THE ANTIOXIDANT ACTIVITY EVOLUTION IN DIFFERENT TECHNOLOGICAL STAGES OF SOME RED WINES

Otilia CHIRIŢĂ[1], Marius NICULAUA[2], Ancuţa NECHITA[1], Valeriu V. COTEA[1]
E-mail: chiritaotilia@gmail.com

Abstract

The studies regarding the antioxidant activity evolution in different technological stages of some red wines was made at the oenology laboratory of U.S.A.M.V. Iasi and closely with the Center for oenology, Iasi branch of the Romanian Academy. 4 local grape varieties for red wines, Feteasca neagra, Negru de Causani, Babeasca neagra and Busuioaca de Bohotin were chosen as study material, the last one more specifically for rose wines than for the red ones, and the Merlot variety in order to compare the antioxidant capacity of the Romanian varieties to the international ones. These wines were produced with the classical technology for red wines using the classical skin contact maceration process. Six important technological moments was supervised. The phenol compounds content of the samples was analyzed using the total polyphenolic index and the antioxidant activity using the photochemiluminescence method, calculated as ascorbic acid equivalents, mmol/L. Firstly the values of the phenolic compounds and of the antioxidant activity of all the five wines have ascending curves, with lower or higher variations of their amplitude according to the specificity of each wine. The technological stage that determined the maximal values of both the TPI and the antioxidant activity was the second one, the one after the maceration process, which is responsible of the extraction of phenolic compounds from grapes. Towards the end of vinification, these values slowly decreased under the influence of the other vinification processes but depending also on the grape variety. Linear correlations between the TPI and AO.A were determined, these correlations varying also depending on the grape variety and can be consider a specific characteristic of the grape sort.

Key words: antioxidant activity, Rumanian grape varieties, technological stages

In human history we find evidence of wine consumption dating from the Neolithic times, but the references about the medical uses of the wine. However, references about using wine as a medicine is much more recent. Differences between wines, depending on the properties and taste, have been made since ancient times. Medical interest for wine-health relationship is part of the history of wine, and the first medical applications of wine have been linked to its antiseptic and anti-inflammatory capacity. It was indicated in treating wounds and infections (Lucia, 1963).

Free radicals have been the subject of numerous studies both in the medical field (Ashok, 1999) and in the oenological one (Vivas 2001; Chiriţa, 2008). Phenolic compounds in wine, especially red wines are characterized by their ability to block the free radicals of oxygen (Elford, 1991), with antioxidant properties (Neacşu, 2008). Free radicals underlie cellular oxidative disturbances (Olinescu, 1994), which lead in turn to serious disfunctions in the body, such as those of cancer cells (Elford, 1991), lipid oxidation that produces arteriosclerosis (Esterbauer,1992) etc.

With its antioxidant compounds, wine helps protect the body, in case of moderate consumption, against harmful action of free radicals (Elford, 1991; Neacşu, 2008).

MATERIAL AND METHOD

To determine the antioxidant action of red wine, four local varieties were chosen, Feteasca neagra (FN), Negru de Causani (NC), Babeasca neagra (BN) and Busuioaca de Bohotin (BB), recommended more for the production of rose wines and the cosmopolitan variety Merlot, to compare the Romanian varieties potential and environmental conditions. These wines were processed by the classical fermentation maceration; during the processes as few treatments and interventions were made as to not influence their natural antioxidant potential. Samples were taken six times during the technological process: m - must, dm - after 3 days maceration , during alcoholic fermentation in the moments a.f. I, a. f. II and a.f. III) and amlf – after malolactic fermentation. Samples were analyzed in terms of phenolic content expressed as total polyphenolic index (IPT) and in terms of antioxidant activity. For the antioxidant activity the

[1] University of Agricultural Sciences and Veterinary Medicine Iasi
[2] Oenology Centre Research, Romanian Academy, Iasi Branch

photochemiluminescence method was used, and the expression was in ascorbic acid equivalent, mmol/L.

RESULTS AND DISCUSSIONS

The 5 varieties of grapes were harvested as soon as they reached technological maturity, with a sugar content of 168 g/L – Babeasca neagra, 198 g/L - Busuioaca de Bohotin, 193 - Feteasca neagra and Negru de Causani and 190 g/L - Merlot. The acidity (expresses in g/L $C_4H_6O_6$) reaches values of 6,91 g/L – Babeasca neagra, 7,68 g/L Busuioaca de Bohotin, 6,51 g/L Feteasca neagra, 7,79 g/L – Negru de Causani and 6,83 g/L for Merlot, specific values for these varieties. Sugars accumulated until harvest allowing the grapes to obtain wines with high alcohol concentration (13,78 % vol. Feteasca neagra), obtaining dry wines. The alcoholic strength of wine at the end of the experiment ranged from values of 10,95 % vol. for Babeasca neagra to 13,78 % vol. for Feteasca neagra. Total acidity had the minimum values of 6,08 g/L for Merlot and the maximum of 7,05 g/L for Busuioaca de Bohotin wine.

Volatile acidity (expressed in g/L $C_2H_4O_2$) ranged between 0.26 g/L for BN and 0.96 g/L for BB (expressed in g/L) ranged between 0.26 g / L to BN and 0.96 g / L on BB; density had values below 1, explained by the lack of residual sugars in wine and the high alcohol level; nonreduced dry extract showed a minimum value for the Negru de Cauşani, of 19,7 g/L and a maximum for the Feteasca neagra wine.

Table 1 and Figure 1 present for each sample individually and for every moment of the experiment, the evolution of the total polyphenols index and antioxidant activity.

For the **Feteasca neagra** samples the IPT values rises from the 19,5 till the maximum, in the second moment of the malolactic, of 35,4.

The antioxidant activity of the must sample is 0,39 mmol/L. The others samples showed values of the AO.A much bigger, up to 1,34, 1,46 and 1,49 during the alcoholic fermentation, and the TPI was 35,4, 33,7, 32,2. The TPI are the biggest comparing with the other four wines, the region of grape origin, Iasi, is well known for the big potential for this variety. The fermentation maceration process determined an increase of the AO.A values of 146 %, but the maximum was registered in the last 2 moment of the alcoholic fermentation when the growth was of 374 and 382 % comparing with the must sample. After, because of the spontaneous fining and of the malolactic fermentation of the wines the AO.A decreased at 1,02 mmol/L, which represent 262 % of the must sample values. The total polyphenolic index reached maximum values of 35,4, immediately after the maceration fermentation.

Compared with maceration stage, for the the finished product we see that the value of A. AO increased up to 178%, almost doubling from stabilization and wine clarification (from 0,57 to 1,02).

To identify the degree of interdependence of the two sizes of the chemical composition of wine the linear correlation index R^2 was used. Hereby the proportionality between antioxidant activity and phenolic content of the variety Feteasca neagra indicated by the value of R^2, of 0.7 (419), may be characterized as high.

For wine Busuioaca de Bohotin, we obtained a AO.A value of 0.76 mmol/L ascorbic acid and the phenolic content expressed with TPI of 10.2 for the must sample; the folowing samples showed values of AO.A much higher up to 3.36 mmol/L for the carbonic maceration sample and a maximum of 3.66 mmol/L in the first stage of alcoholic fermentation. The TPI showed values of 13.3 and 15.5. TPI values are not impressive, this variety is known for its potential to produce rosé wines .

Fermentation maceration process increased the value AO.A with 436%, but the maximum was recorded in the first two phases of alcoholic fermentation when the growth was of 482% and 476% compared with the value obtained for the grape must.

Later, during the process of winemaking, because of the malolactic fermentation and of the spontaneous wine's clarification, the AO.A values dropped to 2,99 mmol/L, wich represent 393 % from the must sample.

The TPI reached maximum values of 19.1 at the completion and stabilization of wine. If we consider that milestone maceration, due to its pivotal role in the extraction of phenolic compounds and antiradical compounds in grapes, we can say that the last AO.A value ofthe finished wine felled by only 10% after stabilization and wine clarification (from 3,32 to 2, 99).

The proportionality between antioxidant activity and phenolic content of Bohotin form Busuioaca variety indicated by the value of R^2 , 0,4(364), can be characterized as midle, think that which can be easily observed in figure 1.

Evolution curve of the two indexes studied for the Negru de Causani wine has the same shape as the Busuioca de Bohotin one: the first wine making moment showed the smallest values, of 1, 71 mmol/L of AO.A and of 14,6 for TPI.

The increase registred after the maceration reached the 4,13 mmol/L value, increasing by

241% compared to the previous time, the maximum antioxidant activity. Since alcoholic fermentation the values of the two indices, TPI and AO.A, began to decline slightly, to 3.77, 3.71, 3.67 and finally to 3.37. The wine has retained a surplus of 82% before the maceration stage and 197 % compared to the must.

Table 1

The TPI for 5 red wines in 6 technological moments

Nr. crt.	Technological stage	Busuioaca de Bohotin	Negru de Cauşani	Feteasca Neagra	Babeasca neagra	Merlot
0	1	2	3	4	5	6
1	must	10,2	14,6	19,5	4,9	13,1
2	am	13,3	34,1	29,5	5,1	55,7
3	af I	15,5	29,5	35,4	28,8	50
4	af II	18,2	25,3	33,7	21,5	42,6
5	af III	19,1	25,6	32,2	21,8	42,9
6	mlf	19,1	25,8	29,2	21	41,3

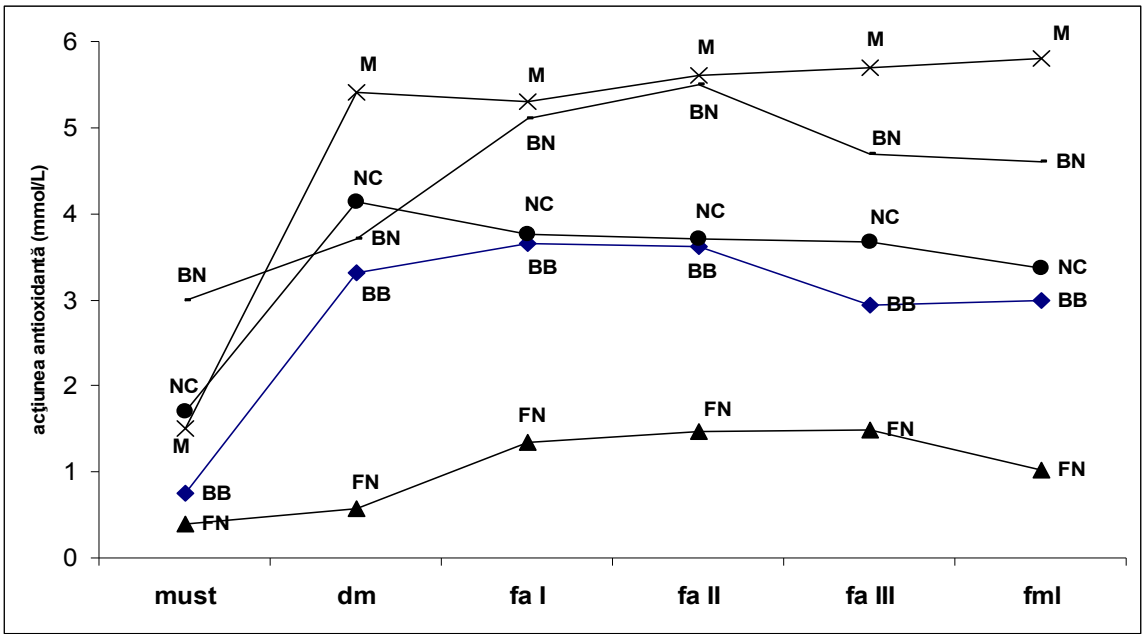

Figure 1 **The evolution of the antioxidant activity for red wines during the wine making process**

Unlike previous varieties this variety remains third in the rankings resulting from comparing the content of phenolic compounds (TPI) and the antioxidant action (AO.A). The proportionality of the measures mentioned above, indicated by the value of R^2 of 0.89, can be characterized as large, even the largest of the five wines.

For the wine of the variety Babeasca neagra, we obtained a AO.A value of 3 mmol/L and the total content of phenolic compounds of 4.9 for must sample; the folowing samples showed much higher AO.A values of 3.7 mmol/L for the sample after maceration on beeswax, 5.1 mmol / L in the first stage of alcoholic fermentation and a maximum of 5.5 A. AO mmol / L in the second part of the alcoolic fermentation (a.f.II).

The maceration fermentation determined an increase of the AO.A with 23%, but the maximum was recorded in the middle of alcoholic fermentation, representing a 83% contribution to the value must obtained.

Subsequently the completion of the alcoholic fermentation and the wine malolactic fermentation decreased the AO.A value to 4.7 mmol/L, respectively 4.6 mmol/L, which represent 157 and 153 % before the phase must. Comparing again the final result, of the wine, with the second sample, the maceration, we can observe that the final AO.A values increased by 23% (from 3.7 to 4.6).

Values are not impressive in any analyses of the two indices, Babeasca neagra being the fourth from the varieties in terms of study. Proportionality between comparable measures, indicated by the value of R2 of about 0.8, is considered to be high.

Last test of the experiment is the Merlot wine. The specifics of this wine comes from the development of the antioxidant action and phenolic compounds values during the winemaking process: for both assesment indexes peaks were obtained after maceration on grape marc, 5.4 mmol/L AO.A

and 55.7 total polyphenolic indeces.

Both in terms of TPI and in terms of antioxidant action Merlot wine has the largest values being the first in the ranking of varieties under study.

Proportionality of compared indices, indicated by the value of R2 of about 0.8, is considered to be also high, as in all other three red wines.

The R^2 value of the correlation between TPI and AO.A for all samples in this experiment is 0.64 indicating an interdependence above average, average for red wines is higher, about 0.8 but is diminished obtained at relatively lower Busuioaca from Bohotin variety (0.4).

CONCLUSIONS

The first observation is that the results of phenolic compounds (IPT) and the antioxidant action (A. AO) from all five varieties have ascending curves, with smaller or larger variations of their amplitude depending on the specific of each variety. The technological stage that determined the maximum value of the IPT and A.AO was the "after maceration fermentation", the stage responsible with the phenolic and antioxidant compounds extraction from the walls of grape berries. Towards the end of vinification these values decrease slightly under the influence of other process steps, but according to each variety individually.

Merlot wine was observed with a higher antioxidant activity compared to local varieties.

Very interesting to note is the degree of interdependence between the values of the two indices studied – antioxidant activity and the total polyphenolic index. For the wines made from red grape varieties the R^2 values were higher than 0.7. Busuioaca Bohotin wine, variety known for its potential for obtaining rose wines had a lower value of R2 index of 0.4. Based on this observation, developed during studies that included white wine, we can set a scale for assessing the antioxidant potential of wines based on their IPT values. Determination of this index is easier and less expensive to make. Knowing the content of phenolic compounds and the correlation equation between the two indices the antioxidant potential and the beneficial effects of the wine on the human body could quickly be assesed, in the case of a moderate consumption.

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