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Toxicological patterns of alcoholic beverages based on congeners gas-chromatographic analysis

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Abstract: We may find a number of volatile congeners in alcoholic beverages, mainly high-class alcohols. Identification and quantification of these substances may reveal certain patterns useful both in drivers' blood alcohol concentration interpretation and in forensic pathology for toxicological evaluations.

Aim of our study is to detect structural patterns in most frequently consumed beverages in Romania, namely brandies, home-made fruit spirits (tuica) and beers. We analyzed 31 samples of beverages using a gas-chromatographic self-validated method for qualitative analysis of congeners (methanol, acetone, 1 and 2-butanol, n-propanol, isobutanol, 2-methyl-1butanol) and quantitative determination. Principal component analysis was the statistical method used for identification of trends in the multidimensional data set.

Beers showed the most homogenous cluster while home-made spirits revealed a large dispersion and variation regarding their chemical structure. Methanol is the principal component of discrimination efficiency among the groups of beverages.

Key Words: volatile congeners, cluster, alcoholic beverages, chemical structure.

During the second part of the last century instrumental analytical methods have developed and alcoholic beverages analysis brought new information about components that accompany ethanol as a consequence of the fermentation process and also about their effect on human body. These substances are collectively called „congeners" [1, 2].

Alcoholic beverages may be divided into three general classes, depending on the manufacturing process and the types of agricultural products used to make them: wines, fermented malt beverages (mainly beers) and distilled liquors also called spirits (e.g. rye, bourbon, blend of brandy, scotch, gin, vodka, rum) [2].

Spirits (vodka, gin) have less congeners than beers or wines because many substances are not distilled due to the boiling temperature [3]. Classification of congeners is made according to the method used for their identification: acidic, basic, volatile, semi-volatile and non-volatile. Some congeners have been identified constantly in alcoholic beverages: acetone, methanol, 1-butanol, 2-butanol, methyl-ethyl-cetone, isobutanol, 2-methyl-1-butanol, 3-methyl1-butanol, n-propanol [2, 3].

In Romania alcoholic beverages have not been described systematically until now in terms of congeners' presence and concentrations. The market is dominated by commercial products but home-made spirits are still widely consumed. Our study aims to:

- identify and measure the volatile congeners in some Romanian commercial and home-made beverages, according to the Commission Regulation (EC) 2870/2000 laying down Community reference methods for the analysis of spirit drinks [4].
- identify alcoholic beverages group patterns (beers, commercial brandies, home-made spirits) based on congener analysis.
- investigate of the medico-legal interpretation possibilities when expertise involving the drivers statements on alcoholic beverages consumption is requested and discuss the relevance of beverages structure for postmortem analysis interpretation.

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MATERIAL AND METHOD

We used a "Konik" gas-chromatograph with flame ionization detector, capilar carbowax column (length 30 m), injection 1 microliter. Carrier gas was nitrogene with a pressure of 10 ml/min, split injection with ratio 1:5; as combustion gas we used synthetic air with hydrogen. Detector temperature was 2600 C and injector temperature 2500 C. For the oven we designed a gradual temperature increase from 70˚ C with a rate of 5˚C/minute up to 110˚C. The resulted signals are processed by a Hewlett Packard data acquisition system with DDS Clarity Master software package.

Prior to beverages analysis we went through the stages of method validation in terms of linearity, detection and quantification limits, accuracy, precision and specificity.

The samples of alcoholic beverages studied included Romanian manufactured brandies found on the market, home-made fruit distilled spirits from different regions (ţuica) and beers.

The statistical method used was principal component analysis (PCA), in order to identify unknown trends in a multidimensional data set [5]. Our aim was to determine if the alcohol congeners may describe a valid pattern of the beverages and to establish the discriminating efficiency of different congeners. Covariance tells us whether changes in any two variables move together, thus we built up the covariance matrix and interpreted the positive or negative associations.

RESULTS AND DISCUSSIONS

The alcoholic beverages samples have been analyzed and alcohol congeners were identified and measured as represented in tables 1 (brandies and home-made fruit spirits = ţuica) and 2 (beers). Applying the PCA method for brandies and ţuica we obtain the matrix of differences between the average value and each sample value (Table 3) and we build up the covariances matrix as represented in Table 4.

High values of a congener x associated with high values of a congener y shows a positive covariance while a negative covariance results from high values of y combined with low x values. Zero covariance means there is no association between x and y [5, 6].

We convert the covariance matrix in square distances matrix according to the formula: $d_{ij} = \sigma^2{}_i + \sigma^2{}_j - 2^*\sigma_{ij}$, where: σ_{ij} is the variables covariance, $\sigma^2{}_i\ \sigma^2{}_j$ -i and j variables variances respectively. Small distances mean small dissimilarities and high covariances. This method is a correlation between variables and the result is represented by recognition and analysis of different patterns in a data set [7]. If we use this method and evaluate data in table 5 we may conclude that methanol on the one hand and the other congeners on the other hand will determine the two beverages groups (brandies and ţuica) to separate into two clusters.

The scatter plot type graphical illustration (figure 1) of the two main components: PC1 – acetone, 1 and 2-butanol, n-propanol, isobutanol, 2-methyl-1butanol) and PC2 – methanol, shows two clusters: one more grouped (brandies) and one more scattered (ţuica).

The difference of homogenity concerning the principal components among our two groups of beverages is justified by the manufacturing methods: brandies are industrial made and certain parameters concerning the raw material and the distillation process are more controlled, while home-made ţuica is more heterogeneous. In figure

Table 1. Congeners concentration in studied beverages (mg/l) and statistics (average, standard deviation, variance)

X=	Acetone	metanol	2butanol	Npropanol	izobutanol	1butanol	2metil1butanol
Jidvei	0	523.31	11.65	144.34	318.35	44.54	216.18
Kvint	5.69	273.2	0	127.83	173.28	15.29	635.93
alexandr	2.79	24.09	0	0	9.74	0	42.1
Unirea	0.61	0.23	0	0	0	9.52	1.45
Miorita	12.31	892.91	60.96	219.16	277.65	17.78	1055.2
Milcov	0.5	258.58	0	74.13	88.09	0.73	403.57
Tequila	52.22	590.16	0	138.43	140.43	0	598.47
tuica am	16.62	2877.97	129.45	520.1	250.88	48.14	578.37
tuicamere	16.71	3015.19	0	209.21	264.49	79.73	1172.16
tuicprunems1	1.04	1802.47	0	166.35	81.16	20.27	294.26
jidvei under	8.61	34.79	0	0	7.65	0	34.05
tuicaprunems2	36	4170	49	1141	581	38	868
tuicaprunems3	30	4028	56	395	309	28	640
tuicaprunemm	37	3558	43	480	390	74	767
tuicaamsj	40.12	3710.32	61.15	700	1092	13	1235
tuicabnam	25	554	134.56	76	390	8	610
Media	17.82	1644.576	34.11063	274.4719	273.3575	24.8125	571.9838
Σ	16.862	1617.777	45.62478	308.8186	271.9921	25.55586	391.3743
Σsqu	284.327	2617203	2081.62	95368.93	73979.7	653.102	153173.9

Table 2. Congeners' concentration in beers (mg/l)

Beer type and ethanol concentration	Acetone	Methanol	2-butanol	n-propanol	isobutanol	1-butanol	2-metil1-butanol
Golden Brau 5%	-	8.87	-	5.29	6.12	-	14.49
Ciuc 5%	-	1.17	-	6.73	7.39	-	28.13
Gosser 5.3%	3.75	7.26	-	6.70	5.63	-	16.92
Silva 5.1%	-	4.26	-	6.58	8.61	-	34.25
Noroc 4.5%	-	5.13	-	5.56	4.18	-	16.85
Bergenbier 5%	-	8.26	-	9.55	7.80	-	41.42
Skol 4.6%	3.49	7.48	-	5.87	5.63	3.68	45.82
Neumarkt 5.2%	-	43.85	-	11.46	7.14	-	25.22
Heineken 5%	-	210.075	-	70.91	21.82	-	40.95
Ciucaş 4.7%	-	15.58	-	8.44	6.63	-	37.22
Timişoreana 5%	3.43	9.73	-	15.61	12.9	-	51.33
Ursus blonde 5%	3.41	7.13	-	6.81	4.46	-	24.06
Ursus dark 6%	4.76	9.85	-	7.02	3.81	-	27.10
Borsodi 4.6%	-	0.98	-	1.62	1.59	-	1.97
Stejar strong 7%	-	29.59	-	18.47	7.38	-	52.41

Table 3. Matrix of differences between average of congeners and each congener.

Y=	aceton	Methanol	2butanol	npropanol	izobutanol	1butanol	2metil1butanol
	-17.82	-1121.27	-22.4606	-130.132	44.9925	19.7275	-355.804
	-12.13	-1371.38	-34.1106	-146.642	-100.078	-9.5225	63.94625
	-15.03	-1620.49	-34.1106	-274.472	-263.618	-24.8125	-529.884
	-17.21	-1644.35	-34.1106	-274.472	-273.358	-15.2925	-570.534
	-5.51	-751.666	26.84938	-55.3119	4.2925	-7.0325	483.2163
	-17.32	-1386	-34.1106	-200.342	-185.268	-24.0825	-168.414
	34.4	-1054.42	-34.1106	-136.042	-132.928	-24.8125	26.48625
	-1.2	1233.394	95.33938	245.6281	-22.4775	23.3275	6.38625
	-1.11	1370.614	-34.1106	-65.2619	-8.8675	54.9175	600.1763
	-16.78	157.8938	-34.1106	-108.122	-192.198	-4.5425	-277.724
	-9.21	-1609.79	-34.1106	-274.472	-265.708	-25.5559	-537.934
	18.18	2525.424	14.88938	866.5281	307.6425	13.1875	296.0163
	12.18	2383.424	21.88938	120.5281	35.6425	3.1875	68.01625
	19.18	1913.424	8.889375	205.5281	116.6425	49.1875	195.0163
	22.3	2065.744	27.03938	425.5281	818.6425	-11.8125	663.0163
	7.18	-1090.58	100.4494	-198.472	116.6425	-16.8125	38.01625

Table 4. Covariance matrix

A=	aceton	metanol	2butanol	npropanol	izobutanol	1butanol	2metil1butanol
Acetone	284.3414	16013.25	268.3455	3025.946	2717.19	70.85932	3897.512
Methanol	16013.25	2617203	27989.7	420563.5	290910.9	26302.47	414262.6
2butanol	268.3455	27989.7	2081.62	5618.036	5714.508	186.7567	6566.451
Npropanol	3025.946	420563.5	5618.036	95368.93	61332.74	3220.583	70787.71
Izobutanol	2717.19	290910.9	5714.508	61332.74	73979.7	1681.369	77152.32
1butanol	70.85932	26302.47	186.7567	3220.583	1681.369	655.5958	4506.995
2metil1butan	3897.512	414262.6	6566.451	70787.71	77152.32	4506.995	153173.9

Table 5. Matrix of dissimilarities. Euclidian square distances

D=	aceton	metanol	2butanol	npropanol	izobutanol	1butanol	2metil1butanol
Acetone	0	2585461	1829.256	89601.37	68829.65	795.7104	145663.2
Methanol	2585461	0	2563305	1871445	2109361	2565251	1941852
2butanol	1829.256	2563305	0	86214.48	64632.31	2361.209	142122.6
npropanol	89601.37	1871445	86214.48	0	46683.15	89580.87	106967.4
izobutanol	68829.65	2109361	64632.31	46683.15	0	71270.07	72848.95
1butanol	795.7104	2565251	2361.209	89580.87	71270.07	0	144813
2metil1butan	145663.2	1941852	142122.6	106967.4	72848.95	144813	0
STDEV	959482.3	912194.7	951617.7	681901.1	777261	951713.7	697362.2

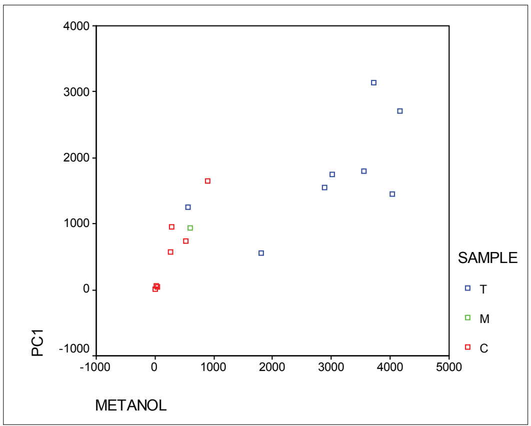

Figure 1. Depict of the beverages clusters (T-ţuica, M-tequila, C-brandies)

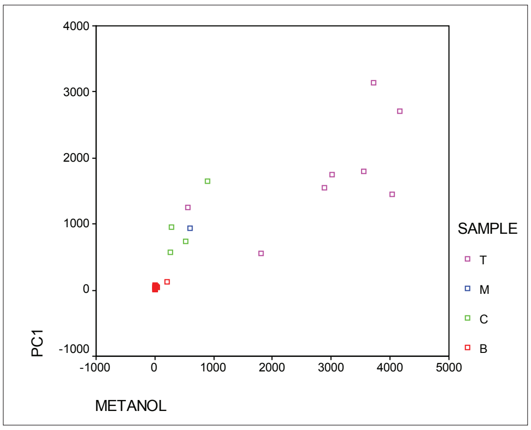

Figure 2. Clusters of all beverages samples (C-brandies, T- ţuica, B- beer, M-tequila)

1 we notice that the product "tequila" manufactured in Mexico is more related to the group of brandies, explained by the industrial manufacturing process. The group of beers represented in figure 2 is the most grouped among all beverages, showing a constant pattern due to the technological process of beer fabrication.

A study concerning irish whiskey authentication concluded that the couple 2-methyl-1-propanol and 3-methyl-1-butanol lead to best separation between irish whiskeys, bourbon whiskeys and scotch single malt whiskeys, the results being explained by the differences in the type of distillation process during manufacturing of these whiskeys [8].

One of the volatile congeners (n-propanol) is considered a good indicator of putrefactive process in tissue, the postmortem production of n-propanol is essentially equal in blood and muscle while ethanol is produced postmortem significantly more in blood [9]. Antemortem ingestion of some home-made distilled beverages with high n-propanol concentration (see table 1) may influence the reasoning of putrefaction based on this congener analysis. On the other hand, it might be considered along with the microbial origin in cases of undetermined or violent death cases with elevated n-propanol concentrations [10].

CONCLUSIONS

Characterization of alcoholic beverages in terms of volatile congeners content and building a monograph of the most frequently consumed drinks is a start point for later blood sample analysis and forensic interpretation of drivers statements regarding type of alcohol consumption.

Principal component analysis is a useful statistical method to define a structural pattern for different types of alcoholic beverages.

Methanol is the main component of discriminating efficiency between industrial manufactured and home-made traditional spirits consumed in Romania.

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