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ISOLATION AND CHARACTERIZATION OF KERATIN IN FEATHERS OF LOCAL AND EXOTIC BIRDS USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR) TECHNIQUE

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ABSTRACT
Keratin wastes are considered as environmental pollutants produced mostly from the poultry farms, slaughterhouses, and leather industries. Keratin wastes are dumped, buried, used for landfilling, or incinerated and all these actions increase the threats of environmental hazards, pollution, negatively influence the public health, and increase greenhouse gases concentration. This study aimed to isolate and characterize keratin in feathers of local and exotic birds from four slaughter houses in Kaduna north, Kaduna State. Keratin extracted from both samples was used for the characterization process using Fourier-transform infrared spectroscopy (FTIR). The result shows that the local specimen exhibits several distinctive hydrogen-bonded hydroxyl (OH) groups, including a broad and weak peak at 3201.4 cm^{-1}, indicating the presence of hydrogen-bonded hydroxyl (OH) groups. The analytical zone (3500 to 1500 cm^{-1}) of the FTIR spectrum for keratin from local turkey feathers revealed the presence of several distinct methyl groups with a peak at 3000.5 cm^{-1} while the FTIR spectrum of keratin from exotic turkey feathers revealed a complex array of functional groups with a broad peak at 3227.9 cm^{-1}. The spectrum of keratin from local duck feathers were observed at a peak of 2851.4 cm^{-1} while the spectrum of keratin from exotic duck feathers exhibited distinct features with peaks at 2847.7 cm^{-1} and 1994.1 cm^{-1} for methylene C-H stretches and isothiocyanate groups, respectively. The findings of this study revealed that local varieties demonstrated a lower concentration of keratin proteins of 52.3±0.73, 57.9±0.35, and 57.7±0.01 for chicken, turkey and duck feathers respectively when compared to exotic chicken, turkey and duck feathers with a higher concentration of keratin proteins of 55.7±0.00, 64.2±0.23, and 60.4±0.40 respectively which aligned with selective breeding aimed at improving feather quality for ornamental and commercial purposes. This increased concentration may enhance the mechanical and biochemical properties of the feathers, making them more suitable for various applications. The successful isolation and purification of keratin from local and exotic bird feathers confirmed that feather type influences keratin yield. This diversity in protein composition may contribute to unique mechanical properties, such as increased strength and flexibility, which are crucial for applications in textiles and biocompatible materials. Notably, the isolation and characterization process yielded keratin with a percentage purity of 96% from exotic bird feathers, compared to 89% from local bird feathers. This difference in purity may contribute to the distinct properties observed in exotic feathers.

Keywords: Keratin, feathers, isolation, identification, Fourier-transform infrared spectroscopy.

INTRODUCTION
Keratin, a structural protein present in the outer covering of vertebrates, is one of the most significant biopolymers in the animal kingdom, second only to collagen. It forms a major part of intermediate filaments and is outstanding among all other members due to its high molecular diversity (Swain *et al.,* 2018). Its various forms exhibit diverse morphologies, ranging from simple waterproof layers like those found in turtle shells to resilient, impact-resistant substances like horn. Keratin demonstrates mechanical efficiency in both tensions, as seen in wool, and compression, such as in hooves (Donato and Mija, 2020). Notably, it shares similarities with collagen, the other major structural protein in animals, which is found in bones, teeth, and connective tissue. Both keratin and collagen consist of α-helix polypeptide chains with well-defined amino acid sequences and a high proportion of glycine and alanine, facilitating the formation of the α-helical structure (Donato and Mija, 2020).

However, there are distinct differences between keratin and collagen. Keratin is formed by two polypeptide chains twisting together to create a coiled coil (α-keratin), while collagen is assembled from three α-helices (tropocollagen) intertwining to form collagen fibrils. Additionally, keratinocytes, the cells responsible for keratin production, perish once they produce keratin, resulting in a "dead" tissue devoid of vasculature, unlike collagen, which is synthesized in the extracellular matrix. Consequently, keratinized materials often exhibit a tiled structure, with polygonal tiles overlapping laterally and stacking atop one another to create a relatively dense layer (Swain *et al.,* 2018).

Furthermore, keratin can be viewed as a composite material comprising a short fiber, known as crystalline keratin, embedded within a polymer matrix, termed amorphous keratin. The amorphous areas can absorb water and swell, while the crystalline component is water insoluble. Keratin typically has a higher Young's modulus than collagen, but it also shows exceptional strain tolerance, indicating excellent toughness levels. Thiol groups (-SH) found in cysteine residues, which are abundant in keratin, provide potent covalent disulfide bonds that bind matrix molecules and polypeptide chains together. This bonding technique is similar to how rubber vulcanizes. Keratins can be classified as 'hard' or 'soft,' with softer types having fewer cross-links and fewer sulfur atoms overall. The epidermis, the skin's outermost layer, is where soft keratin is primarily found (Mahmood *et al.,* 2021).

Recently, there has been considerable interest in utilizing keratin

proteins derived from waste poultry feathers. Keratin is the predominant structural fibrous protein found in various avian components, including feathers, hair, skins, bristles, horns, and hooves. Feather composition typically consists of approximately 91% keratin, imparting structural characteristics associated with high mechanical strength (Ramakrishnan *et al.,* 2018; Vineis *et al.,* 2019). Numerous food industries, such as meat markets, slaughterhouses, and wool producers, generate vast amounts of biomass containing keratin (Gupta *et al.,* 2016). In Ethiopia alone, the poultry industry and slaughterhouses produce billions of tons of poultry feather waste annually, with facilities capable of processing 50,000 chickens able to yield 2-3 tons of dry feathers per day (Tesfaye *et al.,* 2017). Given that chicken feathers can constitute up to five percent of a bird's body weight, their disposal during poultry production for human consumption presents a significant challenge. Notably, chicken feathers may harbor viruses and bacteria, posing potential risks to both human health and the environment (Gindaba *et al.,* 2019). There exists minimal demand for waste poultry feathers, leading most poultry producers to dispose of over five billion tons of feathers annually worldwide through methods like burial, burning, or grinding for inclusion in livestock feed. Burning, the most common disposal method, can release up to 50 times more carbon dioxide than the coal industry, posing a significant environmental concern (Prasanthi *et al.,* 2016). Additionally, burning feathers in specialized facilities proves economically inefficient. Consequently, extracting keratin from feather waste and utilizing it for various bio-products in biomedical engineering, micro and nanoparticles for food, medical, fertilizers, textile and clothing industries, cosmetics, and bio-plastics could substantially mitigate environmental issues and meet current and future keratin demands (Tesfaye *et al.,* 2017; Tesfaye *et al.,* 2018). The keratin protein solution offers diverse applications, including anti-aging creams, shampoos, conditioners, as well as medical uses such as bone replacement and grafting. Presently, there is a growing interest in the creation of environmentally friendly materials sourced from renewable origins (Carrillo, 2016). The main sources of renewable materials include proteins, lipids, and polysaccharides. As polymers made up of various amino acids, proteins promote intra- and intermolecular bonding, which gives resultant materials a variety of functional characteristics. Protein deficiencies provide a serious health danger in underdeveloped nations, increasing the likelihood of disease and possibly death. The search for new protein sources, including waste products, is necessary due to the scarcity of proteins for food and other uses. Feathers from poultry are a bioresource with a high protein content (Mahmood *et al.,* 2021).

Poultry feathers present a significant challenge in waste management due to their resistance to hydrolysis, primarily attributed to the presence of disulfide bonds that protect keratin from degradation. The prevailing practices for collecting and disposing of feather waste vary widely depending on the socio-economic conditions of individual countries. Insufficient infrastructure for waste collection and handling often results in suboptimal utilization of these resources, leading to substantial wastage and environmental harm. Ineffectual management of feather waste not only represents an economic loss but also contributes to pollution.

Therefore, the need for alternative approaches to handling feather waste is paramount. This study aimed to address this need by investigating methods for extracting valuable proteins from poultry feathers for various applications, including anti-aging cream, feather meal, insulation, plastics, paper, pillow stuffing, and more. By converting waste feathers into valuable resources, this research endeavor seeks to reduce the accumulation of poultry feather waste and promote the concept of turning waste into wealth (Ma *et al.,* 2016).

MATERIALS AND METHODS

Collection of Sample

Feather samples from diverse avian species in Kaduna State were collected in sterile containers and transported to the Biochemistry Laboratory at Kaduna State University. Feather samples from various avian species including chicken, duck, turkey, and guinea fowl were procured from multiple slaughterhouses across Kaduna State. These samples were meticulously gathered in sterile containers to ensure the integrity of the specimens during transportation to the Biochemistry Laboratory at Kaduna State University for subsequent analysis.

Pre-treatment of the Feathers

The collected feather samples underwent a thorough cleaning process. They were submerged in ether for a duration of 24 h to eliminate any stains, oils, or grease adhering to the feathers. Following this, the feathers were subjected to successive washes with 0.1% Triton X-100 and distilled water to ensure complete cleansing. Subsequently, the materials were carefully oven-dried at 60 ℃ for a duration of 8 h. The dry weight of the feathers was measured both before and after blending. The dried samples were then ground using either a pulverizer, miller, or blender and stored securely in sealed plastic containers for further experimentation. A sodium sulfide solution was prepared in a conical flask. Ground feather samples were weighed and added to this solution, which was then heated to a temperature of 30 ℃ while maintaining a basic pH level between 10 and 13. The solution underwent continuous stirring for 6 h before being filtered and centrifuged at 10,000 rpm for 6 minutes. The resulting supernatant, free from particles, was collected for subsequent steps, as described by Gupta *et al.* (2012).

Precipitate Preparation

To prepare the precipitate, a solution of ammonium sulfate was prepared and filtered to ensure particle-free status. The previously collected feather filtrate was placed in a beaker and continuously stirred, while ammonium sulfate solution was added drop by drop to achieve a 1:1 ratio with the filtrate. The resulting mixture was then subjected to centrifugation at 10,000 rpm for 6 minutes to collect solid particles. This process was repeated with the supernatant to ensure comprehensive precipitation, as outlined by Gupta *et al.* (2016).

Purification of Keratin (Protein)

The collected solid particles, constituting the precipitate, were dissolved in a solution of 2 M sodium hydroxide (NaOH) and subjected to centrifugation at 10,000 rpm for 6 minutes to collect the supernatant. This process of precipitating, washing, and dissolving was repeated 3 to 4 times to ensure thorough purification, following the methodology proposed by Gindaba *et al.* (2016).

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Fourier Transformed Infrared Spectroscopy Spectrum (FTIR)
The solution obtained after purification underwent analysis using Fourier transform infrared spectroscopy (FTIR), and the resulting wavelength graph was compared with an amino acid standard graph. This involved fully precipitating the solution using ammonium sulfate, separating the solids, and weighing them.

Data Analysis
Data obtained from the study were subjected to statistical analysis using GraphPad-prims (ver. 9.3.1). Independent sample t-test was used to determine the variable in Keratin yield and molecular weight distribution between the local and exotic bird species.

RESULTS

Result of Keratin protein yield
Table 1 shows the result of keratin protein concentration in the various local and exotic bird feather samples measured using the biuret test. The result reveals a higher concentration in all the exotic bird feather samples compared to the local bird samples. Statistically, the significance values obtained from the independent sample t-tests indicate that there are statistically significant differences in protein concentration between local and exotic feather samples for chicken ($p = 0.042$), turkey ($p = 0.004$), and duck ($p = 0.021$). This suggests that the type of poultry (local or exotic) has a significant impact on keratin protein yield across all three types of samples, as all p-values are below the conventional significance level of 0.05.

Table 1: Keratin protein concentration in local and exotic chicken, turkey and duck feather

Keratin Sample	Protein yield (mg/mL)	*P value* (2-tailed)
Chicken Feathers		
Local	52.3±0.73	0.042
Exotic	55.7±0.00	
Turkey feathers		
Local	57.9±0.35	0.004
Exotic	64.2±0.23	
Duck Feathers		
Local	57.7±0.01	0.021
Exotic	60.4±0.40	

Results are presented as mean ± standard error. Probability values (significant values) ≤ 0.05 indicate statistically significant difference between groups.

Result of FTRI analysis of Keratin from chicken feathers
Figure 1 and 2 shows the FTRI spectrum of keratin from local and exotic chicken feathers. The analytical zone (3500 to 1500 cm^{-1}) of each spectrum revealed four FTIR peaks in the local chicken feather keratin specimen and six FTIR peaks in the exotic chicken feather keratin specimen. The FTIR spectrum of the local specimen exhibits several distinctive functional groups, including a broad and weak peak at 3201.4 cm^{-1}, indicating the presence of hydrogen-bonded hydroxyl (OH) groups. This suggests the involvement of hydroxyl-containing compounds in the keratin structure. Small peaks at 2359.4 cm^{-1} and 2109.7 cm^{-1} confirm the presence of C≡C bonds, indicative of terminal alkynes. A small peak at 1994.1 cm^{-1} confirms the presence of isothiocyanate (NCS) groups, which contribute to the biological reactivity of keratin. Additionally, a sharp and intense band at 1648.8 cm^{-1} is associated with the stretching vibration of alkenyl C=C groups, indicating the presence of carbon-carbon double bonds. In contrast, the FTIR spectrum of keratin from exotic chicken feathers revealed the presence of several functional groups. A peak at 2847.7 cm^{-1} indicates the presence of methoxy or methyl ether C-H stretches, suggesting ether linkages in the keratin structure. A peak at 2094.8 cm^{-1} confirms the presence of cyanide or thiocyanate ions, indicative of nitriles or thiocyanates within the keratin matrix. A peak at 1994.1 cm^{-1} points to isothiocyanate groups, known for their active role in the biological activity of keratin. A peak at 1871.1 cm^{-1} suggests the presence of transition metal carbonyl complexes, indicating possible interactions with metal ions. An intense band at 1640.0 cm^{-1} is associated with alkenyl C=C stretching vibrations, highlighting unsaturated carbon-carbon double bonds. Lastly, a peak at 1599.0 cm^{-1} corresponds to the C=C-C stretching vibrations of an aromatic ring, suggestive of aromatic amino acids like phenylalanine, tyrosine, or tryptophan. These functional groups collectively contribute to the unique structural and chemical properties of keratin from both local and exotic chicken feathers.

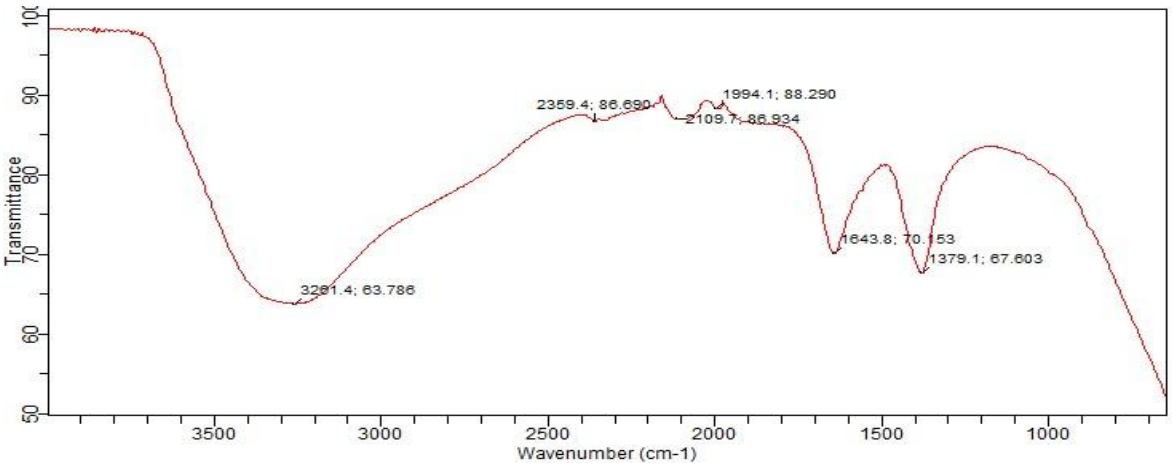

Figure 1: FTRI spectrum of keratin from local chicken feather

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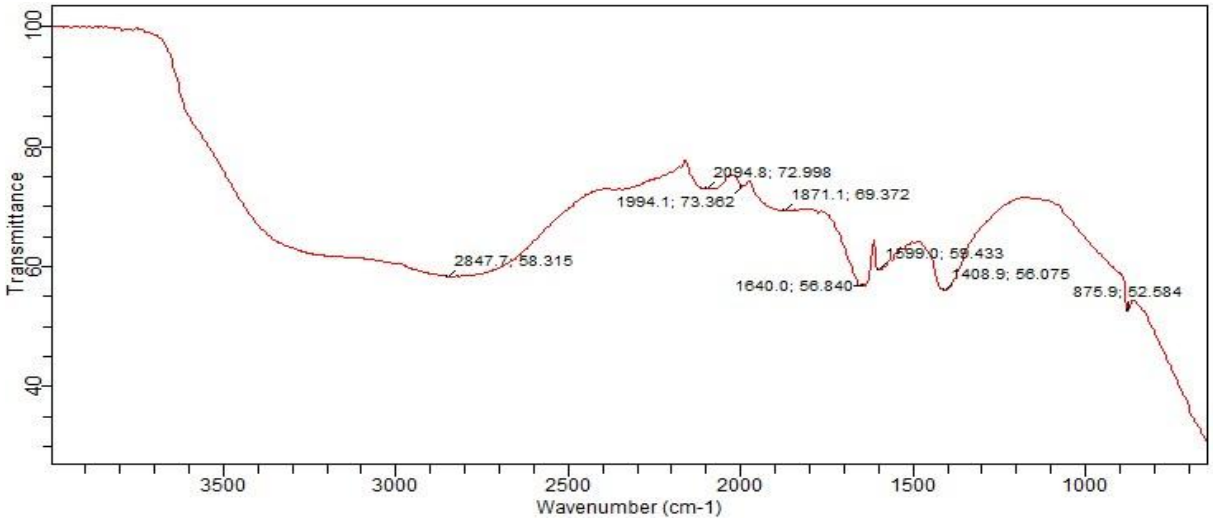

Figure 2: FTRI spectrum of keratin from exotic chicken feather

Result of FTRI analysis of Keratin from turkey feathers

Figure 3 shows the analytical zone (3500 to 1500 cm^{-1}) of the FTIR spectrum for keratin from local turkey feathers revealed the presence of several distinct functional groups. A peak at 3000.5 cm^{-1} indicates C-H stretching vibrations, characteristic of alkyl groups. The presence of asymmetrical and symmetrical methylene C-H stretches is confirmed by a peak at 2851.4 cm^{-1}, suggesting methylene groups contribute to the protein's aliphatic character. A peak at 2079.9 cm^{-1} confirms the presence of cyanide or thiocyanate ions, indicative of nitriles or thiocyanates within the keratin matrix. The peak at 1990.4 cm^{-1} is characteristic of isothiocyanate groups, known for their role in the biological activity of keratin. Transition metal carbonyl complexes are suggested by a peak at 1874.9 cm^{-1}, indicating possible interactions with metal ions. The presence of an amide group, a key component of the protein structure contributing to peptide bonds in keratin, is indicated by a peak at 1640.0 cm^{-1}. A peak at 1576.7 cm^{-1} corresponds to the stretching vibrations of open-chain azo groups, indicating the presence of -N=N- linkages in the keratin structure.

In contrast, the FTIR spectrum of keratin from exotic turkey feathers shown in Figure 4 revealed a complex array of functional groups. A broad peak at 3227.9 cm^{-1} indicates normal "polymeric" OH stretching vibrations, suggesting extensive hydrogen bonding in the keratin. The presence of asymmetrical and symmetrical methyl C-H stretches is confirmed by a peak at 2970.7 cm^{-1}, indicating the presence of methyl groups. A peak at 2873.8 cm^{-1} suggests methoxy or methyl ether C-H stretches, indicating ether linkages within the keratin. The peak at 2083.6 cm^{-1} confirms the presence of cyanide or thiocyanate ions, indicative of nitriles or thiocyanates in the keratin matrix. The isothiocyanate groups, known for their biological activity, are indicated by a peak at 1990.4 cm^{-1}. The peak at 1874.9 cm^{-1} was identified to be transition metal carbonyl complex, responsible interactions with metal ions. An intense band at 1654.9 cm^{-1} is associated with alkenyl C=C stretching vibrations, highlighting the presence of unsaturated carbon-carbon double bonds. Finally, the peak identified at 1591.6 cm^{-1} was found to be bending vibrations of primary amine NH groups, which indicates the presence of amino groups within the keratin.

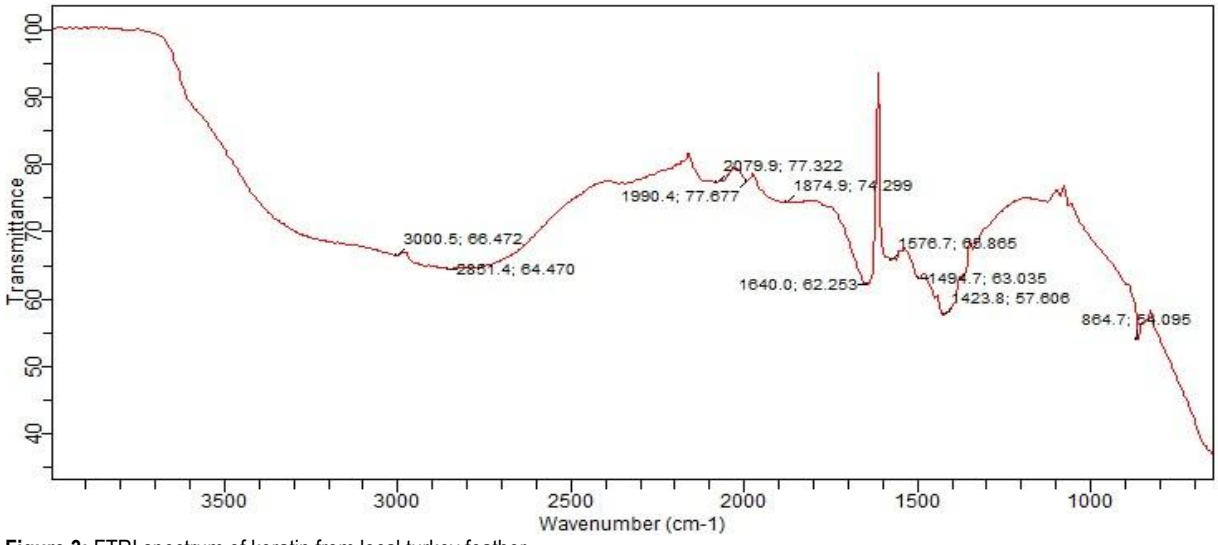

Figure 3: FTRI spectrum of keratin from local turkey feather

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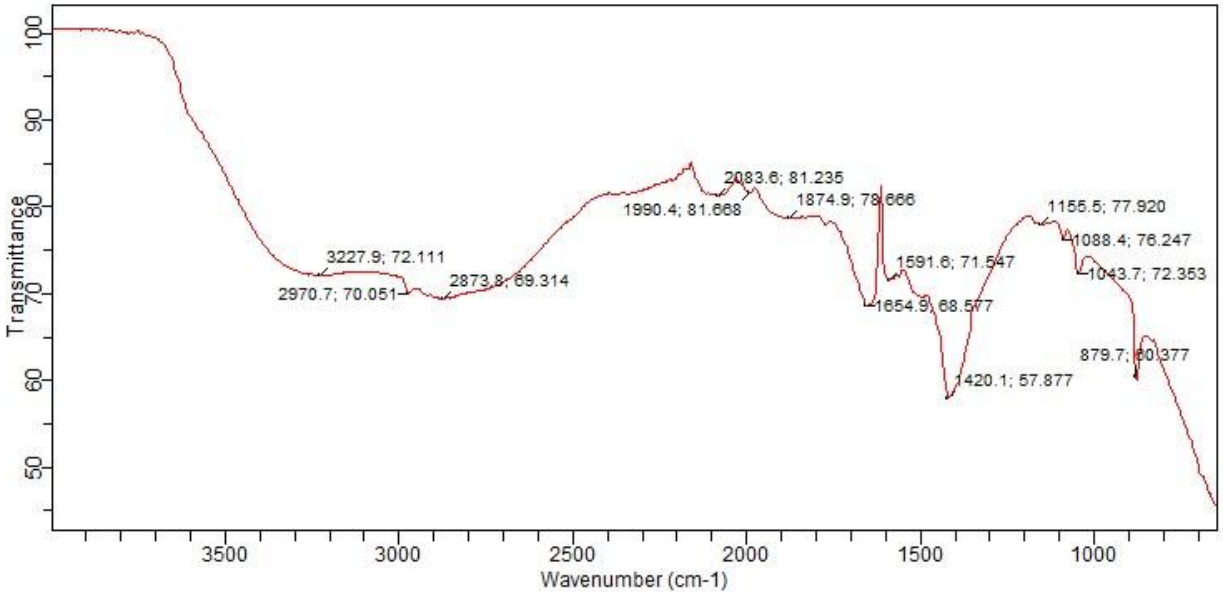

Figure 4: FTRI spectrum of keratin from exotic turkey feather

Result of FTRI analysis of Keratin from duck feathers

Figure 5 shows the FTIR spectra of keratin extracted from local and exotic duck feathers also revealed diverse functional groups that characterize their chemical composition. In the spectrum of keratin from local duck feathers, peaks were observed at 2851.4 cm^{-1} to be methylene C-H stretching vibrations, indicating the presence of methylene groups which contributes to the aliphatic nature of keratin. A peak at 2087.3 cm^{-1} confirms the presence of cyanide, while the peak at 1990.4 cm^{-1} signifies isothiocyanate groups. Likewise, transition metal carbonyl complexes were evident at 1871.1 cm^{-1}, suggestive of metal ions interactions, and an intense band at 1654.9 cm^{-1} indicates alkenyl C=C stretching vibrations, revealing unsaturated carbon-carbon double bonds.

Similarly, keratin from exotic duck feathers exhibits distinct features as shown in figure 6. The spectrum shows peaks at 2847.7 cm^{-1} and 1994.1 cm^{-1} for methylene C-H stretches and isothiocyanate groups, respectively, indicating structural similarities in methylene groups and biological activity roles. Peaks at 2083.6 cm^{-1} and 1874.9 cm^{-1} confirm cyanide or thiocyanate ions and transition metal carbonyl complexes, respectively, indicating potential interactions with metal ions. The presence of an intense band at 1654.9 cm^{-1} indicates alkenyl C=C stretching vibrations, reflecting the presence of unsaturated carbon-carbon double bonds. Additionally, a peak at 1595.3 cm^{-1} corresponds to open-chain azo groups, revealing the presence of -N=N- linkages in the keratin structure.

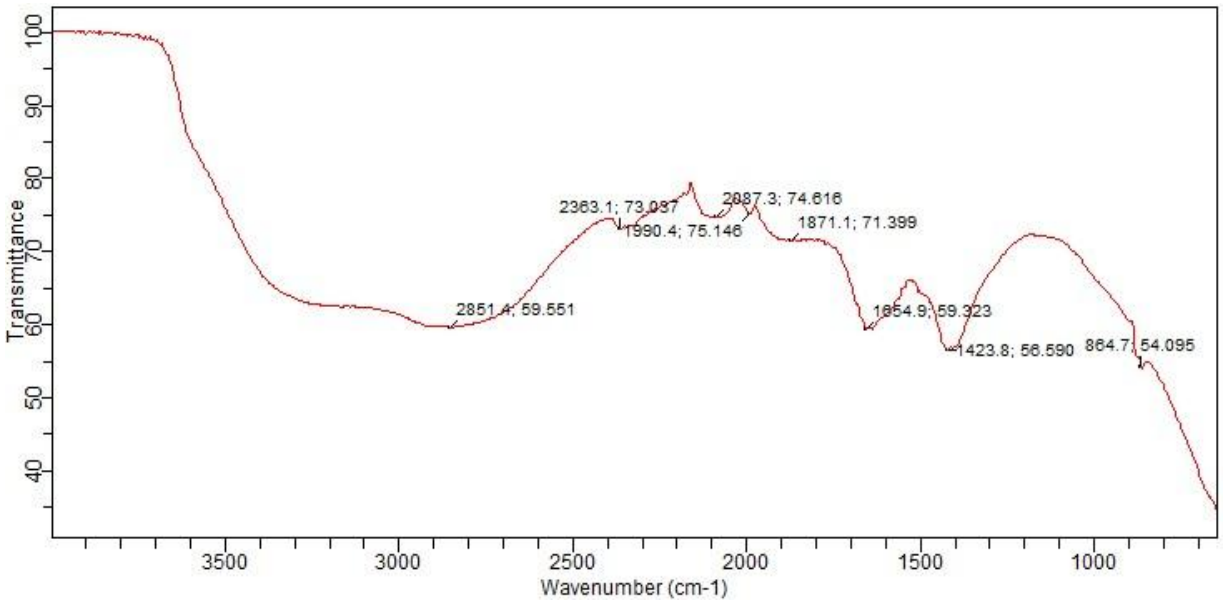

Figure 5: FTRI spectrum of keratin from local duck feather

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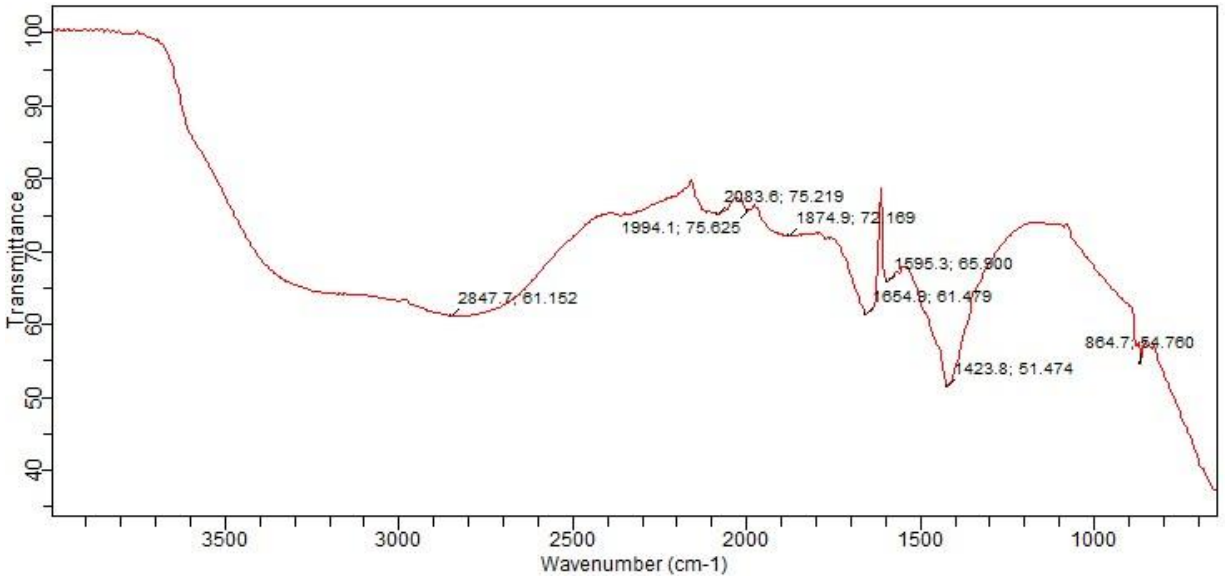

Figure 6: FTRI spectrum of keratin from exotic duck feather

DISCUSSION

The FTIR analysis of keratin from local and exotic chicken feathers provides valuable insights into the structural and chemical differences between these two sources. The distinct peaks observed in the spectra highlight variations in functional groups that may influence the properties and applications of keratin derived from these feathers. The broad peak at 3201.4 cm^{-1} in the local chicken feather keratin suggests the presence of hydrogen-bonded hydroxyl groups. This can indicate a higher affinity for moisture, potentially affecting the keratin's hydrophilicity and its applications in biomaterials or textiles. Both spectra confirm the presence of terminal alkynes (C≡C) and isothiocyanate (NCS) groups, although the peaks are located at slightly different positions. The consistent presence of isothiocyanates in both samples indicates their biological reactivity, which could be significant for applications in biocompatible materials or drug delivery systems. The sharp band at 1648.8 cm^{-1} in the local sample and the intense band at 1640.0 cm^{-1} in the exotic sample both relate to C=C stretching vibrations. The presence of these unsaturated bonds suggests potential reactivity and the ability to form cross-links, which is crucial for enhancing mechanical properties in keratin-based materials. The exotic chicken feather keratin exhibits additional peaks, such as the methoxy/methyl ether C-H stretch at 2847.7 cm^{-1} and the peak at 1871.1 cm^{-1} indicative of transition metal carbonyl complexes. These variations suggest that the exotic feathers may have unique interactions with metal ions, potentially enhancing the material's properties for specialized applications like catalysts or sensors. The peak at 1599.0 cm^{-1} in the exotic sample suggests the presence of aromatic amino acids, which are known to impart UV-absorbing properties. This could be particularly useful in applications requiring UV protection or stabilization (Shavandi *et al.,* 2016; Rahayu *et al.,* 2017). Previous studies on keratin have highlighted its structural diversity influenced by the source and environmental factors. The functional groups identified in this analysis correlate with findings of Badrulzaman *et al.* (2021) which reported that keratin's mechanical properties can be significantly influenced by the presence of hydroxyl groups and the types of bonding present.

Moreover, the unique presence of metal complexes and methoxy groups in the exotic feathers might align with research suggesting that feather-derived keratin can be modified for improved bioactivity and functionalization. For instance, the incorporation of aromatic residues has been linked to enhanced antioxidative properties (García-Morales and Pacheco 2021). The composition of C, H, N, and S of the exotic bird's feathers have high hydrogen and Nitrogen content compare to local bird's feathers which have high carbon and isothiocyanate content. This finding coincides with that of Shavandi *et al.* (2016) which found out that the formation of the sulfhydryl group leads to high hydrogen content since cysteine received hydrogen atom. Besides, Sulphur was present in methionine, cysteine and taurine, but disulfide bonds can only form within cysteine molecules at R-group that attach to a central carbon atom (Rahayu *et al.,* 2017). The high sulfur-containing amino acids in local feather extract solutions are influenced by the presence of a high amount of amino acid cysteine compared to the exotic feathers. Kang *et al.* (2008) reported that 10% of feathers present in a black EBN and contribute 8% of the total protein of the black nest. Cysteine and methionine are sulfur-containing amino acid that essential for the sustainability of structure maintenance (Alashwal *et al.,* 2019). Thus, carbon, hydrogen, nitrogen and Sulphur content in keratin extract influenced by an atom that presence in amino acids structures especially cysteine amino acids.

Conclusion

The findings of this study revealed that exotic chicken, turkey and duck feathers demonstrated a higher concentration of keratin proteins of 55.7±0.00, 64.2±0.23, and 60.4±0.40 respectively when compared to local varieties with 52.3±0.73, 57.9±0.35, and 57.7±0.01 for chicken, turkey and duck feathers respectively which aligned with selective breeding aimed at improving feather quality for ornamental and commercial purposes. FTIR analysis indicates notable differences in functional groups between local and exotic feathers. The presence of hydrogen-bonded hydroxyl groups in local feathers suggests a higher moisture affinity, while exotic feathers exhibit additional peaks associated with methoxy groups

and transition metal complexes, indicating potential for enhanced interactions and functionalities.

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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.

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