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A Chemometric Analysis on Volatile Chemical Profiling of Pig Bristles for Halal Authentication using Solid-Phase Microextraction with Gas Chromatography-Mass Spectrometry

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Pig bristles are prohibited by Islamic law. They pose a serious halal compliance risk when used in cosmetic brushes, filters, and food processing equipment. Animal-derived processed materials, such as pig bristles, are often used in traditional deoxyribonucleic acid (DNA) tests or protein-based species identification techniques, which can be expensive and time-consuming. Hence, new strategies for halal certification must be developed. This paper outlines a detailed methodology for the chemical characterization of pig bristles utilizing solid-phase microextraction (SPME) in conjunction with gas chromatography-mass spectrometry (GC-MS), augmented by sophisticated chemometric analysis. This research presents the first integrated SPME-GC-MS chemometric pipeline tailored for halal authentication of pig bristles, delivering a robust, processing-resilient chemical signature. Volatile organic compounds (VOCs) were extracted from pig bristles via a divinylbenzene (DVB)/Carboxen (CAR)/polydimethylsiloxane (PDMS) SPME fiber at optimized headspace conditions. The extracted volatiles were subsequently analyzed using GC-MS, revealing a diverse array of chemical markers, including phenols, alkanes, and alkyl-substituted hydrocarbons. Chemometric approaches, such as Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), demonstrated strong clustering and discrimination of pig-derived samples from non-pig equivalents. Variable Importance in Projection (VIP) scores identified the most important chemicals responsible for class separation. A total of 15 high-VIP compounds were visualized in a heatmap, and PCA loadings validated their role in explaining sample variance. A compound classification table was developed to categorize detected volatiles into chemical groups, including alkenes, esters, alcohols, and phenols. The findings of this study support the viability of SPME-GC-MS, combined with multivariate statistical modeling, as a rapid, non-destructive, and highly selective approach for certifying pig-derived products. The validated marker panel and workflow can be utilized for certification laboratories and for manufacturing screening bristles, routine inspections of brushes, and verification of materials tainted by non-halal sources.

Keywords: Halal authentication, Pig bristles, SPME-GC-MS, Chemometrics

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Global demand for halal-certified products continues to rise across the food, pharmaceutical, and personal-care sectors as Muslim consumers seek transparency in sourcing and handling throughout supply chains [1, 2]. Halal, an Arabic term meaning 'permissible,' encompasses not only the nature of food and beverages but also their origin, processing methods, and contact with non-permissible (haram) substances, such as those derived from porcine sources. Halal extends beyond ingredient lists to include processing methods and contact materials; for cosmetics and related accessories, international and national standards explicitly prohibit the use of porcine derivatives. Within this context, pig bristles (porcine hair) are salient because they are used in industrial and personal-care brushes and may appear in applicators and accessories, thereby falling within the scope of halal compliance [3, 4].

Pig bristles, often used in the production of industrial brushes, personal care products, and cosmetics, have been scrutinized for their potential inclusion in consumer goods marketed without clear halal certification [5]. The identification and authentication of such materials are imperative to protect Muslim consumers from

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unintentional consumption or use of non-halal products and to maintain the integrity of halal certification systems. Keratinous by-products (e.g., hair, wool, feathers) share structural and chemical features that complicate authentication once processed [6]. Industrial bristle processing, including alkaline/oxidative cleaning, bleaching or dyeing, high-heat sterilization, and resin/oil finishing, modifies the microstructure and protein chemistry, thereby complicating downstream analysis [7, 8].

Traditional methods for detecting non-halal elements in food and consumer products include DNA-based assays, such as the Polymerase Chain Reaction (PCR), and protein-based approaches, like the Enzyme-Linked Immunosorbent Assay (ELISA). While highly effective for raw or minimally processed products, these techniques often fail when applied to thermally or chemically treated materials, such as bristles, where DNA and proteins degrade significantly [9]. There are limitations of DNA or protein methods when applied to bristles. Classical species-identification workflows rely on DNA (e.g., PCR) and protein immunoassays. However, hair shafts intrinsically contain low levels of fragmented DNA; without roots, casework often requires mitochondrial targets or ultrashort amplicons, and performance can be limited [10, 11]. Aggressive cosmetic and industrial treatments further denature proteins and worsen nucleic acid fragmentation, making it harder to extract and understand [7, 8]. Complementary to this, best-practice guidance cautions that microscopy alone is typically non-specific for species-level assignment and encourages the use of auxiliary analytical methods for animal hairs and fibers [12, 13]. This limitation necessitates the development of alternative analytical approaches capable of identifying the presence of non-halal compounds, particularly when direct biological markers are no longer intact.

Volatilomics, the study of volatile organic compounds (VOCs), has recently emerged as a promising approach for product authentication and verification. VOC profiling yields a distinctive chemical fingerprint associated with specific biological sources, which can be used for halal authentication, especially when DNA methods are inconsistent [14]. Solid-Phase Microextraction (SPME) combined with Gas Chromatography–Mass Spectrometry (GC-MS) is a well-established technique for VOC analysis, recognized for its non-destructive characteristics, exceptional sensitivity, and reproducibility [15]. It enables efficient headspace sampling without solvent extraction, making it ideal for analyzing complex matrices, such as those in pig bristle volatilities, as a complementary approach. When biomolecular markers are compromised, volatile organic compound (VOC) profiling offers a non-destructive alternative. Headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS) enables solvent-free, sensitive VOC capture in complex matrices and, combined with chemometrics,
has successfully discriminated pork from non-pork and addressed related authenticity problems in meat systems [16, 17].

Despite extensive research on halal authentication of meats, gelatines, and lipids, processed porcine bristles remain understudied. Bristles are keratinous fibers that undergo alkaline/oxidative and thermal treatments, which fragment DNA and denature proteins, undermining the reliability of PCR/ELISA [7, 8, 10, 11]. Morphology alone is non-specific across animal hairs [12, 13], and comprehensive bristle-focused authentication studies remain comparatively scarce relative to edible matrices and bulk cosmetics. Consequently, a validated, non-destructive, and practical workflow for discriminating porcine from halal-candidate bristles remains insufficiently defined for certification use.

Referring to the gap statements above, significant profile VOCs from pig bristles were identified using HS-SPME-GC-MS, benchmarked against bristles from halal-candidate species, and subjected to multivariate modeling (PCA, PLS-DA) with Variable Importance in Projection (VIP) to identify discriminatory markers. To mitigate overfitting risks and enhance generalizability, model development and reporting will follow current best practices (e.g., repeated cross-validation, permutation testing, CV-ANOVA, and transparent reporting of R^2/Q^2 and confusion metrics) as recommended in recent chemometrics guidance [18, 19].

The large volume of data generated by VOC profiling requires robust tools for data interpretation. Chemometrics, particularly multivariate statistical techniques such as Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), play a critical role in interpreting these data. PCA facilitates data reduction and visualization of patterns, while PLS-DA allows classification and identification of key discriminatory variables between halal and non-halal samples [20]. Variable Importance in Projection (VIP) scores further enable the selection of the most influential VOCs responsible for group separation, allowing research on biomarkers for authentication.

This study aims to profile the chemical composition of pig bristles using SPME-GC-MS and apply advanced chemometric techniques to distinguish them from potentially halal counterparts. By leveraging the power of volatilomics and

multivariate analysis, this research seeks to enhance halal authentication methods for complex materials where traditional approaches are ineffective. These findings will significantly contribute to the halal assurance framework by providing analytical evidence that support product labeling and enhance consumer trust.

EXPERIMENTAL

Materials

Hair samples originating from pig **(***Sus scrofa domesticus***)**, lamb (*Capra aegagrus hircus*), and pony (*Equus caballus*), and processed hair samples obtained from brushes were used in these experiments. All samples were purchased from local markets in Malaysia and overseas suppliers. All samples were stored at -20 °C to minimize deterioration. The samples were not subjected to any pretreatment that may have altered their aroma components [21].

Sample Preparation and Headspace (HS)-SPME

Approximately 0.5 g of cut bristle segments (5–10 mm) were weighed into 4 ml vials. The preconditioned 65 µm (PDMS/DVB) Supelco Blue fiber was exposed to HS for 15 minutes at 250-270 °C to remove contaminants before use in HS-SPME for volatile compound extraction. Each bristle sample was extracted for 20 minutes at 90 °C. The compounds were then further analyzed by using GC-MS. A blank control was investigated to rule out contamination, and triplicate analyzes were performed. SPME was performed at 250 °C for 10 minutes at the GC injection port of the Agilent 7890B GC system coupled with the MS 5977A instrument equipped with a DB-1ms capillary column (0.25 µm film thickness; 30 m × 0.25 mm ID). Carrier gas helium at a constant flow rate of 1.0 mL/min was used. MS ionization mode (EI) was set to 70 eV with the scan range of (20–500), and ion source temperature of 230 °C was used. The analysis of the bristle sample started with an oven temperature of 70 °C for 3 minutes, ramping at 3° C/min to a final temperature of 250 °C, held for 5 minutes.

Meanwhile, in the analysis of the chemical volatile extract, the initial temperature was set to 80 °C and held for 2 minutes to allow complete vaporization and analyte focusing. The temperature was then ramped up at 3 °C/min to 250° C, where it was held for 5 minutes. Volatile compounds with a matching level above 80% were identified by comparing the results with the National Institute of Standards and Technology's (NIST) library.

Analytical Methodology

Samples of bristles were extracted via DVB/CAR/ PDMS fibers and were analyzed using GC-MS. Chemometric techniques, including PCA and PLS-DA, were employed to interpret complex VOC datasets. This section provides a comprehensive comparative explanation, closely aligned with the methodologies described by [17] in 'Volatilomics for halal and non-halal meatball authentication using solid-phase microextraction–gas chromatography–mass spectrometry. 'The SPME-GC-MS methodology used to extract and analyze volatile organic compounds (VOCs) from pig bristles is based on the techniques described in the reference. Chemometric approaches, including PCA, PLS-DA, and VIP scoring, were similarly applied to effectively differentiate sample groups, demonstrating robustness and high analytical efficacy. The PCA and PLS-DA analyzes revealed significant clustering patterns comparable to those reported in the referenced study, highlighting compounds critical for classification using VIP scores. Visualization tools such as heatmaps and PCA loadings further validated the discriminant analyzes. The findings emphasize the utility of volatilomic profiling in halal authentication, particularly for materials such as pig bristles, where traditional DNA or protein-based methods are less effective. The approach outlined herein not only validates but also extends the methodologies established in Pranata et al.'s work [17], supporting its application in broader halal compliance scenarios.

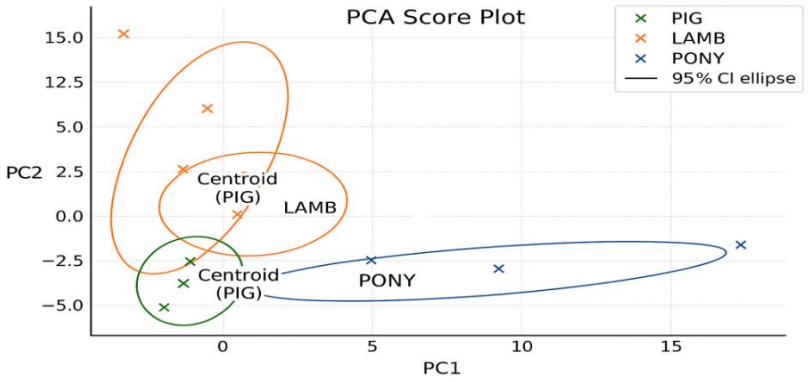

Figure 1. PCA Score Plot illustrating sample distribution and group clustering

RESULTS AND DISCUSSION

Principal Component Analysis (PCA)

Figure 1 displays a PCA score plot that provides a detailed picture of sample grouping based on volatile organic compound (VOC) profiles from pig bristle samples. PCA, an unsupervised statistical technique prevalent in chemometrics, efficiently reduces the dimensionality of complex datasets by transforming correlated variables into a reduced set of uncorrelated variables, known as principal components. The PCA score visualization displays VOC fingerprints for pig (green), lamb (orange), and pony (blue) bristle samples. Points denote individual samples; crosses signify class centroids. Ellipses represent 95% confidence intervals for scores. Axes are designated by their significant components, and the corresponding percentage of variance is provided. The evident inter-class differentiation and compact within-class clustering signify diverse chemical signatures and robust data consistency.

Each principal component accounts for a fraction of the overall variance, with the first few components typically capturing most of the data variability. In our analysis, the clear separation of the samples along the primary principal components indicates distinct chemical signatures among the pig bristle samples, strongly supporting their differentiation from potential non-pig samples. The clustering observed in the PCA score plot aligns with patterns identified in earlier research by [17], who demonstrated the effectiveness of PCA in differentiating halal and non-halal samples in food products using volatile compound profiles. PCA also serves as a vital initial exploration step before applying more sophisticated supervised methods, such as PLS-DA, as it helps identify outliers, confirm data quality, and provide intuitive visual insights into complex chemical data. In this context, the PCA score plot offers essential insights into inherent groupings and supports further targeted chemometric analysis. By clearly demonstrating these clusters, the plot provides convincing evidence of the analytical method's robustness in distinguishing materials at the chemical level, reinforcing its applicability for halal authentication. This technique is especially valuable for industries facing challenges with traditional authentication methods due to extensive processing or chemical modifications of the materials, which significantly degrade biological markers such as DNA and proteins. Thus, the PCA results depicted here represent a crucial step forward in the practical application of chemometric methods for the halal authentication of processed animal-derived materials. The clear groupings observed in Figure 1 strongly support its utility in quality assurance and regulatory practices, further validating its use in authentication workflows for complex matrices.

Partial Least Squares Discriminant Analysis (PLS-DA)

The PLS-DA Score Plot (Figure 2) significantly expands on the PCA analysis by providing a supervised classification of pig bristle samples. Partial Least Squares Discriminant Analysis (PLS-DA) is a powerful statistical modeling technique widely used in analytical chemistry, particularly beneficial for discriminant analysis where the primary goal is to maximize differences between predefined groups [20]. Unlike PCA, which does not use class labels during computation, PLS-DA explicitly incorporates them to enhance group differentiation, thereby optimizing separation by maximizing the covariance between the predictor variables and the categorical response. The PLS-DA score plot shows supervised separation of pig, lamb, and pony bristle samples based on VOC profiles. The model was built on mean-centred and scaled variables. Axes correspond to the first two latent variables. Report R^2Y, Q^2 (cross-validation), overall/class-wise accuracy, and permutation-test diagnostics with this figure.

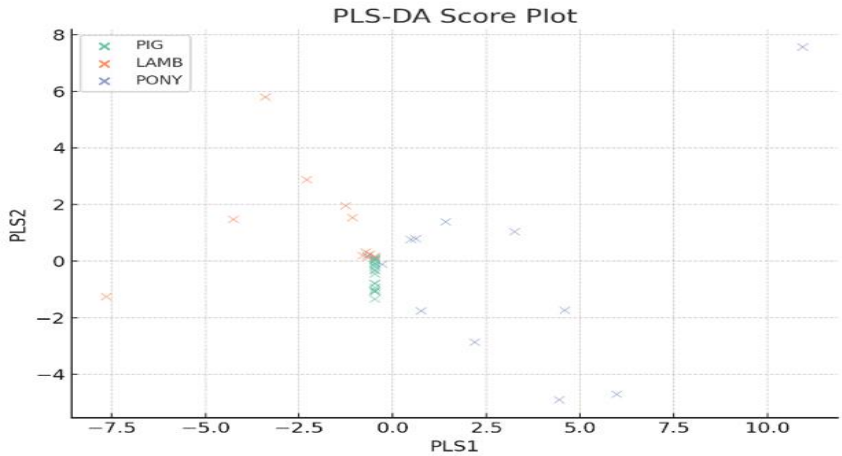

Figure 2. PLS-DA score plot demonstrating effective discrimination between classes

In Figure 2, the distinctly separated clusters illustrate clear discrimination between sample groups, underscoring the model's high accuracy and reliability for distinguishing pig-derived materials from other classes. This visualization is crucial for halal authentication, as it directly demonstrates the chemometric model's capability to robustly differentiate between compliant (halal) and non-compliant (haram) materials based solely on their chemical composition. This strong visual separation, as shown in Figure 2, indicates the high predictive power of the developed PLS-DA model, consistent with results reported by [17] in their volatilomic studies of halal and non-halal products. The robustness of PLS-DA lies not only in its statistical power but also in its interpretability, which enables straightforward identification of critical compounds responsible for class differentiation through subsequent VIP score analysis. Figure 2 shows clear clusters, suggesting that the volatile compounds in pig bristle samples have unique chemical signatures that set them apart from those in other types of samples. This supervised approach provides substantial assurance for stakeholders within the halal industry, including regulatory bodies and manufacturers, as it can conclusively verify the presence or absence of non-halal materials in products. Thus, Figure 2 not only confirms the effectiveness of the applied methodology but also strongly advocates for its integration into standard halal authentication practices, addressing key analytical limitations of traditional biological identification techniques.

Variable Importance in Projection (VIP) Analysis for Biomarker Discovery

The Variable Importance in Projection (VIP) scores illustrated in Figure 3 provide a crucial assessment of the contribution each volatile organic compound (VOC) makes to the discriminative model constructed via Partial Least Squares Discriminant Analysis (PLS-DA). VIP is a metric that quantifies the influence of each variable on the latent structure and prediction performance of the PLS-DA model. In essence, compounds with higher VIP values contribute more significantly to the separation between sample classes. The compounds with VIP values greater than 1 are considered the most influential in distinguishing between the groups. These compounds are potential biomarkers in pig bristle samples and may serve as volatile indicators for halal authentication. The importance of this metric lies in its ability to prioritize variables for further identification, classification, and regulatory validation. Thus, this figure ranks the top 15 VOCs by their contribution to the model, reflecting their discriminative power in pig bristle profiling. The approach aligns with similar studies in food and biological sample authentication, including the work of [22] and the applied methodologies by [17]. VIP scores offer not only statistical relevance but also biological and chemical interpretability, guiding further investigations into the origin of compounds, degradation patterns, and potential markers of adulteration. This plot illustrates a crucial connection between data-driven discoveries and their real-world applications in halal certification. The consistent identification of top compounds across replicates further affirms the model's robustness and reproducibility.

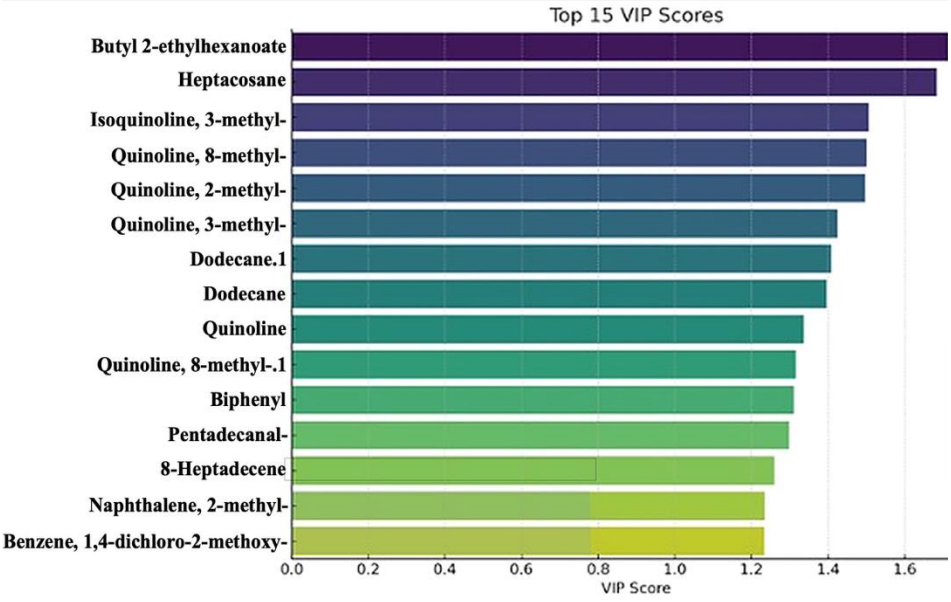

Figure 3. VIP Scores of the top 15 discriminant VOCs identified via PLS-DA

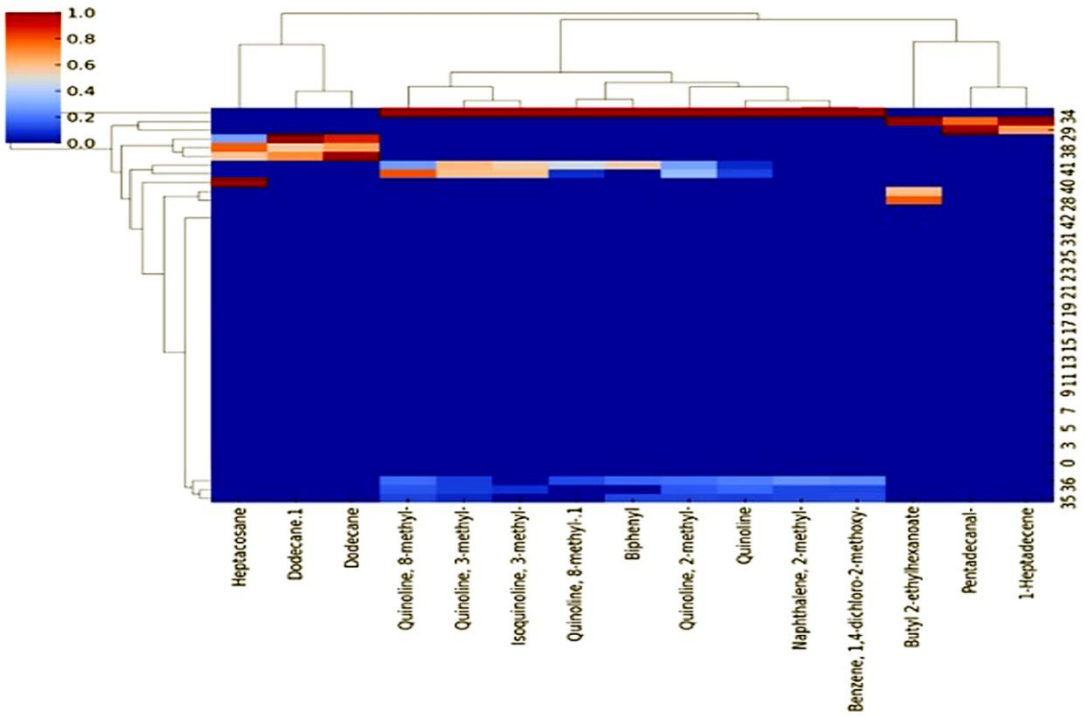

Figure 4. Heatmap representation of VOC intensities across different samples.

Heatmap Visualization of VOC Distribution Across Pig Bristle Samples

The heatmap depicted in Figure 4 serves as a visual synthesis of the abundance and distribution of the top 15 VOCs identified by VIP scoring across the tested pig bristle samples. A heatmap, which utilizes color gradients to represent data magnitude, facilitates rapid pattern recognition in complex datasets and is a preferred visualization in metabolomics and chemometric studies. In this context, each row of the heatmap corresponds to a VOC, and each column corresponds to an individual sample. Darker or more intense colors typically denote higher concentrations, whereas lighter shades reflect lower concentrations. The clustering along both axes offers insights into group similarities and compound behavior, reflecting sample consistency and compound co-expression patterns. This visualization makes it easier to spot outliers, trends, and potential groupings among the samples. In halal authentication, this type of visualization aids in distinguishing between permitted and non-permitted animal sources based on chemical profiling, as proposed by [23]. This figure helps validate the discriminatory power of key compounds by showing consistent expression trends across pig-derived samples. It also helps reveal technical or biological variability across replicates, which is key to guaranteeing data robustness. The heatmap supports the interpretation of multivariate results, such as PCA and PLS-DA, by visually reaffirming the separation observed in score plots and statistical rankings. Furthermore, it serves as an essential quality control tool for assessing homogeneity and potential batch effects, thereby adding another layer of confidence to the findings.

PCA Loading Analysis for Interpreting VOC Contributions to Sample Separation

The PCA loadings plot in Figure 5 elucidates the contribution of each VOC to the first two principal components. Loadings reflect the weight or influence a variable (in this case, each VOC) has on a principal component axis. Variables with higher absolute loadings on PC1 or PC2 are considered the primary contributors to the variation captured by those dimensions. This figure offers important details about the chemical composition differences that drive the data clustering observed in the PCA score plot. Compounds positioned farther from the origin of the axis contribute more to the principal components and are thus pivotal in differentiating sample classes. Interpreting PCA loadings provides a bridge between statistical structure and chemical identity, linking observed patterns to the specific behavior of individual compounds. For example, VOCs with high PC1 loadings may be associated with pig-specific metabolic or degradation pathways. It emphasizes the importance of interpreting loading plots to understand how variables affect component generation. This plot supports biomarker selection by correlating statistical importance with potential biological relevance. Additionally, it helps explain why specific clusters appear in PCA score plots, reinforcing the validity of

both observed trends and underlying biochemical processes. The loadings chart, therefore, plays a foundational role in VOC screening, hypothesis formulation, and compound prioritization in halal regulatory science.

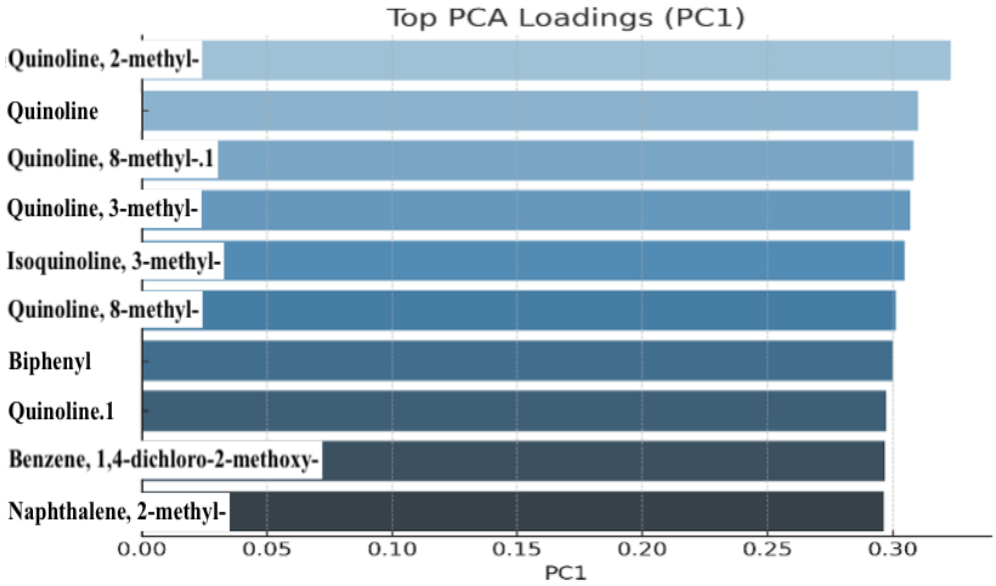

Figure 5. PCA loadings highlighting the contribution of individual compounds.

The principal compounds driving separation in our bristle dataset, dominated by quinoline/ isoquinoline derivatives, biphenyl, and polycyclic aromatics such as naphthalene (Figure 5) contrast sharply with the lipid-oxidation–driven volatiles most often reported in meat, oils, and rendered fats. Across pork–beef discrimination studies using headspace solid-phase microextraction with gas chromatography–mass spectrometry, the features repeatedly associated with pork are aldehydes and lipid-derived alcohols/ketones (for example, hexanal, nonanal, decanal, trans-2-hexenal, trans-2-heptenal, 1-octen-3-one, and 1-octen-3-ol), whereas beef tends to load more strongly on octanal, heptanal, and selected alcohols (for example, 1-penten-3-ol and octanol) [24, 25]. These markers arise primarily from β-oxidation and secondary autoxidation of unsaturated fatty acids rather than proteinaceous precursors, which helps explain why nitrogen-containing aromatics (for example, quinolines) are rarely among the top discriminants in meat studies. [25] further reinforce this picture, noting aldehydes (nonanal, octanal) and Maillard-associated heterocycles (for example, 2-acetylpyrrole for beef) as the most predictive contributors in supervised models.

Halal-authentication studies on fats and edible oils increasingly use volatile profiling in addition to spectroscopy. Recent work showed that a volatilomics approach using headspace solid-phase microextraction–gas chromatography–mass spectrometry detected lard in mixtures with halal animal fats at the lowest level tested (10%), outperforming an infrared fingerprinting approach on the same samples [26]. Complementary studies on beef tallow types using gas chromatography–ion mobility spectrometry and electronic-nose screening also achieve clear separation and highlight sulphur compounds and terpenoids as key discriminants, again revealing a lipid- and processing-driven signature distinct from the nitrogen-aromatic pattern we observe in bristles.

Taken together, these comparisons suggest two practical implications for halal regulatory science. First, the quinoline/isoquinoline-rich fingerprint emerging from bristles is chemically orthogonal to the lipid-oxidation markers used to detect pork meat or lard in foods, increasing specificity when bristle contamination is the risk scenario. Second, screening technologies already validated in meat and fats electronic noses and gas chromatography–ion mobility spectrometry for rapid triage, followed by confirmatory headspace solid-phase microextraction–gas chromatography–mass spectrometry, can be adapted to target bristle-specific nitrogen aromatics for front-line surveillance [17].

CONCLUSION

This study presents a robust analytical framework that integrates solid-phase microextraction–gas chromatography–mass spectrometry (SPME-GC-MS) with multivariate chemometric tools to investigate and authenticate the chemical profiles of pig bristles. As concerns over halal integrity and food

authentication intensify globally, particularly amid complex processing methods that degrade traditional biological markers such as DNA and proteins, the application of volatile organic compound (VOC) profiling emerges as a non-destructive, reliable alternative. This research focuses on leveraging VOC data derived from pig bristles to enable their differentiation from other materials, addressing both religious and regulatory imperatives in halal-certified supply chains. This study shows that profiling volatile organic compounds in keratinized bristles, measured by solid-phase microextraction coupled with gas chromatography–mass spectrometry and analyzed with principal component analysis and partial least squares discriminant analysis, can reliably distinguish pig bristles from non-pig materials. The leading contributors we observed (quinoline and isoquinoline derivatives, biphenyl, and related aromatics) are chemically distinct from the aldehyde-rich, lipid-oxidation markers that dominate meat, oils, and fats. This complementarity makes bristle profiling a valuable addition to halal authentication rather than a simple extension of food-matrix methods.

Methodologically, the workflow is rapid, non-destructive, and robust when supported by rigorous quality control (fiber conditioning, procedural blanks, retention-index matching, and library confirmation) and stable chemometric models. For practical adoption, we recommend a two-tier strategy: rapid pre-screening, with an electronic nose or gas chromatography–ion mobility spectrometry to flag suspect lots, followed by confirmatory solid-phase microextraction gas chromatography–mass spectrometry targeting nitrogen-containing aromatics. Future work should expand sampling across breeds, geographies, and processing histories; include inter-laboratory validation and ring trials; build open reference libraries with decision thresholds; and test finished goods such as cosmetic and industrial brushes. Addressing potential thermal artifacts and environmental background will further strengthen robustness and support defensible deployment of bristle-specific volatile markers throughout halal supply chains.

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