Asian Journal of Chemistry; Vol. 26, No. 19 (2014), 6583-6586

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16674

Simultaneous Determination of Methanol and Ethanol Content in Traditional Chinese Jujube Brandy

JIANFENG SUN[1,2], JIE WANG[1,2,*], KEXIN GONG[1], RAN SUO[1] and BAOYING YIN[1]

[1]College of Food Science and Technology, Agricultural University of Hebei, Baoding 071000, Hebei Province, P.R. China
[2]Engineering Technology Research Center for Processing of Agricultural Products of Hebei Province, Baoding 071000, P.R. China

*Corresponding author: Fax:+86 312 7528189; Tel: +86 312 7528180; E-mail: wj591010@163.com

Received: 11 November 2013; *Accepted*: 22 January 2014; *Published online*: 16 September 2014; AJC-15966

The simultaneous determination of the methanol and ethanol content in traditional Chinese jujube brandy, a simple and rapid method using GC-FID equipped with a semi-polar column (TG-35MS, 30 m × 0.25 mm × 0.50 μm) is presented. Chromatogram showed a retention time of 6.73 min for methanol and 7.38 min for ethanol. The linear ranges of the calibration curve for methanol and ethanol were both wide, range from 31.25 μg/100 mL to 3.20 g/100 mL for methanol (R^2 = 0.9995) and 1.25 to 80 % v/v for ethanol (R^2 = 0.9969). Standard addition of methanol and ethanol to traditional Chinese jujube brandy gained excellent recoveries of 101.8 and 101.3 %, respectively. With this method, pre-treatment of sample is no longer required and less than 19.10 min is taken for one single analysis from direct injection of original samples to obtaining data. Twenty-four samples of traditional Chinese jujube brandy were detected with this method. The results showed that all samples had a high average ethanol content of 53.4 ± 8.4 % v/v, while the average value of methanol content (in 60 % ethanol) was 0.40 ± 0.09 g/100 mL.

Keywords: Traditional Chinese Jujube Brandy, Methanol, Ethanol, GC-FID.

INTRODUCTION

China is the original country of the jujube, also the largest producing country in the world. The cultivation area of jujube was more than 330,000 hectares in 2011, with annual yield up to 1.1 billion kilograms[1]. China's planting area and yield account for more than 98 % of the world and almost 100 % of the international trade of jujube products comes from China, which plays a significant role in the world's jujube industry[2]. In China, Hebei, Henan, Shandong, Shanxi, Shaanxi and Xinjiang and their total production accounts for more than 90 % of China's total yield[1]. Mainly processed products of jujube in China includes jujube series of drinks including fruit wines, vinegars, fruit and vegetable compound beverages, tea beverages and solid beverages, *etc*. Traditional Chinese jujube brandy, jujube milk, dried jujube, preserved fruit, *etc*.[3].

Jujube brandy is brewed with substandard jujube, which is most produced in family wine distilleries with a process of crushing, wetting, straw mixing, naturally fermentation in tank and distilling by steaming. The obtained traditional Chinese jujube brandy shows a high ethanol content, from 40 to 80 % v/v without any blending. The family wine distilleries, whose products are always consumed by themselves, are not certificated by the national production institutes. Traditional Chinese jujube brandy has yellow color, moderate, pleasant jujube and soft sweet flavor, more important retains the jujube's nutritional value and medicinal value, is a typical health wine which can easily and is fully absorbed by human body[3].

Traditional Chinese jujube brandy has a history of thousands years in China, but its manufacture and market is restricted in a large extent reason of high methanol content expect a large-scale industrial production. It is well known that methanol is a toxic and harmful substance to human health (when taken orally at 340 mg/kg of body weight) of whose ingestion or inhalation can cause blindness or death[4]. Newsholme and Leech[5] reported that oxidized methanol produces lactic acidosis which is a metabolic disease causing an increase of lactic acid in blood. Its symptoms lead to weakness, vomiting and finally coma and death. However, methanol quota is deleted in GB/T 11856-2008[6] which raises wholesome requirement and carries out by GB 2757-2012[7]. While GB 2757-2012[7] requires the maximum limit of methanol content is 0.12 g/100 mL in 60 % v/v ethanol (with raw materials of dry sweet potato and substitutes). Therefore, research in methanol formation mechanism and proposing control measures is important and could provide a theoretical basis for large-scale industrial production.

Subjected to the research, a quick and accurate method to determine the content of methanol and ethanol in traditional Chinese jujube brandy is urgently required. A method for determining methanol in edible wine and volatile compounds of bottled wines using gas chromatography was established by Liu and Díaz, respectively[8,9]. Direct injection of samples into a 30 m CP-Wax 58 CB megapore capillary column with FID has proven useful to determine methanol content in a very short time (< 9 min). The present method is not only quick, but also accurate and reliable when examined in the light of the relative errors and coefficients of variation between repeated analyses and recoveries from standard addition tests[10]. A procedure developed for methanol and ethanol determination in alcoholic samples opens new excite citing possibilities for real applications of vapor phase-FTIR, as compared with the classical liquid phase-FTIR and demonstrates, once again, the capability of the FTIR to provide precise and accurate results in the direct analysis of untreated samples[11]. A simultaneous determination of methanol and ethanol content in fuel ethanol using cyclic voltammetry was introduced[12] and a methodology for the determination of methanol and ethanol in transformer oil was also reported[13], however, these methods were not suitable for food.

In order to simultaneous determine methanol and ethanol content in traditional Chinese jujube brandy, a simple and rapid method using GC-FID is presented in this study.

EXPERIMENTAL

Chromatographic grade solvents (purity above 99.9 %), such as methanol, isopropyl alcohol, isobutyl alcohol and ethyl acetate were purchased from Tianjin Shield Specialty Chemical Ltd. Co. And analytical grade solvents (purity above 99.5 %), such as ethanol absolute, 1-propanol, N-butanol, 1-pentanol and Isoamyl alcohol were purchased from Tianjin Guangfu Fine Chemical Research Institute Ltd. Co.

Traditional Chinese jujube brandies, including 24 samples in different wine ages, were purchased from several different family wine distilleries located in Wang Lin Kou township, Baoding county, Hebei province. These 24 samples were named as sample 01 to sample 24, respectively. All samples were obtained and preserved in normal temperature warehouse before measured.

GC analysis operational conditions: The analysis of methanol was conducted in an Agilent Technologies 6820 GC (Agilent Technologies, Santa Clara, USA) equipped with a computer integrator software (Petrochemical industry QA/QC Cerity Network Data System, Agilent Technologies, Santa Clara, USA), a 30 m TG-35 MS semi-polar capillary column (0.25 mm id, film thickness: 0.50 μm; Agilent Technologies, Santa Clara, USA) and a flame ionizing detector (Hydrogen: 0.10 Mpa and Air: 0.28 Mpa). Flow rate of carrier gas nitrogen was set at 0.40 Mpa. The temperatures at injector port and detector were set at 220 and 230 °C, respectively and split injection (about 2 μL for each injection, split ratio = 30:1) was used. In order to avoid cross contamination between samples, the syringe for injection was completely dried out after rinsing the syringe with 60 % ethanol solution. Oven temperature was controlled with a temperature elevation program during analysis, which was initially set at 40 °C for 2 min, elevated to 115 °C at the rate of 5 °C/min for 0 min, then elevated to 220 °C at the rate of 50 °C/min and maintained for 0 min. Each sample was injected in triplicate, qualitative test and quantification was based on retention time and external standard method, respectively.

Establishment of standard curve for methanol and ethanol content: The standard solutions for methanol (in 60 % ethanol) containing a series of concentrations (2^{-10}c, 2^{-9}c, 2^{-8}c, 2^{-7}c, 2^{-6}c, 2^{-5}c, 2^{-4}c, 2^{-3}c, 2^{-2}c, 2^{-1}c and c, c = 3.20 g/100 mL) were analyzed to calculate the response factor. Subsequently, the content of methanol for each sample was determined according to the peak area of methanol from GC analysis mentioned above.

The standard solutions for ethanol (in distilled water) containing a series of concentrations (1.25, 2.5, 5, 10, 20, 30, 40, 50, 60, 70 and 80, v/v, %) were analyzed to calculate the response factor. Subsequently, the content of ethanol for each sample was determined according to the peak area of ethanol from GC analysis mentioned above.

Recovery test and validation of the method: In order to evaluate the validation of the direct GC method, a range of standard methanol and ethanol concentrations were both determined 5 times. Supplements of 0.05, 0.10 and 0.20 g/100 mL methanol standard (in 60 % ethanol) were added to quantified brandy sample, respectively. 1 mL supplements of 20, 40 and 60 % ethanol standard 1 mL were added to 1 mL quantified brandy sample, respectively. Both 2 μL of the mixed samples were subjected to GC analysis mentioned above. Recoveries of the methanol and ethanol were calculated by comparing the original content of methanol and ethanol in the quantified brandy sample and the content with standard addition. Standard deviations of the measured value and relative error of mean were for index of accuracy.

Determination of methanol and ethanol content in traditional Chinese jujube brandy: All samples 2 μL for each injection were subjected to GC analysis mentioned above and each sample was injected in triplicate.

RESULTS AND DISCUSSION

In order to determine the optimum condition for GC analysis of methanol and ethanol, semi-pore capillary columns with film thickness 0.25 μm and 0.50 μm were compared. Preliminary tests suggested that the column with film thickness 0.50 μm (TG-35MS) had the better capability to separate methanol, ethanol and other components in brandy samples. The typical separation profile of known constituents in brandy samples is shown in Fig. 1(A). When compared with other seven authentic compounds, as shown in Fig. 1, the retention time of methanol and ethanol were 6.73 and 7.38 min, respectively, and that of isopropyl alcohol, 1-propanol, ethyl acetate, isobutyl alcohol, N-butanol, isoamyl alcohol and 1-pentanol were 7.80, 8.98, 10.55, 10.64, 11.97, 14.63 and 15.94 min, respectively. Consequently, the methanol and ethanol content in a sample could be determined within 19.10 min.

The peak area value of standard methanol and ethanol were determined. As shown in Figs. 2 and 3, the regression between the peak area and the concentration value of methanol

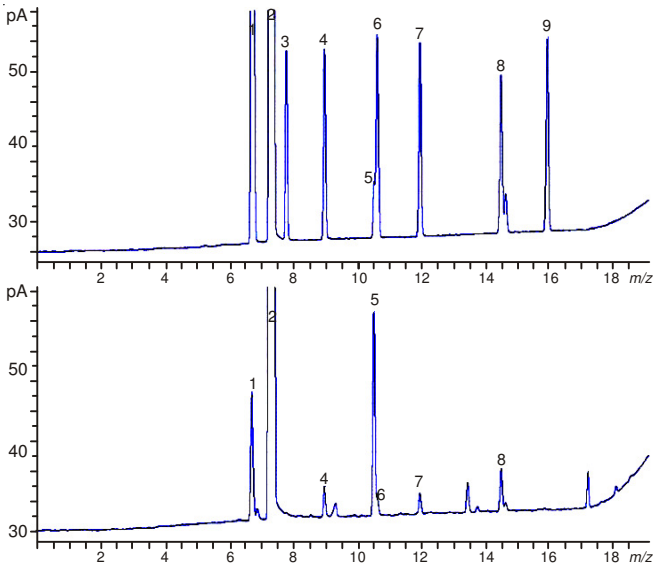

Fig. 1. Gas chromatograms of standard mixture compounds and samples. (A) 1.6 g/100 mL standard mixture compounds, (B) Traditional Chinese jujube brandy sample compounds (keys of numbers above peaks: 1. methanol, 2. ethanol, 3. isopropyl alcohol, 4. 1-propanol, 5. ethyl acetate, 6. isobutyl alcohol, 7. N-butanol, 8. isoamyl alcohol and 9. 1-pentanol)

and ethanol found for peak area (y) to concentration value (x) were y = 257.27x + 3.5596 (R^2 = 0.9995) for methanol and y = 239.19x + 510.32 (R^2 = 0.9969) for ethanol, both fitted a liner relationship well. The linear ranges of the calibration curve for methanol and ethanol were both wide, range from 31.25 µg/100 mL to 3.20 g/100 mL for methanol and 1.25 to 80 % v/v for ethanol. The wide linear ranges of methanol and ethanol detection also suggested that this method might be suitable for determination with samples with highly varied methanol and ethanol content, such as traditional Chinese jujube brandy.

Recoveries from a series of standard addition and variations of detection were also used to evaluate the method. Table-1 shows that indiscriminately spiking Chinese jujube brandy with 0.05, 0.10 or 0.20 g/100 mL of methanol and 20, 40 or 60 % of ethanol, they all displayed a good recovery between 101.5 and 102.2 % with a low coefficient of variation between 0.8 and 1.7 % for methanol and between 99.4 and 103.2 % with a low coefficient of variation between 2.3 and 5.2 % for ethanol.

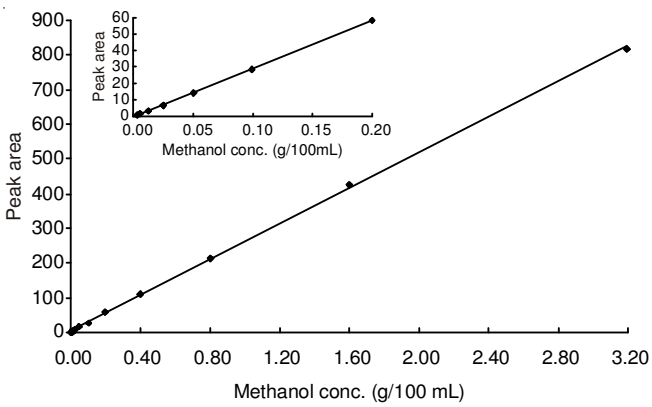

Fig. 2. Calibration curve of methanol

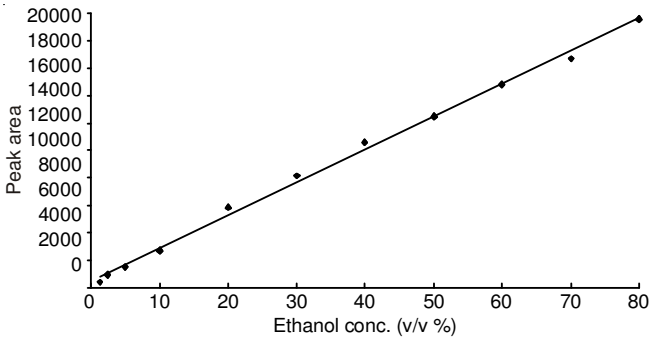

Fig. 3. Calibration curve of ethanol

In addition, varied concentrations of methanol and ethanol standard (from 0.05 to 0.80 g/100 mL for methanol and from 10 to 80 % v/v for ethanol, respectively) were determined three times in a day. As shown in Table-2, the coefficient of variation of detections made within a day ranged from 2.3 to 7.1 % for methanol and 4.5 to 9.3 % for ethanol, for different concentrations of standard methanol and ethanol, suggesting the method performed with high precision. The relative errors obtained from the determination within a day ranged from -7.8 to 6.3 % for methanol and -8.9 to 7.3 % for ethanol, indicating the method also had high accuracy.

This GC method was also subjected to methanol and ethanol content analysis in several traditional Chinese jujube brandies. Table-3 shows that methanol and ethanol content of Sample 01 to Sample 24. In Sample 01 to Sample 24 where

TABLE-1
RECOVERIES OF METHANOL AND ETHANOL IN CHINESE JUJUBE BRANDY

Sample	Methanol (g/100 mL, in 60 % ethanol)			Mean recovery (%)[c]	CV (%)[d]
	Blank[a,b](A)	Added amount (B)	Detected amount[b] (C)		
Chinese jujube brandy	0.1995 ± 0.0132	0.05	0.2506 ± 0.0162	102.2	0.8
		0.10	0.3013 ± 0.0207	101.8	1.7
		0.20	0.4025 ± 0.0198	101.5	1.5
		Mean value		101.8	1.3
	Ethanol (v/v, %)			Mean recovery (%)[e]	CV (%)[d]
	Blank[a,b](A)	Added amount (B)	Detected amount[b] (C)		
	60.0 ± 2.00	20	39.76 ± 3.43	99.4	3.7
		40	51.60 ± 2.89	103.2	2.3
		60	60.84 ± 4.68	101.4	5.2
		Mean value		101.3	3.4

[a]Original content of methanol or ethanol in 1mL quantified brandy sample, [b]Data are mean ± S. D. (n = 5), [c] Recovery (%) = (C-A)/B × 100 %
[d]Coefficient of variation was obtained from quintuplicate tests, [e]Recovery (%) = C/(A + B)/2 × 100 %

TABLE-2
PRECISION AND ACCURACY ANALYSIS OF METHANOL AND ETHANOL DETECTION AT DIFFERENT CONCENTRATION

Methanol conc. (g/100 mL)	Within a day [a]		Ethanol conc. (v/v, %)	Within a day[a]	
	Measured[b]	Accuracy[c]		Measured[b]	Accuracy[c]
0.05	0.0461 ± 0.0043	-7.8	10	10.69 ± 0.96	6.9
0.10	0.1063 ± 0.0061	6.3	20	21.46 ± 1.34	7.3
0.20	0.2108 ± 0.0094	5.4	40	37.40 ± 3.27	-6.5
0.40	0.3804 ± 0.0160	-4.9	60	62.94 ± 5.39	4.9
0.80	0.7752 ± 0.0214	-3.1	80	72.88 ± 9.32	-8.9

[a]Repeat injection for three times in the same day (n = 3), [b]Measured is methanol or ethanol content test value, the data of recovery are mean ± S. D. (n = 3), [c]Accuracy is indicated with the results of relative error (%) of individual detection to the mean value of detection.

TABLE-3
METHANOL AND ETHANOL CONTENT DETECTED IN SAMPLE 01 TO SAMPLE 24

Sample Name	Ethanol conc. (v/v, %)[a]	Methanol conc. (g/100 mL)[b]
Sample 01	52.12.2	0.43 ± 0.02
Sample 02	45.2 ± 1.9	0.19 ± 0.01
Sample 03	44.7 ± 1.9	0.50 ± 0.03
Sample 04	43.9 ± 1.9	0.29 ± 0.02
Sample 05	56.5 ± 2.4	0.48 ± 0.03
Sample 06	47.9 ± 2.1	0.40 ± 0.02
Sample 07	49.8 ± 2.1	0.40 ± 0.01
Sample 08	59.4 ± 2.6	0.46 ± 0.02
Sample 09	45.9 ± 2.0	0.40 ± 0.01
Sample 10	46.8 ± 2.0	0.39 ± 0.02
Sample 11	76.7 ± 3.3	0.31 ± 0.02
Sample 12	48.7 ± 2.1	0.48 ± 0.03
Sample 13	50.9 ± 2.2	0.49 ± 0.03
Sample 14	65.0 ± 2.8	0.38 ± 0.02
Sample 15	56.1 ± 2.4	0.42 ± 0.02
Sample 16	49.8 ± 2.1	0.17 ± 0.01
Sample 17	63.4 ± 2.7	0.50 ± 0.03
Sample 18	53.4 ± 2.3	0.41 ± 0.02
Sample 19	57.7 ± 2.5	0.35 ± 0.02
Sample 20	67.7 ± 2.9	0.40 ± 0.02
Sample 21	42.8 ± 1.8	0.49 ± 0.03
Sample 22	47.0 ± 2.0	0.46 ± 0.02
Sample 23	54.0 ± 2.3	0.48 ± 0.03
Sample 24	55.7 ± 2.4	0.42 ± 0.02
Average Value[c]	51.3 ± 8.2	0.40 ± 0.09

[a] The data of Ethanol conc. are mean ± S. D. (n = 3), [b]The Methanol concentration value was the conversion into Ethanol concentration 60 %,v/v, [c] The data of Average Value according Sample 01 to Sample 24 are mean ± S. D. (n = 24)

ethanol content ranged from 42.8 ± 1.8 % (mean ± S. D., n = 3) to 76.7 ± 3.3 % (mean ± S. D., n = 3) and average ethanol content was 53.4 ± 8.4 % (mean ± S. D., n = 24). Therefore, in general Traditional Chinese Jujube Brandy had a very high content of alcohol. While methanol content (in 60 % ethanol) ranged from 0.17 ± 0.01g/100 mL (mean ± S. D., n = 3) to 0.50 ± 0.03 g/100 mL (mean ± S. D., n = 3) with the average value 0.40 ± 0.09 g/100 mL (mean ± S. D., n = 24). According to GB 2757-2012[8] distilled spirits and liquor preparation hygiene standards (SAC), the average methanol content (in 60 % ethanol) in traditional Chinese jujube brandy is extremely high, even exceeding the maximum legal limits more than 2.3 times. Therefore, studying on the formation mechanism of methanol and proposing control measures is urgent and significant.

Conclusion

A novel method was setup which could determine methanol and ethanol content simultaneous in traditional Chinese jujube brandy by GC-FID. Using this method to detect the samples, it is found that the traditional Chinese jujube brandy had a high ethanol content of 53.4 ± 8.4 % v/v and 0.40 ± 0.09 g/100 mL (in 60 % ethanol) of methanol. Besides, both of ethanol and methanol contents in all samples exceeded the legal limits of GB 2757-20128. The average value even exceeded the maximum legal limits more than 2.3 times.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (NSFC) # 31171725/2011 and 31371815/2013. NSFC had no further role in the study design; in the collection, analysis and interpretation of data; in writing; nor in the decision to submit the manuscript for publication.

REFERENCES

1. W. Song and B.H. Zhao, *J. Anhui Agric. Sci*., **39**, 21475 (2011).
2. P. Liu, J. Zhou, L. Da and S. Yancheva, *J. Mt. Agric. Balkans*, **8**, 872 (2005).
3. Z.X. Lv, P. Liu and X.Y. Wang, *Farm Prod. Process*, **12**, 58 (2011).
4. B. Gnekow and C.S. Ough, *Am. J. Enol. Vitic*., **27**, 1 (1976).
5. E.A. Nessholme and A.R. Leech, Bioquimica Medica, Interamericana and McGraw Hill, pp. 412-414 (1986).
6. SAC, GB/T 11856-2008: Brandy, Standardization Administration of the People's Republic of China, Beijing, China (2008).
7. SAC, GB 2757-2012: China National Food Safety Standard-Distilled Wines and its Mixed Wines, Standardization Administration of the People's Republic of China, Beijing, China (2012).
8. X.P. Liu, H.A. Liu, C. Zhang and L. Zhang, *Sci. Technol*., **5**, 76 (2001).
9. C. Díaz, J.E. Conde, J.J. Méndez and J.P. Pérez Trujillo, *Food Chem*., **81**, 447 (2003).
10. M.L. Wang, J.T. Wang and Y.M. Choong, *J. Food Compos. Anal*., **17**, 187 (2004).
11. J.M. Garrigues, A. Pérez-Ponce, S. Garrigues and M. de la Guardia, *Vib. Spectrosc*., **15**, 219 (1997).
12. P.F. Pereira, R.M.F. Sousa, R.A.A. Munoz and E.M. Richter, *Fuel*, **103**, 725 (2013).
13. J. Jalbert, S. Duchesne, E. Rodriguez-Celis, P. Tétreault and P. Collin, *J. Chromatogr. A*, **1256**, 240 (2012).