ANALYSIS OF THE PHENOLIC COMPOUNDS THROUGH HPLC IN SOME RED WINES IN IAŞI VINEYARD OBTAINED THROUGH DIFFERENT TECHNOLOGICAL METHODS

ANALIZA UNOR COMPUŞI FENOLICI PRIN METODE HPLC LA UNELE VINURI ROŞII DIN PODGORIA IAŞI, OBŢINUTE PRIN DIVERSE METODE TEHNOLOGICE

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Abstract. *In the production year 2010-2011, wines were obtained from four black grape varieties through different wine-making technologies. Basic physical-chemical characteristics were evaluated as well as a series of phenolic compounds: resveratrol, shikimic acid, hydroxycinnamic acids, hydroxybenzoic acids, hydrolysable and non-hydrolysable tannins. Comparing the wines obtained from the 4 technologies, it was registered that the sample from the microwave technology had a higher quantity of phenolic compounds, compared to the other variants.*
Key words*:* resveratrol, shikimic acid, tannins, thermo maceration, microwaves

Rezumat. *În anul de producţie 2010-2011 s-au obţinut dintr-un număr de 4 soiuri de struguri negri din podgoria Iaşi o serie de vinuri prin diferite tehnologii de vinificaţie. La aceste probe, pe lângă caracteristicile fizico-chimice de bază s-au evaluat o serie de compuşi fenolici, precum: resveratrolul, acidul shikimic, acizi hidroxicinamici, acizi hidroxibenzoici, taninuri hidrolizabile şi nehidrolizabile. În urma comparării celor 4 tehnologii s-a observat că variantele obţinute prin tehnologia cu microunde şi cea cu termomacerare,duc la o creştere a cantităţii acestor compuşi în vinurile analizate.*
Cuvinte cheie*:* resveratrol, acid shikimic, taninuri, termomacerare, microunde

INTRODUCTION

The amount of tannins present in wines depends on the duration of its contact with the grapes, skins and seeds, as well as the processing way of the grapes. Depending on the amount that is present in wines tannins contribute positively but sometimes negatively to olfactive and gustative attributes of the product (Cotea et al., 2009).

Tannins are phenolic compounds that are found in all of the vine's organs and are responsible for the wine's astringency. They are soluble in water, form coloured compounds and have the capacity of precipitating proteins and inhibiting

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enzymes' activity, contributing thus, alongside alcohols, to wine conservation (Pomohaci, 2005). Also as antioxidant and antibacterial agent this action has thereby for wines a good preserving property (Țârdea, 2007).

Depending on the version of maceration-fermentation technology used to obtain red wines, the tannins in wine are evaluated by analysing and comparing the results recommending a more efficient version (Moraru, 2011).

Phenolic acids have an important role to interact with anthocyans in acylation reactions and therefore contributing at the wine aging bouquet formation.

Micro-phenolics compounds (resveratrol and shikimic acid) are present in very small quantities in wines. These compounds do not affect the quality of wines but have beneficial effects on human health (Țârdea, 2007).

MATERIAL AND METHOD

Four varieties of grape wines ware studied, two local (Băbescă neagră – noted Bn and Fetească neagră – noted Fn) and two cosmopolit varieties (Cabernet Sauvignon - noted CS and Merlot - noted M). The grapes were harvested at technological maturity from Copou and Bucium area. Grapes were divided into four equal parts and were subjected to several types of maceration technology to assess the chemical composition modifications, especially phenolic compounds. Different technological variants of maceration were performed: classical (code - m), microwave maceration (code - mm), thermo- maceration (code - tm) and rotating tanks (code - rm).

At the thermo-maceration option the working temperature was 70 °C for about 30 minutes and in the microwave tests the samples were subjected to 750 W for 15 minutes. Rotating tanks and classical versions had a maceration period of 5 days before end-test of maceration (skin colour invariants during maceration-fermentation).

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 adding a dose of sulphur dioxide by 40 mg/L per bottle, they were corked with a Mini TS.

For shikimic acid analysis we used the method OIV MA-E-AS313-17-ACSHIK for determining organic acids in wine. Samples were processed on a Shimadzu HPLC system composed of: auto-injector SIL-20AC Shimadzu Prominence series (injection volume: 10 mL, sample temperature 20 °C), quaternary pump LC-20AD Shimadzu Prominence series with five-channel degasser Shimadzu Prominence series DGU-20A5, column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M20A Shimadzu Prominence series (scanning range: 200-440 nm), chromatographic system controller CBM-20A Shimadzu Prominence series and the hole system is connected to a desktop computer unit via LAN (local area network). The separation was made through two C18 columns Grace Organic acids (2×POA) 250×4.6 mm and 5 µm stationary phase. The eluent for the separation is a 0.065 M solution of sulphuric acid (H_2SO_4) with a flow rate of 0.8 mL/min at a temperature of 20°C.

For analysis of phenolic acids and other phenolic compounds in wine we have been using monolithic separation columns (Castellari et al., 2002) using the same chromatographic separation system described in shikimic acid determination.

RESULTS AND DISCUSSIONS

Gallic acid (fig. 1) shows high values in all technology variants of wines made from Cabernet Sauvignon, compared with all other wines considered in this study. Maximum value is recorded in the microwave maceration version obtained (83.93 mg/L) and the next most important value of 79.62 mg/L is for the roto-tanks version. The lowest concentration of gallic acid is registered in Băbească neagră variety wine of 1.39 mg/L obtained by thermo-maceration. Values obtained from Merlot wines are between 24.05 mg/L at classical maceration version and 24.48 mg/L for the thermo version. At wines from Fetească neagră variety, values of 11.97 mg/L were obtained for traditional maceration version and 31.91 mg/L for thermo-maceration version.

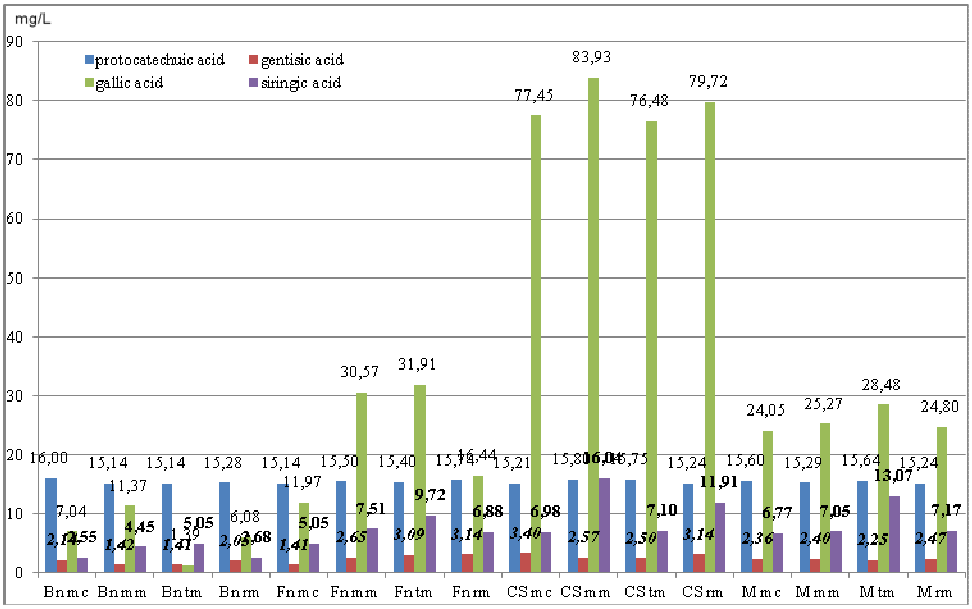

Fig. 1 - Values distribution for *p*-hydroxybenzoic acids at wines in 2010

Protocatehic acid is invariable in all wines studied regardless of variety or technology. The lowest value (15.14 mg/L) is recorded in Băbească neagră wine (microwave or thermo-maceration variants) and at Fetească neagră (classical maceration) and the highest value (16 mg/L) is from Băbească neagră obtained by classic maceration.

Syringic acid values are between 16.04 mg/L to Cabernet Sauvignon (microwave maceration) and 2.55 mg/L to Băbească neagră version with classical maceration. The Băbească neagră variety wines have low values compared with wines obtained from other varieties studied. Whichever technology used, variants with high values for this acid is in all varieties the thermal variants exploited (microwave and thermo-maceration).

Gentisic acid has values that vary between 3.40 mg/L at Cabernet Sauvignon (classic maceration) and 1.41 mg/L in Băbească neagră (thermo-maceration). Also in this case, whichever technology used, it is noted that gentisic acid values are similar in all varieties studied.

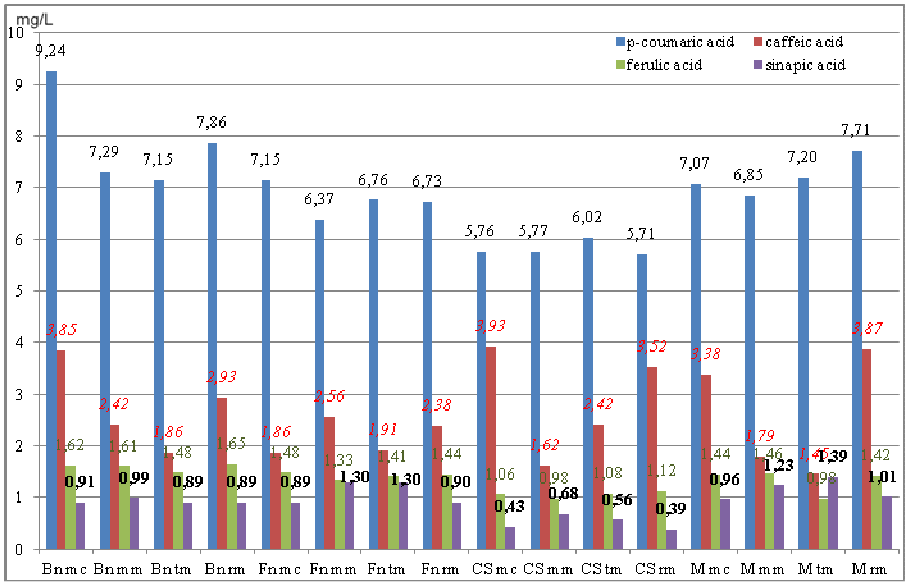

Fig. 2 - Values distribution for cinnamic acids at wines in 2010

The highest value of *p*-coumaric acid (fig. 2) was found in Băbească neagră wine obtained by traditional technology (9.24 mg/L) and the lowest value of 5.71 mg/L at Cabernet Sauvignon (roto-tanks maceration). For all varieties and whatever technological variant used the *p*-coumaric acid values are much higher than the values analyzed for other hydroxycinnamic acids.

The maximum recorded values for caffeic acid (3.93 mg/L) is at Cabernet sauvignon classical maceration version. Lowest value of 1.45 mg/L is found in Merlot obtained by thermo-maceration. Note that the variants have high values obtained by traditional maceration or roto-tanks in detriment to the thermal variations.

Ferulic acid values ranging from 1.65 mg/L obtained by roto-tanks from Băbească neagră version and 0.98 mg/L in Merlot variant obtained by thermo-maceration. In each variety, regardless of technological variant, we see some homogeneity of these values.

Sinapinic acid recorded the highest value of 1.39 mg/L in Merlot thermo-maceration version and the lowest value (0.39 mg/L) at Cabernet sauvignon produced by roto-tanks version. Thermal technology variants recorded higher values in all varieties studied for this kind of substance.

Catechin (fig. 3) has values ranges between 168.42 mg/L at microwave maceration of Băbească neagră variety and 18.6 mg/L in Fetească neagră

(classical maceration). Depending on the variety, the variants obtained by thermo or microwave technology have higher values for this compound.

The highest value for epi-catechin (12.32 mg/L) was achieved in the roto-tanks obtained Cabernet sauvignon. Lowest value for maceration of 3.75 mg/L to Fetească neagră microwave version. From the results we can say that only the variety and less the used technological variant would affect the amount of this compound.

Shikimic acid has values between 75.85 mg/L to Fetească neagră (thermo version) and 18.45 mg/L to Băbească neagră (maceration classic). Shikimic acid variation values are very much influenced by variety and less by technology.

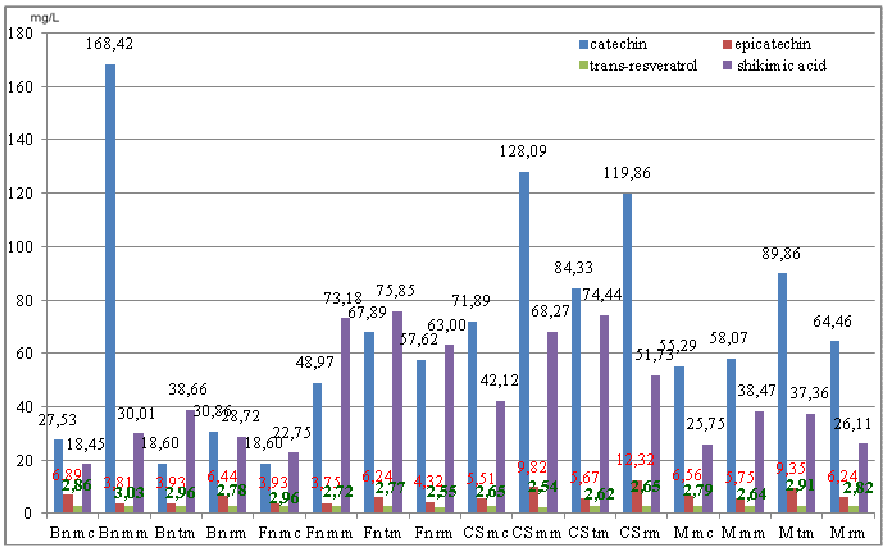

Fig. 3 - Values distribution for catechin, epicatechin, *trans*-resveratrol and shikimic acid at wines in 2010

Trans-resveratrol has very small variation of values, regardless of variety or applied technology. Lowest value of 2.54 mg/L was recorded for Cabernet Sauvignon (microwave version) and the highest value of 3.03 mg/L in Băbească neagră at the same maceration techniques.

CONCLUSIONS

1. There is a technological facilitation for the accumulation of these compounds in wine by various harder extraction methods (thermo-maceration, microwave maceration and roto-tanks).

2. Cosmopolit varieties are richer in phenolic compounds than local varieties but by using modern methods of maceration-fermentation we can enrich the quality and quantity of wines produced and possibly boosting bioactive character of weaker varieties.

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