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Determination of ethanol content in industrial and domestic vinegar samples by headspace-gas chromatography

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Abstract

Background and objective: Vinegar, as a traditional fermented product, plays a significant role in human health and nutrition. This product is produced in different types industrially or domestically. Under production, the vinegar may contain ethanol that its inclusion is banned in the foods by Islam and its content should be adapted to the legislations developed in Islamic countries. Therefore, at this study we measured ethanol concentration of vinegars distributed in Tehran (capital city of Iran).

Materials and methods: Headspace-gas chromatograph equipped with flame ionization detector was developed for determination of ethanol. Acetonitrile and nitrogen were used as internal standard and carrier gas, respectively. For analysis, 140 samples (37 domestic and 103 industrial vinegars) were purchased from local market.

Results and conclusion: Our developed method could successfully determine the ethanol content at low concentration. In this regard, LOD, LOQ, and recovery were 0.0069% v/v, 0.021% v/v, and 100.14%, respectively. In 13 samples, the ethanol content was higher than 0.5% v/v that is the maximum limit determined by Iranian national standard. Out of 13 samples, 10 vinegars were domestically prepared. Evaluation of our results revealed that ethanol concentration in domestic vinegars was higher than industrial products. Therefore, domestic production of vinegars should be controlled and monitored strictly.

Keywords: Ethanol; halal; headspace-gas chromatography; vinegar

1. Introduction

Vinegar has been used as condiment and medicine since its evolution until the 21st century [1]. The old French word “vyn egre” or “vinaigre”, meaning sour wine, is origin of the vinegar word [2]. Babylonians used honey vinegar for food

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preservation for the first time. Health promotion properties of antimicrobial, antioxidant, anti-hypertension, anti-tumor, and anti-diabetic activities were attributed to the vinegar [3]. These functional properties are related to the polyphenolic components such as catechin and epicatechin, and organic acids [4]. Vinegar is a rich source of amino acids, sodium, potassium, vitamin C, and B-group vitamins [5].

In view of side effects, hydroxymethylfurfural (HMF) and furan in vinegar can cause undesirable impact on human health [6]. Osteoporosis, hypokalemia, and hyporeninemia are of the other unfavorable effects of vinegar, which may be observed after high amount ingestion of the product [7]. When using in-home remediation, it may cause burning and gastroparesis [8].

Vinegar, a product of two-step fermentation, is a dilute aqueous solution of acetic acid made by acetic acid bacteria after oxidation of ethanol [9]. Ethanol and CO_2 are also produced from fermentation of the carbohydrates in fruits, grains, honey, and the other carbohydrate-rich substrates. These processes are accomplished by enzymatic activities of the yeasts particularly *Saccharomyces cerevisiae* [10]. Solid-state fermentation (SSF) and liquid-state fermentation or submerged method fermentation (SMF) are two common processes for vinegar production at industrial scale [11].

Ethanol is the product of first-step fermentation and can induce severe damage in human body if swallowed at high concentration. Ethanol is a prohibited (Haram) product in Islam even at small quantities [12]. Side effects of short-term ethanol intake include euphoria, talkativeness, flush reaction, increased pain tolerance, disinhibition, and extraversion (at doses below 100 mg/dl), ataxia, analgesia, spins, mood swings, anger or sadness, nausea, vomiting (at a dose of 100-300 mg/dl), central nervous system depression, pulmonary aspiration, stupor, and coma (at doses of 300-500 mg/dl). Moreover, there is a serious risk of death at doses above 500 mg/dl. Long-term exposure to ethanol leads to the phenomenon of alcoholism, which causes symptoms of hepatitis, pancreatitis, cardiovascular and brain disorders, and a variety of cancers. Due to these problems, several countries have enacted some restrictions with regard to the presence ethanol in foods. According to the Iranian national regulation, the maximum permitted level of ethanol in vinegar is 0.5% v/v [13-17].

To measure and control the concentration of ethanol in vinegar, some analytical and experimental methods have been developed. The oldest method is semi-quantitative ebullioscopic approach based on boiling point of the liquid. Other methods include titration, distillation, high performance liquid chromatography (HPLC), gas chromatography, dichromate oxidation spectrophotometry, near-infrared spectroscopy, nuclear magnetic resonance spectroscopy, enzymatic approaches, and pycnometric density method [18-20]. Some of the mentioned methods have limitations that make them inappropriate for monitoring purposes. For example, distillation and dichromate oxidation spectrophotometry can not determine the ethanol content at low quantities or distillation needs the sample's preparation and pretreatment. Furthermore, low accuracy/reproducibility and low sensitivity is reported for enzyme-based methods and HPLC, respectively [20].

In the current study, we used head space-gas chromatography-flame ionization detector (HS-GC-FID), which is one of the most common methods for analysis of volatile compounds. High speed in analysis, high sensitivity, simplicity, reproducibility, no need for sample preparation, high efficiency, and suitability for measuring the small amount of samples are advantages of this method [21,22]. Compared to the similar studies performed previously, we analyzed more samples in the current work. Through which, we determined the ethanol content within industrial and domestic vinegar samples by using headspace-gas chromatograph.

2. Materials and methods

2.1. Sampling and chemicals

In total, 140 samples of vinegar including 103 industrial vinegars (of 70 brands) and 37 domestic vinegars were collected from supermarkets in Tehran. Pure ethanol and acetonitrile (HPLC grade) were purchased from Merck (USA) and DUKSAN (South Korea), respectively.

2.2. Gas chromatography

Gas chromatograph-flame ionization detector (GC-FID, 7890A, Agilent, USA), equipped with headspace detection facilities (7697A Agilent, USA) and Varian CP-Wax 52 CB column (30 m × 0.53 mm × 0.70 mm) was used for analysis. Flow rate of the carrier gas (nitrogen) was set on 10 ml/min. Temperature of oven, injector, detector, and sample loop were 90, 225, 250, and 100 ℃, respectively. After 2 min, initial temperature of oven (60 ℃) raised to 100 ℃ by flow rate of 5 ℃/min, and then reached final temperature of 210 ℃ with rate of 20 ℃/min. Hydrogen flow rate in gas detector was 30 ml/min and the airflow set on 300 ml/min.

2.3. Sample preparation

One ml of each sample was poured into 10-ml volumetric flask. Then, 50 µl of acetonitrile was added to it as internal standard. The mixture was made up to 10 ml by double distilled water. At the end, 5 ml of each sample was transferred to a headspace vial for analysis.

2.4. Standard preparation

For preparation of stock solution, 10 ml of ethanol was transferred to 100-ml volumetric flask and was further diluted by double distilled water up to 100 ml. Working standard solutions at concentration of 0.05, 0.1, 0.3, 0.5, 0.7, and 1% v/v were prepared from the stock solution.

2.5. Method validation

Validation of this analytical method was done according to the guideline proposed by ICH [23]. Calibration curve was plotted by injection of the standard solutions at six concentrations in triplicate during three consecutive days. Linear range, regression equation, correlation coefficient, limit of detection (LOD), and limit of quantification (LOQ) were calculated after plotting the calibration curve. LOD and LOQ were calculated according to the equations:

$LOD=3.3\ S_y/S$

$LOQ=10\ S_y/S$

(S_y = standard deviation of the intercept in the calibration curve, S = slope of the calibration curve).

For determination of recovery (described as accuracy), the samples were spiked by ethanol up to 0.5% v/v. For relative standard deviation (RSD%), the intra- and inter-day precision were calculated by analysis of a sample three times a day (repeatability) and on three consecutive days (reproducibility), respectively.

2.6. Statistical analysis

Our data were analyzed by SPSS software (version 16). Analysis was done by one-way analysis of variance (ANOVA) for comparison of the means followed by Tukey test. Differences were significant at $p \leq 0.05$.

3. Results and discussion

In the current study, ethanol concentrations of 140 vinegars (103 industrial versus 37 domestic samples) were determined using GC-FID coupled with headspace sampler. Before analysis of the marketed vinegar samples, the system was validated and acceptable results were observed. Figures of merit are demonstrated in Table 1.

Table 1- Figures of merit for analysis of ethanol in vinegars by HS-GC-FID

Regression equation	Linear range (% v/v)	RSD (%)	Recovery (%)	LOQ (% v/v)	LOD (% v/v)
Y = 1.2085 X + 0.067	0.5-1	3.75	100.14	0.021064	0.006951

The accurate linearity was achieved in range of 0.05-1% v/v that covers the maximum permitted level of ethanol in vinegar determined by Iranian national regulation. Standard chromatogram of ethanol and acetonitrile as internal standard is presented in Figure 1.

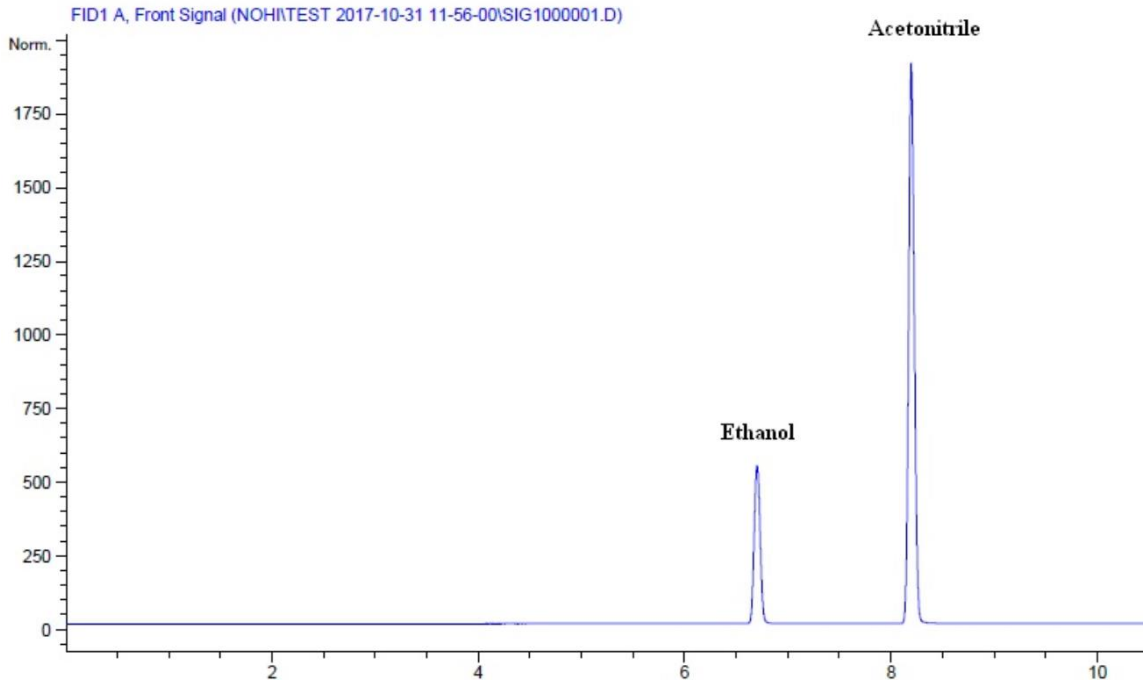

Figure 1- HS-GC-FID chromatogram of ethanol as analyte and acetonitrile as internal standard

Our validated method could successfully determine 13 products violated the national permitted level (ethanol concentration higher than 0.5% v/v), of which 10 vinegars were domestically prepared (Table 2).

Table 2- Concentration of ethanol in industrial and domestic vinegars purchased from local market; n = 140 (37 domestic versus 103 industrial samples of 29 commercial brands in total)

	Ethanol concentration (mean ±SD)
Industrial vinegars (103 samples from 29 commercial brands)	0.209 ±0.106; 0.006 ±0; 0.144 ±0.021; 0.263 ±0.244; 0.16 ±0.033; 0.035 ±0.061; 0.16 ±0.133; 0; 0.021 ±0.02; 0; 0.09 ±0.097; 0.053 ±0.055; 0.317 ±0.119; 0.294 ±0.251; 0.07 ±0.061; 0.095 ±0.021; 0.074 ±0.071; 0.108 ±0.102; 0.013 ±0.029; 0.126 ±0.018; 0.184 ±0.021; 0.366 ±0.074; 0.055 ±0.054; 0.134 ±0.163; 0.029 ±0.008; 0.37 ±0.089; 0.092 ±0.005; 0.314 ±0.018; 0.03 ±0.032
Domestic vinegars (37 samples)	0.336 ±0.369

The maximum concentration of ethanol in domestic vinegars was 4.34% v/v. It reveals that the homemade vinegars are produced under uncontrolled condition and their production should be restricted or strictly controlled by the responsible authorities. In comparison, amount of ethanol in the three inconsistent industrial samples was slightly higher than the permitted level. The average content of ethanol in 37 domestic and 103 industrial vinegars was 1.01% and 0.176% v/v, respectively. According to our results, there was significant difference between industrial and domestic vinegars ($p < 0.05$). In addition, no significant difference was observed among

different brands of the industrial vinegars. Importantly, among different types of the vinegars (grape, red, and white), grape vinegar had the highest concentration of ethanol. Other researchers have done similar studies in this regard. Pulungan et al. (2018) determined ethanol content in two types of vinegar In Indonesia (Arabic vinegar and vinegar x purchased from local market). The average concentration of ethanol was 0.0228% v/v in Arabic vinegar and 0.0117% v/v in vinegar x [22]. Their results were similar to our observations for industrial vinegars to some extent.

Ethanol concentration was determined in some Halal products marketed in Malaysia (including fermented beverages, carbonated drinks, juice, tea, coffee, energy drinks, and vinegars) using gas chromatography-mass spectroscopy, through which ethanol was detected in 58 out of 95 samples. Compared to their study, we used GC-FID for ethanol detection. Applying the flame ionization detector, which successfully determined low concentration of ethanol in the matrix, is more convenient compared to mass spectroscopy for quantitative analysis [24, 25].

Turkucar et al. conducted one study on ethanol concentration of some non-alcoholic beverages (including cola, orange soda, fruit juice, fruit nectar, energy drinks, ayran, and kefir) by titrimetric method in Turkey. This method is time-consuming and non-selective for ethanol, and also requires high quantity of sample. Therefore, it is not comparable to GC for ethanol determination as a high sensitive approach. However, the highest ethanol content was 1.46 mg/l (0.000146% v/v) in fruit juices and the least concentration was determined in cola (0.14 mg/l equals 0.000014% v/v). Ethanol concentration of the samples was as low as expected and did not exceed the Turkish standard (0.3 g/l equals 0.03% v/v) [26].

In a similar study conducted in Iran (2017), ethanol and methanol concentration were determined in 50 samples (of five brands) of non-alcoholic beverages and herbal distillates by HS-GC-FID, and acetonitrile was also used as internal standard. In most of the samples, ethanol concentration was lower than the maximum permitted level. Range of ethanol concentration in non-alcoholic beverages and herbal distillates was 0-2.2% v/v and 0-0.09% v/v, respectively [27]. We quantified ethanol in the range of 0-0.59% v/v and 0-4.34% v/v within industrial and domestic vinegars, respectively. Another study in Turkey (2013) analyzed ethyl alcohol level of vinegars by HS-GC-FID. The concentrations were 0.0038% v/v in grape vinegar, 0.44% v/v in industrial apple vinegar, and 0.0145% v/v in homemade apple vinegar that is in contrast to our results of the highest ethanol in domestic grape vinegars (4.34% v/v) [28]. What is important is that the most validated and common analytical method for quantification of ethanol in food products is HS-GC-FID method that was followed in the current work and acceptable validity criteria were observed in our laboratory.

4. Conclusion

We studied both domestic and industrial vinegars by HS-GC-FID that could accurately determine the least ethanol level in the products. Ethanol content in 10 domestic vinegars exceeded the permitted level determined by Iranian national standard. This observation was due to insufficient control of the homemade vinegars distributed in the local market.

5. Acknowledgement

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6. Conflict of interest

Authors declare that there is no conflict of interest.

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