An Assessment of Differences between Butt and Belly Regions of Indian Sheep Skin

by

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Abstract

Skins and hides undergo changes in biochemical and biophysical properties during the leather processing. Biochemical composition varies with respect to different portions of the skin like butt, belly, shank, and neck. Belly part looseness is the main problem in leather making from sheepskin. To address this issue, the present work focuses on analysis of histology and biochemical properties of butt and belly portion of sheepskin from the southern part of India. Globular proteins are relatively higher in the butt portion than the belly, whereas it is reverse in the case of fibrous proteins except collagen. Interestingly, the presence of proteoglycans content in butt regions is significantly high compared to belly and in case of fat content it is reverse. Acid soluble collagen contribution is higher in butt region than belly and vice-versa in the case of pepsin soluble collagen. In general, collagen content is significantly higher in butt than belly, which may be the major cause for the higher strength characteristics of butt regions. Cr_2O_3 content is higher in butt region than belly, which corroborates with protein content and its interaction with tanning agents. Distribution of pore size influences the breathability property of leather, which has been altered in all the unit operations. Scanning electron microscopy study reveals the morphology of the grain and cross-section of the skin changes during leather processing. Thus, this study aids in better understanding of the butt and belly regions of Indian sheepskin.

Introduction

Traditional leather making has advanced to modern controlled processing through use of efficient treatment chemicals and controlled processing systems.[1-10] These two main key factors are involved in the transformation of the putrescible skin/hide to non-putrescible leather. The hide/skin constitutes non-collagenous protein, leather making protein; meaning collagen, fats and minerals. Operations involved in the transformation of hide/skin into leather are classified into three categories, namely as pre-tanning, tanning and post tanning. Pre-tanning operations are meant for preparing the skin matrix for tanning by removing the proteoglycans, globular proteins, fats and fibrous proteins such as reticulin and elastin. Tanning process imparts stability to the collagen matrix against temperature and collagenolysis. Post-tanning process emphasizes the integration of the fibers and includes operations such as coloring and lubrication of the leather. These three operations alter the biochemical and biophysical characteristics of the collagen matrix.[11-13]

Leather processing does not induce significant changes to structural organization of the skin/hide matrix in the supramolecular level. However, there is change in the composition and physico-chemical properties in different areas of the skin/hide at macromolecular level.[14-16] The chemical composition of skin/hide varies depending on the breed, mode of rearing, age, sex, environmental conditions, etc. Leather made out of skin/hide of different species has different physico-chemical properties.[17-19] Within the same skin/hide, thickness, chemical constituent and fiber orientation also varies in different parts like shoulder, butt and belly.[15] This results in differing strength, elongation and other physical properties of the leather – animal to animal and within the same skin – region to region.[15] Such wide variations influence the unit value realization per skin/hide.

Generally the tanned leather from sheep skin has void spaces and looseness in the belly region than the other regions such

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as butt, neck and shank. Though specific neither to belly nor to butt regions of the skin, it has been experimentally observed that the composition of sheep skin comprises 50-80% collagen and 20-50% non-collagenous components.[20] The non-collagenous protein content is more in sheep skin, particularly in the early age of the animal.[20] The structure of skin is somewhat complicated; and some knowledge of its histology and chemical composition is essential so as to have an appreciation of the extremely complicated structural changes involved during leather manufacturing. Although previous studies have provided adequate information on the histological characteristics of sheep skin, such as the fiber orientation, angle of weave etc., detailed investigation on the characteristic features that distinguish the butt region from the belly has not been carried out so far. In sheep skins, large amount of natural fat is usually found between the corium minor and major and this will give spongy leather when too many fat cells are found in this layer.[14,21] In spite of the fact that goat and sheep skins are almost similar in size, sheep skin is less compact and weaker in texture and strength than goat skin. Generally, the collagenous fiber bundles in the corium areas of sheep skin intertwine at a low angle of weave and are not very compact unlike their fineness in the epidermal area.[13]

Leather quality and increasing the cutting value mainly rely on matching the belly characteristics with the butt portions, through supplementation of phenolic tanning agents during the post-tanning operations. In order to bring the fullness (substance) to the belly regions, filling agents are employed during post tanning operation. Acrylic polymers, melamine-aldehyde based resins and protein based fillers are used in leather processing to enhance the fullness of the skin in the belly regions.[22-26] It has been reported that acrylic acid grafted collagen hydrolysate provides good filling effect.[25] The application of hydrolyzed collagen from cowhide waste trimmings affects the dyeing property with respect to pH and also imparts fullness to the leather.[26] Industry often uses a combination of products to avoid emptiness in the leather, which in turn leads to increase of weight – a major drawback for garment leathers.

The use of a large number of chemical products for increasing the fullness of sheep skin has been worked out through trial and error and no scientific methodology has been worked out so far. To develop an alternative based on the modification in the process and chemicals, there is need for detailed study on biochemical, biophysical and histological analysis of skin/hide matrix during the pre-tanning and tanning processes. The present work focuses on the hair sheep skin and the term sheep represents hair sheep unless otherwise specified. In this study histology, biophysical and biochemical analysis of butt and belly regions of southern Indian sheep skin has been analyzed during and after all the pre-tanning and tanning operations. The non-collagenous proteins, fat, proteoglycans, collagen and chromic oxide have been estimated separately for the butt and belly regions of the sheep skin.

EXPERIMENTAL

Materials

Belly and Butt regions were excised from the three wet salted hair sheepskin with mass range of 1.1-1.3 Kg per skin. At the end of each unit operation, skin samples were analyzed. Chemicals used for the leather processing were of commercial grade. The chemicals used for extraction and estimation of collagen, analysis of spent solution, as well as preservation and preparation of skin specimen for histological examination were of analytical grade.

Sample Preparation

Belly and Butt regions of wet salted hair sheep skin were excised based on the ISO 2418:2002 (IUP 2)[27] sampling procedure and depicted in Figure 1. These two portions were processed separately in a conventional method from soaking to tanning operations. At the end of each unit operation, the spent solution and samples were subjected for further analysis. All the experiments were carried out in triplicate.

Non-collagenous Proteins Analysis

Proteoglycans and other non-collagenous protein contents in the spent solutions obtained after processing of the two portions were analyzed. Prior to analysis, the spent solution was filtered and 1 mL of spent solution was taken and made up to 10ml with distilled water, from which the required amount was taken up for the analysis. Extractable proteins were analyzed from spent solutions after soaking and bating. Spectrophotometric analysis was carried out for the estimation of protein content in the spent solution by Bradford protein assay method.[28] Bovine serum albumin (BSA) was used as

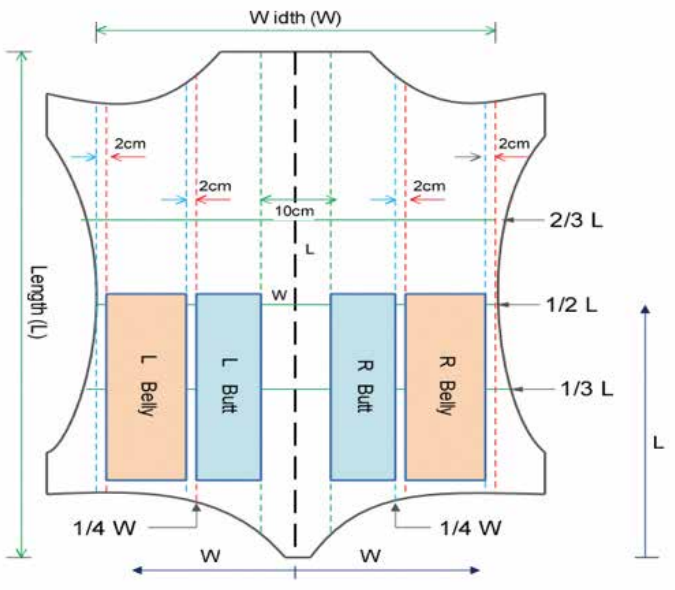

Figure 1. Representation of Butt and belly sampling position in sheep skin.

standard. During the process of liming, proteoglycans get extracted from the skin. The filtered spent liming solution extracted from both regions of the skin was analyzed.. The alkalinity of the liquor was neutralized using 0.5 M Acetic acid (AA) solution. The spectrophotometric analysis for proteoglycan was performed at an absorbance of 555 nm, using periodic acid–Schiff method.[29] Mucin was used as a standard.

Fat Content and Chromic Oxide Content Analysis

The total fat content of both belly and butt regions of skin were estimated after soaking. The hair on the samples was removed using scissor and thereafter the samples were dried to constant weight in an air oven (65 ± 2°C). Standard Soxhlet extraction method[30] was employed for extraction of fat using petroleum ether as a solvent. After distilling the solvent from the flask, the extracted material in the flask was dried for about 7 hours at 65 ± 2°C to constant weight. The fat content was estimated as weight percentage (w/w). Cr (III) content present in the leather was estimated by acid digestion method and reported as % Cr_2O_3.[31] Cr(III) content after tanning was also estimated.

Thermal Stability Determination and Pore Size Distribution

3-5 mg of samples were collected at various stages of leather processing (from soaking to tanning) and blotted on the tissue paper to remove the excess adhered water. Moisture content was maintained around 60-65%. These samples were sealed in a Tzero pan with a Tzero hermetic lid. Pore size distribution and thermal stability were analyzed using DSC-Q200 TA instruments Differential Scanning Calorimeter. Prior to the pore size distribution measurements the samples were cooled to -40°C and at that temperature isothermal for 10 min. The experiments were carried out for -40 – 10°C at a heating rate of 1°C /min. Pore size distribution was determined by the melting temperature of the water as suggested by Fathima et al.[15-16] The thermal stability of the samples was measured by heating the sample from 30°C at heating rate 2°C /min.

Collagen Extraction and Analysis

At the end of degreasing (removal of natural fats from the skin) the samples were washed and cut into smaller pieces, weighed and taken for collagen extraction at 4°C.[32-34] The moisture content of the samples was also measured based on standard procedure (SLC 3).[35] The samples were soaked in 0.5 M acetic acid (AA) solution under stirring at 4°C for 24 h. The mass of residue recovered was taken and soaked in 0.5 M AA and further treated with pepsin powder (100 units/g skin residue) at an enzyme/substrate ratio of 1:100 for 48 h at 4°C under stirring. The pepsin digested solution was filtered and the filtrate was recovered. Both the acid soluble and pepsin soluble filtrates were centrifuged at 16,000 rpm, 4°C for 30 minutes and the supernatant was collected. The supernatant was salted out using 5% (w/v) then incubated overnight. The solutions were centrifuged at 16,000 rpm, 4°C for 30 minutes to recover the pellet. The pellets were re-dissolved in 0.5 M AA and then subjected to dialysis with 20 mM phosphate buffer. After dialysis, the solution was centrifuged again and the obtained pellets were re-dissolved in 0.5 M AA and further dialyzed with 0.005 M AA. Then the solutions were centrifuged for 30 minutes at 16,000 rpm and 4°C and the supernatant (ASC and PSC) were collected and stored for further analysis. The extracted ASC and PSC collagen content was estimated using hydroxyproline assay[36] and its purity was studied using Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using the method of Laemmli (1970).[37]

Conformational Analysis

ASC and PSC conformation were studied using dichroic spectroscopy measurements conducted on a JASCO J-815 CD spectrophotometer equipped with a Peltier temperature control- 423S/15 (JASCO Inc.). CD spectra was recorded in the far UV region (190-250 nm) under constant purging of nitrogen gas employing 1.0 nm bandwidth, 0.1 nm step size, for an averaged time of 1 s.

Scanning Electron Microscopic Analysis

The specimens (approximately same dimensions) were taken from each of the unit operation (soaking to post-tanning) and fixed in 5% formaldehyde and 5% glutaraldehyde solutions. After fixing, the specimen were dehydrated with acetone solution, in increasing concentration of solvent[38] and then coated with gold using Edwards E306 sputter coater after 24 hours drying in a desiccator. The micrographs for both grain and cross-section of the specimens were obtained at high vacuum with an accelerating voltage of 12 kV in varying magnification.

Histological Examination

Samples from both butt and belly regions of the skin were taken after each unit operations (soaking to pickling) and stored in a refrigerator. All the samples were preserved in neutral phosphate buffer formaldehyde solution to prevent autolysis of tissue. Samples were subjected to series of preparations viz. dehydration, clearing, embedding, block preparation, trimming, mounting, sectioning and spreading of ribbons. The specimens were then stained with hematoxylin-Eosin after being de-paraffinised. The specimens were mounted with Canada balsam after clearing with xylene. The microscopic images of the specimens were finally taken and examined.

RESULTS AND DISCUSSION

Skin/hide will undergo several physico-chemical changes during leather processing due to removal of proteoglycans, proteins (albumin, globulin, reticulin, and elastin) and fat. Unwanted material removed from each unit operation is listed in Table I.

Non-collagenous Protein Analysis

Both protein and proteoglycan contents were analyzed in the spent liquor after soaking, bating and liming, respectively. Non-collagenous protein content values (g/mL) were found to be higher in butt region than that of belly region. Fibrous protein (elastin, reticulin), except collagen, was found to be higher in belly region than butt region as shown in Table II.

TABLE I
List of unit operations/processes in leather processing and its corresponding analysis in spent solution and skin matrix.

Unit Operation/ Process	Parameter	
	Spent liquor	Skin matrix
Soaking	Globular proteins	Fat
Liming	Proteoglycans	
Deliming		
Bating	Reticulin, Elastin	
Degreasing		Fat
After degreasing		Collagen
Tanning		Chromic oxide

*end of all the unit operations histology, pore size distribution and thermal stability has been analyzed

The globular proteins removed during soaking were much less in belly, probably attributed to less presence of them in belly region. Proteoglycans are found to be significantly higher in butt portion than the belly; this may be one of the main causes for compactness of butt region. Proteoglycans and dermatan sulphate are known to be distributed over the surface of collagen fibrils in a regular and highly organized manner thereby playing significant role in leather making. This may be one of the reasons for the notion "Leather is made in lime yard."

Fat Content Analysis

In order to compare the effect of fat content in butt and belly regions of the skin, the fat content was analyzed after soaking, which will be removed during degreasing process. The average percentage of fat content (w/w) in the butt and belly was found to be 3.00 and 4.58, respectively. As expected, belly portion contains more amount of fat than the butt, which is the main cause for the void spaces present in the belly region. This leads to looseness and reduces the leather quality and its economic value.

Thermal Stability

The hydrothermal stability of the samples was determined using differential scanning calorimetry. The thermogram of the butt and belly regions after each unit operation have been shown in Figure 2 and the peak temperature and enthalpy has been tabulated in Table IV. A decreasing trend in the peak temperature from soaking to degreasing for both butt and belly region has been observed. The presence of more heterogeneity in butt than belly can also be seen from the thermogram. The thermograms from all operations have been found to be nearly symmetrical except for liming operation, where more shoulders appeared in both regions. Besides, it

TABLE II
Characteristic features of belly and butt regions of sheep skin.

Parameter	Operation	Butt	Belly
Protein (μg/mL)	Soaking	65.15±0.72	13.45±0.42
	Bating	59.66±0.61	81.25±0.73
Proteoglycan (μg/mL)	Liming	3.32±0.08	0.43±0.05
Fat (% w/w)	Soaking	3.00±0.41	4.58±0.32
Chrome (% w/w)	Tanning	3.90±0.07	3.72±0.04
Collagen (mg/g on dry weight)	Acid soluble	34.40±1.03	4.88±0.92
	Pepsin soluble	2.18±0.85	19.94±0.98

TABLE III
Thermodynamic parameters calculated from DSC thermograms of ice crystal melting peak of the sheepskin at various stages of leather processing.

Samples	Butt			Belly		
	Onset (°C)	Melting peak (°C)	ΔH (J/g)	Onset (°C)	Melting peak (°C)	ΔH (J/g)
Soaking	-2.64	-0.50	129.0	-2.52	-0.59	98.71
Liming	-1.79	0.33	201.8	-1.80	0.11	183.4
Deliming	-2.57	-0.51	180.2	-3.24	-0.64	113.3
Bating	-3.63	-0.70	173.1	-4.71, -0.91	-2.07, -0.80	86.0, 1.84
Degreasing	-1.24	-0.58	100.9	-2.44	-0.56	153.6
Pickling	-23.48, -9.39	-22.72, -5.19	3.01, 111.5	-23.96, -10.55, -1.05	-22.58, -5.78, -0.98	2.82, 95.14, 0.39
Chrome Tanning	-5.37	-2.0	181.4	-5.89, -0.88	-2.30, -0.77	152.6, 3.16

*Average values of triplicates

TABLE IV
Thermodynamic parameters of sheepskin at various stages of leather processing from DSC thermograms.

Samples	Butt			Belly		
	Onset (°C)	Melting peak (°C)	ΔH (J/g)	Onset (°C)	Melting peak (°C)	ΔH (J/g)
Soaking	59.94	63.15	10.13	65.91	71.97	15.65
Liming	46.40	50.98	8.01	47.68	52.12	6.27
Deliming	53.52	56.63	8.15	54.37	57.82	7.16
Bating	51.91	56.25	12.61	51.68	55.79	10.30
Degreasing	55.20	62.84	22.76	51.90	58.22	15.77
Pickling	54.17	60.48	8.69	59.50	60.71	10.27
Chrome Tanning	107.48	108.74	11.76	107.97	110.44	9.95

*Average values of triplicates

can be inferred from the liming thermogram that the thermal stability has more effect in butt, than in belly. It has been reported that the removal of proteoglycans, in particular dermatan sulphate, leads to decrease in thermal stability of the skin.[39] The lesser shrinkage temperature of limed butt regions might be due to removal of more proteoglycans in the former, as indicated by proteoglycan content, alongside the effect of its higher swelling during the process.

Pore Size Distribution

The pore size plays a crucial role in leather as it may determine the unique property known as breathability. The pore size distribution gets affected during the various stages of leather processing. The chemicals used in each unit operation and tanning agent have an influence over the pore size. Thermoporometry has been used to determine the pore size distribution in skin matrix (wet condition).[15-16] Pore size distribution has been measured based on the Gibbs-Thomson effect.[40] It mainly depends on the melting behavior of frozen water, pore volume and its size. From the thermogram, the enthalpy of ice crystal melting peak of the skin matrix can be observed (Table III) and the pore size has been calculated using eq.1.

$$R_P = \frac{-33.33}{(T - T_0)} + 0.68 \quad (1)$$

where, Rp, T_0 and T are pore radius, melting temperature of pure water (273 K) and melting temperature of frozen water in different pore size present in the skin matrix. The pore volume distribution has been calculated by using the following eq. 2.

$$\frac{dV}{dR_p} = \frac{\Delta V}{T_i - T_{i+1}} \quad (2)$$

where, ΔV is pore volume, which has been derived from enthalpy and latent heat. T_i is the average temperature interval for the respective pore size. Using the equation 1 and 2 the distribution of pore size and pore volume has been calculated from the thermogram (Figure 3). The pore size distribution of butt and belly regions after each unit operations in leather processing have been shown in Figure 4. Pore size is the main factor for mass and heat transfer used in leather processing as well as its breathability and thermoregulatory properties. The melting peak of ice crystals and its corresponding enthalpy of phase transition in the skin during various stages in leather processing have been tabulated in Table III. The ice crystal melting peak of the thermogram occurred at –0.5 and -0.59°C for the butt and belly regions of soaked skin, respectively. All the operations except liming led to depression of melting temperature, which has been presented in Table III. The enthalpy of phase transition of water in the skin is also presented in Table III. The changes in ice crystal peak and its corresponding enthalpy indicate that the capillary structure of collagen fibers present in the skin matrix may be influenced by the nature of the interaction of the various chemicals used in each process. Soaking is an operation, which brings back to the native condition by the removal of dirt and salt along with globular proteins. After soaking, pore size distribution in butt and belly regions of the skin varies from 3-65 and 5-50 nm, respectively. Next process is liming, which involves the addition of lime and sulfide in order to open up the fibers as well as to remove hair and flesh from the skin. Liming thermogram shows positive ice crystal melting peak for both butt and belly regions, with the larger shifts in the pore size distribution in the range of 3-100 nm. From the results, it can be inferred that, during liming the fibers are opened up as well as removal of proteoglycans occurred which leads to swollen matrix. This positive ice crystal melting may be due to the presence of calcium hydroxide and its higher solubility at lower temperatures. In the case of deliming operation, lime has been removed by usage of ammonium salts and reduces the extreme alkaline pH to slightly neutral pH. After the deliming the pore size distribution ranges from 3-65 and 5-50 nm. Results inferred that, the swelled matrix has been de-swelled. Belly region shows multiple transitions in bating, pickling and chrome tanning. This leads to more drastic changes in the pore structure of the belly region than butt. Bating operation involves in the removal of interfibrilary proteins such as elastin and reticulin. Pore size distribution of the butt and belly regions of the bated pelt significantly varies in the ranges of 3-50 and 3-25 nm, respectively. Belly region

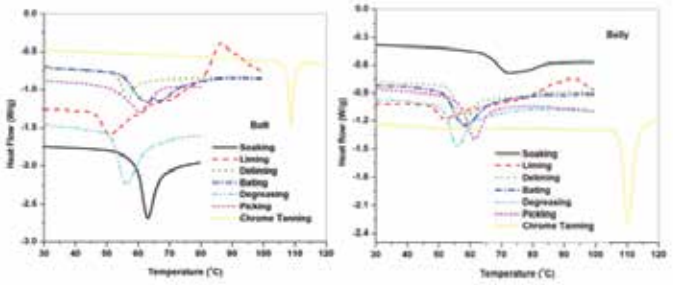

Figure 2. Thermograms of butt and belly portions of sheep skin at various stages of leather processing.

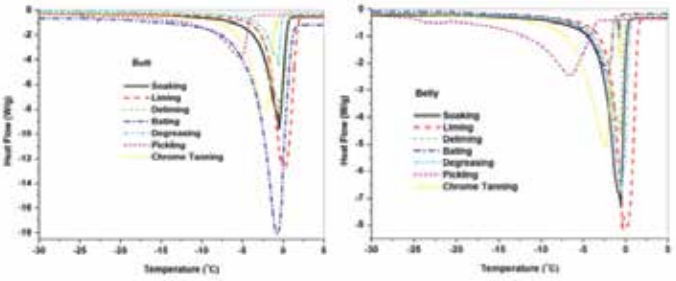

Figure 3. DSC thermograms of ice crystal melting peak of the sheepskin at various stages of leather processing.

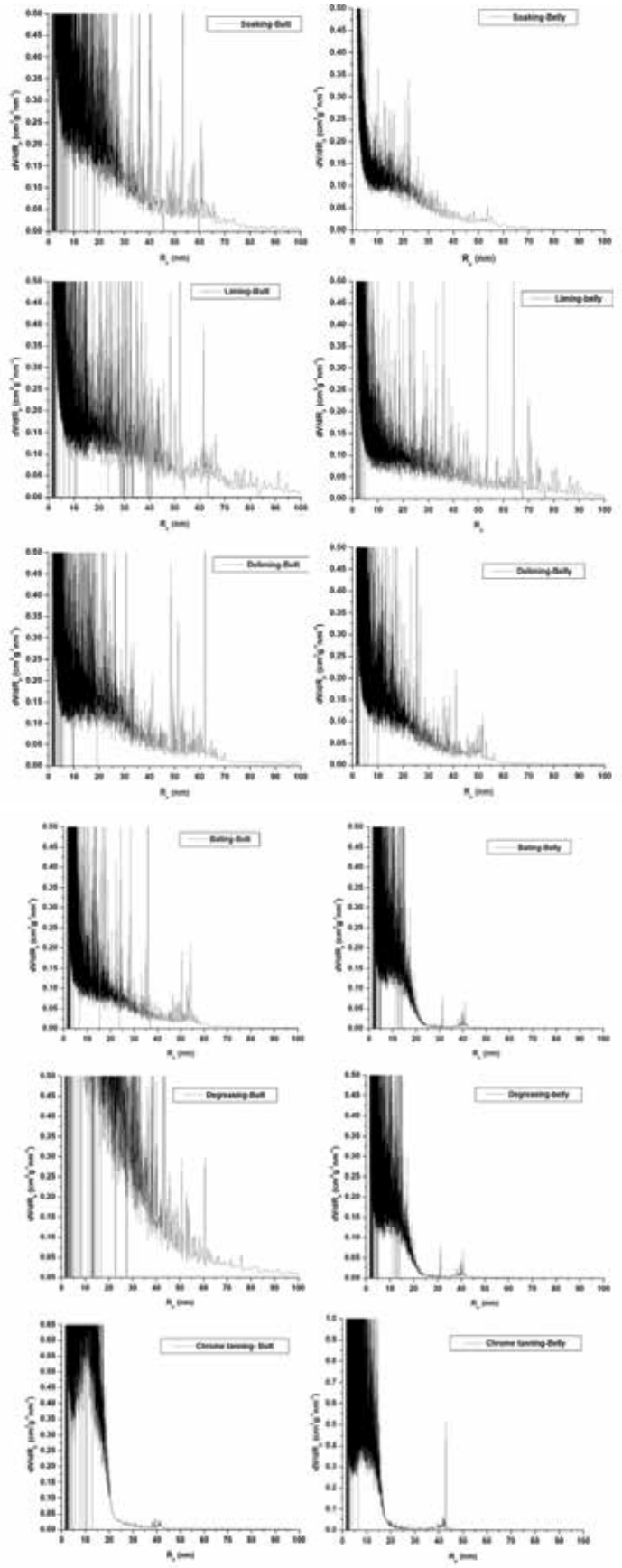

Figure 4. Pore size distribution of butt and belly portions of sheep skin at various stages of leather processing.

shows two transitions for the bated pelt, which may due to the removal of a higher amount of interfibrilary proteins than in the butt. Next operation is degreasing, which removes fat from the skin matrix. After bating and degreasing operation, the skin matrix shows similar pore size distribution. Pickling process involves the addition of acid and salt to the skin matrix to reduce the pH in order to facilitate the diffusion of tanning materials. Pickled pelt shows two transitions in both butt and belly regions, one for the freezing of pore water at –5.19 and -5.78°C and another for salt-water eutectic mixture at –22.78 and -22.58°C [41], respectively. Pickling process induces drastic changes in the pore structure of butt and belly portions of the skin matrix. These results agree with the earlier reports made from goat skin. In tanning process, basic chromium sulfate has been employed to impart thermal stability and resistance to collagenolysis. After tanning, pore size distribution shows similar trend in both butt and belly regions and it ranges from 3-20 nm.

Chromic Oxide Content Analysis

The % (w/w) chromic oxide content is more in butt than in belly (Table II). The value obtained for butt and belly was 3.90 and 3.72, respectively. This might have been due to the presence of less collagen content to absorb the chromium in belly than in butt. However, the hydrothermal stability of the chrome tanned leather samples of both parts were found to be comparable as observed from thermogram values.

Determination of Collagen Content

From hydroxyproline values of both acid-and pepsin collagens of the samples, the collagen content was estimated. Collagen content (mg/g of dry skin weight, Table II) of butt and belly with respect to acid- and pepsin soluble collagen solutions were (34.40, 2.18) and (4.88, 19.94), respectively. From the volume of extracted collagen and hydroxyproline measurement, it was found that pepsin soluble collagen was more in belly than in butt. In contrast, acid-soluble collagen is more in butt than in belly. Earlier studies report that, pepsin soluble collagen content is higher in vertebrates skin than the invertebrates.[32,42-43] In other words, high pepsin soluble collagen in animal skin signifies the presence of higher amount of inter-molecular crosslinks. Results corroborates with the collagen content present in sea animals skin. Sea animal skins possess higher ASC than PSC, which indicates lack of significant amount of inter-molecular crosslinks. From the collagen content, it can be inferred that collagen in belly regions possess more inter-molecular crosslinks than in butt. On the other hand, belly region possess more amount of telopeptides than in butt, which enables an interaction between telopeptides and neighborhood triple helix. These interactions play an important role in assembly and stabilization of collagen fibrils.[44-47] Molecular weight of ASC and PSC from butt and belly regions have been studied using electrophoretic analysis, and shown in Figure 5. Electrophoretic study revealed slight differences in the molecular weight between ASC and

PSC extracted from the butt and belly regions. From the electrophoretic results, it can be inferred that intermolecular crosslinked collagen may be abundant in belly region due to the presence of higher amounts of telopeptides than butt region.

The changes in the secondary structure of extracted collagen (PSC and ASC) with the two methods were examined by CD spectrophotometry in the far UV regions (190-250 nm). Unique CD spectral signature for triple helix will give an intense negative peak at around 197 nm due to π -π* amide transitions and a weak broad positive peak at around 220 nm due to n-π* transition with a crossover point at 214 nm.[48-49] For butt and belly part of the skin, the CD spectra showed a positive maximum peak at 222 nm for both PSC and ASC. Extracted ASC from butt and belly showed a positive maximum peak at 222 nm and a minimum negative peak at 197 nm with a crossover of 214 nm. The spectra indicates that, extracted ASC shows native polyproline II conformation, whereas PSC from butt and belly regions shows red shift in the π -π* amide transitions. PSC shows 9 and 12 nm red shift in the π -π* amide transitions with the 3 and 5 nm blue shift in the crossover point for the butt and belly regions, respectively. This shift may be due to partial cleavage of telopeptide regions by the treatment of pepsin (Figure 6).

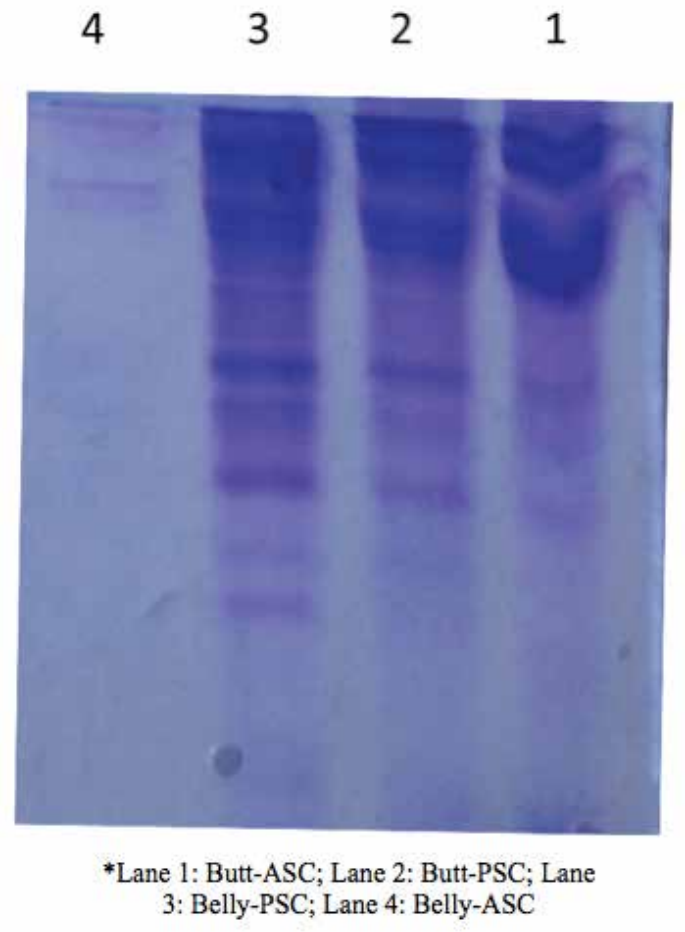

Figure 5. Electrophoretic images of ASC and PSC extracted from butt and belly portions of sheep skin.

Generally, the triple helical structures of both ASC and PSC of both skin regions can appear to be intact as inferred from the CD spectra.

Scanning Electron Microscopic Analysis

The scanning electron microscopic analysis of on the two regions of the skin was performed to investigate the morphology and the effects brought by leather processing unit operations (soaking, liming, bating, degreasing, tanning and post-tanning). From the cross-sectional SEM image (Figure 7), it can be inferred that more fiber opening was observed during liming in butt portion than its counterpart. The images for the delimed samples were in the support of it. The voids observed in butt were also on par, indicating that more non-collagenous proteins were removed as indicated in Table II. After bating, more voids were observed for butt part due to the cumulative removal of non-collagenous proteins from soaking, liming and bating operations when compared with belly part. The non-collagenous protein analysis result at these three operations for butt was found to be more, though bating operations showed more removal of same in belly than in butt. It can also be inferred from the surface analysis that the hair on the butt was uprooted, whereas mainly cut at the surface for belly during dehairing operation. The relative fiber compaction due to chrome tanning was observed to be lesser in belly when compared with butt regions.

Histological Examination

The cross-section butt and belly regions of sheep skin were observed after the unit operations from soaking to degreasing. The imaging was performed under a 12.5X objective. Figure 8 shows images of cross sections for the stated unit operations. The images of cross sections appear to show more compactness in butt than in belly. From the figure it has been observed that, there is a difference in corium major and minor between the

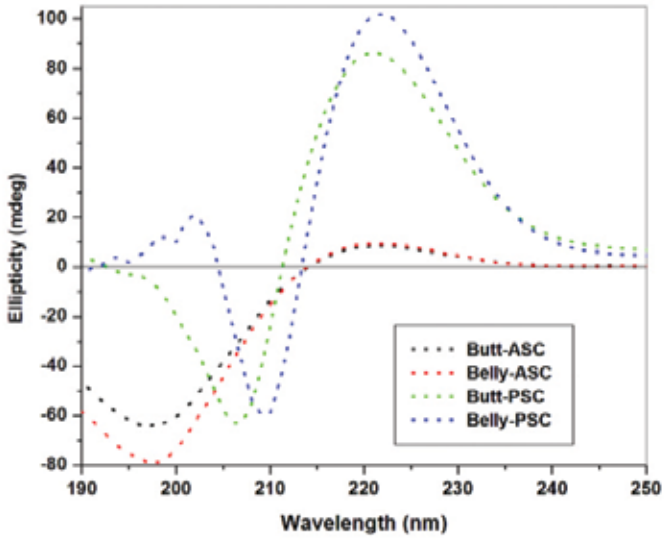

Figure 6. Far-UV Circular dichoric spectra of ASC and PSC extracted from butt and belly portions of sheep skin.

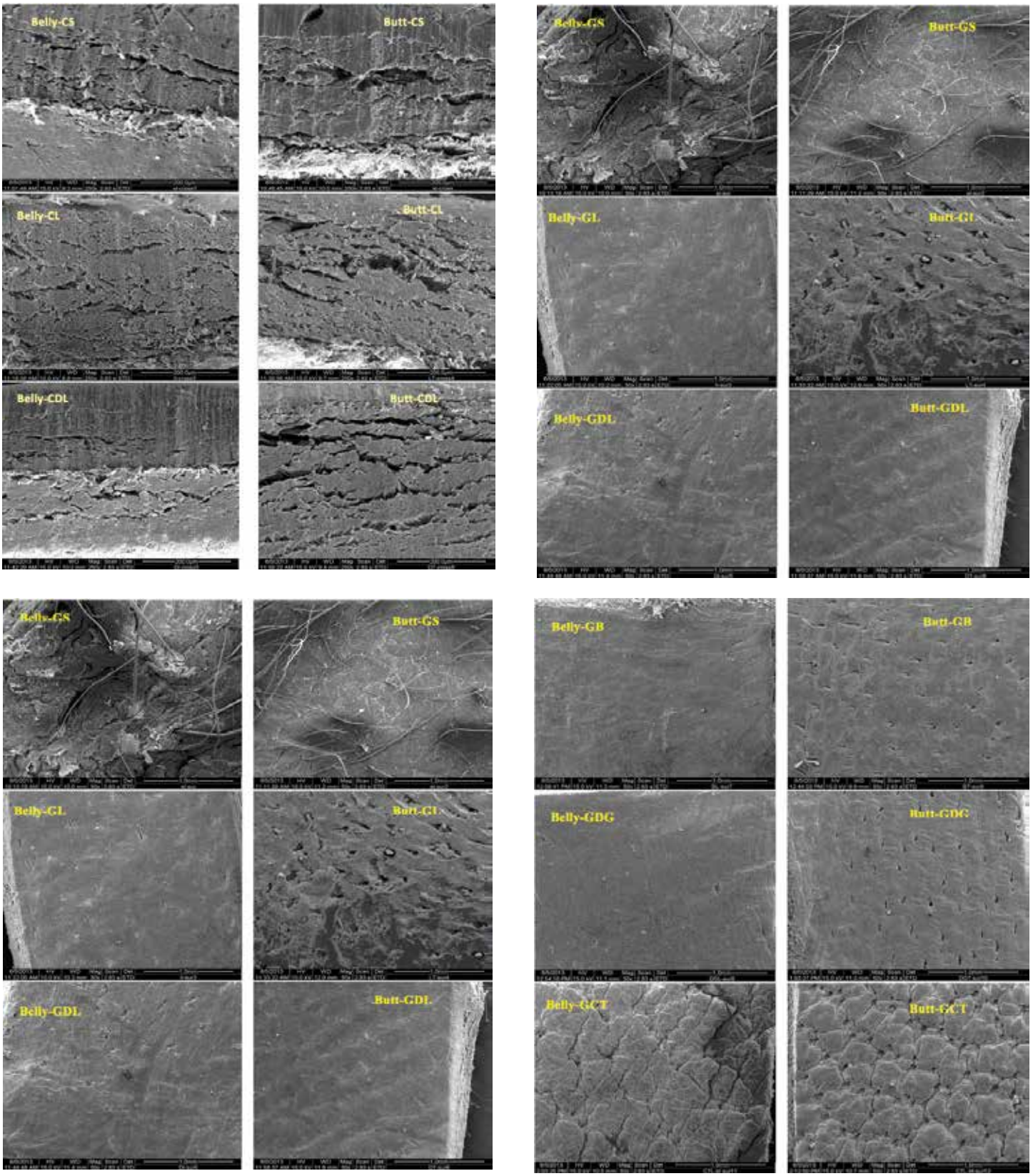

Figure 7. Scanning electron micrographs of butt and belly portions of sheep skin at various stages of leather processing (a) Cross section of sheep skin at 250x (b) Grain surface of sheep skin at 50x. Inset Labels: C- Cross section; G - Grain surface; S - Soaking; L - Liming; DL - Deliming; B - Bating; DG -Degreasing; CT - Chrome tanning.

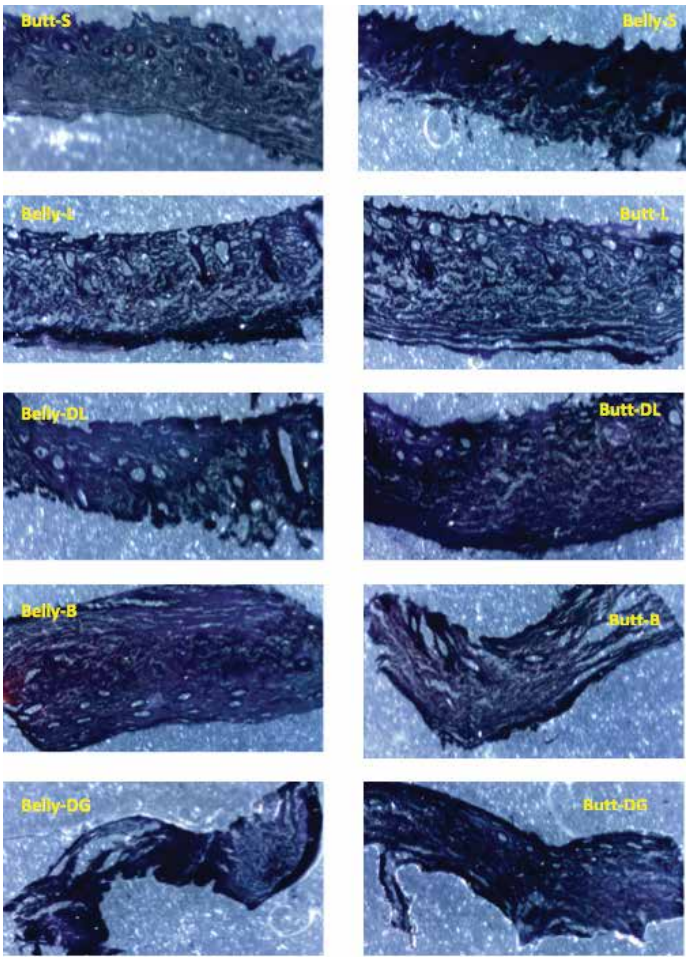

Figure 8. Histological characteristics of butt and belly portions (cross section) of sheep skin at various stages of leather processing. Inset Labels: S - Soaking; L -Liming; DL - Deliming; B - Bating; DG - Degreasing.

butt and belly regions of sheep skins. Similarly, the relative fat pockets in butt appeared to be lesser than in belly, which corroborates with fat content analysis. Angle of weave of fibers is low and its intactness decreases during the various leather processes from soaking to degreasing.

Conclusion

The biochemical analysis of both the butt and belly of sheep skin indicated considerable differences exist between the two regions of the skin. The collagen content and overall non-collagenous proteins are more in butt than in belly. In addition to the difference in collagen content, it appeared to be seen that belly is more soluble by pepsin than acetic acid. This in turn confirmed the difference in cross-linking of collagen fiber between the two regions. The histological examination revealed presence of more voids in belly due to fat pockets. Pore size distribution examination and morphology in the butt and belly regions has been studied during various stages of the leather processing by DSC and Scanning electron microscope, respectively. Difference in pore size distribution, pore volume and intensity has been observed between butt and belly region during leather processing. This directly plays a vital role in uptake of chemicals and breathability property of the leather. Chromic oxide content is more in butt than in belly and there are no considerable differences in the shrinkage temperature in butt and belly regions. In order to increase the value of the leather, the belly characteristics should be like the butt characteristics. This study provided a detailed investigation of butt and belly regions of Indian sheep skin. Future directions may focus on development of protein materials to balance the protein content of butt and belly regions of the sheepskin so as to enhance the value of the leather.

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