ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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SCHOOL OF CHEMICAL AND BIOENGINEERING

ENHANCING UNIT VALUE REALIZATION OF HAIR SHEEP SKIN- AN ASSESSMENT OF CHANGES BETWEEN BUTT AND BELLY REGIONS AND ITS VALUE ADDITION USING COLLAGEN HYDROLYSATE

BY

DAGNEW NEGASA

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A thesis submitted to School of Graduate Studies of Addis Ababa University, Addis Ababa Institute of Technology, School of Chemical and Bio-Engineering in partial fulfilment of the requirements for the attainment of the Degree of Masters of Science in Chemical and Bio-Engineering under Leather Stream.

By

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DECLARATION

Declared that this project entitled **"ENHANCING UNIT VLAUE REALIZATION ON HAIR SHEEP SKIN-AN ASSESSMENT OF CHANGES BETWEEN BUTT AND BELLY REGIONS AND ITS VALUE ADDITION USING COLLAGEN HYDROLYSATE"** is the bona fide work of **Mr. Dagnew Negasa (GSR/4138/04)** who carried out the research under my supervision. Certified further, that to the best of my knowledge the work reported herein does not form part of any other thesis or dissertation on the basis of which a degree or award was conferred on an earlier occasion on this or any other scholar.

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Table of Contents

List of Tables

List of Figures

List of Annex

Acronyms

ASC	Acid Soluble Collagen
A^o	Angestrom (10^{-10} meter)
CLRI	Central Leather Research Institute
CSIR	Center for Scientific Industrial Research
CH	Collagen Hydrolysate
CD	Circular Dichroic
COD	Chemical Oxygen Demand
DSC	Differential Scanning Calorimetric
DG	Director General
Eq.	Equation
GAGs	Glycos amino glycans
Gly	Glycine
kD	Kilo Dalton
LIDI	Leather Industry Development Institute
LMTD	Leather Manufacturing Technology Directorate
nm	Nano Meter
PSC	Pepsin Soluble Collagen
SS	Sheep Skin
Ts	Shrinkage Temperature
RHTH	Right Handed Triple Helix
TDS	Total Dissolved Solid
TS	Total Solid

Abstract

Skin will undergo changes in biochemical and biophysical properties during leather processing. Biochemical composition varies with respect to different regions of the skin like butt, belly, and neck. Supported with the nature of structure and chemical constituents of the skin, leather processing chemicals do have different propensity to different parts of the skin. Belly region's looseness and substance non-uniformity are the main problem in leather making from sheep skin (SS) particularly for shoe upper. Having a back with the leather processing practice and statistical data, an exuberant substance non-uniformity for sheep shoe upper is still recurrent in our country's context. To address same issue, this work focusses on the analysis of histology and biochemical properties of butt and belly portion of hair sheep skin besides the investigation of application collagen hydrolysate on the value addition of dyed crust leather and emission load during pre-tanning.

Globular proteins are relatively higher in the butt region 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 it is reverse. Thus, the effect of liming and bating operations in removing unwanted skin constituents is stronger in butt and belly regions of SS, respectively, which reflects the requisite importance of the two unit operations in the pre-tanning operations. ASC contribution is higher in butt region than belly and the reverse is true in the case of PSC. The overall collagen content is considerably higher in butt than belly which may be the major cause of the higher strength characteristics of butt regions. Cr_2O_3 content is high 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 seen to vary in all the unit operations. Scanning electron microscope study complements same trend with respect to changes in the cross-section of the skin during leather processing. Therefore, the considerable variations observed among the two regions of SS would help the tanners and chemical manufactures to have more information on the parts of skins so as to improve the cutting value of the thin part of skin.

Collagen hydrolysate being prepared under thermal alkaline condition enhances the hide substance of both belly and butt regions of sheep skin during pre-tanning when offered at pH 9.0. Despite the fact that Cr_2O_3 uptake found to be reduced in same way the CH uptake increased in both regions of SS, the Ts still found to be greater than 90^oC thereby strengthening

the application of CH in leather processing. In addition to the pore size distribution of the regions of SS, it has been observed that the fibre density of skin matrix also plays its own part for the uptake and retainment of treatment chemicals. The higher increase in the protein content of butt part of SS than belly augments same. The dyestuff exhaustion is by far better for CH treated leather than commercial protein fillers (CPF) used during the study. The increased in the dye exhaustion and overall physico-chemical properties of CH treated upper leathers would highly help the competitiveness of the tanner for the use of CH particularly coined as wealth from waste. The use of CH would be convenient for the value addition of lower end products. The emission load of tanning process liquor with CH is higher than conventional one and same is true for post-tanning process liquor with CPF.

1. INTRODUCTION

1.1. Background

The application of leather to human life has become versatile as mere body covering to different industrial uses. Leather processing has undergone many changes from traditional leather making approach to a modern controlled processing system. Different chemical treatment and processing systems have been put into practice to utilize the skin/hide to the best, based on the nature of input material and properties pertinent to end product. Therefore, the selection of raw materials and process design plays an ample role for quality production of leather.

Physical properties and chemical composition of skin/hide vary 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. Within same skin, thickness, chemical constituent and fiber orientation also varies in different parts like shoulder, butt and belly and hence results in difference in strength, elongation and other physical properties of the leather. Thus, besides the quality, the source of the input material (sheep, goat, hide, buffalo, camel, etc.) has major role in the type of final leather intended to be produced. For instance, Ethiopia's highland SS is known for its best quality glove and softy type of leather production.

Understanding the histological properties of skins of different animals would primarily dictate the type of possible leather that can be produced out of it. The grain surface pattern, the fibre density or weaving pattern in corium minor and major determines the outward character and bulk properties of the final leather. Layers of skin, from grain to flesh, have different thickness and this varies from animal to animal, indeed. Hair density and types of hair on the skin manifests the grain surface pattern of the leather and such properties varies from one animal to another.

Sheep is one of the major economically important livestock in Ethiopia. There were about 25.5 million sheep[1] in the country, playing an important role in the livelihood or resource-poor farmers. As reported by same[1], about 72.69% were female and about 27.31% were males and close to 99.92 percent of the population comprised indigenous sheep breed with the balance accounted for both hybrid and exotic type. Although many attempts have made to identify and characterize Ethiopia's sheep breeds or types, only recent study succeeded in identifying the breeds. By same study[2], there were reported to be about six sheep breeds viz. Afar or Adal sheep, Arsi-Bale sheep, Horro sheep, Menz sheep and Washera(Dangla) sheep in the country. Ethiopia's high land sheep skin has been known for high quality glove and softy type leather.

1.2. Statement of the problem

Upper leathers made out of sheep skin (SS) mostly have non-uniformity in thickness and looseness in belly areas. Regardless of other skin quality factors, skin from both old and female animal resulted in final leather of inferior quality. Of smaller domestic animals (goat & sheep) in Ethiopia, close to half of sheep population[1] accounted for female sheep of two years & older age which presumably affects the uniformity in leather substance made out of skin of same animal category. Therefore, to exploit the economic value of natural intrinsic quality of Ethiopia's high land hair sheep skin at large extent as possible, there need to be further investigation to seek concert information in this context.

Optimum re-liming and proper combination-tanning process in post tanning operation (acrylic, melamine-formaldehyde, etc.) would presumably reduce belly looseness. Combination-tannage and using post-tanning auxiliaries would help in achieving essential and deserved properties of final leather. Proper fiber opening helps in uniform distribution of chemicals, but too much opening of skin matrix aggravates poor strength of intended final product and associated looseness owing to have fullness in tanning and post tanning treatments. Based on the type of final leather required to be made, auxiliaries like acrylics, melamine-formaldehyde, dicyandiamide, fillers, etc. commonly used to enhance surface and bulk properties of the leather but only with right choice in the order of addition of other treatment chemicals. Nonetheless, the above mentioned problem is still prevailing especially for softy upper leather from SS. Moreover, Thickness uniformity is one of the parameters for upper leather besides the associated discrepancy. The area loss which has direct implication on economic loss needs to be addressed, though in practice has given a little attention. The desire to recover the overall loss of area of finished leather as a result of the aforementioned discrepancy proves to be a better alternative for a tanner.

Apart from the increasing cost of commercial leather chemicals, liquid waste treatment and meeting physical and chemical test limits for final leathers is of great concern. Relatively lower price, availability and biodegradability are triggering criteria for leather chemicals in the tanning industry. The application of collagen hydrolysate (CH) during dyeing was studied which showed the synergistic effect of hydrolyzed collagen. In this study, collagen hydrolysate from solid waste (pelt trimmings) is employed during pre-tanning operation so as to investigate the enhancement in protein content of belly part which is a new approach in CH application. The associated bulk and physical properties of skin pertaining to various regions will also be examined for the production of intended final leather. Therefore, the application of collagen

hydrolysate believed to enhance the protein content of the skin. The increased in protein content has multifaceted advantages for it would improve dye exhaustion and reducing the cost of commercial fillers which ultimately increases the cutting value of leather.

In addition to the reactivity and molecular structure of chemicals being used in the leather processing, the structure of skin and its biochemical constituent has considerable effect on the overall final properties leather comes out of it. Skin's reactive protein content which is mainly of collagen may considerably dictates the final property of the leather. From skin structure point of view, butt part of the skin is more compact than belly part. The angle of weave is an indicative parameter to measure the compactness of the fibre matrix. The less angle of weave, the more the compactness of the fibre matrix. Level of compaction of skin would directly reveal the kind of properties it imparts to the final product come out of it. The fibre orientation and fat accumulation of belly part may be different from its counterpart (butt) within the same skin. Most of post-tanning chemicals do not have similar propensity to the whole parts of the skin. Moreover, the macro-pore size of butt and belly parts may not be similar and hence may induce variation in chemical preference to parts of same skin. As much has not been done so far, examining and comparing the fibre structure of the two parts of the SS will be helpful in providing the type of selective chemicals to be chosen. Moreover, analyzing the difference in chemical compositions such as collagen, fat, proteoglycans and other proteins being removed in the course of pre-tanning operations of the two parts of the SS is another area to be dealt as it also provides fundamental information pertinent to them.

Therefore, it is essential to undertake a study on the histological features and collagen density of both belly and butt regions of SS and investigate the reduction in variation of collagen content of belly regions with its counterpart by applying CH in leather processing operation.

1.3. Research Questions

- How is the removal of non-collagenous proteins in belly part of SS compared with butt part after soaking and bating operations?
- How is the removal of proteoglycans in belly part of SS compared with butt part during liming operations?
- How is the fat content of belly part of SS compared with that of butt part after soaking and degreasing operations?
- How is the collagen content of belly par of SS compared with butt part?

- How is the chromic oxide content of belly part of SS compared with butt part during conventional basic chromium tanning process?
- How is the hydrothermal stability, pore size distribution & cross-sectional properties of belly part of SS compared with butt part during beamhouse and tanning operations?
- What will be the effects of application of collagen hydrolysate from pelt trimmings during pre-tanning on the protein content, associated bulk & physical properties of belly and butt parts of SS?
- How the effects of application of CH during pre-tanning is compared with effects of commercial protein filler (CPF) being employed during re-tanning and dyeing?

1.4. Objectives

1.4.1. General objectives

The prime objective of this thesis is to have well insight on the histological features and quantum of skin matrix (bio-chemical) constituents of the belly part of SS in comparison to the compact portion (Butt) and relate to the properties of leather. Further information as to how well the CH imparts the required physical and chemical properties to this part of SS comparing with relatively compact part to be addressed.

1.4.2. Specific objectives

The specific objectives of the study are:

- To examine and have well insight on collagen density of butt and belly part of SS.
- To examine the histological features (fibre density and fibre orientation) of butt and belly part of SS.
- To determine the amount of non-collagenous proteins (proteoglycans & other proteins) being removed during pre-tanning operations.
- To examine the thermal stability and pore size distribution of the two regions of SS during all pre-tanning and tanning operations.
- To analyze the level of enhancing protein content of belly part of SS with its counterpart (butt) using CH and examine its effect on the final property of the leather.

1.5. Conceptual framework of the study

In this study, histological features, sheep skin's biochemical content and the effects of application of collagen hydrolysate on belly and butt parts of SS is analyzed with the framework indicated in the Figure 1.1. Skin samples of both parts are excised from wet salted hair sheep skin & put into conventional leather processing and the response variables of interest

are measured at the end of each unit operation. Acid soluble and pepsin soluble collagens is extracted from both regions of SS and the overall collagen concentration based on the dry weight is examined. Moreover, CH from limed pelt trimmings is prepared and applied in conventional pre-tanning operation. Optimization of CH with respect to pH and offer is carried out. Ultimately, the effects of application of CH during pre-tanning on protein content enhancement and associated bulk & physical properties of belly region are analyzed.

1.6. Significance of the study

There was no reliable study so far which objectively elucidates the difference between regions of same skin except literally asserting the difference. This study enables to provide well insight as to how the parts (belly and butt) of SS look alike or differ on their structure and composition and help relate to the overall properties of finale leather. Moreover, this study provides information on the effect of application of CH during pre-tanning on protein content enhancement, associated bulk & physical properties of crust dyed leather and emission load.

1.7. Scope of the study

The study encompasses experimental analysis with respect to histological features (fibre density & fibre orientation) and biomolecules (collagen, non-collagenous proteins, fat) content of belly part of SS with that of the butt part and investigation of protein content enhancement of the belly part using CH. Conventional leather making unit operations has been followed where SS samples and spent liquor from various operations are collected and analyzed. CH from limed fleshings and pelt trimmings is prepared under thermally supported alkaline condition and made employed in pre-tanning operation to investigate the enhancement of protein content, emission load and change in associated bulk & physical properties crust leathers between belly region and its counterpart. Experimental and control processes encompassing SS samples of both regions have been used to make the comparison.

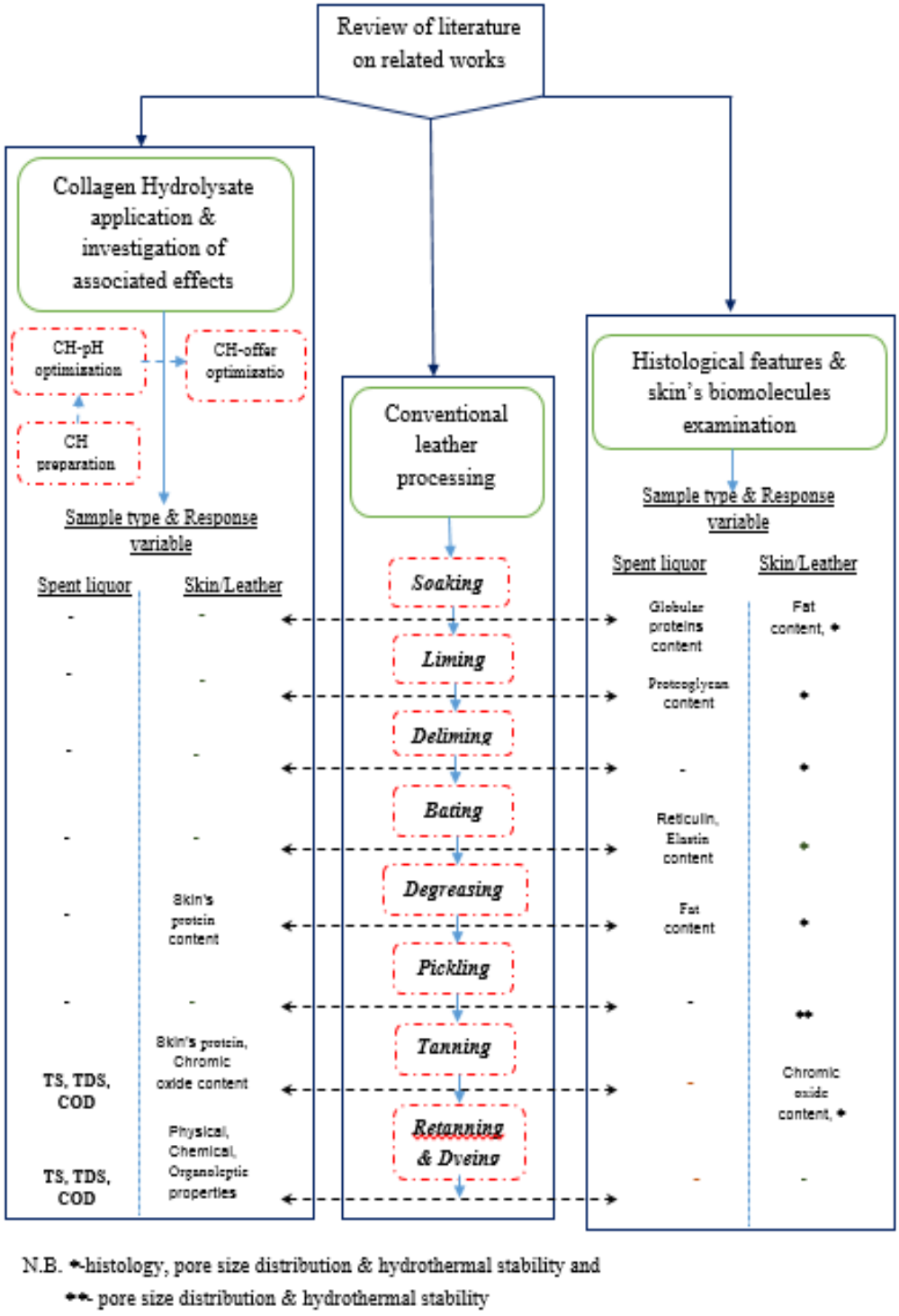


Figure 1.1. Conceptual framework of the study

2. LITERATURE REVIEW

2.1. Histological and chemical composition of skin

Skin is the largest of all organs in majority of living species which is regarded as external integument of the animal. Although skin appears to be a mere covering of the animal, it has unique biological features in that it provides the living species with thermo regulation, sensory set up, holding internal organs and protection from external damage. Skin is made of composite materials where the association and molecular arrangement of them give the skin its own architectural features. It is a finely woven matrix displaying visco-elastic property. It is also a unique porous which is comprised a 3D weave of collagen fibre bundles and other structural protein associated with it.

2.1.1. Anatomical structure

The structure of skin is somewhat complicated; and some knowledge of its histology and chemical composition is essential as to an appreciation of the extremely complicated structural changes involved in the manufacture of leather. The use of the terms 'hide' and 'skin', in the leather industry, is only of conventional, and the distinction is of size, substance or thickness. The word 'hide' usually refers to a large and heavy skin, such as cow, buffalo, horse, etc. There are situation in some species that the deciding factor will be the type of animal rather than the size alone. For the purpose of this work, the term skin is regarded as the outer covering of an animal (domestic) unless specification is made when skin appeared with suffix as sheep skin (SS), goat skin (GS), calf skin and hide for cow hide throughout the document. Moreover, SS is also regarded as hair sheep skin unless distinction specifically made like wool sheep skin. The skin histology of animals normally used in leather production is similar but species differences are observed. There are differences in the relative amounts of the component tissues and their arrangement in different types of skin and in different regions in the skin. The component structures of the skin are capable of flexing, stretching or contracting with the movement of the body.

Skin can broadly be divided into three distinct layers, viz. epidermis (outer most layer), corium or derma (inner layer) and flesh or adipose layer (figure 2.1)[3]. The three layers exist in superimposed manner and the type and distribution of chemical which they constitute could vary accordingly. Major proportion of the skin thickness is accounted by the derma layer. From leather manufacture point of view, it is generally viewed as epidermis, grain, corium and flesh layers. Of the three layers to the skin, the epidermis and dermis are important in the leather making as the third adipose layer comprising meat, fat, etc. will mainly be removed during

mechanical skiving. Dermis, which is the most important layer of leather production, is composed of the grain layer, corium layer and the intermittent layer between the two.

The grain surface pattern of the skin not only varies from species to species but also over the entire parts of same skin. The architectural design in the outer surface of the leather produced has got distinct properties due to the arrangement of hair pores and can duly be used as one means of identification of different leathers. The grain pattern is indeed attributed to the composite of hair follicles openings, presence or absence of nerve papillae and the holistic effects of structures beneath the surface. The depth, diameter, direction and inclination of hair follicles, hair density, parity and disparity in the size of hair pores, etc. are factors that could directly influence the grain pattern of skin.

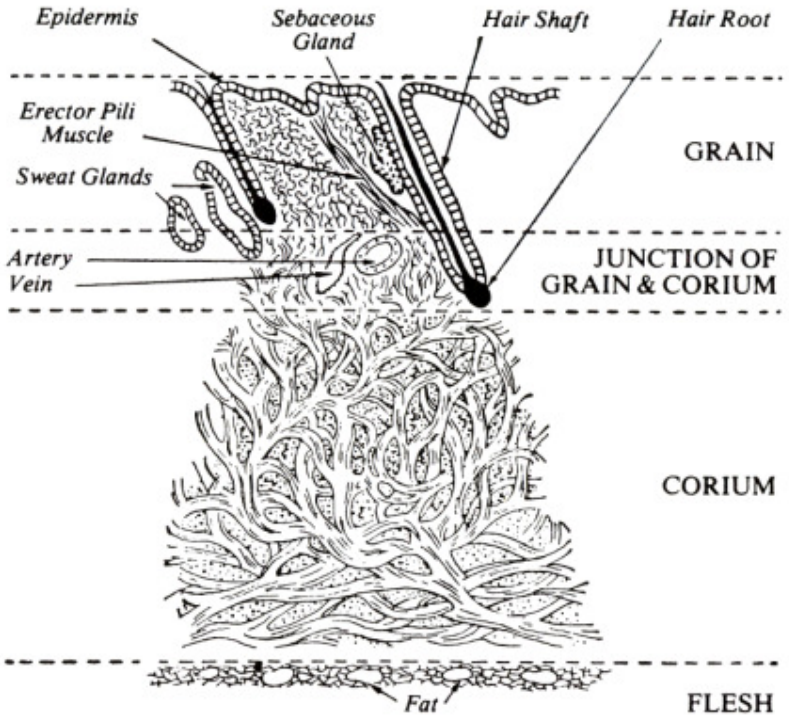


Figure 2.1 A general cross section of calf skin

The collagenous fibre bundles in the corium layer of SS intertwine at a low angle of weave and are not very compact unlike their fineness in the epidermal layer[4]. Wool sheep skin and hair sheep skin are two distinct varieties of sheep skin being provided to tanning industry. From point of view of leather making, tropical and sub-tropical hair sheep and coarse-wool sheep skins are the best material for gloving and clothing leather production[5]. Sheep skin having

lengthy wool is thinner than those with shorter hair. Similarly, the shorter and denser the hairs, the finer and smoother the grain of leather made out of sheep skin. The nature of the hair fibres for wool sheep and hairs sheep differs in that the fibres are straight in the later unlike curly in the former. The absence or less presence of ribs and fatty deposit between the junction of the epidermal area and the corium area are the key aspects that makes hair sheep skin good stock compared with its counterpart.

Though SS and GS are almost similar in size, the former is less compact and weaker in texture and strength than the later. Though, no reliable study made among parts of SS so far, large amount of natural fat is usually found between the corium minor and major and will give spongy leather when too many fat cells found in this layer. On average, cow hide has more hair density (hair per square inch of hide/skin) than skins of any other domestic animals. This renders leather made out of cow hide with fine and smooth grain surface. GS to certain extent exhibits similarity with calfskin (cross-section of hair) and SS (oil glands, fibre bundles through corium, epidermal areas and weave angle but more compact) though it could overall have typical character.

2.1.2. Chemical composition

Skin mainly constitutes water, proteins, fats and mineral salts in the order of percent constitution. Proteins and fats roughly account for 33% and 2-30% of the chemical constituents, respectively[3]. Collagen, keratin, elastin, glycoproteins, albumins and globulins are the prominent proteins constituting skin. Nearly 88% of fibrous proteins present in skin are of collagen and is the one converted into leather, indeed. Proteoglycans are classes of non-collagenous minor proteins but significant constituents of skin substances and are removed together with other inter-fibrillary materials during beamhouse operations. The name proteoglycan was first came into use in 1967 by E.A. Balazs[6] to indicate a protein/polysaccharide complex in which one or more Glycosaminaoglycans (GAGs) are covalently bonded to one or more protein chains. As opposed to glycoproteins, the carbohydrate is the dominant functional component in proteoglycans even though in some of proteoglycans (such as those found in skin), the carbohydrate make up only 40% of the dry weight molecule[7]. Proteoglycans have a variety of biological functions viz. act as tissue organizers, influence cell growth and maturation of specialized tissue, play a role as biological filters and modulate growth factors activities, etc. Proteoglycans also stabilize the skin matrix (fibrous matrix), regulate its tensile strength and render proper filling & right structure to it while the animal is alive. Though specific neither to belly nor to butt regions of the skin, it has

been experimentally observed that composition of SS protein (20-30% by weight) comprise 50-80% collagen and 20-50% non-collagenous components[8]. Besides, the presence of more non-collagenous protein content in the SS, particularly in the early age of the animal, the decrease in its proportion with ageing to certain extent was also indicated. Collagen is mainly found both in the grain and reticular layer where the thinness and fineness of the mesh decreases from grain to reticular layer. The grain layer is an architecture in such a way that thin individual fibres are packed and woven so tightly to give relatively fine and smooth surface after hair removal.

2.2. Collagen

Collagen, from simpler form of sponges to Homo sapiens, is the major protein of animal bodies where it exists in the various forms from skin, tendon, and bone to cornea and basement membrane of the capillaries (A.J. Bailey, *et al*., 1997)[9]. Together with cellulose and lignin, it is one of the three quantitatively dominant biopolymers. Of the many of animals' organs tendon, skin, bone, teeth, vascular system and connective sheaths surrounding muscle are the main ones that are structured with collagen. Collagen is the main protein constituting connective tissue of animals where it is abundant in vertebrates accounting ≈ 30% of total proteins[10]. As the name indicates, it provides structural functions or support to most tissues and cells of the indicated organs. It is mainly responsible for animal's skin strength, elasticity and tissue development and also found in blood vessels, cornea, lens, bone, and teeth, too. Concisely stating, collagen is a fibrous protein well organized in the formation of skin as a building block.

2.2.1. Significance of collagen

As a matter of its incidence, collagen is present throughout the entire animal kingdom with the exception to single-celled organisms and therefore, its significance drives from its numerous functions in living organisms. With respect to mammals, collagen is a primary component of the skeleton and skin and consequently accountable for protecting and supporting the body. Nonetheless, it is also present in many other organs and in fact is not only as part of the extracellular matrix but also paving great significance for cellular biology. Collagen has got many industrial and medicinal applications due its biodegradability, weak antigenicity and superior biocompatibility than other biomolecules.

Collagen molecules assembled and arranged both laterally and longitudinally in a hierarchical fashion making up skin matrix. It has been figured out that about 7000 collagen molecules

(tropocollagens) arranged in a parallel manner to form collagen fibril having 100nm diameter[11]. The arrangement and interwoven collagen fibre bundles render the skin to have its own structure and strength. Being by-products of human foodstuff and wool production, hides and skins primarily from cattle, sheep goats and pigs have been further industrially processed to useable products which in turn recognize collagen's economic importance. The understanding as to where the stability of fibrous structure comes (mainly from natural cross-links) is so important that the following are considered in this aspect:

- Collagen possess significant strength
- It is insoluble in water and major part of it is insoluble in weak acids and alkali where this would be immensely appreciable in aged skin/hide
- It depicts considerable resistance to wet heat to the extent of 65^0c in native state. The Ts is increased remarkably through tanning, indeed.

2.2.2. Types of collagen

There are twenty nine different types of collagens identified based on the variation in the specific amino acid sequence the polypeptides built of[12]. They differ in some cases significantly in their chemical and structural characteristics related to their function in the living organisms. A common feature is their conformance (at least partial) to triple helix domains, their hydroxyproline content, an amino acid which is virtually specific to collagen and their occurrence in the extracellular matrix. Layer of animal's skin (corium) which is processed in the leather manufacture also contains several of these collagens. The different types of collagen are labeled as type I, II, III, … , XXIX and of which the most commonly occurring are type I and type III forming long recognized characteristic fibre bundles being seen in many tissues. Type I collagen contributes more than 98% of the total collagen in mature hide and is highly dominant in corium. The more type III collagen fibres, esp. in growing skin, the more flexible the skin will be. Collagen types in animal hide and their properties summarized as in table 2.1[13]. Studies have shown that sulfur-bearing amino acid such as Cystine would hardly be obtained from hide collagen (in pure state) unless presumably attributed to contamination with keratin for trace amount.

Table 2.1. Collagen types in animal skins

Collagen type	Properties	Remarks
I	Fribril-forming, growth extending to fibres and fibre network	Principal components (> 95%), crucial to the properties
III, V	Forms the fibrils, fibres and fibre strands	Enriched in the grain layer (in particular type III), in some cases microfibers with type I; overall, not crucial to the properties
IV,VII	Not fibrillar (type IV), filamentous	In basement membrane, interface between epidermis and corium, " enzyme-sensitive", (enzymatic dehairing)
VI	Filamentous	Regulatory for fibril assembly; removed in the lime

Depending on the nature or forms in which each collagen types are being aggregated, the identified collagen types are classified into four categories:

i) Fibril forming collagens- eg. Type I,II, III and (V & XI)

Backed by its name, such class of collagen includes collagens of fibrous in nature possessing 300 nm triple helix. The N- and C- globular domains (telopeptides) of such collagen types have been partially removed prior to aggregation of the molecules in the quarter-stager alignment. They possess non-interrupted triple helix structure. Type I is the major collagen type which exists in skin and tendon. It is also exists in bone and dentine which are hard calcified tissues. Type II collagen is the prominent component of cartilage and vitreous humour. Type III collagen forms widely distributed, fine fibres present in high proportions in the tissues such as foetal skin and the vascular systems where a more flexible frame work is required. Type V and XI are almost identical in nature and exist quantitatively as minor components of nearly all tissues comprised of fibrillar collagens. They are believed to form filamentous scaffold or template on which bulk fibrillar collagens copolymerized. For instance, type V and XI copolymerized with type I and II, respectively.

ii) Network forming /or Non-fibrous collagens-eg. Type IV, VIII and X

Collagens of such a class believed to form network. For instance, type IV is the most abundant non-fibrous collagen possessing long (400 nm) and flexible triple helix due to irregularities in the tripeptide sequences. It lacks charge profile required for the formation of quarter-staggered fibres and hence form "a chicken-wire" like network which act as the basic framework for the basement membranes of both vertebrates and invertebrates. Type VIII and X can also form networks and often classified as short-chain collagens.

iii) Filamentous collagen-eg. Type VI and VII

Type VI collagen has been observed as loosely packed filamentous structure with axial repeat of 100 nm short triple helices and with larger globular regions at both N-and C- termini. It has been shown to form end-to-end alignment of tetramers and such collagen type has also been believed to separate and align larger type I collagen.

iv) Fibril Associated Collagen-eg. Type IX, XII, XIV, and XV

Several collagens don not form homotypic fibres or networks but are associated with other fibre forming collagens. Fibril associated collages are found in association with the quarter-staggered fibres of other collagen types and includes the FACIT collagens, i.e. Fibril Associated Collagens with Interrupted Triple helices. For instance, type IX collagen has been reported to decorate the surface of type II fibres (Vaughan et al., 1988), whilst type XII and XIV have been generally associated with type I collagen fibres surface in some tissues. Type IX collagen contains three collagen domains separated by non-triple helical regions which align on type II in anti-parallel manner.

2.2.3. Structure of type I collagen

The primary sequence of collagen is essentially a tripeptide repeat being put as $(Gly\text{-}X\text{-}Y)_{100\text{-}400}$ where X is often proline and Y sometimes Hydroxyproline (Hyp). Glycine is therefore occupies every third residue in a chain which is twisted into a polyproline helix by the pyrrole residues, rather than the more common α-helix of the globular proteins.

Collagen (type I) at molecular level is built from rod shaped smallest collagen molecule called tropocollagen which is 3000 nm long and $15A^0$ thick. Tropocollagen is primarily assembled from three polypeptide chains called α-strands/chains being entwisted about each other giving coiled coil called right handed triple helix. The α chains which combine to form the triple helix exhibit non-helical, short-chain appended peptides (telopeptides) at the N- and C-terminal ends. Two of the α-strands/chains are alike and the third differ from the other in its amino acid sequence. Each chain in the helical region is about 1050 amino acids long[14]. The non-helical

ends of tropocollagen, telopeptide, comprise about 25 amino acid residues and 16 of which is in N-terminus. The amino acid sequence pattern of helical regions with that of non-helical regions of collagen is not similar.

As represented below, collagen is represented by a repeating amino acids triplet or unit $(Gly\text{-}X\text{-}Y)_n$ where glycine amino acid comprise one-third and almost a further third by cyclic amino acids proline and hydroxyproline of the total amino acids involved in the creation of collagen. This is the basic requirement to envisage for the creation of collagen-specific formation of triple helix and the reactivity of collagen to a certain level, too. The amino acid sequence (Gly-X-Hyp) is substantially responsible for the specific helix structure of the collagen.

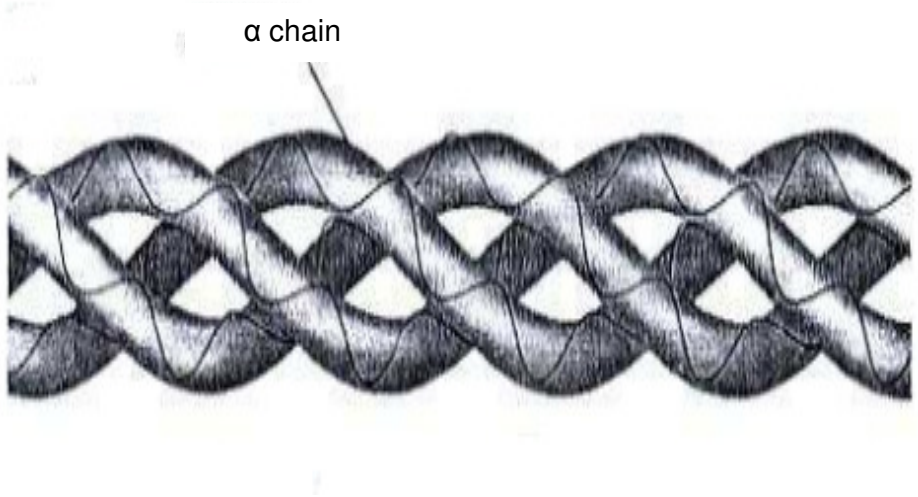


Figure 2.2. Portion of a collagen molecule (RHTH)

Proline and Hydroxyproline amino acids indeed most often take X and Y positions of the corresponding Gly-X-Y triplet, respectively. $(Gly\text{-}X\text{-}Y)_{350}$ is believed to the α chains regular recurrence according to the chain length of type I collagen. Hydroxyproline is virtually specific to collagen being in significant amount, the actual course of event which can be exploited for its quantitative detection and hence the determination of this amino acid is frequently used as an assay of the amount of collagen present in the tissue[15, 16]. It is, in fact, also considerably responsible for the hydrothermal stability of native and fully hydrated collagen.

When it comes to the reactivity of collagen which is the requirement for its transformation to leather, the contents and accessibility of basic and acidic amino acids, amino acids bearing hydroxyl (OH) groups and peptide groups are so crucial and play considerable role in this

regard. The peptide groups essentially involved in the stabilization of triple helix and hence hardly available for hydrogen bonding with other substances.

2.2.4. Levels of order of collagen

Primary structure: primary structure of collagen is manifested by the amino acid profile of each of the three α-chains. The triple helical regions of collagen's chains are built of the repeating unit or triplet, Gly-X-Y, where precisely 1050 amino acids contained in each chains. Next to glycine, proline and hydroxyproline are the most abundant residues accounting 15-30% of amino acids in the sequence.

Secondary structure: It refers to the local configuration of polypeptide chains where the stereo-chemical features of residues in the chains are the determining factors. The rigid in the structure of proline and hydroxyproline residues in the X and Y positions, respectively, provides the rigidity to the collagen structure besides their functional properties. They also prevent the formation of α-helical chain segments. The flexibility (smaller size) of glycine and rigidity of the imino acids all account for the formation of right handed triple helix with the length of 300nm. The occurrence of glycine residue in almost every third position appears to confer a strong propensity for inter-chain, rather than intra-chain hydrogen bonding. There are many stabilizing forces in collagen molecule among which hydrogen bonding, hydrophobic interactions, covalent bonds and salt bridge are prominent ones. Therefore, the three alpha strands form left handed helical conformation which is regarded as secondary structure.

Tertiary structure: The tertiary structure of collagen molecules deals with three dimensional arrangement of atoms in a molecule. The left hand coiled α-strands are entwisted about each other to in a common axis thereby giving supramolecular or coiled coil called right handed triple helix. The triple helix has a molecular weight of 300,000 Daltons per mole. It is stabilized by a number of short-range covalent and non-covalent interactions.

Quaternary structure: It deals with the interactions and arrangement of collagen molecules thereby forming insoluble higher level micro fibril. Five tropocollagen molecules are longitudinally inter-twined in helical twist and hence are staggered by roughly one-fourth their length of the fibre axis[17]. In a" quarter stagger", at least two lateral cross-links are formed between the neighboring collagen molecules form N- and C-termini[11]. In skin collagen, particularly from older animals, it's believed that additional linkages between two molecules emerge from N- and C-termini themselves.

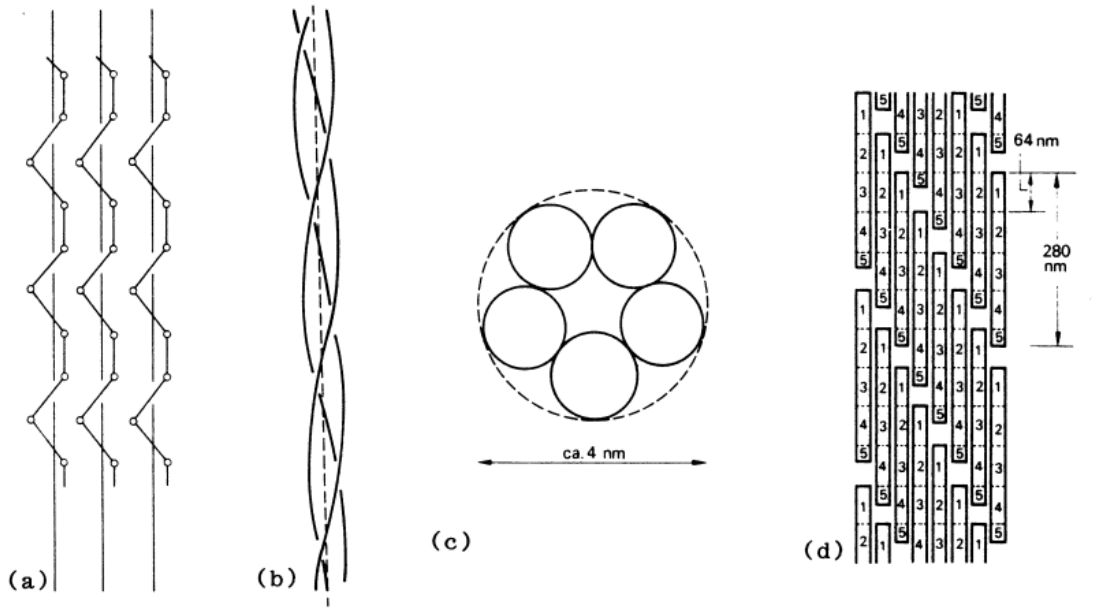


Figure 2.3. Building blocks of collagen fibril

Three polypeptides chains b) three polypeptide chains coiled about common major axis giving triple helix c) five tropocollagen molecules aggregate to form 4nm thick microfibril d) longitudinal staggering of tropocollagen helices along the length of microfibril

2.2.5. The (hydro) thermal stability of collagen

Contraction of the collagen fibre is primarily believed to be attributed to the weakening of interactions crosslinking the fibres. Increasing the kinetic energy of polypeptide chains (through applying heat) and lyotropic swelling are means which contribute mainly to the disruption of the interactions among fibres. A direct cause-and-effect relationship exists between swelling and Ts where an increase in swelling reduces the Ts and vice versa.

In measuring the shrinkage of collagen fibre, the medium in which it has been done would considerably matters. In a more general way, it was shown that raising the dielectric constant of the medium lowered Ts as the salt links thought to be affected by the dielectric constant of the medium. For instance, heat-shrunken collagen fibres in 80% ethanol and fully hydrated with water observed to have average Ts values of 78 and 63^0c, respectively[18]. It would also fair enough to draw the fact that Ts would vary significantly for both moisture free and hydrated collagen fibre. Since long time to most recent studies, it has been validated and established that the Ts is dependent up on the water content of the collagen or leathers samples[13] and the relation of such is shown in table 2.2.

Table 2.2. Relation between the shrinkage temperature (Ts) and the water content

Substrate	Percentage water content	Ts in ^{0}c
Collagen	4	155
	19	121
	30	100
	98	61
	151	61

The type and concentration of electrolytes (acids, bases, salts) and hydrotropes present in fact also have considerable influence on the Ts. Completely shrunken collagen, being losing its orderly structure, can be re-extended to its original length though the low-angle pattern doesn't reappear revealing that the long-range structural alteration in the thermal shrinkage of collagen is irreversible[19] .It has also been proved that Ts values of both alkali and acid swollen collagen is less than its native counterpart. Same holds true for limed, delimed and washed one. Considering the low Ts of limed skin, the following could be taken as possible aspects for considerable distortion in collagen structure[11]:

- Chain splitting
- Formation of isopeptides involving asparagine and glutamine residues and
- Recemization of D-amino acids as a result of hydrogen abstraction due to alkali

According to Borasky and Nutting[20], the Ts of skin, hide and leather can be determined using microscope where the melting stage of the collagen on the microscope was proved to be same as those obtained on gross piece. The shrinkage temperature of animal skin for leather making were studied at different beamhouse and tanning unit operations. It was pointed out in the early studies that the hydrothermal stability at extreme pH conditions (ranging 1 to 4 and greater than 12) was much lower than at its native state. Osmotic swelling and lyotropic swelling were believed to disrupt hydrogen-bond type of linkage thereby lowering the hydrothermal stability of the skin during liming and pickling in the absence of salt addition. However, acidified pelt in brine solution would have better thermal stability due to the dehydration effect of common salt. In the work of Theis (1941)[21] on the shrinkage temperature of pickled skin at pH 3.0 (H_2SO_4) with 0, 5.85, 11.75 and 17.6% NaCl addition, the corresponding Ts (^{o}c) was shown to be 48, 62.4, 71.0, and 75, respectively, manifesting the effect of common salt. Within the pH

ranges of 4.0 to 10.0, the shrinkage temperature of collagen was essentially found to be constant (55 to 57^{o}C) by treating with acid or alkali in the absence of common salt.

Studies also revealed that the thermal denaturation of collagen considerably depends on its water content and cross-linking degree besides the nature of medium particularly pH[22-24]. Fairly enough, an increase in the degree of cross-linking and dehydration substantially enhance the helix-coil transition temperature of collagen for the latter believed to lead exceptional increase in collagen polypeptide chains adhering. Increase in inter-and intra-chain hydrogen bods would considerably back the polypeptide adherence. Less flexibility of collagen polypeptide is commonly accompanied by higher thermal stability of same.

2.2.6. Collagen hydrolysate (CH)

Collagen hydrolysate is a polypeptide composite made by further hydrolysis of denatured collagen. Untanned and tanned skin/hide wastes or scraps are common sources of collagen hydrolysate. It has been pointed out in recent study that only about 20% of collagen was made utilized to useable grain leather in the process of tanning raw hide with the rest, if not converted to useful splits, remained waste[25].

Alkaline hydrolysis, acid hydrolysis either alone or in combination with enzymatic hydrolysis are known methods being used for the production of collagen hydrolysate from skin/hide wastes. Concentration and choice of hydrolyzing material or medium and the duration of hydrolysis are the main factors governing collagen hydrolysis. In general, prolonging hydrolysis time increases the concentration and reduces the molecular weight of collagen hydrolysate. Enzymatic hydrolysis method usually provides hydrolysate having only limited lower limit molecular weight and with relatively uniform molecular weight component. Much smaller molecular weight collagen components were not expected using enzymatic hydrolysis as each enzymes do have specific site for attack. Depending up on the extent to which the hydrolysis conditions are controlled, alkaline and acid hydrolysis followed by enzymatic one would normally results in smaller and uniform molecular weight of collagen hydrolysate.

Alkaline hydrolysis under thermal conditions commonly results in smaller molecular weight than acid hydrolysis but with the nature of functional groups being varied in both cases. For instance, temperature supported alkaline hydrolysis result in higher density of carboxylic groups as the side amide groups believed to be hydrolyzed under such preparative conditions. That means, all amino acids containing hydroxyl and sulphydryl are destroyed by alkali and the final products are racemized which in most cases makes such collage hydrolysate less

suitable for edible or medical purpose. On the other hand, side amide residues remain intact during acid hydrolysis and hence is the reason why the iso-electric point of acid hydrolyzed collagen would relatively be in the basic pH range. In most cases, the rapid reduction in the size of the hydrolysate is observed to be in the early period of hydrolysis after certain initial time. Alkali impregnation is commonly employed when collagen hydrolysate is to be produced from chrome tanned leather wastes. Whenever purity and molecular level is required, acid (Acetic acid) hydrolysis followed by pepsin digestion under controlled condition for collagen extraction from skin is commonly practiced. Pepsin effectively enhances the yield of collagen as it can break down cross-linkages in the telopeptide regions of collagen without harming its secondary structure in such situations.

It has been reported that molecular weight of unmodified CH is relatively small. It also possesses exposed more hydrophilic groups and less hydrophobic groups. This in fact renders the less solubility with oil. In other words, oil absorption ability of CH increases with molecular weight as augmented by recent study[26]. It is fair enough to assert that water retention ability of CH reduces as the molecular weight increases and hence lower molecular weight CH tend to possess more hydrophilic group by same logical reasoning. The low molecular weight of CH, particularly from chrome shavings, deters its functional properties as oil absorption, emulsification capacity and stability.

Modifying CH from such sources may indeed changes some of its functional properties. CH is also known for its higher water absorption. The increase in molecular weight of CH may be accompanied by presence of more hydrophobic groups during its chemical modification. Presence of some innate moisture-keeping species, such as glycine, alanine, asparagine and serine would reflect the moisturizing ability of CH which hardly is influenced by the change in the environmental conditions.

Collagen hydrolysate has better biological compatibility and degradability than other synthetic macromolecular substances. The economic relevance of CH in the field of food, medicine, cosmetics, animal feed, fertilizer and other industrial applications is increasing. The large percentage of residual collagen is mainly used for the production of edible collage casings, gelatin and in the areas of medicine for biomedical application like for drug delivery system using well purified collagen. The manufacture of these collagen derived products has been commonly easy but difficulty arises only when chrome leather wastes like shavings are taken into account. In the production of surfactants, commercially available collagen hydrolysates

are believed to render complication due to their high average molecular weights which lead to lower solubility of final product. Being as one of ample sources of nitrogen, collagen hydrolysate recalls considerable interest in filed or agriculture particularly in the production of organic fertilizers. The exploitation of collagen hydrolysates as biostimulators or modifiers for enhancing plant immunity is also an upcoming area of interest in the field of agriculture.

Molecular weight and molecular weight distributions of collagen hydrolysate believed to play considerable role in determining the application areas of collagen hydrolysate. Unlike discrete molecules which are having well-defined molecular weights, polymers are composed of hundreds to thousands of chains of different molecular weights that result in characteristic molecular weight distribution. The molecular weight distribution depends on the sources and methods of isolation for natural polymers as kinetics, conditions and method of preparation do for syntans. Molecular weight distribution is described in most cases by number average and weight average molecular weight. The magnitude of number average molecular weight is sensitive to the presence of low molecular weight components or species and whereas weight average molecular weight indicates changes in high molecular weight components.

Collagen hydrolysate obtained from leather wastes (shavings, trimmings and fleshings) can be used as filler in post-tanning operations besides its collagen active group supplemental. Hydrolyzing fleshings and pelt trimmings gives collagen hydrolysate (unmodified) and oily paste like material. Though not backed with reliable elucidation, unmodified CH was also shown to have no tanning potency but impart effect on the leather when used during fatliquoring[27]. The application of hydrolyzed collagen from cowhide waste trimmings was also studied during leather dyeing and revealed that the synergistic effect of hydrolyzed collagen was evident but depend on the pH[12]. The presence of many hydrophilic reactive groups viz. amino ($-NH_2$), carboxyl (-COOH), imino (-NH-), and hydroxyl (-OH). The use of collagen hydrolysate in modifying polyurethane finishing agent was also studied and reported[28].

2.3. Non-collagenous skin matrix constituent removal during leather processing

In the beamhouse yard of conventional leather processing, the main underlying aim is to purify skin matrix to be converted to leather. It thus, involves the removal of unwanted skin matrix constituents with the existing leather processing technology. The unwanted skin matrix constituents include interfibrilary materials (i.e. proteins, proteoglycans, glycosaminoglycans (GAGs)) and majority of natural fats in the adipose later. Proteoglycans are complex and multifunctional molecules consisting of a protein with variable number of covalently attached

poly saccharide carbohydrate side chains GAGs. The removal of such unwanted materials escalates the understanding of the corresponding leather processing unit operations and undoubtedly paves ways for better alternative technologies.

Quantitative analysis of these skin matrix constituents has been made on goat skin[27]. Augmented by same[29], the removal of inter-fibrillar materials are mainly during liming and bating operations. Similarly, amount of decorin (pre-dominant & well-understood proteoglycan of skin) removed during beamhouse operation in shoulder, butt and belly regions of hide has been studied and the removal decreases in that order[30]. It was also pointed out that the efficient removal of proteoglycans from hide enhances final leather flexibility and softness. The amount of decorin removed during dehairing doesn't change much in succeeding tanning operations that believed to remove GAGs. The quantification study on the parts of same skin during conventional beamhouse considerably provides further information as the variation among parts of skin which in deed is one of focal point of this work.

Based on the dry weight of skin, the approximate content of fats varies from 0.5 to 4.0% in calf skins, cattle and horse hides; from 3.0 to 30.0% in sheep skins; from 4.0 to 40.0% in pig skins and from 300 to 10.0% in goat skins and of which 75-80% are believed to be triacylglycerol with the balance being waxes and composed of lipids[31]. Among animal skin's being consumed for tanning industry, sheep believed to constitute higher percentage of fat content and the content vary depending on the breed type. The nature of natural fat in the skin makes difficult to remove because of the presence of cerides and a high melting temperature. From the view point of tanning chemistry, fat in skin is a component rendering flexibility, softness and stability.

2.4. Leather Processing

Skin is considered as a semi-permeable membrane in that it only permeable to selective chemicals. Thus, nature of substrate(as to charge, thickness, porosity, etc.), nature of treatment chemicals with process bath condition, mechanical action and duration of processing all matters in governing the penetration and binding treatment chemicals to render the desired properties in the course of leather processing.

Many research studies have been tried to shorten the leather processing steps from overall economic point of view. A three steps tanning techniques have shown to reduce cost incurred as a result of chemical consumption and waste treatment in conventional processes[32]. Having biomaterials of complex histological architecture as an input, leather science and technology is

prone to further study besides the integral environmental concern accompanied tanning industry. In the leather making processes, each preceding unit operation affects all the subsequent operations. Therefore, the sequence of unit operations designed should have subtle purpose on the view of achieving what properties has already been desired. Conventional leather processing steps is addressed concisely hereunder.

2.4.1. Pre-tanning or Beam house operations

Before commencing the first chemicals based treatment called soaking, curing of the raw hides or skins is a crucial step in that they are temporarily preserved so as to avoid or arrest any damage made to the material. Depending on the availability of curing material and the nature of raw material sourcing, different preservation techniques can be employed. Wet salted preservation is the most common type of preservation being practiced. The use of common salt on the other hand has problem in raising total dissolved solids (TDS) in the effluent unless method to recover or reduce the application of it for preservation is taken care. The following are regarded as pre-tanning operations.

a) Soaking

The aim of soaking is to make the raw material regain the moisture content and remove dirt matter, curing material & some of non-fibrous proteins. When environmental concern coming into view, a major portion of TDS in leather processing is contributed by common salt (sodium chloride) coming into the effluent during soaking of salt preserved raw hides and skins. And therefore, the salt discharged into the effluent during the soaking operation is the greatest environmental problem in the tanneries. The degree to which soaking operations is attained has considerable effect on the forthcoming unit operations.

b) Liming

Liming is the second chemical based pre-tanning operation where hair is loosened and removed using lime and other strong alkaline chemicals like sodium sulphide and sodium hydrogen sulphide. Proper & controlled fiber opening and complete hair removal/loosening needs to be maintained at this stage of operation. The degree of fiber opening or swelling, in fact, depends on the type of final leather desired to be made. If for instance, full and soft leather is required, proper fibre opening up (through re-liming) would aid in realizing such properties. During tanning, the thin part of skin better be tanned and filled with tanning materials if optimum fiber opening is ensured at liming stage. It is also worth noticing that re-liming should be in a controlled way as over re-liming may result in poor leather which can be difficult to reverse in

subsequent operations. The emission load attributed to liming effluent is considerably high particularly in terms of chemical oxygen demand (COD) and solid sludge.

Non-structured proteins, pigments, fats to certain, etc. can be removed at this stage of process, too. Decorin is particularly believed to be removed due to harsh alkaline conditions and indirectly ascribes the role of liming operation in affecting final leather to be produced. To reduce, the toxicity of sodium sulphide and salinity & TDS value of lime in the general effluent, enzymatic unhairing in principle can also be possible though its practicability by tanners is less.

c) Deliming

After fleshing and splitting (if required at pelt stage), the alkalinity of the pelt should slowly be brought down so that the pelt made to be at suitable condition in subsequent operations for tanning. This can be achieved by using acidic salts. The extent of through deliming sometimes is adjusted by the tanner depending on the type of leather intended to be made out of the pelt. For firm, tight and slight heavy upper leather, the deliming usually will not be through and through. It is mainly reversing the action of liming operation with regards to alkalinity and suit the material for tanning.

d) Bating

In bating, alkaline proteolitic enzymes are used to digest and remove unwanted protein matter like, short hair, pigment, elastin, etc. so that the pelt become porous for better penetration of chemicals in the next subsequent operations. Relatively, bating would help in softness, grain smoothening and flattening. The degree of bating is considerably dependent based on the nature of input material and the desire of leather intended to come out of it.

e) Degreasing

A considerable amount of fat in raw skins makes their processing difficult for the hydrophobic spaces are then formed thereby repelling water during soaking and forming insoluble calcium soap during liming. Raw skin containing much fat content have to be degreased before further processing. Therefore, the objective of degreasing is to remove and distribute the natural fat in the skin. Patchy defects associated with the fat and non-uniformity in the uptake of chemicals in the succeeding unit operations will be minimized or avoided through controlled degreasing operation. Surface active agents with de-fatting power are commonly used for same in the tannery. Skins which thought to have high fat content (especially sheep skin) require degreasing operation and thus the extent of degreasing depends up the nature of raw material being used.

f) Pickling

Pickling is a stage where the bated and degreased pelt (if required depending on the nature of input material for fat accusation) is made treated either with organic or inorganic or combination of the acids for proper penetration of tanning materials under consideration. The final pH of pelt and bath to be attained is determined as to what type of material for tanning is aimed to be used. For instance, pickle pH is kept to 2.8/3.0 for chrome tanning whereas a little bit higher pH values can be used for vegetable tanning. Pickling can also be used for preservation purpose if kept at a lower pH values. De-pickling followed by re-pickling operations are commonly undertaken whenever pickled pelts are primarily meant for preservation and is customarily happened for sheep skin. From the view point of environment, pickling bath impose considerable TDS to the effluent which triggered many researchers to seek various ways of addressing pickling process.

2.4.2. Tanning

Tanning is one of the process stages in leather making where the putrescible skin material made imparted a property imputrescible using tanning materials. Tanning operation is only one stage in the transformation of collagen into leather and remains an important one. It is after this stage that the pelt is termed as leather. As a result of tanning, the hydrothermal stability and enzymatic stability of the material will be enhanced significantly.

Mineral tanning and organic tanning materials are the two main methods for converting raw skins/hides to leather. Basic salts of Chromium (Cr), Aluminum (Al), Zirconium (Zr), Titanium (Ti) and Iron (Fe) have good tanning power amongst which basic chromium sulphate is the best mineral tanning material so far used. Basic chromium sulphate has good quality in that versatile leather types can be made of chrome tanned blue leather. The fact that chromium (III) salts have the ability to coordinate with carboxyl groups of skin/hide collagen and form crosslinking between collagen fibres were first unraveled by Gustavson et al in 1924. Based on this, Covington et al alter proved that the crosslinking and coordinating reactions mostly mainly occurred between chromium (III) and the jutting carboxyl groups of aspartate and glutamate where the former tended to form bi-point bound chromium complexes while the later revealed a tendency to form uni-point chromium complexes[33]. Vegetable tannins, syntans, aldehydes and oils (fish) are among the class of organic tanning materials. Formaldehyde was found to bring maximum thermal and enzymatic stability among aldehydes tanning agents[34]. Vegetable tannins are commonly used tanning materials giving firm and tight leather types.

Using single tanning material alone cannot achieve the required physico-chimerical properties of leather. Hence, combination of different tanning chemicals can be used to impart the required properties to the leather. For upper leather production, fullness, firmness (degree would vary), roundness, thickness and shade uniformity are amongst the many properties required to be addressed.

2.4.3. Post tanning

As stated above, every tanning material if used alone cannot bring specific properties pertinent to the final leather desired to be made. Some of physical and chemical properties of the leather need to be met and adjusted in carrying post tanning operation. Softness, tightness, coloring, fullness, etc. are all made modified and met using different post tanning chemicals. Skin being containing bio-chemical of exhibiting zwitterion, the relative surface charge of the tanned leather and the nature of post-tanning chemicals to be used determines the end property of the leather besides the effects attributed to the order of addition of chemicals. Therefore, re-tanning and dyeing in particular require carefully designed post-tanning units operations with the right selection of treatment chemicals.

a) Wetting back

Wetting back is the first post tanning operation aiming at to rehydrate the material and washout the contaminants or suspended/unreacted chemicals. In case of pastel shades leather weak acids like acetic or oxalic can be used to remove unbound chrome, chrome stains for long stored wet blues.

b) Re-chroming

It is a pre-processing, before neutralization, so as to aid fine and tight grain as well as deeper shades during dyeing. Basic chrome sulphate and/or syntans of mineral complexes of Cr, Al and Zr are used. Soft and perspiration resistance properties and lubricating effects are also rendered to the leather. Finally the leather is neutralized to pH 3.8/4.0 to aid fixing the mineral tanning materials.

c) **Neutralizatio**n

It is basically aimed to de-acidify (free acids, unbound basic chrome and acids associated with) mineral tanned leather with mild neutralizing salts and syntans. The level of neutralization is, in fact, depends on the type of property the final leather needs to possess. If for instance, softer, level dyed, well lubricated, fuller & round inner fibers are required, through neutralization is a must. Upper leathers usually require medium neutralization (pH 4.8/5.4).As most of re-tanning

materials are anionic in nature, more penetration will be happened at higher pH. If the neutralization pH is lower, the penetration of fat as emulsion will be less possible rather fat emulsion will break giving oils on the surface.

d) Re-tanning

It is a second tanning process followed by first tannage, regardless of the fact that the first tannage is only slight pre-tannage, and is termed as re-tanning. This operation is mainly and generally aimed at to impart the final leather the desired physical and chemical properties. For instance, more filling affects the thickness and weight of the leather. And hence, garment leathers and dress glove leathers don't require much fullness and heavy weight.

Generally re-tanning is a subsequent leather treatment with different tanning agents, following the main tannage (first tannage) in order to give the leather special and optimum property for use. It is a process where combination tannage mainly practiced to impart the leather the required final physico-chemical properties. Different syntans are commonly used where smooth, silky touch, fine and tight grain properties and fullness properties are improved depending on the tanners' preference for the final leather. if fine, smooth, tight, silky grain leather is required resin tanning agents play main role where as if fullness especially in the void area (preferentially a layer between grain and corium junction), acrylic syntans play their part in so doing basically solving break problems. The selective preference of acrylic syntans for compact part of the skin and melamine-aldehyde based resins for belly part has been due to the structural nature of parts of skins. Resin tanning materials, especially melamine formaldehyde, have in general filling power in void areas, preferentially in belly areas. Vegetable re-tanning of chrome blues is commonly practiced for most upper leathers. Protein fillers are commonly used whenever full and even fiber tightness is required.

Leather properties like fullness, grain tightness, softness, degree of shade/dye levelness, color, grain break, buffing properties (short/dense nap, fuzzy or velvety nap), print retention, bulk properties (tensile strength, tear strength, grain distention, substance, etc.), wear properties (color fastness to water/light/perspiration, heat resistance, cold resistance, etc), comfort properties (breathability/water proofness), surface properties (adhesion strength, flexing, grain fineness & smoothness, gloss, etc) mainly dependent on re-tanning operation.

e) Dyeing

As stated above the color is one property desired by the customer and this can be realized using colorants where dyes are main colorant being used at this stage. Dyes are complex, natural or

synthetic, inorganic or organic solvent soluble colored compounds having the properties of imparting their permanent color to other substrates collagen, wool & cotton, etc. fibres.

Dyeing is mainly to improve aesthetic appeal or appearance and make the leather adaptable to fashion style. Dyes can be categorized as *acid, direct (substantive), mordant, and reactive* according to the dyeing behavior towards natural or synthetic fibers. All are either cationic or anionic in nature and acid dyes are the main dyestuffs being utilized by tanning industry. The kind of re-tanning materials being used have impact on the penetration and depth of shade. Thus, right combination of re-tanning materials and dyes should be taken into account. The more phenolic type syntans are being used in re-tanning, the lighter the color shade will be. Dyes can be installed the form of powder, paste or solution. The study on chemical and physical interactions involving leather dyeing revealed that the exact mechanism may involve the interaction of the dye molecules with the reactive functional groups collagen or collagen-tanning agent complex or both[35].

f) Fatliquoring

It is the process of coating surface of fibres (including the interior fibres for soft leathers) of leather with a tinny layer of oils/fats or oil-in-water emulsifiable oils. It gives the leather the desired softness to avoid hardening due to drying. It helps in reducing the density of leather through separation of fibres. Bulk, wear and comfort properties of the leather are also affected as a result of Fatliquoring. Depending on the nature of desired final leather, the amount and types of fatliquors could vary. That is why combination of different fatliquors would normally be employed. For instance, neat's foot oil, lecithin and synthetic vegetable oils are commonly used in shoe upper leather production.

2.4.4. Crust preparation

It involves series of mechanical operations where the moisture level of wet leather brought down and physical properties are altered. The choice and sequence of mechanical operations depends on the properties desired to be imparted to the leather. Before the leathers are taken to finishing, they are well sorted for the quality after which what finishing techniques to be used.

2.4.5. Finishing

The term 'finishing', here, refers to the further processing of leather after the drying operations. It is the last and the most important operation in the tannery as it is the process of adding value to the final leather through improving the surface appearance so much that the leather becomes so attractive to look, to feel and ultimately to upgrade it. These properties are, of course, the

result of chemistry and mechanics modification of the superficial aspects and the properties of the skin; thanks to the anchorage to the derma of a polymeric films and colorants with the aesthetic and covering effects. Truly speaking, any film covering made to the grain surface of the leather negatively affects the natural aesthetic feel of the leather. Thus, finishing is a compromise between covering surface defects through applying finishing chemicals and loosing leather's natural aesthetic feel.

Finishing consists of mechanically softening, smoothening, glossing grooving etc. and chemically covering, coloring, matting/glossing, softening etc. operations of the leather surface. For the latter case, the wide variety of coloring dyes, pigments, binders, waxes, lacquers etc. are applied to leathers which are known as finishes. Colorants (pigments, dye solution), binders, lacquer, handle modifiers, waxes, penetrator and fillers are main finishing chemicals to be used. Based on the type and amount of finishes applied to leather, finishing can be categorized as aniline, semi-aniline, and pigmented/opaque finishes where the higher grade leathers go for aniline finish and the lower end for pigmented finish.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Raw materials and Chemicals

3.1.1.1. Raw Material

Wet salted hair sheep skins were procured from Addis Ababa Abattoirs and skin parts of interest prepared and used as indicated in Figure 3.2. Collagen hydrolysate being prepared from limed pelt trimmings under alkaline-thermal condition was used to investigate the enhancement of protein content in both belly and butt regions of SS.

3.1.1.2. Chemicals

The chemicals used in conventional leather processing (soaking to wet post-tanning) were of commercial grade.

Laboratory Analysis Chemicals:

All chemicals and reagents used for laboratory experiments were of analytical grade. The list of chemicals used were Periodic Acid, Acetic acid (Glacial), Schiff reagent, Mucin, Coomasie blue G, Ethanol, Phosphoric acid (85%, w/v), Bovine serum albumin (BSA), Sulphuric acid, Nitric acid, Perchloric acid, Sodium hydroxide (both flake & solution), Diethyl ether, Sodium chloride, Hydrochloric acid, Ammonium sulphate, Di-Sodium hydrogen phosphate, Mono-Sodium hydrogen phosphate, EDTA, Potassium chloride, Potassium hydrogen phosphate, Formaldehyde solution, Glutaraldehyde solution, Xylene, Paraffin wax, Hematoxylin, Eosin, L-Hydroxyproline (standard), Sodium p-Toluene sulfox chloramide (Chloramine-T), Methyl cellulose, p-Dimethyl amino benzaldehyde (pDAB), Sodium dodecyl sulphate (SDS), Acrylamide, N,N, Methylene bis-acrylamide, Glycerol, Glycine, Tris-Buffer, Ammonium per sulphate (APS), NNN-Tetramethylethylene diamine (NNN-TEMED), β-mercaptoethanol, iso-Butanol, Bromophenol, Methanol, Boric acid, Ammonium nitrate, Sucrose, Copper sulphate (anhydrous), Potassium sulphate (anhydrous), Methyl red, Methyl blue and phenolphthalein, Potassium dichromate, Silver sulphate, 1,10-phenanthroline monohydrate, Iron sulphate (hepta-hydrate), Ferrous ammonium sulphate (hexa hydrate), Mercuric sulphate, Sulfamic acid, Potassium hydrogen phthalate,

3.1.2. Equipments and Apparatuses

Equipment and apparatuses in this work includes leather processing equipments and laboratory instrument & apparatuses as listed below.

3.1.2.1. Leather Processing Equipments

Equipments and apparatus used in the course of leather processing and related works were sample, stainless steel ruler, surgical blade, drum, fleshing machine, shaving machine, sam-setting machine, vibratory staking, rotary staking, weighing balance and graduated cylinder or graduated beakers.

3.1.2.2. Laboratory Instruments and Apparatuses

Instruments, equipment and apparatus used during laboratory analysis were: Analytical weighing balance, sampling tubes, centrifuge tubes, Glass Plate, Filter papers, Measuring cylinders, Refrigerator, Thermometer, Desiccator, Water bath shaker, Burettes, Micropipettes and its tips, Heating mantle, Soxlet apparatus, Hot air heating oven, Microtone, Compound Light microscope, Scanning electron microscope (SEM), Circular dichroic spectropolarimeter, Thermal analyzer, Centrifuge, UV-Vis Spectrophotometer, Dynamic light scattering (DLS) spectrometer, TECHKON SpectroDrive, SDS-PAGE set up, Autoclave, Kjeldahl apparatus (digestion & distillation units), Kjeldahl digestion and distillation glass tubes, Beakers, Standard measuring flask, Membrane tubes, Borosil test tubes, Funnels (glass & Buchner), Crucible, Vacuum pump, Surgical blade, Dynamometer (tensile, tear and elongation at break tester), Glass-fiber filter disks, Membrane filter funnel, Gooch crucible, Blender, Thickness gauge.

Description of major instruments used:

- **Extraction Unit (E-816 Soxhlet)**

  Extraction Unit E-816 Soxhlet is an automated Soxhlet system used to extract non-volatile compound (fat) from analyte under study. It follows the original principle of Soxhlet extraction & the specific extraction chamber being invented by Franz Soxhlet (1879). The extraction has three main steps: extraction (heating up the solvent), rinsing (washing glass sample tubes) and drying (drying solvent remains in the beakers). Being an automated system, *E-816 Soxhlet* has an optical level sensor instead of a siphon which allows to execute more cycles per hour and thus makes the extraction more efficient and faster.

- **Ultra Microtome**

  Ultra Microtome is a microtome capable of producing very fine slices of tissue or sections for examining under compound light microscope. In this work, Rotary Ultra Microtome is used to prepare skin samples for executing specimens cross sectional analysis under the microscope after staining specimens.

- **Compound Light Microscope**

  After staining, thinner specimens or sections of skin from various process stages were examined for their cross sectional or morphological features. SDS-PAGE imaging for extracted collagen was also executed using same instrument.

- **Scanning Electron Microscope (SEM)**

  Surfaces and cross section of skin/leather samples from various process stages were examined using SEM at different magnification power. The examination could yield information about the topography (surface features) and morphology (shape and size of cross-sectional constituents) of skin and leather samples. *Hitachi Scanning Electron Microscope* at high vacuum with accelerating voltage of 12kV in varying magnifications was used to obtain the micrographs of belly and butt parts of skin & leather samples.

- **Sputter Coater**

  As the SEM utilizes vacuum conditions and uses electrons to form an image, special preparations must be made to the samples where their water content is removed and the specimens/samples to be examined made conductive by converting them with a thin layer of conductive materials such as Au, Pt, Pd, their alloys, as well as carbon. This can be done using a device called "sputter coater". In this work, samples of skin/leather of interest were coated with gold using *Edwards E306 sputter coater* following protocol stated afterwards.

- **Hot air Oven**

  It is used to dry the material of interest at the required temperature. Moisture content determination, preparation of analyte (as appropriate), total dissolved solids, and drying of non-volumetric glass wares are handled by heating oven.

- **Circular Dichroic (CD) Spectropolarimeter**

  Circular dichroic spectropolarimeter is a non-destructive scientific instrument used to provide information about the secondary structure of proteins and polypeptides in solution. In CD, equal amounts of circularly polarized light (CPL) of selected wavelength both to the left and to the right, alternatively pass through a sample and the difference in absorption of the components of left and right is measured. In the far UV region (240-180 nm), which corresponds to peptide bond absorption, the CD spectrum can be analyzed to give the contents of regular secondary structures such as α-helix and β-sheet[36]. Conformations of both Acid Soluble Collagen and Pepsin Soluble Collagen being extracted from both parts of hair sheep skin were studied using dichroic

spectroscopy measurements conducted on a *JASCO J-815 CD Spectroscopy* equipped with a Peliter temperature control-*423S/15 (JASCO Inc.)*.

- **Thermal Analyzer**

  Thermal Analyzer is an instrument which enable to study the physical property of analyte under study as a function of temperature, while the sample is subjected to a controlled temperature program (heating, cooling or isothermal). Pores size distribution was determined by measuring the amount of water that has its melting temperature depressed at each step. The enthalpies associated with corresponding each step in 0.1K are used to calculate the pore radii. After further manipulation with volumetric latent heat, graph was finally plotted for pore volume distribution versus pore radius to compare the changes in pore structure in course of leather processing. In this work, thermal stability behavior and pore size distribution of skins/leather samples being collected from various leather processing stages were analyzed with Thermal analyzer under differential scanning calorimetric technique using *DSC-Q200 TA* Differential Scanning Calorimeter.

- **UV-Vis Spectrophotometer**

  Estimating quantum of extracted collagen through measurement of hydroxyproline content, non-collagenous proteins (Globulin, proteoglycan, Reticulin, Elastin) and Chromic oxide in the wet blue were executed using *Thermo SCIENTIFIC EVOLUTION 300 UV-VIS* spectrophotometer at the suitable wave lengths. Standard absorbance curve is used to estimate the concentration of the desired analyte if the analyte or species is not having molar extinction coefficient at the specified wavelength.

- **Dynamic Light scattering (DLS) Spectrometer**

  The basic working principle of DLS is that the sample is illuminated by a laser beam and the fluctuations of scattered light are detected at a known scattering angle by a fast photon detector. The hydrodynamic radii of particles of CH at four different pH values in aqueous solution were analyzed using DLS with high performance particle sizer (*Zetasizer Nano series, Malvern*) at 25^{o}C operating at 4 mW He-Ne laser power, scattering angle of 175^{o} and wavelength of 633 nm.

  Zeta potential (ZP) of CH at four different pH value was found from electrophoretic mobility using the Smulochowski model approximation which is best fitted most commonly in aqueous media and moderate electrolyte solution. Similarly, ZP of CH at four different pH values was also obtained with (*Zeta Nanoseries, Malvern)* from electrophoretic nobilities using the indicated model

- **TECHKON SpectroDrive**

  TECHKON SpectroDrive is a scientific instrument which has a capability of automatic color and density measurement. SpectroDrive can be sued as a scanning device as well as hand-held instrument for single measurements. It is equipped with *TECHKON ExPresso 3* software which offers a comprehensive tool-set comprising measurement of spot colors, support of up to 16 print units, face and reverse-printing, display of color density, dot gain, gray balance, CIE L*a*b*-values and recommendations on how to adjust the ink on the press. CIE L*a*b* values for dyed Crust leather samples were measured and ΔE was also calculated by the software which enabled to investigate the color difference between reference (treated with commercial filler) and experimental (treated with collagen hydrolysate) dyed crust samples.

- **SDS-PAGE Setup**

  The acronym SDS-PAGE represents Sodium dodecyl sulphate poly acrylamide gel electrophoresis. SDS-PAGE setup is a known method for separating proteins by electrophoresis using polyacrylamide gel as a support medium and Sodium dodecyl sulphate (SDS) to denature proteins which is also called the Laemmli method. Protein separations by SDS-PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample, and to determine the distribution of proteins among fractions. Protein markers of known molecular mass are used against which the distribution of proteins among mass fractions are examined after imaging is executed under the microscope. In this work, secondary structure of extracted collagen by acid soluble and pepsin soluble method was examined using SDS-PAGE.

- **Centrifuge machine**

  In principle, the centrifuge machine is a kind of separating machine which is used to separate the useful components in mixtures of liquids and solids or liquids and liquids by applying centrifugal force. The principle of centrifuge is divided into centrifugal filtering and centrifugal sedimentation. Depending on the particular application, centrifuges differ in their overall design and size. However, the common features in all centrifuges is the central motor that spins a rotor containing the samples to be separated. In this work, high speed refrigerated centrifuge was used to purify collagen being extracted from the two parts of hair sheep skin as it provides high speed and temperature control.

- **Autoclaving machine**

  Autoclave is a machine used for sterilizations and protein hydrolysate preparation. *TOMY autoclaves E-215* was used for collagen hydrolysate preparation. It has a microprocessor controlled monitoring system which provides safe sterilization, warming and heating. A digital control panel display enables easy operating parameter setting and real time monitoring of operation status. The three mode programs viz. sterilization mode, sterilization mode and heating mode made easy preparation of culture media and hydrolysate. Collagen hydrolysate for use in the leather processing was made by dissolution of completely delimed pelt trimmings under alkaline condition in *TOMY autoclaves E-215* with the sterilization and heating operation mode at desired temperature and time period.

- **Kjeldahl apparatus**

  Kjeldahl apparatus, named after the inventor of the method Johann Kjeldahl, is an apparatus used for determination of nitrogen. In the thermal catalytic digestion process, the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the digested solution liberates ammonia which is quantitatively steam-distilled and determined by titration using mineral acids. In this work, Kjeldahl apparatus consists of two main units, *BUCHI Digest system K-437* and *VELP Automatic distillation unit* were used for percent nitrogen and crude nitrogenous substance in the CH, CPF and leather samples.

- **Lastometer**

  Lastometer is an instrument which used to determine the grain crack force and distension of leather when used for shoe upper. It consists of a 6.25 mm spherical steel ball head rod to press from flesh side leather to produce distension and two circular rings with 44 mm external diameter and 25 mm internal diameter to secure test specimen not to slip during test.

- **Dynamometer**

  It is a tensile strength testing machine used of physical testing of leather generally working in tension method. Tensile, elongation and tear strength of the leather were measured using *Digital dynamometer IG/DE S.*

a) Ultra microtome

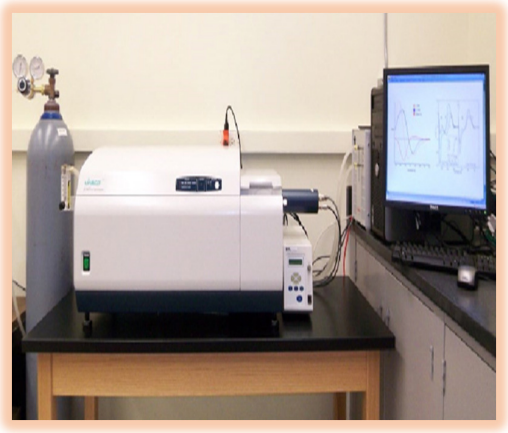
b) Extraction unit (E-816 Soxhlet)

c) Scanning electron microscope

d) Edwards sputter coater

e) Hot air oven

f) Compound light microscope

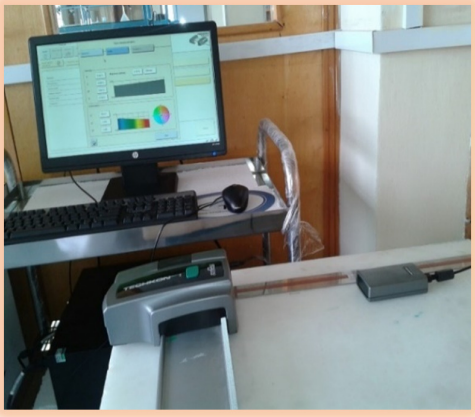


g) Circular dichroic spectropolarimeter

h) Thermal analyzer

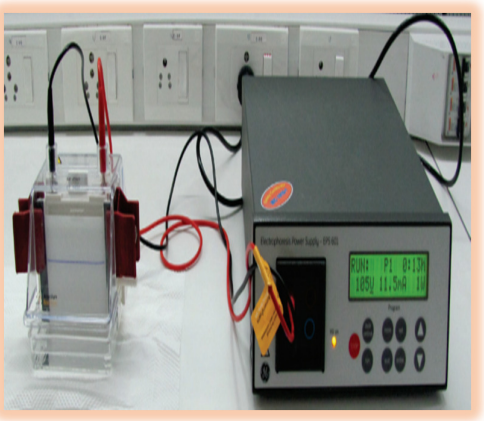
i) Dynamic light scattering

j) UV-Vis spectrophotometer



k) TECHKON SpecroDrive

l) Centrifuge Machine



m) SDS-PAGE setup

n) Kjeldahl digestion and distillation unit

o) TOMMY autoclave

p) Dynamometer

r) Lastometer

Figure 3.1. Major instrument used during the study

3.1.3. **Software**

Edraw Max 7.2 is used to sketch some of the diagrams

Design Expert 7.0.0 is used to analyze some of the statistical data

Origin pro 8 is used to draw some of the graphs

3.2. Methods

The study comprised three phases of experimental work and the methods followed were described below.

3.2.1. Biochemical content and histological features analysis of belly and butt regions of SS

In this section, the following belly and butt skin components and features were analyzed.(i) Non-collagenous (proteoglycans and other proteins) proteins from spent liquor,(ii) fat content estimation, (iii) thermal stability determination, (iv) pore size examination,(v) Chromic oxide content estimation, (vi) morphology examination, (vii) histology examination and (viii) collagen extraction and analysis. The experimental procedures followed were described below.

3.2.1.1. SS samples preparation

In wet salted hair sheep skin, butt and belly regions were marked based on the official method of sampling with slight modification[37] as shown in the figure 3.2. The butt region was excised in such a manner that one side was 5cm away from the backbone and the other side being at mid-point of half part of the SS. The belly region was excised such that one side was 2cm away from other side of butt region and the other side being 2cm away from periphery of the skin. The length of butt region (parallel to the back bone) was cut in way that one of its end was to be located at around of 10/24 L and the other end at around 6/24 L taking tail as a reference. The length of belly was same as that of butt portion. Just out of the researcher's curiosity, both belly and butt regions of skins being excised from the left side of the skin were taken as experimental samples whereas those from the right side of the skin were regarded as control samples throughout the work. Both Belly & butt regions from same side of the skin were processed together in a conventional beam house tanning operations. At the end of each unit operations, samples from spent liquor and both regions of skin were collected and stored in refrigerator at 4^{o} C for analysis. All the experiments were made in triplicates. Unwanted skin material removed from each leather processing unit operations and related analysis being addressed in this work were indicated in Table 3.1.

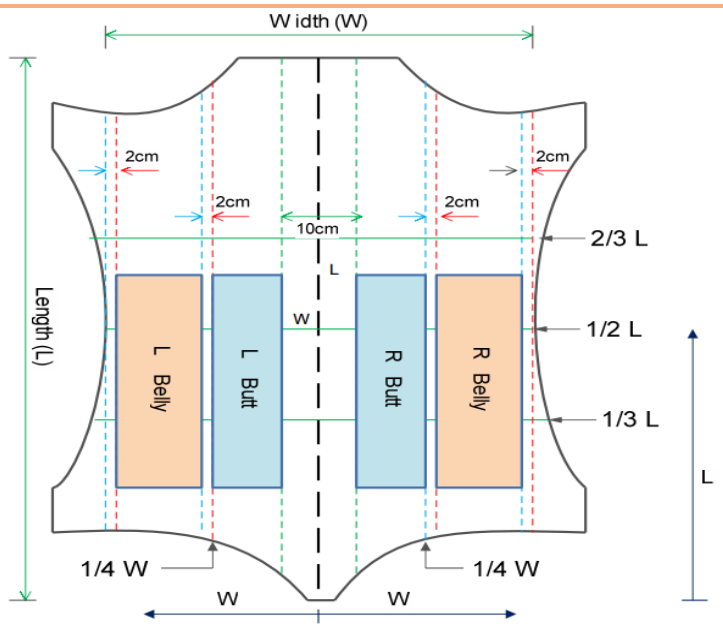


Figure.3.2. Schematic diagram for sampling regions

Table 3.1 List of unit operations/processes in the 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, collagen, *
Tanning		Chromic oxide, *

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

3.2.1.2. Estimation of non-collagenous proteins

Proteoglycans and other non-collagenous proteins such as globular proteins, reticulin & elastin from belly and butt portions of skin were measured by collecting spent liquors from appropriate

unit operations. Prior to analysis, the spent liquors were filtered and 1ml of them was taken and made up to 10ml with distilled water from which the required amount was taken up for analysis. Extractable globular proteins were analyzed from soaking liquors while reticulin and elastin were from bating spent liquors. Spectrophotometric analysis were carried out at an absorbance of 595 nm for the estimation of protein content in the spent solution by Bradford protein assay method[38]. Bovine serum albumin (BSA) was used as standard. The standard curve was prepared by plotting the absorbance against the series of BSA standard concentrations. The amount of extracted protein present in the spent liquor samples were calculated from the slope of standard curve.

During the process of liming, proteoglycans get extracted from the SS matrix and the content extracted from both regions of SS was analyzed from filtered spent liming solution. The alkalinity of the liquor was neutralized using 0.5M Acetic acid (AA) solution. The spectrophotometric analysis for proteoglycan was performed at an absorbance of 555 nm, using periodic acid-Schiff method[39]. Periodate oxidizable glycoconjugates fractions are screened with this Schiff's assay. Mucin was used as a standard. The mucin standard curve was prepared by plotting the absorbance at 555 nm against the series of mucin standard concentrations from which the amount of proteoglycan present in the spent liquor of interest was interpolated or calculated using the slope of the mucin standard graph.

3.2.1.3. Estimation of fat content

The total fat content of both belly and butt regions of skin was estimated after soaking and degreasing. The hair on the samples was removed using scissor and thereafter the samples were dried to constant weight in an air oven (65 ± 2^{o} C). Degreased samples were dried in an air oven at same condition. Fat in the samples was extracted using automated and accelerated Büchi's Extraction Units E-816 Soxhlet. Petroleum ether (40-60^{o} C) was used as extraction solvent.

Known mass of (3-5 gm) dried skin samples were put into glass sample tubes which were to be kept in Soxhlet extraction chamber. Dried and weighed clean beakers were kept at the bottom of extraction setup on the inbuilt heaters and the extraction chamber was fitted at the top of them. The solvent of interest, Petroleum ether, was selected from solvent library with all respective parameters for extraction, rinsing, and drying were being automatically defined. That means, extraction, rinsing & drying times and solvent volume were automatically defied to be 120 min, 5 min, 20 min and 110 ml, respectively. The chosen solvent (Petroleum ether)

added at the top of condensers where it flowed to beakers through inner compartment of condensers. The Soxhlet extraction chamber is emptied when the set level is reached, with the solvent flowing to the underneath heated beaker into which the extracted fat is collected. During each cycle, a portion of fat is dissolved in the solvent. After completion of the extraction, the solvent is evaporated and collected in the water cooled-tank at the back of the instrument which allows to be re-used for the further extraction. After drying off the solvent, beakers containing the extract were removed and dried to a constant weight in an air oven (65±2^{o} C). The mass of extracted fat was calculated and the fat content of samples was estimated in percentage (w/w) basis with respect to the input dry sample mass. The mean values of four replicates were calculated and reported.

3.2.1.4. Thermal stability determination

Samples were collected at various stages of leather processing (from soaking to tanning) and stored at 4^{o}C. 3-5 mg of samples were blotted uniformly on tissue paper to remove excess adhered water. The moisture content was maintained around 60-65%. These samples were sealed in a Tzero pan with a Tzero hermetic lid. The samples were fused in a differential scanning calorimetric cell of a DSC Q 200 TA differential scanning calorimeter. The temperature was calibrated effectively using indium as standard. All the thermograms were recorded by heating the sample from 30^{o} C at heating rate of 2^{o} C/min.

3.2.1.5. Pore size distribution analysis

To examine the pore size distribution of belly and butt regions of the skin, samples were collected at various stages of leather processing (from soaking to tanning) and stored at 4^{o}C. 3-5 mg of samples were blotted uniformly on tissue paper to remove excess adhered water. The moisture content was maintained around 65-68%. The samples were sealed in a Tzero pan with a Tzero hermetic lid. Pore size distribution was analyzed using DSC Q 200 TA differential scanning calorimeter. Prior to measurement, the vessel was cooled to -40 ^{o}C and held at that temperature for 30min.The heating rate was maintained as 1^{o}C/min. All the measurements were carried out between -40 and -10^{o}C at same heating rate. At the end of each measurement, the vessel was dried under vacuum for 24 hours to obtain the mass of dry fibres. The moisture content was maintained at about 65-68% for all samples. Pore size distribution was determined by the melting temperature of the water as suggested by Fathima et al.[40, 41].

3.2.1.6. Chromic oxide content analysis

Cr (III) content present in both control and experimental wet blue leathers was estimated by spectrophotometry. The trivalent chromium is oxidized to the hexavalent state using acid digestion method[42]. The cooled digested sample was diluted to 100 ml with distilled water in standard measuring flask from which 2ml of sample was pipetted out to 100 ml clean and empty standard measuring flask. 50 ml of distilled water was added and to which a few sodium hydroxide pellets were added till the pH greater than 10 was ensured. The content was then diluted to the indicated volume mark with distilled water. 2 ml of the sample was then transfer into a spectrophotometer cell (quartz), which was washed and wiped dry before. The spectrophotometric absorption of the solutions was measured at 372 nm with the ε=4820 $mol^{-1}.cm^{-1}$. The percent chromic oxide content (w/w) was estimated from the absorbance value to the dry weight of the blue leather samples using the relation shown in Eq. (3.1).

$$\%Cr_2O_3 = \left(\frac{Absorbance}{4820}\right)\left(\frac{1}{1000}\right)\left(\frac{152x52}{104}\right)\left(\frac{Dilution\ factor}{Dry\ weight\ of\ wet\ blue\ leather}\right) \times 100 \qquad (3.1)$$

3.2.1.7. Scanning electron microscopic analysis

Specimen samples of approximately same dimensions were taken from each of the unit operation, soaking to post-tanning for morphology examination. All specimens taken from pre-tanning operations (soaking to pickling) were fixed in 5% formaldehyde and 5% glutarldehyde solutions before brought to dehydration. All specimens of wet samples were dehydrated with acetone solution, in increasing concentration of solvent[43] and then coated with gold using Edwards E306 sputter coater after 24 hours drying in desiccator. Prior to drying in desiccator, the dehydrated specimens were mounted on a plate with double face adhesive tape. The micrographs for cross-section of the specimens were obtained using the indicated SEM.

3.2.1.8. Histological examination

Samples from both belly and butt regions of the SS were taken after each unit operations (soaking to pickling). 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. Specimens were cut parallel to the skin surface so as to have histological images revealing well-defined arrangements. 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.

3.2.1.9. Collagen extraction and analysis

a) Collagen extraction

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 extraction at 4°C[16, 44, 45]. The moisture content of the samples was also measured based on the standard procedure (SLC 113)[46]. The samples were soaked in 0.5M Acetic acid (AA) solution under stirring at 4°C for 24 hours. The mass of residue recovered was taken and soaked in 0.5M AA and further treated with pepsin powder (100 units/skin residue) at enzyme substrate ratio of 1:100 for 48 hours at 4°C under stirring. The pepsin digested solution was filtered and the filtrate was recovered. Both 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) NaCl and then incubated overnight. The solutions were centrifuged at16, 000 rpm, 4°C for 30 minutes to recover the pellet. The pellets were re-dissolved in 0.5M 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 0.5M 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.

b) Determination of hydroxyproline

The extracted ASC and PSC collagen content was estimated using hydroxyproline assay[47]. The determination of hydroxyproline in the extracted collagen samples involved hydrolysis of the samples (ASC and PSC) which finally was measured spectrophotometrically at 557nm (Neumann and Logan, 1950)[15,16]. The Hydroxyproline content of collagen solution was then determined using Woessner method. The obtained hydroxyproline value was converted to total collagen concentration using the relation in Eq. (3.2)[48]. Finally, the collagen content was expressed as percentage mg/g of input dry weight of the skin.

$$\textit{Concentration of collagen solution} = \frac{\boldsymbol{Amount\ of\ Hydroxyproline}}{\mathbf{300}} \text{ X } 7.57 \qquad (3.2)$$

c) Electrophoretic analysis

The purity of the collagen (ASC & PSC) solution was analyzed using SDS-PAGE by the method of Laemmli (1970)[49]. High molecular weight markers where HiMark™Pre-Stained Protein Standard previously stored in buffer containing Tris-HCl, Formamide, SDS and Phenol Red were used in order to accurately determine molecular weight of proteins. This standard consists of 9 pre-stained protein bands ranging in molecular weight from 30-460 KDa. 20 µL of sample solution was added with micropipette on the back side of the glass plates of the set up after which 50 volts for staking gel and 100 volts to separating gel was supplied to effect the separation of proteins according to their mobility. The glass plates were then separated to take the gel out and immerse into the fixing solution for one and half minute. The gel was then taken out and immersed in staining solution for one and half hour followed by dipping into the destaining solution. Molecular weight distribution of the samples (ASC & PSC) was studied after taking microscopic imaging of the gel.

d) Circular dichroic (CD) 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.) using acetate buffer (pH 4.2) under the nitrogen atmosphere. For CD data collection, 350 µL of protein was used in a 0.1cm path length quartz cuvette. CD spectra was recorded in the far UV region (190-250 nm) under constant purging of nitrogen gas at 20^{o}C employing 1.0 nm bandwidth, 0.1nm step size, for an average time of 1 s. The raw data in millidegree unit was converted to molar ellipticity [θ].

3.2.2. Collagen hydrolysate characterization and its application

3.2.2.1. Collagen hydrolysate preparation

Collagen hydrolysate (CH) was prepared from limed trimmings using 5% Sodium hydroxide solution in Autoclave at 74^{o}C over a period of 4 hours. Prior to hydrolysis, collected pelt trimmings were completely delimed with ammonium sulphate, degreased using commercial degreasing agent, thoroughly washed with plain water and cut into pieces. After completion of hydrolysis, the content was cooled and the fatty supernatant & precipitated slurry were separated through decantation to recover the hydrolysate layer. The CH was further purified by vacuum filtration. The hydrolysate was then concentrated by evaporating in water bath. The content was cooled and stored in refrigerator at 4^{o}C. 1N HCl solution was used to adjust pH of concentrated CH prior to the estimation of the solid content. The pH was adjusted to four different pH values viz. 9.0, 8.0, 7.0 and 6.0. A known quantity (mass (g) & volume (ml)) of

concentrated and adjusted CH was weighed in a clean & empty crucible and dried at 98.5-100°C to constant weight as per the standard procedure[50]. The percent total solid content of the hydrolysate was calculated based on the percentage ratio of dried weight to its original weight.

3.2.2.2. Particle size distribution and Zeta potential analysis

The hydrodynamic radii of particles of CH at four different pH values in aqueous solution were analyzed using DLS with high performance particle sizer (Zetasizer Nano series, Malvern) at 25°C operating at 4 mW He-Ne laser power, scattering angle of 175° and wavelength of 633 nm. Zeta potential of CH at four different pH values was also obtained with Zeta Nanoseries from electrophoretic nobilities using Smoluchowiski model approximation which is best fitted for aqueous solution containing moderate electrolyte solution.

3.2.2.3. CH application

Tanning process

In the application of CH in pre-tanning, CH-pH and -offer optimization processes were undertaken to investigate the pH and amount of CH rendering relatively higher intake by the experimental belly and butt pelt SS samples. CH at four pH values (9.0, 8.0, 7.0 & 6.0) but at same percentage offer (10%) was used in pre-tanning to investigate maximum intake of the hydrolysate by the pelt. The intake of CH was further optimized by varying percentage offer viz. 5%, 10%,15% & 20% CH at pH where maximum intake was observed. Experimental samples were made tumbled with known amount of CH in a floatless drum for one hour at an ambient temperature before pickling. Conventional pickling and chrome tanning, with same type and percentage offer of chemicals for both experimental and control processes, were then carried out as per the set process recipe (Annex 1. B). Basic chromium sulphate (33% basicity in all tanning trials) was added to both experimental and control processes after the pH of pickle float made adjusted to 2.8/3.2. After penetration of basic chromium sulphate (BSC) was ensured, sodium bicarbonate was used for exhaustion of chrome in all tanning processes. All the process duration, type and percentage chemical offer were kept same for both experimental (treated with CH) and control (without CH) processes. Optimization processes for CH application was carried out in four replicates. After seven hours of chrome tanning duration, chrome liquors of both control and experimental processes were collected for COD, TDS & TS analysis. After ageing of wet blue leathers over a period of 48 hours, belly and butt leathers samples of both experimental processes were taken for nitrogen and chromic oxide content estimation as per the corresponding test methods. The effects of CH application was then investigated with the indicated response variables.

Re-tanning and dyeing process

Using the overall optimized process, CH treated wet blue and CH untreated conventional wet blue were taken for further processing. Conventional re-tanning and dyeing recipe for softy upper was used to further process experimental (with CH) and control (without CH) blue leathers (Annex 1. C). Four different commercial protein fillers (CPF) F_1, F_2, F_3 & F_4 with profile indicated in Annex 2 were used for control blue leathers with same percentage offer (20%) CH being used during tanning. The percentage crude nitrogenous substance of F_1, F_2, F_3 & F_4 were estimated and found to be 28.48, 31.38, 21.10, & 41.54, respectively using the relation in Eq. 3.3 & 3.4. Both control and experimental processes were done in triplicates. Types and percentage offer for re-tanning and dyeing process chemicals were same for both experimental and control leathers. Four process were conducted in a single trial corresponding to number of commercial fillers used. In every process, the control and experimental belly leathers are designated as 1T/1RT, 1L/1RL, 2T/2RT, 2L/2RL, 3T/3RT, 3L/3RL, 4T/4RT and 4L/4RLwhere T/RT representing experimental butt/control butt and L/RL experimental belly/control belly samples. Coefficient of letters in the code indicates process number for which the four CPFs were used. After exhaustion of re-tanning and dyeing bath was ensured, spent liquor solution of both experimental and control processes were collected for TS, TDS & COD estimation. After mechanical operation (Annex 1.C) was completed, color measurement (L*a*b* values), physico-chemical and organoleptic properties were analyzed and compared for both control and experimental dyed belly and butt crust leather samples.

3.2.2.4. Estimation Nitrogenous substance in SS samples

Control and experimental samples of both parts of SS after degreasing and tanning unit operations were taken for nitrogen content estimation. Percent nitrogen content of known quantity of both belly and butt skin/leather samples was estimated through Kjeldahl method (Kjeldahl 1883) using IUC 10[51]. Similarly, the percent cured protein contents of CH & CPF were estimated by using known quantity of samples with the same method. The content of nitrogenous substance of the SS/leather has been calculated from percent nitrogen using the factor indicated in Eq. (3.3). The percent crude nitrogen content of collagen hydrolysate has also been calculated from percent nitrogen content using the conversion factor indicated in Eq. (3.4).

Nitrogenous substance content in percentage = % Nitrogen X 5.62	(3.3)
The crude protein content in percentage = % Nitrogen X 6.25	(3.4)

3.2.2.5. Chemical oxygen demand (COD) and total dissolved solid (TDS) estimation

Spent liquor from control and experimental tanning and post-tanning processes were collected, filtered and conditioned for chemical oxygen demand (COD), total solids (TS) and total dissolved solids (TDS). Chrome tanning and re-tanning & dyeing liquors for TDS & COD analysis were collected just at the end of basification and main re-tanning & dyeing exhaustion duration, respectively (Annex 1.B & C).

COD of the indicated spent liquors was measured using open reflux method[50]. To measure TDS, a well-mixed sample was filtered through a standard glass fiber and the filtrate was evaporated to dryness in a weighted dish and dried to constant at 180^{o}C[50]. The increase in the dish weight was recorded as TDS. Using same[50], TS was estimated by evaporating, a well-mixed known mass of sample in a weighted dish and dried to constant weight in an oven at 103 to 105^{o}C. The increase in weight over that of the empty dish represents the total solids.

3.2.2.6. Sulphated total ash estimation

The amount of mineral substances that can be sulphated out have an effect on the wear and comfort properties of the leather. Samples from both experimental and control dyed crust leathers of belly and butt regions were prepared as per IUP 1 & 2[52]. Sulphated total ash was estimated as per IUP 7[53]. Sulphated total ash was calculated as percentage mass ratio of sulphated total ash to the original sample leather mass.

3.2.2.7. Crust leather samples color measurement

To investigate the color variation among control and experimental dyed crust leather samples, L*a*b* color measurement system was used to measure color of control and experimental belly and butt leather using TECHKON SpecroDrive instrument. The L*a*b* values of each corresponding experimental and control crust leathers were measured. For each leather sample, three spot records were made and the average values were taken. Then, the mean values of triplicate processes were executed and reported. ΔL*a*b* values were calculated by subtracting value of control leather from corresponding experimental leather value. Control leathers taken as reference and experimental ones sample of interest in all measurements. The effects of CH application and CPFs on the color of dyed crust leathers were then examined. In CIE Lab system, any color point can be exactly defined by the three coordinates L, a and b, where, 'L' represents clarity or lightness, 'a' represents the chromatic component green-red and 'b' represents the chromatic component blue-yellow. The total color difference (ΔE),

difference in hue (ΔH) between control and experimental leathers and color chroma (C) were computed, respectively as:

$$\Delta E = \sqrt[2]{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \quad (3.5)$$

$$\Delta H = \sqrt[2]{(\Delta E^2 - \Delta C^2 - \Delta L^2)} \quad (3.6)$$

$$C = \sqrt[2]{(a^2 + b^2)} \quad (3.7)$$

Where, ΔL-shows the variation in clarity or lightness, Δa-shows the redness-greenishness difference and Δb-shows the bluishness-yellowishness difference.

3.2.2.8. Tensile strength and percentage elongation of crust leather samples

Test specimens of belly and butt regions of both experimental and control leathers were prepared and conditioned as per IUP 1 & 3[54]. For each samples, triplicate specimens both along and across the back bone were cut out using oil dynamic micro clicking press machine with a metal die in the standard dumbbell shape. Thickness of each specimen was measured at middle and at two gripping points and average value was considered during tensile strength manipulation. The specimens were tested using Dynamometer as per IUP 6[55] under same constant rate of loading during all the measurements. Tensile strength and elongation at break of the samples leathers calculated as shown below.

$$\text{Tensile strength } (N/mm^2) = \frac{Breaking\ load\ (N)}{(Thickness\ X\ Width)mm^2} \quad (3.8)$$

$$\%\ \text{Elongation at break} = \frac{\text{Change in length between the jaws at the instant of break}}{\text{initial length between the jaws}} \text{ X } 100 \quad (3.9)$$

3.2.2.9. Double edge tear strength of crust leather samples

Tear strength is another bulk property of the leather which is calculated by taking the ratio of maximum load (N) exerted to the maximum up thrust distance the grain moved at the instant of grain burst (Eq.(3.10)). Test specimens of belly and butt regions of both experimental and control leathers were prepared & conditioned as per IUP 1 & 3[54]. For each samples, triplicate specimens both along and across the back bone were cut using oil dynamic micro clicking press machine. Thickness of each specimen was measured to left and right of both ends of the cut slot and average value was considered during tear strength manipulation. The specimens were

tested using Dynamometer as per IUP 8[56]. Tear strength of measured samples were calculate using the relation:

$$\text{Tear strength (N/mm)} = \frac{\boldsymbol{Maximum\ terar\ load\ (N)}}{\boldsymbol{Thickness\ (mm)}} \quad (3.10)$$

3.2.2.10. Distension and strength of grain of crust leather samples

Test specimens of belly and butt regions of both experimental and control leathers were prepared conditioned as per IUP 1 & 3[54]. For each samples, triplicate specimens were cut using oil dynamic micro clicking press machine. The specimens were tested for distension at burst using Lastometer as per IUP 9[57]. The average load (N) and distension (mm) at burst for the specimens were measured and recorded.

3.2.2.11. Organoleptic properties of crust leather samples

Experimental and control dyed crust leathers of both belly and butt parts were assessed for softness, fullness, roundness, grain smoothness, grain tightness, color uniformity and general appearance by hand and visual examination. The leathers were rated on a scale of 0-10 points for each functional properties by three experienced leather experts, the higher point on the rating scale indicating better property.

4. RESULTS AND DISCUSTION

4.1. Biochemical content and histological features analysis

In the course of leather manufacturing processes, skin/hide will undergo several physic-chemical changes due to the removal of proteoglycans, proteins (albumin, globulin, reticulin & elastin) and fat. SS matrix constituents have been analyzed at the end of indicated unit operations. All skin constituents indicated except collagen are removed as the former are integral part of conventional beam house operations. The characteristic features of butt and belly regions of SS with respect to skin matrix constituents have been analyzed and reported as indicated in Table 4.1.

4.1.1. Non-collagenous SS constituent determination

Non-collagenous SS constituents were analyzed in the spent liquor after soaking, liming and batting processes. The overall non-collagenous protein content value (μg/mL) was found to be higher in butt region than belly region (Table 4.1). The globular proteins content removed during soaking was lesser in belly than in butt, probably attributed to often less presence of them in belly region. Proteoglycans were found to be significantly higher in butt portion than in belly which might be the main cause 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 considerable role in leather making. This may be one of the reasons for the notion “Leather is made in lime yard”. The quantum of proteoglycans could be related to the amount of collagen in the given regions of skin and hence their higher observed values in butt regions complement same argument. Fibrous proteins (elastin, reticulin), except collagen were found to be higher in belly region than in butt region. Belly part of the skin is naturally observed to be under higher strain and higher amount of non-collagenous fibrous proteins manifest its elasticity. The variation in the unwanted skin matrix constituents among the parts of SS signifies how important pre-tanning operations are and the requirement of optimum process recipe to obtain the desired quality at each unit operations.

In order to envisage the comparison of fat content effect in butt and belly regions of the skin, the fat content of both portions of the skin was analyzed after soaking, which will be removed to certain level during degreasing process. The average percentage of fat content (w/w) in the butt and belly was found to be 3.98 and 5.42, respectively. As expected, belly portion contains more amount of fat content than 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.

Table 4.1 Characteristic features of butt and belly regions of hair sheep skin

Response variable		Operation	Butt	Belly
Protein (µg/mL)	Albumin + Globulin	Soaking	65.15 ± 0.72	13.45 ± 0.42
	Elastin+ Reticulin	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	3.98 ± 0.43	5.42 ± 0.53
		Degreasing	1.10 ± 0.25	1.41 ± 0.20
Collagen (mg/g on dry mass)	Acid soluble	Degreasing	34.40 ± 1.03	4.88 ± 0.92
	Pepsin soluble		2.18 ± 0.85	19.94 ± 0.98
Chrome (% w/w)		Tanning	3.43 ± 0.77	3.09 ± 0.71

4.1.2. **Determination and analysis of SS's** collagen

4.1.2.1. Collagen content determination

From the hydroxyproline values of acid-and pepsin soluble collagens of the both regions of the skin samples, the collagen content was estimated. Collagen content (mg/g of dry skin mass, Table 4.1) 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 contrary, acid-soluble collagen was more in butt than in belly. Thus, most of collagens in butt were extracted during acid solubilization and only a small portion of cross-links were retained for further pepsin solubilization where the reverse was happened in belly part of skin. Earlier studies report that pepsin soluble collagen content is high in vertebrates' skin than the invertebrates[44, 58, 59]. 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 animals' skin possess high ASC but low PSC, which indicates lack of significant amount of inter-molecular crosslinks. Therefore, it can be inferred from the collagen content that belly region possesses more inter-molecular crosslinks than in butt. Thus, belly region possesses more amount of telopeptides than in butt, which enables an interaction between telopeptides and neighborhood triple helix. Therefore, the result suggested that the covalently cross-linking the at the telopeptide region of belly collagen molecules through the condensation of aldehyde groups as well as inter-molecular cross-linked molecules were tolerant to acid hydrolysis. This interactions play an important roles in assembly and stabilization of collagen fibrils[60-63]. The quantity of collagen content has the direct implication

on the level of uptake of tanning and post-tanning treatment chemicals and hence butt part of SS would relatively uptake more treatment chemicals than belly part of same skin.

4.1.2.2. Electrophoretic analysis

Molecular weight of ASC and PSC from butt and belly regions were studied using electrophoretic analysis, and shown in Figure 4.1.

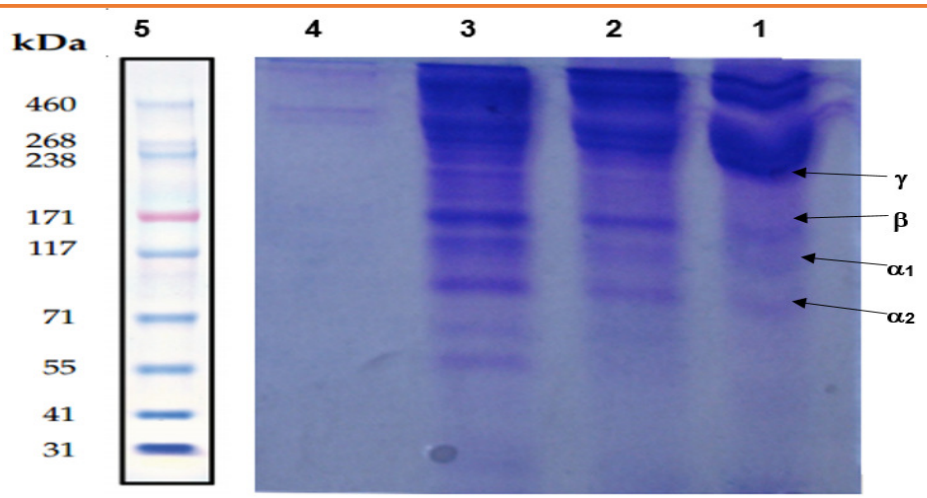


Figure 4.1 Protein pattern of ASC & PSC from butt and belly regions of SS.
**Lane 1: Butt-ASC; Lane 2: Butt-PSC; Lane 3: Belly-PSC; Lane 4: Belly-ASC* and Lane 5: Pre-stained broad protein marker.

SDS-PAGE method helps examine the purity and type of collagen present in the samples of pure collagen. It also reveals about the monomeric, dimeric and higher polymeric forms (α, β, γ sets) of collagen present in the ASC & PSC of both belly and butt regions of the skin. Electrophoretic study revealed that 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 more amount of telopeptides than in butt region. The loosened skin matrix, via swelling mechanism in acidic solution, could also lead to the ease cleavage of telopeptide by pepsin. The presence of more intermolecular crosslinking in belly is manifested by a faint bands in lane 4 revealing relative tolerance to digestion by acid. Furthermore, lower molecular weight components were noticeable in PSC which could probably be due to the cleavage of telopeptide region of tropocollagen by pepsin being used for solubilization. However, in both extraction methods, a similar pattern was observed with the major bands for collagen β-chain and other bands lower than major ones for collagen α_1- and α_2-chains demonstrating that the distribution

of molecular identity of collagens is fundamentally the same among regions of SS and the components are intact. Moreover, high molecular weight components including, β- and γ-components as well as their cross-linked molecules were observed in both ASC & PSC of both regions of collagen molecules. Although there observed a slight difference in relative mobility, particularly for Belly-ASC, the presence of two identical α_1-chains and one α_2-chain in ASC & PSC of both regions of skin would gear to type I collagen.

4.1.2.3. Circular dichroic analysis

CD spectroscopy has been used extensively in protein chemistry for the measurement of secondary structure and the detection of conformational changes. 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[64, 65]. For butt and belly pars of SS, the CD spectra a positive maximum peak at 222 nm for both PSC and ASC. Extracted ASC and PSC from butt and belly showed a positive maximum peak at 222 nm. ASC-belly & PSC-butt has minimum negative peak at 197 nm with a crossover of 214 nm whereas PSC-belly & ASC-butt shows 12nm & 9nm red shift in the π-π* amide

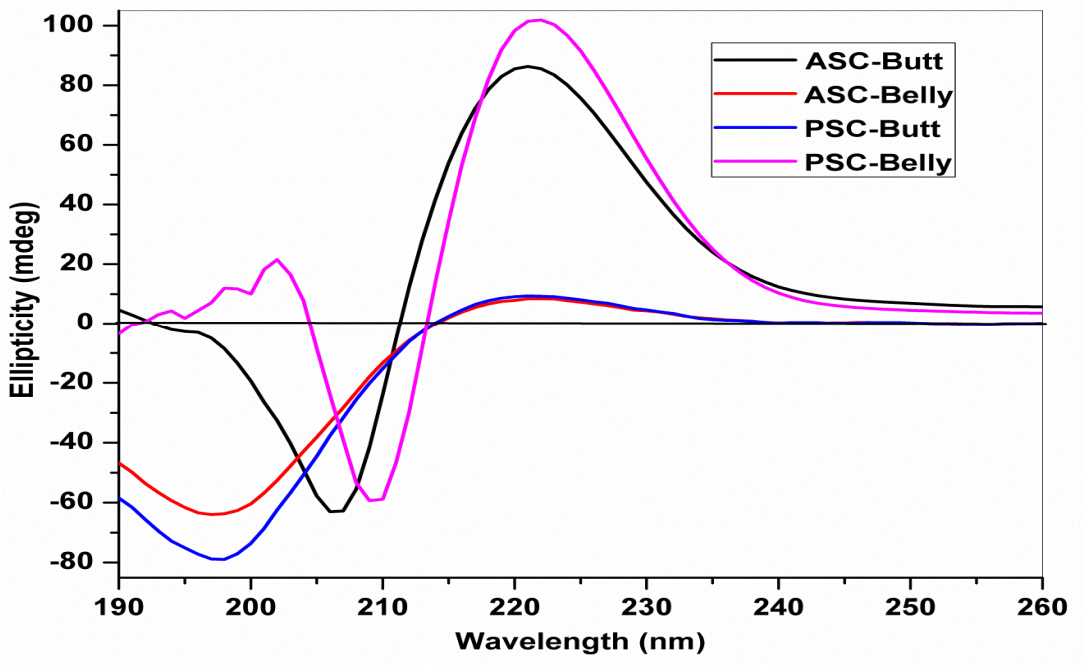


Figure 4.2 CD spectra of extracted ASC & PSC solution of butt and belly regions of SS.

transitions and 3nm & 5nm blue shift in crossover point, respectively. The spectra (Figure 4.2) indicates that extracted ASC shows native polyproline II conformation. This shift may be due to partial cleavage of telopeptide regions by the treatment of pepsin. Generally, the triple helical structures of both ASC and PSC of both skin regions appear to be intact as inferred from the CD spectra showing characteristics of a collagen with triple helix structure.

4.1.3. Hydrothermal stability analysis

The hydrothermal stability of the samples were determined using differential scanning calorimetry. The thermogram of butt and belly regions of samples after each unit operations have been in Figure 4.3 and the temperature and enthalpy has been tabulated in table as indicated in Annex 3.

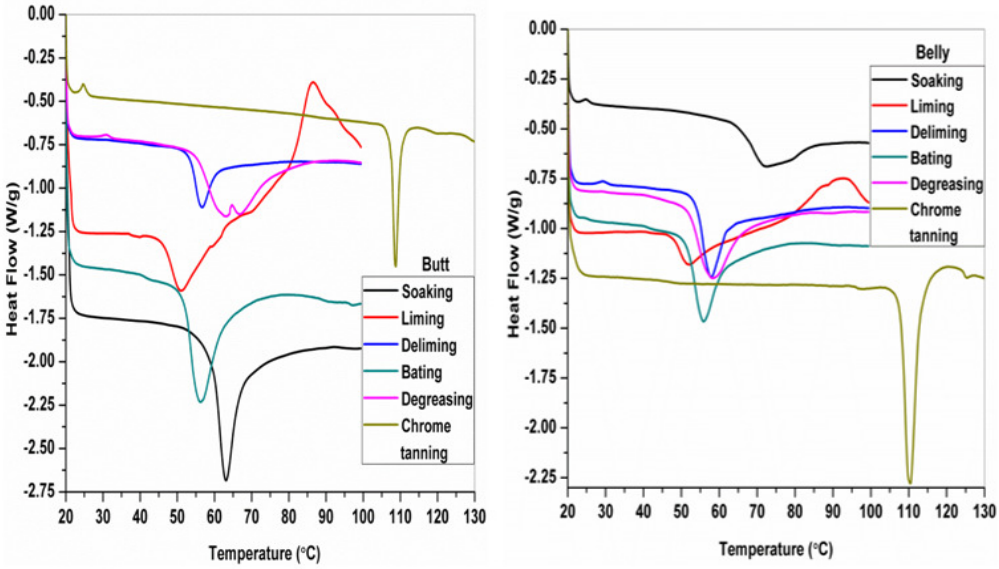


Figure 4.3 Thermograms of butt and belly regions of SS at various stages of leather processing.

A decreasing trend in the peak temperature from soaking to degreasing for both butt and belly regions has been observed. The presence of more heterogeneity in butt and belly can also be figured out 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 can be inferred from the liming thermogram that the thermal stability has more effect in butt than in belly. This might have been ascribable to the fact that more proteoglycans particularly dermatan sulphate were removed during liming in butt than in belly which showed decrease in hydrothermal stability in same operation. Recent studies augmented same[66]. Therefore, the lesser shrinkage temperature of limed butt than belly might be due removal of

more proteoglycans in the former (Table 4.1), alongside the effect of its higher swelling during the process. Relatively lower melting peak of butt in both soaking and liming and that of belly at bating has direct correlation with the quantum of unwanted material being removed at the respective unit operations.

4.1.4. Pore size distribution analysis

The pore size plays a crucial role in leather as it determines the unique property known as breathability. The pore size distribution get affected during the various stages of leather processing. The chemicals used each unit operation and tanning agent have an influence over the pore size. Pore size distribution has been measured based on the Gibbs-Thomson effect[67]. It mainly depends on the melting behavior of frozen water, pore volume and its size. The pore size distribution of butt and belly regions of SS after each unit operations in the leather processing have been shown in Figure 4.5. 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 SS during various stages in leather processing have been tabulated in (Annex 3.A). The ice crystal melting peak of the thermogram occurred at -0.5 and -0.59 ^{o}C for butt and belly regions of soaked skin, respectively, whereas except liming, other operations led to depression of melting temperature (Annex 3.A). The changes in the ice crystal peak and its corresponding enthalpy indicates that the capillary structure of collagen fibres 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 the skin 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 SS varies from 3-65 and 5-50 nm, respectively.

Next process is liming which involves the addition of lime and sulphide in order to open up the fibres as well as to remove hair and flesh from SS. 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. Form the results, it can be inferred that during liming the fibres are opened up as well as removed 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.

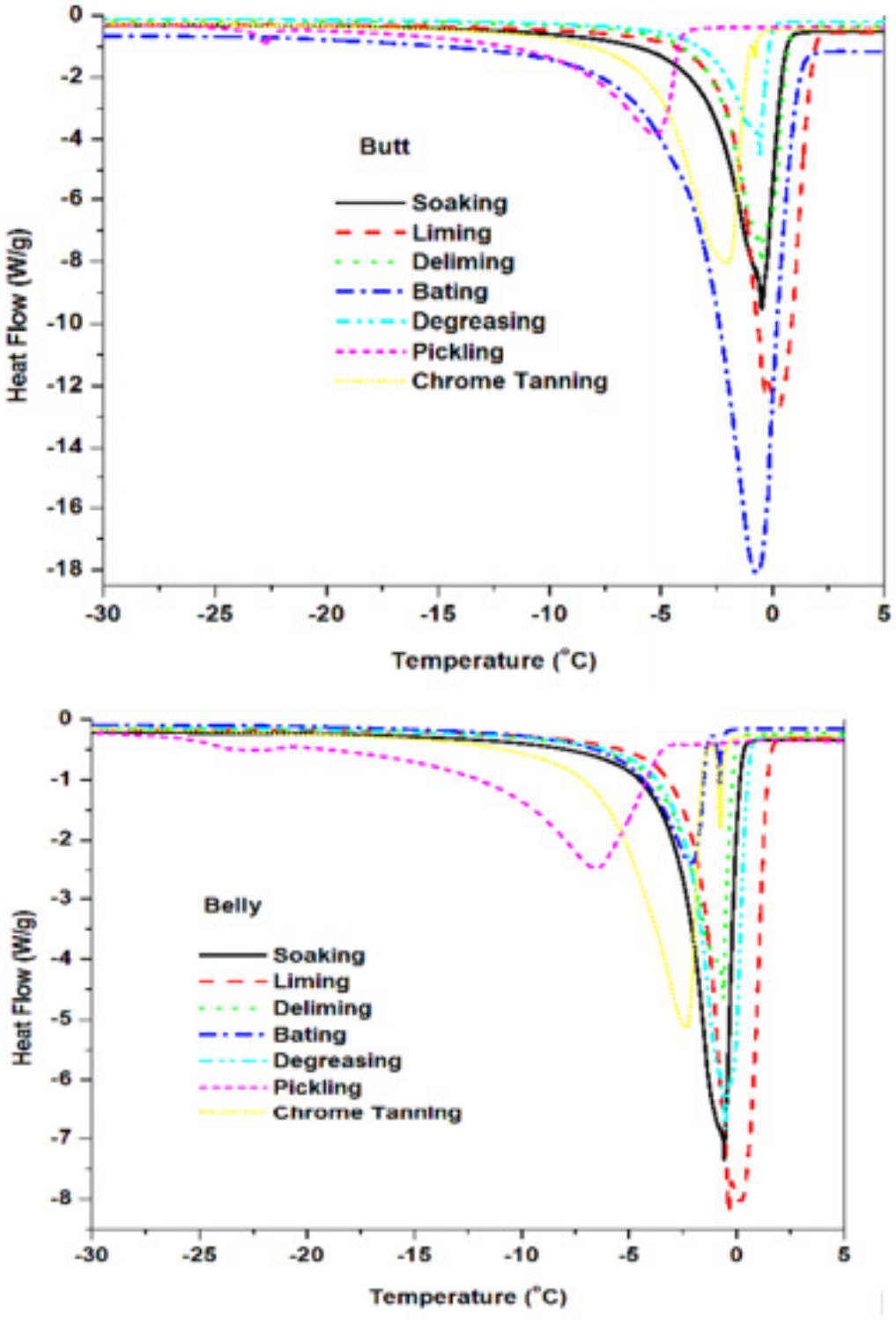


Figure 4.4 DSC thermograms of ice crystal melting peak of SS at various stages of leather processing

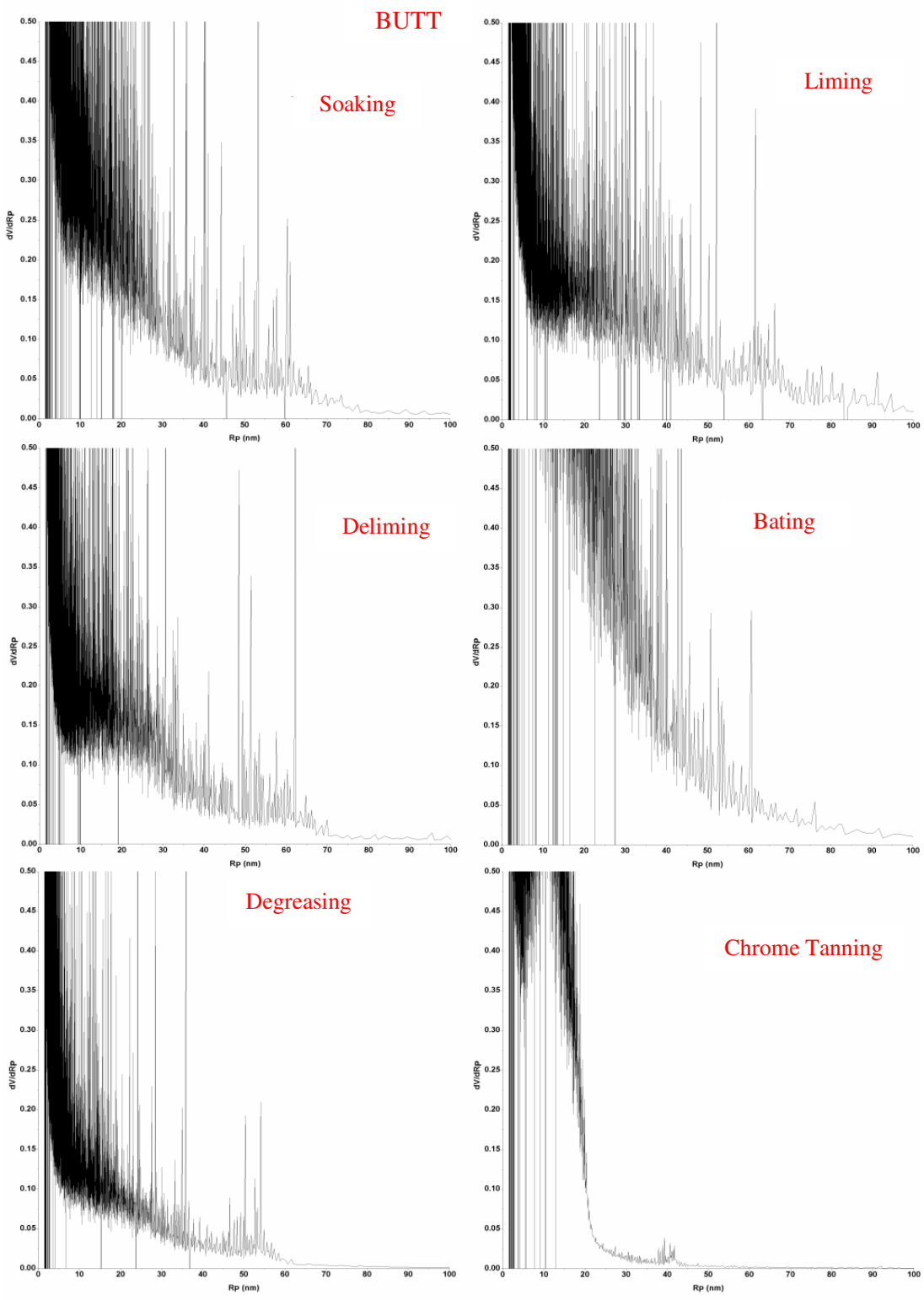


Figure 4.5 Pore size distribution of butt and belly regions of SS-Butt

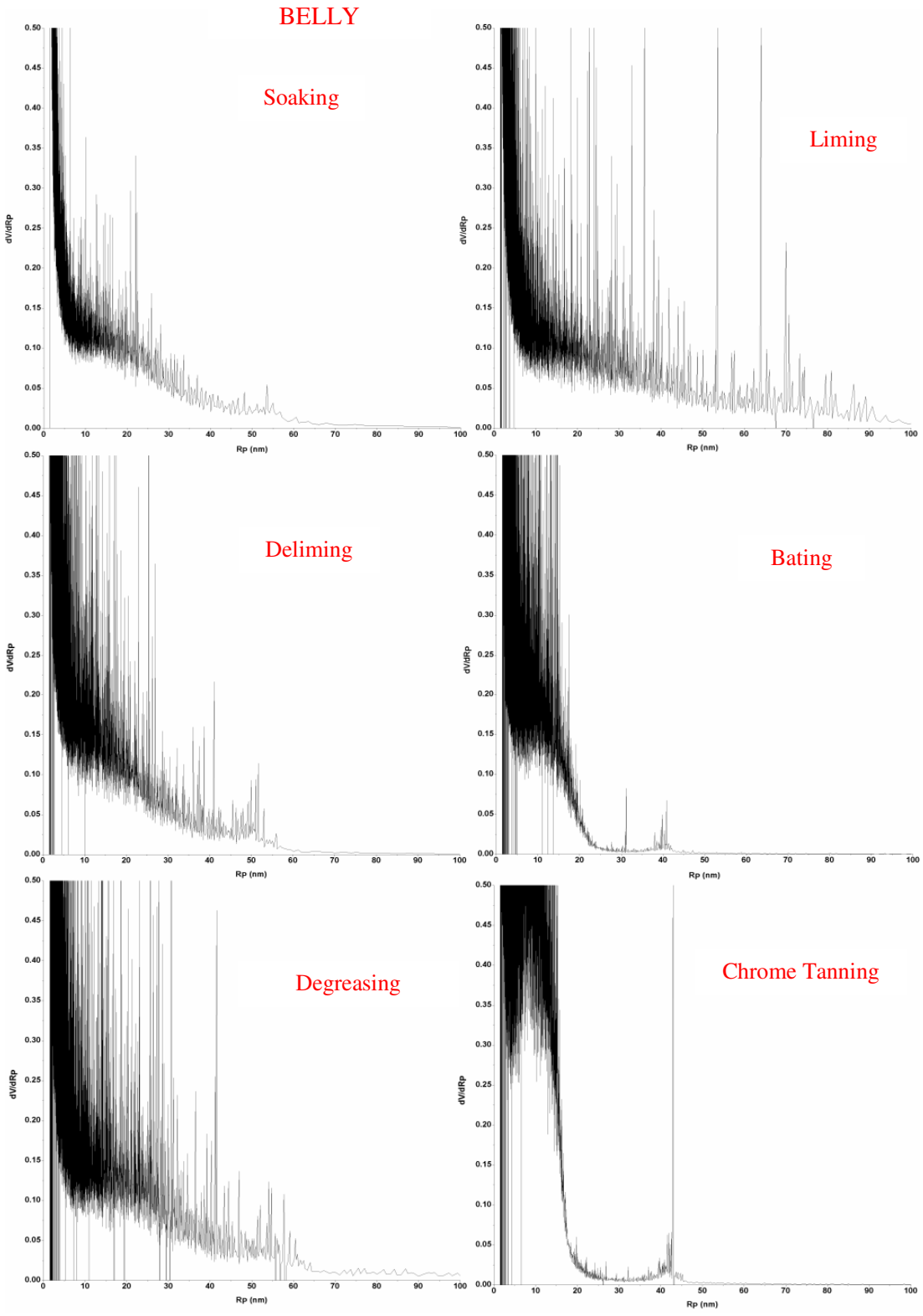


Figure 4.5 Pore size distribution of butt and belly regions of SS-Belly

After deliming, the pore size distribution ranges from 3-65 and 5-50 nm. Results showed that the swollen matrix has been deswelled. Belly region shows multiple transitions in bating, pickling and chrome tanning. This leads to 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 shows two transitions for the bated pelt which may be due to the removal of high amount of interfibrilary proteins than in butt. Next operation is degreasing which removes fat from the skin matrix. After bating and degreasing operations, the SS matrix shows similar pore size distribution.

Pickling process involves the addition of acid and slat to the skin matrix to reduce the pH in order to facilitate the diffusion of tanning materials. Pickled pelts show two transitions in both butt and belly regions, one for the freezing of pore water at -5.19 and -5.78 ^{o}C and another for salt-water eutectic mixture at -22.78 and -22.58 ^{o}C[68], respectively. Pickling process induces drastic changes in the pore structure of butt and belly portions of the skin matrix. These results are agreed with the earlier reports made from goat skin. In tanning process, basic chromium sulphate 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.

4.1.5. Chromic oxide content analysis

The % (w/w) chromic oxide content found to be more in butt than in belly (Table 4.1). The value obtained at 6% basic chromium sulphate offer for butt and belly was 3.43 and 3.09, respectively. Relatively lesser value in belly might have been due to the presence of less collagen content to absorb the chromium in belly than in butt. Surface astringency of chrome has been observed early on belly than on butt as pH increased during basification of chrome tanning. However, the hydrothermal stability of the chrome tanned leather samples of both parts were found to be comparable as observed from the thermogram values.

4.1.6. Scanning electron microscopic analysis

The scanning electron microscopic analysis on the two regions of SS were performed to investigate the morphology and the effects brought by the leather processing unit operations (soaking, liming, bating, degreasing, tanning and post-tanning). Form the cross-sectional SEM image (Figure 4.6), it can be inferred that more fibre opening was observed during liming in butt portion than its counterpart. The images for the delimed samples were in the support of

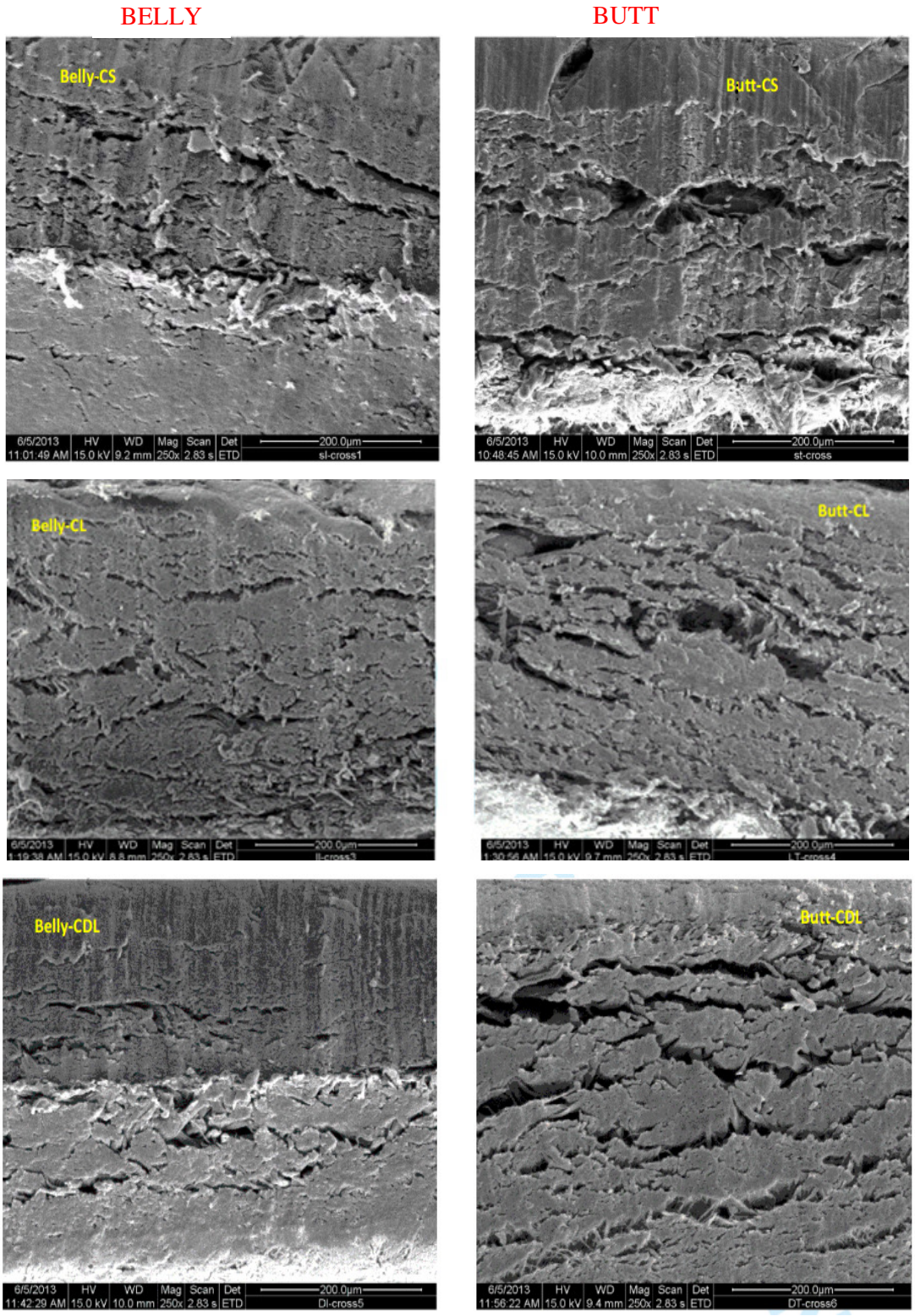


Figure 4.6 Scanning electron micrographs (250x), cross-section of belly and butt regions of sheep skin at various stages of leather processing

**c-cross-section, S-soaking, L-liming, DL-deliming*

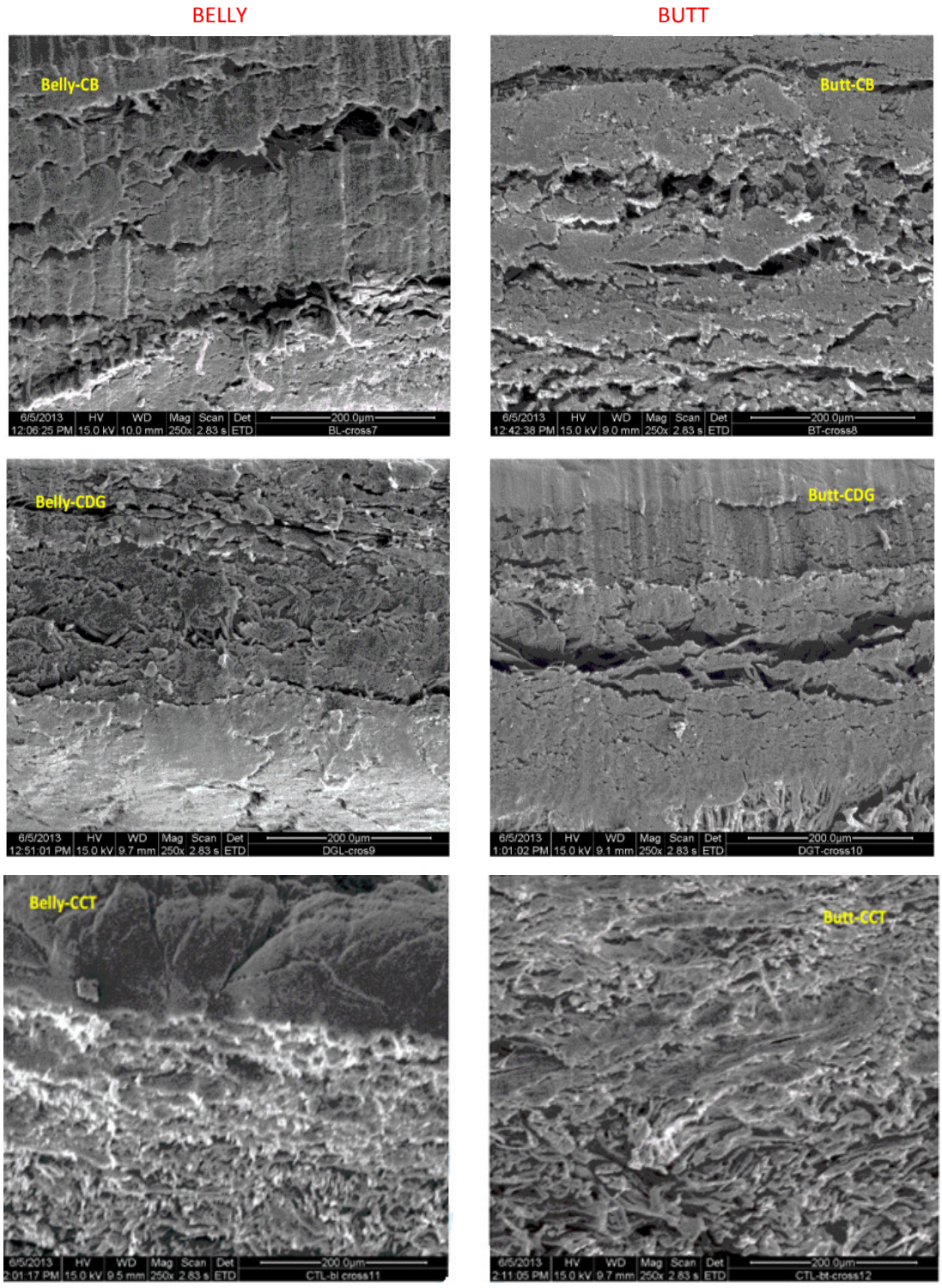


Figure 4.6 Scanning electron micrographs (250x), cross-section of belly and butt regions of SS at various stages of leather processing

**C-cross-section, B-bating, DG-degreasing, CT-chrome tanning*

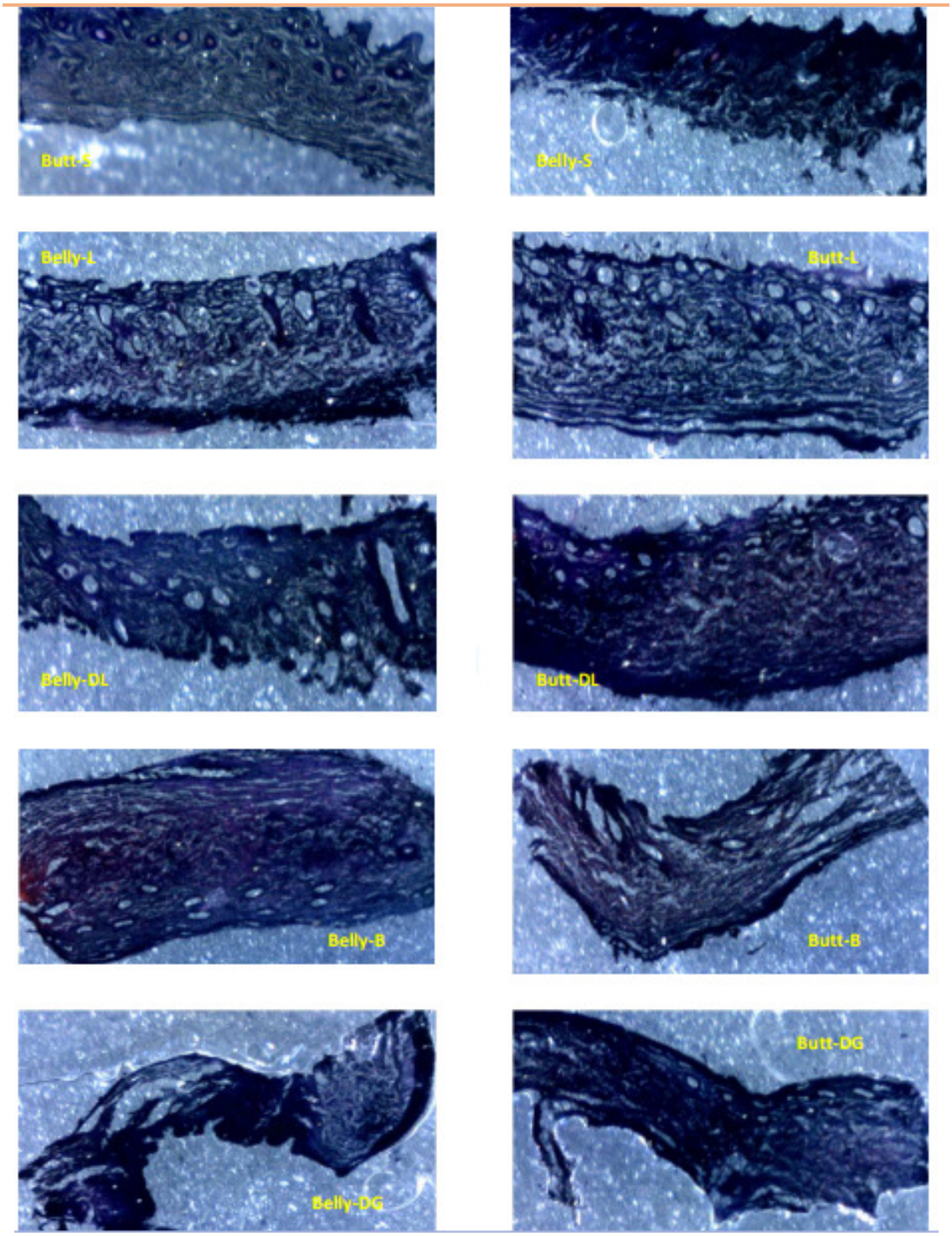


Figure 4.7 Histology images of belly and butt regions of SS at various leather processing operations

*S-soaking, L-liming, DL-deliming, B-bating, DG-degreasing

same. The voids observed in butt were also in parity, showing that more non-collagenous proteins was removed as indicated in Table 4.1. After bating, more voids were observed for butt part due to the fact that cumulative removal of non-collagenous proteins from soaking, liming and bating were high 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. The native fibre compaction due to chrome tanning was observed to be lesser in belly when compared with butt regions.

4.1.7. Histological examination

The cross-section of butt and belly regions of SS were observed after the unit operations from soaking to degreasing. The imaging was performed under 12.5X objective (Figure 4.7). 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 butt and belly regions of SS. Similarly, the relative fat pockets in butt appeared be lesser than in belly which corroborates with fat content analysis. Angle of weave of fibres are low and it is in fact has been decreasing during the various leather process from soaking to degreasing. Moreover, it appeared the follicular density of belly region of skin is more than that of butt regions indirectly revealing the lesser collagen density in the former.

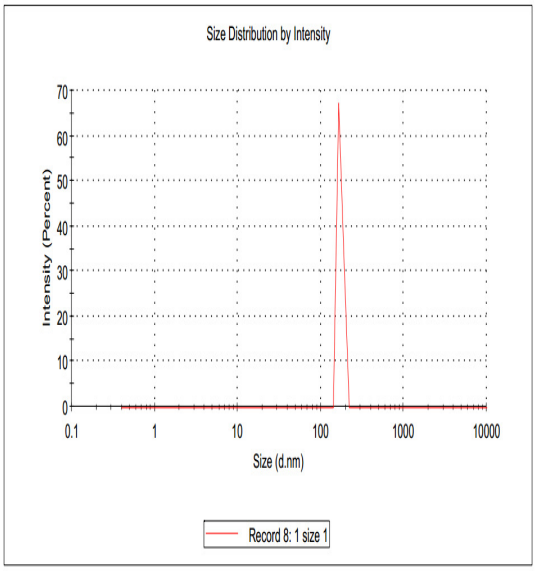
4.2. Application and evaluation of collagen hydrolysate

4.2.1. Collagen hydrolysate analysis

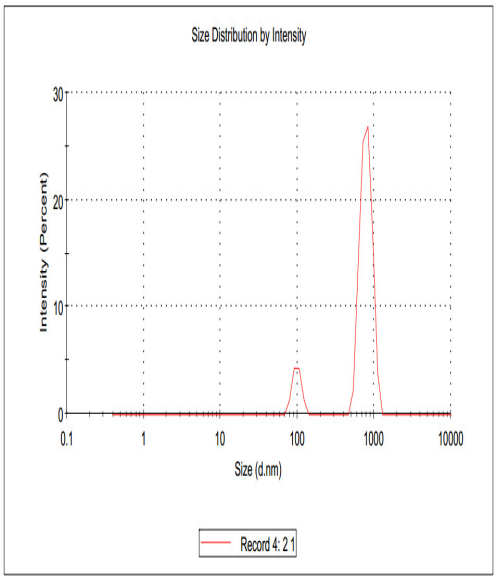
Collagen hydrolysate was prepared from lime pelt trimmings by thermal hydrolysis under alkaline condition. The filtered hydrolysate was concentrated to 20-22% solid content. The crude nitrogen content of the hydrolysate was estimated from organically bonded percent nitrogen content by Kjeldahl method (Eq.(3.4)). The average percentage crude protein content of collagen hydrolysate being used was 78.21 ± 0.63.

Being a biomaterial, CH may behave differently in different pH conditions. To help analyze the amount of CH taken in by the parts of SS, the size distribution and zeta potential of CH at the indicated pH values was analyzed using higher performance particle sizer (Malvern Zetasizer Nano ZS). During hydrolysis process, most of triple helices of CH hand been destroyed and parts of their peptide bonds were also broken out. The components of CH were more complex than native collagen. Therefore, wide distribution and relatively smaller molecular and particle size distribution of the hydrolysate was expected. From size distribution by intensity plots (Figure 4.8), it can be inferred that the size distribution has become wider as

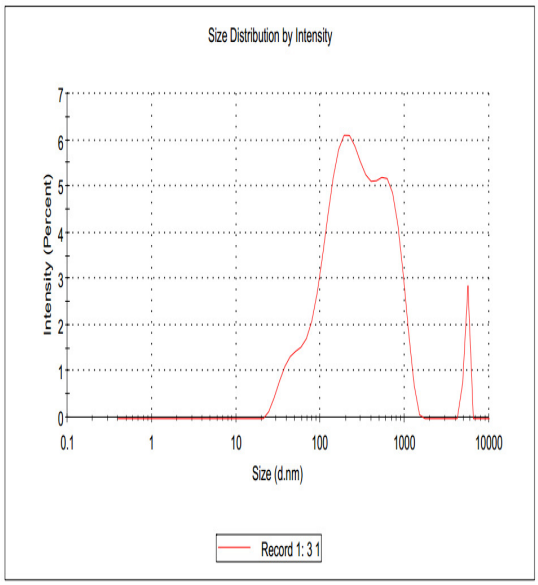
the pH increase to alkalinity. The Z-average (d.nm)/PDI (polydispersity index) values for P_1, P_2, P_3 & P_4 were observed to be 5295/1.000, 1666/1.000, 262.3/0.584 and 638.4/0.638, respectively. From this it can be deduced that the number average particle size which is mainly attributed to lower particle size species is decreasing as the pH decreases. In other words, the size average which is sensitive to large particle size species is increasing in the same trend. Hence, the application of CH (P_1) may not result in its good uptake into meso pores of SS. Moreover, the application of would seem to be preferred for P_2, P_3 and P_4. Particles with intensity distribution showing two or more peaks but with relatively small Z-average and PDI value seem to have better uptake into meso and macropores of skin. The charge distribution of CH at same pH values was also determined using the same instrument (Malvern Zetasizer Nano ZS) with zeta potential dip cell. The ZP values (mV) obtained were -21.0, -25.8, -28.9 and -32.3, respectively as shown in Figure 4.9. The absolute ZP values obviously increases as the pH of the hydrolysate solution increases. If all the particles in suspension have a larger negative or positive zeta potential, then they will tend to repel each other and there is no tendency to flocculate. The decrease in the ZP value from pH values 8.98 to 6.01 may be ascribed to protonation of carboxylic groups of hydrolysate by the acid being used for pH adjustment. The iso-electric point of CH being prepared under thermally supported alkaline condition is normally expected to be in the acid range due to the higher density of carboxylic groups for side amide groups has been hydrolyzed under such preparative conditions. Therefore, CH with ZP at around pH of iso-electric point is less stable. Recent study revealed acidic iso-electric point of CH prepared under such conditions[69]. From the zeta potential values, it can be deduced that the stability increases as the CH pH increases to alkalinity. Generally, particle size alone is not the only determining factor for the extent of uptake of CH by matrix of the skin. The interaction between other treatment chemicals and condition of medium in which the treatment was done all matters. CH at pH 6.01, observed to be having relatively smaller molecular size (172.2 nm), was happened to exhibit smallest percentage of uptake amongst other pH ranges.



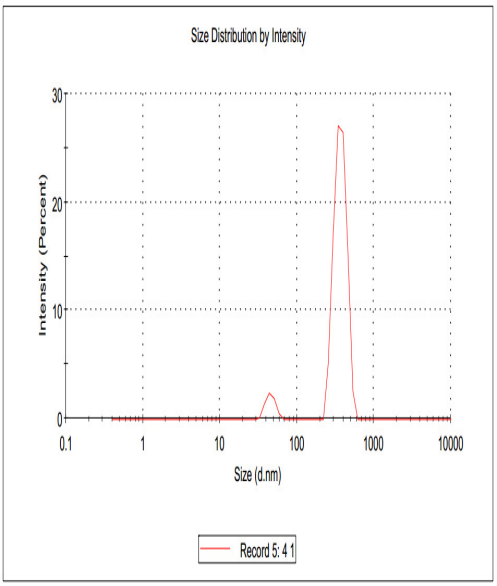
P_1



P_2

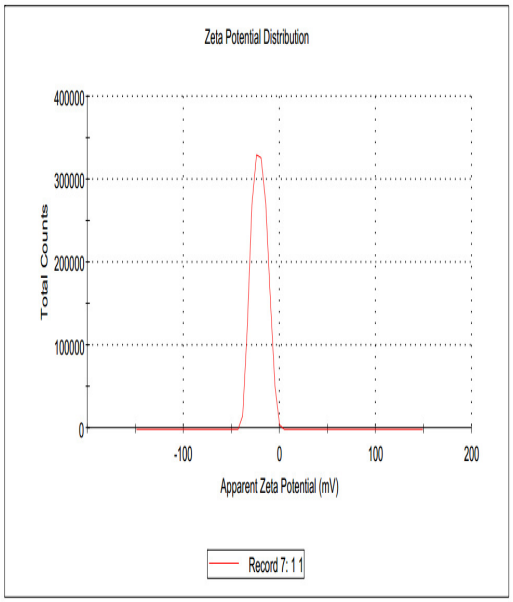


P_3

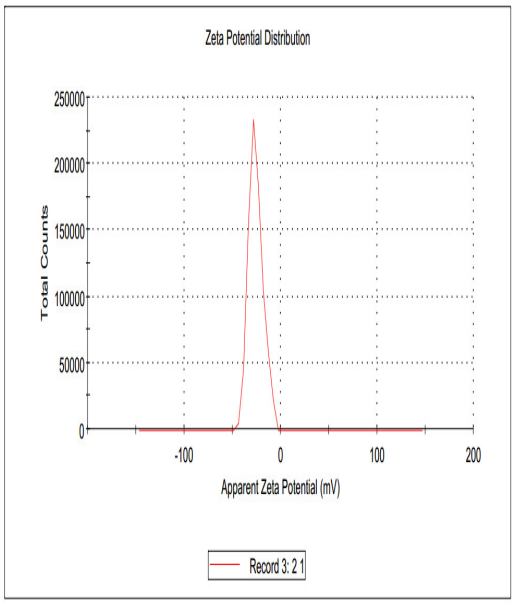


P_4

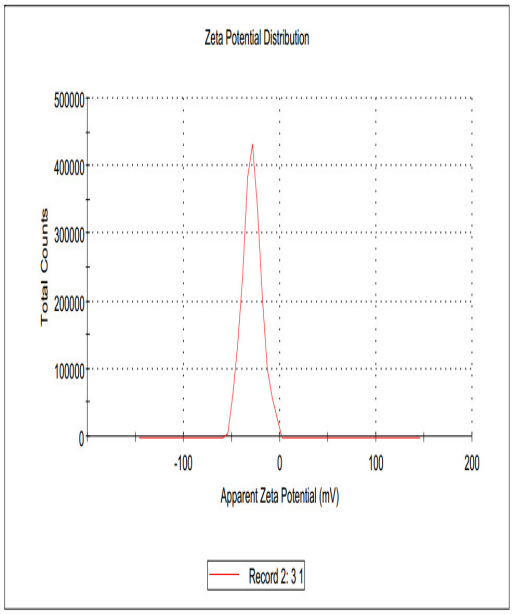
Figure 4.8 Particle size distribution plot of CH at pH 6.01 (P_1), 7.01 (P_2), 7.99 (P_3) and 8.98 (P_4)



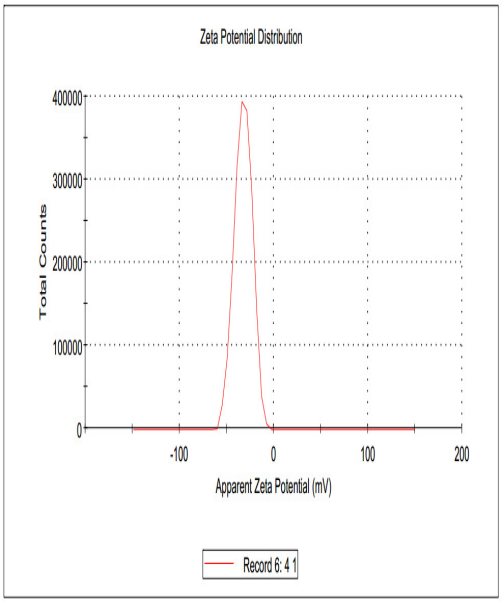
Z_1



Z_2



Z_3



Z_4

Figure 4.9 Zeta potential values of CH at pH 6.01 (Z_1), 7.01 (Z_2), 7.99 (Z_3) and 8.98 (Z_4)

4.2.2. Collagen hydrolysate application evaluation

4.2.2.1. The effect of CH pH & offer on the percent increase in nitrogen and chromic oxide content

In the application of CH during pre-tanning, pH and percentage offer were two parameters considered to investigate the optimum conditions in order to enhance the hide substance of the parts of SS. Collagen hydrolysate at four pH values (6.01, 7.01, 7.89 & 8.98), was applied during pre-tanning and the average increase in percent nitrogen content of belly and butt regions analyzed as presented in graph below (Figure 4.11). Four replicates were made to investigate the maximum increase in percent nitrogen content of belly and butt samples.

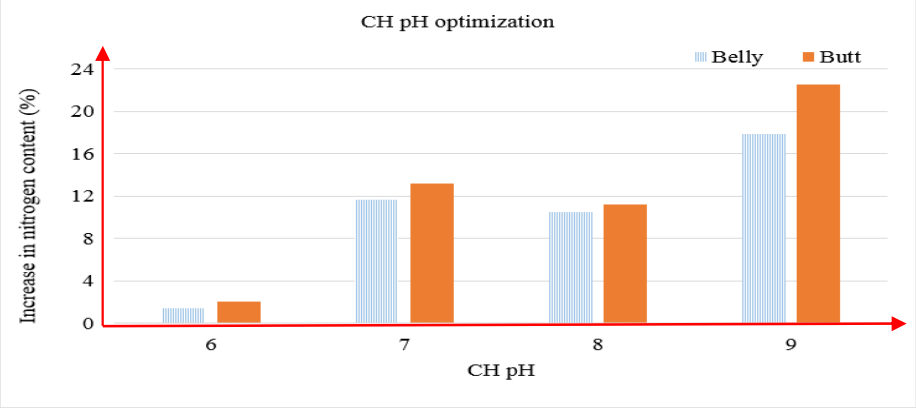


Figure 4.10 Increase in SS's percent nitrogen content vs CH pH

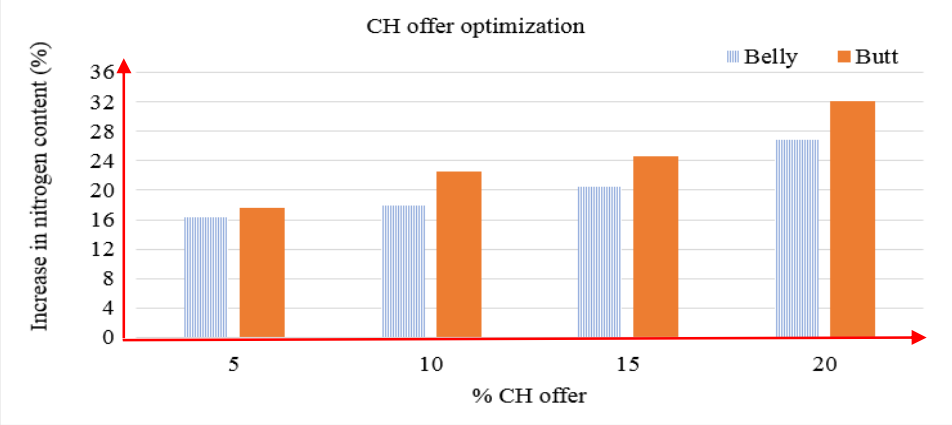
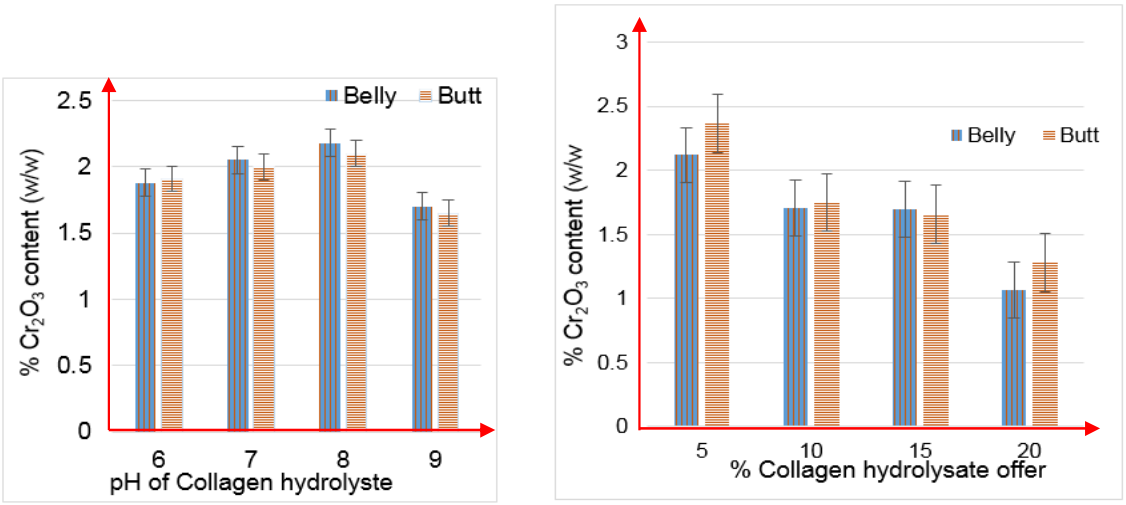


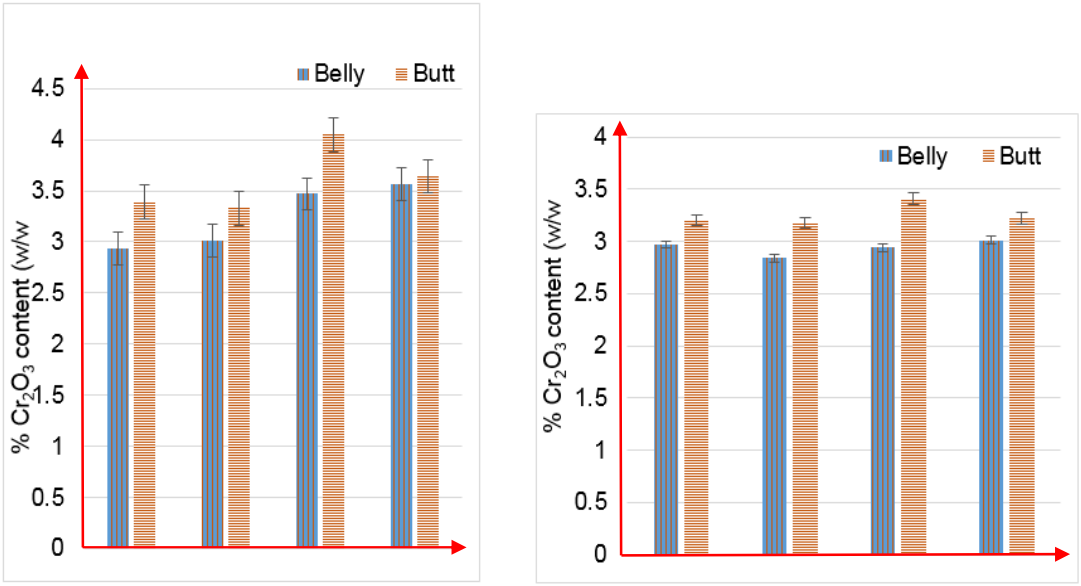
Figure 4.11 Increase in SS's percent nitrogen content vs percent CH offer

Using CH pH rendering maximum increase in nitrogen content, four different percentage CH offer was made and the average values of increase in hide substance for four replicates were presented as in Figure 4.12. The percentage increase in nitrogen of experimental wet blue leathers was calculated with reference to the percent nitrogen content of corresponding control blue leathers. It can be seen from Figure 4.11 that the increase in nitrogen content (%) of experimental belly & butt leather samples was found to be highest at CH pH 8.98 and least at pH 6.01. Similarly, highest increase in nitrogen content (%) was observed at 20% CH offer. On contrary, the percentage chromic oxide content in wet blue leather was observed to be least for highest increase in percent nitrogen content (Figure 4.13). Alternatively, conditions resulting increase in the nitrogen content of skin samples retrograde chrome intake. It was also observed that the nitrogen content of both belly and butt regions of control SS samples slightly get reduced as a result of tanning which might be due to removal of glycosaminoglycans chain. Leather samples of control wet blue leathers were found to exhibit higher percentage chromic oxide content compared to experimental wet blue leathers in all cases. The collagen fibre density may be cause for better uptake or adsorption of CH in butt than belly as observed during both pH and offer optimization processes. The higher uptake of Cr_2O_3 being observed during conventional chrome tanning process (Table 4.1) could supplement the skin fibre density in butt than in belly.



a) b)

Figure 4.12A. Percent Cr_2O_3 (w/w) content of experimental wet blue at various CH: a) pH & b) offer



a) b)

Figure 4.12B. Percent Cr_2O_3 (w/w) content of corresponding control wet blue

Since the nature of chromium species influences the penetration of chromium throughout the SS matrix, CH might have induced change in the polymeric chromium (III) aquo species which resulted in less chromic oxide content in CH treated belly & butt samples. Although subjected to be experimentally justified, CH might have affected the size of polymeric chromium (III) aquo species and/or induced masking effect/or deterred the coordination of carboxyl groups to certain level. The less astringency of basic chrome salt being observed on CH treated belly and butt wet blue leathers would to certain extent support the argument on masking effect of CH. The hydrolysis of side amide groups under temperature supported alkaline preparative condition and higher density of carboxyl groups of CH might have also resulted in chromium (III)-CH carboxyl group complex formation which in turn competitively affected the skin's collagen carboxyl complex formation with Cr^{+3}. During pickle pH adjustment to 2.8/3.2, CH treated skins samples took more sulphuric acid (ca. four times) than the amount offered for control samples which results in higher concentration of sulphate ion in pickle bath. This might induced masking effect for 33% basic chrome tanning solution and hence increased the precipitation pH of chrome sulphate tanning liquor. Study report[70] revealed that introducing sulphate ion to chromium solution causes the creation of stable sulphate complexes and hence the affinity of skin collagen carboxyl group for chromium may remain less at the conventional tanning pH.

The shrinkage temperature (Ts) of belly and butt region wet blue leather of both control and experimental samples were analyzed for CH at pH 9.0 and four different percentage offered values. The mean value of the triplicates were reported and indicated in Table 4.2.

Table 4.2. Ts value for experimental and control wet blue leather

%CH offer	Mean value of Ts (°C)			
	Experimental		Control	
	Belly	Butt	Belly	Butt
5	97 ± 1.5	96 ± 1.2	>100	>100
10	95 ± 1.7	95 ± 1.3	>100	>100
15	93 ± 1.4	93 ± 1.0	>100	>100
20	90 ± 1.5	91 ± 1.0	>100	>100

The mean values of Ts of control belly and butt wet blue leathers samples were found to be higher than corresponding experimental leather samples. The decreasing Ts value was observed as percentage CH offer increased. It can be inferred from Figure 4.12A.b and Table 4.2, the value of percent chromic oxide content has main role for stability of wet blue leathers samples to physically resist heat in water as compared to the presence of CH in the samples.

Statistical analysis

The effect of varying CH pH and percentage offer on the response variable " percent increase in the nitrogen content" for belly and butt regions of skin are statically analyzed with one factor ANOVA technique using Design-Expert 7.0.0 software.

The percentage increase in nitrogen content showed an increasing trend from pH 6.0 to 9.0 except a slight depression at pH 8.0 (Figure 4.13A).

In comparing model variance with residual variance, the ANOVA test result showed F- & p-value for belly/butt 743.52/1118.59 and 0.001/0.001, respectively (Table 4.4). The Model F-value of 743.52/1118.59 implies, the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. "Value of prob. > F" less than 0.0500 indicates CH pH has significant effect on the percentage increase in nitrogen content of both belly and butt regions of SS samples. The smaller residual values also indicate all the data are with the range (Annex 7).

Analysis of Variance for CH pH

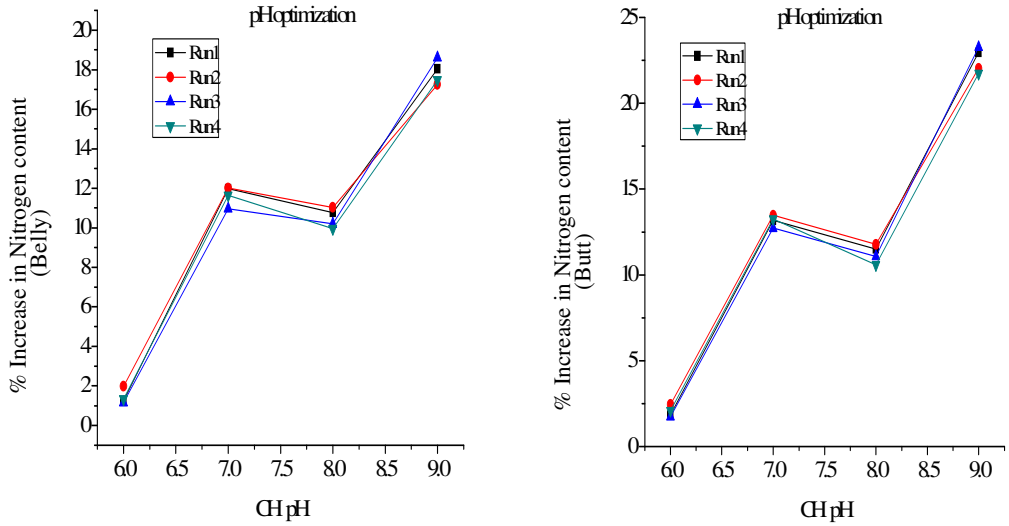


Figure 4.13A. Graph of percent nitrogen content increase vs CH pH

Table 4.3 Diagnostic case statistics for CH pH

Factor	Actual value		Predicted Value		Residual		Run
(CH pH)	Belly	Butt	Belly	Butt	Belly	Butt	order
6	1.25	2.45	1.43	2.03	-0.18	0.42	14
6	1.98	2.09	1.43	2.03	0.55	0.058	2
6	1.16	1.85	1.43	2.03	-0.27	-0.18	12
6	1.34	1.74	1.43	2.03	-0.092	-0.29	6
7	11.98	13.18	11.65	13.16	0.33	0.020	4
7	12.01	13.47	11.65	13.16	0.36	0.31	11
7	10.97	13.26	11.65	13.16	-0.68	0.100	15
7	11.64	12.73	11.65	13.16	-1.000E-002	-0.43	3
8	10.76	10.58	10.49	11.24	0.27	-0.66	1
8	11.03	11.78	10.49	11.24	0.54	0.54	16
8	10.20	11.08	10.49	11.24	-0.29	-0.16	8
8	9.96	11.51	10.49	11.24	-0.53	0.27	9
9	18.04	23.27	17.84	22.50	0.20	0.77	7

Factor	Actual value		Predicted Value		Residual		Run
(CH pH)	Belly	Butt	Belly	Butt	Belly	Butt	order
9	17.25	22.96	17.84	22.50	-0.59	0.46	5
9	18.61	21.73	17.84	22.50	0.77	-0.77	13
9	17.48	22.04	17.84	22.50	-0.36	-0.46	10

Table 4.4 Analysis of Variance for CH pH

	Sum of squares		df		Mean square		F value		p-value prob. > F	
Source	Belly	Butt	Belly	Butt	Belly	Butt	Belly	Butt	Belly	Butt
Model	549.62	845.25	3	3	183.21	281.75	743.52	1118.59	$< 10^{-4}$	$< 10^{-4}$
CH pH	549.62	845.25	3	3	183.21	281.75	743.52	1118.59	$< 10^{-4}$	$< 10^{-4}$
Pure Error	2.96	3.02	12	12	0.25	0.25				
Cor Total	552.58	848.27	15	15						

Table 4.5 Values for reasonable agreements

Parameter	Belly	Butt	Parameter	Belly	Butt
Std. Dev.	0.05	0.05	R-squared	0.9946	0.9964
Mean	10.35	12.23	Adj R-Squared	0.9933	0.9955
C.V. %	4.79	4.10	Pred R-Squared	0.9905	0.9937
PRESS	5.26	5.37	Adeq Precision	66.127	81.564

The model showed an adequate fit in the estimation data (R^2=0.9946/0.9964) as well as good prediction performance in the test data (Rp^2=0.9905/0.9937) for both belly and butt SS samples, respectively. The "Pred R-Squared" of 0.9905/0.9937 is in reasonable agreement with "Adj R-squared" of 0.9933/0.9955 for belly/butt, respectively. This model can be used to navigate the design space since the "Adeq Precision" values, 62.127/81.564, which are greater than 4 in both cases, showed adequate signal.

Analysis of Variance for CH offer

The percentage increase in nitrogen content showed an increasing trend from 5.0 to 20.0, (Figure 4.13B).

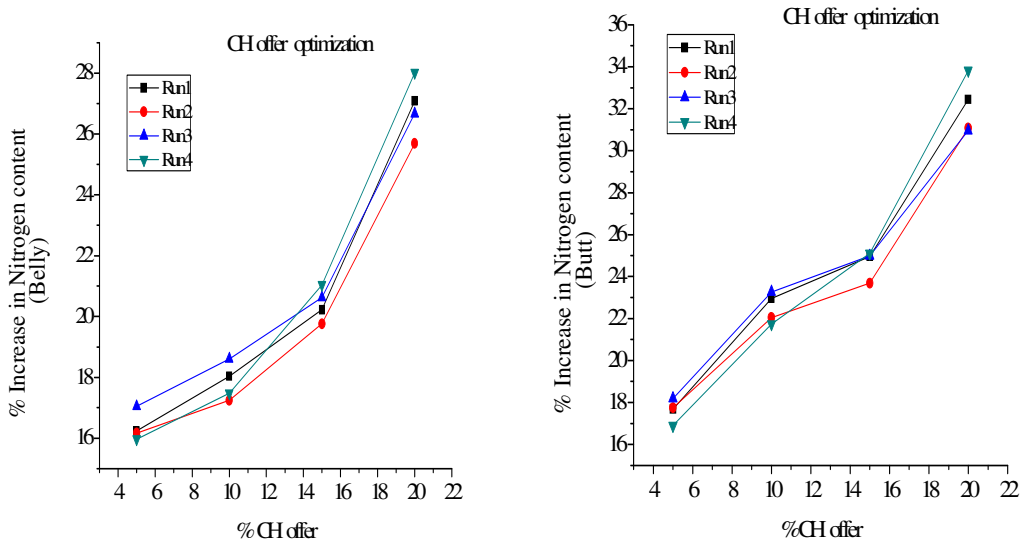


Figure 4.13B. Graph of percent increase in nitrogen content vs % CH offer

In comparing model variance with residual variance, the ANOVA test result showed F- & p-value for belly/butt 189.03/187.44 and 0.001/0.001, respectively (Table 4.7). The Model F-value of 189.03/187.44 implies, the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. "Value of prob. > F" less than 0.0500 indicates CH offer has significant effect on the percentage increase in nitrogen content of both belly and butt regions of SS samples. The smaller residual values also indicate all the data are within the range (Annex 8).

The model showed an adequate fit in the estimation data (R^2=0.9793/0.9791) as well as good prediction performance in the test data (Rp^2=0.9632/0.9629) for both belly and butt SS samples, respectively. The "Pred R-Squared" of 0.9632/0.9629 is in reasonable agreement with "Adj R-squared" of 0.9741/0.9739 for belly/butt, respectively. This model can be used to navigate the design space since the "Adeq Precision" values, 31.117/32.917, which are greater than 4 in both cases, showed adequate signal.

Table 4.6 Diagnostic case statistics for CH offer

Factor (CH offer)	Actual value		Predicted Value		Residual		Run order
	Belly	Butt	Belly	Butt	Belly	Butt	
5	16.24	17.68	16.36	17.63	-0.12	0.055	1
5	16.17	16.89	16.36	17.63	-0.19	-0.73	11
5	17.05	18.19	16.36	17.63	0.69	0.57	8
5	15.97	17.74	16.36	17.63	-0.39	0.11	15
10	18.04	21.73	17.84	22.50	0.20	-0.77	3
10	17.25	22.96	17.84	22.50	-0.59	0.46	13
10	18.61	22.04	17.84	22.50	0.77	-0.46	12
10	17.48	23.27	17.84	22.50	-0.36	0.77	5
15	20.22	24.95	20.41	24.68	-0.19	0.27	4
15	19.76	23.68	20.41	24.68	-0.65	-1.00	7
15	20.63	24.99	20.41	24.68	0.22	0.31	14
15	21.04	25.11	20.41	24.68	0.63	0.43	9
20	27.09	31.08		32.07	0.23		16
20	25.69	32.44		32.07	-0.17		2
20	26.67	33.82		32.07	-0.19		6
20	28.01	30.96		32.07	1.15		10

Table 4.7 Analysis of Variance for CH offer

	Sum of squares		df		Mean square		F value		p-value prob. > F	
Source	Belly	Butt	Belly	Butt	Belly	Butt	Belly	Butt	Belly	Butt
Model	258.65	433.47	3	3	86.22	144.49	189.03	187.44	$< 10^{-4}$	$< 10^{-4}$
CH pH	258.65	433.47	3	3	86.22	144.49	189.03	187.44	$< 10^{-4}$	$< 10^{-4}$
Pure Error	5.47	9.25	12	12	0.46	0.77				
Cor Total	264.12	442.72	15	15						

Table 4.8 Values for reasonable agreements

Parameter	Belly	Butt	Parameter	Belly	Butt
Std. Dev.	0.68	0.88	R-squared	0.9793	0.9791
Mean	20.37	24.22	Adj R-Squared	0.9741	0.9739
C.V. %	3.32	3.62	Pred R-Squared	0.9632	0.9629
PRESS	9.73	16.44	Adeq Precision	31.117	32.917

4.2.2.2.Tensile strength, percent elongation and grain distention test

To ease graphical presentation and comparison, the mean load burst values of all leather samples were taken multiplying the actual value by factor 0.1.

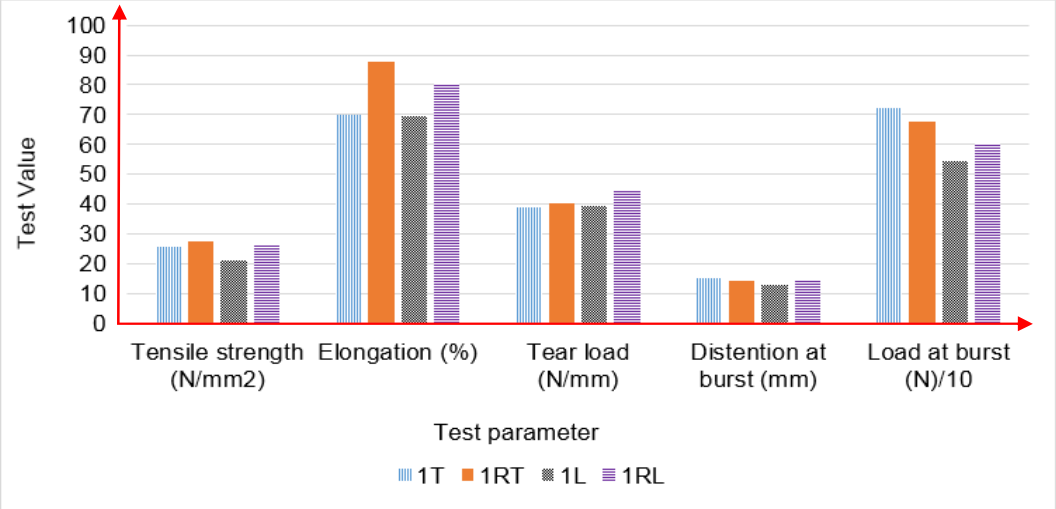


Figure 4.14 Graphical representation of average physical test values experiment, control & blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

It can be seen from the graph (Figure 4.14) that tensile strength values of control belly and butt leathers were a little bit higher than the corresponding experimental leather except it appeared to be lesser for CPF F_2. This might have been due to better compaction rendered by the other three commercial fillers when compared with CH. The lesser tensile strength of experimental belly and butt leathers perhaps be due to lesser uptake of CH by the grain on the relative terms with CPFs. The relative higher load at burst values of experimental leathers complement same argument which in fact obviate their lesser percent extension. Agreeing up on the effect of natural fibre structure of the input material on the strength of the leather, the degree of filling

up of intermittent layers between papillary and reticular regions also dictates the load at burst value. The higher filling up of the regions avoid looseness and generally reduce bursting value. Prone to similar argument, better grain filling effect which could be of smaller molecular size of commercial fillers than CH is ascribed to relatively lesser corresponding distention burst values.

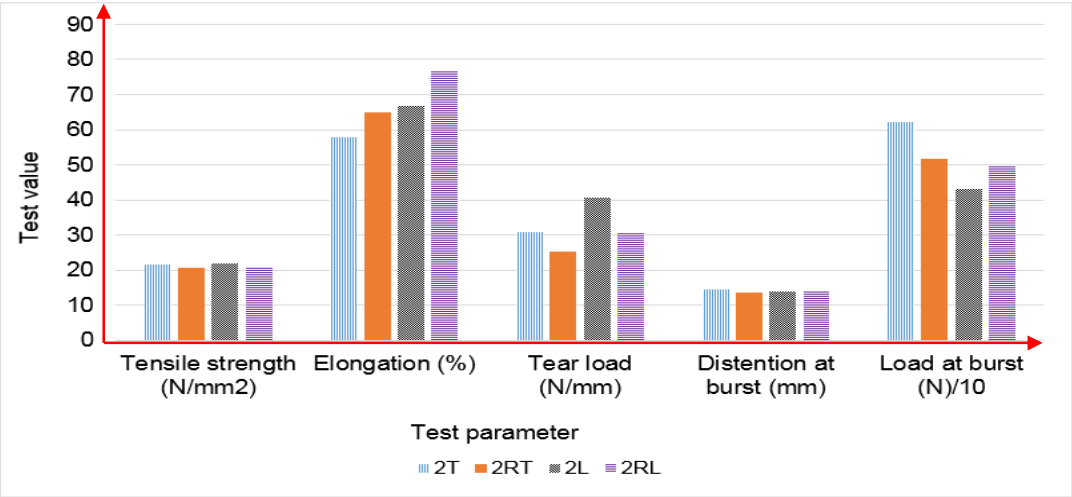


Figure 4.14 Graphical representation of average physical test values experiment, control & blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

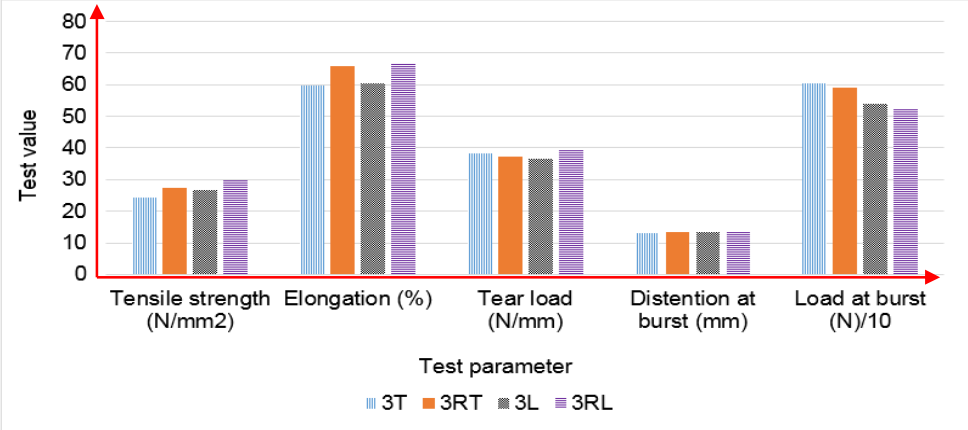


Figure 4.14 Graphical representation of average physical test values experiment, control & blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

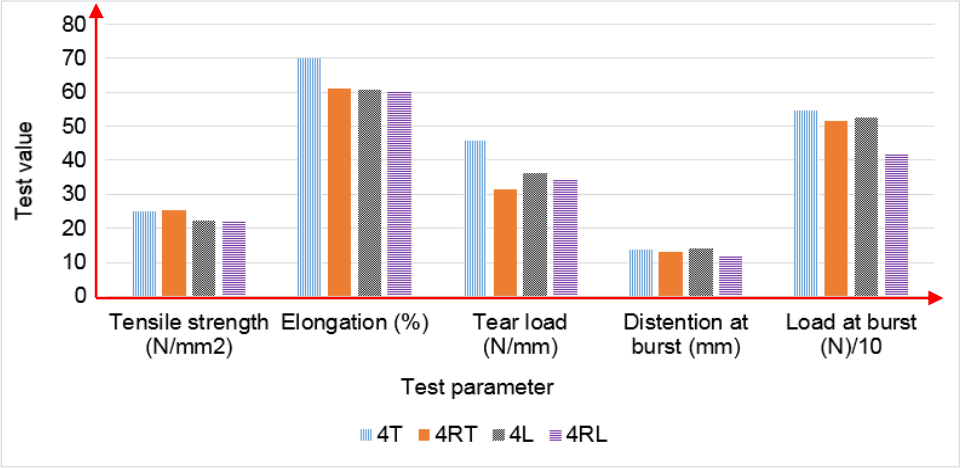


Figure 4.14 Graphical representation of average physical test values experiment, control & blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

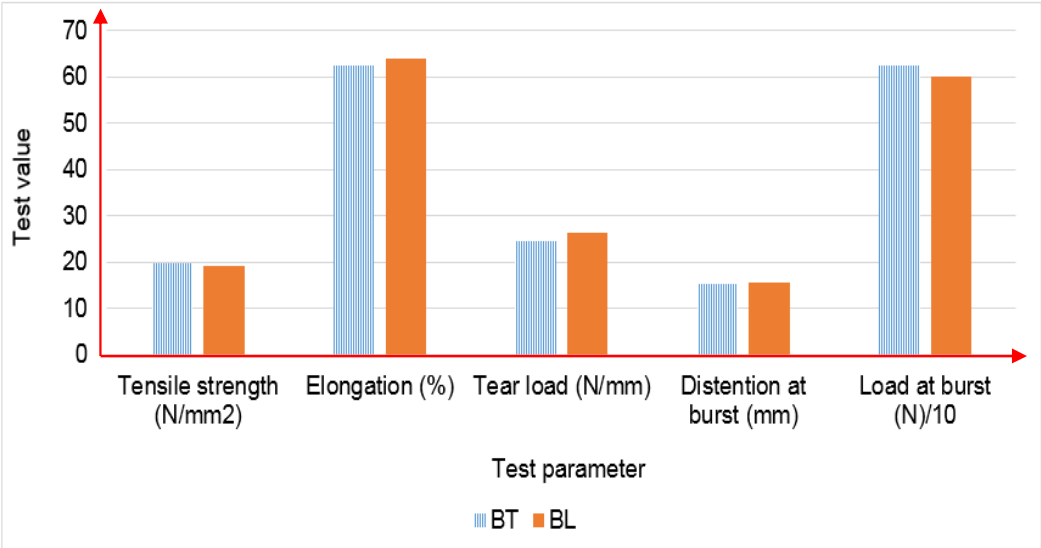


Figure 4.14 Graphical representation of average physical test values experiment, control & blank dyed crust leathers.

**BT/BL- blank experimental Butt & Belly*

It is also seen that control belly and butt leathers showed lesser tear strength except in process number 1 depicting best lubrication power of F_1 among the others. This in turn also indicates that the strength of the leather is primarily dependent on the lubrication power of treatment chemicals. Blank belly and butt dyed crust leathers showed lesser tensile and tear strength

among the control and experimental leathers suggesting lesser fiber compaction and lubrication. The overall physical property rendered by commercial protein filler F_4 is comparable to CH effect and indeed have higher crude protein content. The corresponding physical test values also showed variation among experimental leathers (Annex 5). This can be a fair indication of the fact that bulk properties of leathers are considerably affected by the nature of input raw material which mainly be affected the mode of rearing. In general, it has been shown that CH enhanced the substance of belly part of SS thereby rendering better value addition to same.

4.2.2.3. Organoleptic properties test

Organoleptic properties viz *softness*, *fullness*, *roundness*, *grain tightness*, *color uniformity*, *grain smoothness* and *general appearance* of each control and experimental crust leathers were assessed with three senior leather experts for all three replicates. The average values of triplicates of four processes were taken (Annex 6) and represented graphically as in the Figure 4.15. It is seen that the mean values functional properties, grain tightness, color uniformity and grain smoothness, on average have closeness among experimental and corresponding belly and butt dyed crust leather. This reveals that there has not been deterrence in the uniform distribution of post-tanning chemicals, particularly on the grain, due of CH and commercial protein fillers. The closeness in grain tightness among experimental and control leathers may be due the common effect of acrylic polymer being used and relatively larger molecular size of both CH and protein fillers not to be up taken by the intermediate layer. The softness value of experimental leathers in most cases appeared to be slightly lesser than the corresponding control leathers which may be attributed to less fibre lubrication effect perhaps ascribed to neutral salts. In general, both experimental and control belly and butt leathers showed closeness in fullness properties which reveals that both CH and protein fillers taken up by skin macropores. Observed rate values for fullness and roundness supplement same. Form view point of overall tactile properties, process number 4, commercial filler having higher percentage of crude protein content, showed difference among experimental and control leathers.

Blank belly and butt dyed crust leather found to score less except comparable average rate points observed for color uniformity and grain smoothness properties. It can be inferred that CH and CPFs enhance majority of functional properties.

Figure 4.15 Graphical representation of organoleptic properties of control, experimental and blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

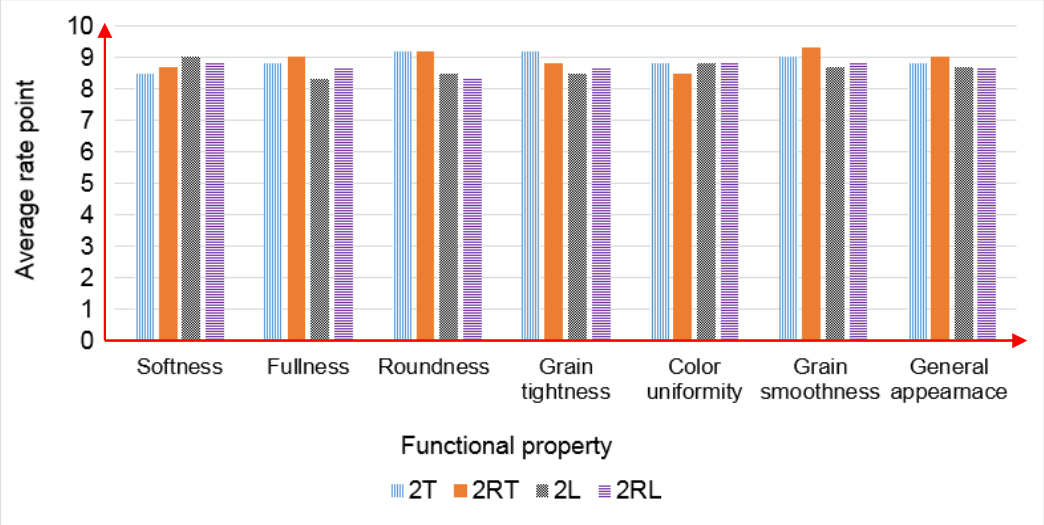


Figure 4.15 Graphical representation of organoleptic properties of control, experimental and blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

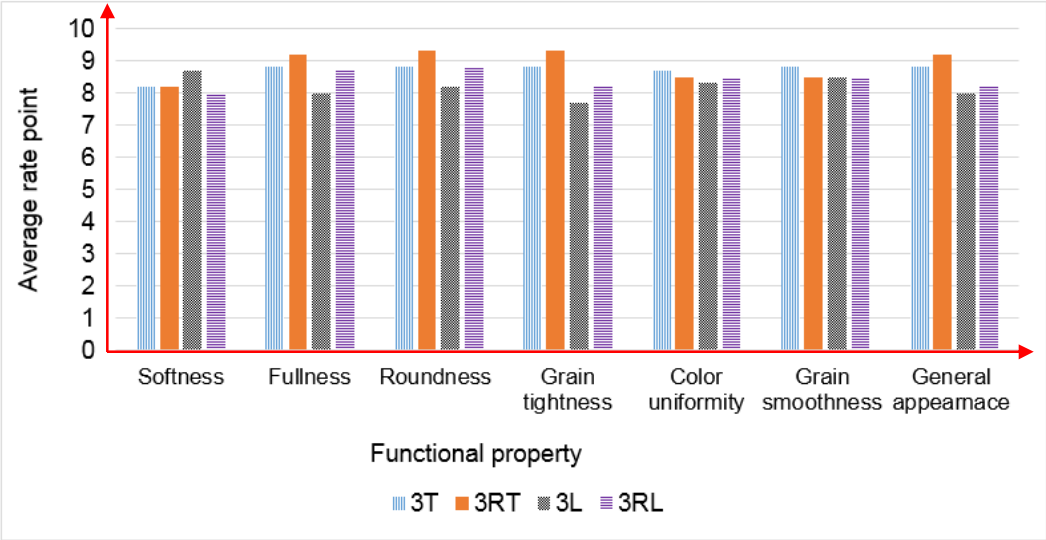


Figure 4.15 Graphical representation of organoleptic properties of control, experimental and blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

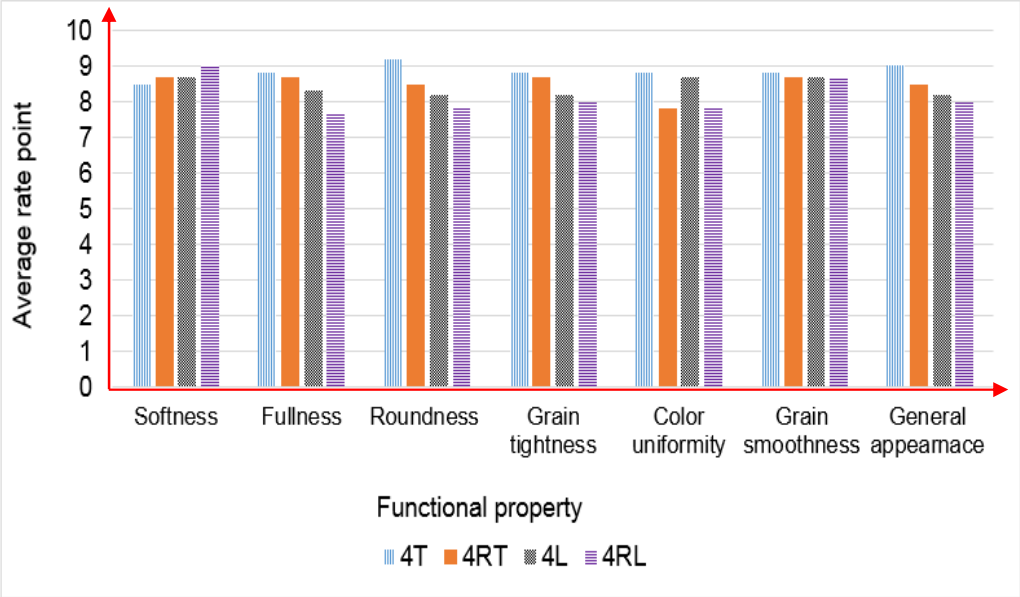


Figure 4.15 Graphical representation of organoleptic properties of control, experimental and blank dyed crust leathers.

**T/L- experimental Butt & Belly; RT/RL- control Butt & Belly*

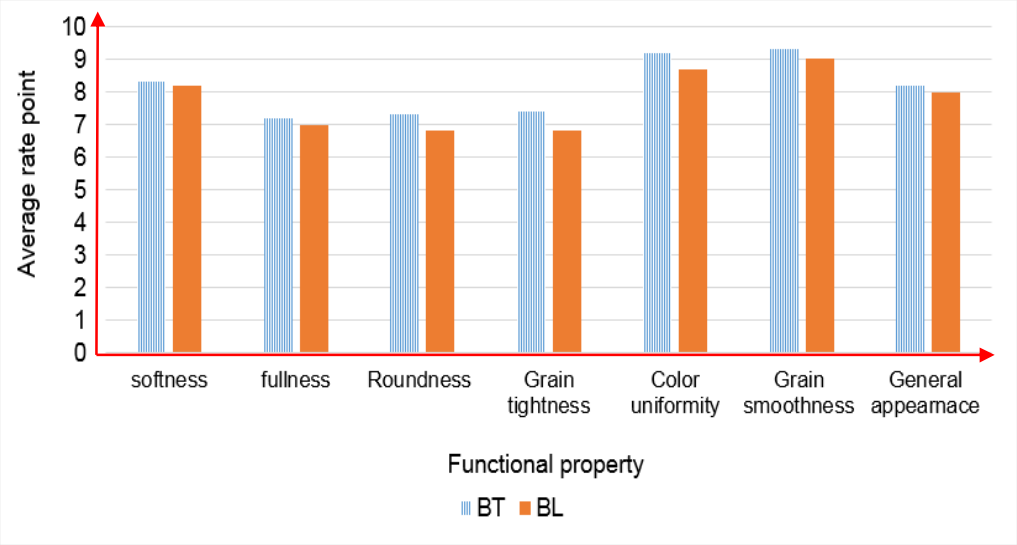


Figure 4.15 Graphical representation of organoleptic properties of control, experimental and blank dyed crust leathers.

**BT/BL- blank experimental Butt & Belly*

4.2.2.4. Color measurement & evaluation

To evaluate the effect of CH treatment with respect to commercial protein fillers, the L*a*b* values of navy blue dyed crust leather samples of four processes were measured using TECHKON SpectroDrive instrument. ΔL*a*b*, total color difference (ΔE^*_{ab}), difference in hue (ΔH), and difference in chroma (ΔC) among experimental and control belly and butt leather samples were recorded and the average values of the triplicates were reported (Table 4.9). The ΔL* values in almost all belly and butt leather samples appeared to be negative or close to zero. This indicates that the darkness of experimental leathers are far more than the corresponding control leather samples. Meaning, control leathers are lighter than experimental one. Values of Δa* and Δb* belly and butt dyed curst leathers found to lie around zero indicating there hardly has been color alteration due to hydrolysate and protein fillers. The highest value of total color difference is also in concurrent with the difference in the darkness between control and experimental leathers. The fact that values of difference in hue and total color difference in all of the leather samples augment same notion. Therefore, both saturation and lightness among control and experimental belly and butt leathers are varied.

Table 4.9 CIE Lab values of experimental and control belly and butt crust leathers

L*a*b* values							
Parameter	Control		Experimental		Deviations		
	1RL	1RT	1L	1T	Parameter	Belly	Butt
L*	24.17	23.21	18.01	20.19	ΔL*	-6.16	-3.02
a*	0.36	0.47	0.79	0.85	Δa*	0.43	0.38
b*	-10.84	-10.93	-8.76	-9.12	Δb*	2.08	1.81
C	10.85	10.94	8.80	9.16	ΔC	-2.05	-1.78
					ΔE^*_{ab}	6.52	3.54
					ΔH	0.55	0.50
	2RL	2RT	2L	2T			
L*	26.99	26.76	19.85	21.30	ΔL*	-7.14	-5.46
a*	0.31	0.38	0.72	0.67	Δa*	0.41	0.29
b*	-11.31	-12.33	-8.00	-7.60	Δb*	3.31	4.73
C	11.31	12.34	8.03	7.63	ΔC	-3.28	-4.71
					ΔE^*_{ab}	7.88	7.23
					ΔH	0.59	0.55
	3RL	3RT	3L	3T			
L*	23.00	19.29	18.70	19.52	ΔL*	-4.3	0.23
a*	0.68	0.90	0.72	0.68	Δa*	0.04	-0.22
b*	-10.03	-8.86	-7.32	-7.74	Δb*	2.71	1.12
C	10.05	8.91	7.36	7.77	ΔC	-2.70	-1.14
					ΔE^*_{ab}	5.08	1.16
					ΔH	0.26	0.11
	4RL	4RT	4L	4T			
L*	31.91	31.29	20.87	21.04	ΔL*	-11.04	-10.25
a*	-0.30	-0.12	0.64	0.78	Δa*	0.94	0.9
b*	-14.96	-14.56	-7.85	-8.29	Δb*	7.11	6.27
C	14.96	14.56	7.88	8.33	ΔC	-7.09	-6.23
					ΔE^*_{ab}	13.17	12.05
					ΔH	1.10	1.12

Control sample being treated with F_3 appeared to have lowest total color difference value which shows its darkness is closest to the corresponding experimental leather compared to the remaining control leathers. This could be envisaged by the fact that the affinity of commercial protein filler F_3 is close to CH. Process number four resulted in largest ΔE^*_{ab} value. This might have been due to less affinity F_4 relative to CH. To further investigate the effect of CH & commercial protein filler on the leather color, the L*a*b* values and differences of experimental and control leathers of process number 4 were executed and compared with dyed belly and butt crust leathers of blank process (without both CH and any protein filler). The mean values of three replicates were presented in Table 4.10. The ΔL* values of control belly and butt leather samples appeared to result in negative but lesser negative value than the ΔL* values of corresponding experimental leathers, both against same blank leathers.

Table 4.10 CIE Lab values of dyed crust leathers from blank, experimental and control processes

	Blank		Experimental[4]		Deviation		
Parameter	Belly	Butt	Belly	Butt	Parameter	Belly	Butt
L*	33.64	31.59	20.87	21.04	ΔL*	-12.77	-10.55
a*	-0.53	-0.21	0.64	0.78	Δa*	1.17	0.99
b*	-15.47	-15.15	-7.85	-8.29	Δb*	7.62	6.86
c	15.48	15.15	7.88	8.33	ΔC	-7.60	-6.82
					ΔE^*_{ab}	14.92	12.62
					ΔH	1.28	1.21
	Blank		Control[4]		Deviation		
Parameter	Belly	Butt	Belly	Butt	Parameter	Belly	Butt
L*	33.64	31.59	31.91	31.29	ΔL*	-1.73	-0.3
a*	-0.53	-0.21	-0.3	-0.12	Δa*	0.23	0.09
b*	-15.47	-15.15	-14.96	-14.56	Δb*	0.51	0.59
c	15.48	15.15	14.96	14.56	ΔC	-0.52	-0.59
					ΔE^*_{ab}	1.82	0.67
					ΔH	0.22	0.08

[4]*process number 4.*

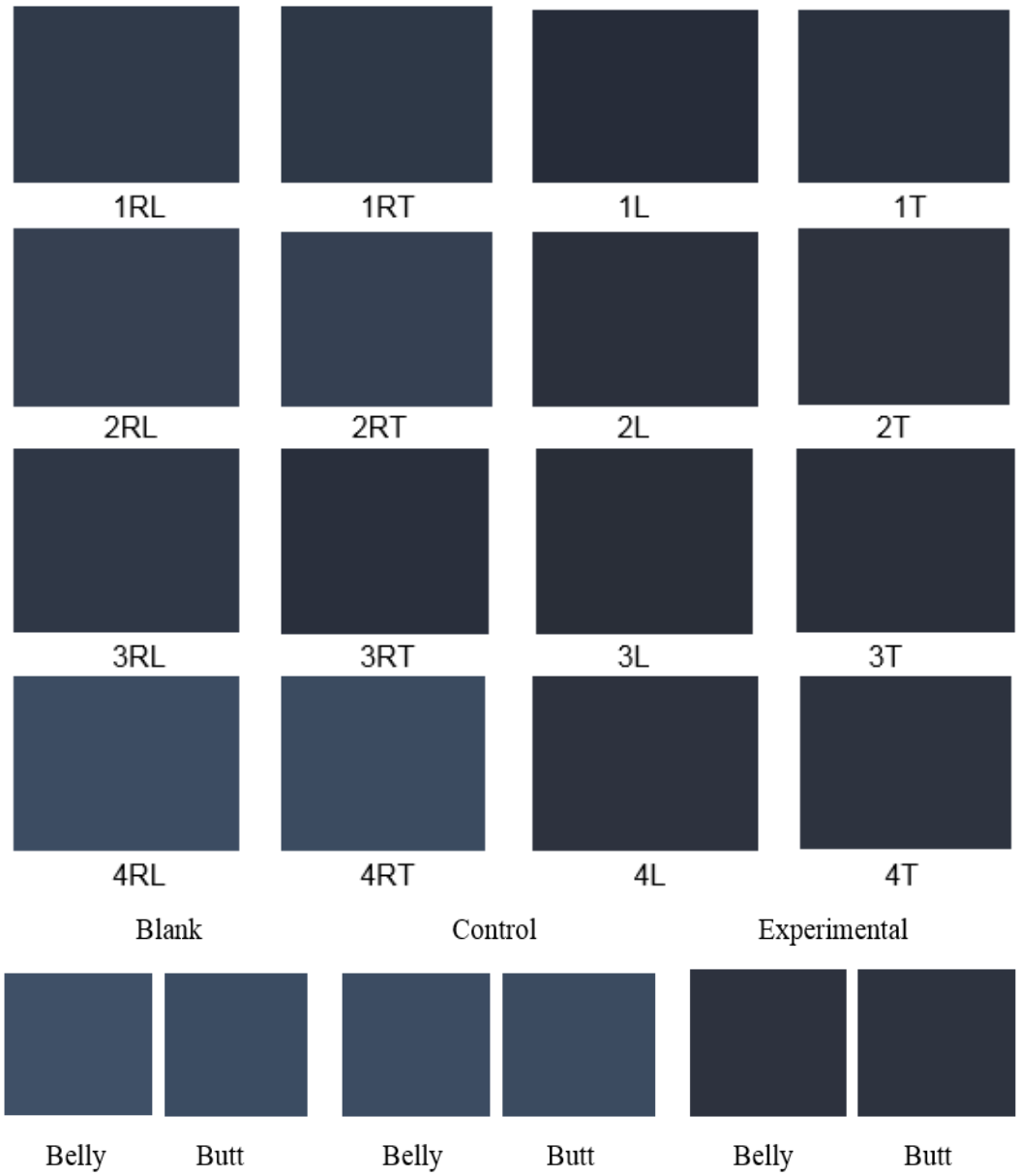


Figure 4.16 Colors of Navy blue dyed crust leathers as per the measured average L*a*b* values.
T/L- experimental Butt & Belly; RT/RL- control Butt & Belly and coefficients representing process number, respectively.

This indicates that blank belly and butt dyed crust leathers have relatively lesser intense color than control ones. This might have been due to the existence of positive affinity of commercial protein filler (F_4) with the dye. The negative ΔL^* values of experimental leathers depict the fact that process with CH has better dye fixation than both blank and control processes.

Based on L*a*b* values being measured, the corresponding color of each leather samples at illuminant D 65 and observer 2^{o} (1931) was obtained and presented as in Figure 4.16. In general, the relative color intensity (depth) experimental leathers was found to be more than the respective control leathers. This is due to the fact that CH provides for additional binding sites for dye, thereby increasing the dye affinity and better uptake. Therefore, the synergistic effect of collagen hydrolysate in fixing the dye than CPFs was manifested which could be helpful in reducing the consumption of dyestuff owing to attain the required color depth. The percent total ash content of both experimental and control crust leathers of fourth process was measured and found to be 3 and 2, respectively. The lesser value of ash content of control leather compared to experimental one may be due to higher sulfuric acid consumption during pickling in the latter which resulted in more sulphated salt content.

4.2.2.5. TS, TDS & COD determination

TS, TDS and COD values of chrome and re-tanning & dyeing spent solutions were analyzed to know the effect of CH (experimental) and CPFs (control) on the pollution load of the corresponding processes. Blank post-tanning process (without both CH & CPFs) was analyzed for same emission parameters. To have an insight on same, process number 4 (with F4) having the highest total color difference was considered. The average values of triplicates were taken and presented in Table 4.11.

Table 4.11 Average TS, TDS & COD values of chrome and re-tanning & dyeing spent liquors for experimental, control and blank processes

	Emission (ppm)			Effluent volume	Emission load (Kg/ton$^{ct/rd}$)		
Process	TS	TDS	COD	(L/ton$^{ct/rd}$)	TS	TDS	COD
CTE	87,351	74,400	3,369.3	1522^{a}	132.9	113.2	5.1
CTC	56,278	48,400	1,206.0	959^{a}	54.0	46.4	1.2
RDE	31,136	25,628	15,267.5	3403^{b}	106.0	87.2	52.0
RDC	41,168	35,736	23,022.4	3407^{b}	140.3	121.8	78.4
RDB	30,551	24,830	13,427.6	3410^{b}	104.2	84.7	45.8

**CT/RD-chrome tanning/re-tanning & dyeing; E/C/B- experimental/control/blank; ^{a}Liquor solution just at the end basification duration, ^{b}Liqour solution just at the end of main re-tanning & dyeing exhaustion duration; ^{ct}Weight of fleshed pelt for CTE/CTC and rdWeight of wet blue for RDE/RDC/RDB, respectively.(Ref. Annex 1.B/C).*

To help have better information on the impact of CH and protein commercial filer on the environment, emission (ppm) values based on TS, TDS & COD of experimental and control processes were converted emission loads (Table 4.10). The concentration of three emission parameters (TS, TDS & COD) for experimental chrome tanning (with CH) is found to be higher than that of control tanning process. Higher amount of sulphuric acid was used during pickling in experimental process and might induced more neutral salts to the tanning bath. Therefore, the presence of CH in the tanning liquor and consumption of higher sulphuric acid perhaps synergistically rise the values of TS, TDS & COD for the experimental process than for control process. Moreover, blank re-tanning & dyeing process found to have relatively lower emission load than experimental process. This perhaps could be due to stripping off physically adsorbed CH and neutral salt in experimental blue leather. The less uptake of basic chromium sulphate observed in experimental blue leather could also augment same analogy. Less quantum of water being used during tanning seems to account for high shooting TS, TDS & COD values for experimental and control tanning processes. Likewise, relatively higher ash content of experimental leather could be in favor of more presence of neutral salt in experimental tanning process. The emission load of tanning process seems to be on par with the existing trend. However, the emission load values of post-tanning process was much higher than that of tanning process as logically expected. Higher values being observed for control process seems to be due to incomplete uptake of protein filler in the bath. In the experimental re-tanning & dyeing process, relatively lower emission values were observed which may be due to better exhaustion of fatliqours and dyestuff which indeed was manifested in better color depth of dyed crust leather of same process. Therefore, the use of CH enhances in the leather substance and color depth and hence is economically beneficial with respect to value addition particularly for thin part of skin.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The type and source of raw materials have considerable roles in the properties of leather produced out of them. The nature of fibre structure and matrix constituents of raw hid/skin in particular has effect on the type of processing to be followed and overall properties of the leather. That is the reason why designing leather processing recipe as per the origin of raw material is technically required.

The biochemical analysis of both the butt and belly of sheep skin indicated considerable differences existing between the two regions of the hair sheep 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 fibre among the two regions. The histological examination revealed presence of more voids in belly due to fat pockets. Difference in pore size distribution, pore volume and intensity has been observed between butt and belly region during leather processing. This is 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 is no significant differences in the shrinkage temperature in butt and belly regions. From the overall biochemical and morphological analysis, the fibre density constituting skin matrix play vital role in the uptake of treatment chemicals as the percent uptake is shown to be more in butt region than belly.

In the investigation of effects of applying CH during pre-tanning, it happened to be seen that CH increased the percent nitrogen content of SS but retrospectively reduce the chrome intake of the skin. This would indicate the unmodified CH has an effect on the polymeric chromium aquo species having better tanning effects. With respect to the bulk properties of the final leather, the effects of CH and protein commercial fillers were assessed. The evaluation indicated that CH has rendered crust leather with overall better physico-chemical properties except raise in ash content and emission load. The depth of dyed crust leathers' color appeared to be enhanced with CH compared to commercial protein fillers. The fibre density of SS plays its part in up taking CH as the increase in nitrogen content of butt part of SS appeared to be more than belly. In order to increase the value of leather belly characteristics should be analogous with the butt characteristics and this study provides detailed investigation of butt and belly regions of sheep skin. Therefore, collagen hydrolysate has economic value addition particularly for lower end leathers.

5.2. Recommendation

- Since the biochemical analysis of both belly and butt parts of the skin revealed considerable difference in matrix constituents, it will be recommendable that chemical manufacturers and tanners give attention to analogously enhance the bulk properties of belly part of the skin with that of its counterpart.
- To cater complete information on belly and butt regions of SS collagen, amino acid sequencing, peptide mapping and solubility trend with both solubilization method need be investigated which could be further area of study.
- The use of CH during tanning was seen to increase the protein content of belly part of the skin. However, the tanning chrome intake was reduced at the same time which might have triggered the change in polymeric aqueous chromium species having better tanning power. Therefore, CH application based on mode of preparation, modification and sequence of addition during tanning may need further investigation to enhance leather bulk property and without hampering the ease of waste treatment associated with the effluent.
- Although CH at pH 9.0 would statistically appeared to give better percent increase in nitrogen content, further investigation may be need to practically assure optimized pH for CH to be used during pre-tanning.
- It has been observed that the intensity of leather color and overall bulk properties are enhanced using CH. Therefore, it is recommendable to use CH for value addition of lower end products for it is easily prepared and availability of limed pelt trimmings.

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Annex

Annex 1. Process recipe used in the experiment

A) Soaking- Liming process recipe

Study Area	Leather Industry Development Institute	**Date:**----------
Type of Leather	Wet blue sheep skin	**Lot no.:**----------
Input material	**Type:** Wet salted hair sheep skin **Size:** Medium **Q'ty:** ---------- Weight:----------	**Actual weight**: ----------
% Based on: Actual wet salted weight		Page: 1 of 1

Process	**%**	**T (°C)**	**Chemicals**	**Time**	**pH**	**Remarks**
Pre-soaking	Load desalted skins into empty and clean drum					
	300	20	Water			
	0.2		Wetting agent	5'		
			Stop	40'		
			wash	20'		Drain float
	300	20	Water	20'		Drain float
Main soaking	300	20	Water			
	0.2		Wetting agent			
	0.2		Anti-bacteria			
	0.4		Soda ash	20'		
	0.4		Soda ash	15'		
			Stop 30' and check pH			pH=9.5/10
Adjust pH			Soda ash	15'		Stop 30'
			Run 3' every 30'	18hr		
Next day	Take samples for analysis! Drain, rinse and unload skins for piling.					
Unharing	Paint skins with paint soln. and pile flat flesh to flesh for 3-4 hrs. Then dewool the skins manually.					
Liming	200	cold	Water			
	0.1		Lime powder	5'		
	Add dewooled pelt to the drum and run			15'		
	0.5		Sodium sulphide flake	15'		
	0.5		Liming auxiliary	15'		
			Run 3' every 30'	18hr		
Next day	Take samples for analysis! and Drain			5'		
	100		Water	10'		
			Drain	5'		
			Discharge for fleshing			
After fleshing, excise belly and butt parts of the pelt as per the sampling designed!						

*-skin samples and spent liquor samples taken for analysis

B) Deliming-Tanning process recipe

Study Area	Leather Industry Development Institute	**Date:**----------
Type of Leather	Wet blue sheep skin	**Lot no.:**----------
Input material	**Type:** Fleshed pelt skin **Size:** NA **Q'ty:** ---------- **Weight:** ----------	**Actual weight**: ----------
% Based on: Actual fleshed pelt weight		Page: 1 of 2

Process	**%**	**T (°C)**	**Chemicals**	**Time**	**pH**	**Remarks**
	Load fleshed skin samples, assigning either sides of the skin as control or experiment.					
Washing	150	37	Water	10'		Drain for 5'
	100	37	Water	10'		
Deliming	1.25		Ammonium sulphate			
	0.4		Sod. metabisulphite	60'		pH=8/8.4
	Take sample for analysis Drain to reduce float					
Bating	0.5		Bating agent	90'		ϕ porosity
	Check porosity and take sample for analysis!					
	150	37	Water	15'		Drain
Degreasing	2.0		Eusapon OD			
	3		Common salt	45'		
	Take sample for analysis! and drain					
	100	37	Water			
	2		Common salt	20'		Drain
	200	25	Water	20'		Drain
	200	25	Water	20'		Drain
	Drain and take skin samples for analysis!					
Only for experimental samples	20		Collagen hydrolysate	40'		
	50	cold	Water			
Pickling	8		Common salt	15'		
	0.4		Formic acid (1:10 cold)	30'		
	0.7		Sulphuric acid (1:20 cold)	120'		pH=2.8/3.2
Adjust pH			Sulphuric acid (1:20 cold)	30'		
Tannning	3		Basic Chromium sulphate	40'		
	3		Basic Chromium sulphate	80'		ϕ penetration

*-skin samples and spent liquor samples taken for analysis

B) Deliming-Tanning process recipe

Study Area	Leather Industry Development Institute	**Date:**----------
Type of Leather	Wet blue sheep skin	**Lot no.:**----------
Input material	**Type:** Fleshed pelt skin **Size:** NA **Q'ty:** ---------- **Weight:** ----------	**Actual weight**: ----------
% Based on: Actual fleshed pelt weight		Page: 2 of 2

Process	**%**	**T (°C)**	**Chemicals**	**Time**	**pH**	**Remarks**
	Load fleshed pelt samples, assigning either sides of the skin as control or experiment.					
Basification	0.25		Sod. bicarbonate (1:20@33°C)	20'		
	0.25		Sod. bicarbonate (1:20@33°C)	20'		
	0.25		Sod. bicarbonate (1:20@33°C)	20'		
	0.25		Sod. bicarbonate (1:20@33°C)	20'		
	0.25		Sod. bicarbonate (1:20@33°C)	20'		
	0.25		Sod. bicarbonate (1:20@33°C)	20'		pH=3.8/4.0
			Run	180'		pH=3.7/4.0
	100	35	water	10'		
	Take liquor samples for analysis!					Drain
	100	35	water	10'		Drain
	150	35	water			
	0.1		Antifungal	20'		
	Drain and pile for 48 hrs					
	Take leather samples for analysis!					

C) Re-tanning and dyeing process recipe

Study Area	Leather Industry Development Institute	**Date:**----------
Type of Leather	Dyed crust softy upper	**Lot no.:**----------
Input material	**Type:** wet blue leather **Size:** NA **Q'ty:** ---------- **Weight:** ----------	**Actual weight**: ----------

% Based on: 60% moisture content wet blue weight Page: 1 of 2

Process	**%**	**T (°C)**	**Chemicals**	**Time**	**pH**	**Remarks**
	Load blue leather samples into empty and clean drum					
Wetting back	200	45	Water			
	0.2		Wetting agent	30'		Drain
	50	50	Water			
	0.4		Eusapon OD	30'		
	200	40	Water	20'		Drain
		cold	Water	wash		Drain
Neutralization	35		Water			
	0.7		Sold.formate (1:5 cold)	20'		
	0.5		Genetan Neutro A2			
	0.5		Sertan PK	30'		
	0.5		Sod. bicarbonate (1:20@33°C	3x10'		
	Check pH= 5.4/5.8 and cross-section with BCG					Drain
		cold	water	wash		Drain
Re-tanning and dyeing	100	40	Water			
	1.5		Electrolyte stable fatliquor	20'		1:10@50°c
	3		Relugan RE	10'		
	1.5		Mimosa			
	3		Retanal SF			
	2		Retanal LSF-100			
	1		Basyntan D			
	1.5		Basyntan SL			
	1.5		Basyntan MLB			
	4		Navy blue dye	40'		Check ϕ
	150	60	Water			
Only for control samples			Protein filler	20'		

C) Re-tanning and Dyeing process recipe

Study Area	Leather Industry Development Institute	**Date:**----------
Type of Leather	Dyed crust softy upper	**Lot no.:**----------
Input material	**Type:** wet blue leather **Size:** NA **Q'ty:** ---------- **Weight:** ----------	**Actual weight**: ----------

% Based on: 60% moisture content wet blue weight Page: 2 of 2

Process	**%**	**T (°C)**	**Chemicals**	**Time**	**pH**	**Remarks**
	Load blue leather samples into empty and clean drum					
Fatliquoring	2		Lipsol J620			
	1.5		Syntol F327			
	2		Synthol DT			
	3		Fosfol AUT C6	1:20 @ 60 °C		
	1		Lipodermliqour LA			
	0.1		Eusapon W			
	0.1		Preventol WB	3X5' + 30'		
Fixation	4.5		Forimic acid (1:10 cold)	2X5' + 30'		
	4.5		Forimic acid (1:10 cold)	2X5' + 30'		pH3.3/3.5
	Take sample liquor for analysis! and drain					
Top dye	200	40	Water			
	0.4		Forimic acid (1:10 cold)	20'		
	0.5		Corelin OT-ITP	5'		
	0.5		Navy blue dye (paste cold and dilute @ 60 °C)	30'		
	2		Forimic acid (1:10 cold)	20'		
	1		Coriamin SA (1:10 @ 60 °C)			
			Drain/wash and pile O/N			
Mechanical operation: setout, overhead drying, vibratory staking and rotary staking						

Annex 2. Profile of CPF used for leather processing

S/N	Product code	Chemical name	Technical properties
1	F1	Tafigal HK	▪ Mixture of organic condensation products and inorganic polymers ▪ Aspect: light powder ▪ Active matter: ca. 100% ▪ pH (1% suspension): ca. 7 ▪ properties help in a selective filling of the loose-structure part of the skin
2	F2	Retanal PNB	▪ Phenol condensate with anionic protein derivative ▪ Physical form: light-colored powder ▪ pH (10%): 6.5 ± 0.5 ▪ is designed particularly for soft items with an excellent tear resistance for skins and hides ▪ is protein based compound containing low-astringency syntan ▪ render excellent light fastness and heat resistance values
3	F3	Nerifill powder	▪ Mixture of inorganic and organic products with anionic resin ▪ Appearance: Light brown powder ▪ Solubility: sparingly soluble ▪ Active matter: ca. 100% ▪ It is particularly suitable for grain leathers which lack fullness in the flank areas of the material ▪ Ensures excellent penetration of the product b/n the fibres
4	F4	Celtan F	▪ Aspect: Off white powder ▪ Basis: selective active proteins

S/N	Product code	Chemical name	Technical properties
			▪ pH (10%): 5.5-7.5 ▪ Solubility: easily dispersible in Luke warm water ▪ Gives fullness and uniform thickness to leather ▪ Helps to solve looseness in sheep skins and all types of splits

Annex 3. DSC thermograms of belly and butt regions of skin

A) Thermodynamic parameters calculated from DSC thermograms of ice crystal melting peak of the sheepskin at various process stages of leather processing.

Process stage	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, 115.5	-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

B) Thermodynamic parameters of sheep skin at various stages of leather processing from DSC thermograms

Process stage	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	56.23	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

Annex 4. Percent chromic oxide content (w/w) of wet blue leathers

A) Chromic oxide content (%) for experimental wet blue

				Cr_2O_3 content (%)	
CH pH	Belly	Butt	CH offer (%)	Belly	Butt
9	1.70	1.65	20	1.07	1.28
8	2.18	2.10	15	1.70	1.66
7	2.05	2.00	10	1.71	1.75
6	1.88	1.91	5	2.12	2.37

B) Chromic oxide content (%) for corresponding control wet blue

	Cr_2O_3 content (%)*			Cr_2O_3 content (%)**	
S/N	Belly	Butt	S/N	Belly	Butt
1	2.94	3.39	1	2.97	3.20
2	3.01	3.33	2	2.84	3.18
3	3.47	4.05	3	2.94	3.41
4	3.57	3.64	4	3.01	3.22

**-control tanning processes against CH pH optimization process and **-control tanning against CH offer processes.*

Annex 5. Average physical test result of dyed experimental and control belly and butt leathers

		Tensile strength and percentage extension (average)		Tear load (double edge, average)	Distension and strength of grain by ball burst (average)	
S/N	Sample	Tensile strength (N/mm2)	Elongation (%)	Tear load (N/mm)	Distention at burst (mm)	Load at burst (N)
1	1T	25.9	70.0	38.9	15.0	720.5
2	1L	20.9	69.6	39.5	12.9	542.5
3	1RT	27.3	87.7	40.1	14.4	675.5
4	1RL	26.1	80.4	44.4	14.7	604.0
5	2T	21.6	57.8	30.9	14.5	623.0
6	2L	21.9	66.8	40.5	14.0	432.0
7	2RT	20.7	64.8	25.3	13.5	517.5
8	2RL	20.9	76.8	30.7	13.9	499.0
9	3T	24.3	59.6	38.4	13.0	604.0
10	3L	26.7	60.3	36.5	13.5	539.5
11	3RT	27.4	66.0	37.1	13.4	591.0
12	3RL	29.6	66.8	39.7	13.5	522.0
13	4T	25.0	70.0	45.9	13.6	545.0
14	4L	22.2	60.8	36.1	14.1	525.5
15	4RT	25.3	61.2	31.4	13.2	515.0
16	4RL	22.4	60.3	34.4	12.2	418.0
17	BT	19.8	62.4	24.6	15.3	625.0
18	BL	19.2	63.8	26.3	15.5	601.5

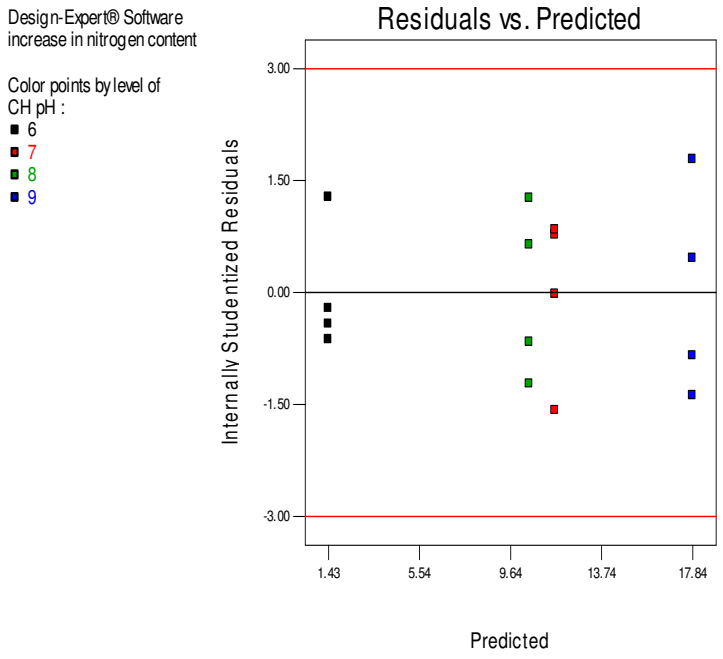
**L/T-experimental Belly and Butt, RL/RT-control Belly and Butt, respectively.*

Annex 6. Average rate values of tactile properties for dyed crust leathers

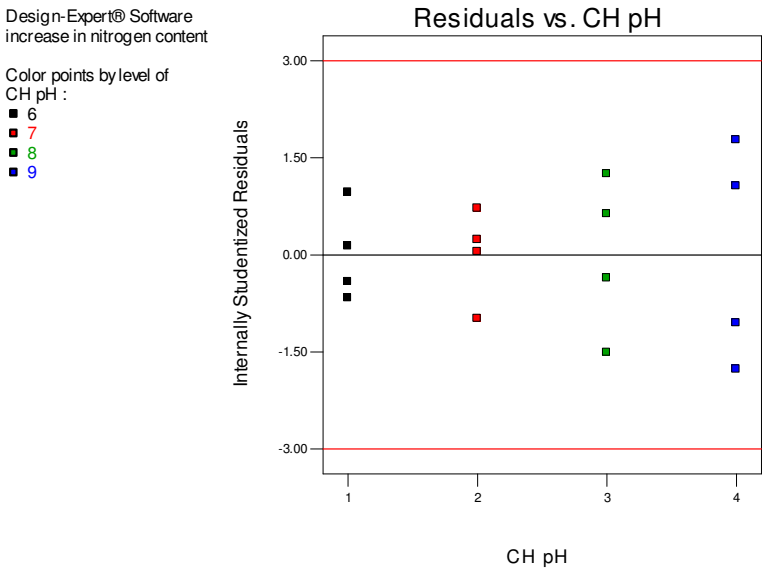
S/N	sample	Average rated point for functional property							Total (%)
		softness	fullness	Roundness	Grain tightness	Color uniformity	Grain smoothness	General appearance	
1	1T	8	9.3	9.0	8.7	8.7	8.8	8.8	87.6
2	1RT	8.8	9.2	9.0	8.8	8.7	8.7	9.0	88.9
3	1L	8.5	8.5	8.2	8.5	8.7	8.8	8.5	85.3
4	1RL	8.8	8.7	8.5	8.5	8.3	8.5	8.5	85.4
5	2T	8.5	8.8	9.2	9.2	8.8	9.0	8.8	89.0
6	2RT	8.7	9.0	9.2	8.8	8.5	9.3	9.0	89.3
7	2L	9.0	8.3	8.5	8.5	8.8	8.7	8.7	86.4
8	2RL	8.8	8.7	8.3	8.7	8.8	8.8	8.7	86.9
9	3T	8.2	8.8	8.8	8.8	8.7	8.8	8.8	87.0
10	3RT	8.2	9.2	9.3	9.3	8.5	8.5	9.2	88.9
11	3L	8.7	8.0	8.2	7.7	8.3	8.5	8.0	82.0
12	3RL	8.0	8.7	8.8	8.2	8.5	8.5	8.2	84.1
13	4T	8.5	8.8	9.2	8.8	8.8	8.8	9.0	88.4
14	4RT	8.7	8.7	8.5	8.7	7.8	8.7	8.5	85.1
15	4L	8.7	8.3	8.2	8.2	8.7	8.7	8.2	84.3
16	4RL	9.0	7.7	7.8	8.0	7.8	8.7	8.0	81.4
17	BT	8.3	7.2	7.3	7.4	9.2	9.3	8.2	81.29
18	BL	8.2	7.0	6.8	6.8	8.7	9.0	8.0	77.86

**L/T-experimental Belly and Butt, RL/RT-control Belly and Butt, BT/BL-blank butt and Belly, respectively.*

Annex 7. Comparison of predicted value vs actual value of experiment for CH pH factor

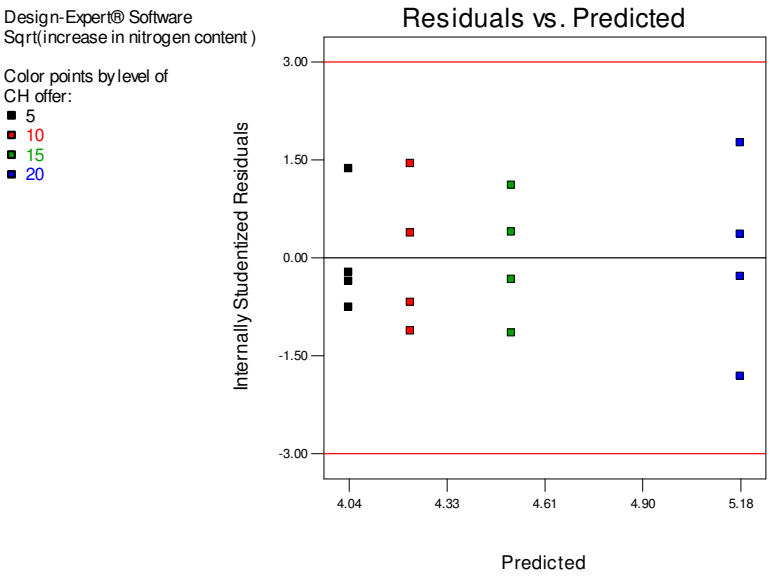


Belly

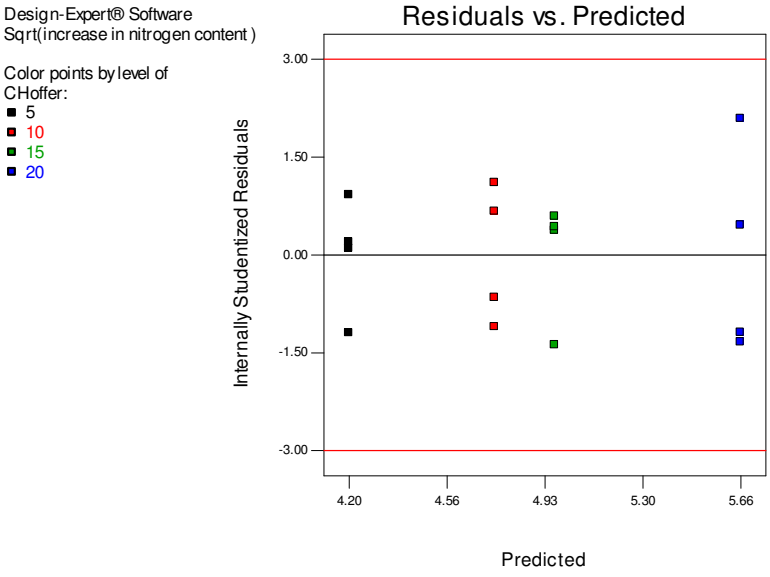


Butt

Annex 8. Comparison of predicted value vs actual value of experiment for CH offer factor



Belly



Butt

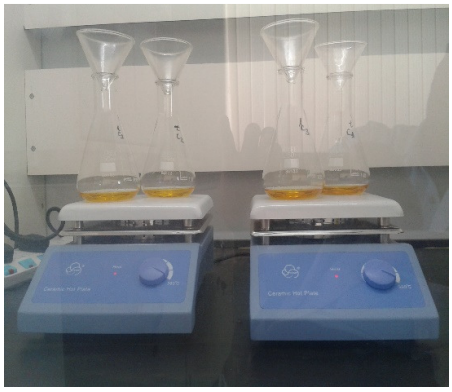
Annex 9. Glimpse on events on some of laboratory analysis works

Dehairing parts of SS samples

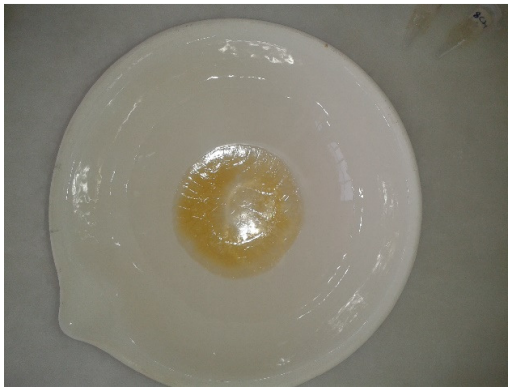
Drum process

Limed pelt trimmings

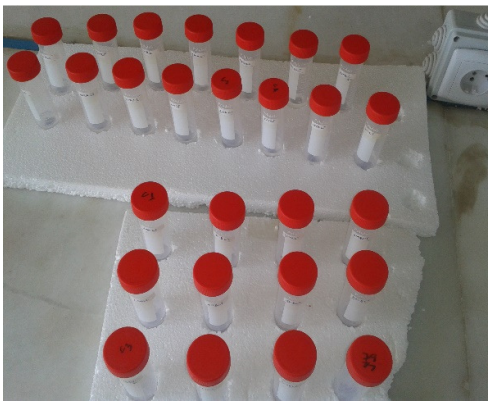
CH under water bath evaporation



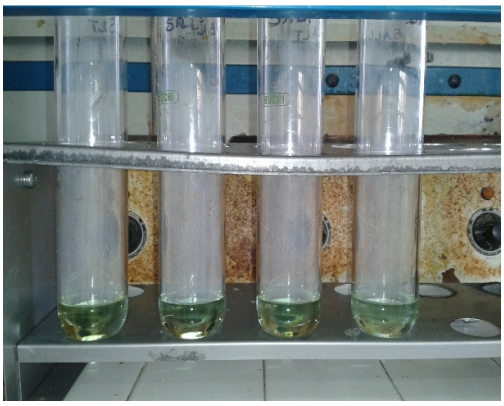
Chrome digestion



CH drying for solid content estimation



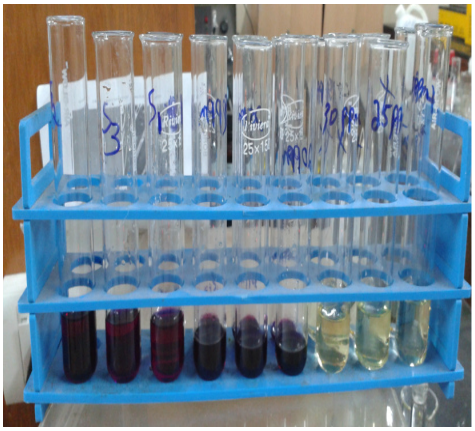
Sample dehydration
for microstructure analysis



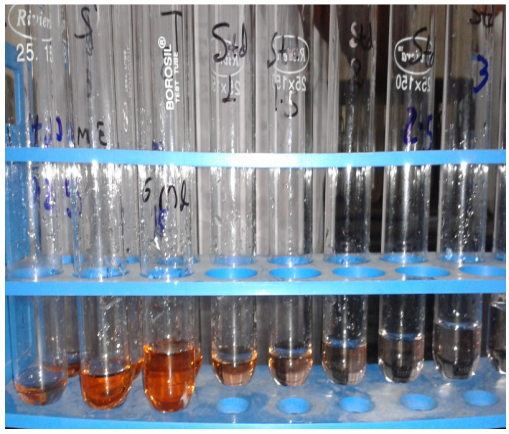
Digested samples for % nitrogen
estimation

Steam distillation for ammoniac nitrogen
estimation

Physical test for dyed crust leathers



Proteoglycan content estimation



Protein content estimation