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ABSTRACT

This study aimed mainly to study acids present in wines obtained from Moldova vineyards, in order to objectively describe as to how these compounds are assigned in wines from different areas of production and also the formation of possible acid function compounds configurations specific to different wines.

The raw material used, the grapes, were harvested from the following vineyards: Iaşi-Copou (19.09.2006, 19.09.2007), Iaşi-Uricani harvested at two different time periods – in 2006 (23.09.2006 and 17.10.2006) and in 2007 (27.09.2007), Cotnari (27.09.2006; 11.09.2007) Huşi (18.09.2006; 07.09.2007); Târgu Bujor (21.09.2006) Panciu (18.09.2006) and Odobeşti (19.10.2006; 22.10.2007). The degree of sugars accumulation in the various studied varieties was specific to the period in question. The evaluation of the wines alcoholic concentrations showed that not all varieties used as raw material had the ability to create superior wines or, in some cases, not even ordinary wines. The need to evaluate these varieties was connected to the possible use as partners in wine blending (with acidity-deficient ones) or to assess the fermentative potential of one or another acid in particular, as an eventual secondary by-product.

In the present study, 101 wines were obtained from indigenous varieties of grapes harvested in 2006 and 2007 from six vineyards from all over Moldova and characterized in terms of total acidity, pH, conductivity, volatile acidity, acids (fixed) tartaric, malic, lactic, succinic, acetic, citric, fumaric, shikimic, protocatehic, p-hydroxybenzoic, vanillic, gallic, siringic, gentisic, caffeic, chlorogenic, p-coumaric, ferulic and sinapinic. Besides these determined quantitative parameters, other qualitative assessments were made on acetic, butyric, isobutyric, hexanoic, octanoic and decanoic acids, constituents of volatile acidity, by using a ITEX-GC-MS technique. The grape sorts analysed were: Amurg, Arcaş, Băbească gri, Băbească neagră, Balada, Bătută neagră, Blasius, Cioinic, Codană, Creață de Banat, Cruciuliță, Fetească albă, Fetească neagră, Fetească regală, Frâncușă, Furmint de Miniş, Gordan, Gordin, Grasă de Cotnari, Mustoasă de Măderat, Negru aromat, Negru de Căuşani, Negru de Drăgăşani, Negru moale, Negru vârtos, Novac, Şarbă, Selena, Vulpe, Zghihară (Iaşi-Copou vineyard in year 2006); Băbească gri, Băbească neagră, Balada, Bătută neagră, Blasius, Busuioacă de Bohotin, Cioinic, Codană, Creață de Banat, Cruciuliță, Fetească albă, Fetească neagră, Fetească regală, Frâncușă, Furmint de Miniş, Galbenă de Odobeşti, Gordan, Gordin, Miorița, Mustoasă de Măderat, Negru de Cauşani, Negru de Drăgăşani, Negru moale, Negru vârtos, Şarbă, Tămâioasă românească (in Iaşi-Copou vineyard in 2007); Fetească regală and Fetească neagră (2006 and 2007) for the Iaşi-Uricani vineyard; Frâncușă, Fetească albă, Grasă de Cotnari, Tămâioasă românească (Cotnari

vineyard - 2006 and 2007 production); Busuioacă de Bohotin, Fetească albă, Fetească regală, Zghihară at Huşi vineyard for the year 2006, Băbească neagră, Fetească albă, Fetească neagră, Fetească regală, Zghihară at Huşi vineyard for the year 2007; Băbească neagră, Fetească albă, Fetească neagră, Fetească regală at Târgu Bujor vineyard for the year 2006; Fetească neagră and Plăvaie at Panciu vineyard for the year 2006; Băbească gri, Băbească neagră, Codană, Fetească neagră Fetească regală, Galbenă de Odobeşti, Mustoasă de Măderat, Plăvaie, Şarbă at Odobeşti vineyard for the year 2006; Băbească gri, Codană, Fetească albă, Fetească neagră, Fetească regală, Galbenă de Odobeşti, Plăvaie, Şarbă, Tămâioasă românească at Odobeşti vineyard for the year 2007.

In addition to the samples of wine, another series of controlled experiments were made and noted, which consisted in the study of acids during the pasteurization processes, cold plasma treatment and during treatments with oenological products: bentonite + casein, gelatine and carboxymethylcellulose CMC (protective colloid, potential substitute for arabic gum).

By assessing the impact of different oenological practices on the composition of wine acids, one observed that the acids in concentrations expressed in g/L had a insignificant change, regardless of the treatment to which the wine was exposed, and those in lower quantities (mg/L range) varied significantly, fact that may depend on the type of wine. This may also indicate that macroparameters such as total and volatile acidity present no significant variations, while each acid has a particular evolution in wine, depending on the acidity balance at the time of treatment. Overall evolution of all the acids in wine is compensatory, thus the mentioned macroparameters do not vary significantly. The clarification and stabilization treatments applied to wine do not significantly influence the composition of organic acids commonly present in wine (with some exceptions), thus that studied treatments can be used further without reservation in oenology.

The analysis methods of major wine organic acids (tartaric, malic, lactic, succinic, acetic, citric, fumaric, shikimic), established in this doctoral thesis is effective, even if it is lengthier in conveniently separate and characterize these compounds in any wine sample.

In this study, an evaluation of all methods proposed by the International Organisation of Vine and Wine was realised, as a variant for the separation and identification of organic acids by using liquid chromatography techniques (LC or HPLC). The two standard methods were not viable, having major drawbacks such as low-resolution in extreme temperature and pH conditions (styrene divinylbenzene type columns S-DVB) or unstable eluent and relatively low resolution (coupling of two columns and using a phosphate buffer eluent). All these shortcomings were resolved with gradual optimization methods and by judicious choice of components that may lead to a convenient separation.

By using a modern technique (liquid chromatography), several wines obtained from all

35 Romanian varieties were analysed in terms of shikimic acid concentration, an extremely important compound in assessing the provenance of the wine in terms of variety, but also with a significant bioactive role. Thus, for white wines, the variation interval is between 6.1 mg/L for Blasius Copou 2007 and 70.5 mg/L for Zghihară Huşi 2006. For the red wines, the minimum values are found in the Fetească neagră 2007 Huşi wine (9.6 mg/L) and maximum - Copou Arcaş 2006 (101.2 mg/L). A notable fact is that the literature data on the composition of this acid in various Romanian wines obtained from different varieties is vague and mainly inexistent, and this study renders a fairly complete case analysis of this compound.

The phenolic acids analysis method has been optimized to obtain information on almost all compounds of interest with proprieties in human physiology (protocatehic acid, p-hydroxybenzoic acid, vanillic acid, gallic acid, siringic acid, gentisic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and sinapinic acid) resulting either from the biosynthetic processes in the plant or during fermentation. In the case of phenolic acids, two methods of separation and dosage were practically established, which can be used in analyzing a large number of compounds in wine and grapes. The differences between the two methods consist in the stability of the eluent used and the simultaneous use of a second detector. When two detectors are used can specifically identify and, of course, quantify several more chemical compounds that have complementary physical properties of the two detectors (DAD and fluorescence). Essentially, the capacity peaks (substances) in such methods can reach up to 120-180 compounds in a time of 90 minutes, during the separation process. Some acids that are known to originate in grapes, produced by the plant biosynthetic processes (protocatehic acid, gallic acid, p-coumaric acid, caffeic acid and ferulic acid) were discovered, by evaluating the technological variants used, to be influenced by a technological bias, more specifically by using more invasive extraction methods (thermo-maceration, roto-tank maceration, colour enhancing technique) - Fetească neagră wines.

The evaluation of volatile compounds in the local wines was innovative because, until now, the scientific literature did not contain relevant data regarding this aspect on those wines. For the first time, a method involving an online concentration technique such ITEX (in tube extraction method), directly coupled to gas chromatograph for analysis of wines (directly) was established. The obtained results were evaluated by using two distinct methods of separation: a first method is the introduction of the wine sample in a vapour analysis vial (headspace) and the concentration under ITEX program, by adding a mixture of NaCl, Na_2SO_4 and KH_2PO_4 in the wine sample enhancing the release of volatile compounds, which are then concentrated according to the established ITEX parameters with the same concentration parameters, but with a more concentrated injection end-product. By using these techniques, a large number of esters

derived from grape seeds and from fermentation (esters of fatty acids) have been identified. The results are comparable in both cases the concentration (with and without salt), but often was found that the fraction of the wine headspace acids are present in free form and, with the application of super-saturation with alkali salts, the wine esters were predominant.

Although quantitative assessment was not performed, several conclusions formulated on the composition of fatty acids, such as the correlation is between hexanoic and octanoic acids for Fetească neagră wines, regardless of year, vineyard or production technology used.

Using gas chromatography coupled to a mass spectrometer, acetohydroxamic acid in Fetească albă white wine (Copou 2006), 2-ethyl-heptanoic acid in Negru de Căuşani wines (sample without salt), Selena (sample with salt) in the Copou vineyard, 2006 and Fetească regală Huşi vineyard, production year 2007 have been identified in some salted samples of wine, with over 80% probability compared to a NIST08 spectral database. 2-methyl-butanoic acid could also be identified in samples with salting out effect from Fetească neagră wines obtained form Huşi vineyard in 2007. The wine from the Panciu vineyard - Plăvaie (2006), contains isopropyl pyruvic acid or 4-methyl-2-oxovaleric acid – a metabolic compound – with a 79% probability to NIST08 database (revealed in salting conditions). Acetohydroxamic acid presence in wines can occur when lacasse is present or when the grapes are affected by *Botrytis cinerea*.

By evaluating the two-year study of different varieties of wines available in the vineyards of Moldova, it was established that in 2006 the acidity of wine is higher than in 2007 (periods of rain during the harvest), regardless of area of origin of grapes.

A particular aspect of the study was the comparison between Fetească neagră wines produced using different maceration-fermentation technologies (blanc de noir, rosé, carbonic maceration, classical maceration, rotating tanks maceration, thermo-maceration, colour enhancing technique), in terms of macroparameters and acid compounds. There are differences between each acid concentration, influenced by the technological method used. Notable is that in the cases of shikimic acid, gallic acid, caffeic acid, synapinic acid, hexanoic acid, octanoic acid, decanoic acid, a clear differentiation in relation to the production of grapes used in the experiments of one side of a dividing line between the north and south of the area studied was present. Influence of the production year is clear for the analysed macroparameters and the qualitative or quantitative assessments of each acid separately.

By evaluating the quantity of malic and lactic acids, one may determine which of the samples of wine were subjected to the malic-lactic fermentation process, fact that often cannot be measured by the quick pH determination. The analysis performed led to the conclusion that the only technology that can easily achieve this process occurs in red wines processed using carbonic maceration. By analysing other acids from wined obtained using the carbonic

maceration process, in most cases it was observed that they decrease significantly in concentration or disappear, or, in some cases, they appear in much higher concentrations (caffeic acid), in comparison with the other technological variants. This quite controversial process modifies very much the natural distribution of acids, deeply altering the tipicity of wine varieties.

Wine's conductivity is a macroparameter influenced not only by the acids present in large numbers or their quantity but, significantly, by other parameters that were not covered by this study: the concentration of various anionic species present in wine, various proteins or enzymes with a protective colloid role and the concentration of metal ions which act as easy-complexing agents.

All the 11 phenolic acids analysed, with high importance for wines, proved to be vanillic acid, gallic acid, caffeic acid and p-coumaric acid with values in the tens of mg/L, and others, like protocatehic acid, p-hydroxybenzoic acid, chlorogenic and ferulic acid concentrations are below or equal to one mg/L. The other remaining acids (siringic, gentisic and sinapinic) are present in the studied wines in very small amounts and often under the detection limits of the method. Red wines have higher amounts of phenolic acids than white wines, but during the climatic conditions of some particular years (2007), the quantities are comparable between the two types of wine (white and red), even in the same vineyard.