Strada Journal of Pharmacy
ISSN: 2776-3544 (print); 2797-9180 (online)
Vol. 7, No 2, October 2025, 97-105

Application of Untargeted Volatilomics for Halal Authentication of Processed Meat Products

Luthfi Nursaudah[1*], Vevi Maritha[2], Weka Shidha Bhagawan[3], Puri Ratra Kartini[4], Desi Kusumawati[5], Avip Kurniawan[6]
[1,2,3,4,5]Program Studi Farmasi, Fakultas Ilmu Kesehatan dan Sains, Universitas PGRI Madiun
[6]Program Studi Teknik Informatika, Fakultas Teknik, Universitas Krisnadwipayana
***Corresponding author: luthfinursaudah003@gmail.com**

ABSTRACT

Food halalness is essential for Muslim communities, including in Indonesia, where the majority of the population is Muslim. Halal food not only means free from prohibited ingredients, such as pork and alcohol, but must also be processed in accordance with Islamic law. With the increasing consumption of processed products such as nuggets, concerns about the authenticity of raw materials have become an important issue. This research aims to determine and classify the volatile compound profile of beef and pork nuggets for halal authentication using a combination of GC-MS and chemometric methods. The analysis data were processed using a multivariate statistical approach in the form of Principal Component Analysis (PCA), Cluster Analysis (CA), and Partial Least Squares Discriminant Analysis (PLS-DA). The research method used was a laboratory experimental study with a volatilomics approach using a combination of GC-MS and chemometric methods. The results showed significant differences in the volatile compound profiles between the two types of nuggets (with pork samples containing 68 volatile compounds and beef samples containing only 45 compounds), which allowed separation and classification based on chemical composition. Further analysis identified specific volatile compounds that have the potential to serve as markers of halal status. The result of the research was volatile compound profiles of beef and pork nuggets were successfully determined using the GC-MS method, showing differences in composition.

Keywords: GC-MS, halal authentication, chemometrics, nuggets, volatilomics

INTRODUCTION

Food halalness is essential for Muslim communities, including in Indonesia, where the majority of the population is Muslim. Halal food not only means free from prohibited ingredients, such as pork and alcohol, but must also be processed in accordance with Islamic law. With the increasing consumption of processed products such as nuggets, concerns about the authenticity of raw materials have become an important issue. Beef-based nuggets are considered halal, while pork-based nuggets are non-halal. Therefore, an authentication method capable of scientifically distinguishing between the two is required.

Halal authentication is the process of verifying the authenticity and halal status of a food product based on its raw materials and production process. Currently, the methods widely used in halal authentication include Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). PCR can detect specific DNA from non-halal ingredients with a high degree of accuracy and sensitivity, while ELISA identifies the presence of specific proteins in samples. However, both methods have limitations. PCR is costly and requires a long analysis time, while ELISA is susceptible to external factors such as temperature and reagent mixing, which can affect the final results.

As an alternative, volatilomic technology has emerged as a promising new approach. Volatilomics is the study of volatile (easily evaporated) compounds produced by a sample. These compounds provide a distinctive profile that can be used to distinguish between different types of food, including the source of meat. Products such as nuggets that have been processed and mixed with various additives require authentication methods that can adapt to the complexity of their composition. Therefore, the untargeted volatilomic approach using Gas Chromatography-Mass Spectrometry (GC-MS) is relevant because it can detect various volatile compounds with high sensitivity.

GC-MS combines two main techniques, namely gas chromatography to separate volatile compounds, and mass spectrometry to identify and measure the concentration of these compounds based on their mass and molecular structure. This combination is particularly suitable for use in food safety, including the authentication of halal meat products. GC-MS analysis typically produces highly complex spectral data. Therefore, advanced statistical methods are required to process and interpret this data. This is where chemometrics plays a very important role.

Chemometrics is a branch of science that uses statistical and mathematical approaches to analyse multivariate data from experimental results. The three main methods of chemometrics used in this study are Principal Component Analysis (PCA), Cluster Analysis, and Partial Least Squares Discriminant Analysis (PLS-DA). PCA enables the visualisation and mapping of data in lower dimensions to see the differences between groups. Cluster Analysis is useful for grouping samples based on similarities in volatile compound characteristics. PLS-DA is capable of identifying specific volatile compounds (markers) that significantly distinguish halal and non-halal products.

Several previous studies have applied a combination of GC-MS and chemometrics in identifying pork contamination in raw food products. For example, PCA has been used to distinguish between beef and pork, while Cluster Analysis and PLS-DA have been used to group and identify key compounds as biomarkers. However, to date, no studies have specifically applied these methods to processed products such as nuggets, which have a more complex material matrix.

This study provides a novel approach to halal authentication, focusing on nugget products as samples. The volatile compound profiles of beef and pork nuggets were analysed using GC-MS and further processed using a chemometric approach. This will yield specific marker compounds that can be used as indicators of a product's halal status. One of the main objectives of this study is to create a halal identification method that is more practical and efficient than conventional methods.

The benefits of this approach are felt not only by consumers, who receive assurance of product halal status, but also by the food industry and halal certification bodies. The industry can use the results of this research as a guideline for product quality control, while certification bodies can consider volatilomics as a complementary or alternative method in the halal verification process.

Thus, halal authentication based on volatilomics using GC-MS combined with chemometric analysis is an innovative and applicable method. This research is expected to make a real contribution to supporting the development of a more scientific, efficient, and reliable halal assurance system, especially in facing the increasingly complex challenges of authenticating processed products.

METHODS

1. Equipment

The equipment used in this study included Whatman paper, filter paper, stirring rods, analytical balances, beakers, vials, GC-MS instruments, Erlenmeyer flasks, funnels, measuring flasks, vortex mixers, centrifuges, and blenders.

2. Materials

The materials used consisted of beef nugget and pork nugget samples as the main materials, as well as methanol, analytical grade distilled water, and nitrogen gas as supporting chemicals.

Data collection techniques were carried out by:

1. Volatilome Compound Extraction

A 100 mg sample of ground beef nuggets 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.

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. 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. GC-MS analysis

Extraction of volatile compounds from beef and pork nuggets produced volatile profiles that differed both qualitatively and quantitatively. The esterification results showed that the volume of beef nugget extract was 50 mL. Meanwhile, the volume of sausage nugget extract was 47 mL. The volume of beef nugget extract was higher than that of pork nuggets.

Some of the main compounds found in corned beef are as follows

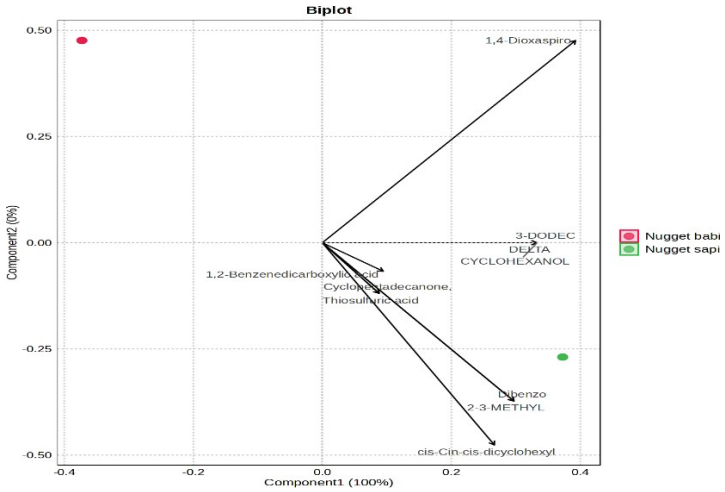
No	Senyawa Volatil	Formula	Retensi Time	BM	Similarity Index	Area Nugget Babi	Area Nugget Sapi	% Area Nugget Babi	% Area Nugget Sapi
1	1,4-dioxaspiro	C8H14O2	18.965	170	77	0	30902	0	15.77
2	3-DODEC	C19H32O3	23.558	212	70	0	12793	0	6.53
3	cyclohexanol	C9H17NO3	21.342	187	72	0	12793	0	6.53
4	1-octen-3-ol	C11H20O2	21.342	184	72	0	12793	0	6.53
5	decanal	C9H17NO3	13.992	270	95	3113209	32600	4.72	16.63
6	nonanal,	C11H20O2	13.992	284	94	213261	32600	0.32	16.63
7	benzeneacetaldehyde,	C17H34O2	15.721	266	93	1996766	66855	3.02	34.11
8	2,3-butanedione	C18H36O2	15.772	298	91	2967531	52829	4.49	26.96
9	phenylethyl alcohol	C17H30O2	26.889	282	95	2284156	52829	3.46	26.96
10	hexanal	C19H36D2O2	22.231	298	77	2393413	30902	3.63	15.77
11	2-methylbutanal	C18H34O2	16.006	326	81	213261	30902	0.32	15.77
12	heptanal	C19H38O2	17.292	176	64	515416	12793	0.78	6.53
13	furfuryl alcohol,	C21H42O2	18.101	294	93	1172443	66855	1.78	34.11
14	toluene	C13H20	18.101	322	92	1996766	66855	3.02	34.11
15	ethyl hexanoate	C19H34O2	15.737	296	96	2967531	52829	4.49	26.96

2. Hemometrics analysis

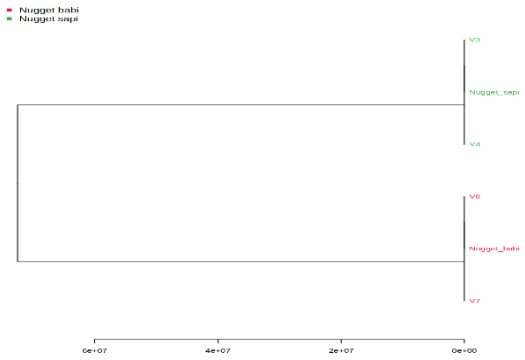
ANOVA analysis showed that there were significant differences ($p < 0.05$) in the intensity of several volatile compounds between the two types of nuggets. Therefore, chemometric analysis was continued using the MetaboAnalyst 6.0 platform.

a. PCA

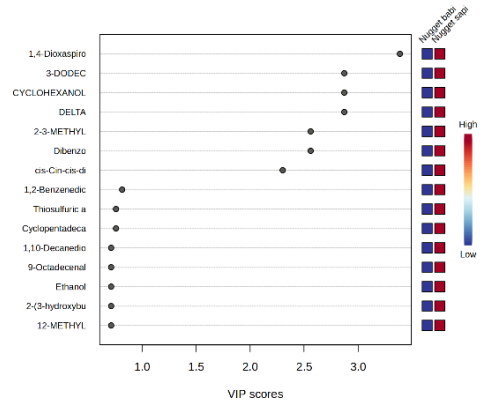
Test data from Figure 1



b. Cluster Analysis
Test data from Figure 2



c. PLS-DA
Test data from Figure 3



DISCUSSION

1. Extraction volatilome

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 volatile compounds. Storage in a freezer is important to prevent the degradation or evaporation of volatile compounds that are sensitive to high temperatures (Nurjuliana et al., 2011).

The esterification process of volatile compounds in beef and pork nuggets produces noticeable differences, particularly in terms of extract colour and volatile compound concentration. After esterification, beef nuggets generally exhibit a clearer, whiter, yellowish colour, while pork nuggets tend to produce a cloudier or more yellowish extract. These differences are influenced by the total fat content and pigment composition of each type of

meat. Pork fat is higher and more soluble in organic solvents such as methanol or ethanol, causing 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 analysis

The significantly different volatile compound profiles between beef and pork nuggets indicate that the type and amount of volatile compounds are greatly influenced by the chemical composition of the meat. Beef nuggets produce more volatile compounds than pork nuggets, which are identified in the form of chromatogram peaks with higher intensity and quantity. The volatile compounds found in beef nuggets 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 nuggets 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. Chemometrics 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 volatile compounds present in beef nuggets and pork nuggets. The results of the PCA biplot analysis in Figure 4.2 indicate that there were significant differences in the compound profiles between beef nuggets and pork nuggets. 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 nuggets are 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 nuggets show 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 beef corned beef and pork corned beef samples into two different groups. Beef nuggets (marked in green) formed their own cluster with variables V3 and V4, while pork nuggets (marked in red) clustered with variables V6 and V7. This pattern indicates a significant difference in the chemical profiles of the two types of nuggets. The clear separation of clusters 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.

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 result between cow nugget and pork in this dendrogram aligns with the study by Putri et al. (2022), which showed that HCA can distinguish between halal and non-halal meat based on volatile compound profiles. Additionally, Chen et al. (2019) also demonstrated the effectiveness of combining GC-MS and HCA in accurately differentiating various meat species. Thus, cluster analysis not only aids in the classification of processed meat products but also provides a scientific basis for authentication systems and halal food product testing.

c. PLS-DA

Based on the PLS-DA analysis results in Figure 4.4, it is known that there are several key compounds with VIP (Variable Importance in Projection) values above the important threshold (VIP > 1), indicating that these compounds play a major role in distinguishing between beef nugget and pork nugget. Hexadecanoic acid and Pentadecanoic acid are the two main contributors to the group separation, with VIP values exceeding 7. This indicates that these two compounds are potential biomarkers for 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 color pattern on the right side of the graph shows a different distribution of compound intensities between beef and pork nuggets, where compounds with high intensity in the pork nugget appear to dominate the top of the VIP list. This indicates that pork nuggets contain more certain saturated and unsaturated fat compounds compared to corned beef. This difference may be caused by natural variations in lipid metabolism between cattle and pigs, as well as possibly different processing methods. The study by Chen et al., 2021, shows that the PLS-DA technique is effective in distinguishing food products based on their volatile and non-volatile chemical profiles and is highly potential for application in quality control and halal traceability of processed meat products.

CONCLUSION

This method can be used for authentication and significant differentiation by identifying the volatile compound profiles, with 60 compounds detected in beef nuggets and 2 compounds in pork nuggets, indicating a clear difference in chemical composition between the two. The method also successfully differentiates classification based on volatile compound profiles, as shown by the PCA and Cluster Analysis results that distinctly separate beef nugget samples and pork nugget samples into different clusters. Additionally, there are 15 specific volatile compounds that can serve as halal markers for beef and pork nuggets.

ACKNOWLEDGEMENT

This research is fully funded by a grant from the Institute for Research and Community Service (LPPM) of Universitas PGRI Madiun under contract number 028.a/H/LPPM/UNIPMA/2025.

REFERENCE

Audifa, W. R., & Syahida, N. (2024). Hukum kandungan babi dalam sediaan farmasi menurut pandangan Islam. *Tadabbur: Jurnal Integrasi Keilmuan*, *3*(01), 61–76.

Amalia, N. A., Sutaryo, & Purnomoadi, A. (2021). *Pengaruh Perbedaan Lama Perendaman dan Ketebalan Daging yang Direndam Asap Cair terhadap Kualitas Fisik dan Sensoris Daging Sapi*. Jurnal Ilmiah Peternakan Terpadu, 9(2), 207–218

Gültekin, F., et al. (2020). Evaluation of food additives in terms of istihalah. *Academic Platform Journal of Halal Lifestyle*, *2*(1), 1–13.

Kuswandi, B., et al. (2022). Colorimetric paper-based dual indicator label for real-time monitoring of fish freshness. *Food Technology and Biotechnology*, *60*(4), 499–508.

Lestari, I., & Syahri, M. (2018). Penerapan teknik volatilomik untuk deteksi kehalalan makanan dengan GC-MS dan SPME. *Jurnal Ilmu dan Teknologi Pangan*, *23*(2), 98–110.

Maritha, D., et al. (2023). Analisis kekurangan metode PCR dalam autentikasi halal produk olahan. *Jurnal Sains dan Teknologi Pangan*, *20*(1), 55–63.

Pranata, Y. D., Nurjanah, S., & Sugiarti, Y. (2021). Volatilomic for halal authentication using SPME-GC-MS combined with multivariate analysis. *Repository UNIDA*. Diakses dari https://repository.unida.ac.id/3208/1/Volatilomic%20for%20halal.pdf

Patriani, P., & Hafid, H. (2023). Efektivitas marinasi menggunakan jus buah asam sihala (Etlingera elatior) terhadap kualitas fisik dan mikrobiologis daging ayam afkir. *Jurnal Galung Tropika*, *12*(1), 119–128.

Purnomo, N., Mansur, M., Nugraha, A., Dagong, M. I. A., & Natsir, A. (2023). Specific species DNA amplification as a halal authentication method in sausage based on genetic markers. *Jurnal Agripet*, *23*(2), 157–163.

Puspitasari, L., Mareta, S., & Thalib, A. (2021). Karakterisasi senyawa kimia daun mint (Mentha sp.) dengan metode FTIR dan kemometrik. *Sainstech Farma: Jurnal Ilmu Kefarmasian*, *14*(1), 5–11.