

**Application of ATR-FTIR spectroscopy and chemometric
techniques for the characterization of animal fiber-based
commercial brushes**

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Application of ATR-FTIR spectroscopy and chemometric techniques for the characterization of animal fiber-based commercial brushes

By

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**Proposal submitted in partial fulfillment of the
Requirement for the degree of Bachelor of Science (Honours) Forensic Science**

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CERTIFICATE

This is to certify that the dissertation entitled “Application of ATR-FTIR spectroscopy and chemometric techniques for the characterization of animal fiber-based commercial brushes” is the bona fide record of research work done by Mr Mohamad Amirul Hakim Bin Mohd Salimi during the period from October 2025 to February 2026 under my supervision. I have read this dissertation and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfillment for the degree of Bachelor of Science (Honours) Forensic Science.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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Mohamad Amirul Hakim Bin Mohd Salimi

Date:20/1/2025

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LIST OF SYMBOLS

C–O	Carbon–Oxygen (Single)
C=O	Carbonyl
C–N	Carbon–Nitrogen
N–H	Nitrogen–Hydrogen
S–S	Disulfide
C–S	Carbon–Sulfur
COO [–]	Carboxylate
CH ₂	Methylene
CH ₃	Methyl
O–H	Hydroxyl
C–O–C	Ether
cm ^{–1}	Square centimeter
%	Percentage

LIST OF ABBREVIATIONS

ATR	Attenuated Total Reflectance
FTIR	Fourier Transform Infrared Spectroscopy
PCA	Principal Component Analysis
SNV	Standard Normal Variate
2D	Two-Dimensional
3D	Three-Dimensional
PCs	Principal Components
USM	Universiti Sains Malaysia
RSD	Relative Standard Deviation
IBM SPSS	Statistical Package for the Social Sciences (IBM)

PENGUNAAN TEKNIK SPEKTROKOPI ATR-FTIR DAN KEMOMETRIK UNTUK PENCIRIAN BERUS KOMERSIAL BERASASKAN GENTIAN HAIWAN

ABSTRAK

Dalam penyiasatan forensik dan analitikal, pengenaltian bahan adalah penting, terutamanya apabila pembezaan gentian yang tepat diperlukan. Berus gentian haiwan kerap digunakan dalam persekitaran perindustrian dan artistik, walaupun ia boleh menjadi sukar untuk membezakan antara jenis yang berbeza kerana persamaannya yang ketara. Tujuan kajian ini adalah untuk menggunakan analisis spektroskopi dan pendekatan kemometrik untuk membezakan produk berus yang diperbuat daripada sampel gentian haiwan yang berbeza seperti kuda (A), kambing (B), kuda poni (C), tupai (D), musang (E), serigala (F), dan babi (G). Semua sampel telah diperiksa menggunakan spektroskopi inframerah (IR) jumlah pantulan dilemahkan - Fourier transform inframerah (ATR-FTIR) dalam julat 4000 cm^{-1} hingga 600 cm^{-1} . Spektrum IR pertama kali dibandingkan secara visual untuk mengenal pasti kumpulan berfungsi biasa yang berkaitan dengan gentian berasaskan protein. Walau bagaimanapun, pertindihan yang ketara antara spektrum menyukarkan untuk membezakan sampel dengan jelas menggunakan analisis visual sahaja. Analisis Komponen Principal (PCA) telah dilakukan pada data spektrum untuk kuasa diskriminasi yang lebih baik. Kawasan cap jari antara 1500 cm^{-1} hingga 600 cm^{-1} telah dipilih untuk dianalisis kerana ia mengandungi maklumat penting mengenai gentian. PCA hampir membezakan sampel berus berdasarkan jenis gentian haiwan. Jumlah variasi data dijelaskan oleh tiga komponen utama pertama, yang menyumbang 90.33%. Iaitu PC1, PC2, dan PC3, yang masing-masing menyumbang 63.96%, 16.77%, dan 9.6% varian. Kajian ini menunjukkan bahawa penyepaduan spektroskopi ATR-FTIR dengan PCA adalah teknik yang mudah, pantas dan tidak merosakkan untuk membezakan berus gentian haiwan, justeru memudahkan aplikasi dalam analisis forensik yang berkaitan dengan jenayah hidupan liar, pengenaltian bahan dan kawalan kualiti.

APPLICATION OF ATR-FTIR SPECTROSCOPY AND CHEMOMETRIC TECHNIQUES FOR THE CHARACTERIZATION OF ANIMAL FIBER-BASED COMMERCIAL BRUSHES

ABSTRACT

In forensic and analytical investigations, material identification is crucial, particularly when precise differentiation of fibers is required. Animal-fiber brushes are frequently employed in industrial and artistic settings, although it can be challenging to distinguish between different types due to their striking similarities. The purpose of this study is to use spectroscopic analysis and chemometric approaches to distinguish and examined brushes product made from different animal fibers samples such as Horse (A), goat (B), pony (C), squirrel (D), weasel (E), wolf (F), and pig (G). All samples were examined using Attenuated Total Reflection–Fourier Transform Infrared (ATR-FTIR) spectroscopy in the range of 4000 cm^{-1} to 600 cm^{-1} . The infrared spectra were first compared visually to identify common functional groups related to protein-based fibers. However, strong overlap between spectra made it difficult to clearly distinguish the samples using visual analysis alone. Therefore, Principle Component Analysis(PCA) was performed on the spectral data for better discriminatory power. The fingerprint region ranging from 1500 cm^{-1} to 600 cm^{-1} was selected for analysis since it contains valuable information regarding the fibers. PCA nearly discriminated the brush samples based on the types of animal fibers. The total variation of the data was explained by the first three principal components, which accounted for 90.33%. They were PC1, PC2, and PC3, which accounted for 63.96%, 16.77%, and 9.6% pf variance, respectively. This study demonstrates that the integration of ATR-FTIR spectroscopy with PCA is a straightforward, rapid, and non-destructive technique for distinguishing animal-fiber brushes, hence facilitating applications in forensic analysis related to wildlife crime, material identification, and quality control.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Animal hair fibers have long served as valuable trace evidence in forensic science due to their distinctive morphological characteristics and resistance to degradation. Referring to Yahmed et al. (2018), the forensic examination of animal hair can provide crucial evidence linking suspects to crime scenes, victims, or specific locations, as the transfer of pet hair or fibers from animal-hair products is common during physical contact. Historically, identification has relied on microscopic analysis of key features such as the cuticle scale pattern (imbricate or coronal), the type and diameter of the medulla (continuous, discontinuous, or fragmental), and the distribution of pigment granules (Yahmed et al., 2018) . For example, a study of domestic animals in Upper Egypt established a reference database using these features, noting that donkey hair has unique coronal scales while sheep hair possesses a fragmental medulla. Beyond criminal investigations, this analysis is vital for diagnosing toxic exposures and combating illegal wildlife trade, where the identification of protected species from hair evidence is frequently required. A prominent historical and ongoing application according to Jose et al. (2024) is in wildlife forensics, particularly involving mongoose hair (from species like the Indian grey mongoose), which is illegally poached to manufacture high-quality painting and shaving brushes. Law enforcement seizures, such as the recovery of over 160,000 mongoose hair brushes in India between 2002 and 2021, underscore the scale of this trade and the forensic need to accurately identify the species of origin from hair samples found in brushes or during investigations (Jose et al., 2024).

According from Polaris Market Research (2023), the commercial hair brush market, valued in the billions of dollars, extensively utilizes animal hair fibers, creating a direct intersection with forensic needs for product verification and wildlife crime investigation. Brushes are manufactured from a variety of materials, with a significant segment dedicated to natural bristles sourced from animals like boar, as well as from protected species like mongoose. The market shows a growing trend toward sustainability and customization, with consumers seeking both synthetic and organic options, the latter including natural fibers like plant-based materials and animal hair (Trivedi, 2023). This commercial demand drives both legal trade and, unfortunately, the illegal poaching of protected animals for their hair. To address the challenge of identifying these often-processed and mixed fibers, advanced chemometric techniques have become essential. According also from Jose et al. (2024), traditional microscopy can be subjective and struggles with processed hairs where roots are absent or fibers are blended. Therefore, he suggested that analytical methods like Fourier Transform Infrared (FTIR) spectroscopy, combined with chemometric tools such as Principle Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) as it offer a rapid, non-destructive, and objective solution. These techniques analyze the biochemical composition of keratin in the hair, creating spectral fingerprints that chemometric models can use to accurately discriminate between species for instance, distinguishing protected mongoose hair from domestic cattle, human, or synthetic fibers in a seized brush (Jose et al., 2024). Hence as summarization for this study, is to practice this combination of spectroscopy and chemometrics in hoping for forensic validation, quality control in the textile and brush industries, and enforcing wildlife protection laws.

1.2 Problem Statement

Although is been used for a long time, a critical research gap exists in developing rapid, accurate, and non-destructive methods for identifying and characterizing animal fiber types, especially in commercial product such as brush, particularly given the prevalence of mixed materials in the product and a certain chance for mislabelling or wrong description on the packet or the website. Some also may be counterfeit product where some supplier may use fake animal hair to the brush manufacturer to cut the cost to make the brush. The challenge of fiber identification also may come from the remarkable similarities in chemical and physical characteristics between different animal fibers, which is being build primarily of the strong disulfide bonds and protein keratin with high sulfur content that make them resistant to various chemical agents and change their solubility in water. Furthermore, the manufacturing process will alter their chemical and physical properties of the hair by various process such as cleaning, bleaching, dyeing, and chemical modifications to enhance their performance.

Currently, identification of fiber are mostly focus on single-method approach and which is widely known and classical The majority of current identification approaches are based on morphological methods that use light and scanning electron microscopy (SEM). These traditional methods are prevalent in small industries as they are the most affordable alternative especially light microscope. However, these methods are criticized for being subjective and needing highly skilled operators (Zoccola et al., 2023). These restrictions are especially noticeable for small and medium-sized businesses (SMEs), who are the foundation of the brush manufacturing industry but do not have access to specialist analytical knowledge. Furthermore, considering the wide pricing differences between various animal fibers and the growing labeling requirements for textile items under laws like EU Regulation No 1007/2011, the legislative and commercial ramifications of precise fiber identification are substantial (Zoccola et al., 2023). Therefore, to counter this problem, Attenuated-Fourier Transform Infrared (ATR-

FTIR) spectroscopy can be suggested for other relevance method because of their significance as a powerful analytical technique for molecular characterization across diverse fields, with recent advancements highlighting particular efficacy in analyzing biological materials including natural fibers (Ali et al., 2025 and Workman, 2024).

The technique operates on the principle that molecules absorb specific frequencies of electromagnetic radiation in infrared region which will influence their vibrational motion corresponding to their chemical structure and bonding, generating a unique spectral fingerprint peak for each material based on vibrational motion that matches the IR radiation transmitted (de Bortoli et al., 2025). Each molecules produce distinct spectral bands that are helpful for identification because they absorb infrared radiation at different frequencies. ATR attachment in FTIR spectroscopy offers several advantages for fiber analysis, including minimal sample preparation, rapid analysis times, can operate effectively with solid materials and non-destructive characterization that preserves sample integrity (Sharma et al., 2024). However, certain limitation involves where ATR-FTIR only is not enough as such been said before like similarities in chemical and physical characteristics between different animal fibers, contaminant from the manufacturing process which can create a complex and blended spectra that may hard to differ by eyes alone.

As ATR-FTIR spectrum produce is highly compact with various of compound, the integration of advanced chemometric methods like Principle Component Analysis (PCA) and Partial Least Square-Discriminant Analysis (PLS-DA) with ATR-FTIR can further enhanced its capability for accurate classification of materials with subtle spectral differences. This making it particularly suitable for distinguishing between closely related animal fibers in commercial brush products (Sharma et al., 2024). PCA is one of the chemometric techniques that has been used by previous studies where it can allows large sample of data sets to be

described using a much smaller number of principal components (PCs). PCA combine with ATR-FTIR technique had enabled the examination of patterns in the examined samples as well as correlations or variations among them in FTIR spectrum as it (Ismail et al., 2021).

1.3 Objectives

General Objective: To utilize ATR-FTIR spectroscopy for characterization of natural fibers in commercial brushes

Specific objectives:

- To acquire IR spectra of commercially available animal based brushes product.
- To determine the chemical composition from the acquired IR spectra.
- To develop chemometric models using including Principle Component Analysis (PCA) to identify fiber types based on their spectroscopic signatures.

1.4 Significance of study

Forensically, this study provides the solely needed reference database for trace evidence. By morphological and chemical profiling of everyday brush fibers, it enables forensic experts to more precisely identify fibers found at a crime scene. This can powerfully link a on the species identification of the animal in certain case such as a unique artist's brush, wildlife crime like illegal import or export of endangered animal species, poaching, poisoning smuggling of rare animal parts or organ, hit and run in certain case to estimate a halal status application of the said product. Besides, certain fiber product that been stolen also may be unique to certain customers since it made from specific animal like Kolinsky sable which said by several internet sources, like Business Insider, reported that the price could reach over \$300. Therefore, this study may help to create a library where it can increase the usefulness of fiber evidence in criminal investigations.

This study has very significant practical value to both consumers and producers of brushes. It offers a fast, certain method to verify bristle material, preserving quality control and avoiding mislabeling. This benefits consumers like artists to make well-informed decisions and make regulatory adherence to labeling laws easier, all through a non-destructive operation that does not compromise product integrity.

Academically, the project encourages materials science by announcing the unique spectroscopic signatures of a variety of animal and man-made bristles. It bridges a gap between industrial practice and analytical chemistry by forming a baseline methodology that can be utilized in coming studies of biological materials and stimulate the use of advanced data analysis for fiber identification in other fields such as skin, bone and nail.

CHAPTER 2

LITERATURE REVIEW

2.1 Fiber

A fibre is a thin, flexible strand which came from natural or synthetic sources and they are widely used in textiles and consumer products. Current advance in technologies has exploit the used of fiber in many application. In forensic science, fibres are important trace evidence because they can transfer between people, objects, and crime scenes. Natural fibres come from plants (cellulose-based) or animals (protein-based), while synthetic fibres are man-made polymers like nylon or polyester. Each type has distinct physical properties (such as diameter, colour, cross-section, and surface pattern) and chemical characteristics (chemical reactivity, molecular composition, functional groups, elemental content, oxidative and thermal stability) that make identification and characterization possible in forensic investigation.

2.2 Animal fiber and application in commercial product

Animal fibre is one type of fiber under natural fiber type which obtained from the protective coats or fleece of mammals, such as sheep, goats, alpacas, or pig, with their primary and defining chemical component being the durable, structural protein known as keratin ,a complex protein built from amino acid chains that form a hierarchical structure with robust chemical cross-links that give it superior elasticity, thermal insulation, moisture regulation, and softness compared to many plant or synthetic alternatives (Lab, 2025). This chemical properties can be use to distinguish animal hair fibres from synthetic counterparts that derived from petrochemicals like polyester or nylon by their biological origin and unique molecular structure. According to Xiao and Hu in 2016, this protein-based foundation provides two critical mechanisms which are permanent disulfide bonds between cysteine amino acids that create a stable, resilient network that maintains the fibre's shape and strength and second, a

multitude of reversible hydrogen bonds within and between protein chains act as molecular switches. These bonds are temporarily broken by moisture (like water or steam), allowing the fibre to be reshaped, and then re-form as it dries, locking in the new form or helping it recover to its original state. This sophisticated, dual-bond system grants animal fibres their characteristic resilience, elasticity, and unique "shape memory" properties, which are absent in the simpler, fully synthetic polymer chains of man-made fibres (Xiao and Hu, 2016).

Despite the development of synthetic-made brush that produce massively, the brush manufacturing industry still relies on animal-based fibers (Nandi, 2025). The observation sourced from online market like Shopee and Lazada had showed several brushes from various species including hog, goat, horse, pony, squirrel, weasel, wolf and some even combine the fibers (natural such as pig and synthetic like acrylic) which improve their properties and can be buy with affordable price. The natural fiber was approved to be still significant as materials to make brush because of their unique mechanical properties and application-specific performance when synthetic fiber are not optimal. These fibers offer distinct advantages over synthetic alternatives, including superior heat resistance, reduced electrostatic charge, biodegradability, and enhanced softness and fineness that enable access to hard-to-reach areas (Carolina Brush, 2025). Plus, this aligning with the growing consumer preference for organic and eco-friendly products where the market is expected to grow significantly as consumers seek hair care solutions that align with their environmental and health-conscious values (Trivedi, 2023).

2.3 Animal fibers as evidence

Animal fibre or animal hairs are is one of trace fibre evidence in forensic investigations since late 1800s because they carry species-specific microscopic, molecular signatures, and chemical signatures that can linked the suspect, victim, or the crime scene to the related investigation. For example, a 2024 study used FTIR spectroscopy combined with chemometric analysis to distinguish mongoose hair from cattle hair, human hair, and synthetic fibres with strong discrimination (high R^2 and low RMSE) for wildlife forensic purposes (Jose et al., 2024). Similarly, Manheim et al.(2016), demonstrated that hair of humans, cats, and dogs can be reliably distinguished non-destructively and rapidly using spectral methods, with zero false positives/negatives for human hair in external validation. This can further help the investigation of crimes especially the collected hair sample that been taken from the crime scene. Such as Alfred Swaine Taylor, a pioneering British toxicologist and medical jurist in 1894 which demonstrated that animal hair evidence was critical in demonstrating a suspect's innocence in a case. His judgment managed to prove the hair discovered on the suspected murder weapon was of animal origin rather than human (Tridico, 2005).

2.4 Current Forensic Analysis of Fiber

2.4.1 Microscopy Technique

According to Zoccola et al. (2023), identification of animal fibers has traditionally relied on morphological methods using Light Microscopy (LM) and Scanning Electron Microscopy (SEM), as standardized in ISO 17751-1:2016 and ISO 17751-2:2016. Overall, These methods focus on characteristics including cuticular cell morphology, pigment distribution, and medulla structure. The LM are benefit from their minimal sample preparation, low cost, easier handling of the instrument. It has good advantages to observe the surface like the color, length, thickness, cross-section and internal structure like the hair pigment distribution, medulla, and cortex. The limitation of LM can be caused by the external light or

poor resolution that interfere the observation of pigment in the hair internal structure, challenge of analyzing blended fibers or finished products where multiple fiber types may be present and lack of analyzation of composition material that make the hair (Zoccola et al., 2023).

Other microscopy method that are well known for this is SEM which work by scanning a sample with a focused beam of high-energy electrons, and then detecting the signals that are produced from the interaction of the beam with the sample's surface which mostly a secondary and backscattered electrons. These observed signal give information of external morphology, chemical composition, and crystalline structure of the sample (Mahadeshwara, 2025). the advantages of SEM are related to high magnification and resolution, which allow for measuring the thickness of cuticular cells greater than 0.6 μm for wool and less than 0.5 μm for fine animal fibers. disadvantage consists of the possibility of examining only the surface characteristics of the fibers without investigating the medulla and pigment distribution. disadvantage consists of the possibility of examining only the surface characteristics of the fibers without investigating the medulla and pigment distribution and their high cost for usage (Zoccola et al., 2023).

2.4.2 DNA analysis

Alternative approaches such as electrophoresis offer high specificity. According to M. Zoccola article, she mention the research conduct by her and her colleagues (2018) and also refer to article conduct by Marshall et al. (1984), this method can be divided into two types which is in one-dimensional gel electrophoresis, where the proteins extracted from fibers by reducing the disulfide bonds are separated according to their molecular mass, while in two-dimensional gel electrophoresis, proteins are separated according to their isoelectric point and in the other dimension according to their molecular mass. Both manage to analyze the different based on their sulphur protein separation pattern. However, the main problems born from the

possibility of low protein extract from many hair samples , extreme weathering and industrial processes which seriously affect their quantitative determination (Zoccola et al., 2023).

2.4.3 Spectroscopy Technique

Spectroscopy relies upon the fact that molecular bonds resonate at specific frequencies. When infrared radiation is absorbed by a sample at frequencies equal to its natural vibration frequencies of its molecular bonds, it stretches, bends, or rotates the bonds. The unique pattern of frequencies absorbed produces a "molecular fingerprint" which is applied in the identification of material. Different regions of the electromagnetic spectrum, such as Near-Infrared (NIR) and Mid-Infrared (MIR) was used to to analyze samples by measuring how light interacts with them,

2.4.3.1 NIR

Recent research has explored spectroscopic approaches to overcome morphological analysis limitations such as in NIR spectroscopy is the most studied and the most promising one (Zoccola et al., 2023). Absorption in the NIR field is associated with overtone and combinations of vibrations of the chemical bonds, mainly R-H, and physical characteristics of a material, such as sample size and surface scattering. spectra absorption intensity at different wavenumbers is another difference, and it is imputable to a different scattering of the NIR radiation with is correlated with physico-morphological characteristics of samples such as the fiber diameter, pigment distribution, the presence or absence of medulla, and the shape and distribution of cuticular cells. Near-Infrared (NIR) spectroscopy combined with chemometric models has shown promise for classifying animal fibers, with one study achieving 100% sensitivity and specificity for identifying sheep cashmere, cashmere, rabbit, and camel fibers using Partial Least Squares-Discriminant Analysis (Chen et al., 2019). NIR spectroscopy has the advantage of being a fast and non-destructive technique, able to be employed directly on

the production line or using portable instruments but is the time-consuming calibration of the methods. Acquired spectra are then evaluated using modern chemometric methods (Zoccola et al., 2023).

2.4.3.2 FTIR

Fourier Transform Infrared (FTIR) is a highly developed form of infrared (IR) spectroscopy that rapidly records a full mid-infrared spectrum. It uses a mathematical process called a Fourier transform to convert the raw data (an interferogram) into the final, accurate spectrum. FTIR typically use MID-IR range and work by using a Michelson interferometer that splits and recombines an infrared light beam, creating an interference pattern that is converted to a spectrum using a Fourier transform. Peets et al. (2017) studies investigating FTIR spectroscopy for textile fiber analysis have demonstrated excellent discrimination capability, with successfully classifying 89 textile samples of 26 varieties using ATR-FTIR and chemometric approaches. Similarly, in recent work as a proof-of-concept study, illustrating the potential of ATR-FTIR spectroscopy in animal fibers identification by Sharma et al. (2024), had obtained a complete differentiation between cashmere, angora and shahtoosh using FT-IR spectroscopy coupled with Partial Least Squares Discriminant Analysis (PLS-DA).

2.5 ATR-FTIR Spectroscopy in Fiber Analysis

According to Gong et al. (2024), there are several methods that are included in the spectroscopy, for example, transmission, reflection, and Attenuated Total Reflectance (ATR) and ATR-FTIR's biggest advantage is its simple workflow where its often requires only placing the sample on the crystal and pressing which makes it faster, easier for inexperienced users, and suitable for a wider variety of samples like pastes, powders, and rub. ATR-FTIR spectroscopy measures how much infrared light a sample absorbs when it comes into contact with a high-refractive-index crystal where a portion of the infrared light is absorbed and the

remainder is reflected back when it contacts with the sample at the crystal-sample interface like be shown in Figure 1. This leaves distinct vibration for information about the sample's composition, molecular structure, and chemical characteristics can be gleaned by examining the ensuing infrared spectrum (Specac, 2023).

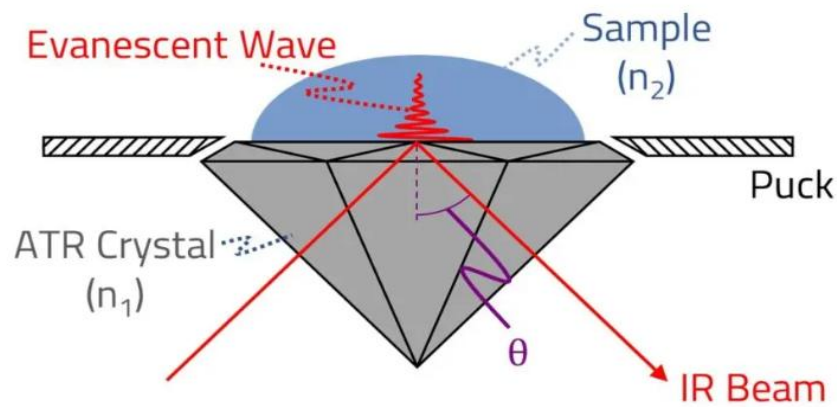


Figure 1: show the work principle of ATR-FTIR

Referring to Ali et al. (2025), FTIR has long been applied to the analysis of fibers, beginning in the 1930s when the technique was first applied to the examination of cellulose hydroxyl groups. In latter decades, scientists continued to develop the identification of absorption peaks in the IR spectra of cellulose and lignin, while hemicellulose absorption peaks were investigated in the 1950s. Recent advances in technology have significantly expanded the capabilities of what can be done by FTIR, with the introduction of new ATR procedures and superior data-processing procedures such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) modelling (Workman, 2024). These developments have made the application of FTIR an extremely useful way of rapidly and non-destructively characterizing fibrous materials, so that both the identification of the kinds of fiber present as well as their composition can be determined (Ali et al., 2025).

There are wide of ATR-FTIR application nowadays that can be used for scientific or industrial or forensic work. An interesting example of the use of FTIR for animal fiber characterization is provided by synchrotron ATR-FTIR microspectroscopy (S-micro FTIR or LUMOS), which has been applied to the analysis of the conformation of individual animal silk fibrers (Ye et al., 2021). The technique provides distinctive strengths in ultrahigh brightness and high spatial resolution to characterize small-sample materials, allowing detailed structure analysis of the animal silk's secondary structure, which is essential to the fibrer's mechanics. Although standard FTIR spectroscopy generally characterizes fiber bundles, the advanced capabilities of the synchrotron-based methods illustrate the potential for yet more detailed characterization of individual animal fibrers as the user also can capture the image proceed to review the structure for further analyzation.

According to Specac. (2023), although ATR-FTIR has the benefit of requiring little preparation, its shallow and inconsistent penetration depth and the need for ideal surface contact are its main drawbacks when it comes to examining fibers or animal hair. Since, it usually only probes a few microns into the sample, the technique is by its very nature a surface analysis method. The signal mostly originates from the cuticle or surface coating of a fiber or hair due to this shallow depth of penetration, which diminishes due to absorption and scattering process at higher penetration (shorter wavelengths), thereby omitting crucial chemical information from the internal cortex or medulla. Furthermore, obtaining a high-quality spectrum requires a flat sample region to be in uniform, intimate contact with the ATR crystal, which can be challenging with the naturally curved, cylindrical, or sometimes uneven surfaces of individual hairs or textile fibers, leading to poor or inconsistent spectral data

2.6 Chemometrics

Chemometrics is a subfield of analytical chemistry that uses statistical and mathematical techniques to extract meaningful information and patterns from chemical data that are not visible through simple observation. It uses methods like pattern recognition, multivariate calibration, and classification to turn complex, overlapping spectral data, like that from animal hair ATR-FTIR analysis, into objective, trustworthy models that can precisely identify the species of origin, quantify components, or classify unknown samples based on their chemical fingerprints (Chu et al., 2022). The core of this method include multivariate data analysis, which is referred to as pattern recognition methods, where the samples will be categorized based on their class with statistical confidence. There pattern recognition that will be used which is unsupervised method (PCA)

2.6.1 Principle Component Analysis (PCA)

Following spectral pre-processing, an unsupervised Principal Component Analysis (PCA) using SPSS since it provides a simple, code-free interface with built-in tests to check data suitability. It also offers standard extraction methods and rotations, while automatically generating publication-ready results .According to Aljannahi et al. (2022) and Ismail et al. (2021), PCA software is used to explore the intrinsic clustering and major sources of variance within the ATR-FTIR spectral data to reduces the high-dimensional spectral data which is absorbance values at numerous wavenumbers into a smaller set of new, orthogonal variables called Principal Components (PCs) that capture the maximum variance in the dataset.

The analysis began by examining the scores plot of the first two principal components (PC1 vs. PC2). In this plot, fiber samples with similar chemical compositions are expected to cluster together. This allows for the identification of natural groupings corresponding to major fiber types without prior labeling of the samples (Geminiani et al., 2022). This step is crucial

for detecting outliers and understanding the natural structure of the data. Subsequently, the loadings plot for each PC will be analyzed to interpret the chemical basis of the separation. Peaks in the loadings plot indicate the specific wavenumbers that contribute most to the variance captured by that component. For instance, a loadings peak might correspond to the C=O stretch which common in synthetic polyesters or the broad O-H and C-O stretches associated with cellulose in natural plant fibers, providing a chemical interpretation for the clustering observed in the scores plot (Misra et al., 2025)

2.6.2 Pre-processing method

SNV (Standard Normal Variate) is a mathematical pretreatment method used in spectroscopy, like ATR-FTIR, to correct for unwanted scattering effects and baseline shifts in spectral data. PCA and SNV collaborate in a sequential manner where PCA firstly identifies the primary sources of variance in your data and condenses them into a small number of "Principal Components" (PCs). The objective is to see distinct groupings in a basic 2D or 3D graphic, such as horse hair versus goat hair. However, PCA will waste its initial and most crucial PCs simply modeling that useless scattering noise if the biggest variation in your raw data is caused by scattering rather than chemistry. SNV is used to "pre-clean" the data before PCA, hence SNV guarantees that the significant variations PCA finds are genuinely connected to the biochemical differences between samples by eliminating the scattering effects (Workman, 2025). This results in much clearer and more dependable clustering and classification.

CHAPTER 3

METHODOLOGY

3.1 Research Design

This study's aims are to use spectroscopic and chemometric analysis to examine, distinguish, and describe type of brush fiber from various animal hair. Both qualitative and quantitative research are included in this study. ATR-FTIR spectroscopy was used to evaluate brush samples from seven type of animal hairs in order to identify the functional groups that were present in the brush. The PCA, an unsupervised pattern recognition method based on data from ATR-FTIR spectroscopy, was then used to distinguish between them.

3.2 Study Scope

The study's scope was restricted to brush samples from the animal fiber. All sorts, brands, and size of brush samples were utilized. Only the plain patch on the brush taken was used for this investigation in order to rule out any potential interference from the ink compound.

3.3 Sampling method

The research employed a comprehensive sampling strategy encompassing both reference materials and commercial brush products. Approximately seven (7) brush samples were bought from online market and from various brand to ensure representation of major synthetic fiber and natural hair fiber types which is horse, goat, pony, squirrel, weasel, wolf, and pig.

3.4 Sample Size

two random samples from each seven brush samples were chosen. Therefore, fourteen (14) samples was be used in this study

3.5 Research tool and Methodology

3.5.1 Sample Collection and Preparation

All of them were labelled as alphabet A, B, C, D, E, F and G respectively and each 3 individuals were labelled with number like shown in the table. Sample authentication followed a two-ways approach. Initially using ATR-FTIR followed by chemometrics analysis. Table 1 show how indication of sample labelling was be done.

Table 1: Labelling of seven different animal types used in this study.

Animal type	Label
Horse	A1-A2-A3
Goat	B1-B2-B3
Pony	C1-C2-C3
Squirrel	D1-D2-D3
Weasel	E1-E2-E3
Wolf	F1-F2-F3
Pig	G1-G2-G3

Sample preparation would strive for minimal processing so that natural fiber properties are preserved while reproducibility ensured. By using scapel and forceps, three of the whole brush fibres from the same type were cut and labelled with number (example A1 for individual 1 for horse fiber or sample ‘A’). Referring to Figure 2, all three individuals were put on the glass slide with a double-sided tape to make sure it mounted hardly on glass slide and avoid the sample been removed when ATR crystal touch the specimen or disturbed the reading. All

of the slide was labelled with their specific alphabet to make writing more easy and avoid error of human error like running the same sample. The glass slides with the samples were stored inside the slide box to avoid any contamination from the environment. Contaminants, residuals from processing, and oils would be eliminated without disrupting keratin structure through cleaned using distilled water. The cleansed samples would then be dry under stable $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ temperature for at least 3 minutes on tissue paper so that standardization of environmental effect on spectral characteristics would be achieved.

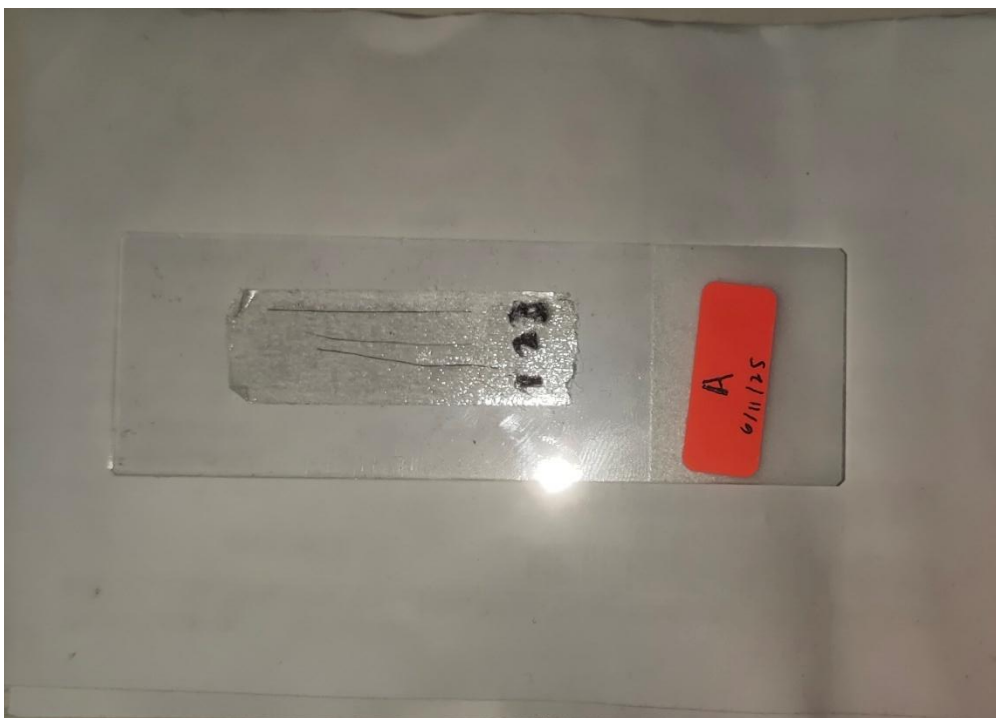


Figure 2: Example of prepared sample A

3.6 Physical Examination

The morphological characteristic, pigment distribution and medulla structure of the animal hair samples were examined using LUMOS microspectroscopy to analyze physical examination that cannot be done by naked eye only. Every trait was noted and observed.

3.7 Data Collection with FTIR Spectroscopy Analysis

Bruker Lumos FTIR spectrometer with a germanium ATR attachment for sampling accessory was used for spectral acquisition; this equipment are include with 3 type of measurement which is reflection, transmission and Attenuated Total Reflection (ATR) which can help to give molecular of organic and inorganic materials of samples provided by command the instruction and view the IR spectra with OPUS software that connected with the instrument. A joystick controller that already been calibrated first, then used to pin seven points each on all 3 sample A in a linear position and the ATR pressure was set to 'medium' mod. In order to guarantee a sufficient signal-to-noise ratio while preserving realistic analysis durations, the spectral range was adjusted to $4000\text{-}600\text{ cm}^{-1}$ (midIR-region) with a resolution of 4 cm^{-1} , accumulating 32 scans per spectrum (de Bortoli et al., 2025), like was shown in Table 2. The benefits of this setup for brush fiber analysis include compatibility with solid fibrous materials, excellent sensitivity, and low sample preparation (Margariti, 2019).

Table 2: ATR-FTIR Spectroscopy Parameters

Parameter	Specification	Rationale
Spectral Range ATR-FTIR	4000-600 cm^{-1}	Comprehensive coverage of molecular vibrations
Resolution	4 cm^{-1}	Optimal balance between detail and scan time
Number of Scans	32	Enhanced signal-to-noise ratio
ATR Crystal	Germanium	Durability and contact efficiency with fibers
Acquisition Mode	ATR	Non-destructive analysis

As a notes. Nitrogen liquid was utilized to lower the instrument's noise, control heat, and cool the detector as thermal processes heated the detector. Filling the FTIR spectrometer with nitrogen liquid into the cooling chamber of spectrometer and allowed it rest to cool for 40 minutes after filling had increased back the performance of this instrument and avoid over heating. The samples that set up on the LUMOS ATR-FTIR spectrometer's stage was be weighted or glued with plasticine clay to make sure it was positioned around the area that would be scanned and to prevent any random error that would affect the spectra's outcome such as glass side moving when touched by ATR crystal, winds or vibrations from surroundings. Plus, the surface picture of the sample that was viewed from the microscope was captured and and to avoid scanning error, the background of the samples was also measured prior to the spot being scanned. The spectrum data collect was viewed in OPUS software and saved in DPT format once the spot was scanned.

After IR spectra been averaged(21 into 7 spectra) and was make sure to covered all spectra range ($4000\text{--}600\text{ cm}^{-1}$)generated , the fingerprint region ($1500\text{--}600\text{ cm}^{-1}$) of taken analysis which concentrated on and functional group region($4000\text{--}1500\text{ cm}^{-1}$) was cut out, as fingerprint region provide significant peaks for natural fibres, such as characteristic peaks of polyethylene glycol, O-H bending in alcohol, C-N and O-H stretching, C-O-C stretching in tertiary alcohols is present at wavenumbers $1414\text{--}1405$, 1365 , 1336 , $1135\text{--}1105\text{ cm}^{-1}$, respectively. Further, prominent peaks at $875\text{--}868$, $728\text{--}718$, and 509 cm^{-1} are also present in natural fibres due to the presence of C-H stretching out-of-plane bending vibration, C-H deformation in alkynes, and metal oxygen binding vibrations, respectively (Sharma et al., 2024). Furthermore, in the case of animal fibers, particular emphasis will be placed on bands for Amide I ($1600\text{--}1700\text{ cm}^{-1}$), Amide II ($1480\text{--}1575\text{ cm}^{-1}$), and Amide III ($1220\text{--}1300\text{ cm}^{-1}$) due to their association with protein backbone shape and keratin-based materials discrimination (Ye et al., 2021).

Other study conducted by Yin et al. (2013) summarized that in their study of feather keratin where the IR spectrum shows characteristic absorption bands at around 1643 , 1527 , and 1237 cm^{-1} , which correspond to peptide bonds (--CONH--) known as amide I, amide II, and amide III, respectively. Amide I, appearing in the $1700\text{--}1600\text{ cm}^{-1}$ range, is mainly due to C=O stretching while Amide II, found between 1540 and 1520 cm^{-1} , arises from N-H bending combined with C-H stretching, and amide III, occurring between 1220 and 1300 cm^{-1} , results from the combined vibrations of C-N stretching and C=O bending. Additional peaks at about 990 and 580 cm^{-1} are characteristic of C-S and S-S bonds, indicating sulfur-containing amino acids. After referring above research and other source material, Table 3 below was created to simplify and helping in identification of IR spectrum in my study. Furthermore, all spectra will be taken seven time at same location to ensure measurement consistency as well as random sampling of fibers from each sample in in 7 different location to ensure reproducibility of the

ATR-Spectra data and the acquired spectra will the undergo data pre-processing for baseline correction and normalization.

Table 3: Related wavenumber range involves from previous study

Wavenumber Range (cm ⁻¹)	Functional Group / Band	Vibrational Assignment
1600–1700	Amide I	C=O stretching (peptide bond)
1480–1575	Amide II	N–H bending + C–N stretching
1250–1500	CH ₂ /CH ₃	Bending
1220–1300	Amide III	C–N stretching + C=O bending
1414–1405	O–H / PEG-related	O–H bending (alcohols, PEG)
1365	C–N / O–H	C–N stretching / O–H bending
1300–750	C–O / C–N	C–O or C–N stretching
1135–1105	C–O–C	C–O–C stretching (3° alcohols)
990/750–550	C–S	C–S stretching
875–868	C–H	Out-of-plane C–H bending
728–718	C–H	C–H deformation (alkyne-related)
580/540–400	S–S	Disulfide bond vibration
509/800–200	Metal–O(excluded) / S–S	disulfide vibration

3.8 Principle Component Analysis (PCA)

There are several software that can be used such as R, Python, SIMCA or Minitab but Statistical Package for the Social Science (SPSS) was used instead all of that to run the PCA for their availability, especially for student with no computer based knowledge. Firstly, the data sets of result analysis of all samples usually was automatically in OPUS format to make it easy to read in spectrophotogram format. The data need to be convert into datapoint format first , where it can be read and edit in Excel software . In Excel, the raw data are in vertical orientation, hence, new sheet will be open to transposed all initial data into horizontal position, hence gave permission for SPSS to read the data and avoid error after it been transfer as usually there is limited row that can be input inside SPSS.

The wavenumber were labelled and the data are categorized by specific labelled based on their type which are natural brush consist of natural hair from animal such as pony, horse, goat, pig, wolf, weasel, squirrel. All initial data are usually covering all infrared region which

consist of functional group region where it used to identify the presence or absence of specific functional groups, such as carbonyl (C=O), and N-H bonds. The bands in this region are usually characteristic of small groups of atoms, and their positions remain relatively constant regardless of the rest of the molecule's structure. Then, fingerprint region where the intricate pattern of multiple absorption bands in this region was unique to the molecule as a whole, similar to a human fingerprint. These bands result from various complex skeletal vibrations, including, C-O, C-N single bond stretches, and bending vibrations. The fingerprint region was used since the focus was on identification of a specific compound by compared its spectrum to a known reference spectrum or library database instead of determined the type of compound being analyzed like in functional group region. Furthermore, to avoid error during reading SPSS the initial data was cut short into fingerprint spectra region where it was ranged between 1500-600 cm^{-1} .

According to Sharma et al. (2024) pre-processing is an important step to be carried out before applying chemometrics. Errors in the spectral data could have an impact on the outcome. So, the data is pre-processed using normalization (SNV technique) in order to prevent spectral inaccuracies and reduce noise on the background. Machine learning methods are then used to classify this processed data.

After the pre-process data was prepared, then its ready to import inside SPSS. Initially, the first row was must contain the wavenumber of finger print region that was included in this analysis. Then, analyse button, dimension and factor analysis was clicked to instruct the SPSS to put and save all the wavenumbers in exception of the string data as the variables. At the most bottom row, unnecessary reading was removed (FAC1_1, FAC1_2, FAC1_3...etc.) in exception of three of it as it needed to change their name into Principle Component (PC1, PC2 and PC3) for better indication. Graph option, scatter plot or 3D scatter plot was then clicked to