COMPUNDS TRAPPED IN THE CO_2 FLOW OF BUSUIOACĂ DE BOHOTIN ALCHOOLIC FERMENTATION

Gabriel Florin LĂCUREANU[1], Valeriu V. COTEA [1], Cintia COLIBABA[1], Marius NICULAUA[1]

e-mail: gabilacureanu@gmail.com

Abstract

The volatile aromas that are lost during the must's fermentation into wine represent a department that is not very much analysed. The capturing and analysing of the volatile compounds that are trapped in the CO2 flow during gas exhaustion of the fermentation stage are the main objective of the present study.The Busuioaca de Bohotin grapes, harvest of 2011, were processed according to the aromatic wine technology. During fermentation, the volatile aroma compounds were captured using SPE cartridges attached to the airlocks of the fermentation vessels. After the fermentation ended, the extracts were obtained by washing the bed of the SPE cartridges with 2 mL diclormethane. Gas-chromatography coupled with mass-spectrometry was used to identify the captured compounds.

Key words: Busuioacă de Bohotin, aroma compounds, exhaust CO_2

Wine organic volatile compounds are probably the most researched and analysed by winetasters and scientists alike. They are responsible for the bouquet and flavour of the wine, being the base of that wine's individuality and specificity. Busuioaca de Bohotin is one of the oldest aromatic Romanian varieties, with flavourful wines, usually sweet, with nuances of „melted rose, honeysuckle and pears". The responsible chemical compounds for these sensorial characteristics are still not very well determined. Wine's birth starts with the alcoholic fermentation, meaning the conversion of sugars into ethanol and carbon dioxide. During this stage, the majority of the aroma compounds are also formed, contributing to the final flavour of the wine. Wine's aroma is influenced by the grape variety used, the maturity degree of the grapes at harvest, yeasts' activity, prefermentative techniques applied and of course, last but not least, the technology used for maturation and aging of wine.

A part of the varietal aroma compounds as well as some of the fermentation aroma compounds are lost during the fermentation phase, being lost together with the evacuated carbon dioxide. It is a scientific acknowledged fact that a part of the aroma compounds are lost (Nechita, 2010), being trapped in the CO2 flow. The monitoring of the volatile compounds formed during certain processes in food products is a difficult and time-consuming procedure. A certain material is needed for trapping the volatile compounds that tend to flow out, as well as a specific analysis method for trace quantities.

This study aims at analysing the volatile aroma compounds lost during alcoholic fermentation (Nasrawi, CW, Wahlstrom, VL, Fugelsang, KC, Miller CG, 1990; Muller CJ, Wahlstrom VL and Fugelsang KC, 1993). The compounds will be trapped by use of Solid Phase Extraction Lichrolut cartridges with a good capacity of retaining volatile aroma compounds.

MATERIAL AND METHOD

Busuioacă de Bohotin grapes were harvested from Pietroasa vineyards in Dealu Mare, in 2011. The grapes were processed according to the methods listed in the specific literature (Cotea et. al., 1986) for aromatic wine production. After destemming and crushing, a 24 hours maceration was applied, for a better extraction into the must of the varietal aroma compounds. Commercial pectolytical enzymes were used for improvement of aroma profile (Zymoclaire M ®). Pressing was achieved by using a hydraulic press, commercial selected yeasts were then added for controlled fermentation (Fermactive Muscat ®). the obtained

1 University of Agricultural Sciences and Veterinary Medicine Iaşi

must was öeft for fermentation in 25 L glass vessels.

Trapping of volatile compounds lost during the fermentation process within the CO2 flow was done thus: the SPE cartrdiges were first conditioned with (6 mL diclormethane, 6 mL ethanol and 6 mL ethanol solution (14%) were passed through the C18 bed) and fixed to the fermentation airlocks so that the exhaust CO2 flow, together with the volatile compounds passed through them. The volatile substances were „trapped" in the SPE device for further analysis.

Figure 1 **Traping aroma compounds within the CO_2 evacuated during Busuioacă de Bohotin must fermentation**

After the fermentation process ended, the attached SPEs were disconnected and analysed. The volatile compounds were obtained by washing the SPE bed with 2 mL diclormethane.The obtained extract was injected into a Shimadzu GC coupled with a QP2010 Plus mass- spectrometer.

1000 μL extract are injected into a Supelco SLB 5 ms GC column, 15 m length, column oven temperature 30 oC, injection temperature 250 oC, in splitless mode, initial temperature 30 oC for 1 minute, then if grows at a rate of 8 oC until 240 oC where it stays for 2.75 minutes. The carrier gas was Helium, column flow 0.75 mL/min, ion source temperature 250 oC, interface temperature 250 oC, detector voltage 0.9 kV. The aroma compounds were determined by means of the NIST 08, Wiley 08 and SZTERP spectrum library. The program lasts for 30 minutes.

RESULTS AND DISCUSSIONS

The volatile compounds perception is important for the wine taster as a high amount of the pleasure is determined by the wine's flavour and bouquet. The wine industry is aware that the percentage of wine consumption depends on its sensorial properties and that even a tiny change in flavour can differentiate wines in superior or inferior quality classes and higher or lower prices. Busuioacă de Bohotin wine is one of the most appreciated Romanian wines, therefore, it was considered necessary to to determine specific aroma volatile compounds from the fermentation gasses. The chromatogram below was obtained as a result of the analysis of the identified volatile compounds from processing the fermentation exhaust gas captured in SPE cartridges during Busuioacă de Bohotin must fermentation and their extraction with diclorومethane.

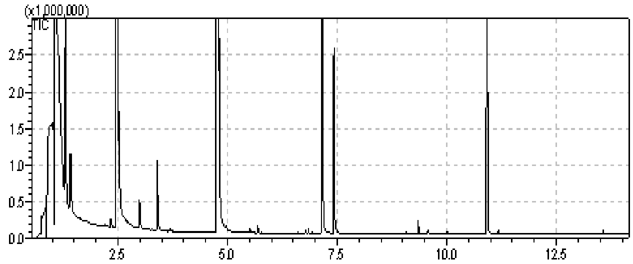

Figure 2 **Chromatogram of volatile compounds identified in the CO_2 flow of Busuioacă de Bohotin must fermentation**

There are thee types of aroma compounds, according to their origin, that can be identified in wines: primary aromas, compounds that are found in the grapes and remain in the must and later wine, during the wine-making process, secondary aromas, generated through fermentation , through the action of yeasts and bacteria, composing, from a qualitative and quantitative point of view, the highest percentage of volatile compounds, and thirdly, the tertiary aromas, formed during maturation and aging of wine, in wooden barrels or in glass bottles.

The volatile compounds identified during the first phase of the wine-making process of Busuioaca de Bohotin are part of compounds specific to wine: alcohols, terpenes, esters. These compounds were identified and characterised according to their peak. The identified compounds were grouped in chemical classes. In the class of esters with fruits and spices odours the following esters were grouped: ethyl acetate, isobutyl acetate, octyl acetate, acetic acid, heptyl ester and ethyl hexanoate.

Although acetic acid is known to be able to produce spoilage in wine in high concentrations, its esters that are o product of its degradation, can impart pleasant aromatic notes to the wine sensorics. Therefore, ethyl acetate, one from the above mentioned esters, is a sub product of ethanol dehydrogenation. It is the most common ester found in wine, being a result of acetic acid's degradation. its aroma is more powerful in young wines and contributes to their fruity aroma. An exaggerated quantity of ethyl acetate is considered a wine fault. Isobutyl acetate is a result of the esterification of isobutanol and acetic acid. It is one of the esters found in wine in high concentrations. It has a floral, fruity aroma in small concentrations. It is naturally found in pears, rasberry and other plants (www.wine-pages.com/guests/tom/taste2.htm). At higher concentrations, the smell can become unpleasant.

Table 1

Volatile compounds identified in CO2 exhaust flow				
No	Retention time	Area	Identified compound	Aroma
Esters				
1	1.303	6323831	Ethyl Acetate	fruits
2	2.997	518969	Isobutyl alcohol	flowers, pears
3	3.406	1404318	Ethyl butirate	pineapple
4	5.523	72359	Isobutyl acetate	pears, bananas
5	5.696	138023	Methyl caproate	floral
6	7.162	12206749	ethyl hexanoate	fruits
7	7.430	3507581	1-Hexyl acetate	aromatic
8	9.352	238459	Acetic acid, heptyl ester	woody
9	9.573	78324	Methyl octanoate	pineapple, apples
10	10.920	6901811	Ethyl octanoate	oranges
11	11.178	53417	Octyl acetate	oranges
Alcohols				
1	1.418	3738858	Isobutyl alcohol	solvent-like
2	2.471	22169067	1-Butanol, 3-methyl-	banana
Terpenes				
1	13.571	58258	Citronellyl acetate	rose, citrsy

Ethyl butirate or ethyl butanoate is an ester with a fruity aroma, similar to that of pineapple. Isoamyl acetate or isopentil acetate is an organic compound, an ester fromed from the reaction of isoamilic alcohol and acetic acid. It has a powerful aroma, similar to that of the chewing gum “Juicy Fruit”, usually described as nuances of banana and pears (www.wine-pages.com/guests/tom/taste2.htm). The term “banana oil” describes the odour of pure isoamyl acetate.

Ethyl hexanoate is a product of hexanoic acid, fatty acid with an umpleasant aroma of old mouldy cheese and barnyard animals. However, the ester has a pleasant fruity smell, of apple peel. Methyl octanoate is an ester with characteristic fruity sensorial properties of green citrus fruits. Ethyl octanoate or ethyl caprilate has a sensorial profile reminiscent of blackcurrants, that, in higher concentrations, forms the main aroma of Pinot noir wine. Hexyl acetate has a fruity sensorial impact, being naturally found in fruits. Heptyl acetate is the ester fromed from the reaction of 1-heptanol and acetic acid. It has a fruity smell, with nuances of wood and rum. The taste is floral, slightly spicy, with a fatty and soapy texture. Isobutylic alcohol is part of the organic alcohols' class with a specific solvent smell, used in the industry as solvents.

3 methyl-1-butanol (known as isopentilic alcohol or isoamilic alcohol) is one of amylic alcohol's isomers. This is the main ingredient in the production of banana oil and is used as a spice in the food industry as well. Only one compound from the terpenes class was identified: citronelyl acetate with a floral, fruity small, of rose-like odour and of lemon grass. It is used in the pharmaceutical industry for its soothing effect.

CONCLUSIONS

During the alcoholic fermentation of must, a part of the volatile aroma compounds are lost, by flying out within the evacuated CO_2 flow. The following compounds were identified: esters (the majority), alcohols and terpenes, subtracting thus from the sensorial profile of the wine. The present study brings its value to the department of volatile compounds identification, especially compounds that are lost during the alcoholic fermentation, from the point of view of trapping and analysis method. In future articles, a quantitative approach will be the main focus, in order to evaluate what percentage of the total aromatic compounds are lost during fermentation

ACKNOWLEGMENTS

The publishing of this article was made possible due to the Oenology team at USAMV Iasi. The author wishes to thank the “Basilescu winery” firm for providing the grapes for the experiment.

REFERENCES

Cotea, V.D., Sauciuc, J.,1988, *Oenology treatise.*

Miller, G.C., Amon, J.M., Simpson, R.F., 1987, *Loss of aroma compounds in carbon dioxide effluent during white wine fermentation*, Food Technology Australia 39, 246-249, 253.

Muller C.J., V.L. Wahlstrom and K.C. Fugelsang. 1993, *Capture and use of volatile flavor constituents emitted during wine fermentatation*. In Beer and Wine Production. American Chemical Society. Washington, D.C.

Nasrawi, C.W., Wahlstrom, V.L., Fugelsang, K.C., Muller C.J., 1990, *Capture and utilization of emission control volatiles*, Presented at Grape Day. California State Univ. Fresno, Nov. 3rd. CATI Publication #901102.

Nechita B., 2010, *"Contribuţii la studiul compuşilor volatili din strugurii şi vinurile obţinute în podgoria Cotnari"*, Teză de doctorat, USAMV Iaşi.

Ron S. Jackson, 2000, *Wine Science: Principles, Practice, Perception,* second edition, 2000, 3Academic Press, California, USA.

Williams, A.A. and Boulton, R.B., 1983, *Modeling and prediction of evaporative ethanol loss during fermentation*, Am.J.Enol.Vitic 34, 234 – 242.

***www.wine-pages.com/guests/tom/taste2.htm

***www.wikipedia.org