



INVESTIGATION OF THE EFFECT OF DIFFERENT FACTORS ON THE LEVEL OF METHANOL IN SAMPLES OF ARAK AND WINE PRODUCED BY LOCAL TRADITIONAL METHODS IN LATAKIA CITY

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ARTICLE INFO	ABSTRACT
<p>Accepted on: 21-05-2016 Published on: 15-09-2016 ISSN: 0975-8216</p>	<p>Wine and Arak are common traditional alcoholic beverages in Syria. However, they are produced with no monitoring especially during processes of fermentation and distillation. Thus their consumers are exposed to methanol poisoning. This research aims at the determination of methanol in two alcoholic beverages of wine and arak that are prepared according to traditional local methods and at checking that they match the Syrian standard specifications. The collected samples were analyzed using colorimetric method (recommended as a reference method) which requires record the absorbance with a spectrophotometer at 575 nm using ultraviolet-visible spectroscopy. Findings were compared to reference values set by Syrian standard specifications numbered 2478 issued in 2003. Furthermore, factors influence on methanol levels was also studied. It was found that methanol concentrations varied due to fermentation methods, distillation times and thermal processing treatment of grape juice. Strict system of monitoring and controlling is recommended to ensure high quality of popular beverage product</p>
<p>Keywords: Alcoholic beverages, arak, wine(dry & sweet), fermentation, distillation, thermal processing treatment, geographic origin, methanol content, ultraviolet-visible spectroscopy</p> <p>Corresponding author: Dr. Marouf Elkheir, Full Professor, Department of Food Chemistry, college of Pharmacy, Tishreen University, Latakia, Syria</p>	

INTRODUCTION

Although alcoholic beverages tend to have few nutrients, they can be a rich source of energy, as ethanol provides high value of nutritional energy (29 kJ/g or 7 kcal/g)(1). Alcoholic beverages are produced from sugar solutions by alcoholic fermentation (2). Simple fermentable sugars are either found or are yielded by raw materials of fermentation process⁽¹⁾. Beer and wine are most important alcoholic beverages that they were known to

early civilizations. Later on, distillation process was introduced to the production of alcoholic beverages (1). Wine is mainly made of grapes and juices (3). Raki or Arak is the distillate product of alcoholic beverages (4). It has been found that excess average of alcohol consumption increases the risk of the major diseases such as mouth and pharyngeal cancer, esophageal cancer, liver cancer, breast cancer, unipolar severe depression, epilepsy. Besides, alcohol use disorders cause

hypertensive disease, liver cirrhosis. Further, it has been found that *alcohol-related diseases predominantly depend on volume and patterns of drinking*. Most effects of alcohol on disease are detrimental, but for certain patterns of drinking, there are a beneficial influences (5). Methanol is found as an additive in adulterated alcoholic beverages (6). Also, Fermentation can generate methanol as a byproduct at increasingly rate due to improper condition of storage (7). Further, methanol can be produced from enzymatic hydrolysis of pectin in particular under the influence of pectin methyl esterase (8,9). Methanol is the simplest alcohol. It is colorless liquid with an agreeable odor and burning taste. Also, *methanol is water soluble and volatile* (4). Methanol poisoning occurs through many routes such as inhalation and dermal exposure but toxication ensues *most frequently via the oral route* that is ingestion 15ml of 40% methanol is a lethal dose. Theoretically, ingestion of 0.25 mL/kg of 100% pure methanol, assuming complete absorption, results in severe toxic consequences (10,11). Methanol is metabolized in liver by alcohol dehydrogenase first to formaldehyde and by then to formic acid by formaldehyde dehydrogenase. Formic acid is responsible for acute toxicity in methanol poisoning (12). the symptoms of methanol poisoning start with drunk status, sedation of central nervous system. At advanced stage when methanol is metabolized into formic acid, other complications appear to include vision (4), nervous system(13) and digestive system (6,11) Concentration of methanol in alcoholic beverages is influenced by many factors such as pH upon fermentation, the factors leading 1)to *fermentation*, time interval between fermentation and distillation(14)and the primary component parts of fermentation reaction, number of distillation times(8) and thermal treatment of grape juice prior to fermentation(15).Many methods were used for the determination of methanol content ⁽¹⁶⁾ for example High Performance Liquid Chromatography HPLC (17,18), Gas chromatography GC (19) enzymatic method (20) and colorimetric method (recommended as a reference method by ISO and AOAC and ISO) (16). In the present study colorimetric method was used to detect methanol by the reaction of chromotropic acid in a sulfuric medium reaction where methanol was

oxidized to formaldehyde. The amount of formaldehyde was determined by the violet colored complex formed. The intensity of the color is determined by spectrophotometry at 575 nm (21).According to Syrian standard specifications issued in 2003 the acceptable concentration of methanol in Arak of alcoholic beverages is 200mg per 100ml of absolute alcohol.

Due to as the result of anaerobic fermentation of sugar stuffs being used in spirits manufacture, the formed methanol has toxic and harmful effects on consumer's health. High levels of methanol have instant damage. The objects of the present study are as follows:

- Determination of methanol alcoholic beverages that were prepared by locally traditional methods.
- Evaluation of fermentation and distillation methods for the production of Arak and wine in terms of methanol content taking into consideration the factors that influence on the result content of methanol

MATERIALS AND METHODS

THE STUDIED SAMPLES

A total of fifty samples of Arak and wine were collected from different rural areas in Latakia countryside. Arak samples were collected at different stages of fermentation and distillation while wine samples were collected during fermentation and at the step of final product ready- to-drink.

In general, the samples were grouped into two phases i.e. at the first phase 26 samples of Arak and wine were collected, while at the second phase 24 samples of Arak and wine were collected. All samples were stored in a refrigerator at 3–4 C until analysis.

CHEMICALS, APPARATUS, AND INSTRUMENTS

- Sensitive balance, with accuracy 0.0001g of type XB 220 APrecisa
- Spectrophotometer (UV -530V Jasco)
- Rotary evaporator(Buchi)
- Water bath (EMKO,ESM-3711-H)
- Density bottle (MC Pycnometer)
- Chromotropic acid sodium salt (QUALIKEMS).
- Potassium permanganate (Archichem)
- Metabisulfite sodium (Riedel-de Haën)
- Phosphoric acid CBDH)
- Concentrated sulfuric acid (CHEMLAB)

- Standard methanol (BDH)
- Distilled water

ANALYTICAL METHODS

In the present study the colorimetric method was used for the determination of methanol content where the absorbance was measured with spectrophotometer at 575 nm wave length. Then methanol concentration in g/100 l or g/ml was calculated using the following equation (Indian standard alcoholic drinks methods of test, 2005. (Dhar *et al.*, 2013)

$$\text{Methanol} = \frac{A_2 \times C \times D \times 1000 \times 100 \times 100}{A_1 \times S}$$

Where

A₂: absorbance for sample standard solution.

C: concentration of methanol standard solution in g/ml.

D: dilution factor for sample solution.

A₁: absorbance for methanol standard solution.

S: ethanol content of sample in percent (v/v)

SAMPLE DISTILLATION AND ETHANOL PERCENTAGE CALCULATION USING DENSITY BOTTLE

Each 200ml sample of wine taken at stages of fermentation and final production line, as well as to each 200ml sample of Arak taken at step of fermentation, were distilled. 150 ml of each distillate was combined and the percentage of ethanol of all samples was measured using density bottle(22), that is, it was weighed cleaned as Wand then filled with alcoholic beverage and weighed (W1). Then, the density bottle was cleaned, dried and filled with distilled water and weighed (W2). The specific gravity was calculated using the following law:

$$\text{Specific gravity} = \frac{W1 - W}{W2 - W}$$

It was taken into consideration the temperature at which the measurement was done. In order to determine the percentage of ethanol content, the findings were compared with reference values set by Syrian standard specifications issued in 2012 in order to determine the percentage of ethanol content. The accuracy of the analytical method was ensured by repeating the analytical procedures on one day or over a number of day As for accuracy demonstration by repeated procedure on one day, ethanol content of a Arak sample which was under

fermentation was measured using pycnometer or density bottle. The trial was repeated 6 times a day as explained previously. Arithmetical mean, standard deviation SD, and relative standard deviation were then calculated.

Whereas, accuracy demonstration by the repeated procedure over a number of days, ethanol content of a Arak sample which was under fermentation was measured over six days using rotary evaporator as describes previously. Arithmetical mean value of the 6 measurements of concentration, standard deviation SD, and relative standard were calculated

METHANOL CONTENT DETERMINATION IN SAMPLES OF WINE AND ARAK

Colorimetric method was applied to the determination of methanol in samples of wine and Arak. 1 ml of each distilled samples was taken, some of them were diluted with distilled water as dilution factor was recorded. 1ml of standard methanol 0.00025 g/ml was added, 2ml of 3% potassium permanganate prepared of 3g potassium permanganate 13 ml Phosphoric acid and volume to 100ml of distilled water. The samples were left for 30 minutes and then they were decolorized by meta bisulfite sodium solution prepared of 10g of meta bisulfite sodium volume to 100 ml of distilled water. 4 ml of chromotropic acid was also added to be followed by cooling prepared of 1.25 g of the mentioned acid, 25 ml of distilled water and volume to 100 ml of concentrated sulfuric acid. 12 ml of concentrated sulfuric acid was gradually added and accompanied with cooling. The mixture was heated in water bath at 80°C. the absorbance was detected at wavelength of 575nm and methanol content was calculated using the previously mentioned equation. The absorbance of the standard was measured by adding 2ml of it as well as reagent according to the previously explained steps. The standard methanol 0.00025g/ml was prepared by adding 1g of standard methanol to 250ml balloon and volumed to standard line with 40% ethanol. 12.5ml of the solution was transferred to 100ml balloon and volumed to standard line with 40% ethanol. Thus, 1 ml of the result solution contained 0.0005g methanol. 50 ml of the solution was then placed in balloon and volumed to standard line with 40% ethanol to obtain methanol 0.00025g/ml. The accuracy of the

analytical method was ensured by repeating the analytical procedures on one day or over a number of days. As for accuracy demonstration by repeated procedure on one day, methanol content of a Arak sample. The trial was repeated 6 times a day as explained previously. The mean, standard deviation SD, and relative standard deviation were then calculated. Whereas, accuracy demonstration by the repeated procedure over a number of days, methanol content of a Arak sample was measured over six days as describes previously. Arithmetical mean value of the 6 measurements of concentration, standard deviation SD, and relative standard were calculated.

It was found that ethanol mean concentration values obtained after 6 trials on one day was $10.82 \pm 0.3\%$ and relative standard deviation was 3.6%, while ethanol mean concentration values obtained after 6 trials over six days was $10.86 \pm 0.3\%$ and relative standard deviation was 5.9%. Based on the repeatability values of analytical method on one day or over six days, it can be suggested that the precision of the adopted method was passable since relative standard deviation for both cases was less than 10%, thus it can be validated for the determination of ethanol content in 50 samples of wine and Arak that were prepared by traditional methods Tab1

RESULTS AND DISCUSSION

Table1: Ethanol content in the given samples of arak and wine

Sample no	Ethanol content%(v/v)	Sample no	Ethanol content%(v/v)
1	10.65 ± 1	26	11.04 ± 1.8
2	40.7 ± 1.5	27	9.03 ± 1.3
3	50.04 ± 1	28	45.08 ± 1
4	60.03 ± 1.8	29	50.04 ± 1.8
5	17.39 ± 1.4	30	59.04 ± 1.4
6	49.09 ± 1	31	16.88 ± 2
7	58.01 ± 1	32	43.06 ± 2.2
8	12.55 ± 1.2	33	56.51 ± 1.4
9	53.08 ± 1	34	11.57 ± 0.8
10	11.95 ± 1	35	41.69 ± 3.1
11	50.86 ± 1.1	36	11.98 ± 0.5
12	58.03 ± 1	37	46.23 ± 2.3
13	70.12 ± 2	38	51.95 ± 2.1
14	13.07 ± 1	39	11.11 ± 0.5
15	47.1 ± 1.2	40	44.46 ± 1.4
16	59.07 ± 2	41	58.13 ± 2.2
17	6.03 ± 0.2	42	70.04 ± 2.3
18	8.15 ± 0.8	43	9.38 ± 0.8
19	7.9 ± 0.9	44	46.45 ± 0.9
20	15.64 ± 1.1	45	4.18 ± 0.6
21	5.21 ± 0.7	46	10.68 ± 0.3
22	12.28 ± 1.4	47	4.98 ± 0.2
23	7.24 ± 0.9	48	11.95 ± 1.3
24	12.71 ± 1.5	49	6.03 ± 1
25	4.03 ± 0.5	50	12.81 ± 0.8

It was found that methanol mean concentration values obtained after 6 trials on one day was $81 \pm 1.01 \text{ mg/100ml}$ of absolute alcohol and relative standard deviation was 1.2%, while methanol mean concentration values obtained after 6 trials over six days was $81.35 \pm 0.7 \text{ mg/100ml}$ of absolute alcohol and relative standard deviation was 0.9%. Based on the repeatability values of analytical

method on one day or over six days, it can be suggested that the precision of the adopted method was passable since relative standard deviation for both cases was less than 10%, thus it can be validated for the determination of methanol content in 50 samples of wine and Arak that were traditionally prepared Tab2.

Table2: Methanol content in the given samples of arak and wine obtained at different stages of preparation with student t values

Sample no	Arak/ wine samples	Methanol content mg/100ml absolute alcohol	studentt value	Sample no	Arak/ wine samples	Methanol content mg/100ml absolute alcohol	student t value
1	Meta-fermentation arak	1034.1±17.6	80.7	26	final wine	68.3±2.2	
2	1 st distillate of arak	239.4±11.4	5.9	27	Meta-fermentation arak	629.0±24.4	29.8
3	2 nd distillate of arak	196.0±5.8		28	1st distillate of arak	129.4±0.4	
4	3 rd distillate of arak	81.2±1		29	2nd distillate of arak	106.6±4.4	
5	Meta-fermentation arak	1095.0±41.2	37.0	30	3rd distillate of arak	49.7±0.8	
6	1st distillate of arak	250.3± 7.8	10.9	31	Meta-fermentation arak	594.5±17.2	39.0
7	2nd distillate of arak	174.9±4.4		32	1st distillate of arak	181.6±7.2	
8	3rd distillate of arak	1064.4±34.2	43.0	33	2nd distillate of arak	124.3±3.5	
9	Meta-fermentation arak	338.2±22.9	10.3	34	3rd distillate of arak	321.3±1.9	111.0
10	1st distillate of arak	643.6±17	44.3	35	Meta-fermentation arak	151.6±4.9	
11	2nd distillate of arak	212.5±24.9	0.9	36	1st distillate of arak	534.7±29.8	19.1
12	3rd distillate of arak	101.5±4.2		37	2nd distillate of arak	199.9± 7.5	
13	Meta-fermentation arak	43.0±1		38	3rd distillate of arak	148.8±6.7	
14	1st distillate of arak	1065.2±212	6.9	39	Meta-fermentation arak	979.5±8	164.6
15	2nd distillate of arak	379.7±23.4	13.0	40	1st distillate of arak	318.1±6.7	30.0
16	3rd distillate of arak	120.9±0.8		41	2nd distillate of arak	156.6±2.8	
17	Meta-fermentation wine	732.9±5.2	173.2	42	3rd distillate of arak	64.4±2.2	
18	Final wine	156.9±1.2		43	Meta-fermentation arak	718.1±5.3	165.0
19	Meta-fermentation wine	550.8±6.5	91.7	44	1st distillate of arak	142.9±10.2	
20	Final wine	117.1±0.7		45	Meta-fermentation wine	295.3±17.4	9.3
21	Meta-fermentation wine	1269.2±71	25.6	46	Final wine	120.1±3.1	
22	Final wine	129.6±6.7		47	Meta-fermentation wine	232.3±42.7	1.3
23	Meta-fermentation wine	612.8±11.8	59.3	48	Final wine	106.2±1.4	
24	Final wine	98.1±1.9		49	Meta-fermentation wine	165.7±8.1	
25	Meta-fermentation wine	543.0± 23.4	24.9	50	Final wine	97.9±3.9	-

EFFECT OF FRUIT TYPE USED IN THE PROCESS OF PREPARATION OF ARAK ON METHANOL CONTENT

It was found that methanol content was higher in samples prepared by using Bunch of grapes during fermentation than in samples prepared by using grape juice Tab 3. This was attributed to the fact that pectin content in bunch of grape was found to be higher than in grape juice, that is, the higher existence of pectin in fruit wooden organs and peels could significantly induce methanol production, thus, methanol resulted under the enzymatic activity of pectin methyl esterase (4) methanol mean content in samples obtained from grape bunch fermentation was

400.68mg/100 ml absolute alcohol (two samples of fig Arak obtained upon fermentation and at first distillation were excluded) while methanol mean content in wine samples obtained from grape juice was 295.05mg/100ml absolute alcohol Tab3 Fig 1

A. ARAK SAMPLES PREPARED FROM WHOLE BUNCH OF GRAPES FERMENTATION

Arak samples were prepared from whole bunch of grapes (meta fermentation samples, 1st distillate samples, 2nd distillate samples, 3rd distillate samples). 11 samples out of 22 had an excess of methanol above the respective limit Fig1

Table 3: Methanol content in the given samples of arak obtained from the whole grape bunch fermentation

Sample no	Methanol content mg/100ml absolute alcohol	Sample no	Methanol content mg/100ml absolute alcohol	Sample no	Methanol content mg/100mg absolute alcohol
1	1034.1±17.6	15	379.7±23.4	38	148.8±6.7
2	239.4±11.4	16	120.9±0.8	39	979.5±8
3	196.0±5.8	31	594.5924	40	318.1±6.7
4	81.2±1	32	181.6223	41	156.6±2.8
5	1095.0±41.2	33	124.3759	42	64.4±2.2
6	250.3± 7.8	36	534.7±29.8	43	718.1±5.3
7	174.9±4.4	37	199.9± 7.5	44	64.4
14	1065.2±212				
Methanol mean concentration mg/100			400.09		

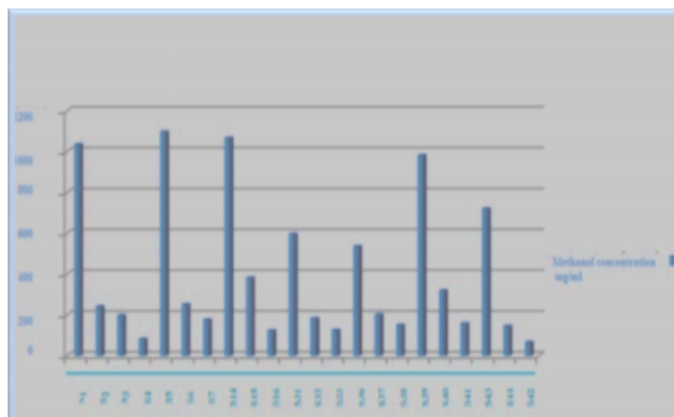


Fig 1: Methanol content (mg/100ml) in the given samples of arak obtained from the whole grape bunch fermentation: arak samples numbers 1, 2, 3, 4, 5, 6, 7, 14, 15, 16, 31, 32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 44.

B. ARAK SAMPLES PREPARED FROM GRAPE JUICE FERMENTATION

Arak samples were prepared from grape juice (Meta fermentation samples, 1st distillate samples, 2nd distillate samples, 3rd distillate samples). Comparatively, it was found that methanol level exceeded the passable limit set by Syrian standard specifications in both

types of arak samples that were prepared from whole bunch of grape and from grape juice with records of 400 and 295mg/ml absolute alcohol respectively Tab4 Fig2,3. Statistical student's t-test was applied on the given samples at significance level of 5% and at confidence level of 95%. 11 samples out of 22 samples were not passable due to

methanol level that exceeded that established by the Syrian standard specifications.

Table 4: Methanol content (mg/100ml) in the given samples of arak obtained from grape juice fermentation arak samples numbers:10,12,17,19,23,25,27,29,34,45,47,21

Sample no	Methanol content mg/100ml absolute alcohol	Sample no	Methanol content mg/100ml absolute alcohol	Sample no	Methanol content mg/100mg absolute alcohol
10	643.6±17	24	98.1±1.9	49	165.7±8.1
11	212.5±24.9	25	543.0± 23	50	97.9±3.9
12	101.5±4.2	26	68.3±2.2	45	295.3±17.4
13	43.0±1	27	629.0±24.4	46	120.1±3.1
17	732.9±5.2	28	129.4±0.4	47	232.3±42.7
18	156.9±1.2	29	106.6±4.4	48	106.2±1.4
19	550.8±6.5	30	49.7±0.8	21	1269.2±71
20	117.1±0.7	34	321.3±1.9	22	129.6±6.7
23	612.8±11.8	35	151.6±4.9		
Methanol mean concentration mg/100			295		

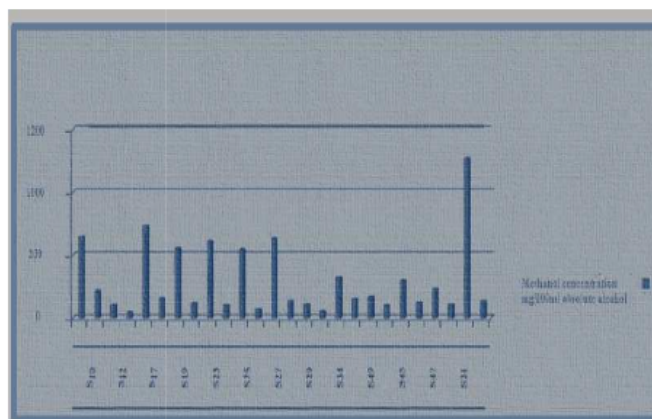


Fig 2: Methanol content (mg/100ml) in the given samples of arak obtained from grape juice fermentation; arak samples numbers:10,12,17,19,23,25,27,29,34,45,47,21

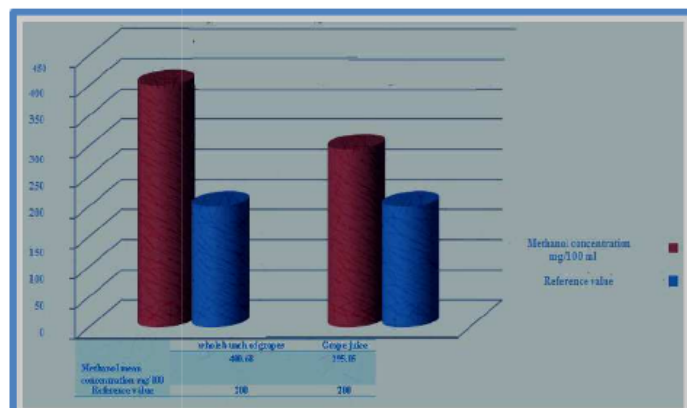


Fig 3: Methanol mean content (mg/100ml) in the given samples of arak obtained from fermentation of grape juice and whole bunch of grapes in comparison with the reference value

EFFECT OF THERMOTHERAPY ON METHANOL CONTENT IN SWEET AND DRY WINE SAMPLES

Different levels of methanol were detected in the given 16 samples of wine. However, wine samples were divided into sweet and dry wine samples that is, sweet wine was exposed to thermal process treatment, dry wine was not. While only two samples of sweet wine obtained upon fermentation exceeded the

allowable limit of methanol. While Methanol content was high in wine samples that were collected during fermentation, it didn't exceed the allowable limit in wine samples that were collected at the step of the final product ready- to-drink Tab5, Fig 4. Four samples of dry wine obtained upon fermentation exceeded the allowable limit of methanol. Regarding to ready- to- drink wine sample,

methanol level was within the allowable range. Methanol level in wine at final step as well as upon fermentation was influenced by thermal processing treatment of grape juice which was being fermented – all studied samples of wine were prepared by fermentation of grape juice Tab 5 Figs 5. Comparatively, it was found that methanol mean concentration in dry wine was above the allowable limit with a record of 423.6mg/100ml absolute alcohol While in the

case of sweet wine, grape juice did undergo boiling prior to fermentation and the mean value of methanol content was below the allowable limit with a record of 238.45 mg/100ml. absolute alcohol Fig 6. Thus, it can be noticed that heat treatment of grape juice lowered methanol content as was demonstrated in previous studies(8). This can be attributed to the fact that yeast fermenting activity is inhibited by heat treatment.

Table 5: Methanol content (mg/100ml) in the given samples of sweet and dry wine

Sweet wine sample No	Methanol content mg/100ml	Dry wine sample No.	Methanol content mg/100ml
45	295.3±17.4	21	1269.2±71
46	120.1±3.1	22	129.6±6.7
47	232.3±42.7	23	612.8±11.8
48	106.2±1.4	24	98.1±1.9
49	165.7±8.1	25	543.0± 23.4
50	97.9±3.9	26	68.3±2.2
17	732.9±5.2	19	550.8±6.5
18	156.9±1.2	20	117.1±0.7



Fig 4: Methanol content (mg/100ml) in the given samples of sweet wine after heat treatment

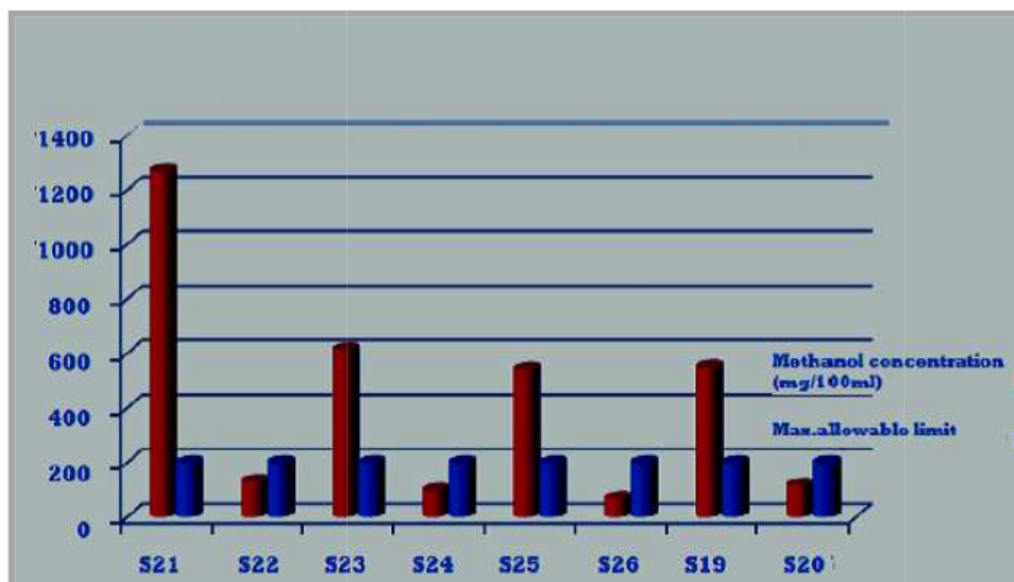


Fig 5: Methanol content (mg/100ml) in the given samples of dry wine without heat treatment

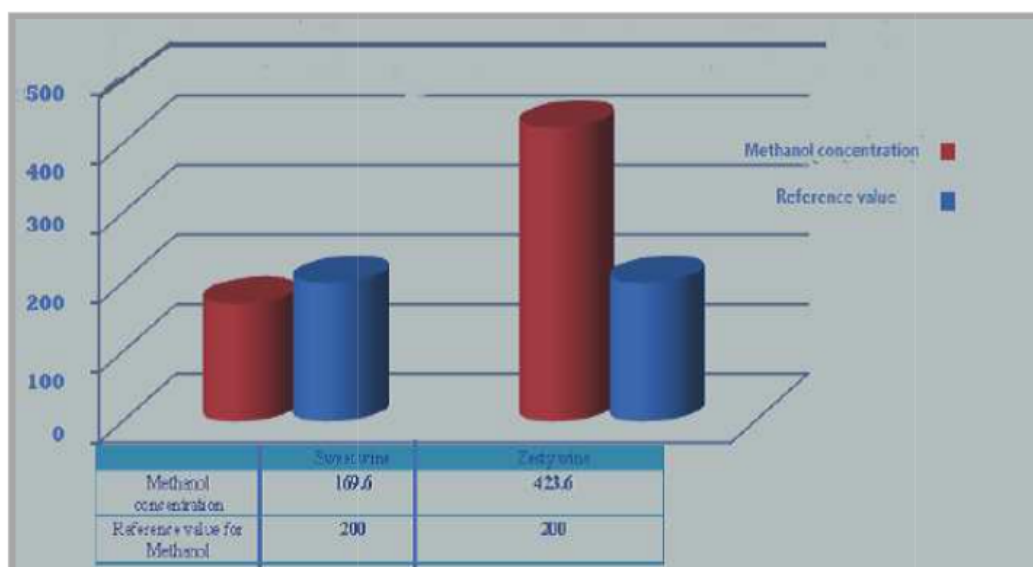


Fig 6: Methanol mean content (mg/100ml) in the samples of sweet and dry wine in comparison with reference value

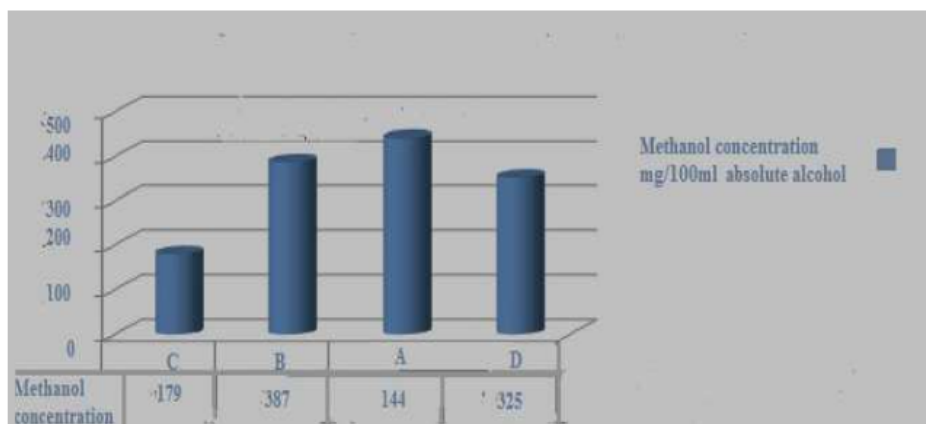
INFLUENCE OF GEOGRAPHICAL ORIGIN ON THE CONTENT OF METHANOL IN SAMPLES OF WINE AND ARAK

The geographical origins, from which wine and Arak samples were collected, were categorized into A, B, C, D. The levels of methanol in the given samples of Arak and wine were compared and the differences were identified at significance level of 5% provided using the same preparation method

for all studied samples in order to avoid the result errors. The comparison involved Arak samples prepared by the fermentation of grape bunches collected from the four suggested geographical regions. It was found that no intrinsic differences in methanol levels in all samples of the four groups that is, methanol level mostly influenced by fermentation process and conditions Tab6 Fig7

Table 6: Methanol content mg/100ml absolute alcohol in the given samples of wine obtained from four different geographical regions (A,B,C,D)

Sample No. from the geographical region A	Methanol content mg/100ml absolute alcohol	Sample No. from the geographical region B	Methanol content mg/100ml absolute alcohol	Sample No. from the geographical region C	Methanol content mg/100ml absolute alcohol	Sample No. from the geographical region D	Methanol content mg/100ml absolute alcohol
5	1095.061	1	1034	40	318.188	31	594.5924
6	250.3244	2	239	41	156.62	32	181.6223
7	174.995	3	196	42	64.49496	33	124.3759
14	1065.273	4	81			43	718
15	379.7315					44	142
16	120.9219						
36	534.7248						
37	199.9878						
38	148.8236						

**Fig 7:** Methanol content mg/100ml absolute alcohol in the given samples of wine obtained from four different geographical regions (A,B,C,D)

CONCLUSIONS

Different methanol levels were detected in samples of wine and Arak that were prepared according to local traditional methods, fermentation techniques and distillation times. It was found that:

- Whole grape bunch based- fermentation is associated with high level of methanol.
- Thermal processing treatment of grape juice designed for wine manufacturing is associated with lower level of methanol.

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