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).

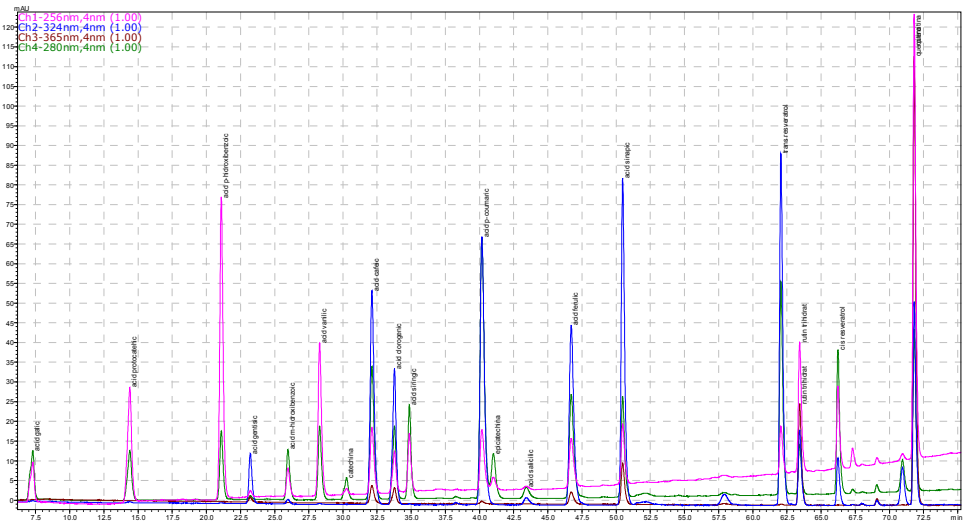

Fig. 2 - Chromatogram of standard mixture of phenolic acids and flavones with the TFA method

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.

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REFERENCES

1. Castellari M., Sartini Elisa, Fabiani Alessandra, Arfelli G., Amati A., 2002 - *Analysis of wine phenolics by high-performance liquid chromatography using a monolithic type column***,** J. Chromatogr. A, vol. 973, pp. 221–227.

2. Valle M. Martínez-Ortega, García-Parrilla M. Carmen, Troncoso Ana M., 2004 - *Comparison of different sample preparation treatments for the analysis of wine phenolic compounds in human plasma by reversed phase high-performance liquid chromatography*, Analytica Chimica Acta, vol. 502, pp. 49–55.

3. María del Álamo, Casado Lourdes, Hernández Victor, Jiménez Juan José, 2004 - *Determination of free molecular phenolics and catechins in wine by solid phase extraction on polymeric cartridges and liquid chromatography with diode array detection*, Journal of Chromatography A, vol. 1049, pp. 97–105.

4. Ortega A. F., Lopez-Toledano A., Mayen M., Merida J., Medina M., 2003 - *Changes in Color and Phenolic Compounds During Oxidative Aging of Sherry White Wine*, Journal of Food Science, vol. 68, nr. 8, pp. 2461-2468.

5. Silva Luís R., Andrade Paula B., Valentão Patrícia, Seabra Rosa M., Trujillo Martha E., Velázquez Encarna, 2005 - *Analysis of non-coloured phenolics in red wine: Effect of Dekkera bruxellensis yeast*, Food Chemistry, vol. 89, pp. 185–189.