

Application of an Unsupervised Chemometric Technique with FTIR Spectroscopy in Leather Material Classification

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This study presents a novel approach for leather classification utilizing Fourier Transform Infrared (FTIR) spectroscopy coupled with an unsupervised chemometric technique. The effectiveness of FTIR in capturing the chemical profiles of various leather types was investigated, and principal component analysis (PCA) was applied to categorize these profiles. Animal leather samples, specifically cow, buffalo, goat, and pig skin, were compared with polyurethane. All samples were analysed by FTIR without any pre-treatment, followed by a principal component analysis (PCA). The combination of FTIR spectral data and advanced multivariate techniques enabled precise differentiation among the leather samples, offering a robust method for quality control and authenticity verification in the leather industry. Our findings demonstrate that this integrated approach significantly enhanced the accuracy of leather classification, paving the way for improved materials analysis and classification standards.

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For centuries, human beings have utilized leather in various daily activities. Early civilizations obtained leather through drying and preserving animal skin, and used it for shelter, clothing, and footwear. Leather preparation methods have evolved over time, and with the advancement of technology. Leather is an expensive material due to the laborious methods it requires for production. Today, leather is widely used for clothing, footwear, handbags, upholstery, automotive accessories, and sports equipment. In modern culture, fashionable apparel and accessories made with premium quality leather are very expensive and considered luxury items. The development of synthetic polymers has resulted in an alternative that is cheaper, easier to manufacture, and customizable [1].

Synthetic polymers can be tailor-made to a specific use or design. For example, artificial leathers are synthetic polymers made to imitate and emulate the appearance, texture, and quality of authentic leather [2]. Despite advancements in synthetic alternatives, consumers continue to greatly value authentic leather products for their unique properties. In contrast to artificial leather, real leather has a breathable and thermal insulating quality that contributes to their durability and longevity [1]. The competition between authentic leather and artificial leather has led to an increase in fraud in the industry. Products made with artificial leather are often claimed otherwise and sold at a higher price for monetary gain [3].

As a consequence, investigations have been ongoing into suitable methods to verify the authenticity of leather products. Methods such as scanning electron microscopy (SEM) and digital microscopy are examples of imaging analyses that provide visual characteristics of the structure of a leather sample [4]. The limitation of image analysis is the subjective interpretation of the result and consideration of surface analysis without details of the chemical composition [5]. DNA analysis is also an option, as it identifies the origin of a leather sample through species determination [6]. However, DNA is prone to degradation during the processing of leather materials, which could lead to insufficient sampling and inaccurate results [7]. Liquid chromatography-mass spectrometry (LC-MS) is an example of a chromatographic method that can be used in leather authentication, through the identification and detection of protein markers present in leather samples [8]. However, there are drawbacks to this method such as the high cost, complexity of operation and data analysis, and extensive sample preparation [9].

Fourier Transform Infrared spectroscopy (FTIR) is also a preferred option for leather authentication when it is coupled with chemometric techniques. The combination of FTIR and chemometric techniques have been shown to successfully differentiate between raw animal skin and leather [10], between different animal leathers [11], and between authentic leather

and faux leather [12]. Results have shown that FTIR can qualitatively differentiate between animal leather and non-leather samples, specifically polyvinyl chloride (PVC) and polyurethane (PU). Samples were discriminated based on the animal origin of the leather samples using discriminator-analysis as a supervised chemometric technique [11-12].

The present study aims to further investigate the combination of FTIR and chemometrics by classifying leather samples based on the species of origin. The objective of this work is 1) to analyse leather samples from different species using FTIR spectroscopy, and 2) to classify the leather samples using an unsupervised chemometric technique, namely principal component analysis (PCA). This work highlights the feasibility of FTIR in classifying different leather samples without any pretreatment. PCA can process unsupervised data which is important in assessing the entire spectral dataset based on intrinsic variation prior to classification. Subsequently, the spectral data may be developed into a reference database for the identification of leather samples. With this method, we hope to simplify the process and reduce analysis time, and thus assist in the confirmation and authentication of leather samples for traceability and halal assurance.

EXPERIMENTAL

Sample Collection and Preparation

This study compared eight animal leather samples and one faux-leather sample. These samples are listed in Table 1, with details of the animal source, type of leather, and colour. The buffalo, cow, and goat leather samples were all obtained from Kulitkraf Sdn. Bhd. and the Malayan Leather Craft, Kuala

Lumpur Malaysia. Pigskin samples were purchased from Saigon Leather Trading & Production Company Limited, Ho Chi Minh, Vietnam. The faux-leather sample was sourced from a notebook provided by Kolej Komuniti Gerik, Perak, which has been certified by the local religious authority (Jabatan Agama Islam Perak) as not containing pigskin, despite exhibiting the typical three-hole pattern characteristic of pigskin. Prior to analysis, each sample was cut into 3 cm squares and labelled.

FTIR Analysis

The Agilent Cary 630 instrument, equipped with a diamond ATR (Attenuated Total Reflectance) accessory, was utilized to collect FTIR data. The ATR crystals were in direct contact with samples placed on a multibounce plate. Each FTIR spectrum was obtained from 16 scans within the range of 4000 cm^{-1} to 600 cm^{-1} , with a resolution of 4 cm^{-1} . The spectral background was analysed prior to the each samples, and subtracted to obtain the final spectrum. To ensure accuracy between analyses, the ATR plate was thoroughly cleaned with methanol and dried with soft tissue. Each background spectrum obtained was compared with the previous one to confirm cleanliness.

Principal Component Analysis

The entire FTIR spectral dataset was compiled and managed in Microsoft Excel for Microsoft 365 (Version 2502). The data matrix was then imported into The Unscrambler X 10.4 (CAMO Software, Massachusetts, USA). Pre-processing techniques included normalization, smoothing (moving average), and derivatization (Savitzky-Golay 2nd derivatives). PCA was calculated with mean centre, cross validation method, and the Singular Value Decomposition (SVD) algorithm.

Table 1. List of different leather and faux-leather samples.

No.	Sample Name	Processing method	Species	Colour	Label
1	Buffalo Leather	Vegetable Tanned	Buffalo	Dark Brown	B1
2	Malayan Buffalo Leather	Vegetable Tanned	Buffalo	Navy	B2
3	Leather Full Grain	Vegetable Tanned	Cow	Black	C3
4	Leather Cowhide	Vegetable Tanned	Cow	Dark Brown	C4
5	Malayan Chevre	Vegetable Tanned	Goat	Dark Grey	G5
6	Malayan Chevre	Vegetable Tanned	Goat	Black	G6
7	Suede Pig Leather	NA	Pig	Gold bull	P7
8	Suede Pig Leather	NA	Pig	Creamy Yellow	P8
9	Faux-leather Notebook	NA	Polyurethane	Light Brown	PU

RESULTS AND DISCUSSION

Qualitative Analysis of Different Leather Samples using FTIR

The FTIR spectra provide information on functional groups present in the leather samples. Based on the wavenumber of a peak, the corresponding functional group can be determined [13]. FTIR spectra for the cow, buffalo, goat, and pig leather samples are shown in Figure 1 and the peaks observed were labelled a ($3600\text{--}3200\text{ cm}^{-1}$), b ($2924\text{--}2920\text{ cm}^{-1}$), c ($2853\text{--}2851\text{ cm}^{-1}$), d ($1638\text{--}1634\text{ cm}^{-1}$), e ($1546\text{--}1544\text{ cm}^{-1}$), f ($1457\text{--}1422\text{ cm}^{-1}$), g ($1339\text{--}1325\text{ cm}^{-1}$), h ($1241\text{--}1235\text{ cm}^{-1}$), i ($1168\text{--}1162\text{ cm}^{-1}$), and j ($1030\text{--}1013\text{ cm}^{-1}$). The FTIR spectra of PU is shown in Figure 2 with the distinct peaks labelled as A ($3000\text{--}2840\text{ cm}^{-1}$), B (1710 cm^{-1}), C (1455 cm^{-1}), D (1407 cm^{-1}), E (1338 cm^{-1}), F (1241 cm^{-1}), G (1088 cm^{-1}), and H (1015 cm^{-1}). From the peaks identified, the corresponding wavenumbers were determined together with the functional groups and possible sources responsible for the peaks, which are listed in Table 2. The determination of functional groups is based on the chemical bonds exhibiting different movements (vibration, stretching, or bending) that absorb infrared radiation at specific frequencies. The specificity of the absorption results in patterns on the spectra that make it possible to determine the bonds and functional groups present in the sample [14].

Authentic leather made from animal skin contains collagen as the main structural protein found in animal tissues [15-16]. Collagen can be observed in an FTIR spectrum as peaks that show up at specific regions and correspond to Amide I, Amide II, and Amide III [17-18]. The cow, buffalo, goat, and pig leather samples all had peaks corresponding to Amide I ($1638\text{--}1634\text{ cm}^{-1}$), Amide II ($1546\text{--}1544\text{ cm}^{-1}$), and Amide III ($1241\text{--}1235\text{ cm}^{-1}$) [12], [19]. Generally, Amide I is positioned in the range of $1700\text{--}1600\text{ cm}^{-1}$, Amide II at $1600\text{--}1500\text{ cm}^{-1}$, and Amide III at $1400\text{--}1200\text{ cm}^{-1}$ [20-21]. Slight shifts in peak position are possible due to changes in the collagen structure from processing and degradation [22]. In an FTIR study on artificially aged leather compared to newly processed leather, changes in the peaks related to Amide I, Amide II, and Amide III were observed [22]. Other notable peaks were the ester peaks f, i and j [23]. Peaks related to lipids were also observed at b, c, and g; this is expected as lipids are naturally present in animal skin [24]. This observation is consistent with other reports of FTIR analysis of animal leather where peaks associated to lipids were observed [25-26]. Visual analysis of the FTIR spectra showed that the samples were leather. However, an FTIR spectrum cannot be used to distinguish between different animal species. As all leather samples come from animal sources, they share similar peaks resulting from the presence of collagen and lipids.

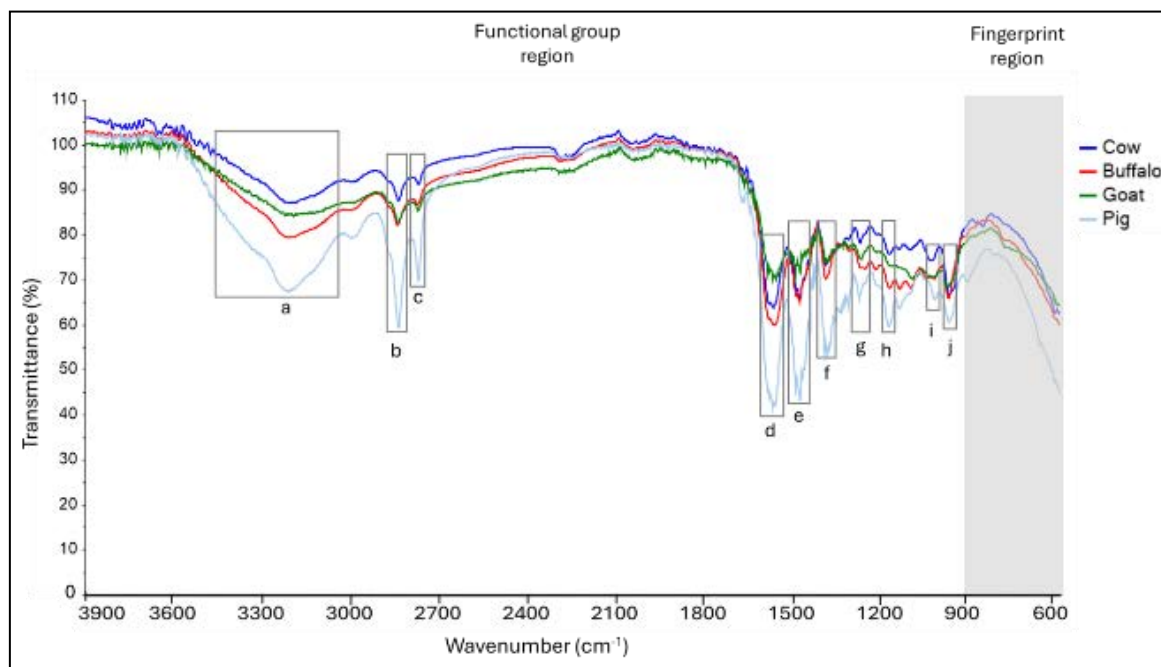


Figure 1. FTIR spectra of cow, buffalo, goat, and pig leather samples showing the functional group region and the fingerprint region. Peaks labelled a-j are peaks that correspond to specific functional groups.

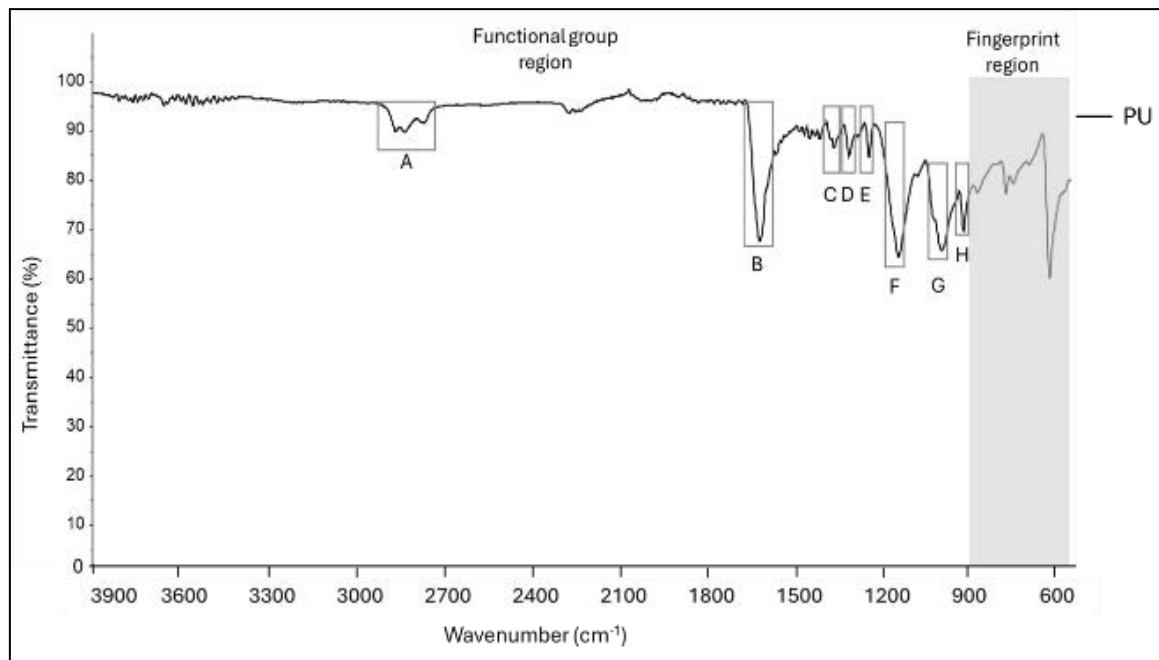


Figure 2. FTIR spectrum of polyurethane showing the functional group region and the fingerprint region. Peaks labelled A-H are peaks that correspond to specific functional groups.

Table 2. List of peaks observed in the FTIR spectra of leather samples.

Sample	Label	Wavenumber (cm ⁻¹)	Functional group	Possible source
Animal Leather	a	3600 - 3200	OH stretching	Adsorbed water
	b	2924 - 2920	CH ₂ stretching	Lipid
	c	2853 - 2851	CH ₃ bending	Lipid
	d	1638 - 1634	C=O stretching	Amide I
	e	1546 – 1541	NH bending and CH stretching	Amide II
	f	1457 – 1442	CO stretching	Esters
	g	1339 – 1325	CH bending	Lipid
	h	1241 – 1235	CN stretching and HNC bending	Amide III
	i	1168 – 1162	CO stretching	Esters
	j	1030 – 1013	CO stretching	Esters
Polyurethane (PU)	A	3000-2840	CH	Aliphatic chain polymer backbone
	B	1710	C=O stretching	Urethane linkage
	C	1455	NH bending and CH stretching	Amide III
	D	1407	CH ₂ stretching	Polymer backbone
	E	1338	CH ₃ bending	Methyl group
	F	1241	CN stretching and NH bending	Amide III
	G	1088	COC	Ether linkage
	H	1015	C-O stretching	Ether or ester linkage in polymer

The FTIR spectra for PU was compared to reference spectra [12, 27]. A strong indicator that confirms PU is not animal leather is the absence of the Amide I peak which appears at $1600 - 1500 \text{ cm}^{-1}$ [19], [28]. This is consistent with previous reports which noted that the FTIR spectra of polypropylene (PP), polyethylene (PE), ethylene vinyl acetate (EVA), polyurethane (PU), polyvinyl chloride (PVC), and polyester (PES) do not have the Amide I peak [11-12]. Amide II and Amide III, however, could still be observed in leather-like polymers [12], as well as peaks C and F. Peak A appeared between $3000 - 2840 \text{ cm}^{-1}$, specifically at 2955 cm^{-1} , 2924 cm^{-1} , and 2857 cm^{-1} . These low intensity peaks likely correspond to the polymer's aliphatic chain backbone [12]. Peaks B (1710 cm^{-1}), C (1455 cm^{-1}) and F (1241 cm^{-1}) are common peaks found in PU [16], [28], [29]. Peak B is associated with C=O stretching and C with NH bending associated with Amide III [12]. PU contains several hydrogen bonds from the presence of the donor NH group and acceptor C=O group which are part of the urethane linkage [30]. Peaks D, E, G, and H Another notable difference found in PU samples is the absence of lipid-based functional groups. Animal skin naturally has lipids as part of the tissue structure [25], as was observed in the spectra of the animal leather samples. Analysis of the leather samples showed that FTIR can provide qualitative information on the nature of the samples through determination of the spectral peaks. FTIR spectroscopy alone can assist in the identification of samples, but it is insufficient to quantitatively measure any differences present in the FTIR multivariate data [31].

Classification of Different Leather Samples using PCA

An unsupervised chemometric analysis, specifically principal component analysis (PCA), was used to analyse the multivariate data obtained in this study. The advantage of using PCA is that the data dimensions are reduced, while important variables are retained [32-33]. FTIR spectral data comprises a large set of multivariate data, with one sample having thousands of variables. Conducting PCA makes the complex data more manageable and easier to understand through visualization using simple data plots that are easier to interpret [34]. The unsupervised nature of PCA makes the approach suitable for preliminary measurements or without pre-defined classification. This is important in exploratory analysis where significant variables, trends or patterns in the data can be identified [35-36].

PCA analysis provides a score plot that visualizes relationships between samples by positioning the samples based on their similarity and significance along the principal component axis [37]. Figure 1(a) is the score plot for the PCA analysis of the cow, buffalo,

goat, and pig leather samples. From this score plot, PC-1 accounted for 53 % of the total variance, and PC-2 an additional 26 %. PC1 and PC2 combined described 89 % of the total variance of the spectral data. Principal components that cumulatively explain more than 80 % of the data are considered optimum and sufficient for the dataset [34, 38]. From Figure 1(a), four distinct clusters were observed for the cow, buffalo, goat, and pig leather samples. The clusters show the classification of samples because the position of the scores indicate whether there are similarities or significant differences between the samples [39]. The cow and buffalo leather samples were located on the positive side of the PC-1 axis, while the goat and pig leather samples were located on the negative side of the PC-1 axis. Along the PC-2 axis, the buffalo and goat leather samples were on the top positive side, while the cow and pig leather samples were located at the lower negative values. Another PCA was performed for the animal leather samples (cow, buffalo, goat, and pig) and PU. The PCA score plot obtained is shown in Figure 1(c) where distinct clusters were observed for the cow, buffalo, goat, and pig leather samples. The PU cluster was clearly distinguished from the animal cluster as it was positioned in the bottom left quadrant. The cow, buffalo, and goat samples were on the positive side of the PC-1 axis, while the buffalo, goat, and pig samples were on the positive side of the PC-2 axis.

In this work, a combination of FTIR analysis and PCA was used to distinguish between leather samples from different animal species and PU. The distinct clusters observed show that PCA can be used as a classification tool for animal leather samples of different species, as well as for animal leather samples against leather-like polymers. Similarly, other reports have also shown that FTIR and PCA can be used to classify leather samples as an unsupervised approach [6, 40]. Although there are observed differences in the FTIR spectral peaks formed by animal leather and PU, quick determination is difficult as the wavenumbers for each peak must be identified, listed, and compared to a reference spectrum prior to any conclusion. In contrast, PCA score plots provide clear visualization of the different clusters that show data classification and differentiation. This feature is important in leather identification or authentication. Past studies focused on leather quality based on spectral differences [8], differentiating between the leather and skin of two animals (i.e. pig and goat) [26], and between cowhide, goatskin, sheepskin, and pigskin compared to PU and PVC [11]. This study continues the investigation by carrying out FTIR analysis on cow, buffalo, goat, pig, and PU leather samples without any pre-treatment, making the process easier and more efficient. The absence of pre-treatment also removes the need for chemicals, which makes the analysis more accessible and user-friendly.

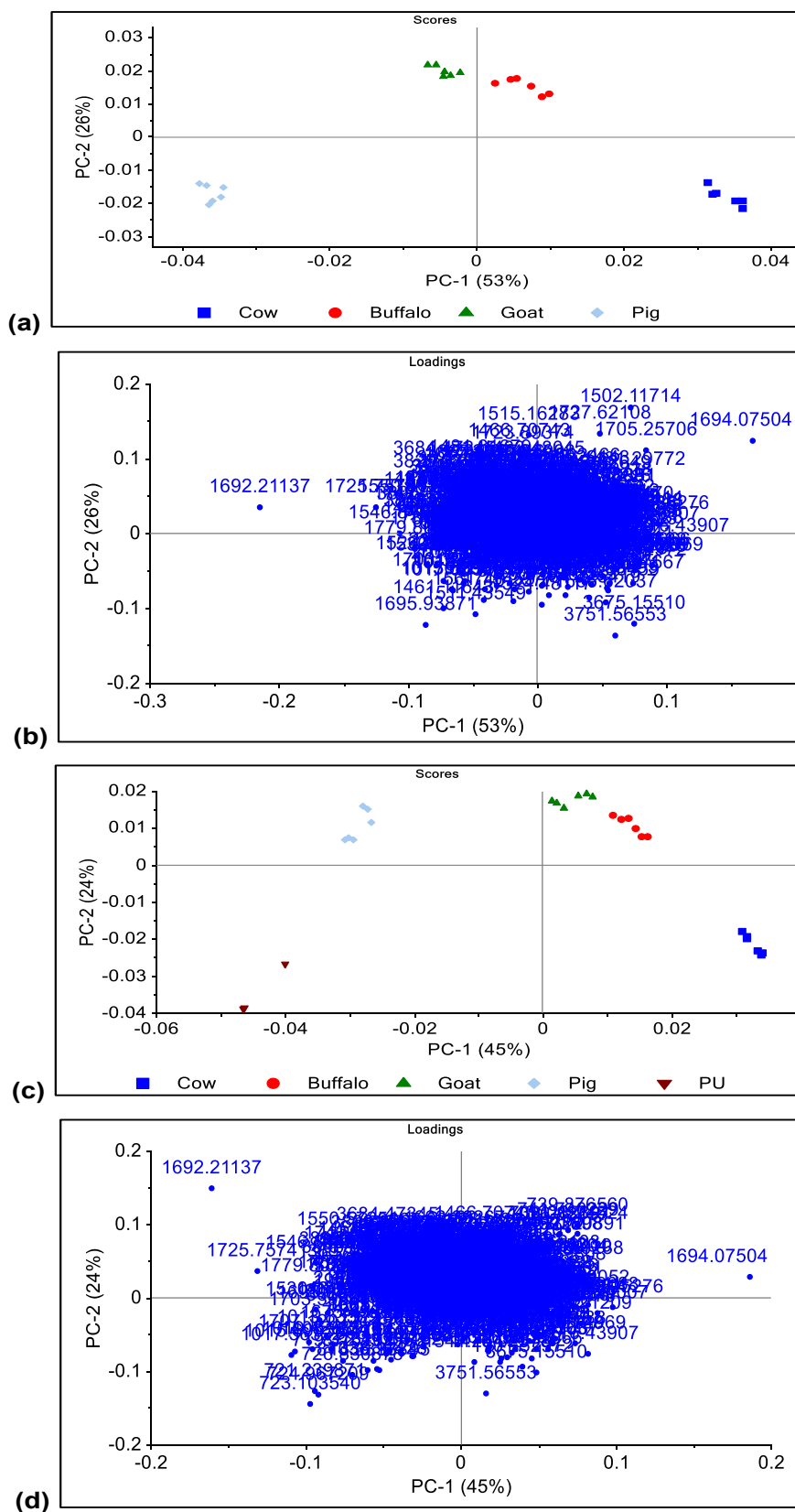


Figure 3. (a) PCA score plot of animal leather from different species, (b) PCA loading plot of animal leather from different species, (c) PCA score plot of animal leather and PU, (d) PCA loading plot of animal leather and PU.

Identification of Significant Variables Contributing to PCA

The PCA conducted in this study used all the spectral data obtained from the FTIR analyses. A loading plot visualizes the relationship of the variables with the samples along the principal component axis [41]. The position of the loading points show the contribution that the variable has on the PCA results and the correlation between variables [41]. Figure 1(b) is the loading plot for the PCA analysis of the leather samples from different animal species, while Figure 1(d) is the loading plot for the PCA analysis of all the leather samples. The loading plot holds valuable information on the variables that influence the principal components; however, it is often overlooked, as the spectral data itself is overwhelming.

The peaks identified in the loading plot of Figure 1(b) were $1692.21137\text{ cm}^{-1}$, $1694.07504\text{ cm}^{-1}$, $1695.93871\text{ cm}^{-1}$, $3751.56553\text{ cm}^{-1}$, and $3675.15510\text{ cm}^{-1}$. In Figure 1(d), the identified peaks were $1692.21137\text{ cm}^{-1}$, $1694.07504\text{ cm}^{-1}$, $1725.75741\text{ cm}^{-1}$, $3751.56553\text{ cm}^{-1}$, $723.103540\text{ cm}^{-1}$. The peaks identified in both Figures 1(b) and 1(d) were not listed as corresponding to the determined functional groups in Table 2. From Figure 1(b) and 1(d), most of the saturated peaks at the origin had no significant influence in differentiating the cow, buffalo, goat, pig, and PU leather samples. This finding agrees with another report which states that variables centred around the origin of the plot had no relation to the direction or position of the scores [42]. Overlapping of loading was also observed when pigskin and goatskin samples were examined using FTIR, making it difficult to identify the exact peaks that contributed to the classification of these samples [26]. In both loading plots, the peaks at $1692.21137\text{ cm}^{-1}$ and $1694.07504\text{ cm}^{-1}$ were observed to be the furthest from the origin on both ends of PC-1. This suggests that these peaks had a significant influence on the differentiation of the samples observed in the score plot [43-44]. It is likely that $1692.21137\text{ cm}^{-1}$ corresponds to the pigskin samples, $1694.07504\text{ cm}^{-1}$ to the buffaloskin samples, $3751.56553\text{ cm}^{-1}$ to the cowskin samples, and $723.103540\text{ cm}^{-1}$ to the PU samples. Unfortunately, there was no peak clearly linked to the goatskin samples.

The peaks at $1692.21137\text{ cm}^{-1}$ and $1694.07504\text{ cm}^{-1}$ seemed to be significant in the loading plot but are not listed in Table 2. Reports have associated these wavenumbers with the C=O functional group that could arise from the presence of carboxylic acids and amides [45]. The carbonyl group was assigned to a peak at $1638 - 1634\text{ cm}^{-1}$ in the spectra of the animal leather samples, and at 1710 cm^{-1} in that of the PU sample. Despite the inconsistency, peak assignments were based on the reference spectra of the same samples analyzed in this work. This finding emphasizes the limitations of using FTIR spectral peaks to assign

corresponding functional groups as a stand-alone method to classify leather samples. Coupling FTIR and PCA showed that PCA was able to highlight the absence of peaks at $1692.21137\text{ cm}^{-1}$ and $1694.07504\text{ cm}^{-1}$ in the FTIR spectra. These findings show that it is important to utilize all the data obtained from both the FTIR and PCA analyses in a preliminary exploration of the data, and highlights the capability of PCA as an unsupervised method to utilise the entire spectral dataset.

CONCLUSION

FTIR analysis was used to analyse samples of cow, buffalo, goat and pig leather, as well as a non-animal leather sample, PU. The spectra of the cow, buffalo, goat, and pig samples all had significant peaks that corresponded to important functional groups relevant to leather samples. These functional groups were Amide I, Amide II, Amide III, and esters from lipids which are inherent in animal skin. In comparing the FTIR spectra of animal leather samples and PU, leather and leather-like PU samples may be qualitatively differentiated. The most notable difference was that the FTIR spectrum of PU did not exhibit the peaks for Amide I and lipids. The combination of FTIR and a chemometric technique, specifically PCA, translated chemical data into spectral data that was visualized in a score plot. The score plot showed clear clusters of samples that could distinguish between leather from different animal species, and between leather and a leather-like polymer. This study demonstrated that FTIR can be used for efficient leather analysis without any pre-treatment. The combination of FTIR and PCA was beneficial in utilizing the entire spectral data. The loading plot also provided insight into specific wavenumbers that could be the source of differences between the samples analysed. Further research in this area should include more animal leather samples and leather-like polymers to determine whether this method provides easy identification and discrimination. Differentiation of leather samples is useful in regulatory work for authentication and traceability purposes.

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