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SOLID PHASE MICRO EXTRACTION-GAS CHROMATOGRAPHY FOR THE ANALYSIS OF OXIDATION PRODUCTS FROM FERMENTATION OF MALT BEVERAGES

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ABSTRACT

In this study, a solid-phase micro extraction (SPME) technique followed by gas chromatography connected with flame ionization detector GC-FID was used for qualitative and quantitative analysis of the produced alcohols in malt beverages. Eight different sorts of malt beverages were collected from the local markets of western province of Saudi Arabia. The studied samples were analyzed via capillary gas chromatography for their alcohol content after exposing to air and in presence of yeast. It has been found that minor concentration of ethanol content was found after exposing malt beverages to air. Considerable variability in the alcoholic strength was found in presence of yeast due to fermentation, overall, the range of concentrations was 0.4621 Vol. % in Budwisers to 3.416 Vol. % in Hillsgurg.

Key words: Solid-phase micro extraction, Gas chromatography, Flame ionization detector, Malt beverages, Fermentation.

INTRODUCTION

Conventional methods such as steam distillation or solvent extraction combined with GC or GC-MS are used as the routine methods for the analysis of the volatile essential oils of aromatic plants. However, these conventional methods have some disadvantages. Steam distillation requires a relative high amount of sample and is a time consuming procedure.

Solvent extraction has the disadvantage that it also extracts non-volatile resinous

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components along with the essential oils, which adversely affect the GC column and the recovery of the volatile components is greatly influenced by the extraction conditions and normally cause pollution problems to the environment and to the technicians[1].

Solid phase microextraction (SPME) technology introduced in 1990 by Arthur and Pawliszyn[2] is a simple and solvent-free technique based in sorption (adsorption and/or absorption, depending on the fiber coating), which is used for extraction and concentration analyses either by submersion in liquid phase or by exposure to a gaseous phase[3]. This technique has become popular in the analysis of volatile and semi volatile chemicals as its superiority over conventional methods has been recognized[4].

Malt beverage is the one of the world's oldest[5] and most widely consumed drink and the third most popular drink overall after water and tea[6]. The production of malt beverages, comprises four main stages: brew house operations, fermentation, aging or secondary fermentation, and packaging[7,8].

The alcohol in malt beverage is produced by the brewing and fermentation of starches, which are mainly derived from cereal grains most commonly malted barley although wheat, maize (corn), and rice are also used[6].

In many countries, people drink malt beverages at lunch and dinner. Studies have found that when food is eaten before drinking alcohol, alcohol absorption is reduced and the rate at which alcohol is eliminated from the blood is increased. The mechanism for the faster alcohol elimination appears to be unrelated to the type of food. The likely mechanism is food-induced increases in alcohol-metabolizing enzymes and liver blood flow[9,10].

Capillary gas chromatography (CGC) connected with flame ionization detector (FID) is a powerful tool in the analysis of alcohols in malt beverage products. Minimal sample preparation, in general, is required. The flavor compounds tend to be volatile in nature, which fulfills one of the main requirements of CGC. In this paper, we have discussed how CGC can be used to (1) monitor alcohol content in alcoholic beverages, (2) determine the volatile profile of a product, and (3) detect trace level impurities.

Our goal in this work was to analysis of eight samples of malt beverages collected from local markets in the western province in Saudi Arabia via solid phase micro extraction-capillary gas chromatography for their alcohol content.

EXPERIMENTAL

Material and methods

Sample collection

Eight malt beverages samples of different international brands were collected from the local markets of western province of Saudi Arabia. The collected samples are given in Table.

Table 1: The origin of the investigated malts beverage samples

S. No.	Sample name	Sample origin
1	Budweiser	USA
2	Efes	Turkey
3	Holsten	Germany
4	Rockers	Gordon
5	Barbican	UAE
6	Hillsburg	KSA
7	Bario	KSA
8	Moussy	France

Solid phase micro extraction procedure

According to Pawliszyn[11], SPME unit consists of a length of fused silica fiber coated with stationary phase (PDMS-DVB), the fiber is attached to a stainless steel plunger in a protective holder. The analysis was carried out by gas chromatography (GC) equipped with flame ionization detector (FID) and fused capillary column.

Analytical procedure

(i) The preparation of stander ethanol solutions with different concentrations (50, 100, 200, 400 and 600 ppm), then stirring for 30 minutes.

(ii) Sampling extraction was performed in 30 minutes by PDMS-DVB fiber over stirred samples.

(iii) After sampling the fiber was withdrawn into the needle of the holder and was immediately placed in the GC injector.

(iv) The desorption temperature was 250ºC and until no carryover was observed after this desorption time.

(v) The malt beverage samples without exposing to air were subjected to the same as the standard sample, the percentage was calculated with respect to standard sample.

(vi) The malt beverage samples after exposing to air were subjected to the same as the standard sample, the percentage was calculated with respect to standard sample.

(vii) 0.1 g of yeast was added to 10 mL malt beverage drink samples. The bottle tightly closed to prevent the aerobic activity of bacteria. The sample with yeast was measured after 24 h.

GC-MS analysis

A Shimadzu 17-A chromatograph equipped with Shimadzu QP-5000 mass spectrometer was used. The separation was achieved using a J&W Scientific DB-1701P column of 30 m x 0.25 mm i. d. and 0.25 ⌈m of film thickness. GC oven temperature was programmed from 40ºC (5 min), to 230ºC at a rate of 5ºC/min and then held 5 min at 230ºC. The carrier gas was helium with a column-head pressure of 1.4 x 105 Pa. Mass spectra were recorded in the electron impact (EI) mode at 70 eV, scanning the m/z 30 to 300. Interface temperature was 250ºC. Data acquisition and data processing were carried using Class5K software.

Peaks in TIC (total ion current) or MIC (Multi Ion Chromatogram) profiles were characterized or tentatively identified from their mass spectral data using National Institute of Standards and Technology (NIST12 or NIST62) and Wiley 229 mass spectrometry libraries. Identification was confirmed using standard compounds, when available.

Gas Chromatography

The studied malt beverage samples were analyzed using Perkin Elmer gas chromatograph of model 580 series equipped with flame ionization detector (FID), using HP-5 fused silica capillary column Packed with 95% dimethyl polysiloxane and 5% vinyl as stationary phase, 30 meter in length, 0.53 mm int. diameter, and thickness film 0.5 μm.

Helium was used as mobile phase, all gas flow rates were set to manufacturer specifications, performing conditioning and standardization of the system. The flow rate was measured from the end of the column with a soap bubble flow rate. Methane as an unretained marker was used to correct the dead volume in the column. Injections were made in split mode with a split ratio of 1 : 15. Glass linear is packed with deactivated glass wool which changed after six injections. The column oven was programmed from 80ºC (hold 1 min) to 300ºC at a rate 10ºC / minute with 190 minute hold at 300ºC. The injector temperature is set at 300ºC and the detector temperature is 320ºC. The data was estimated by integration of the area under the resolved chromatographic profile, using Total Chrom, Ver. 6.2.1 Software, via Interface NCI 900, Manual Injection of 1 μ L of samples after washing syringe with sample's solvent and injected 1 μ L of solvent.

RESULTS AND DISCUSSION

Direct immersion SPME/GC-FID was found to be an attractive technique for qualitative and quantitative analysis of alcohols in malt beverages.

Analysis of alcohols in malt beverages

Malt beverages contain a wide range of volatile compounds, including alcohols. Gas chromatography (GC) a powerful analytical tool in the analysis of these compounds without preliminary extractions. Minimal sample preparation, in general, is required, since the samples are in the liquid state in an alcohol or alcohol/water matrix. For example, AOAC International has published methods for the analysis of fusel oils, methanol, ethanol, and higher alcohols by GC.

In this work, alcohols in malt beverages also can be monitored by capillary GC. Since capillary columns offer efficient separations, capillary GC is especially useful in analyses of structurally similar compounds, such as the fusel alcohols. The unique polarity of the Rtx-5 stationary phase ensures excellent resolution of a range of alcohols. The concentration of alcohol in a beverage is usually stated as the percentage of alcohol by volume. The CGC analysis of the studied samples was done in three steps, the first for the samples after the glass bottle was opened directly, the second at different interval times when the liquid samples were exposed to air during drinking and the third at different interval times in presence of food to see the effect of fermentation.

Firstly, the calibration curve was achieved depending on the relation between the different concentrations of ethanol against the area of each concentration which is summarized in Table 2 and Fig. 1.

Table 2: Concentration of ethanol in the studied malt beverage samples in presence of air after 24 hrs

Sample name	Ethanol (ppm)	Ethanol (Wt. %)	Oxidative product 1	Oxidative product 2
Budweiser	240.0	0.024	96.4	**32.0**
Efes Classic	1940.6	0.194	264.9	81.9
Holsten	290.0	0.290	120.2	**44.3**
Barbican	142.0	0.014	180.2	72.2
Hillsburg	12.7	0.001	601.9	157.6
Bario	73.0	0.007	290.9	98.3
Rockers	551.9	0.055	134.2	56.8
Mossy	70.7	0.007	66.3	21.3

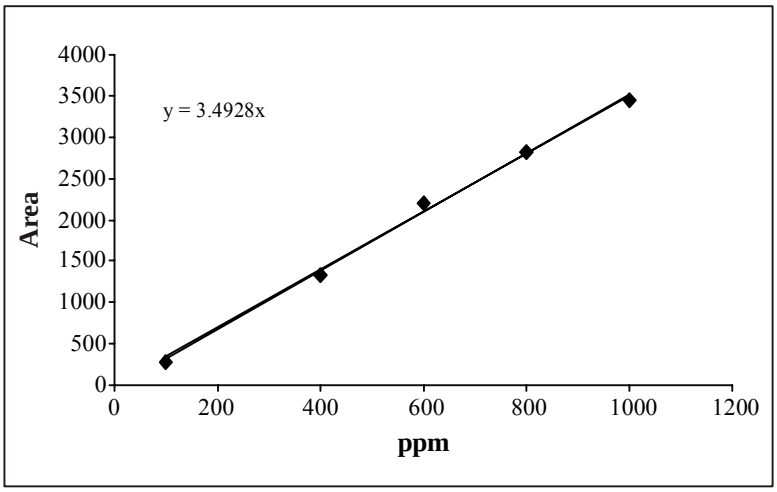

Fig. 1: Calibration curve for ethanol concentration against area

The linear relationship between area and concentration is –

$$y = 3.4928\ x$$

using the linear relationship and the Figure, the minor amounts of the produced ethanol in the studied samples was determined quantitatively.

Directly analysis of malt beverage by CGC

The selected samples were injected directly inside CGC without exposed it to air in order to determine the minor amounts of ethanol if it is present. The quantitative determination of ethanol was achieved depending on the previous internal standard method mentioned above using butanol as internal standard. It has been found that all studied samples have no ethanol contents in their compositions. This result reflecting their agreement with the Islamic religion.

Analysis of malt beverage after exposing to air by CGC

The studied samples were exposed to air after 24 hours. Then the samples were injected in CGC at the same conditions of the standard calibration curve in order to pick up the produced minor amounts of ethanol after exposing to air. The result was given in Table 2. It has been found that all sample produce ethanol after exposing to air but with minor amounts ranging from 12.7 ppm in Hillsburg to 1940.6 in Efes sample. Although the produced ethanol is below the maximum tolerance levels reported by international regulatory standard, the Eslamic so, we advice all moslem people to drink all types of malt beverages without exposing it to air to prevent the production of alcohols. The malt beverages were halal drink when taken directly.

Analysis of malt beverage after fermentation in presence of yeast

Fermentation is a biological process, in which yeast converts sugars and starch into ethyl alcohol (ethanol) and carbon dioxide (CO_2) and is expressed chemically as –

$$C_6H_{12}O_6 + 2\ PO_4^{3-} + 2\ ADP \rightarrow 2\ C_2H_5OH + 2\ CO_2 + 2\ ATP$$

Behind this simplified chemical reaction is a series of complex biochemical reactions. These reactions (known as the Glycolytic pathway or Embden-Myerhof-Parnas pathway) involve a number of enzymes and the reactions take place anaerobically inside the cells of brewing yeast. The presence of oxygen help to stimulate yeast growth and a steady flow of yeast.

The studied malt beverage samples were exposed to air at the same last time intervals in presence of food, then subjected to quantitative analysis by CGC. The process of fermentation occurs in studied malt beverage samples. The produced ethanol contents from fermentation process of the studied samples at different time intervals were given in Table 3.

It has been found that there are five malt beverage samples exhibit minor amounts of ethanol (Vol. % 0.0001) ethanol after 50 minutes of exposing to air in presence of food. These values increase with time and reached to 0.0005 after five hours due to increase the fermentation process with time. All studied samples exhibit minor amounts (Vol. % 0.0001) of ethanol after two hours as a production of fermentation process. The vol. percent of ethanol increases with time and reached 0.004 after five hours.

Table 5: Concentration of ethanol in the studied malt beverage samples in presence of yeast 24 hrs.

Sample name	Ethanol (ppm)	Ethanol (Wt. %)	Oxidative product 1	Oxidative product 2
Budweiser	4621.1	0.462	60.3	-
Efes Classic	9202.4	0.920	188.4	
Holsten	6523.2	0.6523	95.6	-
Barbican	10703.9	1.070	25.6	-
Hillsburg	34160.3	3.416	90.5	43.8
Bario	22328.5	2.233	253.9	20.4
Rockers	5634.3	0.563	60.7	-
Mossy	12799.6	1.280	33.1	-

Although the amounts of ethanol are very minor amounts, the detected ethanol in the investigated samples was found below the maximum tolerance levels (MTLs) reported by the international regulatory standards[10,12]. Although the amounts of ethanol are very minor amounts, the detected ethanol in the investigated samples was found below the maximum tolerance levels (MTLs) reported by the international regulatory standards[10,12].

CONCLUSIONS

(i) The samples Bario, Barbican, Moussy and Hillsburg are more acidic than the other local malt drinks due to their lower pH values.

(ii) The order of conductivity, which is also the order of Total Dissolved Solids (TDS) in this study is Holsten > Budweiser > Efes > Bario > Rockers > Moussy, Barbican > Hillsburg.

(iii) The levels of the mineral and toxic elements measured in all tested samples were found below the maximum tolerance levels reported by the international regulatory standards**.**

(iv) The studied samples have no ethanol contents in their compositions. This result reflecting their agreement with the Islamic religion.

(v) There is a production of minor amounts of alcohols after exposing to air, the production of alcohol content increases in presence of yeast due to fermentation.

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