ANALYSIS OF ORGANIC ACIDS IN GRAPE AND WINE BY PRIOR FRACTIONATION ON SPE

EVALUAREA FRACȚIILOR DE SEPARARE PENTRU ANALIZA ACIZILOR ORGANICI DIN STRUGURI ȘI VINURI

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Abstract. *Analysis of organic acids from wines haze some restriction related to the phenolic compound present in the matrix who can be retain very strongly to the stationary phases. The purpose of the study is the development of efficient separation methods that can be used both for the analysis of organic acids in the wine and grapes. The grapes and wines varieties analysed are Zghihară, Fetească regală, Fetească albă, Busuioacă de Bohotin, Fetească neagră, Merlot and Cabernet Sauvignon. The extraction of acids from grapes was made on activated charcoal, C18 and SDVB materials. Acid separation is done in two ways: one is using two columns with C18 stationary phase and the second one is with an ion exchange stationary phase as a pre separation column. In the case of grapes analysis, the methods are limited by the level of solid material used in extraction that can ranged results from 80 to 105% recovery. These methods can be useful for analysing 10 organic acids with little to no sample preparation.*
Key words: *grape, liquid chromatography, organic acids, wine*

Rezumat. *Analiza acizilor organici din vinuri prezintă câteva vicisitudini, în special, din punctul de vedere al compușilor fenolici prezenți în probe, care pot se pot reține puternic pe faza staționară. Scopul acestui studiu/articol este să dezvolte metod eeficiente de separare, care să poată fi utilizate la analiza acizilor organici atât la vinuri cât și la struguri. Soiurile de struguri și vinurile analizate sunt: Zghihară, Fetească regală, Fetească albă, Busuioacă de Bohotin, Fetească neagră, Merlot și Cabernet Sauvignon. Extracția acizilor organici din struguri a realizată cu cărbune activ, C18 și pe SDVB. Strategia de separare aplicată pentru acizi a fost dual: prima cu utilizarea a două coloane cu fază staționară C18 și a doua cu ajutorul unei coloane de schimb ionic pentru pre-separarea amestecului de substanțe. Experimental s-a observat că în cazul strugurilor metoda este limitată de cantitatea de material adsorbant folosită la extracție, astfel nivelul de recuperare a variat între 80 și 105%. Aceste metode pot fi utilizate pentru analiza a 10 acizi organici, fără eliminarea altor interferenți din probele analizate sau clean-up.*
Cuvintec heie: *struguri, cromatografie de lichide, aciziorganici, vin*

INTRODUCTION

Acid determination has a great importance for the characterisation of wine composition evolution with implication in chemical and biochemical processes.

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The grape contains most of the acids involved in the glycolytic and shikimic acid pathways as well as in the Krebs and glyoxylic acid cycles, the rest remaining unmodified, being transmitted in wine (Ribereau-Gayon *et al.,* 2006).

For the analysis of the acids the are different methods that are all using liquid chromatography, but basically is ion chromatography with litter ionic strength eluents and separation phases, as shown by the methodology of separation know-how at core level (*** OIV, 2015: OIV-MA-AS313-04:R2009; OIV-MA-AS313-17:R2004)

MATERIAL AND METHOD

The grapes and wines varieties analyzed are noted as follows: 1. Zghihară, 2. Fetească regală, 3.Fetească albă, 4. Busuioacă de Bohotin, 5. Fetească neagră, 6. Merlot and 7. Cabernet Sauvignon from the Ampelographic Collection of U.S.A.M.V. Iaşi, Copou centre, Iaşi. The grapes were processed using the classical fermentation for white grape variety and maceration-fermentation technology for red grape variety, as follows: after crushing and destemming, the marc was homogenised, Saccharomyces cerevisiae selected yeasts (30 g/100 kg), and pectolytic and proteolitic enzymes (1.5 g/100 kg) were added in case of red varieties. The white marc was pressed with a hydraulic press and then the must is put in 25 L glass vessel for alcoholic fermentation. The maceration-fermentation was done in 35 L static plastic vessels, for 72 hours, with pumping over the must six times/ day, for 15 minutes. After the end of maceration-fermentation, the marc was pressed with a hydraulic press, the resulted wine ending in demijohns of 25 L for finishing its alcoholic fermentation. A week after the alcoholic fermentation ended, the wine was racked and fined. Bottling was done after filtering with SA-995 plaques.

The extraction experiments for acids content from grapes was made on following material: activated charcoal, C18 and SDVB materials. The cartridges were activated with specific methodology and the final loading is done with 12% ethanolic solution.

For separation of the acids a Shimadzu Prominence LC20 series was used with the following composition: 2 quaternary pumps LC-20AD with DGU-20A5 degassers; SIL-20AC autosampler (20 °C thermostatic controlled sample temperature); columns oven CTO-20AC (at 20 °C); diode array detector SPD-M20A, FCV 20AH valve system; system controller CBM-20A coupled via LAN to an external PC unit where LabSolution 5.3 is controlling, collecting and process the chromatographic information. Two column are used: Prevail Organic Acid 250-4.6-5 (column 1 – pre-separation column) and-or Prevail Organic Acid 150-4.6-3 (column 2 – separation column). The injecting volume is 2 µL. The flow rate is at 0.9 mL/min with some variation for the washing the column 1 (second pump) for economy of solvents. The program developed is isocratic with two pumps: first pump for eluting the compound of interest on a series the 2 columns (loading) and the second pump is for washing the first column. When the acid are eluted from the first column to the second column the valve is switch so the second pump is washing the first column of phenolic compounds and the first pomp is continuing to separate the acids in the second much faster column.At minute 8.8 the position of the changing valve is changed for washing the column 1 with water then acetonitrile (AcCN)and at minute 25 the changing valve is putting the 2 columns in series so the hall system is re-equilibration until 45 min.

RESULTS AND DISCUSSIONS

During the first attempts we have used only one column as the OIV methodology sugest (OIV-MA-AS313-04:R 2009). A Supelco 610H S-DVB 300×7.8 mmcolomn, 7 µm, for an injection of 10 µL, in isocratic mode at 0.5 mL/min. with 10 mMphosphoric acid (H_3PO_4). The optimal temperature is around 30 °C for the common detection at 210 nm for the: 1 –tartaricacid; 2 – malicacid; 3 – lacticacid; 4 –shikimicacid; 5 –fumaricacid; 6 –succinicacid; 7 –citricacid and 8 – aceticacid. We spotted some problems so the method is good, but only at low temperatures around 15-20 °C, but with peak broadening, works at low concentration of acid so you need to guess the best dilution for musts. For wines this method is not good due to overlapping peaks, so we tried the other version with two columns: a Superspher RP-8 250-4 mm and an Prevail Organic Acid 250-4.6 mm both 5µm. The injection is 10 µL elution isocratic at 0.4 mL/min. (which has generatedat 20 °Ca maximum at ~190 bar backpressure) for the elution system KH_2PO_4 70 g/L, $(NH_4)_2SO_4$ 14 g/L, pH 2,1 adjusted with H_3PO_4. The separation is good in this case but the backpressure and the salts are clogging the LC system.

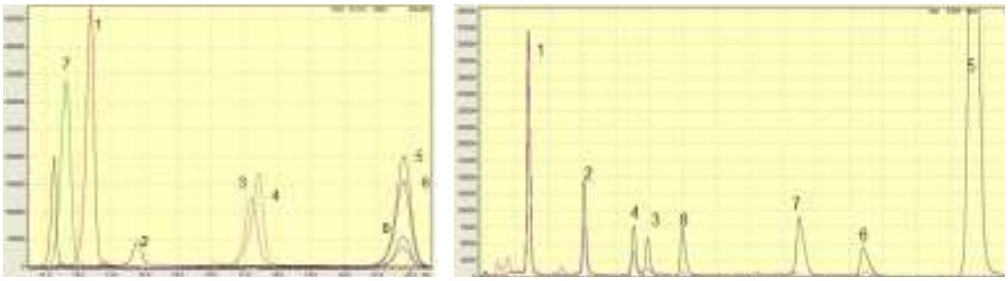

Fig. 1 - Separation of organic acids with well-known methods

This method is good but also remain the problem with SPE clean-up and the long time that is taking for the acid to elute.

Taking this in the consideration the experience (figure 2) we improve the method of separation by 2 columns, but in this case the backpressure is reduce and the elution time due to the shorter and more faster columns is greatly improved. Because the results are now better we introduced other 2 acids that can be separated as well. This method is well suited for grapes (must) or for wines (fig. 3) without the usage of the SPE cartridges (fig. 4). The presence of phenolic compound is showed in figure 4 so the washing of the firs column is a must. In the past methods a large peak is appearing in the chromatogram due to the tannins in red wine so the SPE clean-up in mainly important to red wines. But a problem raised from the utilisation of clean-up cartridges: what is the recovery rate and this can influence the natural distribution in must-grapes.

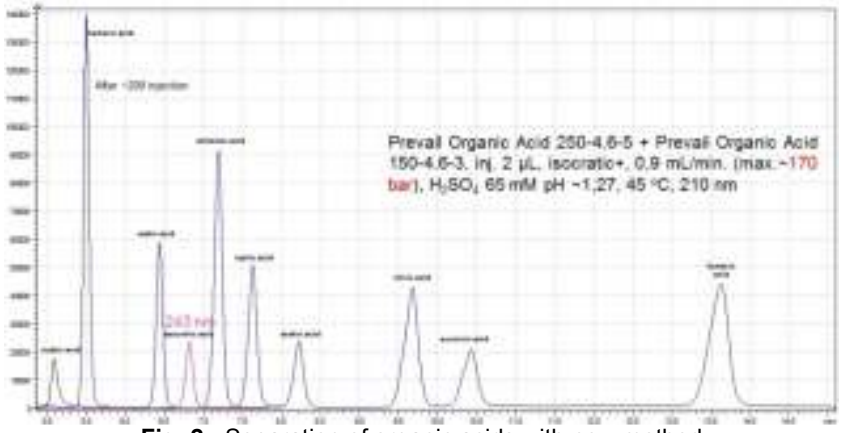

Fig. 2 - Separation of organic acids with new method

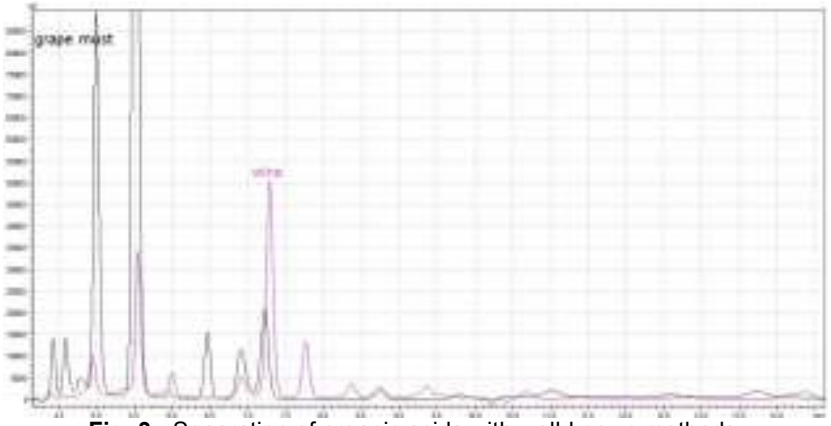

Fig. 3 - Separation of organic acids with well-known methods

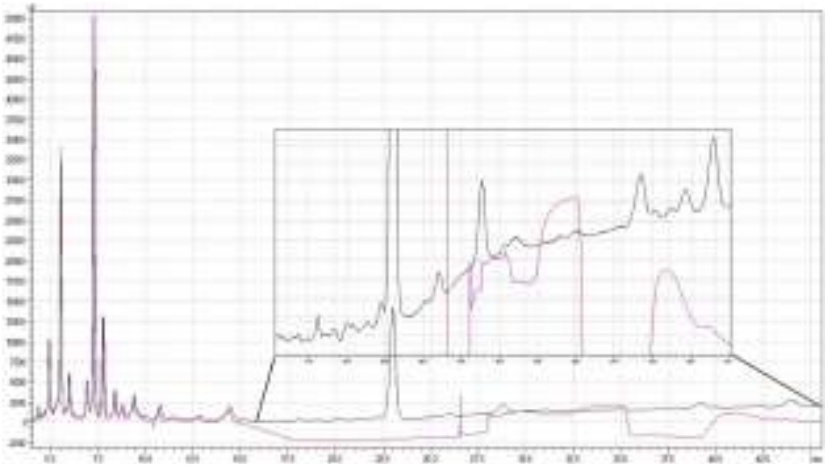

Fig. 4 - Effect of column washing and re-equilibration

In table 1 are the results for the recovery rates in the case of main organic acids. The percentage is the result of three repetition on the same cartridge and a three different cartridges, so we have sufficient information. This is the case of Cabernet Sauvignon grapes and the results confirm that the composition is influenced because the standard deviation varies more than 5% and in some cases of acids the recovery with standard deviation do not reach the initial concentration. Some other factor can be responsible for the results but for now this step can by eliminated if is possible.

Table 1

Recovery of main organic acids2014 harvest

Extraction material for 10 g red grapes / Recovery (%)	tartaric acid	malic acid	lactic acid	acetic acid	citric acid	succinic acid	fumaric acid
activated charcoal 3 g	85±15	87±13	88±9	90±13	95±4	96±9	94±10
C18 3 g/600 mL	88±11	91±11	94±10	85±22	92±10	97±2	92±7
Lichrolut EN (40-120 µm) 3g/600 mL, SDVB	90±17	82±16	89±15	90±15	99±11	91±4	99±8

With this method we analysed directly with-out clean-up the content of organic acids in grapes (table 2) and we saw large amount of malic acid greater then tartaric. Interesting is the presence of shikimic acid in the grapes as a stress related factor in rose and grape grapes.

Table 2

Grape/must organic acids content in the 2014 harvest

value±SD (g/L) from direct must	tartaric acid	malic acid	shikimic acid	citric acid
Fetească regală	7,40±0,20	6,73±0,57	-	0,29±0,05
Fetească albă	6,13±0,17	6,62±0,56	-	0,30±0,05
Busuioacă de Bohotin	5,35±0,14	6,65±0,57	0,026±0,004	0,74±0,13
Fetească neagră	7,31±0,20	8,26±0,70	0,093±0,013	0,31±0,05

After a period of 6 mouths the processed wine is opened and the same procedure without clean-up is used to evaluate the acid content of the samples. The Zghihara wine is the most acidic product because the content of tartaric, malic and also succinic acid is high. The succinic acid is the result of fermentation process and in some cases has the influence of the wine taste attribute. Beside Zghihara this compound is in high concentration at red wines. Another factor that is present an these results is evidence that the bacterial fermentation at red wine from Merlot and Cabernet Sauvignon, kwon as malo-lactic fermentation, is finished do to the larger amount of lactic acid and the low amount for malic acid. The citric and acetic acid have high content at the Merlot based wine do to some secondary fermentation mainly acetic during malo-lactic fermentation. Fumaric

acid is in low concentration (mg/L) but he plays an important role in wine protection metallic hazes.

Table 3

Wine organic acidscontent in the2014 harvest, value±SD (g/L)

tartaric acid	malic acid	lactic acid	shikimic acid	acetic acid	succinic acid	citric acid	fumaric acid
4,50±0,07	3,24±0,28	0,14±0,01	0,071±0,010	1,19±0,13	2,29±0,21	0,18±0,03	0,0001±0,00007
2,30±0,06	0,93±0,08	0,12±0,01	0,011±0,002	1,49±0,16	0,79±0,07	0,15±0,03	0,0007±0,00016
1,93±0,05	0,82±0,07	0,71±0,07	0,008±0,001	2,07±0,23	0,80±0,08	0,15±0,03	0,0047±0,00014
1,55±0,04	1,85±0,16	0,23±0,02	0,007±0,001	1,33±0,15	0,62±0,06	0,37±0,06	0,0012±0,00028
1,71±0,05	1,46±0,12	0,17±0,02	0,022±0,003	1,32±0,15	1,26±0,12	0,15±0,03	0,0051±0,00122
2,67±0,11	0,05±0,01	1,80±0,17	0,042±0,006	3,37±0,31	1,37±0,18	0,47±0,08	0,0004±0,00010
2,78±0,08	0,07±0,02	2,40±0,23	0,028±0,004	2,40±0,27	1,12±0,10	0,19±0,07	0,0003±0,00006

1. Zghihară, 2. Fetească regală, 3. Fetească albă, 4. Busuioacă de Bohotin, 5. Fetească neagră, 6. Merlot and 7. Cabernet Sauvignon

CONCLUSIONS

1. In the present research work, we demonstrated that this methods can be useful for analysing 10 organic acids, directly from wine with little to no sample preparation.

2. Different materials can influence the concentration of organic acids.

3. Characterization of the grapes, must and wine acid composition to make the necessary correction of composition, if they are necessary.

Acknowledgments: *This research was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. Ribereau-Gayon P., Dubourdieu D., Donèche B., Lonvaud A., 2006, - *Handbook of Enology Volume 1 – The Microbiology of Wine and Vinifications***,** 2nd Edition, John Wiley & Sons.

2. *, 2015 -** *Compendium of international methods of wine and must analysis, International Organisation of Vine and Wine*, electronic version, Paris.