

RESULTS OF MEASURING COLLAGEN IN SHEEP SKIN USING ATOMIC FORCE MICROSCOPY

РЕЗУЛЬТАТЫ ИЗМЕРЕНИЯ КОЛЛАГЕНА В КОЖЕ ОВЦЫ С ПОМОЩЬЮ АТОМНО-СИЛОВОЙ МИКРОСКОПИИ

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Using Atomic Force Microscopy, we measured the collagen fibril diameter in the skin of fine-wool KKhantai and semi-fine-wool Orkhon sheep breeds. The proportion of protein in the collagen samples was determined using the Kjeldahl method. The average width of collagen fibrils in Khangai samples was large (12.17 nm), medium (11.48 nm) or small (11.29 nm). In Orkhon samples, the average width was large (10.82 nm), medium (38.06 nm) or small (43.83 nm). Protein contents in Orkhon sheep was large (90.20%), medium (76.30%) or small (69.8%) and in Khangai sheep was large (82.05%), medium (76.40%) or small (70.8%). These results can be used in various areas of research.

С помощью атомно-силовой микроскопии авторами измерен диаметр коллагеновых фибрилл в коже тонкорунных хангайских и полутонкорунных орхонских пород овец. Доля белка в образцах коллагена определена методом Кьельдаля. Средняя ширина коллагеновых фибрилл в образцах хангайской породы распределена на группы: большая (12,17 нм), средняя (11,48 нм) и маленькая (11,29 нм). В образцах орхонской породы средняя ширина для групп составила: большой – 10,82 нм, средней – 38,06 нм, маленькой – 43,83 нм. Аналогично содержание белка у орхонских овец определяется как большое (90,20%), среднее (76,30%) и малое (69,8%), а у хангайских овец эти значения составили: большое – 82,05%, среднее – 76,40%, малое – 70,8%. Эти результаты могут быть использованы в различных областях исследований.

Ключевые слова: тонкорунные породы овец, полутонкорунные, кожа овец, коллагеновые фибриллы, содержание белка в коже.

Keywords: fine-wool sheep breeds, semi-fine-wool, sheep skin, collagen fibrils, protein content in skin.

Introduction

Atomic Force Microscopy (AFM) provides both qualitative and quantitative data on

collagen fibril structure without causing any destruction. This technology is essential for providing a detailed and reliable analysis of

nanomaterials structures and properties. Collagen nanomaterials, skinny collagen films are extensively used in tissue engineering and biomedical fields due to their wide range of applications. Type I collagen is the most abundant extracellular matrix protein widely used as a biomaterial due to its unique properties. Our initial studies on the surface collagen of Mongolian goat skin using AFM began in 2010. Further detailed AFM studies on Mongolian animal skins can serve as foundational materials for nanomaterial research, paving the way for future breakthroughs in tissue engineering and biomedical fields.

Proteins, the most complex and biologically essential organic compounds in living organisms, are composed of carbon, hydrogen, oxygen, and nitrogen, with minor elements like sulfur. Since proteins have a consistent elemental composition, the Kjeldahl protein quantification method plays a crucial role in our research. It determines the nitrogen content, which, when multiplied by a factor of 6.25, provides the protein content percentage in 100 grams of the sample.

Materials and Methods

A small piece (1 cm x 1 cm) of skin was taken from the inner layer, placed on a freshly peeled mica plate without folds, and dried for 30 minutes. The mica plate with the sample was then attached to an aluminum disc using double-sided carbon tape for measurement. The measurements were performed using an SPM AA5000 model Atomic Force Microscope with the following parameters:

AFM Model: SPM AA5000,

Measurement Speed: 1 Hz, Mode: Dynamic,
Probe Material: Silicon

Probe Radius: ≤ 10 nm, Measurement

Area: Khangai – large, medium, small: 200 nm x 200 nm

Orkhon – medium, trim: 400 nm x 400 nm

The collagen fibril width was determined by analyzing 20 fibrils with a 95% confidence interval using "Section Line" analysis.

Kjeldahl Method Principle: The method is based on the nitrogen content of all amino acids constituting proteins. When heated with concentrated sulfuric acid, organic substances

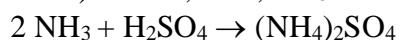
decompose into carbon dioxide, water, and ammonia. Ammonia reacts with sulfuric acid to form ammonium sulfate. To accelerate the decomposition of the organic substrate, a catalyst is added, usually a mixture of CuSO₄ and K₂SO₄, along with a strong oxidizing agent such as H₂O₂. Free NH₃ is released by concentrated alkali in the Kjeldahl apparatus and reacts with sulfuric acid in the receiver flask.

Experimental Procedure:

a) Sample Combustion:

Nitrogen-containing organic matter

(proteins) \rightarrow CO₂, H₂O, NH₃

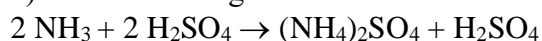


б) During the distillation process:



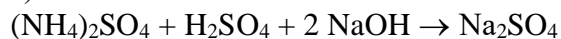
\uparrow + 2 H₂O

в) In the receiving flask:



(residual)

г) In titration:



+ (NH₄)₂SO₄ + 2 H₂O (residual)

To determine the amount of ammonia in the experimental sample, an equal amount of distilled water is used as a control and subjected to the same combustion process. Then the amount of acid that reacted with the ammonia in the experimental and control samples is determined. The difference between these values is used to calculate the total nitrogen content, which in turn is used to determine the protein content.

Research Results

For this study, we selected the skins of fine-wool Khangai and semi-fine-wool Orkhon sheep breeds during the autumn slaughter season. According to the MNS60:2013 standard for leather processing industry organization, we selected large (90 dm²), medium (80 dm²), and small (54 dm²) skins. The prepared samples were analyzed using Atomic Force Microscopy (AFM), and the results are shown in Table 1 (3D images of atomic force microscopy measurements of large, medium, and small areas of skin collagen of KKhongai and Orkhon sheep).

Table 1

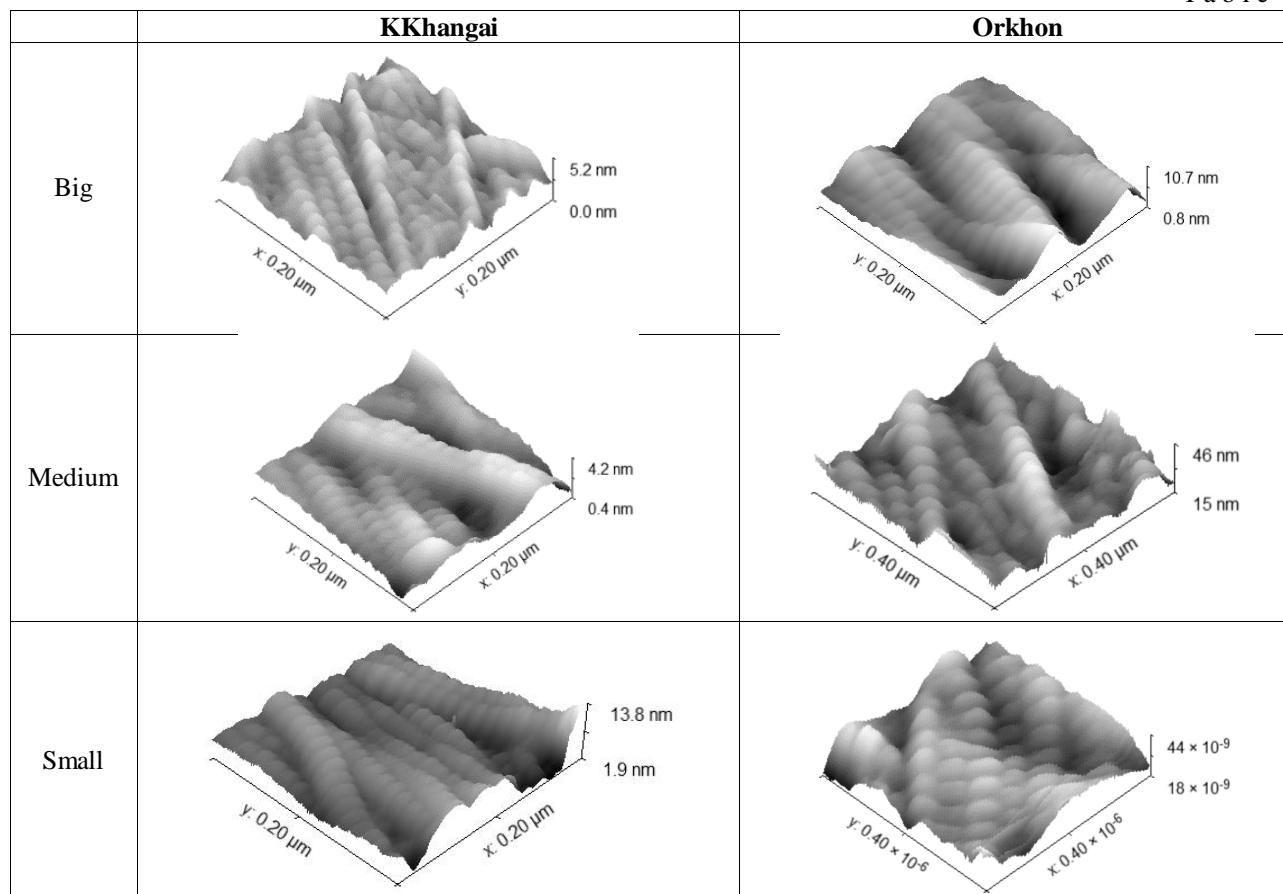


Table 2 shows the measured width of collagen protein, which is the main protein of

the skin.

Table 2

KKhangai			Orkhon		
Big, nm	Medium, nm	Small, nm	Big, nm	Medium, nm	Small, nm
12.17±0.93	11.48±0.59	11.29±0.35	10.82±0.60	38.06±2.08	43.83±2.88

To determine the protein content in the skins of fine-wool Khangai and semi-fine-wool Orkhon sheep, the samples were com-

busted and titrated, and the results were calculated. The findings are presented in Table 3.

Table 3

№	Sample			Control (ml)	Protein Content (%)	
	Name	Combusted Sample Weight (g)	Titrated Volume (ml)			
1	KKhangai	Big	0.5005	9.2	13.25	70.8
2		Medium	0.5008	8.9	13.25	76.4
3		Small	0.501	8.55	13.25	82.05
4	Orkhon	Big	0.502	8.9	13.25	76.3
5		Medium	0.505	9.2	13.25	69.8
6		Small	0.507	8.05	13.25	90.2

CONCLUSION

The average collagen fibril width in the Khangai samples was large (12.17 nm) > medium (11.48 nm) > small (11.29 nm). In contrast, the average collagen fibril width in the

Orkhon samples was large (10.82 nm) < medium (38.06 nm) < small (43.83 nm).

The protein content determined by the Kjeldahl method was as follows:

- Orkhon: small (90.20%) > large (76.30%) > medium (69.8%)

- Khangai: small (82.05%) > medium (76.40%) > large (70.8%)

Therefore, the small Orkhon sample had the highest protein content, and the medium Orkhon sample had the lowest.

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Рекомендована кафедрой товароведения и экспертизы продуктов животноводства Монгольского университета естественных наук. Поступила 13.09.24.