

# 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.<sup>1</sup>, MUSTEA M.<sup>1</sup>, NICULAU M.<sup>2</sup>, NECHITA C.B.<sup>1</sup>,  
COLIBABA Lucia Cintia<sup>1</sup>, LUCHIAN Camelia<sup>1</sup>  
e-mail: kamelia\_luchian@yahoo.com

**Abstract.** *This study determined and correlated the benzoic acids profile of four wines from Cotnari wine area (Frâncușă, Fetească albă, Grasă de Cotnari și 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 gentisic acid than the 2011 samples. At the same time, Cotnari samples register a higher total quantity of benzoic acids (gentisic acid, gallic 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 și 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 gentisic acid than the 2011 samples. At the same time, Cotnari samples register a higher total quantity of benzoic acids (gentisic acid, gallic 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,

---

<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine Iasi

<sup>2</sup> Research Center for Oenology – Romanian Academy Iasi Branch

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).

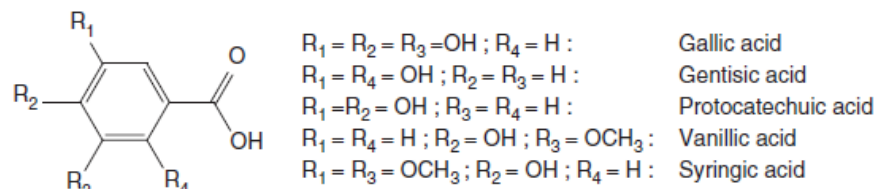


Fig.1. Chemical structures of the hydroxybenzoic acids found in wines.

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: Frâncușă (encoded Frș 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) și 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 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 Frș I 10 - Frâncușă Cotnari 2010).

The grapes were harvested at technological maturity from Iași 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 afterward'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, Iași branch, and at the Oenology Laboratory of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iași. 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 benzoic acids in wines obtained from Cotnari vineyard. The samples were processed in 2010 and 2011. In 2010, benzoic 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 than the 2011 samples.

Table 1

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

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

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

The variation of the benzoic acids content is random according to harvest year and compound. High values for syringic acid were found in 2011 samples: Frâncușă 17.90 mg/L, Fetească albă 21.52 mg/L, Băbească gri 16.62mg/L. In 2010 Frâncușă 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 benzoic 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
<b>RI II 10</b>	7.96	0.38	0.14	<b>1.35</b>	5.12	125.96
<b>Fr II 10</b>	7.96	0.38	0.14	1.35	5.12	<b>125.96</b>
<b>Fa II 10</b>	8.49	1.56	<b>0.51</b>	<b>1.48</b>	0.97	3.29
<b>Bg II 10</b>	8.94	0.92	<b>0.44</b>	1.54	14.28	32.10
<b>RI II 11</b>	7.79	0.56	0.15	0.76	0.89	128.94
<b>Fr II 11</b>	7.96	1.15	0.30	1.74	<b>17.90</b>	35.01
<b>Fa II 11</b>	7.93	0.85	0.27	0.92	<b>21.52</b>	<b>58.54</b>

## 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 benzoic 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 higher quantity 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.

**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.*

## REFERENCES

1. Castellari M., Sartini E., Fabiani A., Arfelli G., Amati A., 2002 - Analysis of wine phenolics by high performance liquid chromatography using a monolithic type column. J. Chromatography A., 973, pp. 221–227.
2. Garrido J., Borges F., 2011 - Wine and grape polyphenols—a chemical perspective. Food Res. Int., 44, pp. 3134–3148
3. Harris C.S., Mo F., Migahed L., Chepelev L., Haddad P.S., Wright J.S., Willmore W.G., Arnason J.T., Bennett S.A.L., 2007 - Plant phenolics regulate neoplastic cell growth and survival: a quantitative structure-activity and biochemical analysis. Can. J. Physiol. Pharmacol., 85, pp. 1124–1138
4. Huang W.-Y., Cai Y.-Z., Zhang Y., 2009 - Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr. Cancer., 62, pp. 1–20
5. Moreno-Arribas M.V., Polo M.C., 2009 - Wine Chemistry and Biochemistry, Springer.
6. Ribereau-Gayon P., Dubourdieu D., Donèche B., Lonvaud, A., 2006- Handbook of Enology Volume 1 – The Microbiology of Wine and Vinifications, 2nd Edition. John Wiley & Sons.
7. \*\*\* 2012 - Compendium of international methods of wine and must analysis, International Organisation of Vine and Wine, Electronic version, Paris.