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Use of chromatographic-based techniques and chemometrics for halal authentication of food products: A review

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ABSTRACT

Halal food products are requisite to be consumed by Muslim communities in the world. The standard methods capable of quantifying non-halal components are very urgent. This review highlights the chromatographic methods and chemometric or multivariate data analysis that offer reliable techniques to provide the separation capacity in halal authentication analysis. This review article was written from reputable worldwide databases including Web of Science, Scopus, and PubMed, between January and February 2022. The keywords were "halal research," "food analysis," "chromatography," "chemometrics" and "authentication." Chromatographic-based techniques in combination with chemometrics of multivariate analysis, a powerful statistical analysis to manage huge data generated from analytical measurement, could be used to identify potential markers to differentiate halal and non-halal samples. Chromatogram and peak separation profiles resulted as the instrument responses can be further evaluated for determination as well as quantification of halal and non-halal components in food products. Combination of chromatographic-based method and chemometrics techniques with some scenarios can be applied for halal research on food products.

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INTRODUCTION

Food and pharmaceutical products are important needs for human beings. In line with the development of science and technology, industrialization and globalization, the halal products may be added or substituted and contaminated with non-halal components such as pig derivatives and alcohols as ingredients or additives to reduce the production cost.[1] In addition, the products available in markets may contain incorrect labeling in terms of ingredient sources making the consumers lost on composition information; therefore, the use of analytical tools to check the presence of non-halal components in the products is a must for assisting the certification processes.[2] In Indonesia, the halal certification is mandatory which means that all halal declared products sold and distributed in Indonesia must be halal certified. In addition, the analysis of non-halal components in post-marketed products is also needed to confirm that the marketed products are not adulterated with non-halal components .[3]

According to Indonesian Act No. 33 (2014), the certification process is carried out by Halal Product Assurance Organizing Agency (BPJPH) and the auditing process is carried out by Halal Examination Agency (LPH). During audit, if the products are supposed to contain non-halal components (pork

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derivatives and alcohols), the laboratory testing using standard analytical methods is needed to confirm that the audited products are free from any non-halal components.[4,5] Today, the Muslim community constitutes for approximately of 25% world's population and is expected to increase further. With the increased awareness among Muslim community to consume the only halal products, the global market of halal products could reach exponentially.[6] Halal is Arabic terms used to any products permissible to be consumed by Muslim community. Today, the term of halal has widely used not only Muslim but also non-Muslim because Non-Muslim community intended to export the products into Muslim community, especially in halal certification issues.[7] Therefore, it is not surprising that halal-related studies are performed not only in majority Muslim countries like Indonesia and Malaysia but also in countries whose Muslims are minority such as the Netherlands, the United States, France and the European Union.[8]

Halal food must be free from non-halal components which are pig and all pig derivatives such as pork, lard and porcine gelatines, carrion or dead animals, blood (flowing or congealed), animals slaughtered not according to Islamic law, animals that were killed accidentally or on purpose through means such as strangling or beating, intoxicants including alcohol and drugs,[9] carnivorous animals, predator birds and certain land animals.[10] Among these, pig derivatives and alcohols are typically found in halal food products; therefore, some scientists are continuously research works on halal-related issues including developing instrumental analytical methods for detecting of non-halal components intended for halal certification.[11] Some countries have obligated the products to be halal certified which can be understood that the products are free from prohibited components. Besides, the products are manufactured using equipment dedicated for halal food.[12] Pork is typically met in meat-based food products such as meatball, sausages, etc.; while lard can be good vehicle in some cosmetics products such as cream, lipstick and lotion. Porcine gelatines are common materials used in food (in candies) and pharmaceutical products (capsule shells).[13] The objective of this review was to provide the integrative information on identification and quantification of non-halal components in food products by chromatographic methods. In addition, chemometrics techniques were reported to be applied to employ the big data evaluation as resulted from the chromatographic detection.

METHODS

This review article was written by identifying, investigating and assembling several review articles, original articles, books and relevant sources on metabolite fingerprinting from reputable worldwide databases including Web of Science, Scopus and PubMed. Literature searching was carried out between January and February 2022. The keywords explored during literature investigation were "halal research," "food analysis," "chromatography," "chemometrics" and "authentication." First, to select the suitable papers, 250 articles were reviewed through the title and abstract. The inclusion criteria to select the papers were (1) studies regarding halal authentication of food products using chromatographic technique between 2005–2022; (2) studies on analysis of non-halal components in food products using liquid chromatography and gas chromatography conducted between 2005–2022; (3) studies on the employment of chemometrics in combination with chromatographic technique for halal authentication of food products; and (4) all papers written in English. The exclusion criteria of the papers were (1) studies on halal authentication of food products using chromatographic techniques published before 2005 and (2) all articles written using language other than English.

Chromatographic-based techniques and chemometrics for analysis of non-halal components

For many years, chromatography has been known as the method of choice to assess the purity and levels of analytes in the laboratories of research, industry and quality control.[14] Gas chromatography (GC) and liquid chromatography (LC) techniques are often used for the analysis of non-halal components in food products. In terms of compound types, GC is more suitable for the analysis of smaller, volatile and stable compounds to heat, while LC is more robust and suitable for larger and less/nonvolatile compounds.[15] Some derivatization techniques are needed in LC in order to convert analytes into detectable derivates with certain detectors, while derivatization in GC for fewer volatile compounds is intended to provide more volatile and stable derivate products, although this derivatization process increases the method complexity and lengthens the sample preparation. In addition, the availability of derivative agents and its steric hindrance in the analyte, and the stability of the derivatized compounds must also be considered.[16]

One-dimensional gas or liquid chromatography using one column is considered as simple and powerful separation techniques for simple and un-complex samples. When the analyzed samples are complex enough, the application of just one-dimension chromatography leads to peak co-elution as well as overlapping and non-resolved peaks; therefore, one dimension chromatography technique is not suitable for separation of large analytes because the peak capacity of one-dimensional analysis is not large enough to achieve the complete separation with acceptable resolution.[17] In last decades, two-dimensional gas chromatography (GC x GC) and liquid chromatography (LC x LC) has been applied in analysis of complex mixture in order to increase the separation speed.[18]

In two-dimensional chromatography, the separation is carried out in two columns with different polarity connected in series by a modulator; as a consequence, the separation capacity of regular one-column in one dimensional chromatography can be considerably increased. The effluent from the first column is transferred to the second column using modulator so that the analytical information obtained (such as retention times, t_R) from the first column can be combined with that from second column, leading to a plot of two retention times.[19] Because of the excellent separation capacity of GC x GC and LC x LC combined with mass spectrometry (MS), both techniques are applied for separation all components in the complex mixtures, especially for metabolomics studies.[18] GC x GC has been widely applied for analysis of metabolites (all fatty acid types) of lard in food samples,[20] while LC x LC is typically used for analysis of peptides,[21] which can be used for identification of pork and porcine gelatines.

Chromatographic-based techniques offered reliable technique in halal authentication analysis. However, due to high number of data covered, the application of chemometrics to treat big data is unavoidable. Chemometrics can be defined as the employment of statistical and mathematical methods to obtain the objective data evaluation by extracting the relevant and meaningful information from related and unrelated responses from chemical measurements. Chemometrics or multivariate data analysis (MDA) is typically applied in numerous aspects including the quality control of halal products, qualitative and quantitative determination of chemical parameters for assessing the products authenticity.[22]

Chemometrics can provide the powerful tools in giving important information extracted from big data obtained from instrumental analyses such as methods based on spectroscopic and chromatographic. The common chemometrics techniques applied in product authentication could be grouped into exploratory data analysis, data pre-processing, description and visualization, discrimination and pattern recognition (classification), regression and prediction and experimental design.[23] Some chromatographic problems encountered during halal authentication analysis included the assessment of separation quality, the evaluation of peak alignment using pre-processing, the optimization of chromatographic systems providing the good separation of all peaks using experimental design, the accuracy of discrimination and classification using pattern recognition and quantitative analysis applying multivariate calibration. Figure 1 showed the correlation between chromatographic responses and chemometrics for certain analytical purposes. In scenario (a), peaks with good separation (good selectivity) in chromatogram was used as variable for the evaluation of compositional analysis (concentration) of analytes assisted by multivariate calibrations. In (b), certain peaks with lack selectivity was used as variable during chromatographic profiling of objects (samples) using discrete datasets (peak area or peak height), while in scenario (c), whole datasets in chromatograms were used as variables during chromatographic fingerprinting of objects. Indeed, the chemometrics of pre-processing was widely applied to obtain the desired analytical modeling.

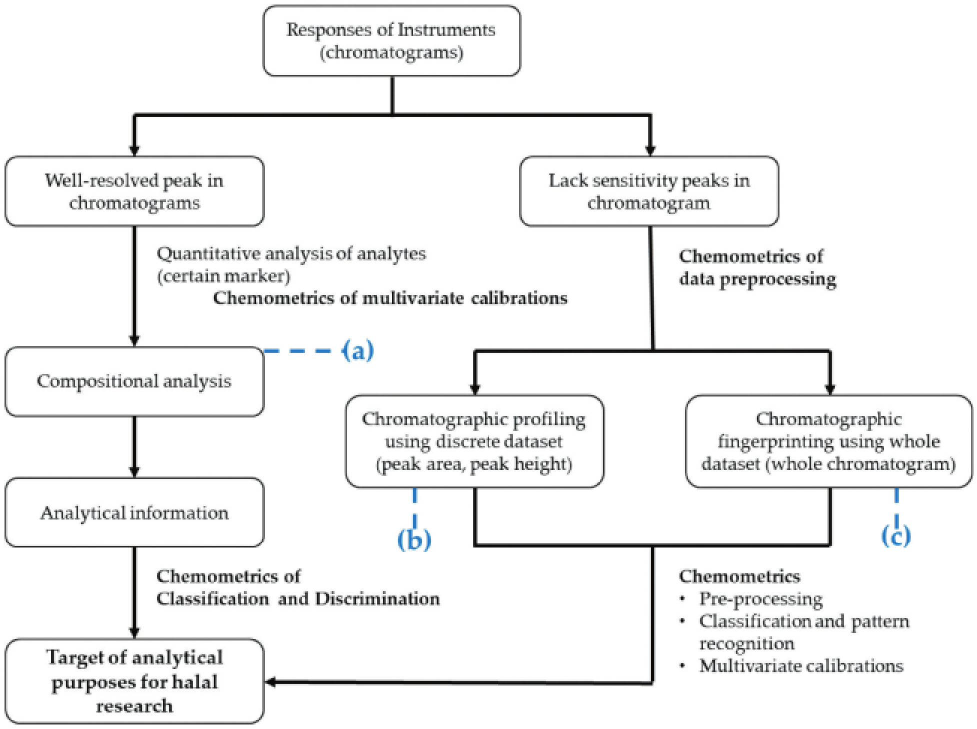

Figure 1. Three different scenarios (a, b, and c) of chemometrics applications employing the chromatograms as variable for obtaining the analytical purposes (classification of halal and non-halal products as well as prediction the levels of non-halal components in the products). Adapted from Ref.[24].

The classification chemometrics was typically carried using (1) exploratory data analysis including principal component analysis (PCA) and cluster analysis (hierarchical cluster analysis and nonhierarchical such as k-means and k-medians), and this technique is typically called as unsupervised pattern recognition and (2) classification and discrimination methods known supervised pattern recognition. There are two types of classification chemometrics methods regardless of the statistical background. The first type is typically employed to assess to which of various predefined classes of samples (objects). The example of this technique is linear discriminant analysis (LDA), orthogonal projection to latent structures – discriminant analysis (OPLS-DA), k-nearest neighbors (KNN) and many others. The second type of classification chemometrics is called as class modeling or one class classifier (OCC) and the example for this group data driven soft independent modeling of class analogy (DD-SIMCA) and Unequal Class-Modeling (UNEQ).[25] Using these chemometrics, someone can answer the question: is the meat belong to pork (non-halal) or beef (halal)? or the question: is the meatball authentic or adulterated?.[26,27]

Analysis of non halal components using liquid chromatography

High performance liquid chromatography (HPLC) using certain detectors have been widely applied for analysis of specific components in non-halal components. HPLC using fluorescence detector has been successfully used for analysis of hydroxyproline and other amino acids in gelatin and collagen samples as initial screening for identification of gelatin types. Hydroxyproline has been known as signature amino acid for gelatin and collagen. The level of hydroxyproline is typically higher in the gelatin samples than that in the collagen samples, except for the samples of fish skin gelatin, and this result could be used as screening tools for identification of non-halal gelatin and collagen in the analyzed samples.[28]

There are three approaches to detect and to identify the presence of non-halal components in food products using chromatographic based methods. The first approach is based on searching the specific markers through analysis of the separated specific components. Indeed, the availability of reference standards is a must. The second approach is used fingerprinting profiles in which the chromatogram profiles of samples with and without non-halal components are compared and evaluated. The third approach involved metabolomics studies either targeting and untargeted techniques by analysis of all metabolites in the analyzed samples. The second and third approaches involved the large datasets; therefore, the chemometrics is employed to perform the analytical tasks (discrimination, classification, etc.) [29]

Table 1 listed the application of HPLC and LC-MS/MS for analysis of halal components in the products. Liquid chromatography using fluorescence detector was also successfully applied for analysis of amino acid (AA) composition non-halal (porcine) and halal (bovine and fish) gelatins. The classification between halal and non-halal gelatins was carried using PCA applying amino acid compositions as variable. AAs with strong fluorescence (Hyp, His, Ser, Arg, Gly, Thr, Pro, Tyr, Met, Val, Leu and Phe) contribute to the classification and become the biomarkers to identify the gelatine sources.[44] Gelatin from three mammalian species including bovine gelatin, porcine gelatin, and donkey gelatin has been successfully identified using liquid chromatography-linear ion-trap high resolution mass spectrometry. Hemoglobin was just found in donkey gelatin. The unique peptide obtained from donkey, bovine and porcine gelatin was GEAGPAGPAGPIGPVGAR, GETGPAGPAGPIGPVGAR and GETGPAGPAGPVGPVGAR, respectively. The unique peptides could be detected either in individual gelatin or in the mixtures of three mammalian gelatins.[45]

Liquid chromatography especially combined with mass spectrometer tandem mass spectrometer (LC/MS-MS) is widely applied for identification of non-halal component in food products including porcine gelatin and pork. Gel-enhanced liquid chromatography-mass spectrometry (GeLCMS) in combination with chemometrics of PCA has been developed for identification of potential protein markers in pork and other meats along with its classification. The myofibrillar protein with weight of 40-kDa such as troponin T, Tropomyosin alpha-1 chain and actin cytoplasmic 1 as well as the thin filament proteins such as actin, troponin and Tropomyosin had molecular weights ranging from 40 to 45 kDa could be used as markers for differentiation of pork from chicken and beef. PCA using PC1 and PC2 accounting of 62% and 35% variances could classify meat types. From MS studies, the potential protein markers for pork meat samples are Troponin T with peptide sequences of [(R)KPLNIDHLSEDK(L)], Tropomyosin alpha-1 chain [(K)EAETRAEFAER(S)], Actin cytoplasmic 1 [(R)HQGVMVGMGQK(D)], COP9 signalosome complex subunit 4 [(R)VLDYRR(K)] and Ribonuclease inhibitor [(R)VLGQGLADSACQLETLR(L)].[46]

The identification of potential biomarkers of gelatin from several sources could be performed using UPLC-MS/MS. Samples used were gelatin from pig, cow, chicken and fish. After the extraction process of proteins from gelatin, proteins were then digested using proteomic grade trypsin for 12 h to obtain peptides. Chemometrics of PCA was used to differentiate partial hydrolysis of gelatin from cow and pig. Result from PCA score plot showed that the sample of cow and pig obtained from digestion process could be well separated. For identification of potential biomarkers from pig, cow, fish and chicken gelatin, PCA employing MPP (Mass Profiler Professional) was applied. Results showed that three unique peptides found only in pig gelatin, seven unique peptides found in bovine/cow gelatin, 22 peptides found only in chicken gelatin and only 1 unique peptide found in fish gelatin. The developed method was also successfully applied to identify species origin of commercial gelatin samples. It indicated that UPLC-MS/MS offers a powerful analytical technique to identify gelatin from different species in food products.[47]

Targeted tandem liquid chromatography-mass spectrometry (LC-MS) using decoy, randomized and concatenated database search program comprising MS-Fit and MS-Tag in combination with chemometrics of principal component analysis and orthogonal partial least square-discriminant analysis (OPLS-DA) was applied for identification of potential peptide markers in non-halal meat (pork) and halal meats (chicken and beef). The peptide markers which are specific to certain species

Table 1. The application of liquid chromatography (HPLC and LC-MS/MS) for analysis of halal components in the food and pharmaceutical products.

Methods	Issues	Results	References
HPLC-UV detection	Identification of pork in meatball products	HPLC-UV in combination with PCA could classify meatballs containing pork and beef in the products using variable of hydrolysis of Triacylglycerols (TAGs). However, the authors did not mention which TAG markers contribute to this classification	[30]
HPLC-Fluorescence detector	Identification of pork through amino acid composition	HPLC using fluorescence detector has been successfully applied for differentiation of pork and other animal meats based on analysis of derivatized amino acids with ortho-phthalaldehyde. The amino acid VAL can be identified as marker for differentiating pork from the other meats studied (beef, chicken mutton, and chevon)	[31]
HPLC-Fluorescence detector	Detection of pig collagen using D, L-amino acids	Pre column derivatization using R (-)-4-(3-isothiocyanato pyrrolidin-1-yl)-7-(*N*, *N*-dimethylamino sulfonyl)-2,1,3-benzoxadiazole [R (-)-DBD-PyNCS] could be used to determine D, L-amino acids in pork collagen. Three amino acids of D-Asp, D-Pro, and D-Hyp were first detected in pork collagen samples	[32]
LC-MS/MS with multiple reaction monitoring (MRM)	Detection of Horse and Pork in Halal Beef	Biomarker peptides were successfully identified by a shotgun proteomic approach using tryptic digests of protein extracts. Pork was identified by peptide markers: TLAFLFAER (from myosin-4) and SALAHAVQSSR (from myosin-1 and myosin-4). The detection limit is 0.55% horse or pork in a beef matrix	[33]
HPLC–MS/MS with MRM	Detection of pork in highly processed food by analysis of specific tryptic marker peptides	HPLC-MS/MS using MRM has been successfully applied for analysis of pork in some processed food products (cooking, frying and baking) based on peptide markers which are specific for pork. The peptide markers of pork identified based on MRM experiment were: marker 1 (YDIINLR) markers 2 (TLAFLFAER) and 3 (SALAHAVQSSR)	[34]
LC-MS/MS	Differentiation of porcine gelatine and bovine gelatine	LC-MS/MS in combination with exploratory data analysis of PCA could discriminate porcine and bovine gelatines. Based on loading plot PCA, peptides appearing in retention time (t_R) 32 min could be identified as peptide markers	[35]
Nano UPLC-Q-TOF-MS	Differentiation of porcine and bovine gelatin in food products	Marker peptide of bovine and porcine gelatin could be detected using nano UPLC-Q-TOF-MS based data dependent technique in yogurt, cheese, and ice cream. The method could be used to detect bovine and porcine gelatin in the mixtures	[36]
Nano UPLC-Q-TOF-MS	Differentiation of porcine and bovine gelatin in food products	Marker peptide of bovine and porcine gelatin could be detected using nano UPLC-Q-TOF-MS based data dependent technique in yogurt, cheese, and ice cream. The method could be used to detect bovine and porcine gelatin in the mixtures	[36]
LC-MS QTRAP	Gelatin speciation (bovine, porcine, and fish)	LC-MS in combination with PCA could differentiate bovine, porcine, and fish gelatin. PLS-DA could be used for classification of pure gelatin and adulterated gelatin (fish and bovine) with porcine gelatin using several concentration levels of porcine gelatin	[37]
LC-MS/MS	Discrimination of raw beef, pork, poultry and their mixtures	Protein of troponin I (TnI), enolase 3, L-lactate dehydrogenase (LDH), triose-phosphate isomerase (TPI), Tropomyosin 1, and carbonic anhydrase 3 could be used as potential markers to distinguish mammals and poultry	[38]

(*Continued*)

Table 1. (Continued).

Methods	Issues	Results	References
LC-Q-TOF-MS	Differentiation between dead-on arrival and normally slaughtered of poultry meat	LC-Q-TOF-MS could be used to differentiate between normally slaughtered and dead-on arrival poultry meat based on metabolic profiles analyzed using multivariate analysis. Using METLIN and analysis of chemical standards, metabolite of sphingosine was found to be potential marker for dead-on arrival poultry meat	[39]
UPLC-TOF-MS	Metabolite's differentiation of broiler chicken slaughtered using different techniques	UPLC-TOF-MS could be used to distinguish between halal slaughtering method and non-halal slaughtering method of broiler chicken based on their metabolite profiles. Non-halal slaughtered method demonstrated high amino acid and high glucose breakdown	[40]
LC-HRMS	Analysis of pork meat in meat mixtures using PRM	Five peptides of myosin were screened and used for PRM analysis using LC-Orbitrap HRMS. Peptide of KLETDISQIQGEMEDIVQEAR was found to be the most sensitive peptide with LOD value of 0.5% in meat mixtures	[41]
UPLC-MS	Detection of pork adulteration in beef using metabolomics approach	PLS-DA using metabolomics data obtained from untargeted measurement could classify pure and adulterated beef samples with pork. There was a significant difference in the metabolism of inositol, glutathione, and sphingolipid between beef and pork	[42]
LC-MS/MS	Detection of pork adulteration in meat samples using carbonic anhydrase 3 as a marker	Three peptides from carbonic anhydrase 3 were found as marker of pork (EPITVSSDQMAK, GGPLTAAYR, HDPSLLPWTASYDPGSAK). Quantification analysis could be performed using those three peptides with perfect quantitative ability and provided good correlation and recovery results	[43]

were identified through shot-gun proteomics. Potential peptide marker identified for raw pork is myosin-2 having sequence of peptide marker of (F)DFNSLE(Q). OPLS-DA using variable of identified peptides could separate halal and non-halal meats.[48]

Targeted proteomic analysis using LC-MS has been developed to investigate the heat stable protein in pork meat. Five heat treatments were applied such as (1) water bath heating at 78°C for 30 min; (2) boiling at 100°C for 30 min; (3) sterilizing at 121°C for 30 min; (4) frying using oil until golden brown color; and (5) baking at 200°C for 30 min. Protein extraction from samples was performed using buffer solution containing 2 M thiourea, 7 M urea and 50 mM Tris-HCl (pH 8.0). Proteins were digested using proteomic grade trypsin added with DTT to reduce disulfide bonds and IAA for alkylation. Incubation was carried out for at least 12 h at 37°C. Result showed that seven heat-stable specific peptides of pork were found such as DQLIHNLLK from l-lactate dehydrogenase A chain, HDPSLLPWTASYDPGSAK from carbonic anhydrase 3, EPITVSSDQMAK from carbonic anhydrase 3, VNVDEVGGEALGR from hemoglobin subunit beta, HPGDFGADAQGAMSK from myoglobin, SLYSSAENEPPVPLVR from carbonic anhydrase 3 and YLEFISEAIIQVLQSK from myoglobin. Commercial samples such as Iberian dried ham, Pasteur dry sausage, import dried ham, lunch meat canned, sandwich sausage and Thuringia flavor sausage were used to identify the presence one or more pig heat-stable peptides. Results showed that the heat-stable peptides of pig could be found in various types of food products with different cooking process methods. It suggested that targeted proteomics analysis using seven heat stable peptides of pig could be used for halal authentication of food products especially meat-based food products containing pork.[49]

Analysis using LC-MS employing MRM (multiple reaction monitoring) technique was successfully used to detect heat-stable peptides in cooked meats including pork meat. Thermal treatment applied was boiling at 100°C, grilling at 150°C and grilling at 180°C. After the protein was extracted, digestion process was performed using proteomic grade trypsin. Identification of homologous protein and potential biomarkers of pork peptide was carried out using UPLC Triple TOF-MS equipped with a C-18 column (2.1 × 100 mm, 1.7 µm; Waters Corporation, Taunton, MA, USA and Wexford, Ireland). The mobile phase used was water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) with flow rate of 0.3 mL/min. On the other hand, MRM analysis was performed using a SCIEX ExionLC AD system (AB SCIEX, Framingham, MA, USA) and an AB SCIEX QTRAP 4500 mass spectrometry system (AB SCIEX PTE. LTD., Marsiling, Singapore) equipped with a column of Waters ACQUITY UPLC BEH C18 (2.1 × 50 mm, 1.7 µm). Results showed that the potential peptide biomarkers in raw pork meat found were GHHEAELTPLAQSHATK from myoglobin, FAGGNLDVLK; ADMVIEAVFEELSLK; TVLGAPEVLLGILPGAGGTQR from trifunctional enzyme subunit alpha, mitochondrial and WGDAGATYVVESTGVFTTMEK from glyceraldehyde-3-phosphate dehydrogenase. Meanwhile, the heat-stable peptide biomarkers of pork were FAGGNLDVLK and TVLGAPEVLLGILPGAGGTQR from trifunctional enzyme subunit alpha, mitochondrial as well as WGDAGATYVVESTGVFTTMEK from glyceraldehyde-3-phosphate dehydrogenase. The MRM analysis confirmed the heat-stable peptide of pork in meat product samples. It suggested that LC-MS employing MRM method could be used as promising analytical technique for halal authentication of meat products.[50]

Application of gas chromatography for analysis of non-halal components

Table 2 listed the application of gas chromatography for analysis of halal components in the food products. GC-MS combined with chemometrics has been proposed as tools for detection of lard as adulterant in olive oil using metabolomic approach. GC separation of fatty acid methyl esters (FAME) was achieved using HP-5 MS nonpolar capillary column. The identification of metabolites of FAMEs was carried out using standard FAMEs and mass spectrometer detector using the WILEY 2007 library. Some FAMEs are specific, i.e., methyl behenate was only present in olive oil and methyl myristate was only detected in lard. PCA using identified FAMEs was successful for separating lard, olive oil and olive oil adulterated with lard for halal authentication study.[66]

Two-dimensional GC combined with time-of-flight mass spectrometer (GC x GC-TOF/MS) is successfully used for analysis of lard as adulterant in virgin coconut oil (VCO) through analysis of sterols. GC x GC system could perform the complete baseline separation of sterol trimethylsilyl ethers derived from cholesterol and cholestanol, which facilitate the detection of lard in VCO. Using GC x GC–TOF/MS, cholestanol trimethylsilyl ether (Cha-TME) and cholesterol trimethylsilyl ether (Che-TME) were separated into some peaks, identified as CHe_1, CHe_{bI}, CHe_{bII}, CHe_2 (Che-TME), and Cha_1, CHa_{bI}, CHa_{bII} and CHa_2 for Cha-TME. Quantification of these compounds could be used as tools for quantification of adulteration levels of lard in VCO.[20]

GC-MS coupled with headspace solid-phase microextraction (HS-SPME) is successful for the analysis of volatile compounds in pork. The profiles of volatile compounds from different meats are different; therefore, the volatile compounds analyzed by GC-HS-SPME/MS could be used as fingerprinting tools for specific meats.[67] In addition, VOCs also contribute to the aroma which can be used for the discrimination tools among animal meats.[68] Analysis of VOCs is very challenging because of different factors, including the high number of volatile compounds, differences in volatility degree and the great amount of functional groups.[69] Chen et al.[70] have identified the key volatile compounds for differentiation of pork from different pig breeding. The volatile compounds contributing to the pork flavor identified during this study were 3-methyl-1-butanol, 1-nonanal, octanal, hexanal, 2-pentyl- furan, 1-penten-3-one, N-morpholinomethyl-isopropyl-sulfide, methyl butyrate, and (E,E)-2, 4-decadienal. Kosowska et al.[71] reported that some volatile compounds namely octanal, nonanal, (E,E)-2,4-decadienal, methanethiol, methional, 2-furfurylthiol, 2-metyl-3-furanthiol, 3-mercapto-2-pentanone and 4-hydroxy-2,5-dimethyl-3-(2 H)- furanone are key features in cooked pork. Thus, the identification of marker volatile compounds in pork can be meaningful for pork identification during halal authentication analysis of products. GC-HS-SPME/MS and GC-MS using simultaneous distillation and extraction (SDE) are

Table 2. The application of gas chromatography (GC-FID and GC-MS) for analysis of halal components in the food and pharmaceutical products.

Methods	Issues	Results	References
GC-FID for analysis of alcohol	Determination of ethanol contents in vinegar	The maximum contents of ethanol in vinegar is 1.0%. GC-FID could determine the levels of ethanol (alcohol) in the marketed vinegar samples. The detection level of ethanol was about 0.4 mg%	[51]
GC-FID for analysis of ethanol in foods	Determination of ethanol in different processed foods and beverages	Extraction technique using aqueous extraction assisted magnetic-stirring could be used to extract ethanol from different foods and beverages. GC-FID successfully used to determine ethanol with good validity. The validated method was successfully used to determine ethanol in 108 food and beverage products	[52]
GC-MS for analysis of alcohol	Determination of alcohol in fermented black tape ketan using GC-MS	GC-MS could be used for quantitative analysis of alcohol content in fermented black tape ketan with good recovery (89%). The alcohol concentrations determined at 3, 10, 17, 24, and 31 days were 4.295, 4.23, 5.005, 4.747, and 5.344% v/v, respectively	[53]
GC-FID for analysis of lard	Differentiation of lard from other edible fats using GC-FID and chemometrics	Lard contains high amount of C18: 2*cis* and low amount of C16:0. Chemometrics of PCA and K-mean cluster analysis could differentiate lard adulteration on chicken fat and beef tallow at low concentrations (0.5%-10%)	[54]
GC-MS for analysis of pork	Analysis of fatty acids a fatty acid methyl esters of pork (non-halal meats) in sausages compared with beef sausages (halal meat)	The dominant fatty acids in pork sausage are palmitic, myristic, oleic acid, and lauric acids. While fatty acids dominating in beef sausage are palmitic, oleic, stearic and myristic acids. The chemometrics of PCA could classify sausages according to meat sources (beef and pork)	[55]
GC-MS for analysis of rat meat	Analysis of rat meat (non-halal meat) and its classification with other meats using chemometrics of PCA	Six fatty acids, i.e. myristic, palmitoleic, palmitic, linoleic, oleic and stearic acids combined with PCA could classify rat meat and other meats	[56]
Headspace GC-MS for analysis of pork	Differentiation of pork (non-halal meat) and pork sausages from beef, mutton and chicken meats	The samples were introduced into GC instrument using headspace, and volatile compounds present in the evaluated samples were separated using GC and detected by MS. The chemometrics of PCA provided good separation between pork-based sausages and halal meat-based sausages	[57]
GC-MS for analysis of lard	Analysis of lard (non-halal fat derived from adipose tissue of pig) in chocolate products	The fatty acid of 11,14-eicosadienoic acid is used as fatty acid marker for identification of lard	[58]
GC-MS-SPME for analysis of wild boar	Volatilomics analysis of non-halal (wild boar) meat ball using GC-MS-SPME and chemometrics	PLS-DA could be used to differentiate volatile compounds of halal meatball and non-halal meatball. Compounds of β-cymene, 3-methyl-butanal, and 2-pentanol were found to be potential markers for chicken meatball. Compounds of 5-ethyl-m-xylene, benzaldehyde, and 3-ethyl-2-methyl-1,3-hexadiene were associated to the potential markers of beef meatball. Compounds of pentanal, 2,6-dimethylcyclohexanone, 1-undecanol, cyclobutanol, 2,4,5-trimethyl-thiazole, and 5-ethyl-3-(3-methyl-5-phenyl pyrazol-1-yl)-1,2,4-triazol-4-amine could be used as potential markers as wild boar meatball	[59]

(*Continued*)

Table 2. (Continued).

Methods	Issues	Results	References
HS-SPME-GC-MS for analysis of minced beef and pork meat	Volatilomics analysis using HS-SPME-GC-MS combined with multivariate analysis to differentiate minced beef and pork meat	GC-MS based on volatilomics analysis and chemometrics of PCA and PLS-DA could be used to differentiate minced beef and pork meat. Heptanal, octanal, butanol, pentanol, hexanol, 1-penten-3-ol, 2-octen-1-ol, 3-hydroxy-2-butanone were associated to the potential markers of beef whereas pentanal, hexanal, decanal, nonanal, benzaldehyde, trans-2-hexenal, trans-2-heptenal could be used as potential volatile compound markers of pork meat	[60]
GC-MS for analysis of pork	Detection of pork in beef meatball using GC-MS and chemometrics	PCA using fatty acid compositions of pure beef meatball and adulterated beef meatball using pork as the variables successfully differentiate pure and adulterated beef meatball. The ratio of SFA: MUFA of pork meatball was 1.0	[61]
GC-MS for analysis of house rat	Detection of rat house in beef meatball by analysis of fat using G-CMS	The fatty acids composition of house rats were myristate (0.19 ± 0.03)%, palmitoleate (2.40 ± 0.29)%, methyl palmitate (27.65 ± 0.32)%, oleate (45.81 ± 3.25)%, and stearate (4.65 ± 0.28)%. Analysis using PCA could differentiate beef meatball and beef meatball containing rat house meat. Further analysis using PCA demonstrated that fatty acids of house rats have high similarity to chicken fatty acids	[62]
GC-MS for analysis of lard	Detection of lard in wheat biscuits using GC-MS and chemometrics	PCA using fatty acids composition could differentiate lard, wheat biscuits, and adulterated wheat biscuits with lard. PLS-DA could be used to find potential marker for differentiation. Fatty acid of C18:3n6 is suggested as potential marker to distinguish pure wheat biscuits and adulterated wheat biscuits with lard	[63]
GC-MS for analysis of dog fat	Detection of dog fat from other animal fats using GC-MS and chemometrics	Nine types of fatty acids in dog fat were found such as lauric, myristate, pentadecanoate, palmitoleate, palmitate, margarate, oleat, stearic, and arachidonic. Analysis PCA showed that dog fat is close to lard	[64]
GC-MS for analysis of rat fat	Detection of Sprague Dawley rat fat in meatball using GC-MS and chemometrics	PCA could differentiate meatball and adulterated meatball with Sprague Dawley rat meats. Further analysis revealed that the Sprague Dawley rat fat is close to beef fat	[65]

also successful for identification of volatile compounds used for the identification of cooking braised pork. There are 109 aroma compounds identified, in which aldehydes were the most predominant in number, followed by alcohols, oxygen-containing heterocyclic compounds, acids and ketones. Methanethiol was the most abundant aroma substance in SPME, while anethole was the most abundant in SDE.[72]

GC-HS-SPME/MS has been developed and validated as reliable analytical method for analysis of volatile organic compounds (VOCs) of minced pork meat during storage. The origin of aromatic hydrocarbons in pork was verified using migration test. Two chemometrics techniques, namely, PCA and OPLS-DA were employed for characterizing and profiling VOCs in pork meat and for identifying the marker VOCs associated with the spoilage of pork. There are 41 VOCs (consisting of 10 alcohols, 7 aldehydes, 7 ketones, 6 aromatic hydrocarbons, 6 linear hydrocarbons, 2 terpenes, 1 acid, 1 ester, 1 furan) were identified during this study. The major VOCs of minced pork are aromatic hydrocarbons, alcohols, aldehydes, linear hydrocarbons, and ketones). From loading plot study, three VOCs namely ethanol, 2,3-butanediol and 2-ethyl-1-hexanol were selected and considered as important variables in the projection values, because these VOCs contribute to the discrimination of pork with different storage times.[73]

Analysis of volatile organic compounds (VOCs) as fingerprinting profiles for identification of dried pork slices from different processing stages have been done using GC coupled with ion mobility spectrometry (GC-IMS). Using LAV software, 54 peaks were selected. During this study, thirty seven VOCs were detected in the evaluated samples, in which aldehydes and alcohols accounted for the largest proportion. 1-octene-3-ol has the flavor of cooked mushroom, is important compound contributing to the VOCs of pork. This compound is considered as the autoxidation product of linoleic acid.[74] GC-MS has been employed for identification of key aroma in pork broth. The multivariate calibration of PLS is used for screening the relatively better flavor of pork broth among different stewing time and applied for assisting the quantitative analysis of VOCs using standard internal of 1,2-dichlorobenzene. From this study, the key odorants of the aroma profile of pork broth were identified namely 4-hydroxy-2,5-dimethyl-3(2 H)- furanone, hexanal, 1-octen-3-ol, (E)-2-octenal, (E)-2-decenal, (E)-2-undecanal, (E, E)-2,4-decadienal, nonanoic acid, decanoic acid, 2-heptanone, 3-hydroxy-2- butanone, δ-decanolactone and 2-acetylpyrrole.[75]

GC-MS coupled with olfactometry (GC-MS/O) and in combination with chemometrics of PCA and PLS-DA was reported to differentiate Chinese marinated pork hocks from four different local brands. The results of PCA and PLS-DA indicated that both chemometrics using variable of VOCs could clearly separate marinated pork hocks according to its groups. There are nine odor-active compounds having the high loading capability for discrimination, namely, heptanal, nonanal, 3-carene, D-limonene, β-phellandrene, p-cymene, eugenol, 2-ethylfuran and 2-pentylfuran. This study concluded that the validated GC-MS/O offered an alternative tools for the differentiation of VOCs profile in different brands of marinated pork hocks.[76]

Analysis alcoholic compounds in products using chromatographic techniques

GC-MS is an excellent method for analysis of alcoholic compounds in foods. Park et al. have validated and reported GC-MS for the simultaneous analysis of five alcohols (methanol, ethanol, propanol, butanol and pentanol) in fermented Korean foods. The separation of alcohols was carried out using silica-based INNOWAX column (film thickness 0.25 mm, i.d. 250 mm, length 30 m) coated with poly-ethylene glycol and applying mass selective detector set to determine the specific selected ions for each alcohol. The LoD and LoQ values ranged from 0.25 to 1.16 mg/kg. The precision and accuracy of GC-MS are acceptable as indicated by intra-day and inter-day RSDs for individual alcohols of below 7%, with recovery values of 90.79–01.50%. The method is valid; therefore, the developed method is suitable for analysis of alcohols in food samples intended in Halal food authentication supporting the certification processes.[77]

Mahama et al. have applied GC with flame ionization detector (GC-FID) for analysis of alcohol (ethanol) in marketed post samples (Fruit and vegetable juices from concentrate, syrups, sauce samples, etc.) in Thailand for identification of non-halal components suspected to be present in the products. The internal standard used is n-propanol. Ethanol, internal standard and others were separated using capillary columns DB-WAXTER (Agilent Technologies, 30 m by 0.32 mm by 1.00 μm) with temperature of FID was set at 250°C. Some certification bodies have different regulation related to the maximum limits of ethanol, and the majority allowed the maximum limit is 1%. The surveillance results indicated that 1 of 24 sauce samples showed an ethanol concentration of 1.0%. Furthermore, an about of 4% of all the concentrated syrup samples exhibited a higher percentage of ethanol than that permitted for Halal products. GC-FID method using a column HP-5 (5% Phenyl 95% Methyl Siloxane) is also valid for analysis of vinegar samples from Indonesia and Saudi Arabia offering reliable technique for alcohol determination.[51]

Šorgić et al. developed gas chromatography coupled with the flame ionization detector and headspace autosampler (HSS-GC/FID) method for analyzing volatile compounds in the wine samples. The HSS-GC/FID method was developed, validated and verified for determining content of methanol, higher alcohols and esters. The developed method was met the validation requirement for linearity, range, sensitivity, accuracy and precision parameters. Two grape varieties namely Merlot and Cabernet Sauvignon were analyzed. It was found that contents of the methanol were 198.0 mg/L and 150.5 mg/L, higher alcohols were 398.5 mg/L and 335.8 mg/L, ethyl acetate were 42.0 mg/L and 55.6 mg/L, and acetaldehyde were 23.3 mg/L and 16.1 mg/L for Merlot and Cabernet Sauvignon varieties, respectively. This study revealed that the higher content of methanol was influenced by type of grape used for preparation as well as maceration duration. Further evaluation wase carried out using PCA to assess the effect of genotypes variation and extraction methods on wine samples.[78]

Gas chromatography combined with PCA and cluster analysis (CA) were successfully applied in determining content of alcoholic compound in Chinese beverages. According to the study, 21 aroma components were found to be important in the aroma profiles of Chinese liquor. Among all the compounds, seven alcoholic compound including methanol, 2-butanol, 1-propanol, isobutanol, *n*-butanol, isoamylol and phenylethanol were detected by validated GC analysis method. Isoamylol, isobutanol and 1-propanol were found as the dominant alcoholic compound with the content of 800.53, 637.67 and 338.84 mg/L, respectively. The dimensionality reduction of PCA was employed in this study to statistically separated young liquor (fresh) and aged liquors. Individual plot was generated as two-dimensional visualization constructed by PC1 and PC2 with total variance of 98.27%. Further separation using CA was built using the Euclidean distance. All liquor samples were clustered into two big groups of young liquor and aged liquors. This results proved the ability of PCA and CA to successfully separate and classify the different ages Chinese liquor samples.[79]

In Indonesia, a majority Muslim country, it was stated by the government that the alcohol content (in percentage) of alcohol-containing drugs, traditional medicines, and supplements have to be declared on the label. Halal evaluation of alcohol content in noni (*Morinda citrifolia* L.) can be performed using gas chromatography method. The GC instrumentation was set as the inlet injection mode split of 2.5:1, injection temperature of 140°C, oven initial temperature FID detector of 40°C and hold for 5 min. The sample of noni herbal medicines was collected from herbal drug stores or online shops in Jakarta, Indonesia. Twenty samples were evaluated and categorized as beverages (18 samples) and herbal medicines (2 samples). It was found that 13 out of 20 samples contained alcohol in the range of 0.04%–1.07%. Unfortunately, none of them were labeled properly according to the regulation.[80]

GC-FID has been used for analysis of ethanol in foods and beverages such as tea-based, fruit-based, cheese-based, milk-based, seaweed-based, instant dried noodle, etc. Ethanol stock solution was prepared (1 mg/mL) and internal standard of 0.1% v/v 1-propanol was used for sample preparation. Sample preparation was carried out using magnetic stirring aqueous extraction. Analysis was performed out using an HP-Innowax (Agilent Technologies) column (30 m x 0.25 mm x 0.25 µm). The sample injection volume was 1 µL using split ratio of 13:1. The developed method was validated according to the requirements of ISO/IEC 17025:2017. Validation result showed that the method had good linearity ($R^2 > 0.999$), good accuracy (recoveries of 96–105%) and good precision (RSD < 5%). The detection limit was low (0.006 mg/g). The determination of ethanol concentration was successfully applied in 108 samples of processed foods and beverages. Therefore, this method could be used as valid method for halal authentication of processed foods and beverages.[52]

GC-MS using static headspace has been applied for determination of ethanol in solid and semi-solid consumer goods such as cakes, ice creams, sauces and powders. Sample preparation was carried out using mechanical homogenization and aqueous dilution of the products. Subsequently, the sample was analyzed using headspace GC-MS. Separation of analytes was performed using a capillary column DB-624 (30 m x 0.25 mm x 1.4 µm) and sample was injected in split mode employing ratio of 1:200. Identification and quantification of ethanol and ethanol-d6 was performed at scan range of 29–250 m/z with a rate of 6.1 scans/s. Result showed that the developed method was specific to detect ethanol and ethanol-d6 at the retention time of 2.65 and 2.61, respectively. The method demonstrated good linearity at the concentration range of 0.1%–2.0% v/v showed by its high R^2 value (>0.998). Additionally, good accuracy as well as good precision was obtained. The accuracy was represented by recoveries value (average recoveries of 99.7%). The precision was demonstrated by its lower RSD value (<1.5%). From the above results, it suggested that headspace GC-MS could be used for identification and quantification of ethanol in a various solid and semi solid food products for halal authentication.[81]

Identification of ethanol using headspace GC-MS has also been applied in Kombucha products. Kombucha is one of fermented beverages consist of sugar, tea, a symbiotic of bacteria and yeast which is commonly known as nonalcoholic beverage. The United States and Canada state that the content of alcoholic compounds in product must be <0.5% and <1.1% alcohol by volume, respectively, to be categorized as nonalcoholic drink. Propan-1-ol was used as internal standard for ethanol quantification. The condition of headspace was incubation temperature at 70°C, syringe temperature at 70°C, incubation time of 300 s, agitator speed at 500 rpm, injection volume of 500 µL and split ratio of 10:1. Analysis was performed using an Agilent J&W DB-624 UI (30 m x 0.25 mm x 1.4 µm) applying flow rate of 1.4 mL/min (constant flow). The developed method was linear (R^2 > 0.995) obtained at a concentration range of 0.025%-2.47%. The accuracy result was good demonstrated by its recovery value (102%) and good precision was also obtained (RSD<4%). The LOD and LOQ values were 0.0002% and 0.002%, respectively. It can be concluded that the method is suitable for identification and quantification of ethanol in Kombucha product. It indicated a rapid and easy integration of analytical method for halal authentication of Kombucha.[82]

The development of GC-MS coupled with headspace and multidimensional (heart-cut) chromatography has been successfully applied to determine ethanol content in medicinal syrups. The aim was to ensure and guarantee the safety of the syrups. Samples used for analysis consist of adult and pediatric syrups. Monitoring and quality control of ethanol content in the products were important due to the efforts of industry to reduce the ethanol content in the food and medicinal products. Sample preparation was directly performed using headspace with condition as follows: heating syringe temperature of 90°C, incubator temperature of 100°C, incubation time of 15 min at 500 rpm, sample volume of 500 µL with split mode using ratio of 1:20. Two dimensional GC analysis was carried out using GC-MS equipped with analytical column of RTX-5 capillary column (Crossbond® 5% diphenyl/95% dimethyl polysiloxane, 30 m × 0.25 mm × 0.25 µm) for the first dimension then for the second dimension used an NST 100 MS column (Carbowax polyethylene glycol, 30 m × 0.25 mm × 2.00 µm). The method was validated according to National Agency of Sanitary Surveillance (ANVISA) with validation parameters of selectivity, linearity, precision, accuracy, LOD, LOQ and robustness. Selectivity test found that isopropyl alcohol was an interfering compound of ethanol determination in syrups. Linearity assay demonstrated linear model at concentration range of 0.25% to 10.00% v/v (R^2 > 0.999). The developed method was sensitive enough as shown by its LOD value (0.03% v/v) and LOQ value (0.06% v/v). The precision was measured for repeatability (CV = 3.04%) and intermediate precision (CV = 3.03%). The recoveries value obtained ranged from 97.28% to 101.38% indicating good accuracy. The robustness test showed that the method remains unchanged with the small changes of several parameters. This developed method could be used as rapid and easy analytical technique for halal authentication of syrups by determining of the ethanol content.[83]

CONCLUSION

Chromatography-based method consist of liquid chromatography and gas chromatography using various detectors has been widely applied for food products authentication including halal analysis due to its advantages. The combination of chromatographic methods with chemometrics of multivariate analysis, a powerful statistical analysis to manage huge data generated from analytical measurement, could be used to identify potential markers to differentiate halal and non-halal samples. It will be very useful for the institutions which have responsibility for halal quality assurance. Chromatogram and peak separation profiles resulted as the instrument responses can be further evaluated for determination as well as quantification for halal and non-halal components in food products. Chromatographic-based methods were successfully carried out to analyze products containing non-halal material such as pork and alcoholic compound. Combination of chromatographic-based method and chemometrics techniques with some scenarios can be applied for halal research on food products.

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No potential conflict of interest was reported by the author(s).

Author contribution

Laela Hayu Nurani: Writing—original draft preparation, writing—review and editing, funding acquisition; **Florentinus Dika Octa Riswanto**: Writing—original draft preparation, writing—review and editing; **Anjar Windarsih**: Writing—original draft preparation, writing—review and editing; **Citra Ariani Edityaningrum**: Writing—original draft preparation, writing—review and editing; **Any Guntarti**: Writing—original draft preparation, writing—review and editing; **Abdul Rohman**: Conceptualization, methodology, writing—original draft preparation, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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