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## Analysis of methanol and its derivatives in illegally produced alcoholic beverages



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#### ABSTRACT

*Introduction:* Illegal alcohol production remains as a common issue worldwide. Methanol poisoning mostly occurs because of the methanol used in production of counterfeit alcohol instead of ethyl alcohol due to its low price or by drinking the liquids containing methyl alcohol. Pectolytic enzymes results in an increase of methanol levels in many fermentation products such as ciders or wines. Methanol poisonings are infrequently encountered in forensic medicine practice. However, sporadic cases due to methanol intoxication as well as epidemic cases have been reported. In this study, we aimed to identify existence of methanol and its metabolites in illegally produced alcoholic beverages used in Antakya region.

*Material and methods:* Twelve legally produced alcohol samples and Fifty-six different illegally produced alcohol samples were collected from the markets and local producers. Existence of methanol, formic acid, methyl amine, methyl formate and trioxan were determined using GC–MS method in these samples.

*Results:* Fifty-six different illegal alcohol samples were analyzed in this study and methanol was detected in 39 (75%) of samples. Formic acid was detected in 3, formamide in 1, methyl amine in 6, methyl formate in 10 and trioxan in 2 samples.

*Conclusion:* Overwhelming majority of illegal alcoholic beverages was detected to contain methanol. Interestingly this study also revealed the presence of trioxane, which has not previously reported among toxic agents in illegal alcohol samples.

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#### 1. Introduction

Unrecorded alcoholic beverage production and consumption is of high importance in Turkey and around the world.<sup>1–3</sup> According to the 2009 report of World Health Organization illegal or unrecorded alcohol is defined as "Homemade or informally produced alcohol (legal or illegal), smuggled alcohol, alcohol intended for industrial or medical uses, alcohol obtained through cross-border shopping (which is recorded in a different jurisdiction), as well as consumption of alcohol by tourists".<sup>4</sup> The consumption of unrecorded alcohol, however, varies from region to region it accounts for approximately 30% of total worldwide alcohol consumption. Illegal alcohol consumption is the highest in Europe, particularly in Eastern Europe, which followed by South America and Africa. Fifty percent of alcohol consumed in Ukraine in 2005 is reported to be unrecorded/illegal<sup>5</sup> (Table 1). According to 2010 World Health Organization Global Information System on Alcohol and Health (WHO-GISAH) nearly thirty percent of alcohol consumed in Turkey is unrecorded.<sup>6</sup>

Pectolytic enzymes results in an increase of methanol levels in many fermentation products such as ciders or wines and especially illegal produced bevarages. Methanol might be added to ethyl alcohol during production of illegal alcohol. Therefore, methanol poisonings may occur due to consumption of unrecorded alcohol. Deaths due to epidemic as well as sporadic methanol poisoning

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#### Table 1

Global distribution of unrecorded adult per capita alcohol consumption 2005 (calculation based on WHO).

WHO region	Unrecorded adult per capita alcohol consumption in l pure ethanol	Total adult per capita alcohol consumption in l pure ethanol	Proportion unrecorded			
Africa	1.93	6.19	31%			
Americas	2.01	8.70	23%			
Eastern Mediterranean region	0.34	0.62	55%			
Europe	2.67	12.20	22%			
South East Asia region	1.52	2.24	68%			
Western Pacific region	1.63	6.23	26%			
World	1.75	6.13	29%			

cases have been reported. Additionally, a number of methanol intoxication related applications to the Emergency Services are noticed.  $^{7-11}$ 

The toxicity of methanol is caused by its metabolites rather than methanol itself. Methanol is firstly converted to formaldehyde. Then, formaldehyde is quickly converted to formic acid, which is mainly responsible for toxic effects.<sup>12–15</sup> Formamide, also known as methanamide, is an amide derived from formic acid. Formamide, is a raw material used in the formation of some pesticide or herbicide formulations and in the production of hydrocyanic acid.<sup>16</sup> Methylamine is an organic compound, of which formula is CH<sub>3</sub>NH<sub>2</sub>. It is a colorless gas derived from ammonia. It has a large industrial use and is found as a solution in methanol, ethanol, or is sold as the anhydrous gas in pressurized metal containers. Methylamine is commercially produced by the reaction of ammonia with methanol in the presence of silicoaluminate catalyst.<sup>17</sup> TheLD<sub>50</sub> (mouse, s.c.) is 2.5 g/kg.<sup>18</sup> Trioxane, cyclic trimer of formaldehyde, is used for the production of highly resistant chemicals with perfect mechanical properties such as poly-oxymethylene polymers. Thus, this polymer is used in heavy duty mechanical parts such as in valves or gear boxes.<sup>19</sup>

This study aims to determine the presence of methanol and its derivates in illegally produced unrecorded alcoholic beverages in south regions of Turkey that is famous with traditional homemade alcohol production. In the literature there are studies about methanol in bogma raki.<sup>20,21</sup> in this study, we analyzed more toxic and lethal derivatives of methanol in Bogma Raki, an alcoholic beverage unique to Turkey and this region in particular.

#### 2. Materials and methods

As alcoholic beverages samples, 56 different illegally produced "Bogma Raki" samples were collected from the local producers and 12 legally produced "Raki" samples were purchased from the licensed producers. Both commercial and bogma raki are produced by same way, however more sensitive distillation method are used in commercial raki. Samples of each product were decanted into sterile glass bottles, allocated codes by one of the investigators (MM) to enable blind testing. Methanol, ethanol, formic acid, formamide, acetaldehytde, methyl amine, methyl formate, acetic acid, iso-amyl alcohol, trans-anethole, propionic acid, 1–butanol, 1propanol, ethyl acetate, 2-propanol and trioxanlevels were analyzed in obtained alcohol samples using GC–MS.

#### 2.1. Chemicals and analytical method

Methanol, ethanol, formic acid, formamide, acetaldehytde, methyl amine, methyl formate, acetic acid, iso-amyl alcohol, transanethole, propionic acid, 1-butanol, 1-propanol, ethyl acetate, 2propanol and trioxan standards were used for qualitative and quantitative analysis of samples. All of the chemicals were obtained from Merck, Darmstadt, Germany.

The samples were analyzed using a GC/MS system (Hewlett–Packard (Palo Alto, CA)) consisting of HP-6890 gas chromatograph, HP-5972 mass selective detector (MSD), and HP-6890 automatic liquid sampler. Separations of compounds as Methanol, ethanol, formic acid, formamide, acetaldehytde, methyl amine, methyl formate, acetic acid, iso-amyl alcohol, trans-anethole, propionic acid, 1-butanol, 1-propanol, ethyl acetate, 2-propanol and trioxan were performed using HP-FFAP (25 m, 0.2 mm i.d., with 0.33  $\mu$ m film thicknesses) cross-linked capillary column (Hewlett–Packard, Palo Alto, CA).

The GC/MS parameter; the pressure of helium, the carrier gas, was 6.0 bar and the split value with a ratio of 1:100. The injection unit temperature was 250 °C and MS quadrupole temperature was 280 °C. The MS quadrupole detector ionization energy was 70 eV. The initial column temperature was 60 °C for the first 4.0 min, then programmed by 6 °C/min increase to final temperature 160 °C and kept at 160 °C for 4 min.

We prepared Synthetic samples (Methanol, ethanol, formic acid, formamide, acetaldehytde, methyl amine, methyl formate, acetic acid, iso-amyl alcohol, trans-anethole, propionic acid, 1-butanol, 1-propanol, ethyl acetate, 2-propanol and trioxan) and spiked in GC–MS. The results were checked compared and verified by accessing Wiley Database.

A comparison between the retention times of the analyzed samples with those of standard mixture (Merck, Darmstadt, Germany; 99.9% purity specific for GLC), run on the same column under the same conditions, is performed to facilitate identification. Afterwards samples' chromatograms verified by accessing Wiley Database.

#### 2.2. Statistical analyses

Statistical analysis was performed using SPSS software version 15.0. Independent student t-test (t) was used to compare the means of methanol, formic acid, formamide, methyl amine, methyl formate and trioxan concentrations in legal products and illegal products. A p value of <0.001 was considered to be significant.

#### 3. Results

Fifty-six different illegal alcohol samples were analyzed in this study. Composition of samples shown that Table 2. Of these, methanol was detected in 39 (75%) samples. Formic acid was detected in 3 samples, formamide in 1, methyl amine in 6, methyl formate in 10, and trioxan in 2 samples. Percentages of methanol and its derivates and LOD and retention time values in analyzed alcohol samples are shown in Table 3. None of the commercial alcoholic beverages produced under the state control contained methanol or its derivates.

#### 4. Discussion

Methanol poisoning is usually caused by methanol containing illegal alcoholic beverages or ingestion of certain fluids containing methanol.<sup>22–24</sup> In Turkey, almost all methanol poisoning-related deaths are caused by use of methanol containing illegal alcoholic beverages and homemade Bogma Raki.<sup>21,24–26</sup>

Raki is the national hard alcoholic drink, in Turkey and it is similar to several other alcoholic beverages available around the Mediterranean, the Middle East, and in Colombia, e.g., pastis, ouzo, sambuca, arak, and aguardiente. Raki is produced by the licensed industries of the state; however, it might be produced at home

Table 2	
Composition of illegal alcohol samples.	

Acetic acid	Methanol	Ethanol	Iso-amyl alcohol	Trans-anethole	Propionic acid	1-butanol	1-propanol	Ethyl acetate		Formamide	Acetaldehyde	Methyl amine	2-propanol	Methylformate	Trioxan	%	Sample no
	0.35	98.57	0.72	0.10		0.07		0.20									1
0.11	0.32	98.72	0.60	0.13												37.00	
0.10	0.32	98.86	0.00	0.12	0.11	0.60										28.00	
0.06	0.30	98.43	0.86	0.09	0.11	0.20										42.00	4
0.21	0.14	98.04 96.72	1.06	0.35		0.20 0.09	0.11	2.01	0.23							43.00	
0.15	0.22	98.65	0.33 0.56	0.44 0.32		0.09	0.11	2.01	0.25	0.10						45.00 44.00	
0.15	0.22	98.65 97.67	0.56	0.52	0.11		0.14	0.38		0.10	0.06	0.12	0.13			49.00	
0.57	0.31	97.67 97.67	0.45	0.50	0.11	0.14	0.14	0.38			0.00	0.12	0.15			45.00	
	0.33	98.38	0.79	0.23		0.14		0.27								38.00	
0.36	0.55	98.99	0.20	0.13			0.12	0.27	0.20							41.00	
0.15	0.30	98.76	0.58	0.22			0.12		0.20							49.00	
0.21	0.13	97.93	0.58	1.01			0.09									55.00	
0.21	0.15	96.48	1.02	1.35		0.21	0.09	0.69				0.12				70.00	
		96.42	1.01	1.39		0.21	0.08	0.03				0.12		0.12		71.00	
0.12	0.34	97.41	0.72	1.16		0.15	0.10	017 1						0112		47.00	
0.12	0.35	97.44	0.73	1.12		0.14	0.10									47.00	
0.12	0.35	97.42	0.75	1.13		0111	0.10	0.11								47.00	
	0.00	97.50	0.73	1.07		0.14	0.10	0.11							0.35	47.00	
0.95	0.25	97.98	0.68	1107		0111	0110	0.1.1							0.50	47.00	
0.00	0.20	98.00	0.68	0.01		0.13		0.94							0.25	47.00	
	0.19	96.73	0.82	1.32		0.13		0.76							0.25	52.00	
	0110	96.33	0.32	1.82		0.11	0.10	1.15						0.17		55.00	
0.57	0.22	97.26	0.26	1.62		0.06	0110							0117		56.00	
0.79	0.22	96.65	0.41	1.77		0.09	0.05							0.22		56.00	
0.25	0.20	96.67	0.11	2.03		0.45	0.07					0.20		0.22		63.00	
1.59		95.81	0.25	1.93	0.11		0.07							0.20		56.00	
0.96		96.65	0.47	1.45	0.11	0.09	0.06							0.21		56.00	
		96.72	0.48	1.83		0.09	0.06	0.59						0.18		60.00	
		96.86	0.22	2.26				0.51				0.16				53.00	
0.56		96.83	0.74	1.59		0.10	0.06							0.13		47.00	
		96.22	0.59	1.85		0.12	0.10	0.29						0.18		50.00	
	0.18	96.03	0.24	1.81	0.11	0.06	0.05	1.49								58.00	
		96.88	0.23	2.14		0.05		0.52				0.19				53.00	
		96.60	0.51	1.86		0.10	0.07	0.63				0.19				60.00	
0.06	0.20	96.36	0.78	1.47		0.14	0.06	0.85						0.19		47.00	
	0.17	98.13	0.79	0.11		0.21	0.06	0.53								36.00	
	0.09	99.39		0.52												22.00	
	0.22	98.71	0.81			0.16	0.07	0.03								24.00	
	0.01	99.24	0.18	0.39		0.07		0.03						0.14		28.00	40
	0.07	99.77		0.16												30.00	41
	0.14	98.35	1.00	0.23		0.16		0.12								40.00	
	0.18	98.34	0.96	0.20		0.25	0.06									30.00	
	0.18	98.03	1.27	0.13		0.16		0.23								34.00	
	0.14	98.39	1.00	0.19		0.15		0.12								35.00	45
	0.15	98.73	0.55	0.12		0.12		0.33								36.00	46
	0.36	98.00	0.74	0.49		0.15	0.10	0.16								38.00	47
	0.34	97.97	0.95	0.37		0.18		0.19								31.00	
	0.29	98.10	0.88	0.24		0.15		0.34								41.00	
	0.18	98.39	0.38	0.42	0.10			0.52								45.00	50
	0.17	97.93	0.77	0.25		0.18		0.70								34.00	51
	0.21	98.26	0.58	0.66				0.30								38.00	
	0.21	97.55	0.50	1.58		0.09		0.08								38.00	53
	0.13	97.36	1.00	1.14		0.13	0.11	0.14								39.00	54
	0.14	98.50	1.13			0.13		0.09								38.00	55
	0.23	98.41	0.52	0.46				0.14	0.24							53.00	56

 Table 3

 Percentages of methanol and its derivates in analyzed alcohol samples.

Uncontrolled produ	ced alcohol	Min	Max	LOD	R.time <sup>b</sup>
Mean (%) ± SD	n:56				
0.220 ±0 .0890	n*:39	0.01	0.36	0.79 <sup>a</sup>	4-5
0.223 ±0 .0208	n*:3	0.20	0.24	1.2 <sup>a</sup>	6-7
0.100± -	n*:1			1.33 <sup>a</sup>	34-35
0.163 ±0 .0361	n*:6	0.12	0.20	2.7 <sup>a</sup>	0-1
0.174 ±0 .03406	n*:10	0.12	0.22	1.9 <sup>a</sup>	3-4
$0.300 \pm 0707$	n*:2	0.25	0.35	1 <sup>a</sup>	27-28
	$\begin{array}{c} \mbox{Mean} (\%) \pm \mbox{SD} \\ 0.220 \pm 0.0890 \\ 0.223 \pm 0.0208 \\ 0.100 \pm - \\ 0.163 \pm 0.0361 \\ 0.174 \pm 0.03406 \end{array}$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$

 $\mathbf{n}^*$ : Sought material or number of the samples containing the metabolite SD: Standard deviation.

<sup>a</sup> ng/ml.

<sup>b</sup> Min.

illegally by the people who live in the villages in Cukurova region, particularly in Hatay, a city nearby the border of Syria. This Turkish traditional homemade raki is called as "BOGMA RAKI" (Arabic name, Arak tini) and it is produced from grapes, figs, or plums. Because of its low cost and special taste, a number of alcohol users especially prefer to consume Bogma Raki. The price of this kind of beverage is fivefold cheaper than recorded commercial products. The homemade producers generally use their own traditional methods; therefore the ingredients are quite different from recorded Rakis and also from each other. For that reason the compositions of these alcoholic beverages are not standard for ethanol levels, methanol and other ingredients. Because of its low cost people consume Bogma Raki in huge amounts, which is responsible for high morbidity and mortality.<sup>21</sup> The deaths due to alcohol intoxication are not uncommon in legal autopsies in our region.<sup>21,26,27</sup> Furthermore, previously conducted studies revealed that the rate of methanol poisoning-related deaths in autopsy series varies between 0.86% and 2.83%, in Turkey.<sup>25</sup>

Ethyl alcohol levels in illegally produced alcoholic beverages are reported to change between 32% and 53%, with an average of 43% of ethyl alcohol.<sup>21</sup> A study conducted in Estonia revealed that among individuals consumed illegal alcoholic beverages, 59% consumed samogon, 23% other substances such as aftershaves, and 18% illegally produced vodka. The same study also claimed that illegal alcohol consumption was more prevalent among people with lower education and less income levels.<sup>1</sup> Among toxic agents, methanol was detected in 39 (75%) samples, formic acid was detected in 3 samples, formamide in 1, methyl amine in 6, and methyl formate in 10. Furthermore, the present analyses also revealed the presence of trioxane in 2 illegal alcohol samples, which is the first in the literature.

In all illegal samples, methanol contents are in acceptale limits according to Turkish Food Codex Communiqué of Distilled Alcoholic Beverages.<sup>28</sup> However other Methanol derivatives which are detected in our samples are not mentioned in this codex similar to EU.<sup>29</sup> But this chemical products should be considered this kind in grape marc spirits.

It was previously reported that besides methanol, the presence of hepatotoxic long-chain alcohols should also be considered in illegally produced alcohols.<sup>26,30</sup> Furthermore, long chain alcohols are also found in detectable levels illegal alcohols particularly in north-east Estonia, as reported by Lang et al.<sup>1</sup> In accordance with these, the incidence of alcoholic liver disease is reported to be higher in users of illegal alcohol produced in rural areas of India compared to those consuming legal drinks.<sup>3</sup> In another study, conducted in Mexico, high mortality rate associated with cirrhosis was attributed to the home-made alcoholic beverages.<sup>31</sup>

Similarly, in Europe cirrhosis-related mortality rates are reported to be the highest in a region including Slovenia, Hungary, Romania and Moldova, in which the consumption of drinks containing long chain alcohols is common.<sup>1</sup>

Hatay, with its large agricultural areas, is a city located in the south part of Turkey on the Syrian border. Homemade alcohol (known as Bogma Raki) production is a highly common tradition for the people in Hatay, for those living in the villages in particular. Being tastier to villagers habitually, very cheap and quite common, obtaining this kind of alcohol is very easy for everyone, which also contributes common use of illegal alcohol.<sup>21</sup>

#### 5. Conclusion

Overwhelming majority of illegal alcoholic beverages was detected to contain methanol that is responsible for rapid toxic effects. This can be explained by the use of fruits as a main component which was the major source for methanol in spirits. Methanol is commonly produced in fruit based spirits by the action of pectin esterase on pectin found in fruit (especially grapes and plumps), explaining its presence in home-produced alcohol. Interestingly this study also revealed the presence of trioxane, which has not previously reported among the agents in illegal alcohol samples. The common use of illegal alcoholic beverages in our region also indicates high risk of chronic alcoholic liver disease in the study area. Furthermore, considering its guite common use worldwide, illegal alcohol consumption still remains as an important public health problem. There are over 100 studies dealing with composition and toxic ingredients of illegal alcohols in the literature.<sup>32,33</sup> Additionally, further studies investigating toxic effects of minor ingredients of illegal alcoholic beverages on human health are needed.

In order to tackle methanol poisonings, preventive measures should be implemented regarding ingestion of methanol containing liquids. Regular controls of licensed alcohol producers and strict regulations should be implemented against illegal alcohol producers, as well.

#### Conflict of interest

We state that neither the author nor any of the co-author has any potential conflict of interests related to the publication of this paper.

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None declared.

#### Ethical approval

Approval for this study was granted by Mustafa Kemal University.

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