

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

NICULAUA M.¹, VĂRARU F.², BUBURUZANU C.²,
MĂLUȚAN G.¹, NECHITA C. B.¹, COTEA V. V.²

e-mail: ccoiasi@gmail.com

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 follows: 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 protocatheic, 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 importanță crucială pentru evaluarea calității vinurilor și permite în cele mai multe cazuri caracterizarea caracteristicilor senzoriale resimțite de consumatori la nivel fundamental. În cazul de față sunt prezentate unele vinuri obținute cu struguri din recolta anului 2010 și 2011 pentru varietățile: Frâncușă, 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 și 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

¹ Research Centre for Oenology – Iași Branch of the Romanian Academy

² University of Agricultural Sciences and Veterinary Medicine “Ion Ionescu de la Brad”

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: Frâncușă, 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 Zymoclar 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 Zymoclar 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-10Axi) in order to achieve a double spectral certification for analytes, 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 Frâncușă 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 și Tămâioasă românească have values somewhat lower than the rest (Table 1).

Table 1

Values of some benzoic acids in wine samples from Copou

Wine variety production year // mg/L	protocatechiuc acid	p-hydroxybenzoic acid	vanillic acid
Frâncușă 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
Frâncușă 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-hydroxybenzoic 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 Frâncușă has 2-3 times higher values than Riesling italian.

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

Table 2

Values of some benzoic acids in wine samples from Copou

Wine variety production year // mg/L	gallic acid	syringic acid	gentisic acid
Frâncușă 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
Frâncușă 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 Frâncușă 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 benzoic 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-hydroxybenzoic 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.

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