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Halal Food / HS GC-FID

Detection and Quantitation of Ethanol in Viscous Liquid and Solid Food by Headspace GC-FID

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❑ Introduction

Ethanol is a component found often in processed food either from additives used in food manufacturing process or from fermentation process [1]. However, halal food must not contain ethanol above the limits specified by the Islamic statutory bodies in respective countries. For example, the maximum allowable ethanol contents in (processed) food are zero or less than 1% and maximum allowable naturally-produced ethanol in (fermented food) food are less than 1% in different countries [1]. This emphasizes the importance of sensitive and accurate quantification of ethanol in food for halal authentication. Methods for quantifying ethanol in liquids such as non-alcoholic beverages and soy sauce had been established previously [2]. In this study, we developed further a method for quantitative analysis of ethanol in various viscous liquid and solid food using static headspace and gas chromatography (HS-GC).

❑ Experimental

Analytical system and conditions

A HS-20 headspace autosampler paired with GC-2010 Plus (Shimadzu Corporation, Japan) was used for this study. Details of analytical conditions of the analysis are shown in Table 1.

Table 1: HS-GC analytical conditions for analysis of ethanol

HS (Loop mode)	
Oven Temperature	85°C
Sample Line Temperature	100°C
Transfer Line Temperature	110°C
Pressurizing Gas Pressure	90 kPa
Equilibrating Time	50 min
Pressurizing Time	2 min
Pressure Equilibration Time	0.25 min
Load Time	0.5 min
Load Equilibration Time	0.1 min
Shaking Level	2
Injection Time	1 min
GC Parameters	
Injection Mode	Split mode Split ratio 15
Carrier Gas	Helium
Gas Flow Condition	Constant linear velocity mode Linear velocity 35cm/s Purge flow 3mL/min
Oven Temperature Programming	35°C (8min) →20°C/min to 250°C (5min)
Column	SH-Rxi-624sil MS 30m x 0.32mm ID x 1.8µm df
Detector - FID	
Detector Temperature	250°C

Chemicals and samples preparation

Ethanol and Isopropyl alcohol (IPA) were purchased from Kanto. Sodium Chloride (NaCl) was purchased from Sigma Aldrich. Ethanol stock solutions of 50, 100, 500, 1000, 5000 and 10000 mg/L were prepared in deionized water. IPA, a surrogate standard, was diluted with deionized water to a concentration of 2000 mg/L. A matrix modifier solution was prepared by adding 180 g of NaCl to 500 mL of deionized water. The matrix modifier solution was used in accordance to EPA 5021A method to reduce the partition coefficient of the analyte and hence, reducing the solubility of analyte in the matrix [3,4]. This was carried out to increase analysis sensitivity.

For preparation of calibration standards, 9.9 mL of the matrix modifier solution was added to a 20-mL headspace vial, followed by 2 mL (approximately 2 g) of ethanol stock solution and 100 µL of IPA (2000 mg/L). The vial was then immediately crimped. Both external standard calibration method and standard addition calibration method were tested and compared in terms of matrix effect with headspace injector. The quantitation recoveries were determined with two spice samples spiked with ethanol at 1000 µg/g (0.1%) and 5000 µg/g (0.5%), respectively.

Fourteen food samples of different types from viscous liquid to solid different forms were purchased from local supermarket. The samples included sauces, spices, fermented product and flavours. For sample preparation, 2 g of food sample was added to the headspace vial, followed by 100 µL of IPA and 9.9 mL of matrix modifier solution.

Students from Nanyang Technological University (*) and National University of Singapore (**) for internship training program

❑ Results and Discussion

HS-GC-FID method

Ethanol and IPA were well separated (Fig.1) using this method. The retention time of ethanol and IPA were 4.868 min and 6.201 min, respectively. Repeatability test using peak area was carried out for both ethanol and IPA in deionized water (Table 2). Percentage relative standard deviation (n=6) of IPA (100 µg/g) and ethanol (50 µg/g) was 4.8% and 2.5% respectively.

Quantitation methods

Two quantitation methods, external standard method and standard addition method, were tested and compared. Percentage recovery of ethanol in the spiked samples was used as main reference to compare the calibration methods.

For external standard calibration, a 6-point calibration curve was plotted (Fig.2). The correlation coefficient (R^2) of the curve was 0.999 across the range of 50 to 10,000 µg/g. IPA (surrogate standard) of 100 µg/g was spiked into each calibration level to monitor matrix effect and extraction efficiency. Based on the recovery of IPA, the sample ethanol concentration derived from the calibration curve was further calculated to obtain the corrected ethanol concentration by following equation:

$$Corrected\ ethanol\ conc. = \frac{C}{Recovery\ of\ IPA\ (\%)} \times 100\%$$

C = concentration of ethanol (µg/g) derived from calibration curve

For standard addition method, a calibration curve was built by spiking the sample matrix with increasing standard concentrations [5]. It was expected that the matrix does not impose or give very little effect to the quantitation result when using standard addition method [5]. Hence, spiking of surrogate (IPA) was not carried out, unlike the external standard calibration.

Chili powder and turmeric powder spiked with 1000 µg/g (0.1%) and 5000 µg/g (0.5%) ethanol were quantitated by both external standard and standard addition methods. The ethanol recoveries determined by the two different methods are compared in Table 3. The ethanol recoveries by external standard method are better (within ±5% differences from the true value) than that by standard addition method. Hence, external standard calibration method was selected for quantitative analysis of ethanol in actual samples. Noted that, however, this finding was made based on the spice samples only. With different matrices, the recovery may exhibit different results. More analysis with different matrices should also be performed in validation of method.

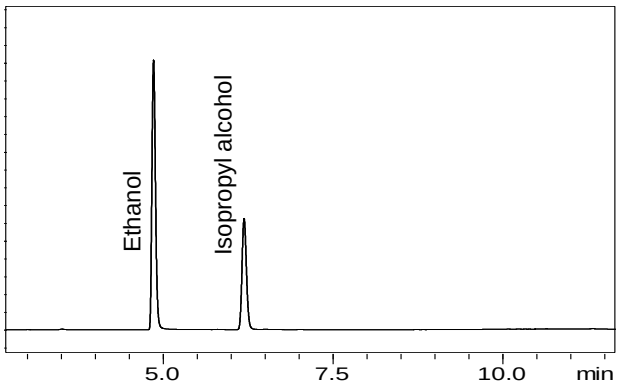

Fig.1 Chromatogram of ethanol and IPA in deionized water

Table 2: Peak area repeatability (n=6) for ethanol and IPA

Inj No.	Ethanol (Area count)	IPA (Area count)
1	33611	219949
2	35414	235604
3	33970	206898
4	34222	233388
5	34008	231192
6	32823	221625
%RSD	2.5	4.8

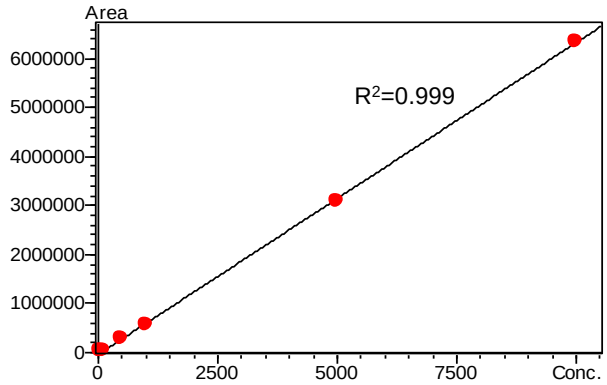

Fig.2 External standard calibration curve for Ethanol

Table 3: Comparison of %recovery for ethanol using different type of calibration method

Sample spiked with ethanol	% Recovery of ethanol using external standard calibration*	% Recovery of ethanol using standard addition calibration
Chili powder (1000 µg/g)	102.3	79.6
Chili powder (5000 µg/g)	100.1	91.0
Turmeric powder (1000 µg/g)	99.2	82.1
Turmeric powder (5000 µg/g)	96.8	89.9

*Note: The recovery of ethanol using external standard is calculated from the corrected concentration w.r.t IPA recovery efficiency.

Table 4: Quantitative results of ethanol in different samples by HS-GC-FID method with external standard method

Sample type	Sample name	Physical Texture	Ethanol Conc. (µg/g)	Recovery of IPA (%)	Ethanol Conc. w.r.t. IPA Recovery (µg/g)
Spice	Cinnamon	Solid stick	75.01	107.8	69.58
	Cumin	Powder	74.78	125.3	59.68
	Curry	Powder	74.70	120.0	62.23
	Mustard seed	Small solid sphere	81.98	108.7	75.40
	Rasam	Powder	76.47	118.4	64.56
Sauce	Barbeque	Viscous liquid	99.62	114.8	86.76
	Chili	Viscous liquid	178.21	113.1	157.62
	Ketchup	Viscous liquid	74.89	111.1	67.40
	Mustard	Viscous liquid	136.56	106.2	128.57
	Sambal chili	Mixture of solid and liquid	75.56	111.4	67.85
Fermented product	Salted soy bean paste	Mixture of solid and liquid	98.61	115.7	85.26
Flavour	Instant noodle seasoning (curry flavour)	Powder	76.99	132.9	57.94
	Instant noodle seasoning (seafood flavour)	Powder	81.02	146.2	55.43
	MSG	Powder	Not Detected	177.2	Not Detected

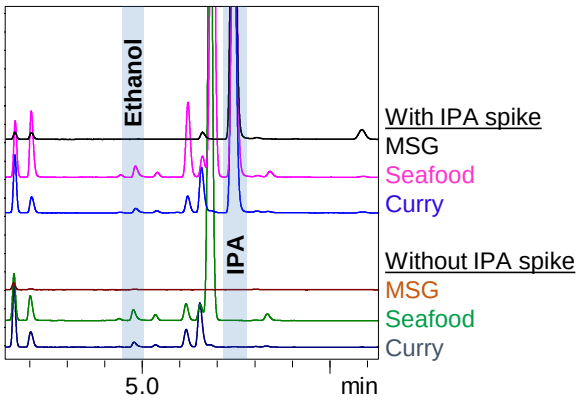

Fig.3: Chromatogram of seasoning samples with IPA spike (top) and without IPA spike (Bottom)

Quantitation of ethanol in samples

Fourteen food samples including spices, sauces, fermented product and flavours, were analysed with the external standard method. The results shown in Table 4 indicate the presence of trace amount of ethanol less than 0.016% in these samples.

The recovery of IPA surrogate was the key indicator of the method in terms of recovery and reliability. Relatively high recovery at 133%~177% were obtained with seasoning powder samples. These samples were also analysed without IPA spike, and IPA was not detected as shown in Fig.3. This result indicates that significant matrix effects in headspace occurred with seasoning powders due to the salt contents in the samples.

❑ Conclusions

A HS-GC-FID method was set up and applied to the quantitative determination of ethanol contents in 14 food samples from viscous liquid form to solid form. External standard method was adopted to establish calibration curve for ethanol at the concentration range of 50~10,000 µg/g. IPA was added as surrogate for checking the recovery. A NaCl based matrix modifier solution following EPA 5021A method was used in the headspace extraction to enhance recovery and sensitivity of the method.

❑ References

1. Jamaludin, et al., *International Food Research Journal. 23(6):* 2737-2743 (2016)
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