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
nurses was reported [9].

Ma-al-shaer, 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-shaer in Iran.

2. Material and Methods

2.1. Sample collection

Eight most used brands of Ma-al-shaer, 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-shaer 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 Milli-pore 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-µm film thickness, 0.32 mm ID x 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 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-shaer (mg/L); WAB is the average body weight (70 kg) and TA is the averaging exposure time for non-carcinogens (365 days/year x ED) [16].

2.6. Statistical Analysis

The values are expressed as means ± 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)

| 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  Intraday validation of method using six different concentrations (mg/L)

| 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 different 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-shaeeer 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|
|              | EtOH      | <10       |
| 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 (figures1 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 foreigns.

4. Discussion

In the current study, we determined the methanol and ethanol contents in different brands of Ma-al-shaer 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 different 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 ±1.22µg/L, respectively. Although methanol content in the Birell brand was 97.7 ±3.76, µg/L, ethanol level was lower than detection range (LOD= 2.5µg/L) [18]. Employing gas chroma-
Figure 1 Typical GC-PFPD chromatogram of methanol, ethanol, and n-butanol detected in standard mixture.

Figure 2 GC-PFPD 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±SD (n=9).

Figure 4  Comparison of methanol concentration among different Iranian Ma-al-shaeer brands. Data are mean±SD (n=9).
Figure 5 Comparison of methanol concentration among different foreign Ma-al-shaeer brands. Data are mean±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.

References

1. Sohrabvandi S, Mousavi S, Razavi S, Malganji S, Khosravi-Darani K, Mortazavian A. The effect of Saccharomyces strain and fermentation conditions on production of Ma-al-Shaeer. Iranian J Nutr Sci Food Technol. 2013;8(3):179-187.
2. Buzrul S. A suitable model of microbial survival curves for beer pasteurization. IWT-Food Sci Technol. 2007;40(8):1330-1336.
3. Bamforth CW. Nutritional aspects of beer: a review. Nutr Res. 2002;22(1):227-237.
4. Fantozzi P, Montanari L, Mancini F, Gasbarrini A, Addolorato G, Simoncini M, et al. In vitroantioxidant capacity from wort to beer. IWT-Food Sci Tech. 1998;31(3):221-227.
5. Ghiselli A, Natella F, Guidi A, Montanari L, Fantozzi P, Scaccini C. Beer increases plasma antioxidant capacity in humans. J Nutr Biochem. 2000;11(2):76-80.
6. Gorinstein S, Caspi A, Zemser M, Trakhtenberg S. Comparative contents of some phenolics in beer, red and white wines. Nutr Res. 2000;20(1):131-139.
7. Nardini M, Ghiselli A. Determination of free and bound phenolic acids in beer. Food Chem. 2004;84(1):137-143.
8. Franco L, Galán C, Bravo R, Bejarano I, Peñas-Lledo E, Rodríguez A, et al. Effect of non-alcohol beer on anxiety: Relationship of 5-HIAA. J Neurochem. 2015;9(2):149-152.
9. Franco L, Sánchez C, Bravo R, Rodríguez AB, Barriaga C, Romero E, et al. The sedative effect of non-alcoholic beer in healthy female nurses. PloS one. 2012;7(7):e37290.
10. Malganji S, Jaliyvand A, Sohrabvandi S. Effects of fermentation temperature and two Saccharomyces yeasts on non-alcoholic Ma-al-shaeer properties. Iranian J Nutr Sci Food Technol. 2013;7(5):335-343.
11. Huige NJ, Sanchez GW, Leidig AR. Process for preparing a nonalcoholic (less the 0.5 volume percent alcohol) malt beverage. Google Patents; 1990.
12. Jammalamadaka D, Raissi S. Ethylene glycol, methanol and isopropyl alcohol intoxication. Am J Med Sci. 2010;339(3):276-281.
13. O’Keefe JH, Bhatti SK, Bajwa A, Dinicolantonio JJ, Lavin CJ. Alcohol and cardiovascular health: the dose makes the poison... or the remedy. Mayo Clin Proc; 2014.
14. Al-Ansari B, Thow AM, Day CA, Conigrave KM. Extent of alcohol prohibition in civil policy in Muslim majority countries: the impact of globalization. Addiction; 2015.
15. Abu-Ras W, Ahmed S, Arfken CL. Alcohol use among US Muslim college students: Risk and protective factors. J Ethn Subst Abuse. 2010;9(3):206-220.
16. Nejabat M, Kahe H, Shirani K, Ghorbannezhad P, Hadizadeh F, Karimi G. Health risk assessment of heavy metals via dietary intake of wheat in Golestan Province, Iran. Hum Ecol Risk Assess; 2017.
17. Sohrabvandi S, Razavi SH, Mousavi SM, Mortazavian A, Rezaei K. Application of Saccharomyces rouxii for the production of non-alcoholic beer. Food Sci Biotechnol. 2009;18(5):1132-1137.
18. Maleki R, Farhadi K, Tahmasebi R. Preparation of a sol-gel titania based coating for HS-SPME of aliphatic alcohols from non-alcoholic beer samples. Chromatographia. 2009;69(7-8):775-778.
19. Morad A, Hikal A, Buchanin R. Gas-liquid chromatographic determination of ethanol in “Alcohol-Free” beverages and fruit juices. Chromatographia. 1980;13(3):161-163.
20. James JT. Spacecraft maximum allowable concentrations for airborne contaminants; 2008.
21. Shirani K, Hassani FV, Azar-Khiai KR, Moghaddam ZS, Karimi G. Determination of methanol in Iranian herbal distillates. J Complement Integr Med. 2016;13(2):123-127.
22. Posnner D, Zimmer T, Kürbel P, Dietrich H. Methanol contents of fruit juices and smoothies in comparison to fruits and a simple method for the determination thereof. Deut Lebensm Rundsch. 2014;110(2):65-59.
23. Karimi G, Hassanzadeh M, Shahidi N, Samie Z. Quantitative determination of methanol in plant water produced in Mashhad by spectrophotometry method. J Med Plan. 2010;339(3):65-59.
24. Wu M-C, Jiang C-M, Ho Y-Y, Shen S-C, Chang H-M. Convenient quantification of methanol in juices by methanol oxidase in combination with basic fuchsin. Food Chem. 2007;100(1):412-418.
25. Lachenmeier DW, Schoedel K, Kanteres F, Kuballa T, Sohnhus EM, Rehm J. Is contaminated unrecorded alcohol a health problem in the European Union? A review of existing and methodological outline for future studies. Addiction. 2011;106(1):20-30.