HALALSPHERE

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Detection of pig bristles in brushes: A halal verification approach using microscopy and chemical methods

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Abstract

The reliable identification of animal-derived bristles, specifically pig hair, in industrial and consumer brushes poses a persistent challenge for halal compliance, as conventional DNA-based methods are often limited by severe genetic material degradation in processed hair samples. This study proposes and validates a dual-platform, non-DNA approach that exploits the intrinsic biochemical and morphological stability of keratin to verify fibre origin. Using a composite brush sample, fibres were sub-categorised by colour (dark brown and white) to account for potential material heterogeneity. The dark brown fibres were identified as animal-derived based on their solubility in NaOH and characteristic sulphurous odour upon combustion. Both are hallmark indicators of the cystine-rich keratinous matrix. High-resolution microscopic analysis (Stereomicroscopy and SEM) further confirmed this origin by the presence of a distinct, broad medulla and imbricate cuticle scales, structural features unique to keratinised mammalian hair. Conversely, the white fibres exhibited chemical insolubility and a lack of organised surface morphology, confirming their synthetic nature. By focusing on the robust, degradation-resistant properties of the keratin protein rather than labile DNA, this integrated methodology offers a rapid, cost-effective, and unambiguous standard for halal verification in the food and cosmetic supply chains.

1. Introduction

Hair is a protein filament predominantly made of keratin and is present in all mammals. Keratin is a fibrous protein characterised by a high concentration of the sulphur-containing amino acid cysteine. These cysteine residues facilitate the formation of extensive covalent disulfide bridges (S-S), resulting in a three-dimensional network with a high density of cross-links that stabilise the hair's internal architecture (Valéria *et al.*, 2023) as illustrated in Figure 1.

Structurally, the hair shaft is composed of three layers: the cuticle, cortex, and medulla, as illustrated in Figure 2. The cuticle is the outermost scaly layer formed by keratinised, dead cells. Beneath the cuticle lies the cortex, which makes up the bulk of the hair shaft. The medulla, the innermost layer, contains scattered cells and empty spaces (Schacker *et al.*, 2018)

Brushes are among the most basic and versatile tools in use today and are broadly categorised into two types: natural bristle brushes and synthetic brushes. Natural bristle brushes are made from animal hair or fur, often from pigs, camels, squirrels, horses, goats, and cows. In contrast, synthetic brushes are made from materials like plastic or silicone (Nurrohmani, 2025). Brushes have widespread applications across various industries, including food production (where they are classified as processing aids), cosmetics (where they are categorised as applicators), cleaning services, and painting. In both the food and cosmetic industries, the integrity of these tools is essential to creating the final product.

The identification of pig-derived bristles is a paramount concern for halal compliance, as the use of pig hair is strictly prohibited (*haram*) under Islamic Law. This prohibition is codified in Malaysia's regulatory frameworks, specifically through the *Fatwa* of the *MKI Muzakarah Committee* (1985), which unequivocally stated that the use of a brush made of pig hair is *haram* (forbidden). In particular, Clause 17.5(c) of the Manual mandates that any equipment derived from animal sources, including bristle brushes, must have a verified halal status. This legal requirement places the burden of proof squarely on manufacturers to demonstrate the non-porcine origin of bristles used as processing aids. Despite these stringent regulations, pig bristles remain prevalent in the food and cosmetic industries due to their superior mechanical properties, posing a significant risk of cross-contamination. While current quality assurance efforts prioritise the status of raw ingredients, ensuring the purity of production tools remains a critical, yet often overlooked, dimension of halal supply chain integrity (Atalgin *et al.*, 2023)

Halal authentication is a critical process in identifying halal-critical ingredients through rigorous laboratory analysis, providing the empirical foundation for raw material and processing tool documentation. The current gold standard for

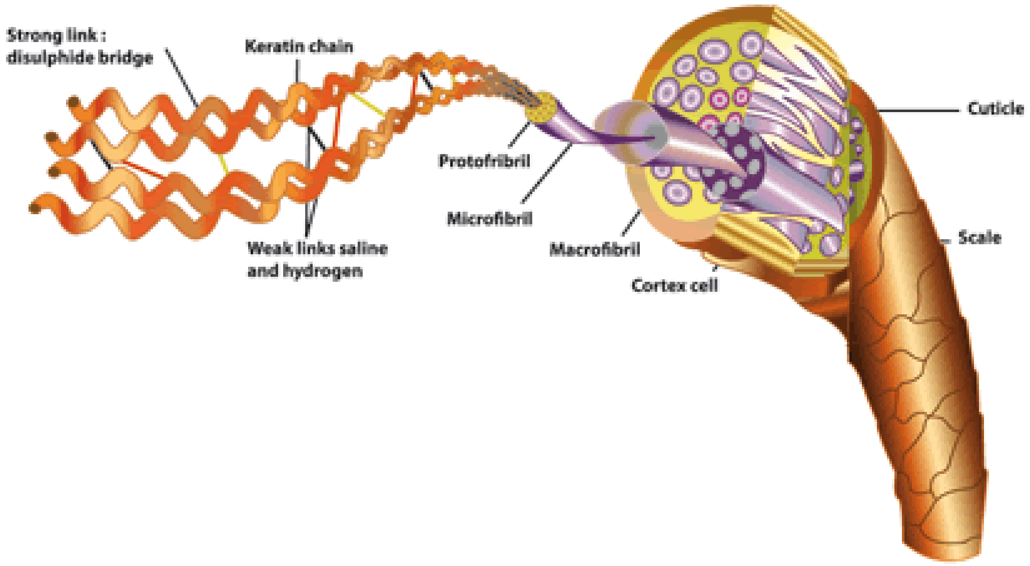

Figure 1: Hierarchical structural organisation of the keratinous hair (Morganti *et al*., 2021).

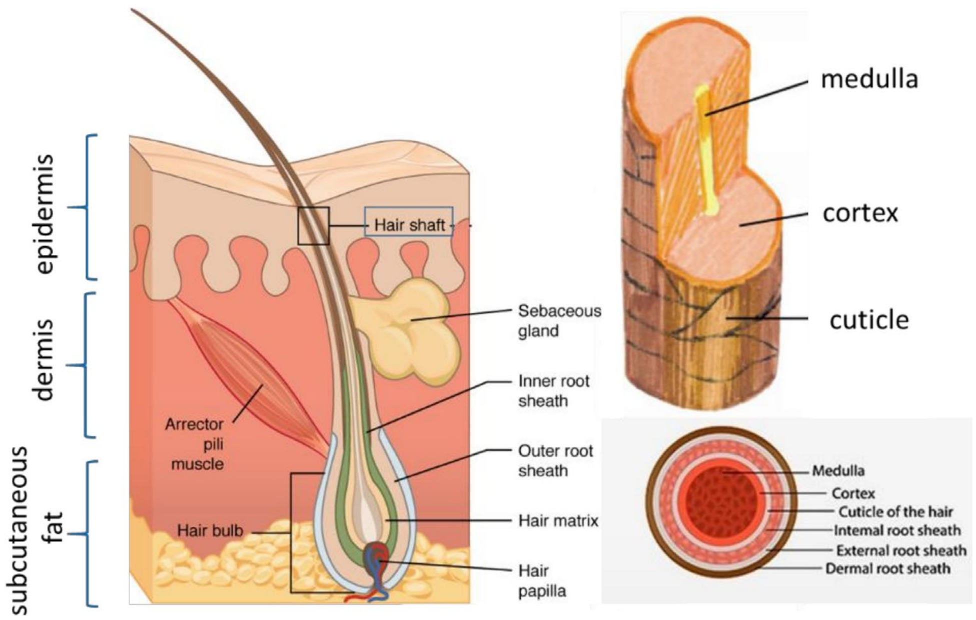

Figure 2: The hair shaft is composed of cuticle, cortex, and medulla (Morganti *et. al*, 2021).

species identification relies on DNA-based analysis. However, in the case of industrial brushes, this approach is often hindered by the liability of genetic material. During brush production, hair undergoes rigorous thermal and chemical treatments that cause extensive genomic fragmentation. While the robust keratin matrix of the cortex and cuticle physically shields cellular remnants, these same protective layers make the extraction of the few remaining, highly degraded DNA fragments nearly impossible.

To address this technical gap, this study proposes a dual-platform methodology that shifts the focus from unstable genetic markers to the permanent, stable morphological and chemical properties of keratin. Chemical and microscopic analyses are exceptionally effective for detecting and differentiating animal-derived components, such as skin (leather), bone, and hair/bristles, which are frequently incorporated into consumer goods, including water filtration media, brushes, and gelatinous materials. According to the Malaysian Standard (MS) 2803:2025, these materials are classified as halal-critical because their permissibility is strictly contingent on species origin and compliance with *Shari'ah* slaughter protocols. Physical authentication by advanced microscopy is particularly indispensable for these sample types; it leverages genetically conserved structural cues and the inherent physicochemical stability of the keratin matrix to distinguish halal from non-halal sources with high diagnostic certainty (Yaacob *et al*., 2025). By utilising Scanning Electron Microscope (SEM) and Stereo Microscopy, we characterise the genetically conserved cuticle scale patterns and medullary structures that remain intact despite industrial processing. Furthermore, we exploit the inherent chemical reactivity of the keratinous disulfide bonds through solubility and burning tests. This integrated, non-DNA-dependent approach, developed at the Malaysia Halal Analysis Centre (MyHAC), provides a practical, forensic-grade solution for rapidly verifying Halal integrity in consumer and industrial tools.

2. Materials and methods

2.1 Sample collection

An unidentified brush sample was procured from a production site by the State Islamic Religious Department (*Jabatan Agama Islam Negeri*) and submitted to the Malaysia Halal Analysis Centre (MyHAC) for definitive species identification. To maintain sample integrity and mitigate the risk of cross-contamination, a single unit was isolated and immediately secured within a sealed polymer zip-lock bag. The specimen was maintained at a controlled ambient temperature during transit and safely delivered to MyHAC for halal verification of the constituent hair fibres.

2.2 Pre-treatment and sub-sampling

To remove external contaminants, the hair and fibre samples were cleaned by sonication for 15 mins in a 70:30 (v/v) ethanol-ethyl acetate solution. The cleaned samples were then retrieved and left to air dry thoroughly at ambient temperature (~25℃) before testing. During visual inspection during the sub-sampling process, the brush sample was observed to contain fibres with variations in colour and visual characteristics. Thus, the fibres were separated and sub-categorised into distinct fractions (dark brown hair and white fibre) for individual analysis.

2.3 Chemical testing

Chemical analysis was performed to determine the chemical reactivity and general nature of the fibres. All chemical tests were conducted on both the unknown hair or fibre fractions and a certified reference standard (porcine bristles and synthetic fibres, if applicable, for comparative analysis).

2.3.1 Burning test

A single strand of the unknown fibre of the reference standard was held with stainless steel tweezers and carefully introduced to a controlled Bunsen burner flame. The thermal decomposition behaviour of each fibre was systematically evaluated based on three critical diagnostic criteria: (i) immediate reaction to heat, specifically monitoring for melting, retraction or ignition; (ii) the profile of volatile odours evolved during combustion, with particular attention to burnt hair odour or sulphurous markers indicative of keratinous proteins; and (iii) the morphology of the post-combustion residue, distinguishing between the friable, brittle ash characteristic of natural hair and the hard, fused beads typical synthetic polymers.

2.3.2 Solubility test

A single strand of the unknown fibre or the reference standard was immersed in a test tube containing 4 mL of a 5% (w/v) Sodium Hydroxide (NaOH) solution. The test tube was subsequently heated in a hot water bath at 80°C for 30 mins. Observations were recorded to determine whether the hair or fibre was soluble (dissolved) or insoluble in the alkaline solution.

2.4 Microscopic examination

Microscopic analysis focused on evaluating morphological characteristics that distinguish mammalian species, specifically the cuticle scale pattern and medullary structure.

2.4.1 Stereomicroscopy

The morphological characteristics, colour, and size of the hair or fibre samples were initially observed using a Stereo Microscope (Nikon SMZ18). Images were captured at 135x magnification to provide an overall structural assessment.

2.4.2 Scanning Electron Microscope

High-resolution imaging was performed using a Scanning Electron Microscope (SEM) (JEOL JSM-6510LV) to confirm the presence and pattern of the cuticle layer.

a- A Sample Preparation and Coating: Hair or fibre strands were mounted onto aluminium stubs using conductive carbon tape. To ensure optimal image quality and prevent charging artifacts, the mounted samples were subsequently coated with a thin layer of platinum using a sputter coater (Auto Fine Coater JEOL JEC-3000FC) for 30 seconds.

b- Imaging parameters: Imaging was performed under high vacuum at an accelerating voltage of 15kV. The entire hair shaft was examined, with focus on the cuticle scale pattern at magnifications typically ranging from 300x to 1,000x for species-specific identification.

3.0 Results and discussion

3.1 Sub-sampling and macroscopic findings

Initial macroscopic examination of the unidentified brush sample revealed a heterogeneous fibre composition, necessitating a systematic sub-sampling approach to ensure analytical accuracy. Based on distinct pigmentation and textural profiles, the specimens were sub-categorised into two primary groups: dark brown hair and white fibre. This sub-sampling strategy proved pivotal, as the observed colour variations directly correlated with disparate chemical and morphological outcomes. Specifically, the dark brown hairs exhibited the chemical and physical traits of animal hair, while the white fibre exhibited characteristics of synthetic materials.

These findings emphasise that macroscopic heterogeneity, particularly variations in fibre colour, must be treated as a primary diagnostic indicator in halal verification, as it significantly influences the final interpretation of the material's origin. Consequently, the results provide conclusive verification of pig-derived components within the composite brush material, validating this non-DNA, keratin-focused methodology as a robust solution for addressing current gaps in halal supply chain assurance.

Table 1: Comparative chemical reactivity of unknown brush fibres

Fiber Type	Solubility in 5% NaOH	Burning Characteristics	Odour Signature	Interpretation
Dark Brown Hair	Soluble	Initially melted, then shrank in the flame	Burning hair	Protein/ Natural origin
White Fiber	Insoluble	Shrank away from the flame	Burnt latex	Synthetic/ Non-protein origin

3.2 Chemical analysis

The chemical analysis successfully distinguished natural animal hair from synthetic fibres based on their intrinsic chemical structures. The chemical reactivity provided definitive preliminary evidence on the fundamental composition (natural vs. synthetic) of the two fibre types, as tabulated in Table 1.

The chemical screening of the unidentified fibres provided distinct results that correlated with their macroscopic categorisation. The dark brown hairs exhibited characteristic responses consistent with a keratinous origin, whereas the white fibres displayed behaviour typical of synthetic polymers. The dark brown samples were fully soluble in a 5% NaOH solution and emitted a characteristic burnt hair or sulphurous odour upon combustion. These reactions are fundamentally dictated by the hair's biochemical composition, which consists of long α-keratin chains stabilised by extensive disulfide (S-S) cross-linking. During pyrolytic combustion, high thermal energy cleaves these crucial disulfide linkages, triggering a reaction between sulphur atoms and the protein matrix to form volatile sulphur compounds, such as hydrogen sulphide (H_2S) (Valéria *et al.*, 2023). This distinctive foul odour serves as a rapid diagnostic marker, clearly distinguishing natural keratin from synthetic materials.

Alkaline hydrolysis of keratinous tissues is typically performed using hydroxyl compounds such as sodium hydroxide (NaOH). This treatment generally leads to a degradation of keratin's mechanical properties, as cysteine residues are the most susceptible sites for chemical attack along the protein chain (Banasaz & Ferraro, 2024). In dark brown hair, solubility in an alkaline medium is driven by the cleavage of these critical disulfide bridges and the subsequent formation of sulphonic groups (Horvath, 2009). Ultimately, the efficiency of this degradation is governed by the specific amino acid composition and the complex hierarchical conformation of the keratin fibre. Conversely, the white fibres remained chemically insoluble in NaOH and produced a burnt-latex odour upon flaming, which reflects the typical behaviour of synthetic analogues such as plastic or latex. This preliminary chemical screening demonstrated that targeting the resilient, cross-linked keratin structure provides a robust, cost-effective method for fibre classification, serving as a vital diagnostic step in the tiered Halal verification strategy.

3.3 Microscopic examination

Following the chemical screening, microscopic analysis was performed to validate the hair's origin and achieve definitive species identification. While chemical assays confirmed the presence of a keratinous matrix, microscopic characterisation provided the essential morpho-structural details required to differentiate a general animal origin from a specific porcine identification.

High-resolution imaging enabled examination of the medulla and cuticle scale patterns, which serve as stable physical biomarkers of the animal's biological lineage. By observing these genetically conserved features of the keratinised shaft, the analysis successfully moved beyond a broad classification, providing the forensic evidence necessary for halal verification.

3.3.1 Stereomicroscopy examination

Stereomicroscopic evaluation revealed a significant structural divergence between the two fibre cohorts, providing physical evidence that validated the prior chemical findings. The dark brown hair samples exhibited a distinct internal channel, identified as the medulla (Figure 3). In contrast, the white fibres were characterised by a solid, homogeneous core entirely devoid of medullary structure (Figure 4).

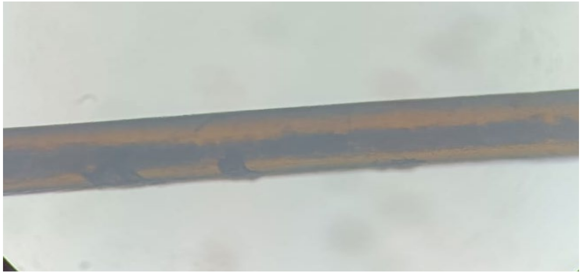

Figure 3: Stereomicroscopy image showing the presence of medulla in dark brown hair (135x magnification).

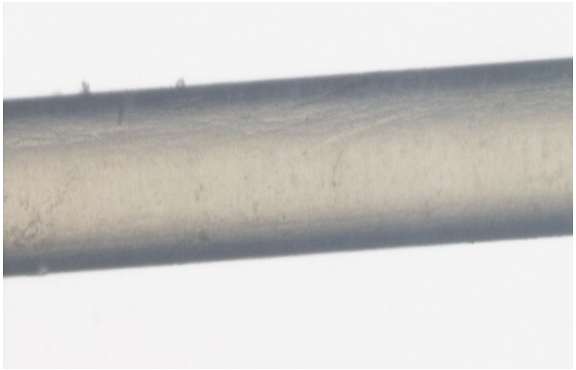

Figure 4: Stereomicroscopy image showing the absence of medulla in the white fibre (135x magnification).

While natural hair and synthetic polymers may appear macroscopically similar in a composite brush, they differ fundamentally at the structure level. Natural hair consists of an outer cuticle and a cortex surrounding a central medulla. The presence of a medulla is a critical diagnostic marker in forensic species identification, as its diameter and continuity vary significantly across mammalian lineages. In this study, the presence of a medulla in the dark brown hairs confirmed their biological origin. In contrast, the absence of such an internal structure in the white fibres verified their synthetic nature. These stereomicroscopic findings align with the earlier chemical reactivity, collectively establishing a robust profile for the keratinous fraction of the brush sample.

3.3.2 Scanning electron microscopy examination

Scanning Electron Microscopy (SEM) provided high-resolution evidence for species identification. The dark brown hair sample displayed a distinct cuticle-scale pattern, as shown in Figure 5, a critical feature for species differentiation. This pattern was observed to be imbricate (overlapping, flattened scales) and exhibited an irregular wave-scale pattern with a heavily rippled scale margin and a close distance between margins. Furthermore, the hair displayed a relatively thick shaft with a large, sometimes continuous medulla (as indicated by Stereomicroscopy), collectively aligning with known morphological characteristics of pig (porcine) hair. Conversely, the white fibre sample showed no cuticle-scale pattern (Figure 6), conclusively confirming its structure as a manufactured synthetic material.

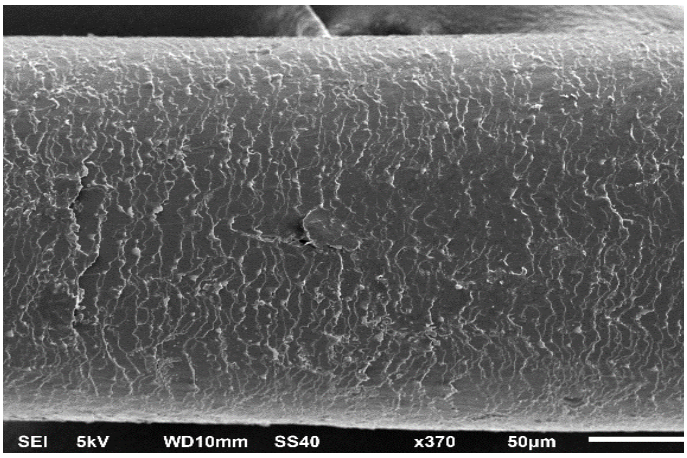

Figure 5: Scanning Electron Microscopy image showing distinct cuticle scale pattern in dark brown hair (370x magnification).

The diagnostic value of the cuticle, specifically the hair's outer layer of transparent, overlapping scales, lies in its species-specific configuration. As illustrated in the classification system by Brunner and Coman (1974), the shapes and arrangements of these scales are genetically determined (Figure 7).

The Scanning Electron Microscopy (SEM) findings on the dark brown hair were unequivocally consistent with porcine characteristics, revealing an imbricate and irregular wave-scale pattern, a heavily rippled scale margin, and a close spacing between margins, consistent with findings for pig hair by De Marinis & Asprea (2006). This detailed morphological profile, combined with the observation of a wide and simple medulla (Raval *et al.*, 2018) It provides unambiguous confirmation of the presence of pig hair. In contrast, the white fibre's smooth outer layer and lack of a distinct scale pattern observed through SEM align with characteristics reported for synthetic hair. (Long *et al.*, 2014), thereby confirming its non-porcine, non-animal origin.

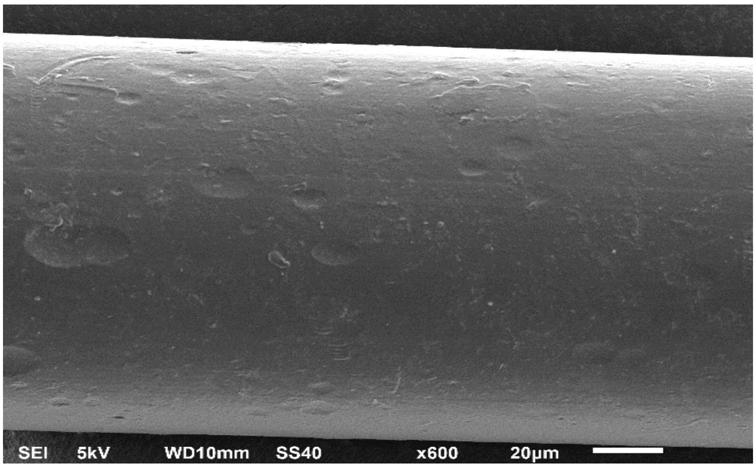

Figure 6: Scanning Electron Microscopy image showing the absence of cuticle scale pattern in white fibre (600x magnification).

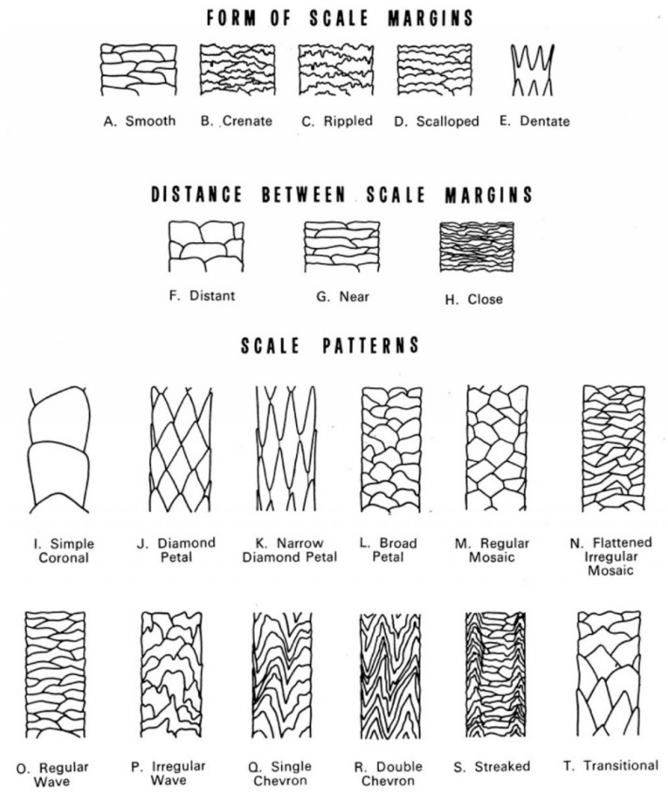

Figure 7: Various shapes and arrangements of hair cuticle scales (Brunner and Coman, 1974).

The successful identification of pig-derived material in this study relied entirely on the inherent stability of the keratin matrix. While industrial processes such as thermal treatment and chemical cleaning often result in severe genomic fragmentation, the structural components of the hair shaft, specifically the cuticle and medulla, remain intrinsically resistant to such degradation. By targeting these resilient morphological markers rather than labile DNA, this integrated approach ensures reliable halal verification even in highly processed or aged samples where genetic testing would be inconclusive.

4. Conclusion

The integration of chemical and microscopic data provides unambiguous verification of the fibre origins within the unidentified brush sample. The white fibres were conclusively identified as synthetic polymers, characterised by their chemical insolubility in NaOH, the evolution of latex-like volatiles during combustion, and a complete absence of medullary or cuticular architecture. Conversely, the dark brown fibers were definitively identified as pig-derived material based on three concurrent lines of evidentary support: (i) biochemical reactivity, evidenced by alkaline solubility and a characteristic sulphurous odour confirming a natural keratinous matrix; (ii) internal morphology, specifically the presence of a broad and distinct medulla; and (iii) surface topography, which displayed an imbricate, irregular wave scale pattern with heavily rippled margins and close inter-margin distances diagnostic of pig hair. Collectively, these findings confirm the presence of pig bristles in the sample submitted to MyHAC, validating this multi-platform methodology as a robust and reliable alternative for Halal compliance verification.

The proposed dual-platform methodology offers significant advantages, directly addressing compliance needs and operational efficiency in the halal sector. For the halal auditors and certification bodies, this method is critical for strengthening legal defensibility. It provides a robust, non-DNA verification tool to achieve definitive species confirmation when traditional DNA tests fail due to processing-induced degradation. This ensures stringent adherence to regulatory mandates (e.g., the Malaysian Halal Manual) and effectively manages the risk of *haram* material contamination in industries that utilise brushes.

For industry users (manufacturers and quality control personnel), this proposed method enables cost-effective and accessible screening. It utilises chemical and stereomicroscopy for rapid, initial differentiation between animal and synthetic fibers, reserving high-cost SEM only for final confirmation. Furthermore, it facilitates decentralised, rapid quality control at the point of manufacture or import, minimising disruptions and providing immediate assurance of raw material conformity to Halal standards.

In conclusion, by offering a reliable alternative to conventional DNA-based analysis for detecting pig hair in brushes, this chemical and microscopic approach establishes a new, practical benchmark for ensuring Halal compliance during food production and cosmetic application.

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6. AI declaration

The authors acknowledge the use of ChatGPT (OpenAI) and Google Gemini solely for language refinement and grammar checking during the preparation of this manuscript. The content, analysis, and interpretation presented are entirely the authors' own.

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