Skin Characteristics of *Cervus elaphus* L. from Republic of Tuva in Russia

by

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Abstract

Characteristics of an animal raw skin are closely related to the physical properties of finished leather obtained from the skin. Although numerous studies have been performed on determination of physical properties of tanned deerskin, characteristics of raw deerskin have not yet been described in literature. In the present study, data on morphological, histochemical and chemical characteristics of wild red deer (*Cervus elaphus* L.) skins obtained from Republic of Tuva within the Russia Federation are presented and the relevant differences between skins of other animal species described. Table Top Scanning Electron Microscopy (TSEM) and Scanning Electron Microscopy (SEM) were used to determine the morphological characteristics of raw deerskins. For histochemical studies the cross section of skins were observed by Research Microscopy (RM). Hydroxyproline content, Total Kjeldahl Nitrogen (TKN), volatile matter, fat content, water-soluble-matter, sulphated total ash and fatty acid contents of raw deerskins were analyzed to further characterize the chemical composition of deerskin. Deerskin has comparatively higher TKN values, lower fat content with higher unsaturated fatty acid ratio, when compared to sheepskin. TSEM and SEM analysis showed that fur fibers and guard hairs of red deer skin were characterized by large cortex and medulla respectively that provides good isolation properties. In addition, high hydroxyproline content, low fat content, and tightly packed collagen fibers revealed by histochemical observations consolidates the long lasting property of deer skin. Assessment of skin characteristics enabled gathering data on physical and chemical properties of red deer skin, which is significant in choosing appropriate potential raw material that will produce the most suitable leather for a specific application.

Introduction

The red deer (*Cervus elaphus*) is one of the largest deer species and has a large global distribution extending from Europe and North Africa through central Asia, the Far East, North America and Siberia.[1-3] Tuva Republic is located in South Siberia within the Russian Federation, on the Mongolian border. The region is characterized by mountain steppe and thin coniferous forest called taiga. The climate is continental, with annual temperature variation from -40°C in winter to +40°C in summer. Herding and hunting are the main livelihood practices, which are important economic activities in the region. According to official estimates there are presently about 12 thousand red deer in the Republic of Tuva. Red deer where its meat is widely used as a food source is of great importance and plays a key role in Tuvan people's subsistence.[4-6]

Deer skin, which is a byproduct of meat production, is heavier and owns a larger surface area when compared to ovine leather.[7-8] It could be considered as an isotropic material that is similar to woven textiles, providing a more uniform area for pattern lay out and cutting due to their less variable physical strength throughout the skin. They are mainly used in clothing industry due to their desirable properties such as soft touch and special grain that offers a suitable material for long lasting garments.[6] It can also be utilized as a raw material for production of high cost, fashion outerwear materials, upholstery, automotive, glove, bag, shoe upper and chamois leathers.[9]

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Earlier studies provided information about assortment, optimal sampling positions, physical, chemical, physico-mechanical properties and possible usage areas of tanned deer skin[6-8, 10-12] and less study has been performed about the characteristic properties of raw deer skins. Elastic fibers surrounding the hair follicles of deer[13] and the hair follicle characteristics of forest deer and mountain reindeer[6] were identified by several researchers. Hartl et al. 1990 investigated the genetic variability and differentiation in Central Europe red deer (*Cervus elaphus* L.)[14] and Fukunaga et al. 2009 reported seasonal variations of morphological and biochemical characteristics of Yeso sika deer (*Cervus nippon yesoensis*).[15] This study is important because it will provide information about raw skin characteristics of *Cervus elaphus* L. which hasn't been reported in literature until now.

In the present work, wild red deer skins (*Cervus elaphus* L.) of Republic of Tuva in Russia are characterized by determination of morphological, histochemical, and chemical properties. Identification of deer skin characteristics is of primary importance to enable a better understanding of fiber type variability that is responsible for the skin's elasticity and mechanical resistance; to exploit raw material for production of long-lasting leather outerwear materials and to provide some basic information about the quality of deer skins.

Experimental

Materials

Two skins of wild male *Cervus elaphus* L. of Artiodactyla class of Cervides family known as Red Deer[16] were obtained from local hunters in autumn season from Republic of Tuva in Russia.

Methods

Samples for histochemical analysis were taken from the croupon region of wild deerskin right after slaughtering and were kept in ice for the first 18 hours and then immediately fixed in formalin (10%; Merck, Darmstadt, Germany). Following the sampling, skins were flayed and cured with salt for further morphological and chemical analyses.

Morphological and Histochemical Investigations of Deer Skins

Morphological Analysis

Tabletop Scanning Electron Microscopy (TSEM) and Scanning Electron Microscopy (SEM) investigations were performed for morphological study in accordance with the previously published procedures; Cadirci et al. 2010[17] and Zengin and Afsar 2011[18] respectively. Magnifications up to 1mm were performed with TSEM and for better visualization of cross-section and higher magnifications SEM was used.

Histochemical Analysis

Deer skin specimens were fixed in formalin solution (10%) and embedded in paraffin. 3 μm thick vertical sections were stained with Van Gieson's staining technique[19] for histochemical study. The images of cross section of deer skin were taken using a research microscope (Leica DM 4000B) equipped with a digital camera (Olympus DP7).

Chemical Characterization of Deer Skins

Preservation salt was removed from flesh side of raw deerskins prior to chemical analysis. The samples were taken from sampling area as described in TS EN ISO 2418[20] and separated into five groups depending on their sampling area such as croupon, neck, shoulder, belly and foot. Test samples were dried at 45°C for 48h in a circulating air-drying oven, milled and prepared in accordance with TS EN ISO 4044.[21]

Determination of Total Kjeldahl Nitrogen

Total Kjeldahl nitrogen values of deerskins were determined in accordance with Kjeldahl nitrogen method.[22, 23]

Determination of Hydroxyproline Content

Hydroxyproline content of deerskins was determined following the previously published method Çolak et al. 2008.[24] The measurement of hydroxyproline was performed spectrophotometrically using chloramin T by a modified version of Taugard method and the results were given as μg/mg.

Determination of Volatile Matter and Fat Content

The volatile matter and fat content of deer skin samples were determined according to the standards of TS EN ISO 4684[25] and TS EN ISO 4048[26] respectively.

Determination of Water-soluble Matter, Water-soluble Inorganic Matter and Water-soluble Organic Matter

The procedures recommended in TS EN ISO 4098[27] were followed for the determination of water-soluble matter, water-soluble inorganic matter and water-soluble organic matter.

Determination of Sulphated Total Ash and Sulphated Water-insoluble Ash

Sulphated total ash and sulphated water-insoluble ash of the samples were determined by following the standard procedure described in TS 4125 EN ISO 4047.[28] Prior to determination of sulphated total ash, samples were washed in 250 ml distilled water and dried at 45°C for 48h in a circulating air-drying oven to prevent the interference of preservation salt.

Analysis of Fatty Acid Methyl Esters by Gas Chromatography (GC)

Analysis of fatty acid methyl esters was performed using a gas chromatography (Agilent 7890) with hexane (GC grade; Merck) as solvent, and data were acquired by solution software. Natural fat of deer skin obtained by TS EN ISO 4048[26] was trans-esterified according to TS EN ISO 12966-2[29] and analyzed following standard TS 4664 EN ISO 5508.[30]

Statistical Analysis

The effects of skin type, region and interaction of these two factors were analyzed. The statistical analysis of data was performed using Minitab for Windows (ver. 14.0, Minitab Inc.,

State College, PA). Analysis of variance was performed on each attribute ($p < 0.01$). Duncan's post hoc test was used for multiple comparisons when significant interactions were observed.

RESULTS AND DISCUSSION

Morphological and Histochemical Investigations of Deer Skins

Morphological Characteristics

The guard hair, fur fiber, cross sections of fibers, epidermis and dermis of wild deer skin are illustrated in Figure 1 and 2. They were composed of epithelial and connective tissues. The medulla proportions of guard hairs (primary hair follicle) were high and the medulla cells had polygonal and/or honeycomb shape. The group of seven or eight fur fibers (secondary hair follicle) was located around one guard hair.

Two types of fibers, fur fiber and guard hair were observed on wild red deer skin surface. In literature, guard hairs of wild animals were described as straight, resilient, and coarse and fur fibers were described as fine, soft and often having a silky hair like form.[31, 33] The SEM images of deer skin samples revealed that the guard hair (A), which was long and had a large diameter and strong hair structure, was located vertically in deep layers of papillary.

Approximately 80-85% of deer guard hair was consisted of medulla, resulting in hollow core. Fur fibers (B), described as the second type of fiber were very fine and closely located to the grain surface (Figure 3). The ratio of medulla in fur fibers was lower in comparison to guard hair. Some parts of cuticle cells were not clearly visible. Accordingly, the fur fibers mainly consisted of cortex, with an approximate ratio of 2/3 to total thickness, which provides flexibility and resistance to fur fibers. In terms of location of hairs through papillar and reticular layer, deer skin shows similarity to sheep skin. However, deer hair is fewer in number and more straight compared to sheep skins.

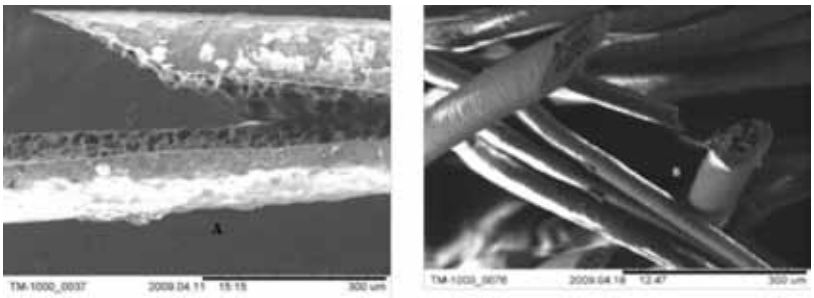

Figure 1: TSEM micrograph of the cross section of the guard hair (A)[31] and fur fibers (B) (*300).[32]

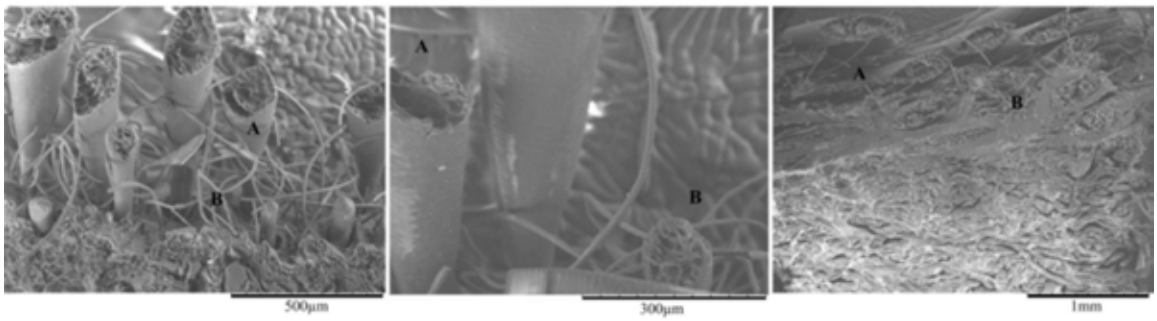

Figure 2: TSEM micrographs of guard hairs (A), fur fibers (B) and the cross section of wild deer skin with magnification factor 200, 300 and 60, respectively.[32]

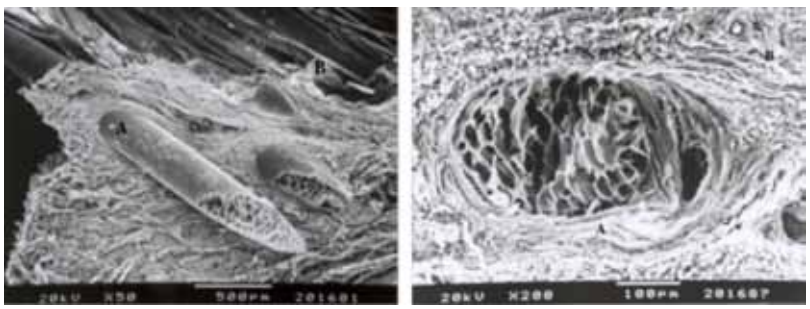

Figure 3: SEM micrographs of the guard hairs (A) and fur fibers (B) of deer skin.[32]

Fukunaga et al. 2009 reported that calf skin and deer skin are similar in size and biochemical composition however collagen fiber and fiber bundle structure of deer skin has unique characteristics.[15]

Histochemical Characteristics

The collagen fibers stained pink-red by Van Gieson's, are loosely packed in the form of fine bundles in stratum papillare layer and oriented parallel to epidermal structures (superficial epidermis and epidermis surrounding the hair roots). In stratum reticulare layer, which is located under the stratum papillare, collagen fibers are arranged densely in coarse bundles and oriented sequentially in longitudinal and transverse directions (Figure 4).

Considerable high extension of deer leather by comparison with both woven textiles and ovine leathers[8] is mainly associated with coarse and rather loosely interwoven corium fiber bundles of deer skin that provide elasticity and well suited to the production of clothing leather.[34]

Chemical Analysis

Samples for chemical analysis were taken from five different regions of wild deer skins that are presented in Table 1. Significant interactions ($p < 0.01$) between deer skins and regions were observed for all chemical analyses, except soluble matter in dichloromethane ($p > 0.01$).

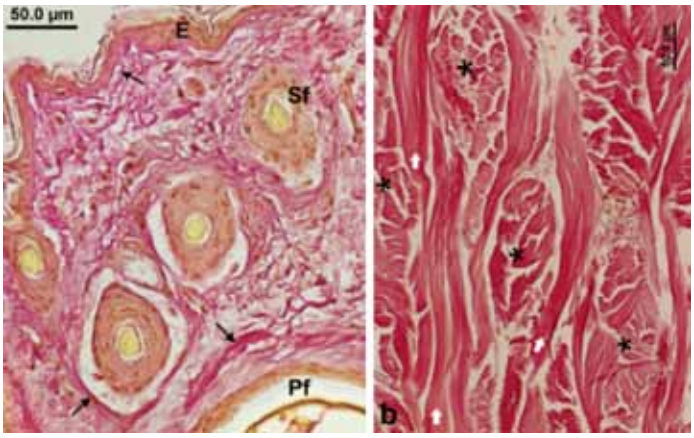

Figure 4: Dispersion of collagen fibers ( ) in stratum papillare (a) and longitudinally ( ) and transverse ( ) localization of collagen fibers in stratum reticulare (b). E, epithelium; Pf, primary hair follicle; Sf, secondary hair follicle; (Paraffin section, van Gieson's stain).

Determination of Total Kjeldahl Nitrogen (TKN)

There were significant differences ($p < 0.01$) among two skins and their different regions in addition to their significant interaction effect. The first deerskin had lower TKN values than second deerskin (Table I). Croupon region had the highest TKN value among other skin regions, while the lowest TKN values were obtained from shoulder and belly of first and second deer skin respectively. Neck and foot regions had the

TABLE I
The chemical properties of wild deerskins depending on five different regions

Results	Deer Skin	Regions of deer skins				
		Croupon	Shoulder	Neck	Belly	Foot
Total Kjeldahl Nitrogen (%)	1	74.62 ± 0.74^{A}	65.93 ± 0.34^{D}	71.26 ± 0.19^{B}	62.25 ± 0.45^{E}	69.25 ± 0.47^{C}
	2	73.78 ± 0.21^{A}	61.41 ± 0.50^{C}	73.16 ± 0.13^{A}	69.08 ± 0.62^{B}	70.42 ± 0.15^{B}
Hydroxyproline content (μg/mg)[24]	1	134.7 ± 1.56^{B}	132.1 ± 0.57^{B}	139.4 ± 0.50^{A}	106.1 ± 0.64^{C}	92.6 ± 1.20^{D}
	2	118.8 ± 1.27^{B}	147.7 ± 0.28^{A}	118.5 ± 2.12^{B}	88.7 ± 0.35^{D}	102.0 ± 0.85^{C}
Volatile Matter (%)	1	5.71 ± 0.02^{A}	4.43 ± 0.07^{B}	4.12 ± 0.04^{BC}	3.82 ± 0.16^{C}	6.17 ± 0.13^{A}
	2	7.02 ± 0.01^{A}	6.12 ± 0.55^{B}	6.92 ± 0.05^{A}	6.56 ± 0.22^{AB}	6.16 ± 0.12^{B}
Water soluble matter (%)	1	4.68 ± 0.24^{A}	5.11 ± 0.20^{A}	5.27 ± 0.13^{A}	3.55 ± 0.10^{B}	0.93 ± 0.06^{C}
	2	4.05 ± 0.09^{A}	3.74 ± 0.18^{A}	0.10 ± 0.21^{C}	3.41 ± 0.07^{A}	2.19 ± 0.10^{B}
Water soluble organic matter (%)	1	1.29 ± 0.22^{A}	1.44 ± 0.22^{A}	1.44 ± 0.41^{A}	0.56 ± 0.15^{B}	0.47 ± 0.07^{B}
	2	2.70 ± 0.42^{A}	1.28 ± 0.47^{B}	0.01 ± 0.03^{D}	1.25 ± 0.05^{B}	0.61 ± 0.07^{C}
Water soluble inorganic matter (%)	1	3.39 ± 0.46^{AB}	3.67 ± 0.02^{A}	3.83 ± 0.15^{A}	2.99 ± 0.14^{B}	0.46 ± 0.02^{C}
	2	1.35 ± 0.39^{B}	2.46 ± 0.42^{A}	0.09 ± 0.01^{C}	2.16 ± 0.03^{A}	1.58 ± 0.02^{B}
Sulphated total ash (%)	1	0.40 ± 0.00^{B}	0.34 ± 0.00^{B}	5.09 ± 0.26^{A}	0.23 ± 0.01^{C}	0.31 ± 0.09^{BC}
	2	0.25 ± 0.02^{BC}	0.33 ± 0.05^{B}	1.08 ± 0.01^{A}	0.19 ± 0.08^{C}	1.11 ± 0.05^{A}

$^{A-D}$ Means in the same row followed by different uppercase letters represent significant differences ($p<0.01$)

next highest TKN values after croupon region for both deer skins. The TKN values obtained from deer skins were higher than Black Anatolian (62.78%), East Anatolian Red (56.05%), and Holstein (63.23%) bovine hides.[35] Similarly to deer skins, variation of TKN values for different hide regions was reported, and croupon provided the highest TKN values 63.94% and 62.58% for Black Anatolian and East Anatolian Red bovine hides respectively.[36]

Determination of Hydroxyproline Content

The hydroxyproline content is one of the important parameter that gives specific information on the quality of leathers, hides and skins. There were significant ($p < 0.01$) differences in hydroxyproline content of skins and regions, their interaction was also found significant (Table I). The first deerskin had statistically higher hydroxyproline content. Among different regions, shoulder had the highest hydroxyproline content whereas neck and croupon had the next highest value for all deerskins. In previously published literature, shrinkage temperature increase of deer skins were reported to be directly proportional to the hydroxyproline content of deer skins and highest results were obtained from shoulder (66.4°C), neck (64.5°C) and croupon (65°C) respectively.[24]

In raw materials hydroxyproline content provide long-lasting and durable leathers in terms of mechanical and physical properties due to the increased stability of collagen triple helix.[37] For this reason, higher hydroxyproline content of raw deer skins produces high quality finished deer leathers with respect to long lasting, resistant and durable properties. In literature, different percentages of hydroxyproline in the total amino acid content were reported for cow (92%), calf (94%), pig (95.5%), shark (79%) and fish (53%) skins.[24]

Determination of Volatile Matter and Fat Content

There were significant differences in volatile matter content of two deerskin samples (Table 1). The second deerskin had significantly higher average volatile matter 6.56% than the first deerskin 4.86%, excluding the effect of regions. This is probably due to different structure of deer skins depending on life habits, season, age, sex, environment and breeding of the animal.[38] The amount of salt used in preservation, type of preservation and relative humidity of air could also affect these results.[39] For volatile matter results significant differences ($p < 0.01$) among five different regions of deer skins were observed. The croupon and foot regions had the highest values, and the lowest values ($p < 0.01$) were determined from belly and neck regions of the first deer skin sample. On the other hand, lowest volatile matter was obtained from foot and shoulder region of the second deer skin, while the highest values were obtained from croupon and neck regions ($p < 0.01$). These regional variations should always be considered as the consequence of inconsistent characteristic of leather material[38] that depends on raw material and processes applied in leather production.[40]

Although various fat contents of different type of raw materials such as sheepskin (5-30%), goat skin (3-10%) and cattle hide (2-4%) have been previously reported,[39, 41] there isn't any published data related to fat content of deer skin. In this study, the first deerskin (1.39%) had lower natural fat than second skin sample (1.64%) when the regional differences were not taken into account. No significant interaction ($p > 0.01$) but differences ($p < 0.01$) between two skins and skin regions was found. The natural fat content was determined as 1.80^{A}, 1.74^{A}, 1.62^{A}, 1.25^{B} and 1.16^{B}% for the shoulder, belly, neck, croupon and foot regions respectively without considering the skin differences. High fat cell content in sheep skins produces tough but spongy leather, because after the removal of fat in degreasing process, the empty fat cells play no part in attaching the corium to the grain layer but only interrupt the weave, resulting in loose skin and reduced mechanical strength.[42] Therefore it can be deduced that low fat content of deer skin provides better structure and higher strength properties.[9] Moreover low natural fat content of deer skins reduces the need for a degreasing process that provides more environmentally friendly leather manufacture resulted in high quality leather products.

Determination of Water-soluble Matter, Water-soluble Inorganic Matter and Water-soluble Organic Matter

The average water-soluble matter of the first deerskin in the form of chloride, sulphate, carbonate, phosphate and silicate[39] was found significantly higher than second deerskin, 3.91% and 2.70% respectively (Table I). The highest results regarding water-soluble matter, water-soluble inorganic matter and water-soluble organic matter were obtained from croupon, shoulder and neck regions while the lowest values were determined from the foot region of the first skin sample ($p < 0.01$). The highest significant value for water-soluble matter and water-soluble organic matter was obtained from croupon region of the second deerskin. The shoulder and belly region had the highest amount of water-soluble inorganic matter. Finally, the lowest significant value was determined from the neck region of the second sample considering all these three analyses ($p < 0.01$).

Determination of Sulphated Total Ash and Sulphated Water-insoluble Ash

Ash content of hides and skins vary from species to species[35] and in several studies, sulphated total ash results of different cattle hides were reported as in a range of 1.81, 2.22, 2.27 and 3.95%.[35, 36, 43] However, the sulphated total ash results of deer skins were found lower than the mentioned values. The difference between the ash content values could be related to leather type and environmental conditions. There was a significant difference in sulphated total ash and sulphated water-insoluble ash results among two skins and skin regions. Besides, interaction of skins with region was significant ($p < 0.01$). The total ash content of the first deerskin was found significantly higher than the second deerskin. The neck region

had the highest total ash content for both skin types, while the lowest value was obtained from belly region ($p < 0.01$). This difference could be derived from skin type and salt used in preservation, which is responsible for the nearly entire ash content.[44]

Determination of Fatty Acid Methyl Esters by GC

The major saturated acids; palmitic (C16:0), stearic (C18:0), tridecanoic (C13:0), myristic (C14:0) and unsaturated acids; palmitoleic (C16:1), oleic (C18:1*cis)*, C18:3 (*trans*), C20:3, oleic (C18:1n9c), α-linolenic (C18:3n3) and long chained nervonic acid (C24:1) determined for deerskins were presented in descending order of abundance in Table II.

TABLE II
Fatty acid composition of different skins

Fatty Acid		Sheep[18]	Pig skin[45]	Deer[32]
C11:0	Undecylic acid	-	-	0.53
C12:0	Lauric	-	-	0.17
C13:0	Tridecylic acid	-	-	6.56
C14:0	Myristic	2.45	-	4.63
C14:1	Myristoleic	0.18	-	0.47
C15:0	Pentadecylic acid	-	-	1.5
C16:0	Palmitic	27.09	1.08	31.77
C16:1	Palmitoleic	3.32	44.91	4.43
C17:0	Margaric	0.18	-	0.80
C18:0	Stearic	42.35	1.53	13.23
C18:1	Oleic	16.55	49.36	8.98
C18:2	linoleic	-	-	1.59
C18:3	Linolenic	-	-	2.80
C20:3n6	Eicosadienoic acid	-	-	1.5
C20:5n3	Eicosapentaenoic acid	-	-	2.03
C22:0	Behenic acid	-	-	1.21
$C_{22:6n3}$	Docosahexaenoic acid	-	-	2.1
C24:0	Lignoceric acid	-	-	1.68
C24:1	Nervonic acid	-	-	2.88

Palmitic, stearic and oleic acid had the highest concentration in fatty acid composition of deerskin samples, which is similar to sheepskins. Deer skin contains less oleic acid and much higher palmitic acid than both sheep and pig skin.[18] Unsaturated fatty acid ratio of deer samples was found nearly 25% of total fatty acids, which primarily affects the melting point of fat, which is lower than sheep skin.

CONCLUSION

In the present study, the characteristics of wild red deer (*Cervus elaphus* L.) skins obtained from Republic of Tuva within the Russia Federation were investigated by morphological, histochemical and chemical methods to exploit this as a raw material for the production of long lasting and high quality leather outerwear. Morphological results revealed that red deer skins have two different hair types; guard hair (primary hair follicle) and fur fiber (secondary hair follicle). The guard hair of deer skin provides a protection against environmental conditions due to their high medulla proportion. The special location of fur fibers around guard hair and their high cortex layer supports this protection and gives flexibility and resistance to fur fibers. The collagen fiber orientation in deerskin was categorized as longitudinally and transverse and the fibers were tightly packed in these two directions. This structural characteristic of collagen resulted in high strength properties and elasticity for resultant leather products. The hydroxyproline and TKN content of deerskins were found higher than the other reported values, which is a good indication of long-lasting properties. In addition, deer skin characteristics showed similarities to calfskin, in terms of low fat and sulphated total ash content. The essential fatty acid methyl esters of deerskin were comparable to sheepskins; however they differ in their amount.

Consequently, the results of this study demonstrated that raw material of red deer skins could be utilized as an ideal raw material for the production of long lasting outerwear materials providing high strength properties. Unique wool characteristic of red deer skins could perfectly promote the utilization of this raw material in other usage areas where also the thermal insulation property is needed.

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