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Profile Volatilome for Halal Authentication of Beef and Pork Corned Beef Using GC-MS

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ABSTRACT

The volatilomic approach to beef and pork corned beef is a method that can be used to provide an overview of the volatile compound profile and has high sensitivity and has been successfully used for halal authentication. The purpose of this study was to authenticate the halal status of beef and pork corned beef using the Gas Chromatography–Mass Spectrometry method. The research method used was a laboratory experimental study with a volatilomic approach using a combination of GC-MS and chemometric methods. The results showed that there were 242 volatile compounds in beef corned beef and 15 volatile compounds in pork corned beef. PCA analysis showed that the volatile compound profiles of beef corned beef and pork corned beef were different. Cluster analysis using dendrograms can classify information and identify patterns of volatile compound profiles in beef corned beef and pork corned beef that are well clustered. PLS-DA analysis results show that there are several volatile compounds that play a significant role in distinguishing between beef corned beef and pork corned beef. The compounds with the highest VIP (Variable Importance in Projection) values were hexadecanoic acid and pentadecanoic acid, which showed a dominant role in separating the two types of corned beef. A high VIP value (>1) indicates that the compound has a large contribution to the model.

Keywords: Halal authentication, GC-MS, chemometrics, cornets, volatilome

INTRODUCTION

Indonesia is the country with the largest Muslim population in the world. According to data from the Ministry of Home Affairs' Population and Civil Registration Agency, 87.08% or 245,973,915 of Indonesia's population is Muslim in the first half of 2024. One of the advantages of a Muslim country is the availability of halal-certified food products. One halal food product currently in the spotlight is processed meat products, including corned beef. Corned beef is a highly popular product among the public due to its convenience and long shelf life. Corned beef can be made from beef or pork (Mardiyana, 2021). Corned beef made from beef is halal, while that made from pork is non-halal (Sherly and Anna, 2016). Therefore, to ensure that the corned beef product is made from beef or pork to determine its halal status, an authentication process is required.

Halal authentication is a process to ensure the halal status of products, especially those that may contain non-halal ingredients. According to Mortas et al. (2022), halal authentication analysis methods can be carried out using Polymerase Chain Reaction (PCR), Enzyme Linked Immunosorbent Assays (ELISA), and Fourier Transform-Infrared Spectroscopy (FTIR).

The publication by Hendrik et al., 2018 explains that PCR is capable of identifying the presence or absence of pork content in corned beef products using specific P14 pig DNA primers based on cell DNA information. This PCR method has limitations, such as the denaturation of soluble proteins due to thermal treatment, which can reduce the potential of protein-based approaches (Rahman et al., 2019). The ELISA test is an immunological test commonly used to measure antibodies, antigens, proteins and glycoproteins in biological samples. Tukiran et al., 2016, mentioned that a method had been developed for the rapid detection of pork gelatin in edible bird's nests using an ELISA test. However, this ELISA test has weaknesses, namely its non-specific antigen immobilisation method and relatively long testing time (Budi Santosa, 2020). Analysis using FTIR can be used to determine the halal status of a food product by looking at the spectrum pattern in its animal fat. Millen et al., 2019, explained in their research that FTIR was able to analyse the pork fat content in meatball samples. One of the limitations of FTIR is that overlapping molecular absorption spectra in samples may make visual and direct interpretation difficult (Gad et al., 2013). Therefore, an alternative method is required, one of which is volatilomics.

Volatilomics is a term for volatilome analysis aimed at the detection, characterisation, and quantification of volatile organic metabolites. The volatilome is defined as the group of all volatile organic compounds produced by living organisms, including animals, ecosystems, or substrates, and includes exogenous organic and inorganic compounds (Lytou et al., 2019). Pavlidis et al., 2019, used volatilomics on beef and pork but it has never been done on processed products such as corned beef. The instrument used for volatilomics is Gas Chromatography–Mass Spectrometry (GC-MS). GC-MS provides more detailed information because the spectrum shows specific mass spectra, enabling the detection of volatile compounds (Zanin et al., 2019). The results from GC-MS yield a large amount of spectral data, so chemometric analysis is required to simplify the authentication process.

Chemometrics is a combination of statistical analysis and chemical analysis. Chemometrics is an analytical method that combines mathematics and statistics to process multivariate data. The chemometric methods used in the authentication process are Principal Component Analysis (PCA), Cluster Analysis, and Partial Least Squares-Discriminant Analysis (PLS-DA). PCA is used to group beef corned beef and pork corned beef, but PCA cannot provide conclusions that will then be followed up with Cluster Analysis (Kuswandi et al, 2017). Cluster Analysis is used to group objects based on the characteristics of each object (Puspitasari et al, 2019). PLS-DA is used to predict the volatilome that will become a marker for halal authentication (Cahyaningsari et al., 2019). This review focuses primarily on various chemometric methods used to process volatilomics data generated from GC-MS.

Based on the above description, halal authentication of beef and pork corned beef using a combination of GC-MS and chemometric methods is necessary. This study will produce a distinctive volatile compound profile of beef and pork corned beef, which can be used as a reference for the authentication process. This will provide consumers with a guarantee of the halal status of corned beef products on the market. This study could also serve as an alternative method for the halal authentication process of corned beef products.

METHODS

1. Equipment
   The equipment used in this study included a stirring rod, analytical balance, beaker, vial, GC-MS instrument, Erlenmeyer flask, funnel, measuring flask, and blender.
2. Materials
   The materials used consisted of beef sausage and pork sausage samples as the main ingredients, as well as methanol and n-hexane as supporting chemicals.

3. Data collection techniques were carried out as follows:
   3.1 Volatile Compound Derived from Fatty Acids Extraction
   100 mg of ground beef corned beef sample was added to 2 mL of BF_3 solution in 14% methanol, then 2 mL of n-hexane was added as a non-polar solvent by gently shaking and left until two layers formed. The upper phase was then collected using a pipette and filtered using a 0.22 µm filter membrane to remove particles or solid residues. The clear extract obtained was placed in a vial and analysed using a GC-MS instrument.
   3.2 Volatilomic Analysis Using GC-MS
   The analysis was performed using gas chromatography-mass spectrometry (GC-MS). Fibres exposed to the sample were injected into the GC-MS injection port using split mode (ratio 1:2) at a temperature of 250°C. Separation was performed using a DB-WAX capillary column (30 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies, USA). The temperature programme started at 40°C for 5 minutes, followed by an increase of 4°C/minute until reaching 150°C, then increased again to 250°C at a rate of 30°C/minute and maintained for 5 minutes. The interface temperature was set at 280°C, while the ion source and quadrupole temperatures were set at 230°C and 150°C, respectively. The mass spectrometer operated in electron ionisation mode with an energy of 70 eV and a scan range of 29–350 m/z (speed of 4.37 scans/second). This method refers to Pavlidis et al. (2019), with modifications to the type of column used, namely DB-WAX replacing HP-5MS as described by Pranata et al. (2021).
   3.3 Chemometrics Analysis
   The volatile compounds obtained were then statistically analysed using one-way variance (ANOVA) with Minitab. If the results were significant ($p<0.05$), they were followed up with chemometric analysis using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). The chemometric analyses performed included PCA, cluster analysis, and PLS-DA.

RESULTS

1. Volatilome Extraction

Extraction of volatile compounds from beef and pork corned beef produced qualitatively and quantitatively different volatilomic profiles. The esterification results showed that the volume of beef sausage extract was 50 mL. Meanwhile, the volume of pork sausage extract was 36 mL. The volume of beef sausage extract was highr than that of pork sausage.

2. GC-MS Analysis

The volatile compounds used as results of GC-MS analysis are those with a similarity to the database of 70% or higher. Some of the main compounds found in corned beef are as follows

No	Volatile compound	Formula	Retensi Time	BM	Similarity Index	Are Pork cornet	Area Beef cornet	% Pork corned	% Beef cornet
1	Nonadecanoic Acid, Methyl Ester	C20 H40 O2	16.006	312	82	0	30902	0	15.77
2	Methyl Arachidonate	C21 H34 O2	17.292	318	66	0	12793	0	6.53
3	4,7,10-Hexadecatrienoic Acid, Methyl Ester	C17 H28 O2	17.292	264	64	0	12793	0	6.53
4	5,8,11,14-Eicosatetraenoic Acid, Ethyl Ester	C22 H36 O2	17.292	332	64	0	12793	0	6.53
5	Hexadecanoic Acid, Methyl Ester	C17 H34 O2	13.992	270	95	3113209	32600	4.72	16.63
6	Hexadecanoic Acid, 15-Methyl-, Methyl Ester	C18 H36 O2	13.992	284	94	213261	32600	0.32	16.63
7	Methyl-9,12-Hexadecadienoate	C17 H30 O2	15.721	266	93	1996766	66855	3.02	34.11
8	Methyl 9,9-Dideutero-Octadecanoate	C19 H36 D2 O2	15.772	298	91	2967531	52829	4.49	26.96
9	Octadec-9-Enoic Acid	C18 H34 O2	26.889	282	95	2284156	52829	3.46	26.96
10	Heptadecanoic Acid, 16-Methyl-, Methyl Ester	C19 H38 O2	22.231	298	77	2393413	30902	3.63	15.77
11	Eicosanoic Acid, Methyl Ester	C21 H42 O2	16.006	326	81	213261	30902	0.32	15.77
12	Cyclohexane	C13 H20	17.292	176	64	515416	12793	0.78	6.53
13	Octadeca-9,12-Dienoic Acid Methyl Ester	C19 H34 O2	18.101	294	93	1172443	66855	1.78	34.11
14	11,14-Eicosadienoic Acid	C21 H38 O2	18.101	322	92	1996766	66855	3.02	34.11
15	9-Octadecenoic Acid	C19 H36 O2	15.737	296	96	2967531	52829	4.49	26.96

3. Chemometrics Analysis

ANOVA analysis showed that there were significant differences ($p < 0.05$) in the intensity of several volatile compounds between the two types of cornet. Therefore, chemometric analysis was continued using the MetaboAnalyst 6.0 platform.

a. PCA

The PCA results show that beef corned beef and pork corned beef cluster separately. This indicates that the volatilome profiles of beef corned beef and pork corned beef are different. The results of the PCA chemometric analysis based on the volatile compounds of corned beef and corned pork are shown in Figure 1 below.

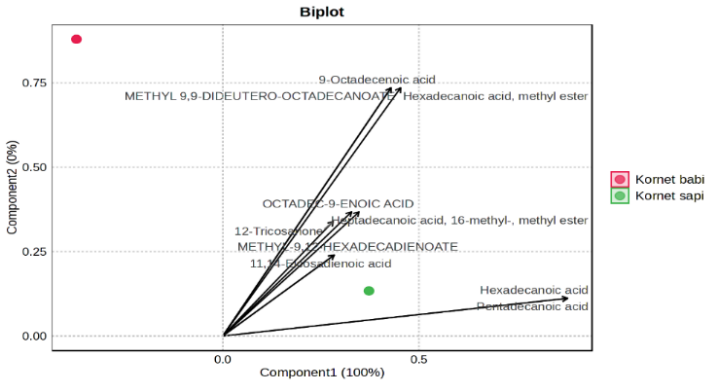

Figure 1. PCA beef corned and pork corned using volatilomic methods

b. Cluster Analysis
Cluster analysis was used to group beef corned beef and pork corned beef based on the similarity of their volatile compound profiles. The results shown in Figure 2 indicate that beef corned beef and pork corned beef can be effectively clustered

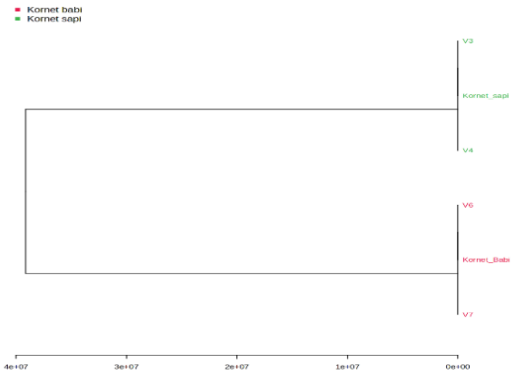

Figure 2. Cluster analysis beef corned and pork corned using volatilomic methods

c. PLS-DA
The PLS-DA model demonstrates high classification capability as shown in Figure 3 The PLS-DA results indicate that 15 volatile compounds could serve as potential markers for authenticating the halal status of beef corned meat and pork corned meat.

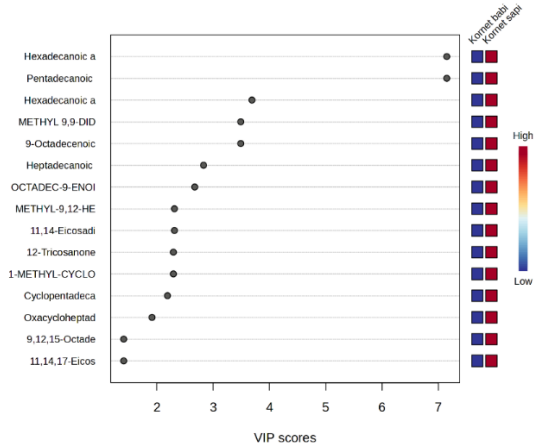

Figure 3. VIP score beef corned and pork corned for halal authentication using volatilomic methods

DISCUSSION

1. Volatilome Extraction

The extraction process using the esterification method was carried out to increase the volatility of free fatty acid compounds in the sample, so that it could be analysed more effectively using GC-MS instruments. From 100 mg of sample, the final results were 50 mL for beef corned beef and 36 mL for pork corned beef, indicating that although the sample mass was the same, the fat content or compounds that could be esterified in the two samples were different. This difference is likely influenced by the composition of the raw materials, particularly the type and content of fat contained in each type of meat. After the esterification process, the samples were stored at -20°C before analysis to maintain the stability of the volatilome compounds. Storage in a freezer is important to prevent the degradation or evaporation of volatilome compounds that are sensitive to high temperatures (Nurjuliana et al., 2011).

The esterification process of volatile compounds in beef and pork corned beef produces striking differences, particularly in terms of extract colour and volatile compound concentration. After esterification, beef corned beef generally exhibits a paler or clear yellowish colour, while pork corned beef tends to produce a cloudier or darker yellowish extract. These differences are influenced by the total fat content and pigment composition of each type of meat. Pork fat, which is higher and more soluble in organic solvents such as methanol or ethanol, causes the extract to appear more concentrated. In addition, processing and heating can release pigments and lipophilic compounds that contribute to the colour. A study by Amalia et al. (2021) noted that pork corned beef extract samples appeared darker and cloudier than beef after derivatisation through esterification.

2. GC-MS

The significantly different volatile compound profiles between beef and pork corned beef indicate that the type and amount of volatile compounds are greatly influenced by the chemical composition of the meat. Beef corned beef produces more volatile compounds than pork corned beef, which are identified in the form of chromatogram peaks with higher intensity and quantity. The volatile compounds found in beef corned beef generally consist of aldehydes, ketones, alcohols, and short-chain esters, which are formed from the degradation of amino acids and the oxidation of unsaturated lipids during the canning process. The diversity of these compounds contributes to the complex aroma characteristic of beef-based products.

In contrast, pork corned beef showed fewer volatile compounds, with lower GC-MS peak intensities. Although pork has a higher total fat content, the type and reactivity of its fats in forming volatile compounds appear to be different. This is indicated by a simpler volatile profile and fewer total compounds identified. This contrasts with the study conducted by Pavlidis et al. (2019), which stated that there were 53 volatile compounds identified in fresh beef and 53 volatile compounds identified in fresh pork. Meanwhile, Pranata (2021) successfully identified 69 volatilome compounds in beef and 62 volatilome compounds in pork. This difference could be due to the different analysis techniques used, both of which employed the SPME (solid phase micro extraction) technique. Additionally,

other differences may also be contributing factors, such as differences in genotype, sex, habitat, and feeding patterns of the animal samples analysed. Therefore, this volatile compound profile can be used as an authentication marker for meat products, particularly in the context of halal supervision (Denny Arfarus, 2024).

This significant difference reinforces the importance of the volatilomic approach in the halal authentication of food products. Unique compounds found only in beef corned beef, especially those that appear in high concentrations, have the potential to be markers in the classification and authentication process. The use of chemometric analyses such as PCA (Principal Component Analysis), Cluster Analysis, and PLS-DA (Partial Least Square Discriminant Analysis) can group and separate samples based on their respective volatilome profiles. This is also supported by research by Nurjuliana et al. 2011, which states that the combination of GC-MS and chemometrics is very effective for detecting the presence of non-halal ingredients in processed meat products. Thus, the GC-MS method has been proven to be effective as a tool for identifying the source of animal-based ingredients and can be integrated into a science-based halal control system.

3. Chemometric analysis
   a. PCA

One of the chemometric analyses used in this study was PCA. This analysis was used to determine whether there were differences in the volatile compounds present in beef corned beef and pork corned beef. The results of the PCA biplot analysis in Figure 4.2 indicate that there were significant differences in the compound profiles between beef corned beef and pork corned beef. The first principal component (PC1), which explains 100% of the data variation, clearly separates the two sample groups. This indicates that the detected compounds have very different distribution patterns between the two types of corned beef. Pork corned beef is more closely related to compounds such as 9-octadecenoic acid and hexadecanoic acid methyl ester, which tend to dominate the composition of pork fat. Conversely, beef corned beef shows a strong relationship with compounds such as pentadecanoic acid and hexadecanoic acid, which are commonly found in beef fat. These differences reflect the specific chemical characteristics of each meat, which can be used as a basis for the authentication and labelling of meat species-based products.

In addition to showing chemical differences, these PCA results also reinforce the function of chemometric methods as a supporting tool in testing the authenticity and halal status of processed meat products. PCA's ability to reduce complex data into informative two-dimensional visualisations is very helpful in identifying specific marker compounds. Research by Putri et al. (2022) proves that a similar approach is effective in distinguishing between halal and non-halal meat products. Similarly, Sahilah et al. (2019) used PCA analysis of fatty acid profiles to detect the presence of pork in processed food products. Both studies support PCA as a reliable method for species-based meat authentication, particularly in the context of quality control and halal assurance systems in the food industry.

   b. Cluster Analysis

Cluster analysis is used to classify information and identify patterns (Of et al., 2024). In this study, cluster analysis was performed using a dendrogram, as shown in Figure 4.3. The results of the cluster analysis on the dendrogram show a clear separation

between the beef corned beef and pork corned beef samples into two distinct groups. Beef corned beef (marked in green) formed its own cluster with variables V3 and V4, while pork corned beef (marked in red) clustered with variables V6 and V7. This pattern indicates a significant difference in the chemical profiles of the two types of corned beef. The clear cluster separation shows that the chemical data from the GC-MS results are able to represent the unique characteristics of each type of meat, thus proving that the cluster analysis method is effective for classification based on species.

The cluster analysis method works by calculating the similarity or distance between samples based on the intensity of detected chemical compounds, then grouping the most similar samples into one branch. The separation of beef and pork in this dendrogram is consistent with the research by Putri et al. (2022), which shows that HCA is capable of distinguishing halal and non-halal meat based on volatile compound profiles. In addition, Chen et al. (2019) also proved the effectiveness of combining GC-MS and HCA in accurately distinguishing various meat species. Thus, cluster analysis not only assists in the classification of processed meat products but also provides a scientific basis for the authentication and halal testing of food products.

c. PLS-DA

Based on the results of the PLS-DA analysis in Figure 4.4, it is known that there are a number of main compounds that have VIP (Variable Importance in Projection) values above the significance threshold (VIP > 1), indicating that these compounds play a major role in distinguishing between beef corned beef and pork corned beef. Hexadecanoic acid and Pentadecanoic acid are the two main contributors to group separation, with the highest VIP values reaching more than 7. This indicates that these two compounds are potential biomarkers in the authentication of processed meat products. The presence of medium to long-chain fatty acids such as 9-Octadecenoic acid and Heptadecanoic acid further strengthens the indication that fat composition is a distinguishing component between the two types of meat.

Meanwhile, the colour pattern on the right side of the graph shows the distribution of different compound intensities between beef and pork corned beef, where compounds with high intensity in pork corned beef appear to dominate the top of the VIP list. This indicates that pork corned beef contains more saturated and unsaturated fatty compounds than beef corned beef. This difference may be due to variations in natural lipid metabolism between cattle and pigs, as well as possible differences in processing. The results of a study by Chen et al., 2021 show that the PLS-DA technique is effective in distinguishing food products based on their volatile and non-volatile chemical profiles, and has great potential for application in quality control and halal tracking of processed meat products.

CONCLUSION

This method can be used for authentication and to identify significant differences in the profiles of volatile compounds, namely 242 compounds in beef corned beef and 15 compounds in pork corned beef, indicating a clear difference in chemical composition between the two. This method is also capable of distinguishing classification based on volatile compound

profiles, as demonstrated by the results of PCA and Cluster Analysis, which successfully separated beef corned beef and pork corned beef samples into distinct clusters. In addition, there are 15 specific volatile compounds that can be used as markers of the halal status of beef and pork corned beef. Hexadecaoic is the primary volatile marker compound for determining the halal status of beef and pork corned beef

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