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RESEARCH ARTICLE

Determination of alcohols in various fermented food matrices using gas chromatography-flame ionization detector for halal certification

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Abstract

In this study, an analytical method for the determination of alcohols by gas chromatography-flame ionization detection was applied to fermented foods. The limit of detection and limit of quantitation were 0.79 and 2.40 mg/kg for methanol, 0.55 and 1.66 mg/kg for ethanol, 0.51 and 1.56 mg/kg for *n*-propanol, 0.35 and 1.05 mg/kg for *n*-butanol, and 0.38 and 1.16 mg/kg for *n*-pentanol, respectively. The recoveries from the matrices of *gochujang,* soy sauce, and *kimchi* were 93.80–102.03%, 93.27–99.69% and 89.06–102.17%, respectively, and the corresponding intra- and inter-day relative standard deviations were below 5.33%, 5.35% and 4.10%. In 95 fermented foods, ethanol showed the highest mean, median and maximum values among the five alcohols. The detection rate of ethanol was 86.3% among all samples and 100% in *gochujang* and *gochujang*-based sauces. A total of 22 samples had an alcohol content above 0.5%, of which 16 were *gochujang*.

Keywords Alcohol · Fermented food · Halal · Method validation

Introduction

Halal is an Arabic term meaning permissible and lawful. *Haram* is the opposite, meaning prohibited. Alcoholic drinks that cause intoxication (*khamr*) are *haram* according to the *Quran* because of the harmful effects on the nervous system, causing anti-anxiety, short-term memory loss, decreased concentration and impaired judgement (Regenstein et al., 2003). In general, a small amount of ethanol, the main ingredient in alcoholic drinks, is acceptable if the amount is insufficient to cause intoxication (Wan Nadiah et al., 2009). However, the standards for ethanol content in *halal* food industries differ by country and certification body. The permissible ethanol level is less than 0.1% according to the Islamic Food and Nutrition Council of America (IFANCA), 0.5% according to the Korea Muslim Federation (KMF) and the Department of Islamic Development Malaysia (Jabatan Kemajuan Islam Malaysia [JAKIM]), and 1.0% according to the Indonesian Ulema Council (Majelis Ulama Indonesia [MUI]), respectively (Pauzi et al., 2019; Wan Nadiah et al., 2009).

Ethanol is produced commercially by both fermentation and chemical synthesis procedures. Fermented ethanol is produced along with carbon dioxide as by-products of hydrolysis of a carbon source (cellulose (e.g., starch, sugar, cellulose) during fermentation by yeast or bacteria. Synthetic ethanol is produced from ethylene, a by-product of petroleum manufacture. Ethanol is present in food/drink due to natural fermentation or because it has been added as a processing aid in the food and beverage industry (Alzeer and Abou Hadeed, 2016). Many fermented foods include fruits and legumes. Both soy sauce (produced through the fermentation of soya beans) and yoghurt (fermented milk) are types of fermented food (Pauzi et al., 2019). As a food processing aid, industrial ethanol is widely utilised as a

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food preservative, sanitiser and solvent in manufacturing spices or extracts (Mat Hashim et al., 2009).

The Association of Official Analytical Chemists (AOAC) has published standard methods (984.13, 983.14) for determining ethanol content in wine (AOAC, 1988a, 1988b) and beer (AOAC, 1988a, 1988b). The AOAC method employs distillation method, which is not suitable for the simultaneous analysis of various alcohols because the peak of water interferes with those of *n*-propanol and *n*-butanol in the gas-chromatography chromatogram (Park et al., 2016). Many previous studies have reported alcohol content using gas chromatography-mass spectrometry (Ding et al., 2016; Jamaludin et al., 2016), gas chromatography-flame ionization detection (GC-FID) (Gunduz et al., 2013; Najiha et al., 2010), gas chromatography-time-of-flight-mass spectrometry (Mat Hashim et al., 2009) and electronic nose (Ordukaya and Karlik, 2016; Park et al., 2017). However, most of them are limited to liquids, such as beverages, vinegar and alcoholic beverages, with only a few studies reporting on Korean traditional fermented foods.

Korean traditional fermented foods are widely consumed as condiments and used as raw materials in various processed foods, such as sauces and home meal replacement products. However, alcohol is an obstacle to *halal* certification when exporting fermented food in which alcohol is produced as a result of the natural fermentation process. In addition, some commercial fermented foods add ethanol to enhance flavour, remove odour and prevent microbial overgrowth (Gil et al., 2016). Accordingly, for fermented foods to obtain *halal* certification, it is essential to analyse the residual alcohol in the food.

This study performed the dimethyl sulphoxide (DMSO) extraction method and a GC-FID validation to detect and quantify alcohols in solid, semi-solid and liquid Korean traditional fermented foods. It is expected to be used as basic research data for halal certification and export.

Materials and methods

Chemical reagents and materials

Methanol (99.9%), ethanol (100%), *n*-propanol (99.9%), *n*-butanol (99.7%), *n*-pentanol (99.0%), *n*-hexanol (99.0%) and DMSO were sourced from Sigma–Aldrich of Merck Co. (St. Louis, MO, USA). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Commercial *kimchi*, *gochujang*, soy sauce, sauces based on *gochujang or* soy sauce were purchased from local markets in Seongnam-si, South Korea. All samples were stored in the dark at 4 °C until they were analysed.

Preparation of standard solution

The stock solutions of alcohols were prepared in DMSO at 2.54–2.65%. These solutions were diluted with DMSO to provide working standard solutions (1.95, 3.91, 15.62, 62.50, 250.00 and 1000.00 mg/kg) for calibration curves. A solution of 250 mg/kg *n*-hexanol diluted with DMSO was used as the internal standard. All solutions were kept at 4 °C until analysis.

Sample preparation

Alcohols were analysed following a DMSO extraction procedure reported in a previous study (Park et al., 2016). An aliquot (0.5 g) of the sample was weighed in a 20-mL vial containing a polydimethylsiloxane-coated stirring bar (Twister, Gerstel GmbH, Mülheim a/d Ruhr, Germany), followed by 1.0 mL *n*-hexanol (2500 mg/kg), used as an internal standard, and 8.50 mL DMSO. The vial was closed, and then the sorptive extraction technique was carried out at 1300 rpm, 25 °C for 1 h. After removing the Twister, the extract was centrifuged (Union 32R, Hanil Science Industrial, Incheon, Korea) at 1300 rpm, 25 °C for 10 min. The supernatant was filtered through a 0.45-µm syringe filter (Whatman, Maidstone, UK), and the filtrate was analysed by GC-FID.

Method validation

For method validation, the limit of detection (LOD), limit of quantitation (LOQ), linearity, accuracy and precision were measured. Linearity of the six-point calibration curves (1.95, 3.91, 15.62, 62.50, 250.00 and 1000.00 mg/kg) of standards was tested by regression analysis and expressed as the correlation coefficient (r^2). LOD and LOQ were calculated as $\text{LOD} = 3.3 \times [SD / S]$ and $\text{LOQ} = 10 \times [SD / S]$, where SD is the standard deviation of the intercept and S is the slope of the calibration curve. Accuracy and precision were determined by spiking solid (*kimchi*), semi-solid (*gochujang*) and liquid (soy sauce) matrices at three concentration levels of the alcohols (62.50, 125.0 and 250.0 mg/kg) in three replicates on the same day (intra-day accuracy and precision) and on three consecutive days (inter-day accuracy and precision). Accuracy was expressed as the recovery (Eq. 1), while precision was reflected by the relative standard deviation (RSD) value.

$$\text{Recovery (\%)} = \left(C_S - C_B\right)/C_{cer} \times 100 \quad (1)$$

where C_S represents the total concentration measured after spiking, C_B represents the initial measured concentration before spiking, and C_{cer} represents the spiked concentration.

GC-FID condition

GC-FID was performed using a GC-2010 equipped with a flame ionization detector (Shimadzu, Kyoto, Japan) and a DB-WAX column (internal diameter = 320 μm, length = 60 m, film thickness = 0.25 μm; Agilent Technologies, Santa Clara, CA, USA). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The detector temperature was 240 °C, and the injector was operated at 160 °C in split mode (split ratio 30:1). The oven temperature was held at 40 °C for 5 min and then increased to 240 °C at 10 °C/min and held for 9 min.

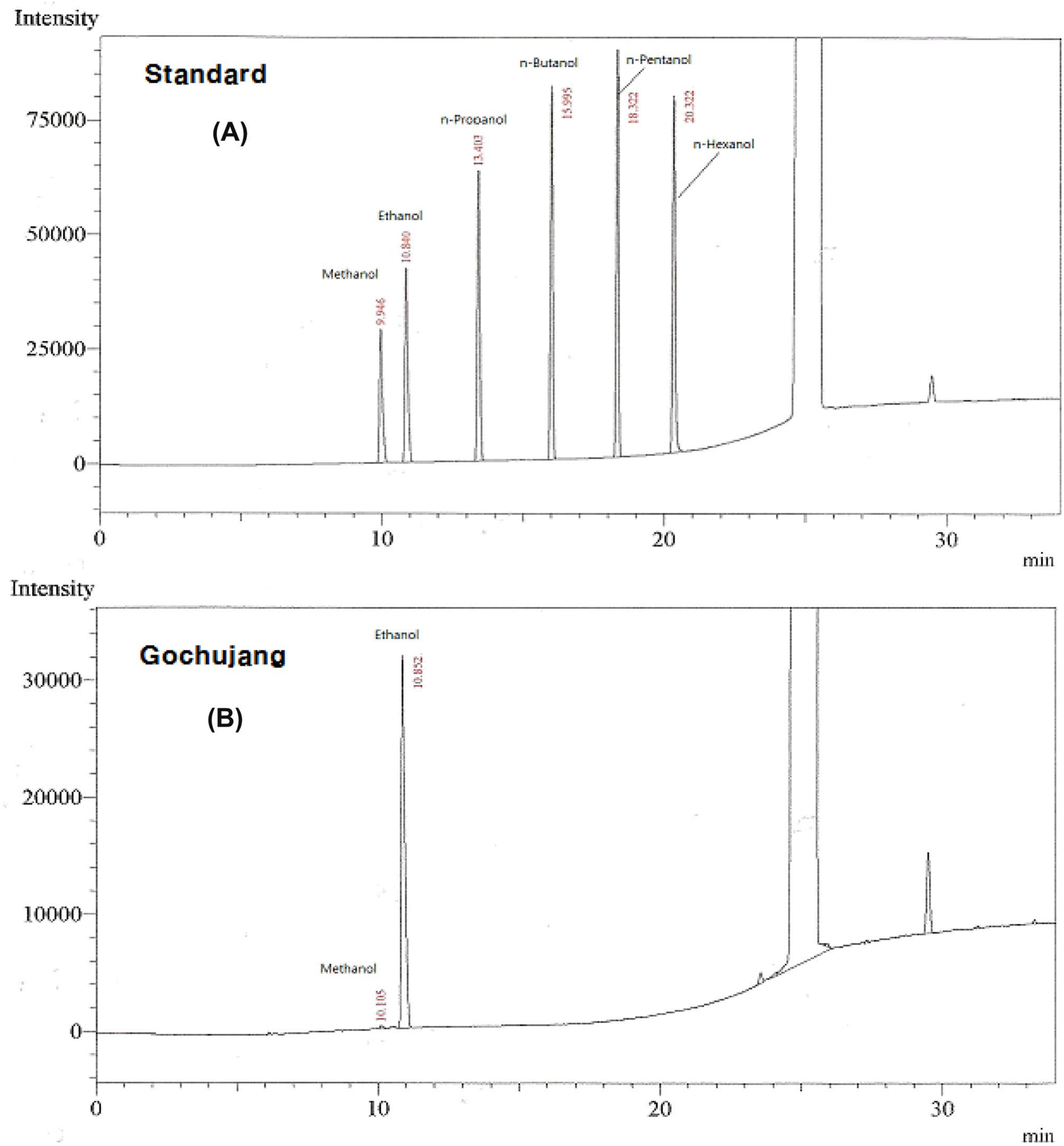

Fig. 1 GC-FID chromatograms of alcohols. (**A**) Standard solution, (**B**) *Gochujang* sample

Results and discussion

Validation of analytical method

The chromatograms of standard solutions and *gochujang* samples are shown in Fig. 1. The standard calibration curves of five different alcohols contained six concentration points. Individual solutions were injected ten times, and the values are presented in Table 1. Good linearity was achieved ($r^2 > 0.999$). The LOD and LOQ were 0.79 and 2.40 mg/kg for methanol, 0.55 and 1.66 mg/kg for ethanol, 0.51 and 1.56 mg/kg for *n*-propanol, 0.35 and 1.05 mg/kg for *n*-butanol, and 0.38 and 1.16 mg/kg for *n*-pentanol, respectively.

Accuracy and precision were investigated by analysing *gochujang*, soy sauce and *kimchi* spiked with three concentrations of the alcohol (62.50, 125.0 and 250.0 mg/kg) in triplicates on the same day (intra-day) and three consecutive days (inter-day). The data are presented in Table 2. The recoveries were 93.80–102.03% in *gochujang*, 93.27–99.69% in soy sauce and 89.06–102.17% in *kimchi*. Intra- and inter-day RSD values were < 5.33% in *gochujang*, < 5.35% in soy sauce and < 4.10% in *kimchi*. All values showed sensitivity, accuracy and repeatability values acceptable according to the Food and Drug Administration (FDA) guidelines (Food and Administration, 2019).

Analysis of alcohols in fermented foods

It is reported that the export of traditional fermented foods as *halal* certified products poses difficulties because of the naturally occurring alcohol (Gil et al., 2016). Accordingly, the validated method in this study was applied to quantify five alcohols (methanol, ethanol, *n*-propanol, *n*-butanol and *n*-pentanol) in *gochujang* (25 samples), soy sauce (23 samples), fermented soybean paste (24 samples) and sauces based on *gochujang* or soy sauce (23 samples). These fermented foods were targeted because they are produced in Korean traditional manufacturing processes without ethanol addition. Commercial products may contain ethanol added as a preservative after heating or sterilization and detected at a level unsuitable for *halal* certification (Gil et al., 2016). The contents of the alcohols are displayed in Table 3.

From the 95 samples analysed, ethanol was the alcohol detected the most frequently (82/95, 86.3%) in each food matrix. Ethanol content (mean, median and maximum) was highest in *gochujang* at 0.14–2.7%, followed by sauces based on *gochujang* (0.52–0.77%), sauce based on soy sauce (0.04–0.46%), fermented soybean paste (ND—0.23%) and soy sauce (ND—0.12%). A previous study also found that *gochujang* had the highest ethanol content among the fermented foods examined (Gil et al., 2016). It is reported that fermentation temperature, initial sugar content and starch source are major factors that can affect the ethanol content (Choo and Shin, 2000; Najiha et al., 2010). Accordingly, it is assumed that ethanol content of *gochujang* is high due to the various starch sources and high sugar content used in the manufacturing of *gochujang*. Park et al. (2016) detected ethanol using DMSO extraction with GC-mass spectrometry and *gochujang* contained 1.73–1.95% ethanol. Gil et al. (2016) analysed ethanol using aqueous extraction combined with GC and reported ethanol levels 1.24 ± 1.97% in *gochujang*.

The distribution of alcohol content among the samples is shown in Table 4. The detection rate of ethanol was 100% in *gochujang* and sauce based on *gochujang*. The detection rate of *n*-propanol, *n*-butanol and *n*-pentanol was below 24.0%, respectively, in each matrix, and none of these alcohols were detected in the sauces. A total of 22 samples had an ethanol content above 0.5%. These included 64% of the *gochujang* samples and all (100%) of the sauces based on *gochujang*. The remaining (44%) *gochujang* samples were found to contain more than 1.0% ethanol. If the KMF's certification standards are applied, 64% of the *gochujang* samples will be inadequate for *halal* certification. Therefore, when *gochujang* is manufactured by a Korean traditional process, it is necessary to check whether it complies with the standards for alcohol content set by the exporting countries if *halal* certification is desired.

In this study, DMSO extraction combined with GC-FID analysis was successfully applied to analyse five different alcohols in solid, paste and liquid foods. The validated method demonstrates good sensitivity, linearity, accuracy

Table 1 The linearity, r^2, LOD and LOQ of alcohols by GC-FID

Type of alcohols	Linearity	r^2	LOD[a] (mg/kg)	LOQ[b] (mg/kg)
Methanol	y = 190.0x – 384.7	0.9999	0.79	2.40
Ethanol	y = 262.4x – 433.3	0.9999	0.55	1.66
n-propanol	y = 349.7x – 751.3	0.9999	0.51	1.56
n-butanol	y = 392.1x – 883.9	0.9999	0.35	1.05
n-pentanol	y = 415.4x – 827.5	0.9999	0.38	1.16

[a]LOD = 3.3 × standard deviation /slope

[b]LOQ = 10 × standard deviation/slope

Table 2 The precision and accuracy of alcohols by GC-FID

Type of matrices	Type of alcohols	Intra-day precision (RSD %)			Inter-day precision (RSD %)			Recovery (%)		
		62.5 (mg/kg)	125.0 (mg/kg)	250.0 (mg/kg)	62.5 (mg/kg)	125.0 (mg/kg)	250.0 (mg/kg)	62.5 (mg/kg)	125.0 (mg/kg)	250.0 (mg/kg)
Gochujang	Methanol	1.75	1.01	1.20	5.33	2.44	2.26	94.63 ± 6.01	95.40 ± 2.52	96.56 ± 2.17
	Ethanol	1.59	1.51	2.88	1.98	3.67	3.55	102.03 ± 2.35	102.00 ± 4.45	101.52 ± 3.98
	n-propanol	2.13	0.94	0.83	3.46	1.96	2.08	96.61 ± 3.34	97.20 ± 1.90	97.55 ± 2.03
	n-butanol	1.76	0.85	1.01	4.15	1.82	1.98	93.95 ± 3.90	95.10 ± 1.73	96.01 ± 1.90
	n-pentanol	1.15	1.00	0.84	4.05	1.76	2.35	93.80 ± 3.79	95.02 ± 1.67	95.79 ± 2.25
Soy sauce	Methanol	0.38	0.56	0.71	0.51	0.63	0.98	95.74 ± 0.94	94.59 ± 0.70	95.42 ± 1.03
	Ethanol	0.74	1.31	1.09	1.01	1.31	1.53	99.69 ± 1.89	99.13 ± 1.67	99.69 ± 1.32
	n-propanol	0.87	0.61	2.88	1.22	0.60	5.35	95.10 ± 1.16	93.90 ± 0.56	97.61 ± 5.22
	n-butanol	0.89	0.51	0.65	0.83	0.57	0.66	95.66 ± 0.79	94.50 ± 0.54	95.58 ± 0.63
	n-pentanol	0.97	0.47	1.82	1.12	0.69	3.04	94.30 ± 1.05	93.27 ± 0.65	95.25 ± 2.89
Kimchi	Methanol	0.93	0.54	0.57	2.11	1.25	1.86	92.77 ± 1.77	91.48 ± 1.07	91.22 ± 1.62
	Ethanol	1.33	1.35	3.44	1.47	1.57	4.10	102.17 ± 5.87	101.11 ± 5.85	97.83 ± 4.83
	n-propanol	1.19	0.33	0.69	1.93	1.07	1.59	92.20 ± 1.51	91.86 ± 0.48	92.21 ± 0.80
	n-butanol	0.92	0.56	0.52	1.49	0.99	1.57	91.27 ± 1.36	90.59 ± 0.89	90.66 ± 1.43
	n-pentanol	1.35	0.64	0.83	1.87	0.97	1.80	90.46 ± 1.69	89.20 ± 0.86	89.06 ± 1.60

Table 3 Content of alcohols in fermented foods

Food group	Type of alcohols	Content of alcohol				
		Number of samples	Mean (mg/kg)	Median (mg/kg)	Minimum (mg/kg)	Maximum (mg/kg)
Gochujang	Methanol	25	245.47	246.22	137.30	345.08
	Ethanol		11,255.54	9155.15	1354.37	26,957.92
	n-propanol		38.76	38.69	ND	62.86
	n-butanol		40.39	39.84	ND	49.61
	n-pentanol		22.81	22.32	ND	33.73
Soy sauce	Methanol	23	25.30	25.30	ND	25.30
	Ethanol		253.47	88.12	ND	1223.57
	n-propanol		355.17	485.76	ND	527.08
	n-butanol		34.62	34.62	ND	52.97
	n-pentanol		43.20	43.20	ND	50.28
Fermented soybean paste	Methanol	24	58.82	59.19	ND	83.73
	Ethanol		815.08	602.79	ND	2343.08
	n-propanol		38.69	51.35	ND	58.33
	n-butanol		43.70	32.99	ND	67.86
	n-pentanol		21.12	12.96	ND	35.35
Sauce based on *Gochujang*	Methanol	6	131.37	112.68	ND	212.83
	Ethanol		6316.68	6116.53	5297.63	7701.55
	n-propanol		ND	ND	ND	ND
	n-butanol		ND	ND	ND	ND
	n-pentanol		ND	ND	ND	ND
Sauce based on soy sauce	Methanol	17	ND	ND	ND	ND
	Ethanol		2275.61	2453.98	425.42	4646.47
	n-propanol		ND	ND	ND	ND
	n-butanol		ND	ND	ND	ND
	n-pentanol		ND	ND	ND	ND

ND Not detectable

and precision and proved to be a reliable method for detecting a low-alcohol concentration. Methanol, ethanol, *n*-propanol, *n*-butanol and *n*-pentanol in 95 fermented foods produced by Korean traditional manufacturing processes were analysed. In all samples, ethanol showed the highest mean, median and maximum values among the five alcohols, with a detection rate of 86.3%. Ethanol was detected in all tested samples of *gochujang* and sauces based on *gochujang* and displayed levels of 0.14–2.7% and 0.52–0.77%, respectively. Few studies have simultaneously analysed the residue of five alcohols in Korean traditional fermented foods. Accordingly, this study is expected to be used as basic research data for *halal* certification and alcohol reduction research in fermented foods. Further studies are required to monitor the alcohol content of various fermented export foods and to reduce ethanol in *gochujang*.

Table 4 Distribution of alcohol content in fermented foods

Food group	Type of alcohols	Content of alcohol (detection rate, %)				Total
		X ≥ 1.0%	0.5 ≤ X < 1.0%	0.0 ≤ X < 0.5%	Not detectable	
Gochujang	Methanol	N.D (0.0)	N.D (0.0)	25 (100.0)	0 (0.0)	25
	Ethanol	11 (44.0)	5 (20.0)	9 (36.0)	0 (0.0)	
	n-propanol	N.D (0.0)	N.D (0.0)	6 (24.0)	19 (76.0)	
	n-butanol	N.D (0.0)	N.D (0.0)	6 (24.0)	19 (76.0)	
	n-pentanol	N.D (0.0)	N.D (0.0)	6 (24.0)	19 (76.0)	
Soy sauce	Methanol	N.D (0.0)	N.D (0.0)	1 (4.3)	22 (95.7)	23
	Ethanol	N.D (0.0)	N.D (0.0)	12 (52.2)	11 (47.8)	
	n-propanol	N.D (0.0)	N.D (0.0)	3 (13.0)	20 (87.0)	
	n-butanol	N.D (0.0)	N.D (0.0)	2 (8.7)	21 (91.3)	
	n-pentanol	N.D (0.0)	N.D (0.0)	2 (8.7)	21 (91.3)	
Fermented Soybean paste	Methanol	N.D (0.0)	N.D (0.0)	18 (75.0)	6 (25.0)	24
	Ethanol	N.D (0.0)	N.D (0.0)	22 (91.7)	2 (8.3)	
	n-propanol	N.D (0.0)	N.D (0.0)	5 (20.8)	19 (79.2)	
	n-butanol	N.D (0.0)	N.D (0.0)	5 (20.8)	19 (79.2)	
	n-pentanol	N.D (0.0)	N.D (0.0)	5 (20.8)	19 (79.2)	
Sauce based on *Gochujang*	Methanol	N.D (0.0)	N.D (0.0)	6 (100.0)	0 (0.0)	6
	Ethanol	N.D (0.0)	6 (100.0)	N.D (0.0)	0 (0.0)	
	n-propanol	N.D (0.0)	N.D (0.0)	N.D (0.0)	6 (100.0)	
	n-butanol	N.D (0.0)	N.D (0.0)	N.D (0.0)	6 (100.0)	
	n-pentanol	N.D (0.0)	N.D (0.0)	N.D (0.0)	6 (100.0)	
Sauce based on soy sauce	Methanol	N.D (0.0)	N.D (0.0)	1 (5.9)	16 (94.1)	17
	Ethanol	N.D (0.0)	N.D (0.0)	17 (100)	0 (0.0)	
	n-propanol	N.D (0.0)	N.D (0.0)	N.D (0.0)	17 (100.0)	
	n-butanol	N.D (0.0)	N.D (0.0)	N.D (0.0)	17 (100.0)	
	n-pentanol	N.D (0.0)	N.D (0.0)	N.D (0.0)	17 (100.0)	

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Declarations

Conflict of interest The authors declare that they no conflict of interest.

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