Heliyon 9 (2023) e16273

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Monitoring the curing efficiency of a plant-based green engineered additive on caprine skin: a spectroscopic and chemometric novel approach

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ARTICLE INFO

Keywords:
Collagen chemistry
Green chemistry
P. hydropiper
ATR-FTIR spectroscopy
Goatskin preservation
Chemometrics
Leather industry
Environmental sustainability

ABSTRACT

Environmental sustainability in industrial processing is one of the most fundamental requirements for sustainable development. The leather industry is known for its vast environmental pollution. But, green engineering may occur a paradigm shift in this sector. Plant-based goatskins curing is a cutting-edge green technology that holds the concept of pollution reduction through prevention on the upper stream of leather processing. The successful and rapid monitoring of the efficiency of this technology is the foremost demand for mass-level applications. In this study, ATR-FTIR spectroscopy was employed to monitor this technology's efficiency using a plant *Polygonum hydropiper*. Chemometrics was also applied to extract information from spectral data leading to an understanding of the inherent effect of studying preservatives on goatskins collagen chemistry. 10% plant-paste +5% NaCl, 10% plant-paste +10% NaCl, and 15% plant-paste +5% NaCl on goatskin were assessed by ATR-FTIR on 0th, 10th, and 30th days of preservation. The Spectral peak fitting ($R^2 = 0.99$) area of amide I and II of collagen peptide bands revealed 2.73 to 1.33 times more structural suitability of studied goatskins than the control. Principal component analysis and Hierarchical cluster analysis showed that, after 30 days of curing, 15% paste +5% salt-rubbed goatskin collagen matrix significantly (around 50%) interacted with *P. hydropiper*. The interaction was superficial, as it happened before the opening up of collagen fibers. In conclusion, ATR-FTIR spectroscopy with Chemometrics can be an effective tool in evaluating the efficiency of goatskin curing and understanding the entire effect on collagen chemistry quickly.

1. Introduction

The global leather industry builds upon the products of shoes, upholstery, bags, belt, and leather garments that are processed from raw hides and skins after a series of chemical and mechanical processes [1]. Hides and skin preservation or simply curing is the foremost step before the beginning of the leather-making process. Though 40–50% NaCl is being used as a popular scale for curing, the hypersaline effluents (1–10% of salinity) from soaking liquor during leather processing [2] accelerate the focus of academician in searching for curing agents from sustainable sources [1].

Abbreviations: P. hydropiper, *Polygonum hydropiper*; ATR-FTIR, Attenuated total reflectance-Fourier transform infrared spectroscopy; PCA, Principal Component Analysis; HCA, Hierarchical Cluster Analysis; PC, Principal Component.

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https://doi.org/10.1016/j.heliyon.2023.e16273
Received 30 January 2023; Received in revised form 6 May 2023; Accepted 11 May 2023
Available online 13 May 2023

Plant-based curing agents have been considered the most dominant eco-friendly curing source in academia during the last few decades. Powder, extract, and paste of different plant parts are applied to preserve goatskin for a maximum of one month [3]. The plants which were successfully investigated are *Acalypha indica* [4], *Semecarpus anacardium* [5], *Tamarindus indica* [6], *Azadirachta Indica* [7], *Cassia fistula, Psidium guajava* [8], *Rumex abyssinicus* [9], *Aphanamixis polystachya* [10], *Sesuvium portulacastrum*, *Salicornia brachiata*, *Citrus sinensis* peel [1], *Clerodendrum viscosum* [11], *Moringa oleifera* [12], *Sphagneticola trilobata* [13], *Acacia bussei, Acacia albida* [14], Lemon [15], *Calotropis gigentea* [16], *Aegle marmelos* [17], *and Polygonum hydropiper* [18,19]. Plant-based curing agents are effective in reducing the generation of pollution in the leather industry. Hossain et al., 2022 reported that *Polygonum hydropiper*-based goatskin curing agent can prevent the generation of a maximum of 81% salinity during the soaking process [18]. The salt-free or less salt bio-additive-based green technology significantly prevents the generation of harmful pollution in the leather industry by minimizing the harmful chemical use. "*Prevention instead of treatment*" is one of the basic principles of green chemistry and green engineering [20,21]. According to UNIDO, pollution prevention and minimization is the most effective condition for sustainable leather processing [22].

During hides and skins curing experiments, a series of tests, for example, moisture content, hydrothermal stability, bacterial count, nitrogen content, and hydroxyproline content, were done in regular intervals of an average of one month, e.g., 5–6 times to understand the efficiency of applied preservatives [4–19]. These frequent experiments are very laborious and time-consuming. The successful and rapid monitoring of the efficiency of this practice is the foremost demand for accelerating research and mass-level applications. In this study, we applied Fourier Transform Infrared Spectroscopy (FTIR) to monitor the efficiency of a curing agent in a fast way.

The collagen in the hides and skin interlayer is mainly the polymer of amino acid having peptide linkage. Specific bacteria-secreted proteolytic enzymes attack peptide linkage to fragment amino acid chains into small pieces [23]. The amide group (CO–NH) in peptide bonds gives vibrational stretching and bending in the different areas of IR spectroscopy [24–26]. Any degradation of amino acid chains in the collagen triple helix will reflect in IR spectra. FTIR can easily detect degraded collagen or the structural fitness of collagen. Hu et al., 2022 studied the structural fitness of the tanned leather [27], and Belbachir et al., 2009 studied the collagen type [28] by applying the spectral peak fitting tool to FTIR spectral data. So, monitoring the collagen essential structural fitness of preserved goatskins using this tool can be implemented as a suitable parameter for moisture content, bacterial count, nitrogen content, hydroxyproline content, and hydrothermal stability. This process would be faster and less laborious.

Additionally, plants contained different flavonoid groups involving tannin and polyphenolic compounds [29], having a coincidental penetrating on the skin collagen matrix during preservation. As a result, the collagen chain of the preserved skins can interact with those compounds resulting in full or partial tanning before entering the tannery yard. The interaction of different bioactive compounds in the collagen matrix of the preserved hides and skins will change the intensity and pattern of FTIR spectra compared to the usual conditions. Therefore, considering the tanning effect of plant-based preservatives, this is one of the most concerning points. There is no study that assessed the extent of the interaction of plant-based preservatives with the collagen matrix of the skin during the preservation period. Understanding that possible interaction with collagen fibers can reveal many unknown scientific causes affecting this practice.

Chemometrics can effectively reveal the cause using two tools Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). PCA and HCA are applied for pattern recognition. Naturally formed, a similar set or group can be verified by these unsubstantiated techniques. The PCA is done by manipulating the raw data mathematically to get the newest variables with a lined arrangement of the core variables, which are orthogonal to one another, titled principal components (PCs) [30]. By figuring the distance amid samples, the HCA way shows their resemblance and difference [31]. The application of Chemometrics has been reported in leather-making processes [32,33], distinguishing the origin of skin and leather [34] and the artificial deterioration of vegetable-tanned leather [35].

For goatskin preservation, we employed a recently reported [18,19] plant, *Polygonum hydropiper* (local name "Bishkatali," and the English name "Water piper"), where Hashem et al., 2022 [19] and Hossain et al., 2022 [18] monitored the goatskin preservation following usual preservation efficacy parameters and reported comparable physical properties after leather processing. This plant has been reported as having antimicrobial and antifungal activity [36]. This study aimed to monitor the efficiency of plant-based goatskins preservation only by applying ATR-FTIR spectroscopy through spectral peak fitting and Chemometrics (PCA and HCA). Applying ATR-FTIR spectroscopy in combination with multivariate statistical analysis would be an effective quick way to monitor the goatskin preservation efficiency. According to the best of our knowledge, this is the first study that applied ATR-FTIR spectroscopy and Chemometrics in monitoring the goatskin preservation process.

2. Materials and method

2.1. Collection of plant sample

Plant sample (exclude root) was gathered from Dinajpur district, Bangladesh at 25.740°N, and 88.671°E of longitude and latitude, respectively.

2.2. Extraction of P. hydropiper

Air-dried plant sample was ground to form powders and 25 g was taken in conical flasks with 250 ml of methanol (99.9%), 80% methanol (v/v), and water (distilled). The mixtures were placed in a shaker with 180 rpm at room temperature for 24 h for extraction and then filtered with Whatman no. 1 filter paper. Evaporation was done to remove the solvents from filtrates using a rotary evaporator

(Heidolph Vaporota 4000; Fisher Scientific; France), maintaining minimized pressure. Finally, the extracts were placed in a freeze-dryer at −55 °C at 2.7 Pa for 24 h to form powders and stored at 4 °C for subsequent experiments.

2.3. Preservation experiment

2.3.1. Collection and preparation of goatskin

Freshly flayed goatskins were collected from a local abattoir at Kaptan Bazar, Dhaka, Bangladesh, to explore the applicability of the *P. hydropiper* plant for skin preservation. The goatskins were the byproduct of that slaughterhouse. The skins were rapidly brought to the leather processing lab, washed with tap water to get rid of adhered dirt and blood, and then oscillated for 10 min to get rid of surplus water.

2.3.2. Skin preservation experiment

The standardized combinations of plant paste and salt (NaCl) were adopted from Hossain et al., 2022 [18] as (Table 1) 10% *P. hydropiper* paste +5% salt (w/w), 10% *P. hydropiper* paste +10% salt (w/w), 15% *P. hydropiper* paste +5% salt (w/w). A goatskin was cut into four portions respecting the vertebral line and IUC sampling location (Fig. 1). The standardizations and 50% salt (w/w) were applied on the flesh side of each one-fourth cut. The goatskin preservation experiment was repeated three times. Then, the freshly cured goatskins were arranged flesh to flesh and stored at 30 ± 2 °C for 30 days of preservation. During preservation, organoleptic properties like rotting odor, physical feel, hair sliding, and white patches were observed regularly to assess any deterioration.

2.4. ATR-FTIR spectroscopy of preserved goatskin

Sampling was done from the butt portion indicated in Fig. 1. The size of the samples for ATR-FTIR spectroscopic analysis was maintained 1.5 cm × 1.5 cm. Then, the flesh side of each samples were cleaned by cotton swab to remove adhered plant paste before spectroscopic analysis. The preserved investigational and control skins (raw skin, 10th day, and 30th day) were investigated via an FTIR (IR-prestige-21, Shimadzu Corp., Japan) spectroscopy with single-bounce ATR. Each sample was scanned 32 times to record the spectrum with a 4 cm^{-1} resolution in the wavenumber between 400 cm^{-1} and 4000 cm^{-1} at 20 ± 2 °C. Every sample was examined four times (raw skin one time from each cut). SNV (Standard Normal Variate) and unit vector normalized average spectra were studied between 4000 cm^{-1} and 500 cm^{-1}. SNV and unit vector normalization was performed using Unscrambler X version 10.4 (CAMO Software AS, Oslo, Norway). The methanol, 80% methanol, and water extracts of *P. hydropiper* were also analyzed using ATR-FTIR spectroscopy in the same way.

2.5. Spectral peak fitting

The spectral peak fitting was accomplished to understand the bands of amide I and amide II along with the bands area using OriginPro software 2018 (Origin Lab Corp., USA) version 95E [37] using the second derivative curve as guidance in the area of 1500 cm^{-1} and 1690 cm^{-1}. The full width at half maximum (FWHM) version of the Gaussian function and Levenberg-Marquardt algorithm were used for peak fitting. Required iterations were done for the best spectral fitting. Deconvulated peaks with spectral fitting were presented with a second derivative curve. Before the spectral fitting, baseline correction was done using the peak analyzer tool from the same software with manual recalculation of user-defined baseline mode.

2.6. Chemometrics

The Principal Component Analysis (PCA) [30] and Hierarchical Cluster Analysis (HCA) [38] were accomplished by using the OriginPro software 2018 (Origin Lab Corp., USA) version 95E [37] after the spectral SNV, and unit vector normalization of ATR-FTIR data of raw skin (0^{th} day), 10th, and 30th day of preservation period along with *P. hydropiper* (average spectra of three extracts) in the region between 4000 and 500 cm^{-1}. SNV and unit vector normalization was performed using Unscrambler X version 10.4 (CAMO Software AS, Oslo, Norway). Primarily selected four numbers of PCA decomposition were done by analyzing the correlation matrix with simple descriptive statistics. Score plots were presented with two-dimensional graphs.

Group average cluster method and Euclidean distance type were selected for HCA accomplishment while the sum of distances method was utilized to find out clusteriod. Finally, a horizontally oriented two-dimensional dendrogram was presented as a graph.

Table 1
Standardization of plant dry powder paste and salt for the final experiment was adopted from Hossain et al., 2022 (Ph-1, Ph-2, and Ph-3 were investigational samples, and Ph-C was the control sample) [18].

SL. No.	Standardization	Sample ID
1.	10% *P. hydropiper* Paste +5% salt	Ph-1
2.	10% *P. hydropiper* Paste +10% salt	Ph-2
3.	15% *P. hydropiper* Paste +5% salt	Ph-3
4.	50% salt	Ph-C

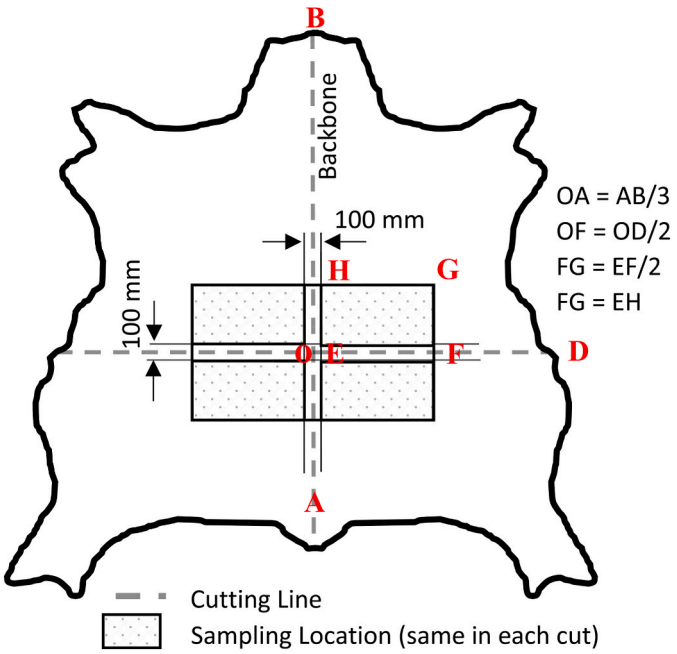

Fig. 1. The sampling position of goatskin for ATR-FTIR spectroscopy Analysis.

3. Result and discussion

3.1. ATR-FTIR spectroscopy of P. hydropiper

ATR-FTIR spectra of *P. hydropiper* extract of water, methanol, and 80% methanol were shown in Fig. 2, revealing the number of peaks as water extract > 80% methanol extract > methanol extract order. One flavonoid compound, fisetin, gives IR bands around 1619 cm^{-1} [39] of carbonyl groups. The bands between 1704 cm^{-1} to 1722 cm^{-1} correspond to steroids C═O stretching. This C═O stretching also can be for steroidal alkaloids and terpenoids aldehyde of polygodial. The presence of –OH in phenolic compounds is indicated by the bands above 3300 cm^{-1}. The C–O stretching of flavone and hydroquinone is exhibited at 1362 cm^{-1} [40]. The peaks 1612 cm^{-1} and 1616 cm^{-1}, 1439 cm^{-1}, 976 cm^{-1}, and 1207 cm^{-1} indicate aromatic C═C stretching, aromatic C–O stretching, out-of-plane C–H asymmetric bending, and OH in-plane deformation, respectively attributed the presence of tannin, phlobatannins, and hydroquinone [41,42]. Besides, peaks at 675 cm^{-1} and 679 cm^{-1} are responsible for out-of-plane C–O bending and aromatic C–C–C bending [41]. In addition, the peaks near 2900 cm^{-1} are due to the skeletal C–H stretching indicate the possibility of the presence of confertifolin and polygodial. Therefore, the presence of different bioactive compounds in *P. hydropiper* is reflected in

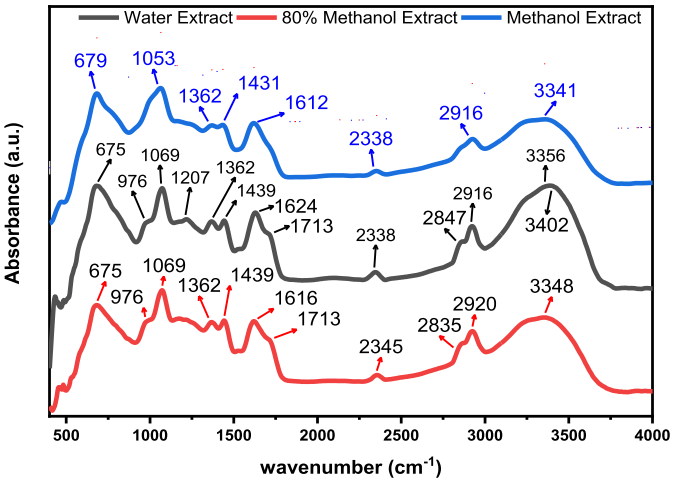

Fig. 2. ATR-FTIR spectra of water extract, methanol extract, and 80% methanol extract of *P. hydropiper*.

ATR-FTIR spectra.

3.2. Organoleptic properties of preserved skin

During the final preservation, organoleptic properties were observed regularly. Later one month of preservation, all the situations of the investigational samples stayed normal in appraisal to the control. Thus reflect the preservatives visual effectiveness concede with previous studies [18,19]. However, in the investigational sample Ph-3, detected the white patches to some extent on the flesh sideways as an indication of fungal development.

3.3. ATR-FTIR spectroscopy of preserved goatskin

The collagen protein of animal hides and skin is the fundamental component for leather making process. The structural integrity of collagen is maintained during the hides and skin preservation stage. This study assesses of the curing effect of the plant paste-based preserving goatskin using ATR-FTIR spectroscopy and compares of the outcomes between investigational and control samples over a 30-day period. Primarily, the structural integrity was assessed by recognizing the characteristics bands and alterations in vibrational spectra.

The raw skin was tested for ATR-FTIR to assess the structural fitness of collagen protein of preserved goatskins and presented in Fig. 3 with their spectral assignment in Table 2. The leading characteristic bands were designated in Fig. 3, and other related bands were assigned in Table 2. Amide A, amide B, and amide I to amide V-related bands are the main features of collagen protein assessed by ATR-FTIR techniques [24]. ATR-FTIR signals at 3350 cm^{-1}, 2931 cm^{-1}, 1620 cm^{-1}, 1550 cm^{-1}, 1245 cm^{-1}, and 690 cm^{-1} are responsible for amide A, amide B, amide I, amide II, amide III, and amide IV, V respectively [24,26,43].

The peptide linkage breakdown by any microbial activity will be reflected in these band areas during skin preservation. Goatskin has 4%–5% fat, which is reflected in ATR-FTIR bands 1708 cm^{-1} and 1390 cm^{-1} presented in Table 2. During the fleshing process before or after soaking, these fatty materials are removed and not present in the final leather. Again, other minor peaks at 2345 cm^{-1}, 1340 cm^{-1}, and 1165 cm^{-1} are for S–H vibration of cysteine, CH_2 side chain vibrations of collagen, and stretching vibration of C–O of C–OH groups of serine, threonine, and tyrosine residues protein, respectively [39,44].

In Fig. 4, the ATR-FTIR spectra of the investigational and control sample at the 0^{th} (raw skin), 10th, and 30th day were presented and compared. All the experimental samples showed major collagen peaks near 3350 cm^{-1}, 2931 cm^{-1}, 1630 cm^{-1}, 1538 cm^{-1}, 1240 cm^{-1}, and 640 cm^{-1} representing amide A, amide B, amide I, amide II, amide III, and amide IV, V of the peptide bond, respectively. Therefore, the spectrums revealed the presence of all peptide bands in experimental samples in the different intervals of the preservation period until the last day. This result reflects no deterioration of the collagen chain by microbial actions. The monitoring of the curing efficiency of the studied plant assessed by two groups of researchers previously concluded the same claim [18,19].

Again, the shifting of amide I to the higher frequency (for Ph-1, Ph-2, Ph-3, and Ph-C) compared to raw goatskin was due to the visibility of secondary structure, which was bound with hydrogen bonding in raw skin, but after 30 days of preservation, the amount of this bond decreased upon decreasing moisture content (Table 5). The amide I band of protein usually appeared in 3300 cm^{-1} due to N–H stretching [24], which appeared to have a higher frequency in increasing preservation days. In the case of skin collagen, the –OH stretching band shifted higher (nearly 3350 cm^{-1}) due to the presence of hydroxyproline. For the decreasing of moisture content after breaking hydrogen bonding to some extent, the entire OH group of hydroxyproline becomes more visible in IR spectra, thus increasing the bands to a higher frequency. On the other hand, the amide B bands remain at the same frequency throughout the preservation period. Amide III bands appeared in all experimental samples strongly but were comparably weak in control after 30 days of curing.

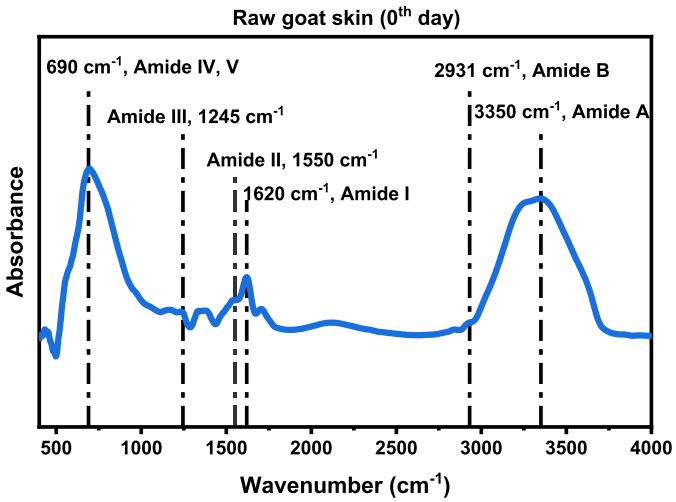

Fig. 3. ATR-FTIR spectra of raw goatskin (0^{th} day) with the assignment of main amide bands of collagen.

Table 2
ATR-FTIR spectral assignment of minor peaks of raw goatskin (0^{th} day).

Bands	Assignment	Designation	Reference
1708 cm^{-1}	*v* C═O	Fatty acid, side chain COOH of collagen	[39,44]
1340 cm^{-1}	CH_2 side chain vibrations	Collagen	[44]
1390 cm^{-1}	*v* COO^- symmetric	Fatty acid	[44]
2345 cm^{-1}	S–H vibrations	Cysteine	[39]
1165 cm^{-1}	*v* C–O	C–OH groups of serine, threonine, and tyrosine residues proteins	[44]

v = stretching vibration.

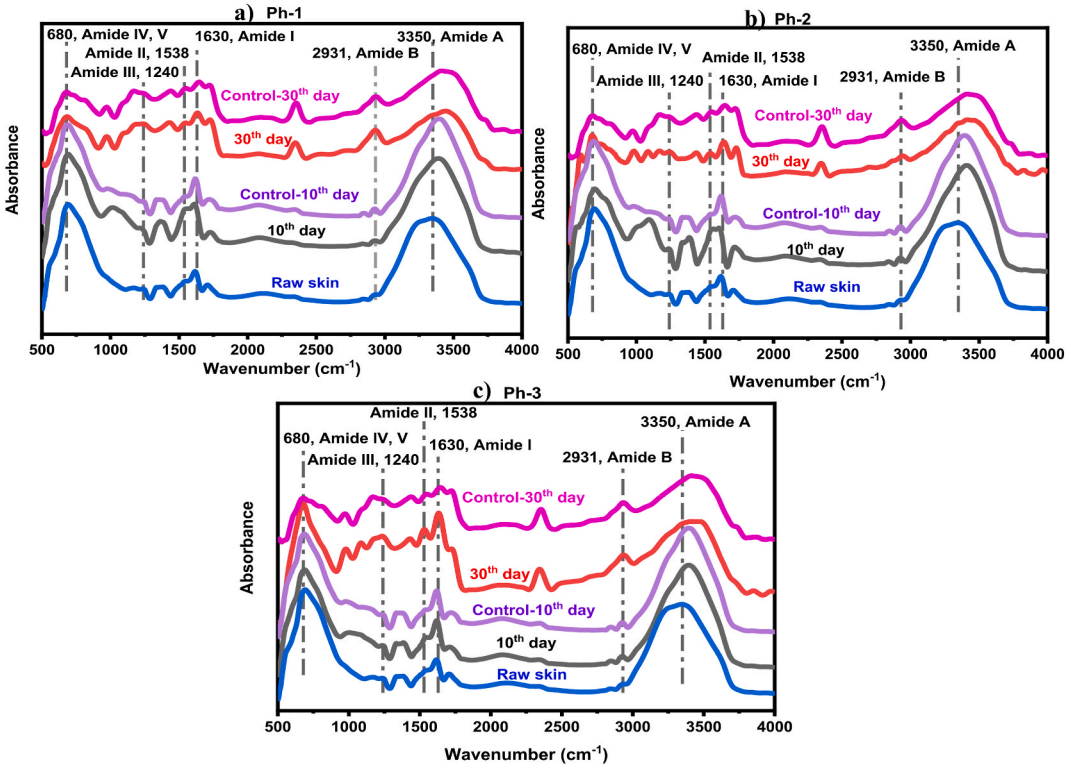

Fig. 4. ATR-FTIR spectra of experimental sample (a) Ph-1 (10% P. hydropiper Paste +5% NaCl), (b) Ph-2 (10% P. hydropiper Paste +10% NaCl), and (c) Ph-3 (15% P. hydropiper Paste +5% NaCl) with the assignment of main amide bands of collagen in comparison to control sample and raw goatskin. All the bands related to amide indicates their presence up to last day of preservation.

Amide IV and V showed strong bands throughout the curing days. The peak intensity between 1000 cm^{-1} and 1700 cm^{-1} of experimental skin Ph-3 appeared higher than others on the last preservation day, probably due to the mild effect of plant polyphenols.

The structural fitness of amide linkage of entire goatskin collagen reflected in vibrational spectroscopy successfully described their originality after 30 days of preservation. Hossain et al., 2022 reported the effectiveness of *P. hydropiper* by assessing the goatskin preservation efficacy parameters (Table 5), which are also reflected in the ATR-FTIR spectra [18].

3.4. Spectral peaks fitting of preserved goatskins

Amide I represents the C═O vibrational stretching, while amide II represents the *C*–N vibrational stretching and N–H out-of-plane bending of the vibrational spectroscopy [24]. Therefore, Amide I and amide II are the main structural state of collagen peptide linkage. The spectral peaks fitting between 1500 cm^{-1} to 1690 cm^{-1} range were studied of both experimental and control samples in the 0^{th} day and 30th days of preservation to understand the bands of amide I and amide II along with peaks area. The results are shown in Fig. 5 (a-e) with related secondary structural assignments in Table 3. In addition, the comparison of peaks area is also tabulated in Table 4. Each fitted peak was guided by second derivative spectra resulting in $R^2 = 0.99$ for all spectral fitting. The deconvoluted peaks assignment in Table 3 Depicted more secondary structure after the 30th day of curing than on the 0^{th} day. 1610 cm^{-1} - 1628 cm^{-1}, and 1540 cm^{-1} - 1550 cm^{-1} are more representative area in this study. Ph-1 displayed peaks at 1657 cm^{-1}, 1614 cm^{-1}, 1641 cm^{-1}, and 1544 cm^{-1} while Ph-2 showed at 1620 cm^{-1}, 1665 cm^{-1}, 1686 cm^{-1}, 1645 cm^{-1}, 1522 cm^{-1}, 1540 cm^{-1}. Again, Ph-3 exhibited

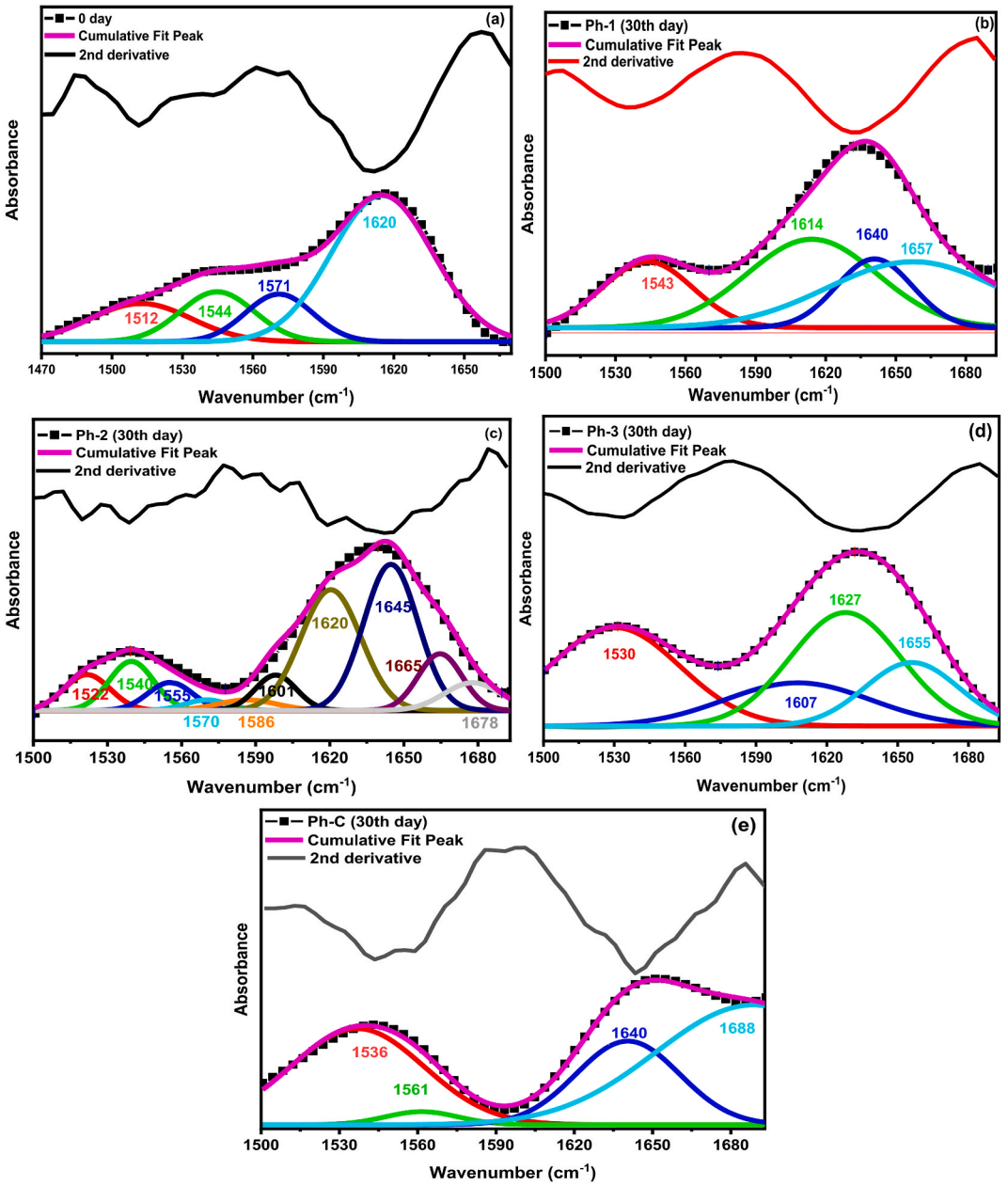

Fig. 5. Spectral fitting peaks ($R^2 = 0.99$) of investigational **(b–d)**, and control **(e)** preserved goatskin of 0^{th} day **(raw skin-a),** and 30 days **(b–e)** of preservation in the range of 1500 cm^{-1} to 1690 cm^{-1}.

Table 3
Fitting spectral peaks assignment of the secondary structure of collagen amide linkage.

Lit. value[a] (cm^{-1})	Ph-1	Ph-2	Ph-3	Ph-C	Raw	Assignment [25,44]
1648–1660	1657		1656			β-sheet or, α-helix
1625–1640			1628	1640		β -turns
1610–1628	1614	1620	1608		1620	Aggregated strands, aggregated β-sheet
1660–1696		1665, 1686		1688		β-turns, β-sheet
1641–1647	1641	1645				Random coil/unordered (occasionally overlays with α-helices)
1520–1545		1522		1536		Random (unorder)
1540–1550	1544	1540			1545	β-sheet
1520–1530			1530			β -turns

[a] 1600 cm^{-1}-1690 cm^{-1} amide I, and 1480 cm^{-1} – 1575 cm^{-1} amide II.

Table 4
Area of peaks of fitting spectral peaks of amide I and amide II (1500 cm^{-1}- 1690 cm^{-1}) of experimental, and control goatskin samples before and after 30 days of preservation.

Amide types	After 30 days				0^{th} day
	Ph-1	Ph-2	Ph-3	Ph-C	Raw
Amide I (1500-1575 cm^{-1})	1.53	1.34	2.76	1.01	3.09
Amide II (1600-1690 cm^{-1})	0.33	0.39	1.22	0.31	0.76

All values represent $R^2 = 0.99$.

Table 5
According to Hossain et al., 2022 the change of moisture content, shrinkage temperature, and bacterial load during 30 days of preservation using *P. hydropiper* powder paste [18].

Samples	Moisture Content (%)		Shrinkage Temperature (°C)		Bacterial Load (CFU/g)	
	1st Day	Last Day	1st Day	Last Day	1st Day	Last Day
10% *P. hydropiper* Paste +5% salt	55.30	44.30	68	68	1.0×10^6	1.0×10^6
10% *P. hydropiper* Paste +10% salt	55.10	31.90	65	70	3.0×10^6	1.0×10^7
15% *P. hydropiper* Paste +5% salt	57.90	41.0	66	72	8.0×10^5	7.0×10^6
50% salt	51.0	36.10	65	72	2.0×10^5	5.0×10^6

signals at 1656 cm^{-1}, 1628 cm^{-1}, 1608 cm^{-1}, 1530 cm^{-1} while Ph-C showed peaks at 1640 cm^{-1}, 1688 cm^{-1}, and 1536 cm^{-1}. The deconvolution result of experimented skin Ph-2 showed more peaks due to more solid content as reporting least moisture content (Table 5) [18]. In addition, the bands at 1601 cm^{-1} also can be ascribed to amide I. Most of those bands represent the presence of α-helical and β-sheet structure of collagen indicating their stability during preservation. The other peaks like 1555 cm^{-1}, 1562 cm^{-1}, and 1562 cm^{-1} were in the range of the amide II region, probably due to primary structural bands.

The area of deconvoluted peaks corresponding to amide I and amide II was tabulated in Table 4. For amide I, the area of Ph-1, Ph-2, Ph-3, and Ph-C after 30 days were 1.53, 1.34, 2.76, and 1.01, respectively, while in raw goatskin it was 3.09. Besides, for amide II, the area of Ph-1, Ph-2, Ph-3, and Ph-C after 30 days were 0.33, 0.39, 1.22, and 0.31, respectively, while in raw goatskin it was 0.76. The results revealed a significant reduction referring to 10%–56% of the amide I area of experimental samples followed by a 67% reduction for the control sample after the 30th day of preservation compared to raw skin (0^{th} day). In the case of amide II, the area reduction was 49%–56% for two experimental samples (Ph-1 and Ph-2), followed by 59% for the control. After comparing the area to the control, all the experimental skin showed higher results, especially Ph-3, which revealed the significant probabilistic effect of the *P. hydropiper*-based curing agent. The area of amide II of experimental sample Ph-3 after the 30th day of curing showed 1.6 times higher than raw goatskin. The area loss of amide I and amide II for experimental samples and even control samples compared to raw goatskin was for the change of moisture content and hydrothermal stability (Table 5.) but not for microbial deterioration, according to the report [18, 19]. This result also revealed that NaCl-based curing was not entirely ideal, there occurred some changes in collagen chemistry, and experimental skins were inherently in better condition than the control. The highest shrinkage temperature, second least moisture content (Table 5.), and halophilic bacterial action in control skin may be liable for this.

3.5. Principal component analysis (PCA) and hierarchical cluster analysis (HCA)

A sample's functional group or chemical structural fitness is related to the absorbance intensity of ATR-FTIR spectra. The strong correlation of intensity variances of wavenumbers in multiple samples reveals their chemical structural harmony. PCA and HCA correlate and group multiple samples based on their intensity variances in different wavenumbers.

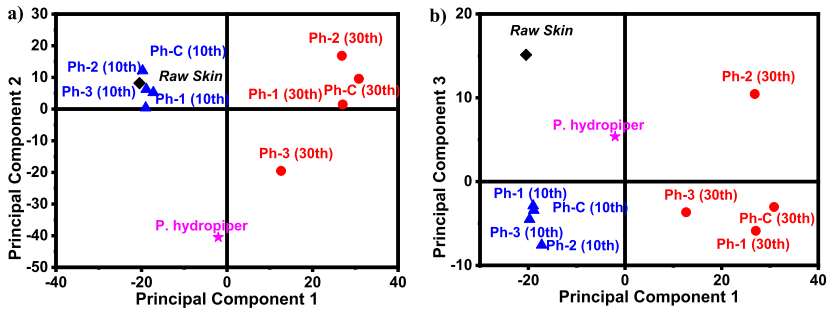

Fig. 6. Principal Component Analysis (PCA) of preserved goatskin using *P. hydropiper*-based curing agent and conventional technique on the 0^{th} day (raw), 10th day, and 30th day. **a)** PC1 vs PC2 **b)** PC1 vs PC3.

The results of PCA, and HCA analyses of ATR-FTIR spectra of raw (0^{th} day), 10th day, and 30th day preserved skin of control, and experimental samples, along with *P. hydropiper* were presented in Figs. 6 and 7, respectively. Fig. 6 shows the principal component 1 (PC1) versus principal component 2 (PC2) score plot of observational data of ATR-FTIR representing 53.21% and 31.23% variables, respectively. In PC1, from raw skin to the 10th day, all samples scored nearly similar negative values attributing their 53.21% similarity to the overall variable. However, all the 30th-day samples showed positive scores for PC1. Fig. 6a successfully separated raw skin and 10th-day samples from 30th-day samples except for Ph-3. Around 84% of spectral data revealed the changes in 30th-day samples from raw skin reflecting the output of spectral peaks fitting area in Table 4. The score of Ph-3 in PC1 was half of the other 30th-day samples. As Ph-3 was combined with 15% of *P. hydropiper* paste, the highest of other combinations, PC1 reported around 58% dissimilarity of that sample to others. *P. hydropiper* variance loading was minimal (−0.9) in PC1. PC1 successfully attributed the distinction of raw and 10th-day samples to 30th-day samples revealing the change of absorbance intensity over time due to moisture content and shrinkage temperature alteration (Table 5.) [18]. In PC2, *P. hydropiper* loaded the highest negative score (−40.48), and Ph-1 (10th) loaded the lowest positive score (0.39). The second-highest negative score was for Ph-3 (30th), around 50% of *P. hydropiper*. So, the 58% dissimilarity of Ph-3 to the other 30th-day samples in PC1 was mainly for the effect of *P. hydropiper*. The interaction with the bioactive compounds (mainly phenolic compounds) or the presence of bioactive compounds in the collagen matrix can be inferred from the dissimilarity. This can be estimated as a tanning effect due to the interaction of the collagen chains with *P. hydropiper*. Again, other samples scored positively on PC2, but Ph-2 (30th) and Ph-C (10th) showed higher scores. In PC3, only raw skin (15.13), Ph-2 (30th) (10.45), and *P. hydropiper* (5.37) scored positively, revealing only a 6.20% similarity of the variables among their observational ATR-FTIR data. In Figs. 6b, 10th-day samples and 30th-day samples were distinguished properly except Ph-2 (30th).

The dendrogram in Fig. 7 shows 9 clusters at different distances. At 0.1 distance level, all the observable samples are distinguishable. After that, Ph-3 (10th) and Ph-C (10th) were more similar next to Ph-1 (10th). At the same level, Ph-1 (30th) and Ph-C (30th) are similar in another cluster. The Ph-2 (10th) sample connection to other 10th-day samples makes a cluster of 10th-day samples describing their similarity, just like the PCA result. All those samples were clustered with raw skin at nearly 0.3 levels of distance. The 30th-day samples of Ph-1, Ph-C, and Ph-2 show another cluster connected to a higher distance with the Ph-3 and *P. hydropiper* cluster. PCA reports also reflected two groups of 10th-day samples with raw skin and 30th-day samples in the dendrogram. Therefore, the 30th-day samples in the same cluster with *P. hydropiper* revealed the presence of bioactive compounds in the collagen matrix with different extents for preventing microbial growth. Nevertheless, forming a cluster of Ph-3 with the studied plant at nearly 0.5 distance level revealed its significant interaction with phenolic compounds of *P. hydropiper*. So, 15% paste-based goatskin collagen after 30 days of preservation showed a significant interaction with polyphenolic groups, which supports the PCA result. Additionally, the development of fungus on the surface of Ph-3 (30th) to some extent indicates the probability of tanning, as Orlita, 2004 reported that fungus growth starts in tanned leather, not preserved hides and skin [45]. Plant polyphenols interact with collagen triple helix by creating hydrogen bonds during vegetable tanning [23], thus giving leather good physical properties and strength. Nevertheless, Hossain et al., 2022 reported that 15% *P. hydropiper* paste combined with 5% NaCl preserved goatskin has comparable physical properties and fiber structure in final leather [18]. Though around 50% of the entire collagen matrix of Ph-3 interacted with the studied plant during preservation, there was no impact on the final leather quality [18]. These interactions happened with collagen before the splitting of collagen fibers structure in the fibrils bundle level. But the operation of opening up of the fiber's structure or simply liming is considered an essential pre-tanning process [23]. As a result, this interaction can't be stable during the beam-house processes. However, this fact may not be the same for all plant-based preservatives. Therefore, exploring the degree of interaction is needed for future studies.

4. Conclusion

In this study, the applicability and efficiency of the *P. hydropiper*-based curing agent were assessed through spectroscopic and multivariate analysis. Application of two chemometric tools PCA and HCA can monitor skin preservation by utilizing *P. hydropiper* paste. Spectral peak fittings also be effective to evaluate and compare the structural fitness of the preserved skins. The results of this study suggest that the application of ATR-FTIR spectroscopy in quick monitoring of the curing efficiency of plant-based preservatives on goatskin will facilitate the development of green chemistry as well as sustainability in the leather industry.

Furthermore, the ATR-FTIR spectroscopy as well as Chemometrics of rehydrated skin after soaking operation could be a significant comparison factor in future research. In addition, future studies could investigate more research by utilizing other analytical techniques at the molecular level for better understanding. Finally, the outcomes of this study will be helpful to relate this technique to other hides and skin preservation method.

Author contribution statement

Md. Mokarom Hossain: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Uttam Kumar Roy: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

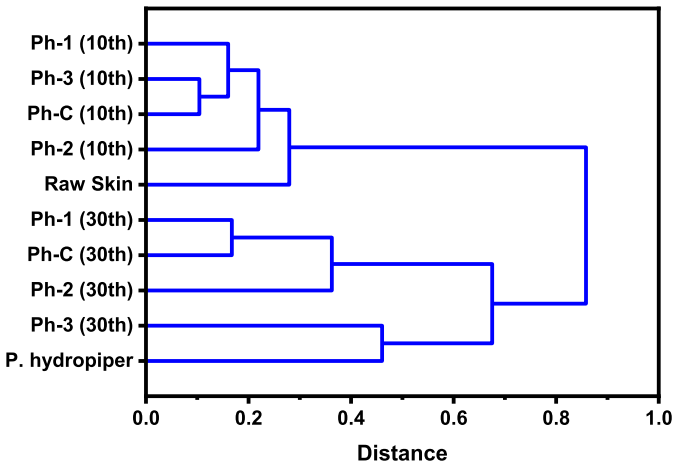

Fig. 7. Hierarchical Cluster Analysis (HCA) of preserved goatskin using *P. hydropiper*-based curing agent and conventional technique on the 0^{th} day (raw), 10th day, and 30th day of the preservation.

Research ethics

The animal skins that were subjected to experiment in this research were the byproduct of a small meat industry and these byproducts are being used as raw material for the tannery. There was no possibility that the author's slaughtered goats only for collecting skins to carry out this research.

Funding

This work was supported by the Ministry of Science and Technology, Government of the People's Republic of Bangladesh (Group Reg. No. 785/EAS No. 39.00.000.012.002.06.21.179 Date: 30/12/2021).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the people of Dinajpur, Bangladesh for their kind cooperation in collecting the plant sample. They are also grateful to the Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Bangladesh for the assistance in carrying out the ATR-FTIR test; Dr. Md. Latiful Bari, Food Nutrition and Agriculture Research Laboratory, Center for Advanced Research in Science, University of Dhaka, Bangladesh and Dr. Md. Zakir Sultan, Drug Analysis and Research Laboratory, Center for Advanced Research in Science, University of Dhaka, Bangladesh for their technical assistance in carrying out this research.

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