USING GAS - CHROMATOGRAPHY TO DISTINGUISH THE WINE AND STILL FERMENTED BEVERAGES

UTILIZAREA GAZ-CROMATOGRAFIEI ÎN VEDEREA DIFERENȚIERII VINURILOR ȘI BĂUTURILOR FERMENTATE LINIȘTIT

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Abstract. *For several years, in order to harmonize the Romanian legislation with the European one, was introduced the notion of "still fermented beverages". This is a beverage obtained by fermentation, but it is not explanained what raw materials are used in the production process and their contribution to the alcoholic strength of the final product. For this reason, some manufacturers are tempted to sell such drinks as the wine. We conducted a series of refermentation of pomace from red and white winemaking and volatile compoundsfrom these drinks were analyzed by gas chromatography coupled with mass spectrometry. The mass spectra of the compounds were compared with the spectra from Wiley and other libraries. We made an identification of volatile components comparing them with those identified in wine.*
Key words: *wine, fermented beverages, gas chromatography*

Rezumat. *De câțiva ani, în vederea armonizării legislației românești cu cea europeană, a fost introdusă noțiunea de "băutură fermentată liniștit". Conform definiției este o băutură obținută prin fermentare, dar nu există precizari din punct de vedere al materiilor prime utilizate în procesul de producție și de aportul acestora în concentrația alcoolică a produsului finit. Din acest motiv, unii producători sunt tentați de a comercializa aceste băuturi sub numele de vin. Pentru diferențierea acestora s-au efectuat o serie de refermentări ale boștinei provenite din vinificația în alb și în roșu, iar compușii volatili din aceste băuturi au fost analizați prin gaz-cromatografie cuplată cu spectrometria de masă. Spectrele de masă ale compușilor au fost comparate cu librăriile de spectre Willey și NIST. S-a efectuat o identificare a componenților volatili și compararea lor cu cei identificați în vinuri.*
Cuvinte cheie: *vin, băuturi fermentate, gaz cromatografie*

INTRODUCTION

Being an enjoyable beverage, wine enjoyed its fair share of attention from people, either positively or negatively. The positive aspect has resulted in optimization of wine making technologies, focusing especially on storage conditions, maturation, stabilization and conditioning.

The negative aspect materialized in producing fake wines for money purposes. The authors Baxter et. al, 1997; Mihalca et al, 2002; Nămăloşanu et al, 2005; Bulancea et al, 2009 present the most common used practices in faking wines, both

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in our country and in other countries with traditions in this aspect.

If until half a century ago forgery was achieved simply by adding sugared water, nowadays forgery methods have improved. The fake alcoholic beverages now have the flavor, aroma, color of original wine, but also the physical-chemical characteristics recorded on the analysis sheet, within the parameters of a correct wine.

Since 2007, when Romania entered the European Union, production of still fermented beverages was legalized. This led to the marketing of many drinks that virtually contain almost no wine. In most cases, on the label one finds written either fermented ceverage with flavor, synthetic sweetener, citric acid, colouring substances, etc. Unfortunately there is no trace of wine. However, the labels use suggestive words and ideas that there would be wine in that beverage, as a way of bypassing the law that prohibits the use of viticultural symbols,

Legally not every product can be marketed as wine. The definition of wine as specified in the Vine and Wine Law no. 224 from 2002, Annex 2 states: "Wine is a beverage produced exclusively through the complete or partial alcoholic fermentation of fresh grapes, crushed or uncrushed, or grape must, while its alcoholic strength may not be less than 8.5 % volumes".

Unfortunately, by definition, a still fermented beverage is a beverage obtained by fermentation, but there still are no explanations in terms of raw materials used in the production process and their contribution to the alcoholic strength of the finished product. Thus, the producers' temptation to market the drink as wine is understandable .

Accordingly, the Laboratory of Enology of USAMV Iaşi initiated a series of preliminary tests regarding the possibility of data differences between the volatile content of fermented wines and still fermented beverages in the presence of fermentation yeasts as well as bread yeasts (Kohn et. al., 1961; Salim–ur Rehman et.al., 2006; Poinot et.al., 2008).

MATERIAL AND METHOD

To attain the proposed objective, refermentations of the pressed grape marc from white winemaking were set up. In this respect, yeasts already used in the original must fermentations as well as commercially available bread yeast were applied.

The obtained variants are noted thereby

- ✓V1 - Wine yeasts, wine, inverted sugar;
- ✓V2 - Wine yeasts, water, inverted sugars;
- ✓V3 - Bread yeasts, wine, inverted sugars;
- ✓V4 - Bread yeasts, water, inverted sugars.

Upon completion of fermentation, the volatile compounds of the resulted beverages were extracted by liquid-liquid extraction and were analyzed by gas chromatography coupled with mass spectrometry, the mass spectra of the compounds being compared with spectra libraries Willey and NIST to certify the identity of the identified chemical compounds.

The liquid - liquid extraction was carried out with a mixture of dichloromethane and pentane (1:1) from the wine distillate obtained by entrainment with water vapours for determining the alcoholic strength.

A Shimadzu 2010 MS Plus gas-chromatograph coupled with QP-2010 mass spectrophotometer detector and AOC 5000 PAL Combi autosampler with liquid injection

system was used for separation and identification of the volatile compounds.

The column used was formed from two connected columns. First column was AT-Wax from Alltech-Grace – polar (lenght 30 m, diameter 0,25 mm, film thickness 0,25 μm) and second was SolGel mS from SGE – non-polar (lenght 60 m, diameter 0,25 mm, film thickness 0,25 μm).

The GC conditions were as follows: injection temperature 250°C; oven: initial temperature 35°C, equilibration time 5 minutes, temperature program 2 °C/minute up to 100°C, equilibration time 15 minutes, temperature program 3 °C/minute up to 150°C, equilibration time 15 minutes, temperature program 4 °C/minute up to 200°C, equilibration time 15 minutes, temperature program 4 °C/minute up to 220°C, equilibration time 60 minutes; flow controller: splitless. MS and qualitative parameters were as follows: EI acquisition: 0.85 kV, mass range 50-300 m/z, in order to be able to confirm the identified heavy compounds.

RESULTS AND DISCUSSION

Identification of volatile compounds in the four variants of beverages was based on chromatograms. A compound was considered identified if the probability indicated by the software was higher than 90%, and the confirmation of the compound's identity was made by AMDIS software, provided by NIST library.

As it is registered, and as it was expected, in the case of variants V3 and V4, where bread yeasts were used, a lower number of compounds were identified than in the variants where selected yeasts for winemaking were used. Also, the intensity of the signals in the chromatogram was much lower in the variants with bread yeast.

In the variants V2 and V4, where bread yeast, water and sugar were used, the number of identified compounds was lower than the variants where water was replaced with wine.

The identified compounds have been divided into classes for ease of analysis. Their quantification was performed by using the area percentage of each compound (Tables 1, 2, 3 and 4).

The alcohols composition of the samples, Table 1 proves the fact that the refermented wines, regardless of the yeast type (V1 and V3) will ultimately lead to the production of a wider range of alcohols, in contrast to the variants in which only fermentation of yeast bread with water and sugar occurred.

Among these, 3-methyl-1-butanol and 2-methyl-1-butanol are identified, as alcohols that appear always during fermentations. Benzylic alcohol was identified only in the case of fermentations with bread yeast, while leaf alcohol (3-hexen-1-ol) was recorded only in the case of refermented wines. This aspect can lead to finding a first difference between refermented wines and fermenting a mixture of yeasts, water and glucose/fructose.

Analysing the acid composition (table 2) of the studied samples, one registers the major component represented by acetic acid in the case of fermented mixture of water and glucides (V2 and V4) while in the case of refermented wines, this acid is barely detectable.

Table 1

Identified alcohols in the chromatograms of the 4 variants (area percentage)

Compound name	V1	V2	V3	V4
propanol	0.91		0.64	0.88
iso butyl alcohol	4.61	0.45	1.06	1.32
n-butanol	0.51			
3-methyl-1-butanol	16.15	5.33	44.01	24.63
1-pentanol	0.19	0.32		
2-methyl-1-butanol		2.06	3.48	2.44
3-ethoxy-1-propanol				0.68
3-methyl-1-pentanol	1.39		0.55	
3-ethoxy-1-propanol			0.43	
3-hexen-1-ol, leaf alcohol	0.49		0.41	
1-hexanol	3.09			
1-butoxy-2-propanol			0.62	
1-heptanol	0.16			
2-ethyl-1-hexanol	0.44		0.10	
3-(methylthio)-1-propanol	1.39	0.05		0.23
ho-trienol	0.22			
benzyl alcohol			0.18	0.04
phenethyl alcohol	1.98	37.31	17.65	47.37
4-ethyl-2-methoxy-phenol	0.54			

Table 2

Identified acids in the chromatograms of the 4 variants (area percentage)

Compound name	V1	V2	V3	V4
acetic acid		42.63	1.24	14.80
propionic acid				0.32
isobutyric acid		0.43		0.34
2-methyl-propanoic acid				0.31
butanoic acid	0.48		0.83	0.15
pentanoic acid, valeric acid				0.63
3-methyl-butanoic acid	3.53		3.32	2.20
2-methyl-butanoic acid	2.64		3.57	
hexanoic acid	3.29		2.35	
2-ethyl-hexanoic acid			0.26	
caprylic acid	16.49		3.53	

In addition, a number of acids, 2-methyl-butanoic acid, hexanoic acid, 2-ethyl-hexanoic acid, caprylic acid, n-decanoic acid, could be identified only in case of refermented wines (V1 and V3) but not in the variants V2 and V4. It is noteworthy that caprylic acid is found in greater proportion in the case of refermented wines in the presence of yeasts (V1).

Although, from a percentage point of view, identified esters are inferior to identified alcohols or acids, their number is much higher (Table 3). As in the previous cases, a greater variety of esters are found in the refermented wines compared to the mixture of inverted sugar and water. This can be explained by the

presence of a majority of esters before the refermentation begun.

It is worth noting that, independent of the variables (V1 - V4), towards the end of the analysis, a number of phthalates, which normally should not be registered, were identified: 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, diisooctylphthalate, dibutyl phthalate.

Table 3

Identified esters in the chromatograms of the 4 variants (area percentage)

Compound name	V1	V2	V3	V4
ethyl acetate	1.67	0.20	0.91	1.24
propyl acetate			0.07	
ethyl propanoate	0.34		0.17	
ethyl isobutyrate			0.09	
isobutyl acetate	0.15			
ethyl 2-methylbutyrate			0.04	
ethyl isovalerate			0.05	
isoamylacetate	1.16		0.26	
propanoic acid, propyl ester		1.35		
ethyl hexanoate	2.03		0.70	
butanoic acid, 2-hydroxy-3-methyl-, ethyl ester				0.06
butanoic acid, 3-hydroxy-, ethyl ester	0.87	0.06	0.07	
ethyl 2-hydroxycaproate	0.53		0.07	
octanoic acid, ethyl ester	8.57		0.24	
ethyl 4-hydroxybutanoate			1.33	0.28
butanedioic acid, diethyl ester	10.36		1.45	1.94
benzoic acid, 2-hydroxy-, methyl ester			0.20	
acetic acid, 2-phenylethyl ester	0.53		0.60	0.04
ethyl caprate	2.66		0.12	
ethyl phthalate		0.17	0.20	0.01
methyl myristate	0.23		0.13	
isobutyl phthalate		0.08	2.20	0.03
hexadecanoic acid, methyl ester	0.69		0.56	
ethyl palmitate	0.70			
dibutyl phthalate	0.49		1.05	
octadecanoic acid, methyl ester	1.24		0.92	
1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester		9.26		
diisooctylphthalate			1.88	

In addition to the above compounds (alcohols, acids, esters) other components were recorded as well (Table 4). Thus, the observations regarding the higher content of components identified in the case of refermented wines compared to the other variants are confirmed. It was also noticed that, in the case the refermented wine is aromatic (V1), some terpenes present previous to the refermentation can be found.

Table 4

Other identified compounds in the chromatograms of the 4 variants (area percentage)

Compound name	V1	V2	V3	V4
acetal	0.14			
decane			1.24	
butyrolactone		0.12		0.03
benzaldehyde	0.18	0.04		
limonene	0.29			
2-furanmethanol				0.01
1,3,6-octatriene, 3,7-dimethyl-, (z)-	0.21			
linalool l	1.04			
3-cyclohexene-1-methanol, .alpha., .alpha. 4-trimethyl-	0.31			
n-(3-methylbutyl) acetamide	0.24			
l-citronellol			0.42	
nerol	0.77			
3,7-dimethyl-1,5-octadien-3,7-diol	0.64			
5-hexyldihydro-2(3h)-furanone, γ-decalactone			0.18	
2,4-bis(1,1-dimethylethyl)-phenol	0.49			
5-(hydroxymethyl)-2-furancarboxaldehyde		0.13		

CONCLUSIONS

The varied content of volatile compounds, the presence or absence of some as such as leaf alcohols, make possible to clearly differentiate between a refermented wine and a beverage resulted from the fermentation of a mixture of water and inverted sugar. At this level, the results do not allow distinguishing between wines refermented with bread yeast and those refermented with yeast remaining from fermentation, which becomes necessary proof for further studies in this regard.

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