting of limestone, marle, sand oolithe, sandstone, and fossile-rich elements (Rotaru Liliana et al., 2010).

Observations were conducted at Rotila Farm which belongs to SC Cotnari SA, 233 m above sea level, 3-4% slope and the ground water level at 15 m depth. A soil profile was dig (Figure 1) before the tillage treatments and soil samples were collected in every layer to analyze the most relevant properties to their soil types. The pit was dug perpendicularly to the tillage direction. It was used both for bulk density measurements and for morphological characterisation. On the soil profile (0-100 cm) the pH is weak-moderately alkaline (7.22-8.5) and the content of organic matter range form 1.62 to 2.16%.

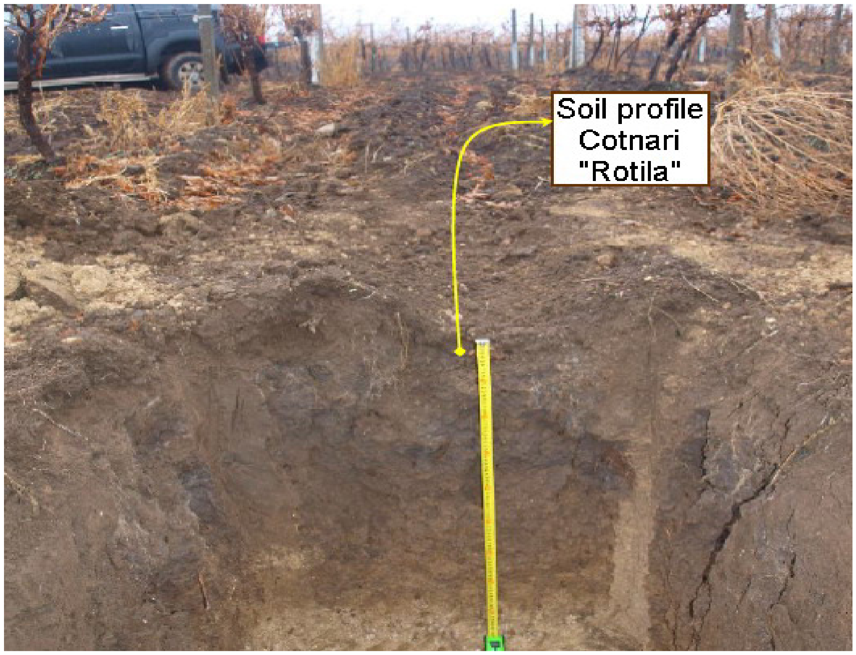

Fig. 1 - Profile no. 1 – Cotnari Rotila Farm, 47º57’46” N latitude, 26º56’2” E longitude (16th December, 2011)

The average annual temperature reaches approximately 9.1-9.3°C; the amount of active temperature (°C>10) exceeds 3200°C each year, sunshine hours are more than 2100 and 340-390 mm rainfall/growing season (Barbu et al., 2002). In the last decade it can be seen that in general the temperature regime increased and the rainfall was reduced.

The vineyards of the study area are planted in straight rows separated by three-meter side unvegetated lanes by 1.2 m between plants on row, semi-high training, bilateral cordon. The survey was performed in December 2011 and October 2012 that being most critical period for machinery traffic;

Vineyards in this area are normally managed through tillage, using the commonest farming techniques in the region. Tillage and other management practices are carried out following the direction of the contours. Tractors use the lanes to plough (one time in fall and one time in spring at 18-22 cm depth), harrowing to remove any spurious vegetation, spread fertilizer, apply herbicides and pesticides.

Then, the stainless steel pan containing the soil derived from the sieving was dried at 105°C until water evaporated. Aggregate stability was only measured on the smaller soil fraction as this particle size is usually responsible for sealing the soil. The water stable soil aggregates (WSA) were measured using the procedure of Kemper and Rosenau. Briefly, replicate 4 g samples of soil aggregates per subplot were moistened with deionised water by capillary action for 10 min. Air-dried sieved soil (1-2 mm diameter) was placed in a 0.250 mm sieve and immersed in an stainless steel pan with distilled water for 3 min with a stroke length of 1.3 cm and a frequency of 35 cycles min^{-1}. The soil retained in the sieve was immersed again in a sodium hexametaphospate solution (2 g L^{-1}) for 15 min and 35 cycles min^{-1} and the stainless steel pan was also dried. After drying, aggregate fractions (from water and hexametaphospate) were weighed to obtain the $WSA_{1\text{–}2\ mm}$ percentage. The initial and final weights of aggregates were corrected for the weight of coarse particles using the formula: final weight = initial weight - coarse particles weight. Coarse particles were fraction of soil contained in the last sieve of the manipulation.

The $WSA_{1–2\ mm}$ was calculated by weighting the mass of soil aggregates remaining after wet sieving and expressed as a percentage of the total mass of aggregates at the beginning of the experiment.

Bulk density was determined by the core method (Blake and Hartge, 1986). Undisturbed soil cores (100 cm^3) were taken horizontally at 10 cm depth intervals from 0 to 50 m on the soil profile. Three replications were performed per pit (Figure 2).

Fig. 2 - Core sampling for bulk density determination

The penetration resistance of the soils was determined using a digital penetrologger *(Eijkelkamp Equipment, Model 0615-01 Eijkelkamp, Giesbeek, The Netherlands*) which had a cone angle of 30^0 and a base area of 1 cm^2. It was carefully inserted into the soil profiles in 1 cm increments from the surface to a depth of 50 cm by the same person. 30 parallel records performed in each plot and averaged, on both wheel tracks and in the middle of an alleyway.

RESULTS AND DISCUSSIONS

Neither runoff nor soil erosion were measured in the field, but the proportions of water-stable aggregates were determined as a relevant soil erodibility indicator. The highest percentage (80.10%) of $WSA_{1–2mm}$ was recorded on 30-40 cm depth fallowed by 20-30 cm layer, probably caused by absence of influence of tillage (figure 3). The poorest aggregate stability was measured on the top layer (53.00%), indicating a serious problem and a poor soil physical quality.

Generally, the higher the index value the better the soil's capacity to transmit water and air and to promote root growth and development. The differences in aggregate stability were more pronounced in the surface soil samples than in the 30–40 cm samples.

Several studies have shown that the proportion of water-stable aggregates is related to soil loss measured in fields varying in scale from square metres to hectares (Bradford et al. 1987; Barthes & Roose 2002, Goulet E., et al., 2004).

On vineyard hillsides, the frequency index of erosion features was relatively high and was negatively correlated with the topsoil content of water-stable aggregates .200 mm, especially in the absence of conservation practices (Barthes B. and Roose E., 2002).

Alterations in soil structure due to topsoil and subsoil compaction by vehicular traffic influence many soil properties which control crop production and quality of yield.

The PR is an empirical measure of soil strength and it is widely used for assessing the compacting and loosening effects of agricultural implements. Root elongation and growth decreased by increasing soil penetration resistance.

With regards to grapevine growth, at values higher that 3 MPa the root growth is retarded except through cracks and old root channels.

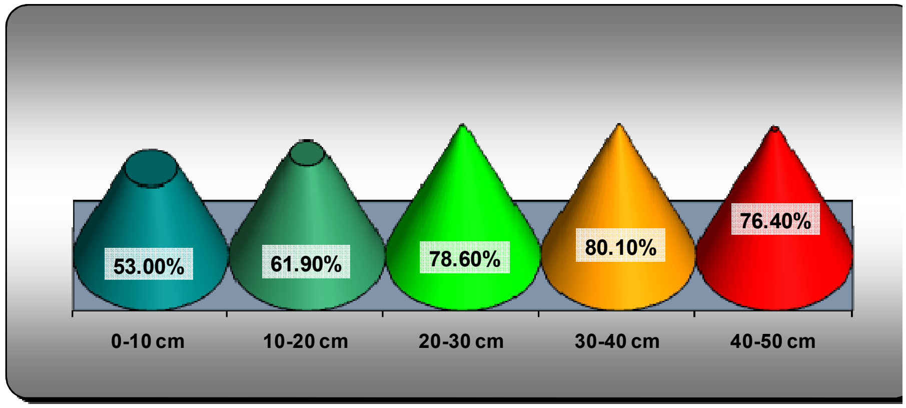

Fig. 3 – Water stable aggregates – mean values 2011-2012

From the comparison of values shown in Figure 4 results that penetration resistance varied in a wide range and is very clear influenced of passing of machinery in wheel tracks.

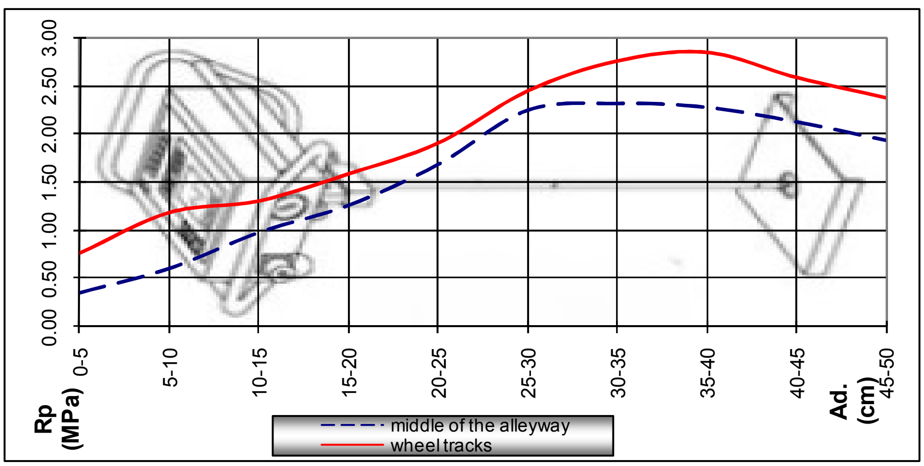

Fig. 4 – Soil penetration resistance on the middle of the alley and on the wheel tracks – mean values 2011-2012

The critical values (2.85 MPa) achieve penetration resistance at 35-40 cm depths on wheel tracks, while in the middle of the alleyway almost all measurements were not close to this value. The highest value, 2.31 MPa, was recorded at 30-35 cm depths (due to a plough pan). On 40-50 cm depth the values ranged from 1.93 in the middle of the alley to 2.59 Mpa on wheel tracks.

The strongly compacted zones were also identified on soil profile thanks to their specific features, i.e. no visible macropores, massive structure and smooth breaking surfaces.

Many authors have shown that compaction of soil particularly just below the cultivated layer, limit the penetration of roots and restrict the ability of plants to absorb water and nutrients from the subsoil (Chan et al., 2006).

Bulk densities and penetration resistance generally increased with depth within the profiles. The bulk density values ranged from 1.17 g/cm^3 in 0-10 cm depth to 1.51 g/cm^3 at 30-40 cm, as average in 2011-2012. On the last layer analyzed the bulk density slightly decreased (1.48 g/cm^3). Increases in soil strength and bulk density are a common characteristic of hard-setting soils during drying (Mullins et al., 1990).

CONCLUSIONS

The unfavourable soil compaction in the wheel track of the tractors and the machines can be eliminated by using looseners corresponding to the wheel track, controlled traffic, deep ripping and conservation tillage practices are recommended for increasing the soil physically status. Another suggestion would be to apply organic mulch, in order to decrease erosion without decreasing yields

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