DETERMINATION OF ORGANIC ACIDS CONTENT IN MOLDAVIAN WINE BY HPLC

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Abstract: Wines are often falsifiable drinks. In recent years, there are found quite "accurate" counterfeits, when drink's basic physical and chemical indexes meet the requirements of standards and conditions, declared on the labels. Therefore, fighting for the quality of the product it's necessary to develop effective methods for comprehensive assessment of wines, their authenticity, origin... Collection of statistical data can help in the evaluation of the received data after samples' analysis. This article presents statistical data analysis of a number of Moldavian wines on the composition of the main organic acids in wine, their content, ratio. Thus, was marked characteristic diapason of some organic acids ratio, for example: ratio tartaric acid/sum malic, lactic acids – 1,0-2,0 g/dm^3 for red dry wines, and 0,5-1,5 g-dm^3 – for white dry wines. The data were obtained by HPLC method of analysis.

Key-words: HPLC, wine, organic acids

Introduction

The standard documentation provides a list of physical and chemical indexes needed for analysis of identification and confirm the safety of wines. Thus, according to the Technical Regulations (TR) №356 of 11.05.2009 and SM 117, for the certification of wines mandatory parameters are: the volume fraction of alcohol ethylic, the mass concentration of sugars, total extract, the mass concentration of titratable acids, volatile acids, the mass concentration of total sulfurous acid, the mass concentration of citric acid, mass concentration of malvidin diglucoside, the mass concentration of iron, copper and toxic elements according to SanPin 2.3.2.1078.

In the framework of contracts between economic agents can be specified and other additional requirements for some indexes. For example: limit of the content of citric acid in the wine (the maximum permitted – 1,0 g/dm^3); the ratio of glucose / fructose <0,5 (recommended by the OIV the ratio of glucose / fructose should be <1).

To protect consumers from counterfeit products there are a number of indicators aimed at defining the quality of the "drink". For example: Blarez' rule, Blarez' ratio, Gautier' number, etc., requiring complex sample analysis [1].

Wine is a multicomponent system. The content of wine ingredients varies widely depending on the species and varieties of grape, climatic, geological, agronomical and other conditions [2]. In their qualitative and quantitative content in wines we can estimate the authenticity and correctness of technological process [3].

Organic acids make major contributions to the composition, stability and organoleptic qualities of wines. Their preservative properties also enhance wines' microbiological and physicochemical stability [4,5]. Analysis of published data showed that natural wines contain a rich and varied set of organic acids. The main wine acids are aliphatic organic acids. Mostly tartaric, malic and lactic acid, in smaller amounts -

succinic acid, acetic acid [6]. Some of them runs in the wine from the original material (grape juice), the part appears by the fermentation process [7].

Method and Materials

Modern scientific methods of analysis are needed to achieve the purpose of complex wine investigation. High performance liquid chromatography is one of these. HPLC is an important analytical tool for separating and quantifying components in complex liquid mixtures.

All data analysis were obtained by liquid chromatograph LC-20AD (*Shimadzu*) with spectrophotometer SPD-20AV on the wave length 210nm, using the next conditions: stationary phase – NUCLEODUR C18 Pyramid (*Macherey-Nagel*), with the next technical characteristics: 250x4 mm, pore size 100Å, particle sizes 5 μm, pH stability 1–9 [8]; oven temperature 35ºC; flow rate – 0,5ml/min; eluent – 0,2% H_3PO_4 [9].

Results and Discussion

In order to determine the characteristic concentrations of main aliphatic acids in grape wines were analyzed wines produced in Moldova in 2010 and 2011 years. In the series of tests were included dry red and white wines.

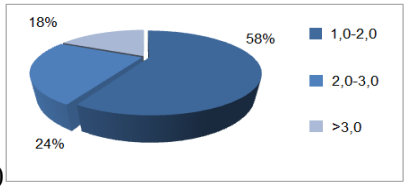
According to the results of analysis content of tartaric acid in red dry wines was 1,5-3,7g/dm^3, and 0,7-3,9 g/dm^3 in white dry wines, respectively.

Because of the fact that tartaric and malic acids have the biggest content in wine, and in the case of malolactic fermentation, lactic acid too, there were calculated concentrations ratio of these acids. Results are represented in Table 1.

Table 1. The results of Moldavian wines analysis (2010-2011 y.), obtained in the next conditions: liquid chromatograph LC-20AD (*Shimadzu*), stationary phase NUCLEODUR C18 Pyramid (*Macherey-Nagel*) 250x4mm, 35ºC, flow rate – 0,5ml/min; eluent – 0,2% H_3PO_4.

Acid	**Red dry wines**			**White dry wines**		
Tartaric acid, g/dm^3	1,5-3,7			0,7-3,9		
∑(mal+lact), g/dm^3	58%	24%	18%	45%	32%	23%
	1,0-2,0	2,0-3,0	other	1,0-2,0	2,0-4,5	other
Tartaric acid / ∑mal+lact	46%	33%	21%	45%	34%	21%
	1,0-2,0	0,5-1,0	other	0,5-1,5	<0,5	other
Citric acid, g/dm^3	0,10-0,91			0,13-0,88		
Tartaric acid / citric acid	59%	22%	19%	51%	26%	23%
	5-10	>15	other	2,0-4,5	6-10	other

Also in presented samples were found ratio of content of tartaric acid to citric acid (concentration of citric acid, together with tartaric and malic acids, depends on the maturity of the grape). Results of investigation are shown in Fig.1-3.

a) 

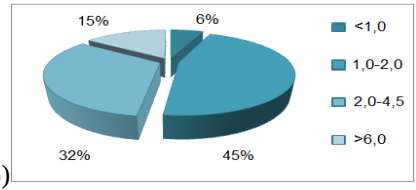
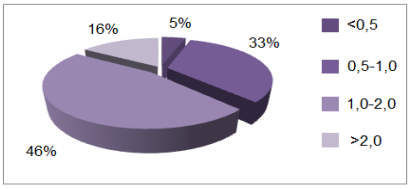
b) 

Fig. 1. a) The diagram of results of the sum malic and lactic acids in red dry wines (g/dm^3); b) the diagram of results of the sum malic and lactic acids in white dry wines (g/dm^3).

a) 

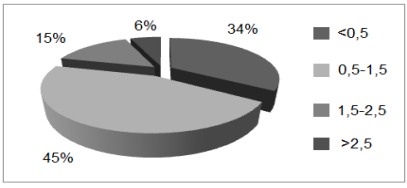
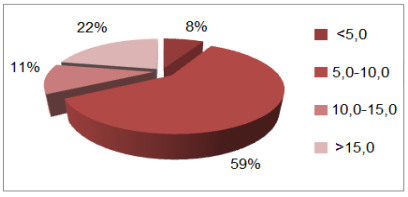
b) 

Fig. 2. a) The diagram of results of the ratio: tartaric acid to the sum malic and lactic acids in red dry wines; b) the diagram of results of the ratio: tartaric acid to the sum malic and lactic acids in white dry wines.

a) 

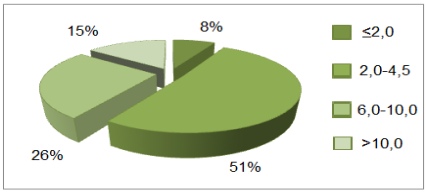
b) 

Fig. 3. a) The diagram of results of the ratio: tartaric acid to citric acid in red dry wines; b) the diagram of results of the ratio: tartaric acid to citric acid in white dry wines.

Practical work also shows that the original (authentic) and counterfeit products differs significantly on qualitative composition of organic and amino acids. In the laboratory of National Center for Quality Testing of Alcoholic Beverages were investigated near 100 arrested samples with doubtful origin using HPLC as reliable, exact and relatively express method of analysis, which doesn't require difficult sample preparation.

Most of investigated wines had the low degustation appraisement, as the confirmation had the extremely high level of citric or tartaric acid as a demonstration of artificial acidification of wine; or extremely low level of natural tartaric and malic acids that can show the fact of using raw with not grape origin or diluted materials [10].

The results of some investigation with HPLC method are represented in *Fig.4*, *5*.

In addition, in counterfeit products was found content of sugars and glycerin by HPLC. Example of chromatogram is shown in *Fig.6*. High level of glycerin content (more than 8,0g/dm^3 for white wines, and 10,0g/dm^3 for red wines) also talks about falsification of wine, as well as the ratio glucose/fructose >1,0 (according to the recommendation of OIV ratio glucose/fructose needs to be less than 1,0), presence of synthetic colorants (use of which is not permitted in natural grape wines, according to

the Government Decision RM Nr.229 of 29.03.2013, SanPin 2.3.2.1293-03). Synthetic colorants can also be determined by HPLC method of analysis (Fig.7).

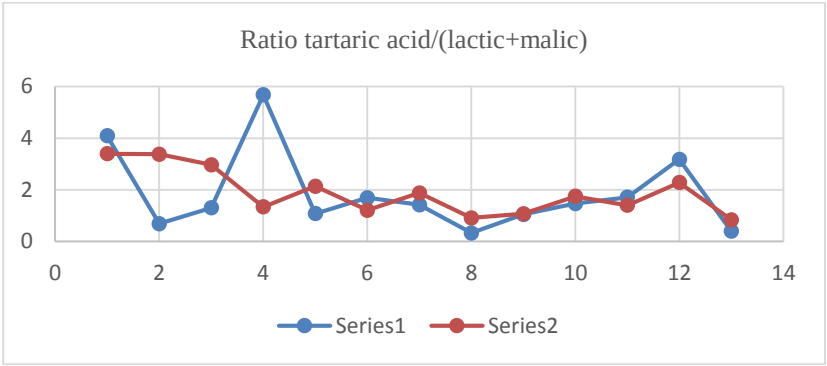


Fig. 4. Comparative characteristics (ratio: content of tartaric acid to sum lactic acid, malic acid) of two lines of wine samples – with low degustation point (series1) and high one (series2).

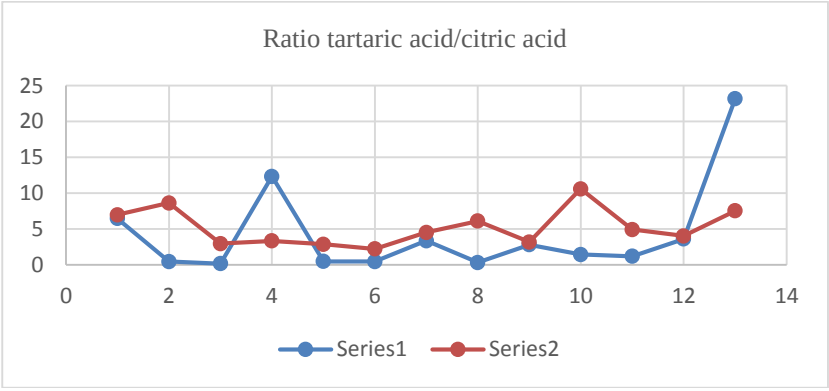


Fig. 5. Comparative characteristics (ratio: content of tartaric acid to citric acid) of two lines of wine samples – with low degustation point (series1) and high one (series2).

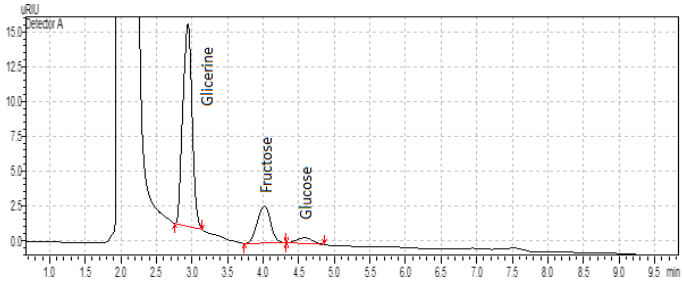


Fig. 6. Chromatogram of red dry wine, obtained in next conditions: chromatographic column – Nucleodur NH2-RP 250/4,6mm; eluent – 80:20 acetonitrile-water; flow rate – 1,7ml/min; 38°C; detector – RID 10A.

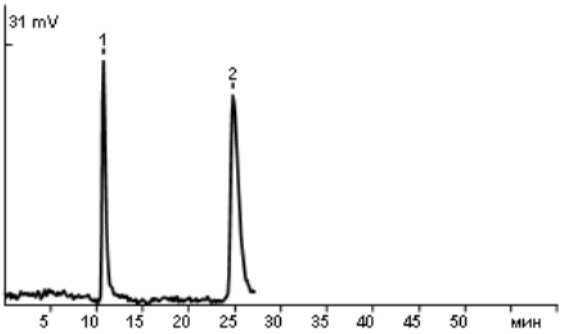


Fig. 7. Chromatogram of standard solution of tartrazine (1) and indigo carmine (2). Luna C8 150x4,6 mm; eluent – phosphate buffer pH 4,2:acetonitrile – 95:5; flow rate – 1,0 ml/min; 20°C; 500nm.

On the issue of determination of wine quality and origin can also be used such time-consuming and relatively expensive methods of analysis like GC-MS, micro-elemental analysis (ICPE). In complex with HPLC these modern methods of analysis can give us full information about natural origin of the wine sample.

Conclusions

Taking into account obtained data it may be noted that Moldavian wines have some characteristic features, so content of tartaric acid in red wines was determined in diapason 1,5-3,7 g/dm^3, and 0,7-3,9 g/dm^3 in white wines, which corresponds to the data about the grape growing region [11].

The results of citric acid content were 0,10-0,91 g/dm^3 for red wines, and 0,13-0,88 g/dm^3 for white wines respectively. The ratio tartaric acid to the sum malic acid with lactic acid was 1,0-2,0 for the most red wines, and 0,5-1,5 for the most white wines. The ratio tartaric acid to citric acid were 5-10 and 2,0-4,5 for red and white wines respectively.

Certainly, this direction of investigation needs more statistical dates for identifying characteristic profiles of separate wine variety, of wine made from grape grown on different vineyard belonging to different wineries. Such statistical data together with other evaluation criteria can help in purpose of further eliminating counterfeit products, traceability of quality production.

Taking into account that HPLC as a complex method of analysis can give us answers on many parameters of wine such as acids content, concentration of sugars, presence of saccharose, maltose, synthetic colorants, etc., relatively low cost of this method, and fact that by HPLC can be determined nonvolatile components in contradistinction to GC, we can propose HPLC like independent complex method of determining the quality and natural origin of wines.

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