Attenuated Total Reflection Fourier Transform Infrared Spectroscopy and Chemometrics for the discrimination of Animal Hair Fibers for the Textile Sector

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Abstract

Analyzing the composition of animal hair fibers in textiles is crucial for ensuring the quality of yarns and fabrics made from animal hair. Among others, Fourier Transform Infrared Spectroscopy (FTIR) is a technique that identifies vibrations associated with chemical bonds, including those found in amino acid groups. Cashmere, mohair, yak, camel, alpaca, vicuña, llama and sheep hair fibers were analyzed via Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy and Scanning Electron Microscopy (SEM) technique aiming the discrimination among them to identify possible commercial frauds. ATR-FTIR, being a novel approach, was coupled with chemometric tools (PLS-DA), building classification/prediction models which were cross-validated. PLS-DA models provided an excellent differentiation among animal hair of both camelids and eight animal species. In addition, the combination of ATR-FTIR and PLS-DA was used to discriminate the cashmere hair from different origins (Afghanistan, Australia, China, Iran and Mongolia). The model showed very good discrimination ability (accuracy 87%), with variance expression of 94.88 % and mean squared error of cross-validation (MSECV) of 0.1525.

Keywords: animal hair, textile fibers, ATR-FTIR, infrared spectroscopy, multivariate analysis, SEM

Introduction

Specialty animal hair fibers, such as cashmere, mohair, yak, camel, alpaca and vicuña, are valuable natural raw materials used by the fashion industry for manufacturing high-quality luxury textiles, and are distinct from sheep's wool fibers.[1] Although they have a very similar composition from a chemical point of view (keratin proteins), the main characteristics that confer high value to these fibers are warmth, finesse, softness, lightness, luster and, also, their rarity.[2] For these reasons, the cost of these fibers is high, but pretty variable depending on the specific type, and the detection of eventual adulteration is therefore essential to guarantee quality maintenance and traceability within the supply chain up to the final consumers. For instance, some features of fine yak fiber are similar to those of cashmere (i.e., in terms of the mean fiber diameter), but its price is only a quarter. Thus, the bleaching of naturally pigmented fine yak hair is highly attractive economically, potentially leading to commercial fraud.[3]

Furthermore, for example in the case of cashmere, fibers obtained in different countries have different prices in relation to their qualities.[4] Due to the complexity of the textile supply chain, natural fibers that are often produced in one country and subsequently processed elsewhere are more subjected to risks of label falsification.[5]

The traditional and most widely employed techniques for the identification of animal fibers in the textile sector involve microscopies. On the one hand, optical microscopy allows the study of the internal fiber structure, such as pigmentation and medulla, whereas, on the other hand, scanning electron microscopy (SEM) shows the surface morphology and the arrangement and fine structure of the cuticle cells at high resolution.[2,6] Further improvements in microscopy-based techniques include

automatization and application of deep learning and artificial intelligence,[7–10] and also the use of innovative equipment such as the digital holographic microscope.[11]

The investigation of the thermal behavior of fibers can be used, as well, to distinguish different animal hair, in particular, through Differential Scanning Calorimetry (DSC). Still, such analyses can be practically utilized only from a qualitative point of view.[12] The most important drawbacks of the techniques described above are that they are often time-consuming, subjective and operator-dependent. In order to enhance the objectivity and accuracy of the identification of animal hair fibers, even in blends, alternative methods have been proposed, analyzing protein fractions, external/internal lipids and DNA. Another method was based on specific selectivity antigen-antibody, applying monoclonal antibodies. Nevertheless, the results obtained were often affected by the influence of the chemical treatments to which the fibers have been subjected during manufacturing processes, such as bleaching, depigmentation and dyeing.[13]

Among these efforts, validated studies for the identification in mixed samples of yak, wool and cashmere fibers exploited specific molecular markers that could be unequivocally linked to individual species, bringing about an excellent qualitative and quantitative identification of cashmere, wool and yak. The procedure includes the enzymatic digestion of keratin extracted from the fibers and the peptide analysis by ultra-performance liquid chromatography – electrospray mass spectrometry (UPLC-ESI-MS). This method has become an international standard “ISO 20418-1 Textiles - Qualitative and quantitative proteomic analysis of some animal hair fibres Part 1: Peptide detection using LC-ESI-MS with protein reduction”.[14,15] A similar proteomic approach was also adopted for South American camelid hair fibers.[16] However, so far, only some types of animal hair fibers can be objectively recognized and distinguished from each other with the proteomic method and, additionally, as other techniques mentioned, it is quite expensive and destructive.

In recent years, spectroscopic methods coupled with chemometric analyses have been developed to overcome issues in textile material identification (both natural and synthetic), even in cases of very similar compositions.[17–23] Recently, Deep Neural Networks (DNN) and Support Vector Machine (SVM) machine learning techniques

were able to discriminate the hair of five animal species with an accuracy of more than 95%.[24]

In this work, the aim is to propose a method for different animal hair fiber recognitions by Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) spectroscopy as a fast and practical tool, as it can be used in a non-destructive way for the analysis of various samples shaped as powders, fibers, fabrics, etc. without any pretreatment. Such analyses were assisted by chemometrics, in order to set a more and more objective methodology for the discrimination of keratin-based samples, at least for a preliminary screening in the first steps of the supply chain, when the fibers are initially provided. Indeed, it is worth specifying that spectroscopy in ATR mode can be affected by chemical modification of the sample's surface, due to its intrinsic working system based on the phenomenon of total internal reflection. While research activities demonstrate the feasibility of this method, its true significance lies in the potential reduction of operating times and quality control costs of the textile industry. Recent case studies indicate that these savings can amount to hundreds of thousands of euros per year.[25,26]

The fibers considered in this research were chosen on the basis of the taxonomic classification of animals or their geographical origin to simulate different operating scenarios.

Materials and Methods

Sample collection and ATR-FTIR measurements

Hair fibers were collected from eight different animal species belonging to six animal genera in total: *Ovis* (sheep), *Capra* (cashmere goat and Angora goat, from which mohair originates), *Bos* (yak) *Camelus* (camel), *Lama* (llama), *Vicugna* (vicuña and alpaca), where the last three genera all belong to camelids (family Camelidae). In addition, white cashmere hair samples were delivered to the laboratory from five different countries around the world (Australia, Afghanistan, China, Iran and Mongolia). The fibers were supplied by leading companies in the textile sector and

were stored in a glass vacuum desiccator with silica gel until analysis, to control moisture levels. Hair samples were used as provided by the suppliers without any pretreatment (e.g., cleaning with solvents)[27,28] in order to detect the global infrared profile of the hair. Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) spectroscopy analysis was performed with a diamond crystal ATR-FTIR equipment (Spectrum Two FT-IR Spectrometer adjusted with UATR Two Accessory, Perkin Elmer, Waltham, MA, USA), in a wavenumber range of 4000 to 450 cm^{-1}, at 4 cm^{-1} resolution with 32 scans. At the beginning and before every measurement, the diamond crystal was cleaned meticulously with isopropyl alcohol. During the conduction of the measurements, an amount of animal hair, approximately 0.3 g, was placed on the surface of the crystal (2.0 × 2.0 mm).

Spectra pre-processing and data analysis

The spectra pre-processing step was carried out with Spectragryph software v. 1.2.15. All animal hair spectra were baseline corrected, followed by multiplicative scatter correction (MSC). Multivariate analysis was performed with MATLAB (R2022a; The Mathworks, Natick, MA, USA), based on a previously reported work,[29] to build a classification with a PLS-DA model (supervised technique). In order to validate the PLS-DA model, each dataset was randomly split into a training set and a test set with a ratio of 70%:30%. The classification/prediction model was built from the training dataset of samples, and its efficiency was confirmed via the classification of the "unknown" test dataset samples. In order to avoid an overfitting effect on the upcoming classification model, 10-fold repeated stratified cross-validation (100 repeats) was performed.

Data management

Following ATR-FTIR measurements, a high amount of spectra and data were obtained. The data management process for the series of studies is displayed in Figure 1. In the initial study, infrared spectra were collected for each of the eight animals (discrimination among species) to identify patterns and correlations. Then, four animals from the family of camelids were chosen to investigate in detail the discrimination ability of ATR-FTIR within the same animal family. The final deepening

was focused on one selected animal fiber (cashmere) from the initial eight-animal group, studying variations and adaptations of cashmere goat hair derived from five different geographical origins. This robust data management system ensured that the data from these diverse sources was integrated seamlessly, allowing for comprehensive comparisons and insightful conclusions.

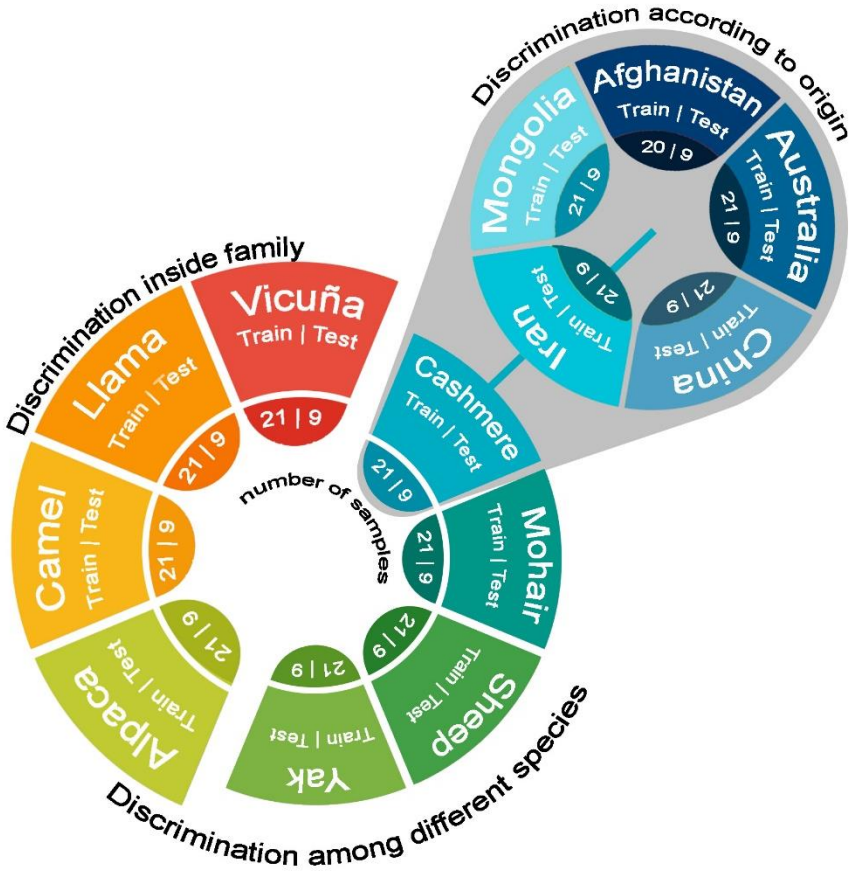

Figure 1: Data management visualization and number of samples used (split in training and test datasets). The colorful circle indicates the samples used for the initial discrimination among different species. Warm-color semicircle indicates the sub-group used for the discrimination among camelids. The small blue-color palette circle indicates the dataset used for the discrimination of cashmere samples based on their origin.

Morphological analyses

Scanning Electron Microscopy (SEM) measurements were performed using an EVO10 instrument (Carl Zeiss Microscopy GmbH), according to the standard methods IWTO

TM 58-00 and AATCC 20A-2017, specific for fiber analyses. The acceleration voltage was set at 20 kV. Prior to the analyses, the samples were sputter-coated with a 20 nm-thick gold layer in rarefied argon, using a Quorum Q150R ES plus Sputter Coater.

Results and discussion

Hair fiber discrimination for different animal species and within the camelid family

SEM images of the different animal fibers taken into account in this part of the study are reported in Figure 2. Generally, animal fibers are similar on the basis of their morphological features. Some distinctions are possible by carefully observing the shape and thickness of the scales and the profile of the fiber.[30] Due to thicker cuticle scale edges and higher scale frequency, the wool fibers are simple to identify compared to other animal fibers, assuming a significantly higher surface roughness (Figure 2a). On the contrary, the cashmere fiber scales are regular, thin with smooth surface and every scale envelopes the fiber shaft flatly and evenly. The distance between adjacent scales is large (Figure 2b). The mohair fibers exhibit an even diameter, straight fiber shaft and smooth surface. Many cuticle scales are lance-shaped, but usually, scales overlap flatly and regularly on the fiber shaft with tile shapes (Figure 2c). Finer mohair shows similar scale patterns to those of cashmere. Therefore, sometimes, it is difficult to distinguish these fibers from each other. The yak fibers have a high scale frequency and a high diameter evenness in the fiber's axial direction. Scales are thin and they encircle the fiber shaft tightly with an irregular ring-shaped pattern (Figure 2d). In the camel fibers, the scales are thin and arranged obliquely along the fiber axis. Some fine fibers have scale patterns resembling those of cashmere (Figure 2e). The alpaca fibers show a high scale frequency with less clear and ripple-crenate edges (Figure 2f). These characteristics are very similar to those of llama fibers (Figure 2g); therefore, it is not straightforward to distinguish with certainty these two kinds of fibers. Finally, the vicuña fibers have fine diameters and show high-scale frequency and characteristic axial furrows (Figure 2h).

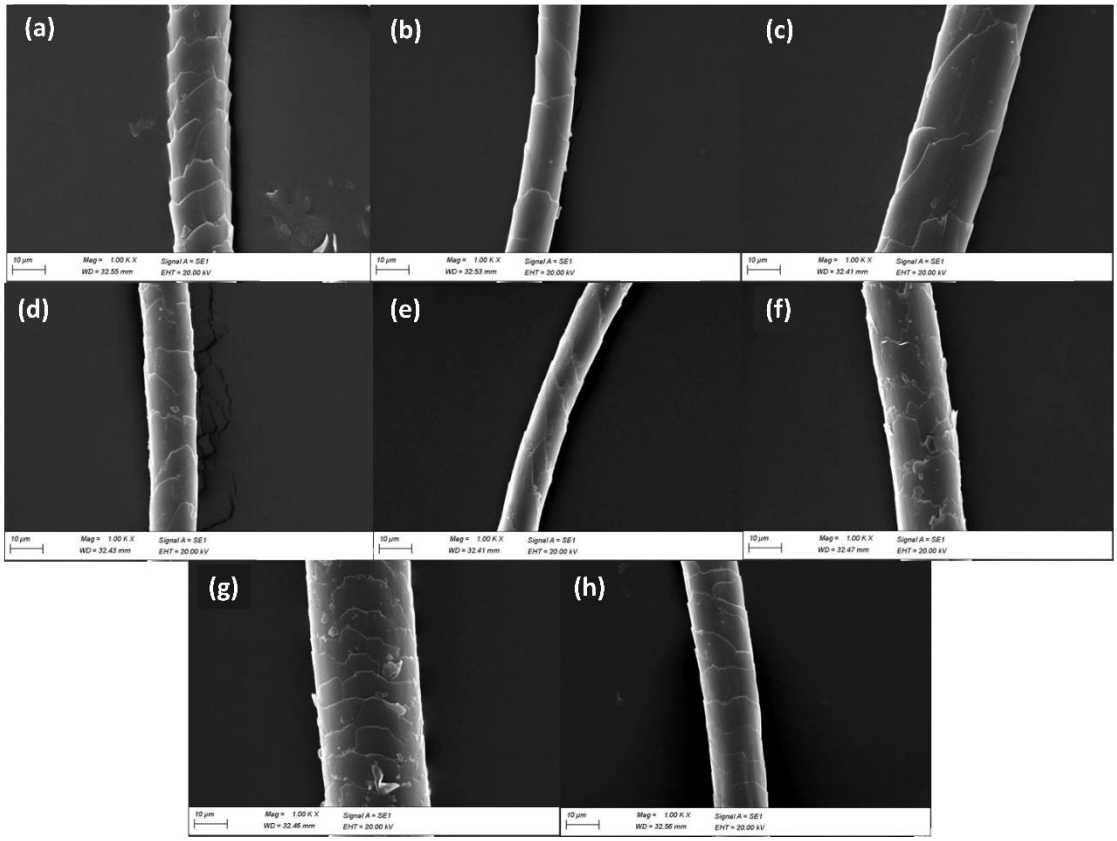

Figure 2: SEM images of the different animal fibers: Wool (a), Cashmere (b), Mohair (c), Yak (d), Camel (e) Alpaca (f), Llama (g) and Vicuña (h). Scale bar: 10 µm.

In Figure 3, infrared spectra of hair fibers from the eight animal species considered are reported.

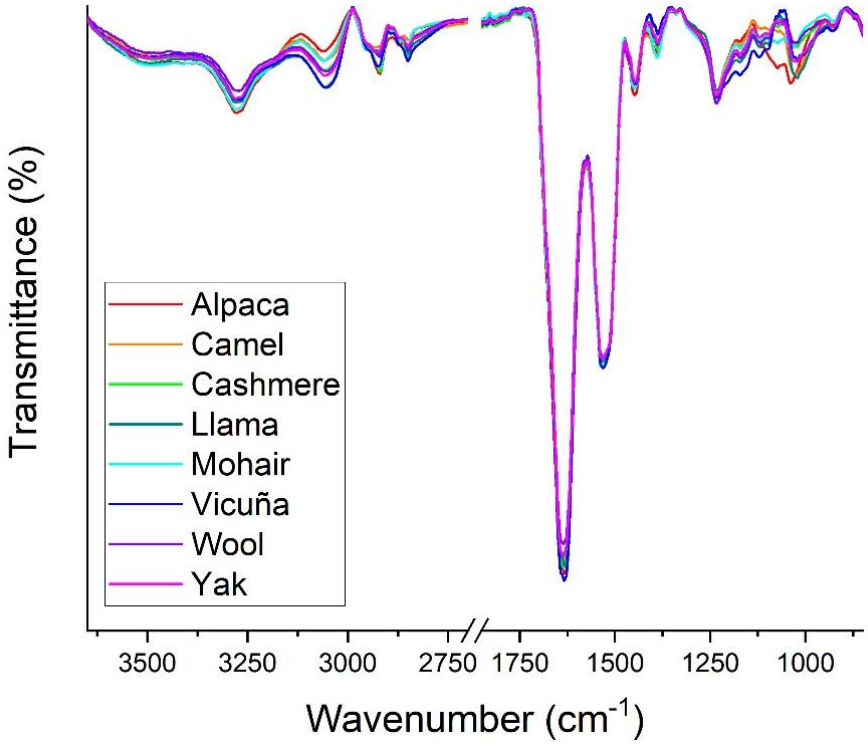

Figure 3: Averaged infrared spectra of hair fibers from eight animal species.

The typical spectra of hair fibers consist of some macro-regions that can be summarized as follows:

- The broad zone with centers at 3500 and 3275 cm^{-1} due to the O-H and N-H stretching vibrations (amide-A band)[31], plus the peak at 3060 cm^{-1} of Amide B;[32,33]

- The signals around 2920 and 2850 cm^{-1} due to asymmetrical and symmetrical stretching of the CH_2 and CH_3 groups;[34–37]

- The peaks at 1634 cm^{-1} (Amide I) and at 1530 cm^{-1} (Amide II) corresponding, respectively, to C=O stretching vibration and the coupling of the N-H bending with C=N stretching;[34]

- The complex Amide III band at 1234 cm^{-1} attributed to the in-phase combination of C-N stretching and N-H bending, with some contribution from C-C stretching and C=O bending vibrations;[38]

- The peaks between 1200 and 1000 cm^{-1} (fingerprint region) belonging to the S-O stretching vibration band (hair keratin contains a high amount of intra- and intermolecular disulfide bonds of cystine amino acids).[23,39]

First of all, the absence of significant absorptions around 1760-1720 cm^{-1}, where the signals of esters and organic acids of lanolin can lie, is the index of a sufficient cleanness of the fibers, making the ATR-FTIR attributions mostly relatable to the inherent characteristics of the keratin-based components.[40] Indeed, lanolin is the wool grease that is derived from animal skin secretion.[40]

Some of the main differences among spectra can be detected by zooming in on the spectrum in the high wavenumber region (Figure S1 in Supporting Information). Between 3250 and 3150 cm^{-1}, a more pronounced shoulder in llama and vicuña spectra than in other samples can be ascribed to differences in the presence of primary and secondary amide functionalities.[35] However, in the range 3400-3100 cm^{-1}, also residual adsorbed moisture might influence the spectral features. In the region of C-H vibration modes, the band centered at 2920 cm^{-1} is particularly diverse for yak (less sharp), camel (less intense and flat) and llama (additional shoulder at 2934 cm^{-1}), while, at 2893 cm^{-1}, a small peak appeared for vicuña and sheep wool. This region seems particularly affected by the sample variation, probably not only for the C-H modes, but also for the contribution of NH_3^+ stretching vibrations and the specific differences in keratin composition, as already seen in the FTIR spectra acquired in Dai et al.[41] Moreover, between 2885 and 2825 cm^{-1}, the positions of the peaks slightly varied depending on the sample type and the intensity ratio of the signals 2851/2872 cm^{-1} (CH_2/CH_3) resulted in being reduced in the cases of camel and alpaca. The explanation for these discrepancies likely lies in the aminoacidic composition -creating polypeptide chains- of the animal hair deriving from different species and exemplars.[42] Indeed, variable combinations of alanine, arginine, aspartic acid, cysteine, glutamine, glutamic acid, histidine, leucine, lysine, methionine, serine, threonine, tyrosine and valine are usually contained in keratins.[43–45] Regarding the cases in which the diminishing of the CH_2/CH_3 ratio happens, for instance, it is possible to hypothesize a decrement of alanine, leucine and valine that possess a methyl group with respect to other amino acids where only -CH_2 are present, like glutamic acid or cysteine that are among the most abundant ones in keratins.[46]

If the amide I and amide II bands can be considered substantially equal among the specimens, the fingerprint zone clearly highlighted other variations (Figure 3). The

transmittance values for vicuña, from 1233 to 1097 cm^{-1}, were higher than other fibers, probably implying a difference in the secondary structures of keratin, where β-sheets were favored.[47]

For alpaca, the signal intensity enhancement occurred from 1138 to 966 cm^{-1}, together with a shift of the peak at 1030 cm^{-1} to 1040 cm^{-1}. On the contrary, the peak at about 1030 cm^{-1} showed a decreased intensity in mohair, cashmere and wool samples. This latter signal is assignable to cysteine -S-sulfonate (-S-SO_3-) and cysteic acid (-SO_3H), whereas between 1055 and 1200 cm^{-1}, cystine oxides can be detected.[36,48–51] Again, these discrepancies can be typical of specific amino acid presence, particularly in terms of cysteine amount and, although to a lesser extent, methionine, or an index of the oxidation state of the fibers. The oxidation state of the fibers can be related to multiple factors, namely the exposure of the animals from which the hair originates to oxidative stress sources,[52–54] or modifications potentially occurring in the supply chain. In this excursus, the sample that showed various peculiarities was the alpaca, which is in accordance with the literature. We suggest, indeed, that in this kind of fiber, sulfur-based and –CH_2-containing amino acids, such as cysteine, are predominant at the expense of other amino acids. Atav and Türkmen have already found that Suri alpaca fibers had higher sulfur and cystine content compared with sheep wool.[55]

Given the time-expensive procedures and the necessity of experienced operators to punctually distinguish the physical-chemical characteristics of each fiber sample, multivariate analysis was applied to the infrared spectra and a PLS-DA model was built to discriminate them by a chemometric tool. In order to choose the optimum number of components to build a model expressing the maximum variance among animal species but with minimum estimated error, diagnostics and 10-fold cross-validation were performed at the training set (168 samples). As depicted in Figure 4a, a high number of components (18 latent variables, LVs) were required to achieve a variance expression of 99.10 %. Indeed, a model with 18 LVs showed the minimum mean squared error of cross-validation (MSECV, 0.4851, Figure 4b), while the LVs' increase did not add any features to the model. Figure 4c displays the score plot of the first three LVs, indicating the discrimination propensity of the model. Finally, the

discrimination ability of the model was evaluated with the classification of "unknown" test dataset (72 samples). According to the confusion matrix, the 18-LVs model succeeded with 100 % accuracy (Figure 4d), indicating very good discrimination ability. The discrimination of alpaca, vicuña, llama, mohair and Cashmere via ATR-FTIR spectroscopy and machine learning techniques has recently and successfully been carried out in the work of Quispe et al.[24]

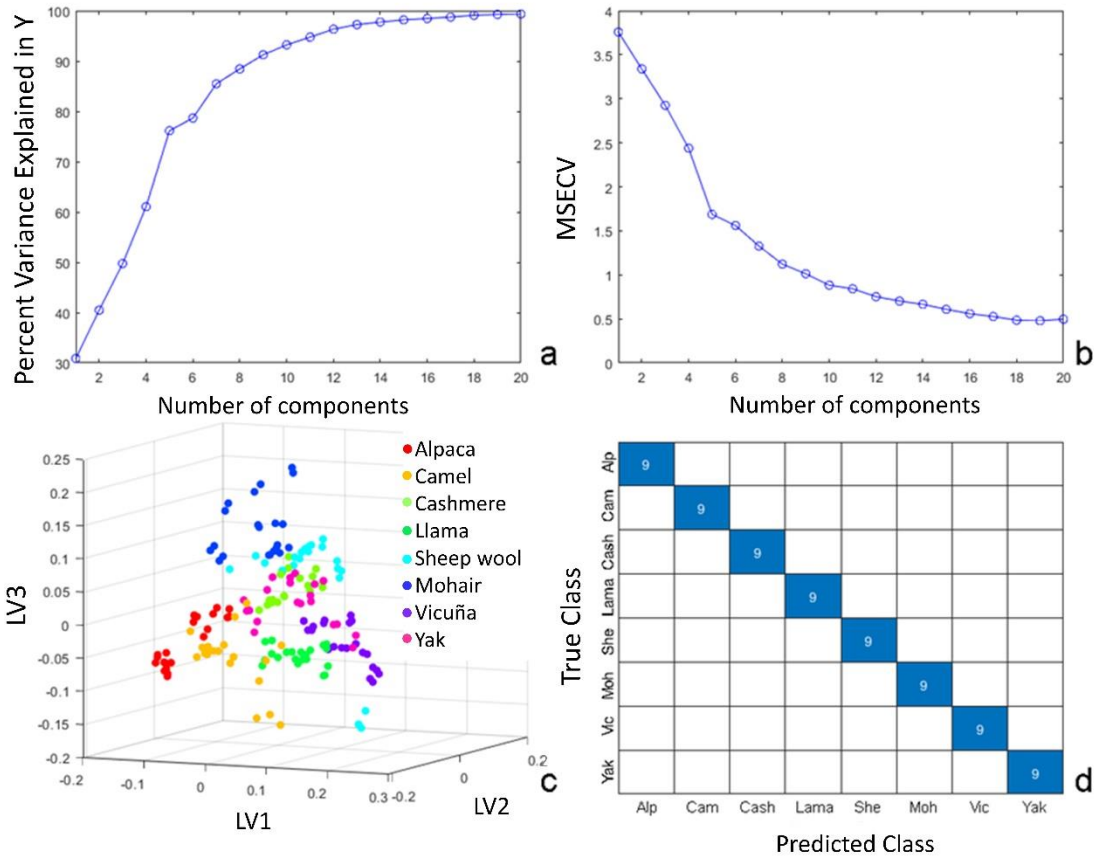

Figure 4: Diagnostics of the PLS-DA model among eight animal species. (a) Percentage of variance explained in Y (groups of animal species), (b) 10-fold cross-validation of the model, (c) score plot of the first three components (LV1, LV2, LV3) and (d) confusion matrix (using 18-LVs) of the predicted "unknown" test dataset. In (d), Alp=alpaca, Cam=camel, Cash=cashmere, Lama=llama, She=sheep wool, Moh=mohair, Vic= vicuña, Yak=yak. Such order is followed from left to right in the "Predicted Class" axis and from top to bottom in the "True Class" axis.

However, herein because of the large number of groups that were analyzed, a high number of components (18 LVs) were required. In order to assess the discrimination ability of infrared spectroscopy, the study was repeated, including only the subgroup of camelids (84 samples as the training dataset). It is evident in Figure 4c that the four

subgroups of camelids (alpaca, camel, llama, vicuña), at the lower part of the plot, were slightly separated from the other groups of animals. Indeed, by reducing the number of groups, PLS-DA could separate the four classes of camelids with only ten LVs. According to Figure 5, a 10-LVs model was able to classify the "unknown" samples (36 samples as test dataset) correctly with 100 % accuracy, while the expressed variation and MSECV (99.43 % and 0.0437 in Figure 5a and 5b, respectively) were much better than previously.

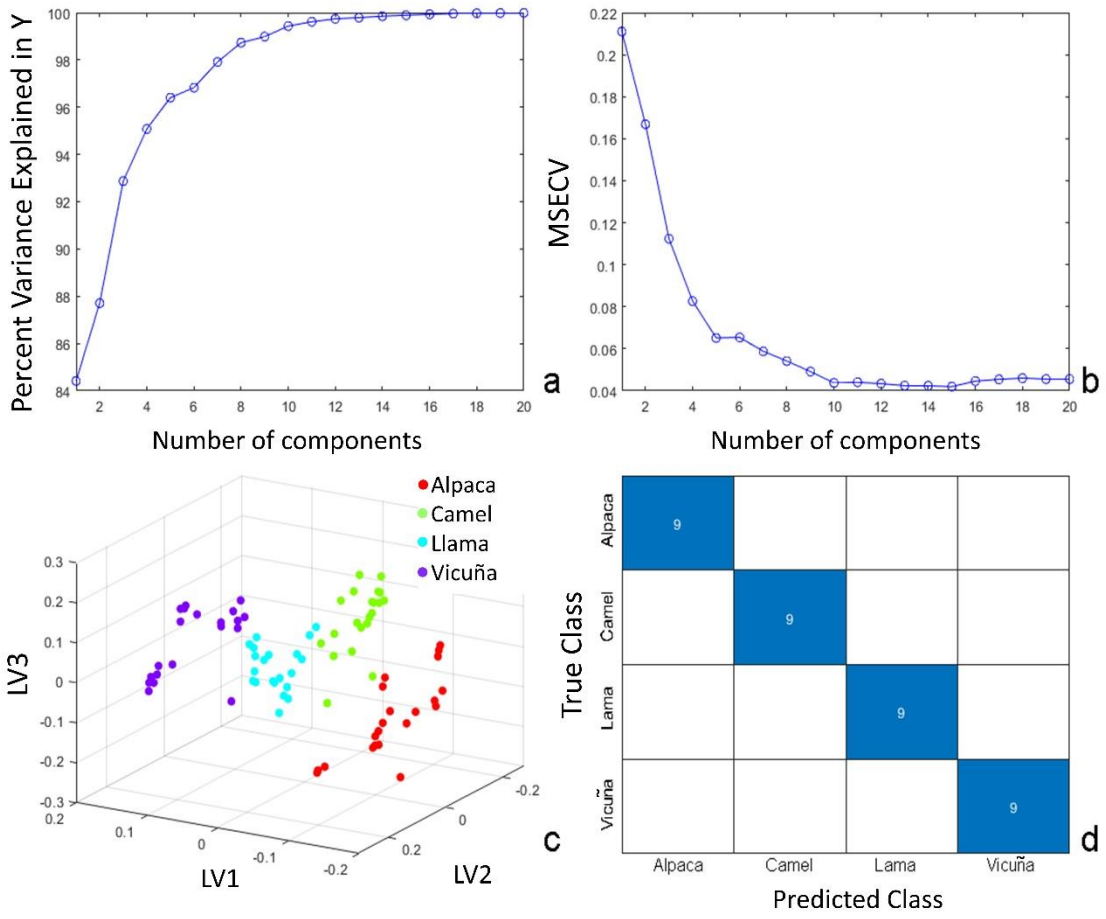

Figure 5: Diagnostics of the PLS-DA model among four camelids (alpaca, camel, llama, vicuña). (a) Percentage of variance explained in Y (groups of camelids), (b) 10-fold cross-validation of the model, (c) score plot of the first three components (LV1, LV2, LV3) and (d) confusion matrix (using 10-LVs) of the predicted "unknown" test dataset.

These analyses confirm not only the ability of ATR-FTIR to separate animal hair from different animal species, but also the ability to discriminate hair from animals belonging to the same family.

PLS-DA model for cashmere fibers from different origins

At this point, delving further into this research, only one animal fiber type was studied, but this time, hair samples were collected from different countries (Afghanistan, Australia, China, Iran and Mongolia). It is important to point out that fibers coming from the same animal can show slight differences in morphological characteristics according to their geographical origins. However, these differences are difficult to recognize by microscopical analysis and to associate them with one origin rather than another. In Figure 6, SEM images of cashmere fibers coming from goats of different origins are reported and they are all identified as "fibers having the morphological characteristics of cashmere".

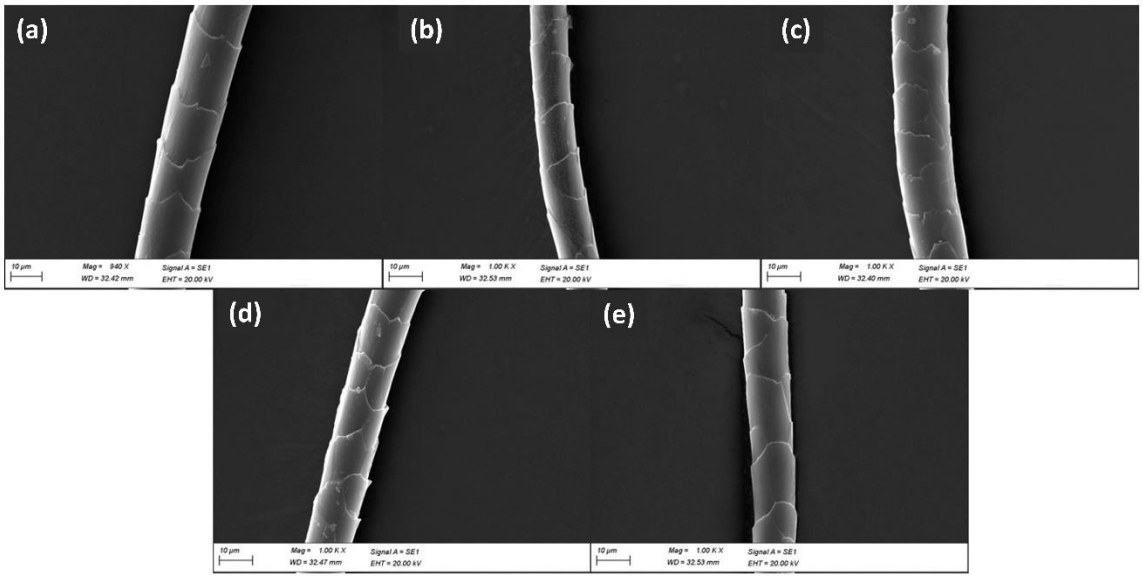

Figure 6: SEM images of cashmere fibers with different geographical origins: Mongolia (a), China (b), Iran (c), Afghanistan (d) and Australia (e). Scale bar: 10 µm.

Regarding the multivariate analysis, since it began with a maximum number of ten latent variables, 10-fold cross-validation was performed to further minimize the LVs' number (Figure S2 in Supporting Information). A compromise between a low MSECV and a minimum number of LVs was selected as an optimum prediction model without overfitting: thus, the analysis proceeded by choosing only six LVs (MSECV 0.1525) and obtaining an excellent discrimination ability.

The differentiation among the training dataset samples associated with the five countries of origin of the cashmere hair can be detected distinctly in the score plot of PLS-DA (Figure S3 in Supporting Information). On the one hand, Australian samples showed some variability; still, this group could be discriminated by the model. On the other hand, Mongolian samples had the best separation from the other groups. Consequently, 6-LVs PLS-DA model with an expressed variation of 94.88 % and excellent fitting ability (R^2 = 0.9488) can be used as a suitable chemometric tool for the discrimination among the five countries of origin of the cashmere hair.

The analysis continued by investigating the variables associated with the discrimination. A detailed inspection of ATR-FTIR spectra (Figure 7a and 7b) together with the regression coefficients plot of the first two major components (Figure 7c) indicates all spectral characteristics from which the clustering of the samples mainly derives. As anticipated in the previous section, the detection of the peak at 1735 cm^{-1} in the Mongolian fibers could give an idea of a lower degree of cleanness of that sample, which can have influenced the discrimination, bringing about additional information regarding the skin secretion of the animal species. However, analyzing other more PLS-DA "sensitive" regions, between 2980 and 2825 cm^{-1}, assigned to C-H asymmetric and symmetric stretching vibrations, the most visible event was the band intensity variation (the Mongolia sample was the most intense, whereas the Afghanistan sample showed the smallest signals). In the case of Mongolian fibers, this fact can also be associated with the superimposition of alkyl chain groups of lipids [35] with those of aminoacids. This hypothesis is corroborated by the presence of a broadened band comprised between 1280 and 1130 cm^{-1} (Figure 7a and 7b), which can subtend the vibrational modes of C-O stretching, related to fatty acids.[56]

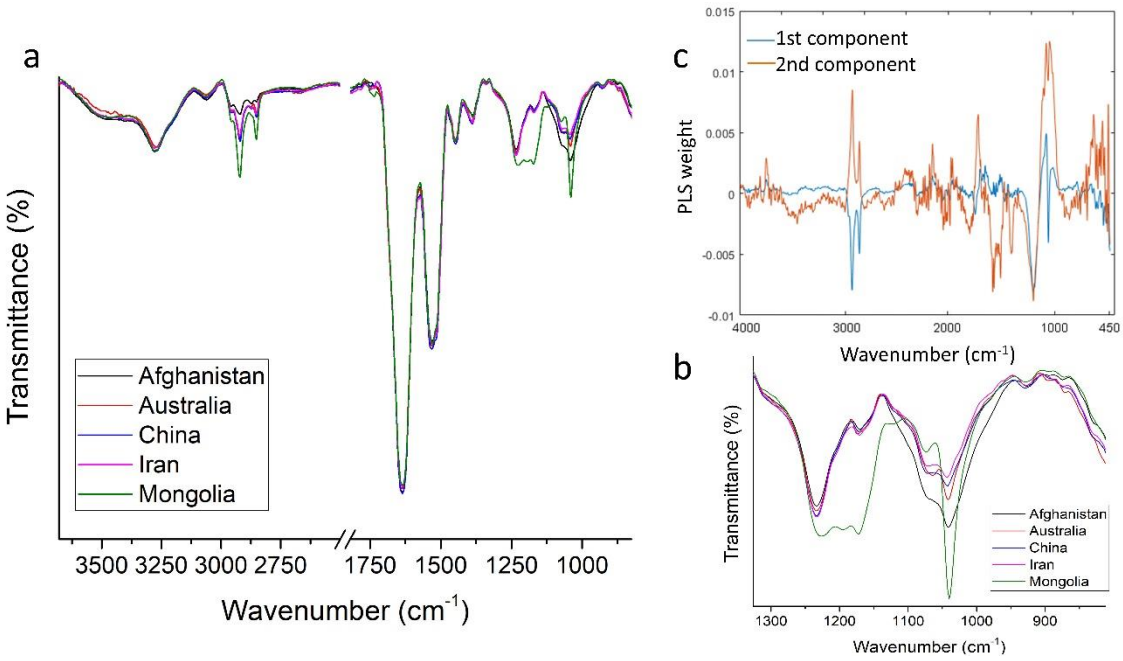

Figure 7: (a) Infrared spectra of cashmere fiber from different countries, (b) zoom of the region 1325-810 cm^{-1} (c) PLS coefficients plot.

Additionally, a strong peak at 1182 cm^{-1} (appeared in both LV1 and LV2), and two peaks at 1056 cm^{-1} and 1036 cm^{-1} (the strongest among LV2's variables which were associated with the discrimination, but in LV1 not so strong) were present in the loading plot (Figure 7c). As already mentioned, these wavenumbers were connected to S-O stretch vibration. More specifically, the 1200-1000 cm^{-1} region is the infrared window of cystine dioxide (-SO_2-S-) (1182 cm^{-1}), cystine monoxide (-SO-S-) (1056 cm^{-1}) and cysteic acid (-SO_3H) (1036 cm^{-1}) vibrations.[49–51] Looking at Figure 7b, the most diverse band centered at about 1040 cm^{-1} was that from Afghanistan, with its stronger intensity. The differences observed in spectra of cashmere fibers could be correlated with their different amino acid composition, in turn, linked with the origin and the diet (grazing, administration of feeds, use of protected proteins) and also by the energy intake.[57] Glycine, phenylalanine, serine, and tyrosine content has been reported to be affected by the country of origin; alanine, histidine, isoleucine, proline, valine, aspartic acid and phenylalanine levels depend on the energy nutrition and management.[57]

Multivariate analysis was ended by utilizing the developed 6-LVs PLS-DA model to assess the test dataset samples of cashmere hair indicated as "unknown" for the model. The confusion matrix of the test dataset is reported in Figure 8. The accuracy

of the model was 86.67 %, which indicates an extensive classification of the "unknown" country of origin of the samples. Australian cashmere hair was the group with the highest misclassification capacity, due to the fact that five out of nine "unknown" samples were classified elsewhere. Overall, only 6 out of the total 45 samples of the test dataset were misclassified, giving the model sensitivities and specificities presented in Table 1. These results demonstrate that ATR-FTIR coupled with PLS-DA could satisfactorily discriminate the cashmere hair samples from different origins.

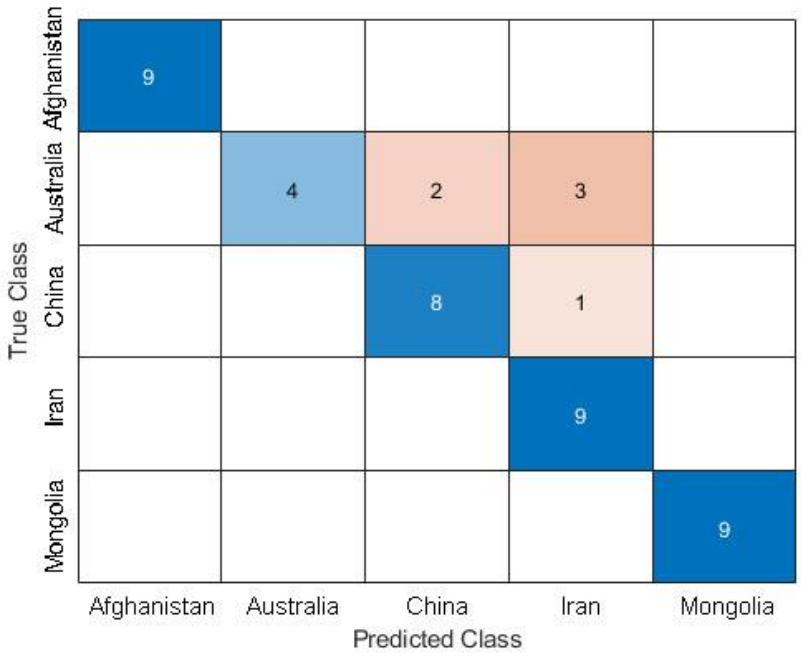

Figure 8: Confusion matrix of the cashmere hair origin predictive PLS model.

Table 1: Sensitivity and specificity of country origin of cashmere hair.

Country origin	Sensitivity	Specificity
Afghanistan	1	1
Australia	0.44	1
China	0.89	0.94
Iran	1	0.89
Mongolia	1	1

Conclusions and perspectives

In summary, our study represents a significant advancement in the field of animal hair fiber discrimination and origin classification. We investigated the utility of Attenuated Total Reflection (ATR) Fourier Transform Infrared (FTIR) spectroscopy coupled with a chemometric tool, specifically Partial Least Squares Discriminant Analysis (PLS-DA), for discriminating various animal hair fibers (cashmere, mohair, yak, camel, alpaca, vicuña, llama and sheep) and differentiating cashmere hair samples from different origins (Afghanistan, Australia, China, Iran and Mongolia). Scanning Electron Microscopy (SEM) technique was used to analyze the surface morphology of the different sample categories.

Our findings underscore the effectiveness of ATR-FTIR coupled with PLS-DA in accurately distinguishing between eight animal hair groups and camelid subgroups. Specifically, it achieved variance expressions of 99.10 % (18 LVs) and 99.43 % (10 LVs) when discriminating between eight animal hair groups and camelid subgroups, respectively. The robustness of our discrimination approach was supported by low MSECV values, 0.4851 and 0.0437, for eight animal hair groups and camelid subgroups, respectively.

Particularly, ATR-FTIR coupled with PLS-DA effectively classified cashmere hair samples from different countries, with Mongolian samples demonstrating superior separation compared to Australian samples, which exhibited higher misclassification rates. The discrimination seemed to be influenced by the superimposition of alkyl chain groups of lipids, when detected, and cystine oxides and cysteic acid vibrations also contributed with a strong influence.

Although this study successfully demonstrates the qualitative identification of animal fibers using PLS-DA, it does not encompass quantitative analysis, which is useful for blends. Additional research should explore the integration of quantitative methods to enhance the robustness and applicability of the findings. Moving forward, future research efforts could focus on addressing some of the limitations encountered in our study, such as sample size constraints or the exploration of additional discriminant

factors beyond amino acid compositions. Additionally, there is potential to further optimize the ATR-FTIR technique and refine chemometric models to enhance discrimination accuracy and reliability, as well as investigate other animal hair types, such as cashmere, whose origins hold significant importance in the fashion industry and potential susceptibility to commercial frauds.

To conclude, our research contributes to advancing the understanding and application of analytical techniques for discriminating animal hair fibers, taking part in the preliminary stages of the textile manufacturing process, or as a complementary tool together with other identification techniques.

Author contributions

Conceptualization: Maria Laura Tummino, Vasilios Sakkas; Formal analysis and investigation: Christoforos Chrimatopoulos, Maria Laura Tummino, Eleftherios Iliadis, Cinzia Tonetti; Writing - original draft preparation: Christoforos Chrimatopoulos, Maria Laura Tummino; Writing - review and editing: all the authors; Supervision: Vasilios Sakkas.

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