Food Research 7 (5) : 190 - 203 (October 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Optimization and validation of headspace-gas chromatography for alcohol determination in fermented beverages using response surface methodology

[1,2]Nurul Ain, R., [1]Siti Roha, A.B., [3]Andoyo, R. and [1,*]Adi, M.S.

[1]*Department of Food Science and Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[2]*Food Division, National Public Health Laboratory, Ministry of Health Malaysia, Lot 1853 Kampung Melayu, 47000 Sungai Buloh, Selangor, Malaysia*
[3]*Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km. 21, Jatinangor, Bandung 40600, West Java, Indonesia*

Article history:
Received: 2 July 2022
Received in revised form: 26 August 2022
Accepted: 16 May 2022
Available Online: 5 October 2023

Keywords:
Ethanol,
Methanol,
Isopropanol,
Tert-butanol,
Response surface methodology,
Method validation

DOI:
https://doi.org/10.26656/fr.2017.7(5).354

Abstract

Date palm sap is a clear sweet drink originating from the date palm tree, which is potentially fermented into toddy. Beer and wine are a product of alcoholic fermentation of malted grain and grape juice, respectively. This study aimed to optimize and validate the method for the determination of ethanol, methanol, isopropanol, and *tert*-butanol in date palm sap, beer, and wine using headspace-gas chromatography with a flame ionization detector. The volume of sample, oven temperature, and equilibrium time for headspace conditions were optimized at 2.5 mL, 102°C, and 31.7 mins, respectively, using the response surface methodology. The study showed that the model is satisfactory with no significant lack of fit ($p>0.05$). This method was specific with no overlapping peaks and good chromatography separation for all analytes. The limit of detection and limit of quantification was 0.01% (v/v) and 0.03% (v/v) for ethanol, 0.3 mg/L and 1.0 mg/L for methanol, and 0.1 mg/L and 0.5 mg/L for both isopropanol and *tert*-butanol, respectively. All analytes demonstrated good linearity with correlation coefficients (R^2) greater than 0.990, good recovery (87.8–114.1%), and acceptable precision for repeatability (0.7–4.3%) and reproducibility (1.5–10.2%). This study suggested that the method was successfully optimized and validated as an appropriate technique for verifying alcohols in palm saps and alcoholic beverages for the surveillance of Halal status and inedible alcohol contamination, respectively.

1. Introduction

Date palm sap (DPS) locally known as *Nira Kurma* is a product of the date palm tree (*Phoenix dactylifera* L.) (Makhlouf-Gafsi *et al*., 2016). It is a sweet liquid that is consumed fresh after being collected from the tree trunk through the tapping process. Fresh DPS gained its popularity in Malaysia as a refreshing drink by Muslims for Iftar during the month of Ramadhan (Noornasrin Salsabila, 2021). However, DPS can also be consumed as an alcoholic beverage known as toddy after undergoing a natural fermentation process. The fermentation takes place 5–12 hours after collection, mainly caused by *Saccharomyces cerevisiae* at ambient temperature (Ben Thabet *et al*., 2010; Joshi *et al*., 2017). The ethanol concentration of fermented DPS could reach up to 5% (Barreveld, 1993), which exceeds the permissible level for non-alcoholic beverages as stipulated by Halal governing bodies in Malaysia, Indonesia, and Brunei (Pauzi *et al*., 2019), which could lead to religious issues. Beer is a liquid produced from the alcoholic fermentation of malted grains, while wine is a product of grape juice fermentation (Laws of Malaysia, 2017). According to Wachełko *et al*. (2021), the types and quality of the beer depends on the sweetness, bitterness, acidity or buttery flavour. The concentration of ethyl alcohol determines the strength of beer as well as has an impact on the sensory perception of wine. However, in wines, the amount of ethanol is directly proportional to its sweetness, while the degree of bitterness is reduced (Panovská *et al*., 2008).

The presence of alcohol in fermented beverages is common. Ethanol can be either naturally formed during the fermentation process or intentionally added as a

*Corresponding author.
Email: *adisikin@uitm.edu.my*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

solvent for flavouring or colouring in beverages such as juices and cordials (Law *et al*., 2009). Methanol, which is inedible alcohol, could potentially be added as a substitute for edible alcohol in making alcoholic beverages due to its low price (Giovanetti, 2013). However, it can still be detected at an exceptionally low amount in some fermented beverages because of the acetic fermentation process due to the hydrolysis of the pectin methoxyl group (Zhang *et al*., 2015). Although there are no reported cases of alcohol poisoning in fermented DPS, methanol poisoning cases were reported in Malaysia that caused 45 deaths due to adulteration of alcohol into commercial and homemade liquor (Rosli, 2018). *Tert*-butanol and isopropanol are mostly used denaturants for ethanol. *Tert*-butanol is normally used as a solvent or perfume carrier in cosmetic industries (Andersen, 2005), while isopropanol is used as a sanitiser (Garg and Ketha, 2020). *Tert*-butanol is an anthropogenic source and there is no information about its natural occurrence, therefore it should not be present in food containing alcohols (Destanoğlu and ATEŞ, 2019). A high oral dose of *tert*-butanol may lead to ataxia and hypoactivity, with a threshold limit value of 100 ppm for 8 hours in humans. There is no reported data for *tert*-butanol oral lethal dose in humans, but its oral lethal dose in a rat is 3384 mg/kg (McGregor, 2010). On the other hand, isopropanol has a lower minimum lethal dose of 100 mL for an adult compared to that of methanol (15–30 mL) (Ambranson, 2010). The presence of isopropanol in beverages may lead to the risk of cancer and hepatic disorders as it is more hepatotoxic than ethanol (Lang *et al*., 2006). In this regard, these four alcohols are considered important analytes to be monitored in fermented beverages as well as alcoholic beverages due to their impact towards food safety and toxicological issues.

There are several methods for quantifying alcohol in fermented beverages and alcoholic beverages. Both enzymatic and titrimetric methods produce poor reproducibility and accuracy results. Besides being time-consuming, the densimetric and colourimetric methods have poor sensitivity and are prone to interferences due to the colourants in beverages, respectively (Zhang *et al*., 2015). High-performance liquid chromatography (HPLC) and gas chromatography (GC) are frequently used to quantify different types of alcohol due to their capability to separate analytes with high sensitivity. However, samples' pre-treatment such as filtration and solvent extraction before injection of samples into HPLC is often complicated and time-consuming. As a result, the quantitation of the analytes is prone to significant errors (Zhang *et al*., 2015). GC combined with the flame ionization technique (GC-FID) is commonly used for routine alcohol analysis in alcoholic beverages (Tiscione *et al*., 2011). GC-FID equipped with a direct injection port has been used to detect methanol in alcoholic beverages, but the rinsing process of the syringe after each injection is required to avoid cross-contamination between samples (Wang *et al*., 2004). Alternatively, headspace-GC-FID (HS-GC-FID) was used in the detection of methanol in black liquor (Li *et al*., 2007), ethanol in fermentation liquor (Li *et al*., 2009), soy sauce (Liu *et al*., 2014), kombucha (Ebersole *et al*., 2017), craft beers, wines, and soft drinks (Wachełko *et al*., 2021), and both methanol and ethanol in olive oil (Gómez-Coca *et al*., 2014), and wines (Zhang *et al*., 2015). This technique involves taking out samples from the vapour phase that has reached equilibrium (headspace area) above the liquid or solid samples in a closed sealed vial and hence reduces the interference of non-volatile analytes in the sample (Kolb and Ettre, 2006). It is an efficient injection technique in quantifying volatile analytes in samples with complex matrices without the requirement for pre-treatment of samples (Zhang *et al*., 2015).

The development of a new method is crucial as a sophisticated technique is needed for the quality and safety control of the food. The optimization of headspace conditions was conducted for the determination of ethanol in fermentation liquor (Li *et al*., 2009), and both ethanol and methanol in wines (Zhang *et al*., 2015) involving three variables: equilibrium time, equilibrium temperature and sample volume. However, the optimization was conducted conventionally without any statistical analysis by focusing on one variable at a time. In addition, the interactive effects between all those variables were not taken into consideration. This conventional approach also prevented the variables from generating the maximum analytical signal of analytes and was time-consuming when many analytes needed to be focused (Ma *et al*., 2013; Bokhon *et al*., 2021). Response surface methodology (RSM) is one of the most helpful statistical optimization tools that has been progressively used in the optimization process. RSM identified the effects of the independent variables towards responses and the relationship between the independent variables and responses of this study (Bokhon *et al*., 2021). It ensures that the maximum responses of all analytes are generated with minimum time on the optimization. For example, RSM was used in optimizing headspace conditions for benzene in beverages (Kim *et al*., 2019) and volatile compounds in high-fat dairy powders (Salum and Erbay, 2019), margarine (Dadalı and Elmacı, 2019) and baked confectionery products (Garvey *et al*., 2020). To the best of our knowledge, there is no published study to optimize headspace conditions using RSM for alcohol determination in fermented beverages let alone the

https://doi.org/10.26656/fr.2017.7(5).354

detection of alcohol in fresh or fermented DPS. Therefore, this study applied RSM in the optimization of the headspace conditions for alcohol determination involving three independent variables, which are the volume of the sample, oven temperature, and time of equilibrium in getting the maximum responses, which is the peak area of all the analytes.

The optimization and validation of an analytical method are normally based on the suitability of the working conditions of the intended analytes to ensure the method is fit for purpose. An established method is normally validated concerning selectivity, sensitivity, linearity, range of determination, limit of detection, precision and accuracy (Ornelas-Soto *et al*., 2011). Previously published studies of palm sap focused on the physicochemical changes of coconut palm (*Cocos nucifera*) sap after fermentation (Xia *et al*., 2011; Singaravadivel and Hariharan, 2012) and the responsible analytes for the aroma of the *nipa* palm (*Nypa fruticans*) sap (Nur Aimi *et al*., 2013). Due to the limited number of studies on the detection of alcohol in palm sap, especially in the DPS, this study aimed to optimize a simultaneous determination of ethanol, methanol, isopropanol, and *tert*-butanol in the DPS using RSM and validate the optimized method in three different samples, which are DPS, beer, and wine.

2. Materials and methods

2.1 Samples

DPS was randomly harvested from a date farm in Kelantan, Malaysia. The sap was collected at midnight for its low temperature to reduce spontaneous fermentation. The samples were obtained by cutting the stalk of matured date palm trees. The open cut of the trunk was wrapped with sterile plastic bags to preserve its hygiene. The collected DPS was combined and then transferred into 200 mL of plastic bottles and quickly stored at below 0°C during transportation. The samples were kept in the freezer at a temperature of -20°C before analysis. The beer and wine were purchased from commercial stores in Selangor, Malaysia and kept unopened at room temperature before analysis.

2.2 Chemicals and reagents

Certified standards of ethanol, methanol, isopropanol, and *tert*-butanol (purity >99%, Dr Ehrenstorfer GmbH, Germany) and internal standard of pentanol (purity 99.5%, Dr Ehrenstorfer GmbH, Germany) were used in this study. All mixed, single standard solutions and their additional dilutions were prepared using Ultrapure water (Milli-Q® IQ 7003 Pure and Ultrapure Water System, Merck KGaA, Germany) and were stored at 4°C. Helium (He), Nitrogen (N_2), Hydrogen (H_2), and ultra-high purity zero air (99.99%) (Poly Gas Sdn. Bhd., Selangor, Malaysia) were used for HS-GC-FID analysis. All chemicals and reagents used in this validation were analytical reagent grade that was purchased from certified sources.

2.3 Sample preparation

All samples were homogenized by using a stirrer prior to analysis. During the optimization stage, a range of 0.01 to 6.01 mL samples were pipetted into a 20 mL headspace vial (Perkin Elmer Inc., USA) before being spiked with a certain volume of pentanol as an internal standard (target concentration of internal standard is 5 mg/L). The vial was immediately sealed using a 20 mm aluminium seal cap with polytetrafluoroethylene/silicon septum (Perkin Elmer Inc., USA) and injected into HS-GC-FID.

2.4 HS-GC-FID analysis

The alcohol (ethanol, methanol, isopropanol and *tert* -butanol) content in the samples was analyzed by using an automated headspace sampler Turbo Matrix HS40 (Perkin Elmer Inc., USA) interfaced to the Agilent GC 6890N (Agilent Technologies, CA, USA) equipped with FID. The headspace oven temperature and equilibrium time were varied during the optimization step within a range of 65–145°C and 2–60 mins, respectively. The sample loop temperature was set at 110°C and a transfer line (0.25 mm ID silica tubing) with a temperature of 120°C was used to connect the automated headspace sampler and injector of the GC-FID. Pressurization pressure, carrier gas pressure, vial pressurization time, sample loop fill time, and transfer time were set at 29.0 psi, 25.6 psi, 1 min, 5 s and 15 s, respectively.

An Elite-BAC1 Advantage column (30 m, 0.32 mm ID, 1.8 um df, Perkin Elmer Inc., USA) was applied in this study. Helium gas was used as a carrier gas with a flow rate of 1 mL/min. Hydrogen and air were employed for the FID at a flow rate of 40 and 400 mL/min, respectively. Split mode with a ratio of 50:1 was used in this study. The temperature of the column was set at 40° C and held for 5 mins, increased to 60°C at 5°C/min, then increased to 150°C at 75°C/min and held for 3 mins. The injector and detector temperature were set at 150°C and 250°C, respectively. For the data acquisition, MSD ChemStation (Agilent Technologies, CA, USA) software (v.E.02.02.1431) was used to process the obtained data.

2.5 Design of experiment for response surface methodology

RSM was employed in this study to optimize the headspace conditions for the optimum detection of ethanol, methanol, isopropanol and *tert*-butanol in the

https://doi.org/10.26656/fr.2017.7(5).354

DPS. Central Composite Design (CCD) was used to investigate three independent variables; volume of sample (X_1), oven temperature (X_2), and equilibrium time (X_3) towards the responses of this experimental design, which was the peak area of ethanol (Y_1), methanol (Y_2), isopropanol (Y_3), and *tert*-butanol (Y_4) at five different levels (-α, -1, 0, 1, and α) as shown in Table 1. As shown in Table 2, this CCD experimental design involved 20 runs ($2^k + 2k + C_p$), where k represents the number of independent variables in this study, which is 3 and C_p is replications of the centre point, which is 6 (Garvey *et al*., 2020). All experimental runs were analyzed in three replicates and the mean of the data was used. Then, the second-order polynomial model was used to assess the relationship between the independent variables and responses of the design as shown in Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X_i^2 + \sum_{i \neq j=1}^{k} B_{ij} X_i X_j \quad (1)$$

where Y is the predicted response, X_i and X_j are independent variables, k is the number of independent variables, β_0 is the intercept, and B_i, B_{ii}, and B_{ij} are regression coefficients for the linear, quadratic, and interactive effects, respectively (Yang *et al*., 2020).

Table 2. Central composite design (CCD) matrix of independent variables in the date palm sap.

Run	Independent variables		
	X_1 (mL)	X_2 (°C)	X_3 (mins)
1	1.01	85	16.5
2	3.01	85	16.5
3	1.01	125	16.5
4	3.01	125	16.5
5	1.01	85	45.5
6	3.01	85	45.5
7	1.01	125	45.5
8	3.01	125	45.5
9	0.33	105	31.0
10	3.69	105	31.0
11	2.01	71	31.0
12	2.01	138	31.0
13	2.01	105	6.6
14	2.01	105	55.4
15	2.01	105	31.0
16	2.01	105	31.0
17	2.01	105	31.0
18	2.01	105	31.0
19	2.01	105	31.0
20	2.01	105	31.0

Effects of the independent variables towards the dependent variables of the DPS were discovered using one-way analysis of variance (ANOVA), while the coefficient of determination (R^2) and lack-of-fit value were analyzed to assess the capability and fitness of the CCD model. The three-dimensional response surface plots were visualized to describe the interactions between independent variables and dependent variables (Yolmeh and Jafari, 2017). Furthermore, a *t*-test and prediction of error were performed to verify the model by comparing the predicted and experimental values of the optimized model. The percentage of prediction error was calculated by using the Eq. (2) according to Sapkal and Jagtap (2018):

$$Percentage\ of\ prediction\ error\ (\%) = \frac{Experimental\ results - Predicted\ results}{Experimental\ results} \times 100 \quad (2)$$

2.6 Method validation

Validation of the method was performed by applying the optimized method of HS-GC-FID according to the standard operating procedure (SOP) No: A02-023 for Method Validation for Chemical Analysis owned by the Ministry of Health (MOH), Malaysia. This SOP was primarily referred to by Eurachem Guide (Magnusson and Ornemark, 2014), the Association of Official Analytical Chemists (AOAC, 2002) and the Official Journal of the European Communities (European Commission, 2002). An in-house validation was conducted for all parameters such as specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, precision (repeatability and within-laboratory reproducibility), and accuracy (recovery). The validation involved three types of samples, which are DPS, beer, and wine for low, medium, and high concentrations of samples, respectively.

2.6.1 Specificity

Specificity was conducted to find out the suitability of the method in measuring all targeted analytes. It was performed by analyzing the blank DPS (DPS without being spiked with any analyte), the DPS sample spiked with internal standard, known as an internal blank sample, and the DPS spiked with both internal and external standards; pentanol, ethanol, methanol, isopropanol, and *tert*-butanol, known as internal-external blank sample. The samples were then injected to identify the retention time of all targeted analytes (pentanol, ethanol, methanol, isopropanol, and *tert*-butanol) by

Table 1. Independent variables and their correspondence coded levels for response surface methodology (RSM) with central composite design (CCD).

Independent variables	Coded	Level of correspondence				
		-α (-1.68179)	-1	0	1	α (1.68179)
Volume of samples (mL)	X_1	0.33	1	2	3	3.69
Oven temperature (°C)	X_2	71	85	105	125	138
Equilibrium time (mins)	X_3	6.6	17	31	46	55.4

https://doi.org/10.26656/fr.2017.7(5).354

comparing each chromatogram.

2.6.2 Limit of detection and limit of quantification

LOD and LOQ were established by calculating the standard deviation from the calibration curve. Ten levels of mixed standard solutions were prepared with a concentration of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 4, 7, and 10% (v/v) for ethanol and 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 mg/L for methanol, isopropanol and *tert*-butanol. The solutions were analyzed for three batches using different freshly prepared standards. The LOD and LOQ were computed using Eq. (3) and Eq. (4), respectively as follows:

$$LOD = \frac{3 \times SD}{b} \quad (3)$$

$$LOQ = \frac{10 \times SD}{b} \quad (4)$$

where *SD* is the standard deviation of the y-intercept and *b* is the sensitivity or slope of the regression line according to the International Conference on Harmonization (ICH) of Technical Requirements (2005).

2.6.3 Linearity

Linearity was performed by preparing six levels of calibration solutions starting from the LOQ level. The mix standard solutions were prepared at a concentration of 1.0, 3.0, 5.0, 10, 30 and 50 mg/L for methanol and 0.5, 1.0, 5.0, 10, 30 and 50 mg/L for isopropanol and *tert* -butanol. While 0.03, 0.1, 1.0, 5.0, 20, and 40% (v/v) of concentration were prepared for ethanol. The standard solutions were analyzed for three different batches at different times. The coefficient of determination (R^2) was determined to assess the linearity following Wachelko *et al*. (2021). The linearity was verified by conducting a lack-of-fit test and regression test.

2.6.4 Precision and recovery

Precision and recovery were obtained by spiking the blank DPS, beer, and wine with LOQ, 5% (v/v), and 30% (v/v) of ethanol and LOQ, 5 mg/L, and 30 mg/L of methanol, isopropanol, and *tert*-butanol, respectively, to represent the low, medium, and high-level concentration of these alcohols. Precision studies of DPS, beer, and wine were carried out by using repeatability (r) and within-lab reproducibility (R), which are well-known precision measures for in-house validation (AOAC, 2002). For repeatability, ten batches of duplicate DPS, beer, and wine for each spiked concentration of all analytes were analyzed by the same analyst. To determine the within-lab reproducibility, ten batches of duplicate DPS, beer, and wine for each spiked concentration of all analytes were analyzed by three different analysts at a different time. The performance of precision was evaluated by using the Horwitz equation and Horrat ratio. The acceptance criteria relative to the standard deviation of r (RSD_r) and R (RSD_R) were evaluated by using Eq. (5) and Eq. (6), respectively (AOAC, 2002), where *C* is the concentration conveyed in a decimal fraction. The equations are as follows:

$$RSD_r\ Horwitz = C^{-0.15} \quad (5)$$

$$RSD_R\ Horwitz = 2C^{-0.15} \quad (6)$$

According to the AOAC Guidelines for Single Laboratory Validation of Chemical Methods (2002), the calculated RSD_r for ethanol is 4%, 2%, and 1.5% for low, medium, and high-level concentrations, respectively. The calculated RSD_r is 8% for low-level and 6% for both medium and high-level concentrations of methanol, isopropanol, and *tert*-butanol. The calculated RSD_R is 8%, 4%, and 3% for low, medium, and high-level concentrations of ethanol, respectively. The calculated RSD_R is 16% for low-level and 11% for both medium and high-level concentrations of methanol, isopropanol, and *tert*-butanol. Horrat ratio was calculated by using the value from Eq. (5) and Eq. (6) with the calculated RSD_r or RSD_R as follows:

$$Horrat_r = \frac{Eq.(5)}{calculated\ RSD_r,\%} \quad (7)$$

$$Horrat_R = \frac{Eq.(6)}{calculated\ RSD_R,\%} \quad (8)$$

Recovery was performed by spiking low, medium, and high concentrations of analytes into the blank DPS, beer, and wine, respectively. The recovery must fall within an acceptable range of 85–110%, 92–105%, and 95–102% for all levels of concentration of ethanol. The recovery must fall within an acceptable range of 75–120% and 80–115% for low and both medium and high concentrations of methanol, isopropanol, and *tert*-butanol as stipulated by AOAC (2002). The recovery was calculated by using Eq. (9) as mentioned by the European Commission (2002):

$$Recovery = \frac{concentration\ in\ spiked\ samples - concentration\ in\ blank\ samples}{concentration\ of\ analyte\ added} \times 100 \quad (9)$$

2.7 Statistical analysis

Each analysis was conducted in triplicate and the average of the data was used in the calculation. The ANOVA, multiple regression analysis, lack-of-fit test, response surface analysis, *F*-test, and *t*-test was calculated by using Minitab software (version 17). *F*-test and *t*-test were performed to find significant differences between results. All data were analyzed statistically at the confidence level of 95% ($p < 0.05$).

https://doi.org/10.26656/fr.2017.7(5).354

3. Results and discussion

3.1 Optimization of HS-GC-FID using response surface methodology

3.1.1 Establishment of HS-GC-FID conditions for alcohol analysis

The operating conditions for the headspace were optimized based on previous literature involving several factors that have effects on the recovery and sensitivity of the method. The conditions were identified as independent variables, such as sample volume, oven temperature and equilibrium time (Li *et al*., 2009; Zhang *et al*., 2015; Kim *et al*., 2019; Bokhon *et al*., 2021). Based on Li *et al*. (2009), Zhang *et al*. (2015) and Kim *et al*. (2019), different ranges of sample volume were set for optimization of headspace conditions. It was found that when using the full evaporation technique of headspace for the determination of ethanol and methanol in wines (Zhang *et al*., 2015), the optimum sample volume for methanol and ethanol is 30 µL and 60 µL, respectively. The range of sample volume used in that study is 0 µL–60 µL and the optimization was conducted conventionally, without application of RSM. According to Nur Aimi *et al*. (2013), 0.1 mL and 3 mL of nipa palm sap were used to determine the amount of ethanol and other alcohol, respectively. Tipler (2013) also reported that the concentration of ethanol reached equilibrium when the sample volume was 4 mL and there was no increment in terms of ethanol concentration even though the volume of the sample was increased constantly at the interval of 2 mL up to 12 mL. A larger sample size is desirable in obtaining better detection sensitivity, but some of the volatile solutes may remain in the condensed phase if the sample size is too large. In that case, full evaporation of the analytes will not be reached. Besides, the sample size of headspace analysis is varied and depends on what types of targeted analytes as well as the nature of the sample matrices (Li *et al*., 2009). In this regard, the sample volume within the range of 0.33 mL and 3.69 mL was selected to be optimized in determining the optimum concentration of alcohols in the DPS.

The high temperature of the headspace oven is needed to help the analytes turn into the vapour phase from the liquid phase, but too high of a temperature can cause high pressure, which will lead to sample leaking or vial explosion (Li *et al*., 2007; Zhang *et al*., 2015). Due to that, the selection of range considered all analytes' boiling points, including the internal standard used to ensure full evaporation had taken place. The boiling point of ethanol, methanol, isopropanol, *tert*-butanol, and pentanol is 78°C, 65°C, 82°C, 83°C, and 138°C respectively (LibreTexts Chemistry, 2020). Therefore, the range of 71–138°C for headspace oven temperature was decided for this study. The necessary equilibrium time needs to be established to ensure that the analytes have reached their saturation in the vapour phase because once reach their saturation, the response will be no longer increased (Tipler, 2013). The shortest and longest equilibrium times used by the previous researchers were 2 mins (Li *et al*., 2007) and 60 mins (Zhang and Guo, 2017), respectively. Therefore, the range of 6.6–55.4 mins of equilibrium time was selected for this study.

The peak area of each analyte was chosen as the dependent variable as it represents the corresponding concentrations of the analytes in the samples (Zhang *et al*., 2015). The optimization was conducted to obtain the best combination values of independent variables for the maximum response of each analyte. The selection of GC peak area as a dependent variable was reported in the determination of methanol and ethanol in industrial oils (Bokhon *et al*., 2021) and wines (Zhang *et al*., 2015), benzene in beverages (Kim *et al*., 2019) and ethanol in fermentation liquor (Li *et al*., 2009). The ethanol was spiked at a concentration of 5%, while other alcohols (methanol, isopropanol, and *tert*-butanol) were spiked at a concentration of 5 mg/L for the whole optimization process.

3.1.2 Interactions between independent variables and response variables

Table 2 summarizes the CCD matrix of independent variables for the determination of ethanol, methanol, isopropanol, and *tert*-butanol in the DPS. The linear, quadratic, and interaction effects of the independent variables towards response variables were analyzed using ANOVA and tabulated in Table 3. According to Yang *et al*. (2020), a *p*-value is considered significant when it is less than 0.05, while the *F*-value represents the influence of the factor on the evaluation index. A larger *F*-value indicates a higher impact on the index. The model was found to be satisfactory with a coefficient (R^2) of 0.833, 0.857, 0.940, and 0.912 for response variables of Y_1, Y_2, Y_3 and Y_4, respectively. As shown in Table 3, no significant lack-of-fit was observed ($p>0.05$) for all response variables, suggesting that the model is fit and satisfactory.

The volume of the sample showed significant values for all response variables except for the peak area of isopropanol. The sample's volume also has a good interaction with the oven temperature for all peak areas of analytes but has only a positive interaction with the equilibrium time for the peak area of ethanol. Larger sample volumes can increase the sensitivity of the detection method (Kolb and Ettre, 2006). Therefore, a higher volume of samples will generate a higher peak area of the analytes. However, if the sample volume is too large, some of the volatile contents may remain

https://doi.org/10.26656/fr.2017.7(5).354

Table 3. ANOVA of central composite design (CCD) for independent variables and response variables.

Source	Peak area of ethanol (Y_1)		Peak area of methanol (Y_2)		Peak area of isopropanol (Y_3)		Peak area of *tert*-butanol (Y_4)	
	F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Model	5.55	0.007*	6.67	0.003*	17.38	≤0.001*	11.55	≤0.001*
X_1[a]	6.69	0.027*	5.07	0.048*	2.43	0.150	6.40	0.044*
X_2[b]	7.31	0.022*	9.73	0.011*	46.58	≤0.001*	50.13	≤0.001*
X_3[c]	2.18	0.170	0.97	0.348	4.99	0.049*	0.00	0.991
X_1^2	7.10	0.024*	1.81	0.209	8.73	0.014*	16.52	0.002*
X_2^2	26.14	≤0.001*	41.25	≤0.001*	94.28	≤0.001*	36.50	≤0.001*
X_3^2	2.03	0.185	5.70	0.045*	6.99	0.025*	0.01	0.942
X_1 X_2	5.76	0.037*	5.73	0.039*	5.22	0.038*	5.95	0.038*
X_1 X_3	4.69	0.042*	0.11	0.749	0.11	0.752	0.22	0.647
X_2 X_3	0.83	0.384	0.11	0.748	5.93	0.035*	0.05	0.828
Lack of fit	2.40	0.160	4.70	0.053	0.85	0.479	3.99	0.083

X_1 = Volume of sample (mL); X_2 = Oven temperature (°C); X_3 = Equilibrium time (mins).
*p-value < 0.05 = significant.

inside the sample and will prevent the full equilibrium and evaporation of analytes, thus leading to reducing the peak area of those analytes (Zhang *et al*., 2015). Larger sample volumes also need higher temperatures and a longer equilibrium time to ensure the complete transfer of analytes occurs in the headspace area (Liu *et al*., 2014). This was clearly demonstrated in Figures 1 (a) and (b), as well as in Figures 1 (g), (h), and (j).

According to Table 3, the oven temperature showed significant values for all linear and quadratic response variables (p-value <0.05), except for the quadratic response of methanol. As shown in Figures 1 (c) and (f), as well as in Figures 1 (i) and (l), increasing the temperature of the oven will ensure volatile solutes inside the liquid phase of the sample enter the headspace phase thus increasing the peak area of analytes (Zhang *et al*., 2015). However, too high of temperature may lead to the degradation of analytes, which will cause a decrease in the peak area of analytes (Câmara *et al*., 2006). Also, it may increase the risk of sample leaking or even bursting the headspace vial (Zhang *et al*., 2015). These research findings were in accordance with what has been reported by Ma *et al*. (2013) and Kreutz *et al*. (2018).

Equilibrium time can be defined as the time needed for each analyte to fully transfer from its original state of samples into the headspace area, as well as reaching its equilibrium to ensure high recovery of analytes (Garvey *et al*., 2020). According to Table 3, equilibrium time showed only significant values for linear response (X_3) of isopropanol and has good interaction with oven temperature for peak area of isopropanol. However, equilibrium time shows a positive interaction with the volume of the sample for the peak area of ethanol. The equilibrium time of the headspace was not a relevant factor to *tert*-butanol possibly due to its high molecular weight as compared to other analytes, which takes a much longer time to achieve (Kreutz *et al*., 2018).

Based on the optimization study using CCD, the optimum headspace conditions for all the analytes in the DPS were 2.5 mL of the volume of the sample at 102°C of oven temperature for 31.7 mins of equilibrium time. The desirability function was applied in this study to ensure the optimization of headspace conditions is achieved. This function is applicable in combining all optimum conditions for each response, and then providing one best condition towards all responses. The value of desirability is between 0 to 1, where 1 is the most ideal value (Garvey *et al*., 2020). The optimization desirability value of this study is 0.927, which is close to 1. Hence, the headspace conditions of this study are the most suitable to be applied for the determination of ethanol, methanol, isopropanol and *tert*-butanol.

3.1.3 Verification of model

The proposed optimum headspace conditions of sample volume (2.5 mL), oven temperature (102°C), and equilibrium time (31.7 mins) were applied in an experiment (n = 5) to verify the proposed HS-GC-FID method. The average experimental results and predicted results for peak area of ethanol, methanol, isopropanol, and *tert*-butanol, p-value, and the percentage of prediction error were presented in Table 4. There is no significant difference (p-value > 0.05) between the predicted and experimental results for all the response variables. The prediction of error also shows that the responses are satisfactory as the overall percentage of prediction error is 0.773%, which the experimental results are close to the predicted results. Therefore, the optimized headspace conditions from CCD are fit to be used for further validation.

https://doi.org/10.26656/fr.2017.7(5).354

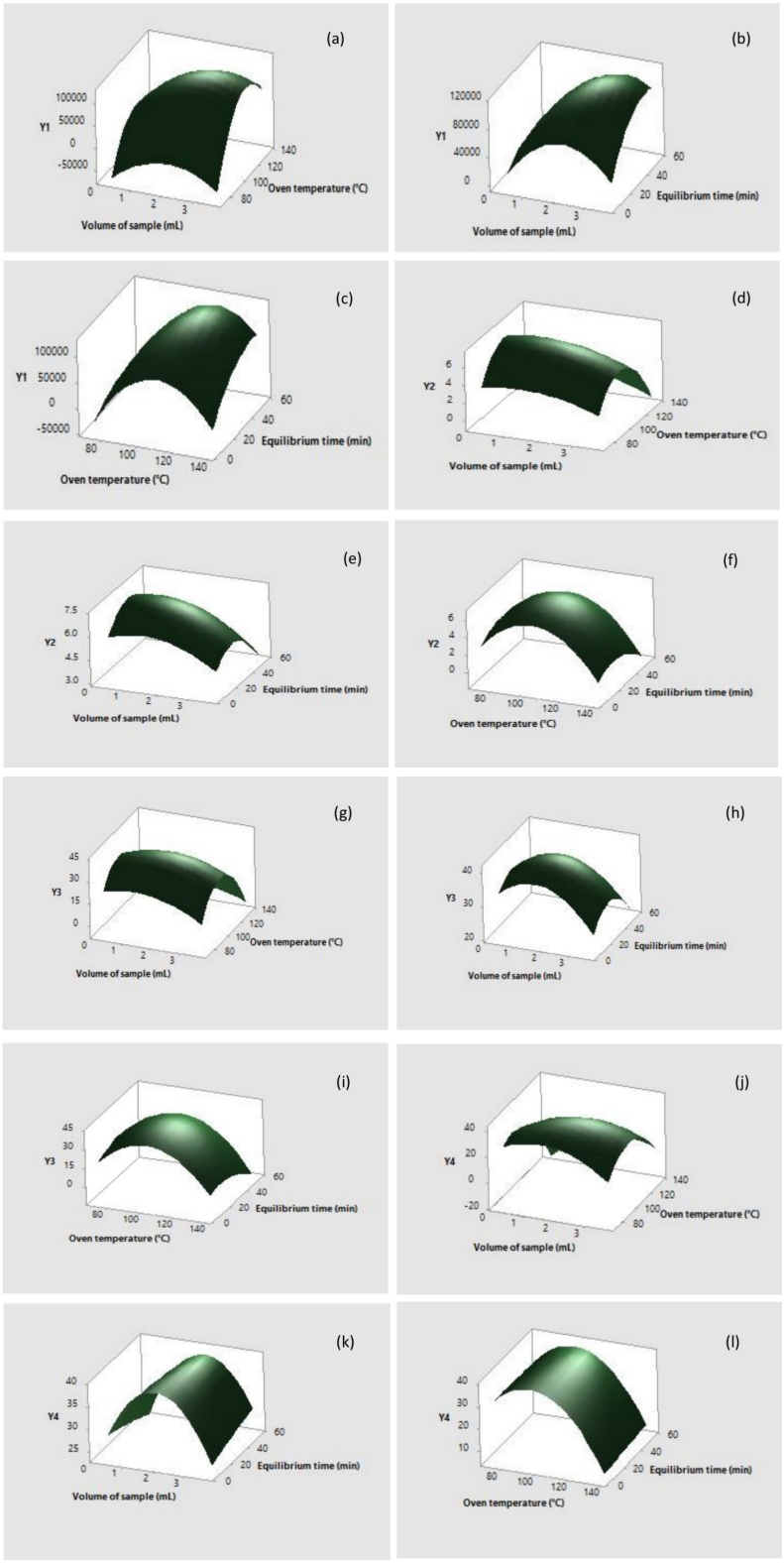

Figure 1. Response surface plot (3D) of interactive effects between the volume of sample (mL), oven temperature (°C), and equilibrium time (minutes) towards the peak area of ethanol (Y1), peak area of methanol (Y2), isopropanol (Y3), peak area of *tert*-butanol (Y4).

Table 4. Comparison between the experimental values and predicted values of RSM.

Responses	Experimental values[a]	Predicted values	*p*-value	Percentage of error (%)
Peak area of ethanol	110543.831±153.789	110500.000	0.542*	0.040
Peak area of methanol	6.874±0.300	6.712	0.261*	2.360
Peak area of isopropanol	38.836±0.257	38.670	0.188*	0.426
Peak area of *tert*-butanol	38.180±0.725	38.078	0.761*	0.267
Overall percentage error				0.773

[a]Experimental values were presented as mean ± standard deviation (n = 5).

**p*-value > 0.05 = not significant.

https://doi.org/10.26656/fr.2017.7(5).354

3.2 Method validation

3.2.1 Specificity

Specificity is defined as the capability of a particular method to distinguish and quantify the target analytes in the existence of other analytes or interferences (AOAC, 2002). Figure 2 shows the chromatogram of alcohol determination in the blank DPS and spiked blank DPS. The peaks were consistent with no splits, shoulder, or overlapping for all analytes between the retention time of 3 and 8 min for ethanol, methanol, isopropanol, and *tert*-butanol. Pentanol was located at the retention time between 11 and 12 mins. This indicated that well separation of peaks and a good resolution were obtained for all the analytes as no interferences were observed. Even though the peak of ethanol and isopropanol was close to each other, it was not a major drawback since high concentrations of both analytes are infrequent in non-alcoholic beverages (Rollman *et al*., 2021). This was in accordance with Ebersole *et al*. (2017) who reported a good separation of ethanol and propanol in kombucha without any overlapping peak.

3.2.2 Limit of detection and limit of quantification

LOD is defined as the minimum amount of a target analyte, which can be detected by a particular method, but not certainly can be quantitated as a definite value, while LOQ is described as the minimum amount of a particular analyte, which can be determined quantitatively with acceptable precision and accuracy (ICH, 2005). The LOD and LOQ of ethanol, methanol, isopropanol, and *tert*-butanol were established according to the first point of the calibration curve and evaluated based on the linearity study. As shown in Table 5, the LOD of 0.01% (v/v) was detected for ethanol, 0.3 mg/L for methanol, and 0.1 mg/L for both isopropanol and *tert* -butanol. While the LOQ of 0.03% (v/v), 1.0 mg/L, and 0.5 mg/L was detected for ethanol, methanol, and both isopropanol and *tert*-butanol, respectively. Unlike other alcohols, a percentage was used to express the amount of ethanol in declaring the alcohol in alcoholic beverages in Malaysia. For example, Malaysia's Food Regulation 1985 (Laws of Malaysia, 2017) stipulated that the lowest level of ethanol in alcoholic beverages should not be less than 2% (v/v). Furthermore, the permissible limit of alcohol content in beverages to be considered Halal in Malaysia is 1% (v/v) (JAKIM, 2011). It is noteworthy that the limit of concentration for methanol, isopropanol, and *tert*-butanol in beverages was not mentioned in Malaysia's Food Regulation 1985. However, the lowest detection and quantification limit possible were recommended for these alcohols due to their inedible status (Giovanetti, 2013). Therefore, the unit of milligram per litre (mg/L) was used to report the concentration of these analytes.

The LOQ of ethanol in the current study is well below the permissible limit of ethanol due to the high sensitivity of GC-HS-FID used. Therefore, this validated

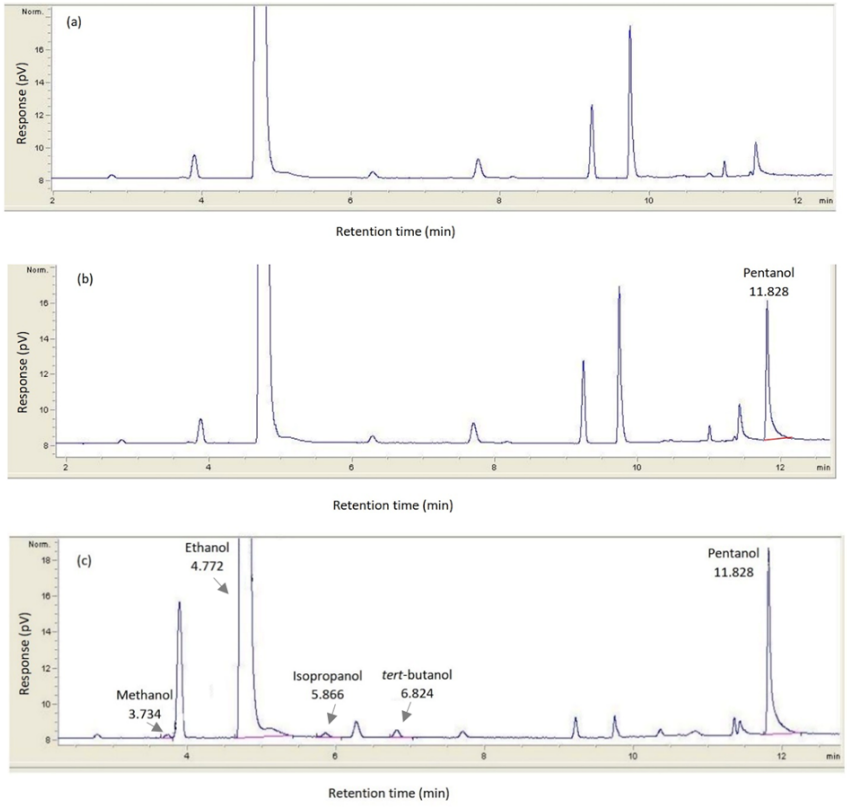

Figure 2. Headspace gas chromatography (GC-HS) chromatogram of (a) blank date palm sap; (b) date palm sap spiked with internal standard (pentanol); (c) date palm sap spiked with ethanol, methanol, isopropanol, and *tert*-butanol, together with internal standard (pentanol).

https://doi.org/10.26656/fr.2017.7(5).354

Table 5. Limit of detection (LOD), limit of quantification (LOQ), and linearity for ethanol, methanol, isopropanol, and *tert*-butanol in date palm sap, beer, and wine.

Analytes	LOD	LOQ	Linear range	Equation	R^2
Ethanol (%)	0.01	0.03	0.03–40	y = 8474.5x + 953.5	0.998
Methanol (mg/L)	0.3	1.0	1.0–50	y = 0.6271x – 0.6775	0.990
Isopropanol (mg/L)	0.1	0.5	0.5–50	y = 1.3830x + 2.3016	0.990
Tert-butanol (mg/L)	0.1	0.5	0.5–50	y = 3.2288x + 4.6862	0.990

LOD = limit of detection; LOQ = limit of quantification; R^2 = coefficient of determination

method can be used to determine the ethanol concentration in both non-alcoholic and alcoholic beverages for Halal and food fraud surveillance purposes in Malaysia. The LOD and LOQ of methanol in the present study were lower than those reported in alcohol-free malt beverages, energy drinks, and fruit juices (Sirhan *et al*., 2019) as well as in wine (Zhang *et al*., 2015), which is 5.74 mg/L and 13.0 mg/L, respectively. On the other hand, a previous study reported the same value of LOD but a lower value of LOQ of isopropanol and *tert*-butanol in beer, fruit wine, rice wine, and spirit (Kim *et al*., 2017) as compared to this research. Another study mentioned the detected concentration of these alcohols in alcoholic beverages, but the LOD and LOQ of isopropanol and *tert*-butanol were not identified (Destanoğlu and ATEŞ, 2019). These analytes are denaturants for ethanol and are always used as a substitute for alcoholic beverages to increase the alcoholic strength of the products (Lachenmeier, 2016). The human tolerable content of ethanol and methanol varies between individuals, depending on their physical and health conditions (Ou *et al*., 2019). However, it was reported that methanol and ethanol lethal doses were 25–75 mL and 384 mL, respectively, for 60 kg of body weight (Tulashie *et al*., 2017).

3.2.3 Linearity

Linearity is expressed as the capability of the method to acquire results directly proportional to the concentration of analytes in the sample, according to its designated range (ICH, 2005). As displayed in Table 5, the calibration curves displayed a good correlation coefficient with R^2 of 0.998, 0.990, 0.990, and 0.990 for ethanol, methanol, isopropanol and *tert*-butanol, respectively. A high value of correlation coefficient of more than 0.99 is normally suggested as a goodness of fit (AOAC, 2002). The lack-of-fit and regression test was accepted for all the analytes within the linear working range. The acceptable working range of ethanol and methanol is 0.03–40% (v/v) and 1.0–50 mg/L, respectively. The linear working range for both isopropanol and *tert*-butanol is 0.5–50 mg/L. The linearity of this study was in accordance with that reported in black liquors (Li *et al*., 2007), *nipa* palm sap (Nur Aimi *et al*., 2013), soy sauce (Liu *et al*., 2014), kombucha (Ebersole *et al*., 2017), alcohol-free beverages, energy drinks and fruit juices (Sirhan *et al*., 2019), and non-beverage alcohol (Rollman *et al*., 2021) with the R^2 ranging from 0.982 to 0.999.

3.2.4 Precision and accuracy

The precision of a particular method is described as the closeness of the experimental data to each other when being collected from the same homogenous sample under specified conditions (ICH, 2005). The parameters used for precision are repeatability (r) and reproducibility (R). Repeatability (r) is the precision with the same operating conditions over a certain period, while reproducibility (R) is the precision between different analysts, instruments, or times. On the other hand, accuracy is the closeness of the collected data towards reference value (ICH, 2005). Table 6 shows the precision data of relative standard deviation for repeatability (RSD_r) and reproducibility (RSD_R), as well as the accuracy (i.e., recovery) of the spiked DPS, beer, and wine. The obtained values of RSD_r for ethanol, methanol, isopropanol, and *tert*-butanol ranged from 0.7–2.8%, 1.4–3.7%, 2.2–4.3%, and 1.4–2.5%, respectively. These values were lower than the acceptable values of RSD_r from Eq. (5) for ethanol (1.2–3.4%), methanol (4.8–7.9%), and both isopropanol and *tert*-butanol (4.8–8.8%) (AOAC, 2002). The obtained values of RSD_R for ethanol, methanol, isopropanol, and *tert*-butanol ranged from 1.5–6.5%, 3.4–4.9%, 2.5–10.2%, and 3.0–8.9%, respectively. These values were lower than the range of acceptable values of RSD_R from Eq. (6) for ethanol (2.4–6.8%), methanol (9.5–15.9%), and both isopropanol and *tert*-butanol (9.5–17.6%) (AOAC, 2002). This indicated that the performance of this method was satisfactory. The precision obtained in this study was higher than the previous author, who reported a range of 1.2–4.8 % for ethanol in craft beers, wines, and soft drinks (Wachełko *et al*., 2021) but lower than what was observed in alcohol-free beverages, energy drinks, and fruit juices for ethanol and methanol with 5.3–9.2% (Sirhan *et al*., 2019).

As shown in Table 6, the recoveries of ethanol, methanol, isopropanol, and *tert*-butanol in the spiked DPS, beer, and wine were between 99.9–104.7%, 99.5–114.1%, 87.8–101.5%, and 100.0–108.5%, respectively. The values were within an acceptable recovery range for specific concentrations according to AOAC (2002). The

https://doi.org/10.26656/fr.2017.7(5).354

recovery of ethanol and methanol in this study was higher than what was reported in energy drinks and malt beverages with a range of 84.9–112.8% and 83.0–110.2%, respectively (Sirhan *et al*., 2019). However, the recovery of ethanol in this study was similar to what was reported by Nur Aimi *et al*. (2013) in nipa palm sap and Ebersole *et al*. (2017) in kombucha beverages with 97.5% and 99.6–100.4%, respectively. Zhang *et al*. (2015) reported recovery of ethanol and methanol in wines ranging from 96.1–104%. Table 5 shows the Horrat ratio for repeatability ($Horrat_r$) and reproducibility ($Horrat_R$) of the spiked DPS, beer, and wine. In this study, the values of $Horrat_r$ ratio were within the range of 0.8–0.9 for ethanol, 0.8–1.0 for methanol, and 0.8–1.1 for both isopropanol and *tert*-butanol. The values of $Horrat_R$ ratio were within the range of 0.8–0.9 for ethanol and 0.9–1.1 for methanol, isopropanol, and *tert*-butanol, respectively. These values were within an acceptable range of 0.5–2.0 as stipulated by AOAC (2002).

4. Conclusion

A HS-GC-FID method for the determination of ethanol, methanol, isopropanol, and *tert*-butanol in DPS, beer, and wine was successfully optimized by using RSM and validated. The optimized headspace conditions obtained were 2.5 mL of the volume of the sample, 102° C of oven temperature, and 31.7 mins of equilibrium time. The validated method demonstrated an acceptable and satisfactory performance regarding specificity, LOD, LOQ, linearity, precision, and accuracy. Therefore, the entire procedure combining headspace sample injection and analyte detection using GC-FID is fit for its intended purpose and can be applied in detecting ethanol, methanol, isopropanol, and *tert*-butanol in DPS, beer, and wine. Due to its much wider range of analytes, the application of this method can be potentially extended to other fermented and alcoholic beverages for products' quality, safety, and Halal assurance purposes with further validation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors sincerely acknowledge the Faculty of Applied Sciences, Universiti Teknologi MARA Shah Alam (Selangor, Malaysia), National Public Health Laboratory (Selangor, Malaysia), and Food Safety and Quality Division, Ministry of Health, Malaysia for the laboratory facilities and technical supports.

References

Ambranson, S. (2010). Chapter 51: Extracorporeal Treatment of Poisonings. In Himmerfarb, J. and Sayegh, M.H. (Eds). Chronic Kidney Disease, Dialysis and Transplantation, p. 700–719. Philadelphia, USA: Saunders Elsevier. https://doi.org/10.1016/B978-1-4377-0987-2.00051-0

Andersen, F.A. (2005). Amended final report of the safety assessment of t-butyl alcohol as used in cosmetics. *International Journal of Toxicology*, 24 (2), 1–20. https://doi.org/10.1080/10915810590953833

AOAC. (2002). AOAC guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals. Arlington, USA: Association of Official Analytical Chemists.

Table 6. Precision and accuracy data for the determination of ethanol, methanol, isopropanol, and *tert*-butanol in spiked date palm sap, beer, and wine at three different levels of concentrations, respectively.

Analytes	Concentration	Precision						Accuracy	
		RSD_r (%)	RSD_R (%)	RSD_r Horwitz (%)	RSD_R Horwitz (%)	$Horrat_r$	$Horrat_R$	Recovery (%)	SD[e] (%)
Ethanol (%)	0.03	2.8	6.5	3.4	6.8	0.9	0.9	104.7	8.6
	5	0.9	2.9	1.6	3.1	0.8	0.8	102.9	1.5
	30	0.7	1.5	1.2	2.4	0.8	0.8	99.9	2.7
Methanol (mg/L)	1	1.6	4.6	7.9	15.9	1.0	1.0	114.1	1.0
	5	3.7	4.9	6.2	12.5	1.0	1.1	101.5	5.7
	30	1.4	3.4	4.8	9.5	0.8	0.9	99.5	2.7
Isopropanol (mg/L)	0.5	2.4	10.2	8.8	17.6	1.1	1.1	87.8	4.9
	5	4.3	2.5	6.2	12.5	1.0	1.1	101.5	2.2
	30	2.2	3.4	4.8	9.5	0.8	0.9	97.0	3.5
tert-butanol (mg/L)	0.5	2.5	8.9	8.8	17.6	1.1	1.1	108.5	9.9
	5	2.4	6.4	6.2	12.5	1.0	1.1	100.0	2.5
	30	1.4	3.0	4.8	9.5	0.8	0.9	101.6	2.1

RSD_r = relative standard deviation for repeatability; RSD_R = relative standard deviation for reproducibility; $Horrat_r$ = Horrat ratio for repeatability; $Horrat_R$ = Horrat ratio for reproducibility; SD = standard deviation.

https://doi.org/10.26656/fr.2017.7(5).354

Barreveld, W.H. (1993). Date palm products. FAO Agricultural Services Bulletin No. 101. Retrieved on April 16, 2021 from FAO website: http://www.fao.org/3/t0681e/t0681e00.htm#con

Ben Thabet, I., Francis, F., de Pauw, E., Besbes, S., Attia, H., Deroanne, C. and Blecker, C. (2010). Characterisation of proteins from date palm sap (*Phoenix dactylifera* L.) by a proteomic approach. *Food Chemistry*, 123(3), 765–770. https://doi.org/10.1016/j.foodchem.2010.05.024

Bokhon, F., Daryanavard, S.M., Gholamshahzadeh, A. and Tezerji, A.K. (2021). Application of experimental design for determination of methanol and ethanol in transformer industrial oils using headspace single-drop microextraction. *Analytical and Bioanalytical Chemistry Research*, 8(1), 27–38. https://doi.org/10.22036/abcr.2020.233914.1510

Câmara, J.S., Arminda Alves, M. and Marques, J.C. (2006). Development of headspace solid-phase microextraction-gas chromatography-mass spectrometry methodology for analysis of terpenoids in Madeira wines. *Analytica Chimica Acta*, 555(26), 191–200. https://doi.org/10.1016/j.aca.2005.09.001

Dadalı, C. and Elmacı, Y. (2019). Optimization of Headspace-Solid Phase Microextraction (HS-SPME) technique for the analysis of volatile compounds of margarine. *Journal of Food Science and Technology*, 56(11), 4834–4843. https://doi.org/10.1007/s13197-019-03945-w

Destanoğlu, O. and Ateş, İ. (2019). Determination and evaluation of methanol, ethanol and higher alcohols in legally and illegally produced alcoholic beverages. *Journal of the Turkish Chemical Society, Section A: Chemistry*, 6(1), 21–28. https://doi.org/10.18596/jotcsa.481384

Ebersole, B., Liu, Y., Schmidt, R., Eckert, M. and Brown, P.N. (2017). Determination of ethanol in Kombucha products: Single-laboratory validation, First Action 2016.12. *Journal of AOAC International*. 100(3), 732–736. https://doi.org/10.5740/jaoacint.16-0404

European Commission. (2002). Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities*, 221, 8–36.

Garg, U. and Ketha, H. (2020). Chapter 5- Alcohols: volatiles and glycols. In Ketha, H. and Garg, U. (Eds). Toxicology Cases for the Clinical and Forensic Laboratory, p. 37–49. Cambridge, United Kingdom: Academic Press. https://doi.org/10.1016/B978-0-12-815846-3.00005-3

Garvey, E.C., O'Sullivan, M.G., Kerry, J.P. and Kilcawley, K.N. (2020). Optimisation of HS-SPME parameters for the analysis of volatile compounds in baked confectionery products. *Food Analytical Methods*, 13(6), 1314–1327. https://doi.org/10.1007/s12161-020-01740-4

Giovanetti, F. (2013). Methanol poisoning among travellers to Indonesia. *Travel Medicine and Infectious Disease*, 11(3), 190–193. https://doi.org/10.1016/j.tmaid.2013.03.013

Gómez-Coca, R.B., Cruz-Hidalgo, R., Fernandes, G.D., Pérez-Camino, M.D.C. and Moreda, W. (2014). Analysis of methanol and ethanol in virgin olive oil. *MethodsX,* 1, 207–211. https://doi.org/10.1016/j.mex.2014.09.002

International Conference on Harmonization (ICH). (2005). ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology Q2(R1), presented at International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Geneva. Switzerland.

JAKIM. (2011). Alkohol dalam makanan, minuman, pewangi dan ubat-ubatan. Retrieved on April 17, 2021 from e-SMAF Website: http://e-smaf.islam.gov.my/e-smaf/index.php/main/mainv1/fatwa/pr/10281

Joshi, V.K., Attri, B.L., Panesar, P.S., Abrol, G.S., Sharma, S., Thakur, A.D., Selli, S., Kelebek, H. and Reddy, L.V. (2017). Specific Features of Table Wine Production Technology. In Kosseva, M.R., Joshi, V.K. and Panesar, P.S. (Eds). Science and Technology of Fruit Wine Production, p. 295–461. Cambridge, United Kingdom: Academic Press.

Kim, E.M., Kim, D.A., Kwon, S.W., Jin, Y., Lee, H., Kang, S. and Lee, J. (2019). Headspace conditions and ingredients can affect artefactual benzene formation in beverages. *Food Chemistry*, 293, 278–284. https://doi.org/10.1016/j.foodchem.2019.04.089

Kim, H.M., Yang, G., Kim, J.Y., Yoon, S.J., Shin, B.K., Lee, J., Park, J.H. and Kwon, S.W. (2017). Simultaneous determination of volatile organic compounds in commercial alcoholic beverages by gas chromatography with flame ionization detection. *Journal of AOAC International*, 100(5), 1492–1499. https://doi.org/10.5740/jaoacint.17-0006

Kolb, B. and Ettre, L.S. (2006). Chapter 3: The technique of HS-GC. In Kolb, B. and Ettre, L.S. (Eds). Static Headspace-Gas Chromatography: Theory and Practice, 2nd Edition, p. 51–163. New Jersey, USA: John Wiley and Sons, Inc. https://doi.org/10.1002/0471914584.ch3

Kreutz, T., Lucca, L.G., Loureiro-Paes, O.A.R., Teixeira, H.F., Veiga, V.F., Limberger, R.P., Ortega, G.G. and Koester, L.S. (2018). Optimization, validation and application of headspace solid-phase microextraction gas chromatography for the determination of 1-nitro-2-phenylethane and methyleugenol from *Aniba canelilla* (H.B.K.) Mez essential oil in skin permeation samples. *Journal of Chromatography A*, 1564, 163–175. https://doi.org/10.1016/j.chroma.2018.05.073

Lachenmeier, D.W. (2016). Advances in the detection of the adulteration of alcoholic beverages including unrecorded alcohol. In Downey, G. (Eds). Advances in Food Authenticity Testing, p. 565–584. Duxford, United Kingdom: Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100220-9.00021-7

Lang, K., Väli, M., Szucs, S., Ádány, R. and McKee, M. (2006). The composition of surrogate and illegal alcohol products in Estonia. *Alcohol and Alcoholism*, 41(4), 446–450. https://doi.org/10.1093/alcalc/agl038

Law, S.V., Mat Hashim, D., Abu Bakar, F. and Radi, N.A. (2009). Ethanol content of beverages and condiments in Malaysia, presented at 3rd IMT-GT International Symposium on Halal Science and Management 2009, Malaysia.

Laws of Malaysia. (2017). Food Regulations 1985. Retrieved on March 17, 2021 from Food Safety Quality Division, Ministry of Health Malaysia Website: http://fsq.moh.gov.my/v6/xs/page.php?id = 72

Li, H., Chai, X.S., Deng, Y., Zhan, H. and Fu, S. (2009). Rapid determination of ethanol in fermentation liquor by full evapouration headspace gas chromatography. *Journal of Chromatography A*, 1216(1), 169–172. https://doi.org/10.1016/j.chroma.2008.11.024

Li, H., Zhan, H., Fu, S., Liu, M. and Chai, X.S. (2007). Rapid determination of methanol in black liquors by full evapouration headspace gas chromatography. *Journal of Chromatography A*, 1175(1), 133–136. https://doi.org/10.1016/j.chroma.2007.10.040

LibreTexts Chemistry. (2020). 14.3: Physical Properties of Alcohols. Chem 100 General Chemistry (O'Connor). Retrieved on February 5, 2021 from LibreTexts Chemistry Website: https://chem.libretexts.org/Courses/Mount_Aloysius_College/CHEM_100%3A_General_Chemistry_(O'Connor)/14%3A_Organic_Compounds_of_Oxygen/14.03%3A_Physical_Properties_of_Alcohols

Liu, M., Li, H. and Zhan, H. (2014). A novel method for the determination of the ethanol content in soy sauce by full evapouration headspace gas chromatography. *Food Analytical Methods*, 7(5), 1043–1046. https://doi.org/10.1007/s12161-013-9711-8

Ma, Q.L., Hamid, N., Bekhit, A.E.D., Robertson, J. and Law, T.F. (2013). Optimization of headspace solid phase microextraction (HS-SPME) for gas chromatography mass spectrometry (GC-MS) analysis of aroma compounds in cooked beef using response surface methodology. *Microchemical Journal*, 111, 16–24. https://doi.org/10.1016/j.microc.2012.10.007

Magnusson, B. and Ornemark, U. (2014). Eurachem Guide: The fitness for purpose of analytical methods - a laboratory guide to method validation and related topics. Retrieved from Eurachem website: http://www.eurachem.org/images/stories/Guides/pdf/valid.pdf

Makhlouf-Gafsi, I., Mokni-Ghribi, A., Bchir, B., Attia, H., Blecker, C. and Besbes, S. (2016). Physico-chemical properties and amino acid profiles of sap from Tunisian date palm. *Scientia Agricola*, 73(1), 85–90. https://doi.org/10.1590/0103-9016-2015-0041

McGregor, D. (2010). Tertiary-butanol: A toxicological review. *Critical Reviews in Toxicology*, 40(8), 697–727. https://doi.org/10.3109/10408444.2010.494249

Noornasrin Salsabila, O. (2021). "Nira Kurma" juice much sought after during Ramadan. Bernama News. Retrieved on June 15, 2021 from http://ramadan.bernama.com/2021/news.php?id = 1955480

Nur Aimi, R., Abu Bakar, F. and Dzulkifly, M.H. (2013). Determination of volatile compounds in fresh and fermented Nipa sap (*Nypa fruticans*) using static headspace gas chromatography-mass spectrometry (GC-MS). *International Food Research Journal*, 20 (1), 369–376.

Ornelas-Soto, N., Barbosa-Garcia, O. and Lopez-de-Alba, P.L. (2011). Procedures of food quality control: Analysis methods, sampling and sample pretreatment. In Shoyama, Y. (Eds). Quality Control of Herbal Medicines and Related Areas, p. 64–90. IntechOpen E-Book. https://doi.org/10.5772/23206

Ou, T.H., Wang, Y., Fang, D., Narayanan, S.R. and Wu, W. (2019). Detection of fake alcoholic beverages using electrolyte-free nanogap electrochemical cells. *ACS Applied Materials and Interfaces*, 11(6), 1–24. https://doi.org/10.1021/acsami.8b18729

Panovská, Z., Šedivá, A., Jedelská, M. and Pokorný, J. (2008). Effect of ethanol on interactions of bitter and sweet tastes in aqueous solutions. *Czech Journal of Food Sciences*, 26(2), 139–145. https://doi.org/10.17221/2466-cjfs

Pauzi, N., Man, S., Nawawi, M.S.A.M. and Abu-Hussin, M.F. (2019). Ethanol standard in halal dietary product among Southeast Asian halal governing bodies. *Trends in Food Science and Technology*, 86, 375–380. https://doi.org/10.1016/j.tifs.2019.02.042

Rollman, C.M., Sanderoff, R.K. and Bezabeh, D.Z. (2021). Simultaneous quantification of ethanol and propylene glycol in nonbeverage alcohol products using gas chromatography flame ionization detection. *Chromatographia*, 84(1), 97–102. https://doi.org/10.1007/s10337-020-03981-1

Rosli, J. (2018). Methanol poisoning: Health Ministry tracking down source as deaths hit 45. Malay Mail. Retrieved on April 16, 2019 from http://www.malaymail.com/news/malaysia/2018/10/02/methanol-poisoning-health-ministry-tracking-down-source-as-deaths-hit-45/1678523

Salum, P. and Erbay, Z. (2019). Optimization of headspace solid-phase microextraction for the analysis of volatile compounds of high-fat dairy powders. *Food Analytical Methods*, 12(10), 2216–2225. https://doi.org/10.1007/s12161-019-01576-7

Sapkal, S.U. and Jagtap, P.S. (2018). Optimization of micro EDM drilling process parameters for titanium alloy by rotating electrode. *Procedia Manufacturing*, 20, 119–126. https://doi.org/10.1016/j.promfg.2018.02.017

Singaravadivel, K. and Hariharan, B. (2012). Physicochemical properties of fresh and stored coconut palm toddy. *Open Access Scientific Reports*, 1(8), 397. https://doi.org/10.4172/scientificreports.397

Sirhan, A.Y., Wong, R.C.S., Abdulra'uf, L.B., Aljabar, J.A., Mostafa, A. and Talhouni, A. (2019). Simultaneous determination of ethanol and methanol in alcohol free malt beverages, energy drinks and fruit juices by gas chromatography. *Asian Journal of Agriculture and Biology*, 7(2), 183–189.

Tipler, A. (2013). An Introduction to Headspace Sampling in Gas Chromatography. PerkinElmer, Inc. Retrieved from http://www.perkinelmer.com/PDFs/downloads/GDE_Intro_to_Headspace.pdf

Tiscione, N.B., Alford, I., Yeatman, D.T. and Shan, X. (2011). Ethanol analysis by headspace gas chromatography with simultaneous flame-ionization and mass spectrometry detection. *Journal of Analytical Toxicology*, 35(7), 501–511. https://doi.org/10.1093/anatox/35.7.501

Tulashie, S.K., Appiah, A.P., Torku, G.D., Darko, A.Y. and Wiredu, A. (2017). Determination of methanol and ethanol concentrations in local and foreign alcoholic drinks and food products (Banku, Ga kenkey, Fante kenkey and Hausa koko) in Ghana. *International Journal of Food Contamination*, 4, 14. https://doi.org/10.1186/s40550-017-0059-5

Wachełko, O., Szpot, P. and Zawadzki, M. (2021). The application of headspace gas chromatographic method for the determination of ethyl alcohol in craft beers, wines and soft drinks. *Food Chemistry*, 346, 128924. https://doi.org/10.1016/j.foodchem.2020.128924

Wang, M.L., Wang, J.T. and Choong, Y.M. (2004). A rapid and accurate method for determination of methanol in alcoholic beverage by direct injection capillary gas chromatography. *Journal of Food Composition and Analysis*, 17(2), 187–196. https://doi.org/10.1016/j.jfca.2003.08.006

Xia, Q., Li, R., Zhao, S., Chen, W., Chen, H., Xin, B., Huang, Y. and Tang, M. (2011). Chemical composition changes of post-harvest coconut inflorescence sap during natural fermentation. *African Journal of Biotechnology*, 10(66), 14999–15005. https://doi.org/10.5897/AJB10.2602

Yang, Y.N., Liang, M., Yang, Y., Zheng, F.P., Wang, X.P. and Yu, A.N. (2020). Optimization of a headspace solid-phase microextraction method for the gas chromatography-mass spectrometry analysis aroma compounds of *litsea mollis hemsl*. Immature fruit. *Food Science and Technology*, 40(4), 786–793. https://doi.org/10.1590/fst.20319

Yolmeh, M. and Jafari, S.M. (2017). Applications of response surface methodology in the food industry processes. *Food and Bioprocess Technology*, 10(3), 413–433. https://doi.org/10.1007/s11947-016-1855-2

Zhang, C.Y. and Guo, M.Q. (2017). Solvent-saturated solid matrix technique for increasing the efficiency of headspace extraction of volatiles. *Journal of Chromatography A*, 1511, 9–14. https://doi.org/10.1016/j.chroma.2017.06.060

Zhang, C.Y., Lin, N.B., Chai, X.S., Zhong-Li and Barnes, D.G. (2015). A rapid method for simultaneously determining ethanol and methanol content in wines by full evapouration headspace gas chromatography. *Food Chemistry*, 183, 169–172. https://doi.org/10.1016/j.foodchem.2015.03.048

