

Ethanol and Methanol Concentration in Commonly Used Brands of Ma-al-shaeer in Iran: Estimation of Dietary Intakes and Risk Assessment

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Key Words

ethanol, methanol, Ma-al-shaeer, non-alcoholic beer, gas chromatography, risk assessment.

Abstract

Objectives: Ma-al-shaeer is a popular beverage in Islamic countries. The aim of this study was to determine the concentrations of methanol and ethanol in most consumed brands of Ma-al-shaeer in Iran.

Methods: Eighty-one Ma-al-shaeer samples which commonly used in Iran were provided. Methanol and ethanol contents were determined by gas chromatography with flame ionization detector.

Results: The mean methanol concentrations in Iranian and foreign brands was 129.84 ± 205.38 mg/L and 110.157 ± 135.98 mg/L, respectively. Although mean ethanol contents of Iranian brands was 1.2 ± 2.41 mg/L, ethanol level in foreign ones was lower than LOQ.

Conclusion: Since the most Ma-al-shaeer brands had methanol pollution at different levels establishment of a

definitive relationship between the methanol content and toxicological effects seem to be vital. EDI of methanol for Iranian people through consumption of Ma-al-shaeer was determined 0.023mg/kg bw/day.

1. Introduction

Beer is one of the most favorable beverages in the world. Owing to adverse effects of alcoholic beer on athletes, those with cardiovascular diseases, and pregnant women, there has been increasing tendency to consume non-alcoholic beer [1, 2]. Findings have shown the beneficial effects of different components of beer [3]. Phenolic compounds in beer exhibited useful biological effects including elevation of plasma antioxidant capacity, prevention of atherosclerosis and cancer, and modulation of enzymatic activity (i.e. superoxide dismutase and glutathione peroxidase) [4, 5]. These elements not only inhibit the oxidation of low density lipoprotein (LDL) but also affect activation of transcription factors and gene expression [6, 7]. Lysine, an essential amino acid in non-alcoholic beer, revealed anxiolytic effect in human. This is refers to partial 5HT4 antagonist role of L-lysine [8]. Recently, sedative effect of alcohol-free beer in healthy female

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nurses was reported [9].

Ma-al-shaeer, a non-alcoholic beer, is among the most consumed drinking in the world [10]. Nowadays, different methods are developed for production of non-alcoholic malt or low alcohol beers. Fermentation with *saccharomyces* strain, dialysis, and reverse osmosis are example of them. It should be note that production of alcohol (i.e. methanol and ethanol) is inevitable in most techniques [11]. Due to mass production of it, quality assessment of this product is an important issue.

Methanol, as a colorless liquid, is freely miscible with water and easily cross the blood-brain barrier. Along with lethal dose of 1-2 mL/kg, methanol poisoning is common in clinical practice. Death and blindness have been shown with as low as 0.1 mL/kg. After oral intake, methanol is absorbed rapidly and metabolized in liver. Formic acid, a main toxic metabolite of it, disrupts cytochrome C oxidase activity. Methanol poisoning can result in optic nerve lesion, hypotension, CNS damage and anion gap metabolic acidosis [12].

Similarly, it has been shown that ethanol consumption is correlated with violence, cirrhosis, stroke, and poisoning. In addition, association between ethanol use and malignancies such as liver, breast, and colon cancers are exhibited. World Health Organization (WHO) estimated that alcohol is responsible to approximately 2.5 million deaths each year [13]. On the other hand, ethanol consumption, selling, and transport is prohibited in Islamic sources (i.e. Qur'an) [14, 15]. Hence, in current study, we aimed to determine the levels of ethanol and methanol in commonly used brands of Ma-al-shaeer in Iran.

2. Material and Methods

2.1. Sample collection

Eight most used brands of Ma-al-shaeer, comprising of five Iranian brands and three foreign ones were collected from different markets in Iran. Within a given brands, the most popular flavors were selected. Then, three different samples from each flavor were provided. Finally, eighty one most commonly used Ma-al-shaeer samples were tested.

2.2. Reagents

Methyl alcohol and ethyl alcohol were obtained from Sigma-Aldrich (Steinheim, Germany). 1-butanol and 2-propanol were purchased from Merk (Darmstadt, Germany). Distilled deionized water (DDW) was supplied from a Millipore Milli-Q water system (Bedford, MD). All other chemicals and reagents were of the highest available purity and used as purchased.

2.3. Chemical Analysis

Involving GC-PFPD, analyte concentrations were determined. Analysis was carried out on a Varian CP-3800 GC

directly coupled to a Varian PFPD detector (Varian, Inc., Lexington, Massachusetts, USA). The GC column was a DB-5 capillary column (0.25- μ m film thickness, 0.32 mm ID \times 30 m in length), obtained from J&W Scientific (Folsom, California, USA).

The operating conditions were as follows: the carrier gas was N₂ with a linear velocity of 30 mL/min and air flow rate of 300 mL/min. In isothermal state, the injection port, column and detector temperatures were 65 and 200 °C, respectively. Along with Split ratio 20, retention time was 12 minutes.

2.4. Evaluation of Accuracy and repeatability

Quantification was performed by the use of external calibrations which were obtained with methanol and ethanol solutions at six concentration levels. The r² values for the calibration lines for the methanol and ethanol were calculated 0.9924 and 0.9975, respectively. Interday and Intraday tests were employed to evaluate accuracy and repeatability.

2.5. Tolerable daily intake (TDI) and estimated daily intake (EDI)

The tolerable daily intake (TDI) is indicative of safe exposure levels and is used to predict the amount of chemical substances, ingested over a lifetime without important risk.

The daily intake of methanol not only depends on daily food consumption but also related to methanol levels in foods. Body weight is another key factor can affect the tolerance of pollutants. Based on upon factors estimated daily intake (EDI) can be determined. Consequently, According to the following equation (1), EDI was calculated:

$$EDI = \frac{EF \times ED \times FIR \times C}{WAB \times TA}$$

Where EF is exposure frequency (365 days/year); ED is the exposure duration (70 years), equivalent to the average lifetime; FIR is the food ingestion rate (13.7 mL/person/day); C is the mean methanol concentration in Ma-al-shaeer (mg/L); WAB is the average body weight (70 kg) and TA is the averaging exposure time for non-carcinogens (365 days/year \times ED) [16].

2.6. Statistical Analysis

The values are expressed as means \pm SD. ANOVA (analysis of variance) and the Tukey posttest were employed to determine significant differences in the data of various groups. P values less than 0.05 were considered significant.

Table 1 LOD and LOQ values (mg/L) of the analytical method for methanol (MeOH) and ethanol (EtOH).

| Analytes | LOD | LOQ |
|----------|-----|-----|
| MeOH | 5 | 25 |
| EtOH | 2.5 | 10 |

Table 2 Interday validation of method using six different concentrations (mg/L)

| | | Interday validation | | | | | |
|----------|---------|---------------------|--------------|--------------|--------------|-------------|-------------|
| | | Concentration (n=6) | | | | | |
| Analytes | | 50 | 75 | 100 | 125 | 150 | 200 |
| MeOH | Mean±SD | 0.198±0.001 | 0.407±0.0025 | 0.630±0.0012 | 0.763±0.0008 | 0.950±0.009 | 1.26±0.012 |
| | %CV | 0.655 | 0.615 | 0.15 | 0.104 | 0.094 | 0.995 |
| EtOH | Mean±SD | 0.354±0.0018 | 0.524±0.0012 | 0.729±0.0015 | 0.918±0.001 | 1±0.011 | 1.31±0.0222 |
| | %CV | 5.07 | 2.29 | 2.05 | 1.1 | 1.07 | 1.706 |

Abbreviations: MeOH: methanol, EtOH: ethanol, SD: standard deviation, CV: coefficient of variation

Table 3 Interday validation of method using six different concentrations (mg/L)

| | | Interday validation | | | | | |
|----------|---------|---------------------|--------------|--------------|-------------|-------------|-------------|
| | | Concentration (n=6) | | | | | |
| Analytes | | 50 | 75 | 100 | 125 | 150 | 200 |
| MeOH | Mean±SD | 0.204±0.0144 | 0.419±0.0226 | 0.628±0.0238 | 0.759±0.413 | 0.936±0.05 | 1.26±0.0954 |
| | %CV | 0.037 | 5.379 | 5.789 | 5.422 | 5.312 | 7.571 |
| EtOH | Mean±SD | 0.347±0.0396 | 0.564±0.044 | 0.752±0.0439 | 0.956±0.069 | 1.708±0.079 | 1.47±0.203 |
| | %CV | 11.40 | 7.79 | 5.83 | 6.89 | 7.74 | 1.90 |

Abbreviations: MeOH: methanol, EtOH: ethanol, SD: standard deviation, CV: coefficient of variation

Table 5 The levels of methanol (MeOH) and ethanol (EtOH) content (mg/L) in defferent foreign Ma-al-shaeer brands.

| Brands | Flavor | Analytes | Mean±SD |
|---------|-------------|------------|----------------|
| Bavaria | Peach | MeOH | <25 |
| | | EtOH | <10 |
| | Apple | MeOH | <25 |
| | | EtOH | <10 |
| | Pomegranate | MeOH | <25 |
| | | EtOH | <10 |
| Malt | MeOH | 39.44±9.17 | |
| | EtOH | <10 | |
| Efes | Malt | MeOH | 18.2±12.97 |
| | | EtOH | <10 |
| Baltika | Malt | MeOH | 302.24±126.75* |
| | | EtOH | <10 |

Abbreviations: *: amount higher than legal limit

Table 4 The levels of methanol (MeOH) and ethanol (EtOH) content (mg/L) in different Iranian Ma-al-shaeer brands.

| Brands | Flavor | Analytes | Mean±SD |
|------------|-------------|-------------|-----------------|
| Bit malt | Equatorial | MeOH | 26.6±0.50 |
| | | EtOH | <10 |
| | Apple | MeOH | 30.24±2.36 |
| | | EtOH | <10 |
| | Lemon | MeOH | 28.35±2.26 |
| | | EtOH | <10 |
| Malt | MeOH | 35.17±2.98 | |
| Istak | Peach | MeOH | <25 |
| | | EtOH | <10 |
| | Cantaloupe | MeOH | 32.61±2.86 |
| | | EtOH | <10 |
| | Strawberry | MeOH | 10.25±14.51 |
| | | EtOH | <10 |
| | Mango | MeOH | 30.40±1.95 |
| | | EtOH | <10 |
| | Pineapple | MeOH | 19.81±14.04 |
| | | EtOH | <10 |
| | Lemon | MeOH | <25 |
| | | EtOH | <10 |
| | Coffee | MeOH | 18.24±13.90 |
| | | EtOH | <10 |
| | Pomegranate | MeOH | 9.70±13.72 |
| | | EtOH | <10 |
| Malt | MeOH | 8.59±13.15 | |
| | EtOH | <10 | |
| Equatorial | MeOH | 29.40±5.15 | |
| | EtOH | <10 | |
| Hey day | Peach | MeOH | 13.73±19.45 |
| | | EtOH | <10 |
| | Lemon | MeOH | 18.93±13.39 |
| | | EtOH | <10 |
| Equatorial | MeOH | 19.73±13.95 | |
| | EtOH | <10 | |
| Lemon-Mint | MeOH | 35.77±11.72 | |
| | EtOH | <10 | |
| Petrovich | Malt | MeOH | 540.27±157.546* |
| | | EtOH | <10 |
| Holstein | Peach | MeOH | 35.39±5.54 |
| | | EtOH | <10 |
| | Apple | MeOH | 55.14±2.17 |
| | | EtOH | 16.26±22.99 |
| | Lemon | MeOH | 40.56±0.98 |
| | | EtOH | 16.42±3.22 |
| Malt | MeOH | 32.59±3.08 | |
| | EtOH | <10 | |

Abbreviations: * amount higher than legal limit

3. Results

3.1. Calibration

Calibration curves were obtained by use of 6 different concentrations of each analyte including 50, 75, 100, 125, 15 and 200 mg/L, separately. In the range of 50 to 200 mg/L, response versus the amount of alcohol injected showed a good linearity. LOD (limit of detection) and LOQ (limit of quantification) values of analytical method are shown in table 1.

Ruggedness of method and instrument were assessed using the intra- and interday variance. To achieve this, six different concentration were prepared and injected to the GC. Results are shown in tables 1 and 2.

3.2. Determination of alcohol content in samples

Based on GC chromatograms (figures 1 and 2), methanol and ethanol concentrations were calculated. Results are shown in table 4 and 5. Results showed that mean methanol concentration in Iranian and foreign brands were 129.48 ± 205.34 and 110.15 ± 135.98 mg/L, respectively. Statistical analysis showed that there is no significant difference between Iranian and foreign brands ($P=0.889$).

When Iranian brands were compared together, it was found that malt flavor of Petrovich brand contains the highest amount of methanol (540.27 ± 157.546 mg/L). In addition, among the foreign ones malt flavor of Baltika brand contains the highest methanol concentration (302.24 ± 75.126 mg/L). Although mean ethanol contents of Iranian brands was 1.2 ± 2.41 mg/L, ethanol level in foreign ones was lower than LOQ. Ethanol levels in apple and lemon flavors of Holstein brand were 16.26 ± 22.99 and 16.41 ± 3.22 mg/L, respectively. No significant difference was observed when mean ethanol concentrations of Iranian brands compared with that of foreigners.

4. Discussion

In the current study, we determined the methanol and ethanol contents in different brands of Ma-al-shaeer available in Iran. Results of this work revealed that approximately all brands are contaminated with methanol at different levels. Fortunately, ethanol concentrations were lower than Maximum Residues Levels.

Legal definition for alcohol-free beer may differ from country to country. Iran, Germany, and England accepted 0.5% (v/v) as maximum ethanol level. However, US and Arabic countries determined 0.05% (w/v) and 0.1% (v/v), respectively [17].

In one study, ethanol and methanol contents of non-alcoholic beer samples were determined. Findings were revealed that methanol and ethanol concentration of classic Delester samples were 19.3 ± 1.34 and 21.8 ± 1.22 μ g/L, respectively. Although methanol content in the Birell brand was 97.7 ± 3.76 μ g/L, ethanol level was lower than detection range (LOD= 2.5 μ g/L) [18]. Employing gas chroma-

tography, a rapid and sensitive method was developed for determination of ethanol in alcohol-free beverages and fruit juices. Herein, five brands of beer, four brands of apple juice, and seven brands of grape juices were analyzed. It was revealed that ethanol content is ranging from 0.009% to 0.385% v/v [19]. In another study, Hämmerle et al. provided a biosensor to determine alcohol (including ethanol and methanol) concentrations in apple juices. The sensing system was based on amperometric detection method and catalytic activity of alcohol oxidase. They showed that alcohol content of three apple juices were in the range 0.30–0.67g/L. When these findings are compared together, it was observed that in some cases ethanol contents are higher than permissive levels. Although almost all products contain ethanol level lower than legal limits, these products are not actually alcohol-free.

Result of our study is in accordance with these findings and all Ma-al-shaeer brands contained ethanol levels in permissive ranges.

American standard is permitted 120–460mg/L of methanol in canned and fresh juices [20]. In addition, International Office of Vine and Wine (OIV) sets 150mg/L as a maximum acceptable limit for methanol in white wines. The maximum allowable level of methanol recommended by the Iranian standard is 100 mg/L in the herbal distillates [21]. In this study, malt flavor of Petrovich and Baltika brands contained methanol in excess from standard.

Possner et al. determined the methanol content in fruit juices and nectars by GC-MS. In this study apple, pear, grape, elderly berry and multivitamin juices and pear, cherry, and black currant nectars were analyzed. Results showed that the highest methanol concentrations were found in black currant nectars and elderly berry juices (160.5 and 149.5mg/L, respectively) [22]. Using spectrophotometry, Karimi et al. evaluated the amount of methanol in ten plant water. Their findings revealed that the highest methanol level was in dill distillate water (1477.7 ± 23.8 mg/L). However, Egyptian willow water samples contained minimum methanol content (79.4 ± 3 mg/L) [23]. Wu et al. quantified methanol content in fresh fruit juices by means of methanol oxidase in combination with basic fuchsin. Methanol content in carrot, tomato, and papaya were determined to contain 36, 42, and 38 mg/L, respectively [24].

Methanol is present in vegetable and fruit juice and related product. Pectin, a heteropolysaccharide component in the cell wall, composed of linear chains of α -1, 4-galacturonic acids. In nature, usually carboxyl groups of galacturonic acid are esterified with methanol. During fruit and vegetable process, enzymatic activities (i.e. Pectinesterase) are resulted in methanol and pectin release. Hence, methanol is found in those product bonded and freely. On the other hand, fermentation of cellulose may lead to methanol production [21, 22]. Different factors including type of yeast strain, fruit varieties and species and oenological practices can influence methanol level. In addition, various process of food production in different factories is another key factor influence methanol concentration in final product [22]. High methanol levels in different Ma-al-shaeer brands may be illustrated by these factors. Methanol is a poisonous volatile liquid which potentially can produce

Maashaer figure

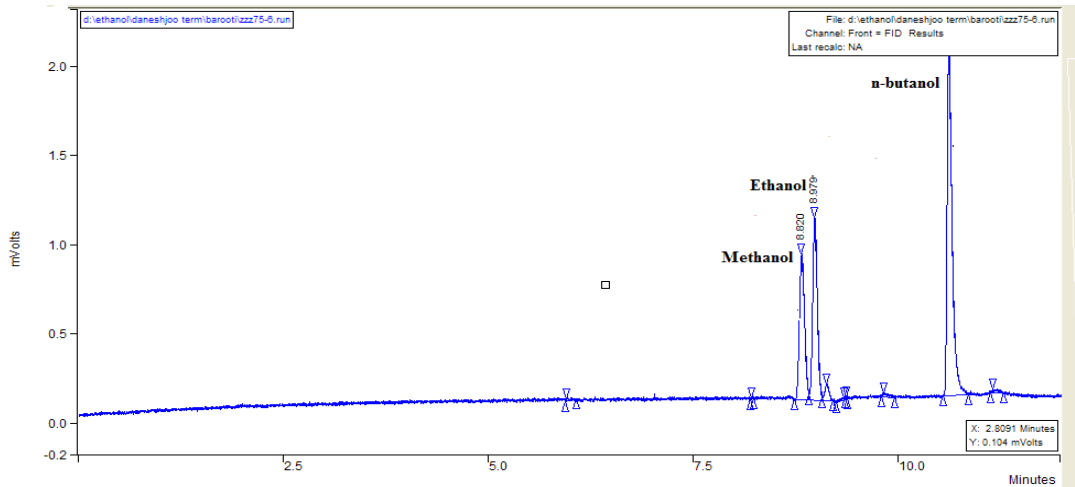


Figure 1 Typical GC-PPFD chromatogram of methanol, ethanol, and n-butanol detected in standard mixture.

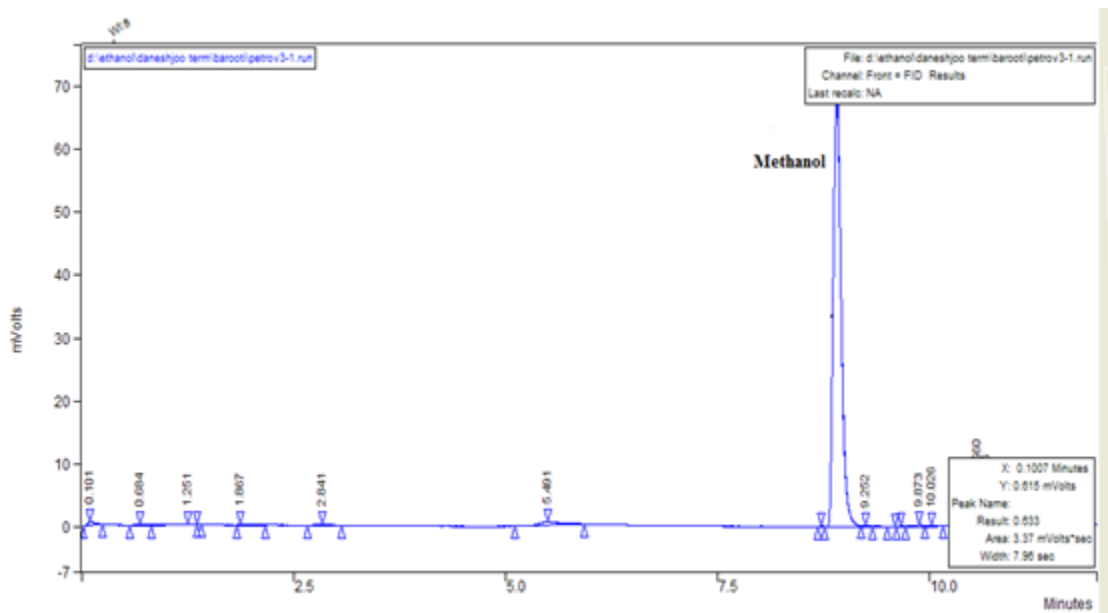


Figure 2 Figure 2 GC-PPFD chromatogram of methanol in Petrovich brand (malt flavor).

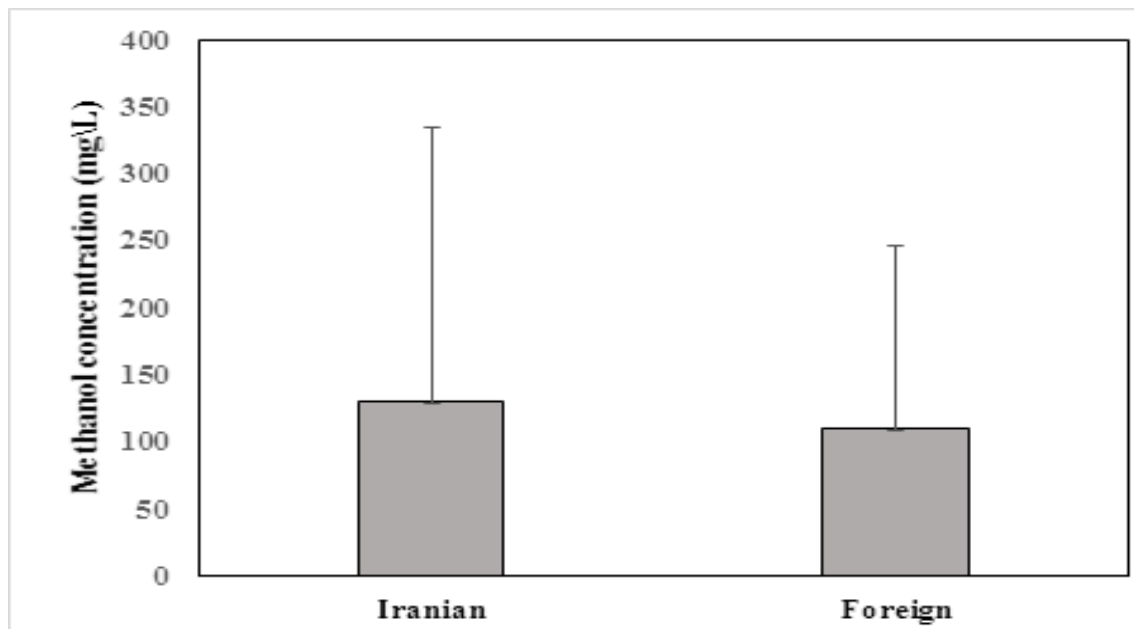


Figure 3 Comparison of methanol concentration between Iranian and foreign Ma-al-shaeer brands. Data are mean \pm SD (n=9).

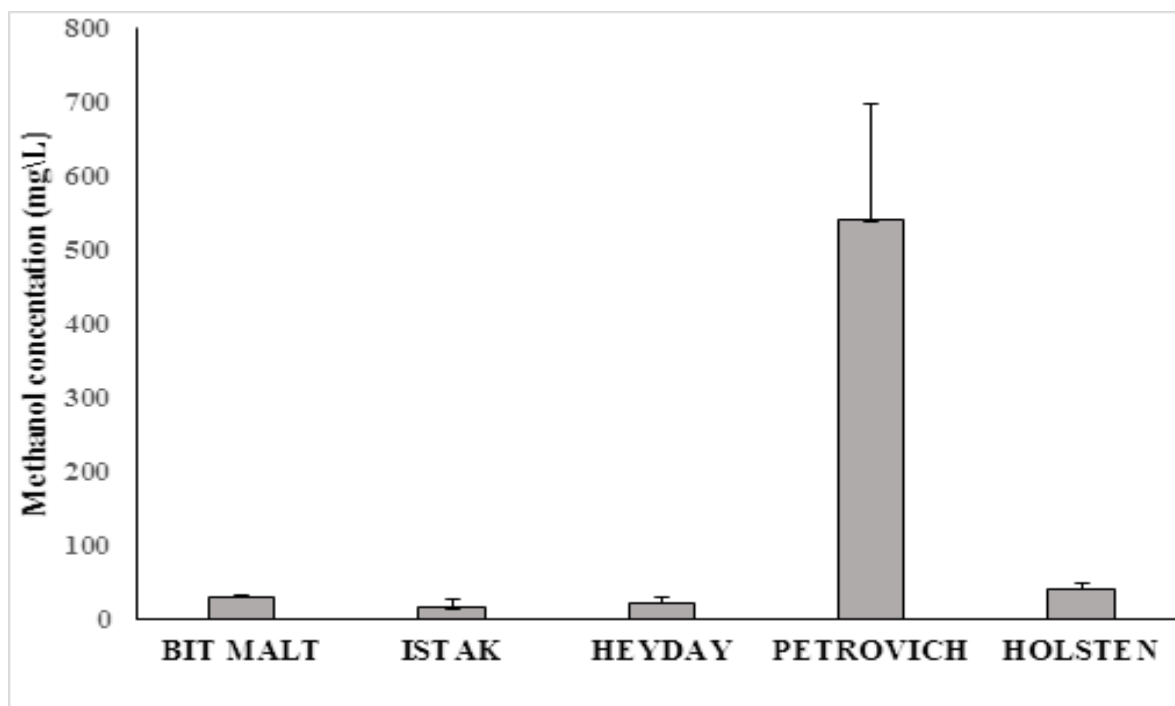


Figure 4 Comparison of methanol concentration among different Iranian Ma-al-shaeer brands. Data are mean \pm SD (n=9).

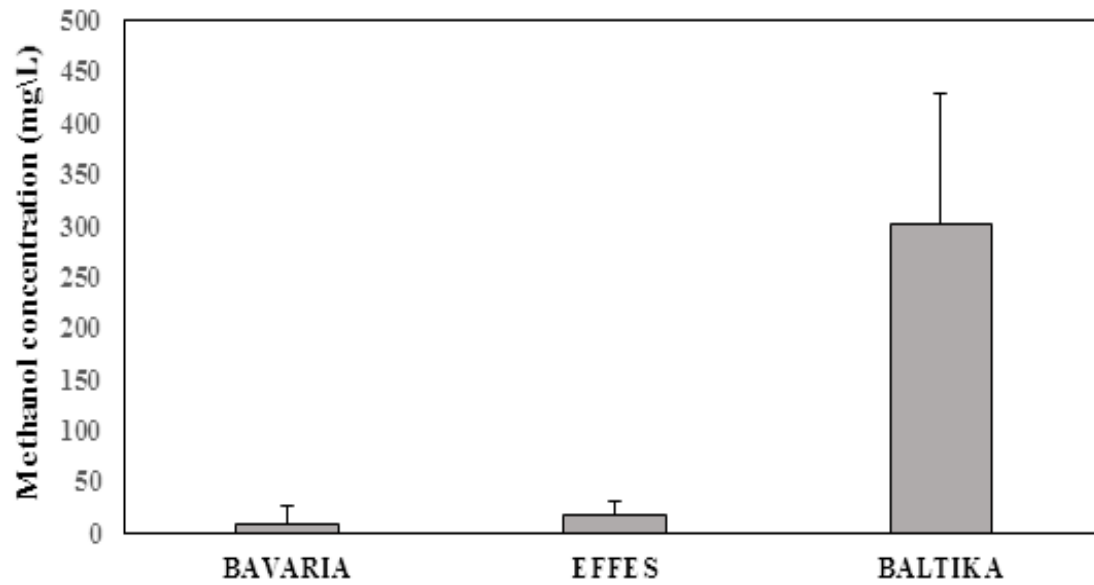


Figure 5 Comparison of methanol concentration among different foreign Ma-al-shaeer brands. Data are mean \pm SD (n=9).

symptoms such as optic nerve damage, diarrhea, abdominal pain, hypotension, and anion gap. Metabolic acidosis, seizures, and coma are associated with blood methanol concentration above 500 mg/L. Concentration above 1500–2000 mg/L will result in death. Owing to its major toxicity, quality control of Ma-al-shaeer products is greatly recommended.

5. Conclusion

Using Eq. (1), EDI of methanol for Iranian people through consumption of Ma-al-shaeer was determined 0.023 mg/kg bw/day. In regard to TDI of methanol (20 mg/kg bw/day), it is clear that methanol concentration in Ma-al-shaeer brands was much lower than its TDI value (25). It was assumed that all Iranian people were used mentioned brands. Our finding revealed that there is no health risk to methanol by Ma-al-shaeer consumption.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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