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Measuring Ethanol Content in Kolesom, a Javanese Wine, and Traditional Wine of Bali by Gas Chromatography (GC): Ensuring Halal Label for Traditional Herbal Medicine

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ABSTRACT

Wine is an alcoholic drink that results from the fermentation of grape juice with the help of microbes. One of the variants of wine circulating in Indonesia is wine Kolesom. Kolesom, also known as ginseng wine. Gingseng/kolesom/temulawak is classified as grapes wine with other food ingredients as an addition. It contains EtOH in between 7-24 %v/v according to the quality standard of Kolesom in The Regulation of the National Agency of Drug and Food Control of Republic Indonesia (BPOM) No. 5/2021. Kolesom extracts, considered nutritious for health, are added to grape juice, though it is Khmer and haram. In traditional herbal medicine, kolesom usually use as an addition, giving the body a strong taste, warm effect, and vitality. Adding kolesom in Jamu is a choice depending on the customer's favor. Kolesom wine is classified as B-class liquor, containing 5-20% v/v ethanol. Considering that most Indonesian citizens are Muslim, it is necessary to analyze and cross-check information to the customer about the level content of the alcohol in Javanese wine, kolesom. Ensuring the correct information to the customer and halal lifestyle. Gas chromatography equipped with a flame ionized detector (GC-FID) is proposed as a method for analyzing the ethanol (EtOH) content in kolosem, Javanese wine, as well as the traditional wine of Bali. The results showed that the proposed GC-FID method was well-validated. The method fulfilled all the requirements: specificity with resolution (Rs) ≥ 2, linear correlation (is Y = 5,760X – 1,093.3, r = 0.9992, r^2 = 0.9986, and Vx_0 = 2,34%) and low level of LOD and LOQ. They

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are 0.322% and 0.975% for LOD and LOQ, respectively. All the Javanese wines studied are poorly labeled and informed about the EtOH content; however, they are still in the B-class of alcoholic drinks. In contrast, the traditional wine of Bali brand X contains high EtOH, 40% v/v, and is classified in the C-class. It is suggested that BPOM tightly controls both alcoholic drinks and beverage circulates in the market. Muslim consumers advise being more careful with product labels and choosing products, ensuring the halal lifestyle is tightly maintained.

Keywords: *Ethanol, Gas Chromatography, Jamu, Kolesom, Traditional Drinks, Wine.*

1 Introduction

Wine is an alcoholic drink. Wine results from the fermentation of fructose and glucose from grapes extract into alcohol with the bits of help of microbes [1]. Grape wine from *Vitis sp*. usually contains alcohol of 7-24% v/v. Wine has many variants. One of the variants of wine circulating in Indonesia is wine Kolesom. Kolesom, also known as ginseng wine, is a fermented drink made from grape juice. In terms of the manufacturing process, it is the same as making wine or liquor derived from grapes. However, in kolesom wine, kolesom extracts that are considered nutritious for health are added. In traditional herbal medicine, kolesom is an additional addition, giving the body a strong taste, warm effect, and vitality [2]. However, depending on the customer's favor, adding kolesom in Jamu is a choice.

Kolesom (*Tallinum triangulare* Willd.) is in the Portulaceae family and has equivalent properties to ginseng. Kolesom extract's efficacy empirically uses for diarrhea, anti-inflammatory, aphrodisiac, and increased vitality. Kolesom has the chemical content of saponins, triterpenes/steroids, polyphenols, and essential oils. Ginseng extract and traditional ingredients are very effective for increasing appetite, maintaining health and freshness, improving blood circulation, helping the body's metabolism smooth, and increasing stamina and strength for men.

Moreover, kolesom wine is quite popular in Indonesia and has excellent demand by Indonesian consumers as herbal medicine. This kolesom is also a commodity with a sizeable market share in foreign countries because of its savor [3]. The legal status of kolesom is the same as wine or other liquor. It is a Khmer. Usually, kolesom contains mid to high levels of alcohol, such as>5%. Generally, kolesom wine labeled contains ca. >14.7% v/v of ethanol (EtOH). Hence, it is relatively high and may result in intoxicants therefore classified as Khmer. The Regulation of the National Agency of Drug and Food Control of Republic Indonesia (BPOM) No. 5/2021, appendix 1, Gingseng/kolesom/temulawak classified as grapes wine contain other food ingredients; the EtOH based on the quality standard is in between 7-24 %v/v.

Moreover, the Regulation of the National Agency of Drug and Food Control of Republic Indonesia (BPOM) No. 5/2021, article 1 concerning safety and quality standards for alcoholic beverages; the definition of alcoholic beverages is a beverage that contains ethyl alcohol or ethanol (C_2H_5OH) processed from agricultural products containing

carbohydrates through both fermentation and distillation process or fermentation process without any distillation. The definition is the same as in Standard National Indonesia (SNI) 99004:2021, general requirements for halal food. Regarding the halal products assurance system, Sistem Jaminan Produk Halal (SJPH), which is regulated in the decision of the Head of the Halal Product Assurance Organizing Agency (BPJPH) No. 57/2021, alcohol in food and beverages is allowed when alcoholic beverages and food may contain EtOH when medically safe and not harmless for human consumption, as well as the EtOH content in final products is less than 0.5%. In addition, it is emphasized that EtOH used in Halal Product processes (Proses Produk Halal, PPH) should not come from Khmer industries and do not involve raw materials from pigs and their derivates. Apart from being produced from the fermentation process, EtOH can be produced by non-Khmer industries such as chemically synthesized (from petroleum), by direct hydration using ethylene (ethylene hydration), or other alkanes from the cracking process of distilled petroleum. This type of alcohol is not Khmer and not haram. Therefore, industrial alcohol is usually not absolute alcohol; impurities such as methanol are added to make it not misused as a liqueur.

Both beverages and drinks that are intoxicated are Khmer and haram. Therefore, when herbal medicine mixed with kolesom wine or other liquids contains alcohol and is consumed as medicine, even though not drunk, it is still haram. Nevertheless, it is better not to use herbs/medicine that contain alcohol if still a choice of herbs/medicines whose properties and efficacy are similar because Allah does not make cures from haram things [4]. Regarding the Islam rule, every this which is intoxicant is haram. Related to al Hadist, H. R. Al-Tirmidhi, Rasulullah, the messenger of Allah, said, "Everything that if a lot is intoxicating, then even a little is also haram." Khmer is haram as Allah ta'alla avows it at the Qur'an QS Al Maa-idah, 90-91.

Whereas according to Minister of Health regulations of Republic Indonesia (Permenkes RI) No. 86/Menkes/Per/IV/77, liqueurs are classified into three groups: Class A contains alcohol 1-5% v/v, Class B contains alcohol 5-20% v/v and Class C contains alcohol 20-55% v/v. Considering the classification, commercial kolosem wine in Indonesia contains 17.5% v/v of EtOH. Hence, kolesom wine is classified as a B-class liqueur, containing 5-20% v/v ethanol. Therefore, it is necessary to analyze and cross-check information to the customer about the level content of the alcohol in Javanese wine, kolesom, ensuring the correct information to the customer and halal lifestyle.

Ethanol, with the chemical formula of C_2H_5OH, is the main ingredient in alcoholic beverages. It is a volatile organic compound with a carbon value range of C1 to C5 and a low boiling point of 60-70 ^oC [5]. Ethanol has a detrimental effect on the body; at low levels. It also acts as a stimulant resulting in several effects, such as relaxation, anxiety, loss of balance, and loss of control. However, EtOH can be nerve-wracking at high consumption levels and lead to addiction among consumers, including hallucinations, headaches, hypertension, insomnia, excessive sweating, etc. [6].

Measuring the alcohol content in beverages and fermented foods is mandatory for halal certification. Therefore, the gas chromatography (GC) method for ethanol analysis is proposed; since ethanol has a low boiling point, then suitable for detection by GC [7-10]. Ethanol (EtOH) and butanol (BuOH) are commonly used as internal standards for determining alcohol in traditional fermented foods and beverages by the GC method [8,9]. In addition, GC analysis is also suitable for analyzing EtOH in traditional wine [7,10].

Therefore, in this study, the validated GC method is used to determine the ethanol content in Javanese wine, i.e., wine brand X variant kolesom (in 620 mL package), red wine brand X variant red, red wine brand X variant gold, red wine brand X variant white, and traditional wine of Bali brand X. The results values were then compared to the ethanol level on the packaging labels. In addition, this study may provide consumer protection against the alcohol content contained in Javanese wine following safety standards based on BPOM and Permenkes RI regulations.

2 Materials and methods

2.1 Materials

Samator Gas commercially provides helium gas of high purity and is used. Ethanol absolute high purity purchased from Sigma Aldrich while the variance of the sample such as wine brand X variant kolesom (in 620 mL package), red wine brand X variant red, red wine brand X variant gold, red wine brand X variant white, and traditional wine of Bali brand X. All samples are purchased from the e-commerce platform and should fulfill the random sampling technique based on inclusion criteria. By means, each bottle of sample should be sealed, not yet beyond the expiration date, and produced with the batch/lot number. Therefore, four bottles are provided for each sample. Moreover, glass lab apparatus such as measuring cup, beaker glass, pipette volume, separating funnel, volumetric flask, stick glass stirrer, and micropipettes were also used.

2.2 Methods

Ethanol Analysis by Gas Chromatography. Ethanol (EtOH) content in samples was analyzed by gas chromatography method. Gas chromatography (GC) with a flame ionization detector (GC-FID) is applied. A capillary column of HP-PLOT Q (Divinylbenzene/Styrene Polymer) 40.0 µm x 30 m, Helium gas as mobile phase with a constant flow rate of 8.0 mL/min, and constant makeup flow, 75.0 mL/min are used. The GC-FID is programmed to have a temperature injection of 275 ^{o}C, temperature detection of 275 ^{o}C, and initial temperature of column (max.) 290 ^{o}C. A 1 µL of aliquot sample is injected with a split ratio= 1:15.

Sample Preparations. Before analysis, each sample of and traditional wine of Bali were homogenized by gently shaking each bottle. Dilution was performed on each sample. A 1000 µL of each sample was taken using a micropipette and put into a 10.0 mL volumetric flask. Subsequently, a mineralized aquadest was added up to mark the volumetric flask. Gently shake the diluted solution, ensuring it is homogenously

diluted. The diluted solution is filtered using a Millipore filter holder and 0.45 µm Whatman filter paper.

Calibration curve preparations. The standard ethanol solution is prepared at concentrations of 1, 2, 4, 5, 6, and 7% v/v using absolute ethanol p.a 99.8% as the primary standard. Paralleled dilution is applied to prepare the solution.

Linearity. Linearity is determined using a minimum of 5 different concentrations. The linearity is determined by the correlation coefficient (r). The data, e.g., the calibration curve, is called linear when it has a correlation coefficient r ≥ 0.9990 [7]. However, other parameters are also needed to show linearity, such V_{x0}, a function of the coefficient of variance. Mathematically calculations of V_{x0} follow:

$$Sy = \sqrt{\frac{\sum(y-yi)^2}{N-2}} \quad (1)$$

for a linear regression equation of Y = mX + b (2)

$$\mathrm{Sx_0} = \frac{\mathrm{Sy}}{\mathrm{b}} \quad (3)$$

$$Vx_0 = \frac{Sx_0}{\bar{x}} \text{ x } 100\% \quad (4)$$

Limit of Detection (LOD) and Limit of Quantification (LOQ).

The limit of detection (LOD) can be determined based on the regression equation data (Y = mX + b), with the formula: LOD = (3.3 SD) / m (5)

SD is the standard deviation of the five replication standard solution series, and m is the slope value of the linear regression (Y = mX + b). While the limit of Quantitation (LOQ) is determined based on the formula: LOQ = (10 SD) / S (6)

Accuracy and precision. Each sample was pipetted at three different variances of volume, i.e., 1000, 2000, and 3000 µL, using a micro syringe. Then put it into a 5.0 mL volumetric flask. Added a 2000 µL of 7% v/v of a standard solution of EtOH into each 5.0 mL volumetric flask. Subsequently, add mineralized aquadest till the final volume reach of 5.0 mL. Gently shake the volumetric flask to make sure a homogenous solution is diluted. Hence, a 2.8% v/v of the final solution is obtained. The final solution is then ready to be analyzed by GC. The accuracy parameter is calculated as follows:

$$\%\, recovery = \frac{Ethanol\ analyzed}{Ethanol\ added}\ x\ 100\% \quad (7)$$

While precision is expressed with the coefficient of variance (KV) using the equation (8) below:

$$KV = \frac{SD}{\bar{X}} \text{ x } 100\% \quad (8)$$

3 Results and discussion

3.1 Results

Before further analysis of the samples, a method validation of GC-FID was carried out. Specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ) are determined.

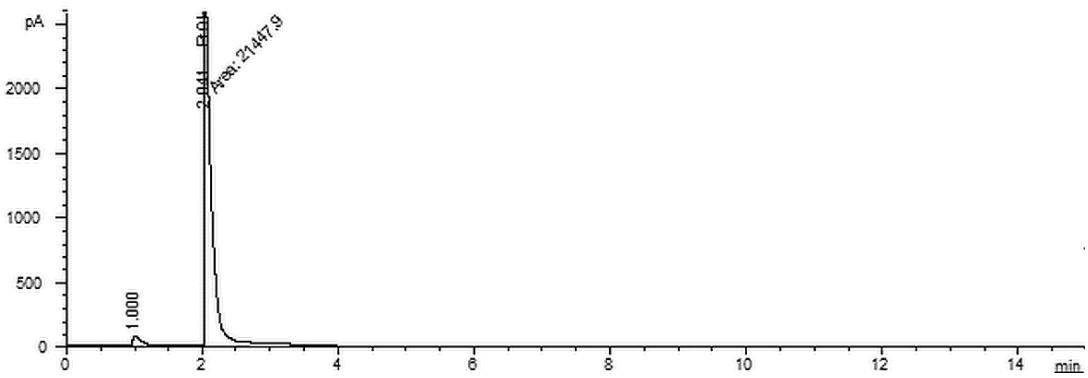

Fig. 1 Chromatogram of a standard ethanol solution by GC-FID.

Specificity is the resolution value (Rs). Using the data as shown in Fig. 1, the resolution value is $Rs = \frac{2\Delta t_R}{(W_1+W_2)} = \frac{2\,(2{,}041-1{,}000)}{(0{,}2+0{,}3)} = 4.164$. It indicates that the separation is good since it meets the requirements of Rs ≥ 2 [11,12].

The calibration curve shows the linearity of the data between the ethanol concentration (% v/v), X axes, and peak area, Y axes (Fig. 2). Linearity was determined through six different concentrations of standard ethanol solution, i.e., 1, 2, 4, 5, 6, and 7 % v/v.

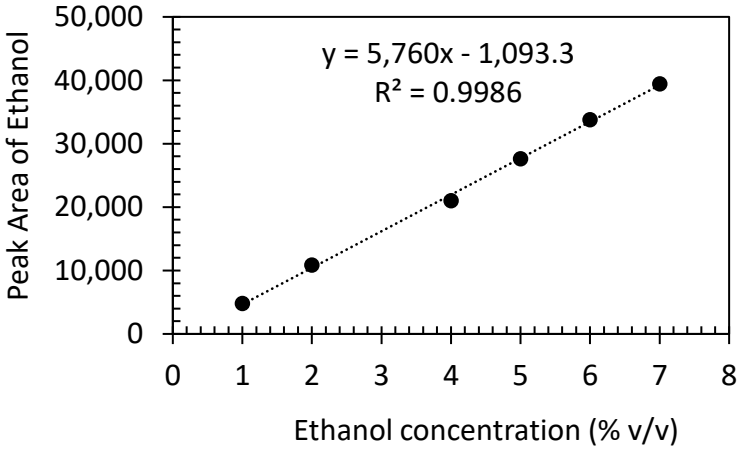

Fig. 2 Calibration curve of ethanol analyzed by GC-FID.

The regression equation is Y = 5,760X – 1,093.3 (r = 0.9992 and r^2 = 0.9986) and Vx_0 = 2,34% (Table 1). The ethanol peak has a retention time of 2.041 minutes (Fig. 2). The Linearity meets the requirements for r ≥ 0.9990 [13], as well as Vx0 = < 5%. The data has a good correlation of linearity.

Table 1. Data for calculating LOD and LOQ values.

No	EtOH concentration (% v/v)	y	ŷ	(y-ŷ)	(y-ŷ)²
1	1	4,776.6	4,666.7	109.9	12,078.01
2	2	10,842.0	10,426.7	415.3	172,474.10
3	4	20,989.0	21,946.7	-957.7	917,189.30
4	5	27,608.0	27,706.7	-98.7	9,741.69
5	6	33,800.0	33,466.7	333.3	111,088.90
6	7	39,424.0	39,226.7	197.3	3,8927.29
				Σ (y-ŷ)²	1,261,499.00
				S y/x	561.58
				LOD (%)	0.322
				LOQ (%)	0.975

Following equations (1)-(6), the calculation of the detection limit and quantitation limit, this GC-FID method is only able to detect the minor level (LOD) of 0.322% and a quantitation limit (LOQ) that can be detected by the tool is 0.975%. The LOD is the minor concentration the instrument can detect, while the LOQ is the lowest sample content that could be quantified. Therefore, EtOH in the samples cannot be detected and quantified when it is below both the LOD and the LOQ limit.

Table 2. Accuracy and precision data (spiked with 2 mL of 7% v/v of standard EtOH).

Sample volume (mL)	replicate	Peak area	*recovery* (%)	RSD* (%)
1	1	15,081.9	90.68	4.22
	2	16,245.2	98.51	
	3	15,963.5	96.14	
2	1	17,122.1	93.27	1.77
	2	17,644.2	96.63	
	3	17,379.9	94.87	
3	1	18,106.8	90.15	3.36
	2	18,522.2	92.73	
	3	18,942.7	95.34	

*RSD = relative standard deviation

Accuracy is the percentage of recovery of the added analyte; it is a ratio between the EtOH analyzed by GC-FID and the amount of the added EtOH. At the same time, precision is measured as the relative standard deviation (coefficient of variation, KV). In this study, an accuracy test was carried out using the standard addition method by adding a 7% v/v standard ethanol solution to the samples with 5.0 mL of fixed final volume (Table 1). Table 1 shows the analysis data for having accurate and precise values. The analytical method meets the accuracy parameter if the percentage

recovery value is 80% - 120% [13]. Hence, the proposed GC method has fulfilled the requirement of accuracy.

Regarding the specificity, linearity, LOD, and LOQ obtained above, the GC-FID method proposed in the methods section is valid for EtOH analysis in kolesom wine samples and the traditional wine of Bali.

3.2 Discussion

The validated GC-FID method is used further for analyzing EtOH content at wine brand X variant kolesom (in 620 mL package), red wine brand X variant red, red wine brand X variant gold, red wine brand X variant white, and traditional wine of Bali brand X.

The chromatogram in Fig. 3 shows that all samples give EtOH at a similar retention time, R_t = 2.041 minutes. The peaks are well specificity and repeatable consistency. The consistency of GC analysis is also shown with repeatable data (Table 3).

Table 3. Ethanol content (% v/v) in different variances of wine samples.

	Ethanol content (% v/v) in different samples of wine				
wine brand X variant kolesom		**Bottle-1**	**Bottle-2**	**Bottle-3**	**Bottle-4**
	Replicate-1	18.60	18.65	18.59	19.20
	Replicate-2	18.65	18.61	18.63	18.62
	Replicate-3	18.57	18.60	18.61	18.63
	average	18.60	18.62	18.61	18.62
red wine brand X variant red		**Bottle-1**	**Bottle-2**	**Bottle-3**	**Bottle-4**
	Replicate-1	15.61	15.67	15.66	15.54
	Replicate-2	15.67	15.51	15.51	15.56
	Replicate-3	15.73	15.51	15.65	15.65
	average	15.67	15.56	15.60	15.58
red wine brand X variant gold		**Bottle-1**	**Bottle-2**	**Bottle-3**	**Bottle-4**
	Replicate-1	20.48	20.43	20.47	20.53
	Replicate-2	20.47	20.33	20.49	20.49
	Replicate-3	20.47	20.30	20.52	20.52
	average	20.47	20.53	20.49	20.51
red wine brand X variant white		**Bottle-1**	**Bottle-2**	**Bottle-3**	**Bottle-4**
	Replicate-1	15.36	15.59	15.48	15.41
	Replicate-2	15.56	15.48	15.42	15.59
	Replicate-3	15.50	15.57	15.54	15.59
	average	15.47	15.54	15.48	15.53
traditional wine of Bali brand X		**Bottle-1**	**Bottle-2**	**Bottle-3**	**Bottle-4**
	Replicate-1	39.11	39.47	39.74	38.69
	Replicate-2	38.90	39.00	38.85	38.79
	Replicate-3	38.82	38.68	38.91	39.13
	average	38.94	39.05	39.17	38.87

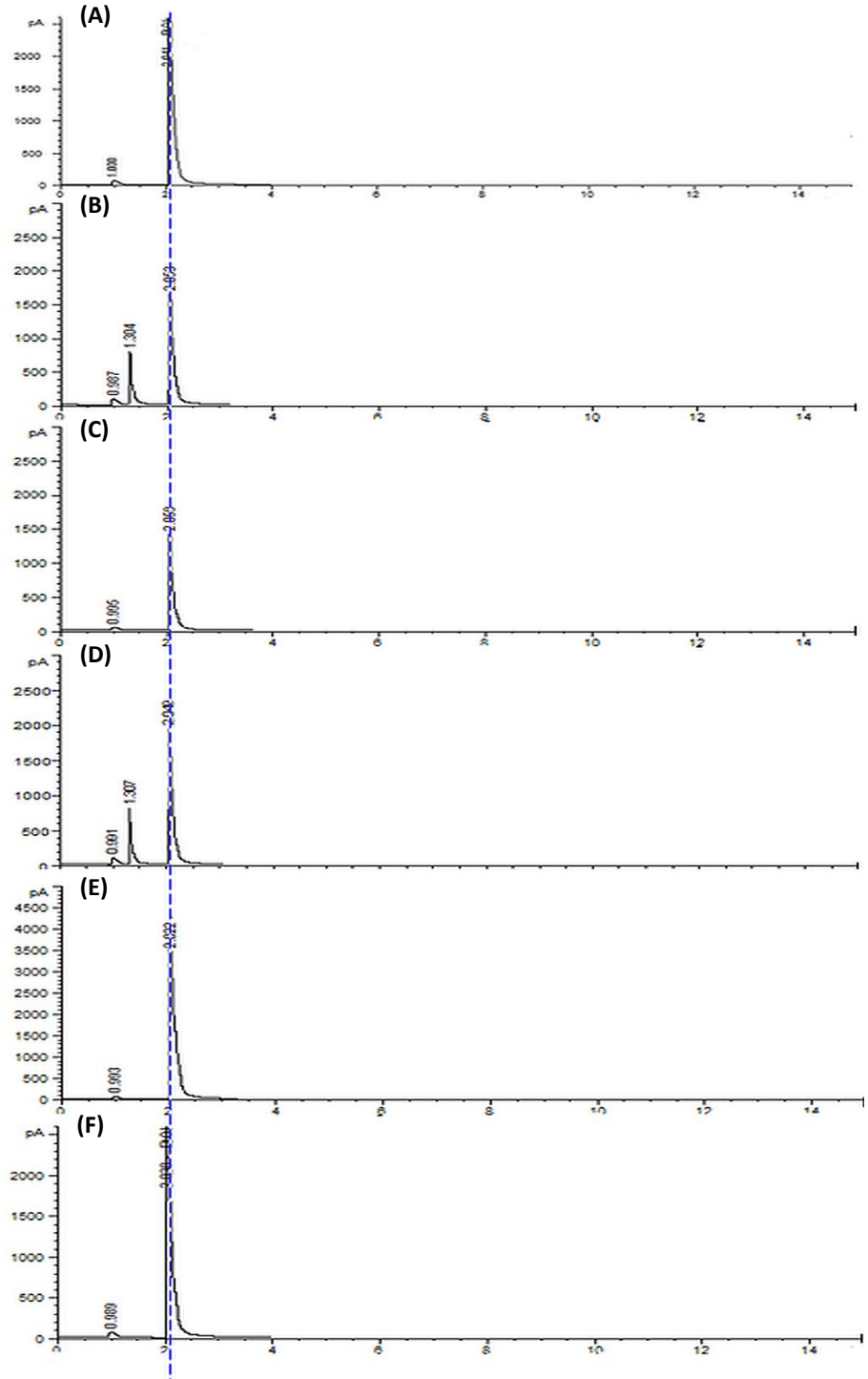

Fig. 3 Peak of ethanol which was detected in all samples: (A) standard solution of EtOH, (B) wine brand X variant kolesom (in 620 mL package), (C) red wine brand X variant red, (D) red wine brand X variant gold, (E) red wine brand X variant white, and (F) traditional wine of Bali brand X.

Table 4. Ethanol content (% v/v) in different variances of wine samples.

sample	Ethanol content (% v/v)		Content discrepancy (%)**	Class of beverages***
	Analyzed (SD)*	Content in label		
wine brand X variant kolesom	18.66	17.50	6.63	B
red wine brand X variant red	15.61	14.70	6.19	B
red wine brand X variant gold	20.46	19.70	3.86	B
red wine brand X variant white	15.51	14.70	5.51	B
traditional wine of Bali brand X	39.01	40.00	2.48	C

*SD = standard deviation; **discrepancy between the labeled EtOH and analyzed EtOH; ***According to Minister of Health regulations of Republic Indonesia (Permenkes RI) No. 86/Menkes/Per/IV/77. Class A = 1-5% v/v, Class B = 5-20% v/v, and Class C = 20-55% v/v.

The EtOH level of wine brand X variants kolesom meets the safety and quality standards of alcoholic beverages according to BPOM No. 5/2021; Gingseng/kolesom/temulawak classified as grapes wine contain other food ingredients; the EtOH based on the quality standard is in between 7-24 %v/v. While in Permenkes RI No. 86/Menkes/Per/IV/77, “alcoholic beverages fermented from grape juice (*Vitis sp*.) contain EtOH 7-24% v/v” as well as Class B with an alcohol content of 5-20%. That kolesom is still classified as safe to consume due to the EtOH levels falling within the range of the requirements criteria. However, the side of alcohol drinks class B (moderate) can cause visual disturbances, sensory loss, ataxia, and slow reaction time. At the same time, Bali's traditional wine contains high EtOH, ca. 40% v/v, classified in C Class. The definition of Arak is an alcoholic beverage produced from the distillation of fermented liquid with sorghum, rice, molasses, concentrated sugar juice, and fruits with a unique character of smell and taste. Arak itself contains EtOH, not less than 30% v/v. The GC-FID analysis of EtOH in Arak is appropriate.

The proposed GC-FID method for analyzing the EtOH content in kolosem, Javanese wine, as well as the traditional wine of Bali, was well validated. All the Javanese wines are poorly labeled and informed about the EtOH content. Therefore, it is suggested that BPOM tightly controls both alcoholic drinks and beverage circulates in the market. Customers are advised to be more careful with product labels and choosing products, especially Muslim consumers, ensuring the halal lifestyle is tightly maintained.

4 Conclusion

The proposed GC-FID method for analyzing the EtOH content in kolosem, Javanese wine, as well as the traditional wine of Bali, was well validated. The method fulfilled all the requirements: specificity with resolution (Rs) ≥ 2, linear correlation (is Y = 5,760X – 1,093.3, r = 0.9992, r^2 = 0.9986, and Vx_0 = 2,34%) and low level of LOD and LOQ. They are 0.322% and 0.975% for LOD and LOQ, respectively.

All the Javanese wines studied are poorly labeled and informed about the EtOH content; however, they are still in the B-class liquor. In contrast, the traditional wine of Bali brand X contains high EtOH, 40% v/v, and is classified as C-class liquor.

It is suggested that BPOM tightly controls both alcoholic drinks and beverage circulates in the market. Customers are advised to be more careful with product labels and choosing products, especially Muslim consumers, ensuring the halal lifestyle is tightly maintained.

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