GELATIN PROCESSING FROM CHROME-TANNED LEATHER TRIMMINGS AND SHAVINGS

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GELATIN PROCESSING FROM CHROM TANNED TRIMMINGS AND SHAVINGS

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CERTIFICATION

The thesis titled "Gelatin Processing From Chrome-Tanned Leather Trimmings And Shavings " submitted by Fahmee Asif, student no- 0417042505, session April 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science in Environmental Engineering on 1st March 2022.

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DECLARATION

It is hereby declared that except for the contents where specific references have been made to the work of others, the studies contained in this thesis are the result of investigation carried out by the author. No part of this thesis has been submitted to any other university or other educational establishment for a Degree, Diploma or other qualification (except for publication).

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ABSTRACT

The chromium toxicity of solid waste created by the tannery industries is a subject of worldwide concern. Solid tannery waste is high in protein, yet it is dumped in the environment also putting human health at risk. Chromium exists in different oxidation states, having Cr^{3+} and Cr^{6+} being the most prevalent and stable in nature. Trivalent chromium is considered vital for human health in trace amounts, whereas Cr^{6+} is a recognized carcinogen and hazardous compound.

The current study shows that utilizing a combined hydrolysis process, industrial grade gelatin can be extracted from chrome-tanned leather trimmings and shavings by dechroming the leather and used in a variety of industrial applications. The isolated shavings gelatin has 1.1 mg/kg chromium and trimmings gelatin have 7.2 mg/kg chromium that were below the standard limit 10 mg/kg making it suitable for use as industrial grade gelatin.

Hydrolysis of chrome tanned leather waste was tested under atmospheric pressure and alkaline-acidic conditions to obtain dechromed leather for gelatin extraction. To obtain the required dechroming degree, four steps of combined hydrolysis operations were performed. Dose studies, time effects, and temperature effects were applied to improve various steps. Chemical analysis techniques and experimental procedures (AAS, FTIR, Viscosity test, SEM, XRF, TCLP) were utilized to characterize dechromed leather, gelatin extracts, dry gelatin and chrome cakes. The SEM analysis helped to ensure the dechromed condition of tanned leather waste and the FTIR analysis evaluated the chemical bonding of the dechromed leather trimmings and shavings.

Gelatin samples recovered in this study met a variety of gelatinous properties including 99.95% and 99.99% Chromium free level with 68% and 64% aqueous yield, had no Pb, were better at gel formation, had a suitable viscosity and contained low levels of Cd, Zn and other heavy metals after dechroming optimization.

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ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
BCSIR	Bangladesh Council for Scientific and Industrial Research
Conc.	Concentration (mg/kg)
Cr	Chromium
FTIR	Fourier Transform Infra-Red
RPM	Revolutions per minute
SEM	Scanning Electron Microscopy
TCLP	Toxicity Characteristic Leaching Procedure
XRF	X- Ray Fluorescence

Chapter 1
INTRODUCTION

1.1 General

Leather is a popular product being used worldwide from the ancient period of time. From the beginning to the present, the leather sector has experienced unstoppable growth because it produces products that are both natural and long-lasting. Leather is one of the few materials that can combine beauty, comfort, and utility all at the same time. Putrescible skin/hides are converted into non-putrescible leather during the leather manufacturing process, which creates about 600,000 tons solid leather wastes worldwide annually, becoming a threat to the environment. Generally, out of 1000kg raw skin/hide, 200kg is converted into product and 800kg is wasted (Catalina et al., 2006). From 1000kg raw skin, 65kg chrome splits and 95kg chrome shavings, 30 kg wet blue trimmings, 35 kg crust trimmings are produced with up to 4.5% chromium content. Finally, 60-75% of the chromium used remains in the collagen structure (Yorgancioglu et. al., 2020). For a number of reasons chromium 3^+ basic sulphate is the predominating tanning agent used in the leather industry worldwide (Beghetto et al., 2013). Chromium is carcinogenic and hazardous when it is converted into chromium 6^+ in the environment (Matyasovsky et. al., 2011). About 78000 ton/year solid wastes are generated in Bangladesh including chrome-tanned wastes from tanneries and they are dumped into open spaces (Nahar et. al., 2018). This is a rather expensive and environmentally inappropriate way of handling a waste material that has the potential for naturalization (Paul et. al., 2013). Landfill cost is increasing and incineration in air atmosphere results in other forms of pollutants (gas and ash discharge). Also, there is a practice of direct use of solid leather wastes, including chrome-tanned wastes as poultry feed which is very alarming (Cabeza et. al., 1998). Chromium in the tanned leather can be removed by various methods and collagen protein of hides can be denatured to convert into other by products (Catalina et. al., 2007). During the past decades, some methods of reusing these wastes have been proposed. Acid hydrolysis, alkali hydrolysis and enzymatic hydrolysis have been widely used for separation and recovery of chrome and collagen material from waste. Gelatin is the first degradation product of collagen which is clear and lighter in color (Mariod and Adam, 2013). It is a bio polymer product with potential industrial value and can be derived from wide ranges of organic sources (Thakur, 1979). Although gelatin processing from solid leather waste is a good

project practiced worldwide, it is not so popular in Bangladesh. In this study, the problem of handling chrome-tanned solid leather wastes generated from tanneries can be mitigated by dechroming and recycling the wastes and making value added products like gelatin and others. Chrome tanning is currently used in over 90% of leather manufacturing processes. In tanneries, transforming chrome tanned crust leather into finished leather requires operations such as splitting, shaving, and trimming due to the in-homogeneity of thickness and breakage. In those processes, considerable amounts of chrome tanned solid wastes are produced. According to some statistics, approximately 80% of the crust leather (based on the first layer and splitting leather weight) is used for valuable leather, while approximately 20% of chrome leather shavings and splits are abandoned as solid waste (Kolomaznik et al., 2008). In chrome tanned solid leather waste, chromium exists as a trivalent form Cr^{3+}, which is nontoxic to the human body, but it is easily oxidized to Cr^{6+} in natural conditions, which is highly toxic to all forms of living organisms, mutagenic in bacteria and carcinogenic in humans and animals (Losi et al., 1994). The chrome tanned trimmings and shavings can be processed in such a way that after treatment, the Cr^{3+} is removed by oxidation to Cr^{6+} in an alkali media. Cr^{6+} can be reduced to Cr^{3+} in acid medium after filtration and washing. By Repeating of this cycle chromium can be almost eliminated (Matyasovsky et. al., 2011). Researchers all over the world are searching for ways to reuse this protein source. However, dechroming leather wastes and making value added product from it is a rare effort in Bangladesh. With proper chemical treatment and modification, solid leather wastes can be turned into many value added products. This waste has a negative impact on our ecosystem as well as all humans.

1.2 Statement of the problem

Chrome-tanned leather shavings and trimmings are one of the potential solid wastes generated by the leather industry, and their disposal is becoming increasingly difficult for tanners due to the presence of heavy element chromium. The leather tanning process is composed of several batch stages associated with the generation of large amount of solid waste. Although tanning can be performed according to different procedures, most of the leather is obtained with chromium salts as the tanning agent (Cui and Qiang, 2019). The tanning agent is characterized by significant organic load and remarkably high concentrations of inorganic compounds such as chromium, chloride, ammonia, sulfide, and sulfate. Shifted from Hazaribagh in Dhaka to protect Buriganga river from pollution, the

tannery is now polluting Dhaleshwari river and Savar area, as its waste management systems are not fully functional yet. It's been 17 long years since the relocation was planned. Large amounts of solid leather waste were previously disposed of in landfills (Hossain et. al., 2009). This resulted in chromium leaching from the soil, rendering it unfit for farming and other uses. Increased environmental regulations and rising disposal costs have prompted the leather industry to create cleaner technology by reducing waste and maximizing reuse. As a result, to limit the amount of chrome leather pieces that end up in landfills, they are now used as either chicken feed or fertilizer (Hossain et. al., 2009). Dhaka University and the Bangladesh Council for Scientific and Industrial Research (BCSIR) conducted a study in 2007 that found greater levels of chromium in eggs and poultry meat above the tolerated limit. The largest amount of chromium found in solid waste was 3.2037% (Mazumder et. al., 2013). There are rules for effluent treatment and sludge handling management in Bangladesh but unfortunately no active rules and regulations has set up yet for the solid leather waste handling management. It is prohibited to use tannery waste as poultry feed in Bangladesh. On July 21, 2011 the High Court ordered stopping tannery waste being used as feed for chicken, fish and ducks, after hearing the petition from the human rights and environmental organization (bdnews24.com, 2017). It was again reported that these wastes are still in use as poultry feed in Bangladesh (daily sun, 2019). These wastes are being dumped or incinerated in open places creating hazardous impacts on the environment. Toxic leather wastes are also good source of protein which can be used for various purposes. Given the circumstances, this problem necessitates a unique approach, not only nationally but also globally. In this paper, reuse of the leather waste by dechroming has been studied to make another value-added product, gelatin.

1.3 Objectives of the study

The main objective of the study is to find out an effective chemical treatment option for removal of chromium from chrome tanned leather trimmings and shavings and make gelatin from it. The specific objectives of the study are:

1. Optimization of dechroming process of chrome tanned solid leather waste by combined alkali-acid hydrolysis method.

2. Extraction and physico-chemical property characterization of gelatin from chrome-tanned solid leather waste.

The objectives of the study would help to assess the problem of mitigating the solid waste handling problem around the world and rather make a value added, nontoxic, eco-friendly product from it. The possible outcomes of the study include:

1. Detailed physico-chemical characterization of chrome tanned splits and trimmings.

2. Stability and efficiency determination of gelatin extraction from chrome-tanned solid leather waste.

1.4 Scope of the study

The goal of the research was to figure out how to dechrome chrome tanned leather trimmings and shavings using a combination acid-alkali hydrolysis method. Setting up some dechroming stages with alkali and acid treatment helped to achieve this goal. To obtain optimal dechromed leather, dose, time and temperature optimization was performed. The optimum value determined by considering both the chromium concentration and the sample yield.

In dechroming process different alkali and acids were used alternately in four steps. Chromium concentration were determined by Atomic Absorption Spectroscopy (AAS). The objective of gelatin extraction and characterization was done by heating the dechromed leather. Fourier Transform Infra-Red spectroscopy (FTIR), Scanning Electron Microscopy (SEM) analysis, chromium concentration measurement, viscosity analysis, X-Ray Fluorescence (XRF) measurement, Toxicity Characteristic Leaching Procedure (TCLP) were done to characterize the product. The main scope of the study was to find out a way to make the leather wastes (trimmings and shavings) reusable and extract another value-added product out of the wastes.

1.5 Organization of the thesis

The thesis is divided into the following Chapters:

Chapter one presents the background of the thesis research and the necessity of this study. For this study various relevant research papers on dechroming of chrome tanned solid leather wastes have been studied. A lot of published journals on dechroming treatment by acid/alkali hydrolysis process and gelatin extraction process were reviewed. Efforts have been made to make a detailed understanding of the relevant problem and solution to the regarding topic.

Chapter Two presents the literature review of studies conducted by other researches of past to present. It focuses on the chemistry of the respective dechroming stages. This Chapter emphasizes the advantages of using acids and alkalis as chemicals and illustrates the applications of gelatin. This Chapter also presents the collagen behavior upon hydrolysis operation. The use of gelatin and its extraction process from different literature also have been discussed in this chapter.

Chapter Three presents the methodology applied to achieve the objectives of the thesis. It discusses the experiments to dechrome the leather wastes and optimization of the dechroming process. Same procedure was used to dechrome both chrome tanned trimmings and shavings. It also consists of procedures to extract gelatin from the dechromed leather wastes.

Chapter Four presents the results obtained from the experimentation. It consists of discussion of the results and presents summarized scenarios for the results. The sample characterization test results are combined in this chapter.

The thesis is brought to a close in chapter Five. It makes observations, draws conclusions, and makes suggestions.

Chapter 2
LITERATURE REVIEW

2.1 Introduction

This chapter provides an overview of the tanning process for leather. This chapter also emphasizes the need of solid leather waste management, the environmental challenges created by solid leather waste dumping, and the possibilities of creating value-added products from toxic solid leather waste. Here are the various forms of dechroming techniques, as well as their benefits and drawbacks. For a better knowledge of the leather-collagen-gelatin chemistry, this chapter also covers dechroming theory and gelatin processing.

2.2 Solid leather waste problem and possibilities in Bangladesh

Bangladesh has experienced remarkable economic growth in recent years. The government is optimistic about the expansion of the leather industry, which has allowed for the restructuring of previously unstructured leather sectors. Though Bangladesh handled the COVID-19 circumstances admirably, there has been some volatility in export profits of leather and leather-made goods, which are currently about $1.10, according to the Export Promotion Bureau (Pocket Export Statistics Book 2018-2019, 2020). It is now regarded as one of Bangladesh's most important industries. In a single day, Bangladesh processes 400 metric tons of hides and skins. Because of the degradation of skin proteins and the formation of gases such as NH_3, H_2S, and CO_2, the leather business is one of the most polluting sectors due to the massive volume of liquid and solid waste it generates (Beghetto et. al., 2013). Raw trimmings, fleshings, chrome shavings, polishing dusts, and keratin wastes are examples of solid waste. The accumulation of these wastes causes a sludge problem and clogging of treatment pipes, resulting in a decline in treatment plant efficiency (Beghetto et. al., 2013).

Nike, Puma, CK, H&M, Hugo Boss, Armani, Timberland, and many others source leather from Bangladesh. 56% of the leather is sourced from cows, 30% from goats and the remaining from buffalo. Only 15 percent to 18 percent of total leather supplies are required to meet domestic demands, nearly 76 percent of tanneries are export-oriented, and exported products include shoes, bags, wallets, belts, and finished leather (Bliss, 2017). Treatment of

solid wastes are likewise inefficient, putting a financial strain on tanners. The leather business produces a significant amount of chromium-containing leather waste, such as trims and shavings from chrome tanned leather. Because of their high chromium content, these wastes are rarely used directly (Barasan et. al., 2008). Leather industry in the developing countries is facing lot of solid wastes problem and many tanneries closed for not meeting Bio-chemical Oxygen Demand (BOD) and Total Dissolved Solids (TDS) norms. These tanneries produce 118 metric tons solid wastes. Among these solid wastes, major portions are shaving and buffing dust, trimmings, fleshings and fats. In Bangladesh, yearly 20.1×103 MT fleshing was generated only from cow hide and goat skin where 10.3x103 MT for cow hide and 9.8×103 MT for goat skin (Beghetto et. al., 2013).

The majority of the leather wastes are dumped on the ground and burned. Landfill costs are rising, and incineration in the atmosphere produces other pollutants (gas and ash discharges). Cr^{3+} is generally oxidized to Cr^{6+} in the presence of a strong oxidizing agent in an acidic environment. Conversely, it can also occur at high pH in the presence of mild oxidizing chemicals. Liming, pickling, and neutralization are the stages in the leather production process where such situations can occur (Barasan et. al., 2008). In Bangladesh, there is only one recognized glue and gelatin industries. To meet national demand, a large amount of gelatin is imported (Naher, 2018). Because they do not have a trade license, certain small-scale glue, gelatin, fish, and poultry feed production units (unnamed) conduct their operations in complete secrecy. They use chrome tanned leather trimmings and shaving dust as raw materials without making them chrome-free (Naher et. al., 2018).

Mia explained (as cited in Naher et. al., 2018) Bangladesh produces about 1/5th of the world population of hides and skins. Instead of being thrown away, tannery waste materials can be sold to generate additional cash and reduce disposal costs. There are numerous opportunities to use tannery solid wastes to manufacture gelatin, soap, animal feed, fish feed, compost, chicken feed, and direct use in soil (agricultural recycling), among other things. However, due to a lack of planning and management of the leather industry's solid wastes, a lack of awareness and investment in tanneries by product manufacturing units, a lack of government facilities, a lack of appropriate technology, old methodology, health issues, and other factors, these essential protein sources go unused (Naher et. al., 2018). Wet blue trimmings and raw trimmings can be used to make glue and gelatin. They're called value-added items.

Leather is based on collagen; it is in principle possible to extract the protein free of chromium or other potentially objectionable compounds and obtain pure collagen. Such collagen sample find extensive and value-added applications like bone implants-controlled drug delivery system, collagen sheet, bare clarifying agent, surgical sutures and other biomedical products. Use of protein in cosmetic application is of established. Since collagen obtained some unique amino acid the hydrolysis and isolation of protein maybe one good source of such residues. Application can be of high value specially because of costs of the final product (Naher et. al., 2018).

2.3 Leather tanning chemistry

Chromium tanned leather can be regarded as a mixture of chromium 3^+ complexes. Prior to the introduction of the basic chromium species in tanning, several steps are required to produce a tannable hide. These steps include: Scudding (removing the hair), Liming (the introduction of alkali agents such as sodium hydroxide), Deliming (restoring neutral pH), Bating (softening the skin with enzymes), and Pickling (lowering pH of the hide with salt and sulfuric acid). The pH is very acidic when the chromium is introduced to ensure that the chromium complexes are small enough to fit in between the fibers and residues of the collagen. Once the desired level of penetration of chrome into the substance is achieved, the pH of the material is raised again to facilitate the process. This step is known as "basification" (Kolomaznik et. al., 2008). In the raw state chrome tanned skins are blue and therefore referred to as "wet blue". Chrome tanning is faster than vegetable tanning (less than a day for this part of the process) and produces a stretchable leather which is excellent for use in handbags and garments.

The mechanism of chrome tanning relies on the formation of a trimeric chromium aquo complex in un-bound water before cross-linking with the carboxyl group of the collagen (Kolomaznik et. al., 2008). Chromium is known to interact with the telopeptidyl aspartic and glutamic acid residues in collagen affecting its intermolecular structure. Collagen molecules are aligned in a quarter stagger structure, resulting in repeating gap/overlap regions within the fibrils (Petruska and Hodge, 1964). The most important mineral cross-linking agents are hydrated basic chromium 3^+ sulfate complexes. These compounds form extended polynuclear coordination complexes containing hydroxol, oxo, and sulfato bridges into which ionized carboxyl groups on collagen enter readily as coordinating ligands accomplishing crosslinking.

On pH adjustment and partial drying, highly stable complexes are formed with oxo bridges predominating and protein amide groups entering the coordination complex (Harlan and Feairheller, 1977).

There are a number of ways in which the tanning action could take place, and although an ionic compound may be formed initially, it seems certain that the main chemical action is by means of coordination complexes or covalent bonds. It also seems certain that the most important coordinators are the free carboxyl groups of the collagen structure, but the amino groups and the imido groups of the peptide linkage are not excluded. This theory is implied mainly by tanning actions at various pH's. Tanning action increases as pH increases, and no tanning occurs at all when all -CO_2- groups of the collagen molecules are protonated. The basic component of the skin is collagen, a fibrous protein (Mann and McMillan, 2017).

Collagen is characterized by a high content of glycine, proline, and hydroxyproline, usually in the repeat -gly-pro-hypro-gly- relative to other proteins. Glycine has a small molecular footprint, which adds stability to a chain and permits close orientation with other chain. The amino acid structures of proline and hydroxyproline allow twisting of a chain, and the hydroxyl group of hydroxyproline stabilizes the triple helix structure at body temperature (Holwerda and Loon, 2021). It was claimed by Heidemann (as cited in Repository, Sudan University of Science and Technology, 2021) that collagen's high content of hydroxyproline allows for significant cross-linking by hydrogen bonding within the helical structure. Ionized carboxyl groups (RCO2-) are formed by hydrolysis of the collagen by the action of hydroxide. This conversion occurs during the liming process, before introduction of the tanning agent (chromium salts). The ionized carboxyl groups coordinate as ligands to the chromium 3^+ centers of the oxohydroxide clusters.

The process of tanning imparts the advantage of resistance to heat. This is animportant factor in many of the uses of leather. In conjunction with chemical processing, the tanner imparts colour, texture and finish to the leather, to enhance its appearance and suit it to today's fashion requirements. The latest research indicates that the basic collagen structure consists of twined triple units of peptide chains of differing lengths **(**Mann and McMillan, 2017). The collagen molecule is a triple helix consisting of three peptide chains, held together through hydrogen bonds. The chains consist of a sequence of tripeptides containing one glycine residue. The amino acid glysine residues are joined together by peptide links. The peptide chains within the triple helices are held together by hydrogen bonding (Figure 2.1). The term

amino acid might mean any molecule containing both an amino group and any type of acid group; however, the term is almost always used to refer to an carboxylic acid. The simplest a-amino acid is aminoacetic acid, called glycine. Peptide bond contains partial positive charge groups and partial negative charge groups. The N-H and C=O bonds are polar, i.e., partially positive H and partially negative O (Figure 2.1). The amino acid residues of poly peptide in collagen of leather are joined together by cystein chain linkages. The sulphur-sulphur linkage in cystein is susceptible to the reaction of alkali and breaks down quite readily in the presence of alkali and a reducing agent. They are not broken by heating or high salt concentration. They can be broken by exposing them to strong acid or base for a long time at elevated temperature. Also by some specific enzymes (digestive enzymes) **(**Mann and McMillan, 2017).

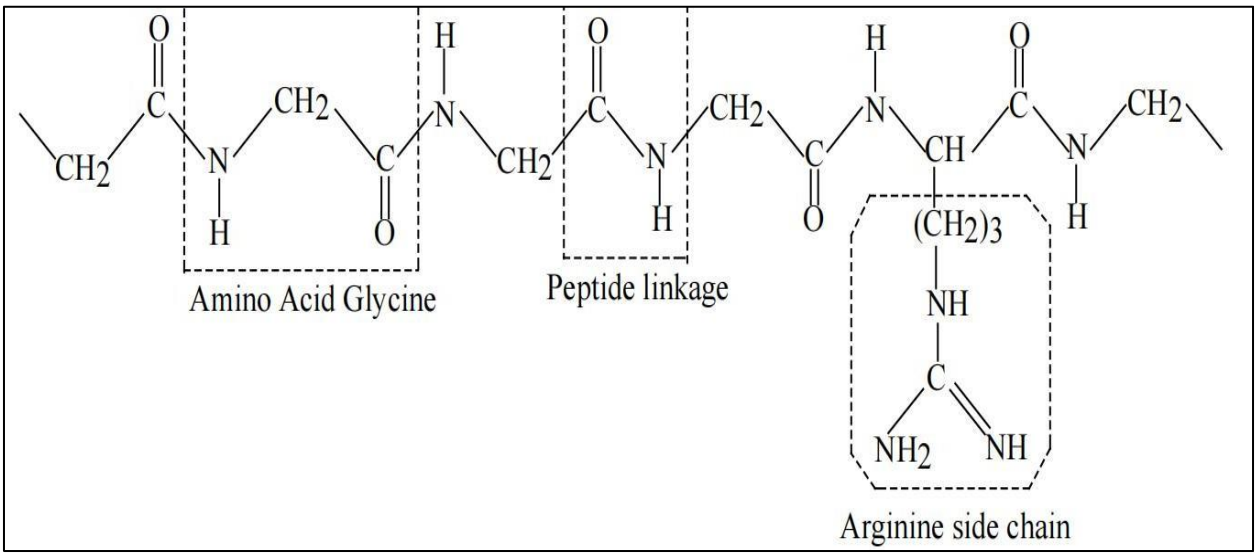


Figure 2.1: Fundamental structure of a peptide (Mann and McMillan, 2017)

Cr, also loosely called chrome, is the twenty-first element in relative abundance with respect to the earth's crust, yet is the seventh most abundant element because it is concentrated in the earth's core and mantle. It has atomic number 24 and belongs to group (VIB) of the periodic table. On a tonnage basis, chromium ranks fourth among the metals and thirteenth among all mineral commodities in commercial production (Poulopoulou et. al., 1998). Chromium is used vastly to tan leather because of its good reactivity with the collagen protein. Chromium, a transition element which forms co-ordination complexes by using 3d orbitals to

accommodate extra electrons, has an unrivalled position as a tanning agent (Mann and McMillan, 2017). Chromium Leather tanning is a key step of leather processing in prevailing industrial treatment which transforms animal skin into leather, is tanning with Cr^{3+} salts, mostly sulfates. During this process a stable and inert poly nuclear Chromium-collagen complexes are formed (Poulopoulou et. al., 1998).

There are two main features of chromium chemistry which enable it to act as a tanning agent. Firstly, the complexes formed are of intermediate stability, and thus exchange of coordinating ligands can take place comparatively easily. Secondly, chromium has the ability to form polynuclear complexes in which Cr-O-Cr bridges are involved (Mann and McMillan, 2017). Hydrolysis is conducted under mild conditions. Typically, an acidic Cr^{3+} solution is added to the pretreated skin, and pH and temperature are gradually increased. The tanning solution contains olated soluble oligomers of Cr^{3+} notably the dimer, trimer and higher structures. Formation of sulfate bridges has been also postulated (Poulopoulou et. al., 1998).

Mammal skin and hides are used in the leather industry to make usable products from them. The basic component of the skin is collagen, a fibrous protein. The basic collagen structure consists of twined triple units of peptide chains of different lengths. The amino acid residues are joined together by peptide links. The peptide chains within the triple helices are held together by hydrogen bonding while tanning. The complex is basic by nature and will contain hydroxyl groups associated with the chromium nuclei (Mann and McMillan, 2017). The positively charged chrome tanning agent, i.e., hydrated chromium ions, forms a coordinate bond with the negatively charged collagen carboxyl group during the chrome tanning process, fixing the structure of the collagen fiber and providing tanning effects such as thermo stability, chemical resistance, and flexing endurance (Beghetto et al., 2013).

The chemistry of the chrome tanning process was broken down into three stages: hydrolysis, olation (metal ions form polymeric oxides in aqueous solution), and precipitation. To do so, chromium sulfate is first ionized in water to produce the Cr^{3+}ion, then the Cr^{3+} ions combine with available water molecules to generate hydrated chromium ions (as shown in Figure 2.2) (Oruko et. al., 2020).

Menderes elaborated that the pH is normally kept low throughout this process to allow Cr^{3+} species to penetrate deeper into the opened collagen lattice. With an increase in molecular weight and reactivity, the consequent increase in pH makes collagen more reactive and the chromium species more basic, facilitating strong complexation between the acidic side chains

of collagen and the chromium species via covalent bonding (as cited in Oruko et. al., 2020). Furthermore, the chromium nuclei self-polymerize via hydroxyl bridges to produce thermodynamically stable chromium collagen cross-linked complex bridges between the protein chains (Figure 2.2).

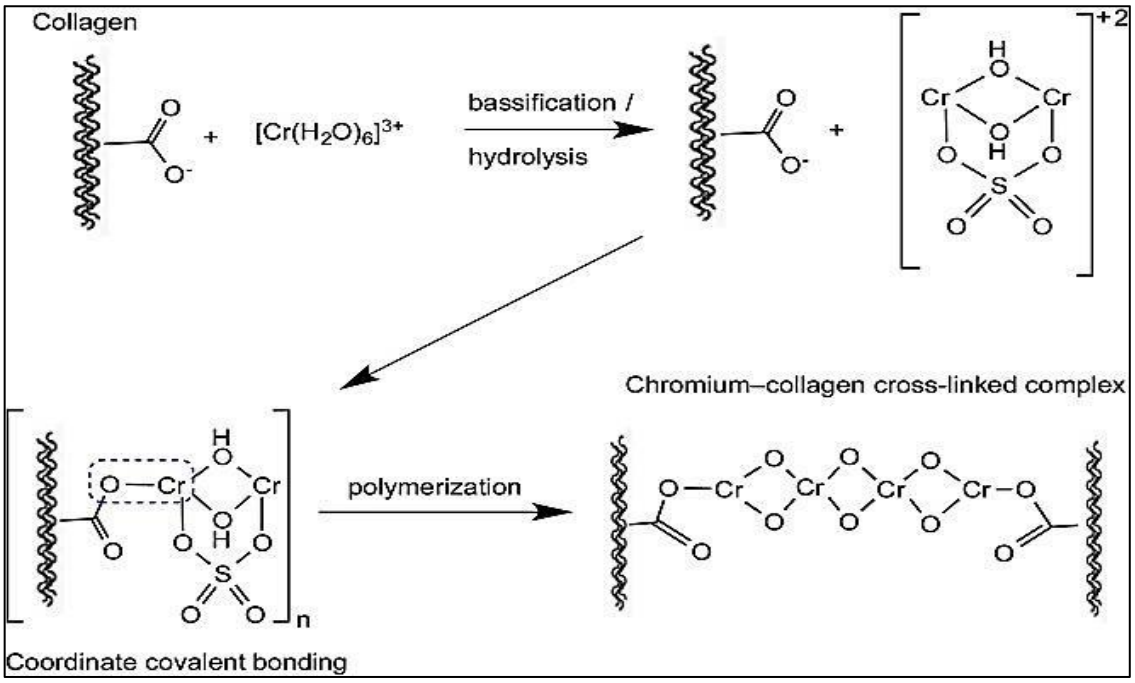


Figure 2.2: Collagen chromium reaction during tanning (Oruko et. al., 2020)

When the tanning process is over, the chromium salts that have not reacted with the skin fibers are released into the environment as chromium waste. It is estimated that 30–40% of the chromium salts used in the tanning process do not react with the skin/hides and are consequently discharged as spent tanning solution around the world (Liu et al., 2016). Lots of leather trimmings, shavings and sludge wastes are also generated during tanning process. Despite the Cr^{3+} species from these wastes showing higher stability in the environment, they may undergo various specifications and transformations in terrestrial ecosystem resulting into high chromium pollution.

2.4 Protein denaturation

Chromium tanned leather wastes fibers are difficult to manage by chemical and biological processes due to the strong bonds established between collagen and chromium. Therefore, it

is of great interest to develop treatments that disrupt recalcitrant bonds and may open new perspectives to materials valorization (Wikipedia, Denaturation, 2021). Denaturation is a biological process that alters a protein's molecular structure. Many of the weak connections, or bonds (e.g., hydrogen bonds), inside a protein molecule that are responsible for the highly organized structure of the protein in its natural (native) state are broken during denaturation. The structure of denatured proteins is looser and more random, and most of them are insoluble. Denaturation can be achieved in a variety of ways, including heating, alkali, acid, urea, or detergent treatment, and vigorous shaking (Britannica, 2021). By denaturation proteins or nucleic acids lose the quaternary structure, tertiary structure, and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), agitation and radiation or heat (Wikipedia, Denaturation, 2021). Ionization of carboxylic groups, amino acid groups, or phenolic groups are some of the modifications related with denaturation. Rearrangements of the molecules may occur, resulting in the release of sulfhydryl or disulphide groups. Denaturation or disruption of the total protein molecule can occur as a result of these modifications (Acharya and Chaudhuri, 2021). Denatured proteins can exhibit a wide range of characteristics, from conformational change and loss of solubility to aggregation due to the exposure of hydrophobic groups. Denatured proteins lose their 3D structure and therefore cannot function. Protein folding is key to whether a globular or membrane protein can do its job correctly; it must be folded into the right shape to function. However, hydrogen bonds, which play a big part in folding, are rather weak and thus easily affected by heat, acidity, varying salt concentrations, and other stressors which can denature the protein.

In quaternary structure denaturation, protein sub-units are dissociated and/or the spatial arrangement of protein sub-units are disrupted (Wikipedia, Denaturation, 2021).

Tertiary structure refers to the unique three-dimensional shape of the protein as a whole, which results from the folding and bending of the protein backbone. The tertiary structure is intimately tied to the proper biochemical functioning of the protein (Chemistry Libertexts, 2021). Tertiary structure denaturation involves the disruption of: Covalent interactions between amino acid side-chains (such as disulfide bridges between cysteine groups). Non-covalent dipole-dipole interactions between polar amino acid side-chains (and the surrounding solvent). Van der Waals (induced dipole) interactions between non polar amino acid side-chains (Wikipedia, Denaturation, 2021; Yorgancioglu et. al., 2020).

A protein molecule is not a tangle of polypeptide chains thrown together at random. Instead, the chains are organized in a variety of distinct but unique conformations. The stable arrangement of the polypeptide backbone is referred to as secondary structure. Proteins or portions of proteins twist into a spiral or a helix. This helix is known as a right-handed-helix because it is maintained by intrachain hydrogen bonding between the carbonyl oxygen atom of one amino acid and the amide hydrogen atom four amino acids up the chain (placed on the following turn of the helix) (Chemistry Libertexts, 2021). In secondary structure denaturation, proteins lose all regular repeating patterns such as alpha-helices and beta-pleated sheets, and adopt a random coil configuration (Wikipedia, Denaturation, 2021).

The primary structure of a protein is the quantity and sequence of amino acids in its polypeptide chain or chains, starting with the free amino group and maintained by peptide bonds joining each amino acid to the next. (Chemistry Libertexts, 2021). Primary structure, such as the sequence of amino acids held together by covalent peptide bonds, is not disrupted by denaturation (Wikipedia, Denaturation, 2021).

Loss of function: Most biological substrates lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site, and because amino acid residues involved in stabilizing substrates' transition states are no longer positioned to be able to do so.

Denaturation can also be caused by changes in the pH which can affect the chemistry of the amino acids and their residues. The ionizable groups in amino acids are able to become ionized when changes in pH occur. A pH change to more acidic or more basic conditions can induce unfolding. Acid-induced unfolding often occurs between pH 2 and 5, base-induced unfolding usually requires pH 10 or higher (Wikipedia, Denaturation, 2021).

2.5 Process of protein denaturation

Denaturation happens when the secondary structure (hydrogen binds to amides) and tertiary structure bonding connections are disturbed. Hydrogen bonding, salt bridges, disulfide bonds, and non-polar hydrophobic interactions are four forms of bonding interactions between "side chains" in tertiary structure that can be broken (Virtual Chembook, 2021; Yorgancioglu, 2020). The monomeric units that make up the molecule's sequence may not change. Peptide bonds between amino acids in a sequence are less vulnerable to denaturation than hydrogen

bonds. The structural folding of denatured proteins is thereby disrupted by denaturation. Only the original 3D structure of the biomolecule is disturbed during denaturation (Bioligy Online, 2021). As a result, denaturation can be caused by a wide range of chemicals and circumstances. When proteins are exposed to denaturing chemicals, they unfold and lose their active site (e.g. strong acids or bases, heat, solvents, and salts) (Virtual Chembook, 2021). Hydrogen bonds and non-polar hydrophobic interactions can be disrupted with heat (as shown in Figure 2.3). Heat raises the kinetic energy of the molecules, causing them to vibrate so quickly and violently that the bonds are broken (Virtual Chembook, 2021). The peptide bonds between the amino acids in a sequence are not as susceptible to denaturation as the hydrogen bonds. Thus, what denaturation disrupts is the structural folding of denatured enzymes. Disruption of the structural folding means that their 3D shape is damaged and lose their function as protein (Biology Online, 2021). The newly-synthesized protein will appear as a linear polymer of amino acids. Then, it folds accordingly to become a 3D molecule. The 3D shape is achieved by forming hydrogen bonds within its structure (Biology Online, 2021).

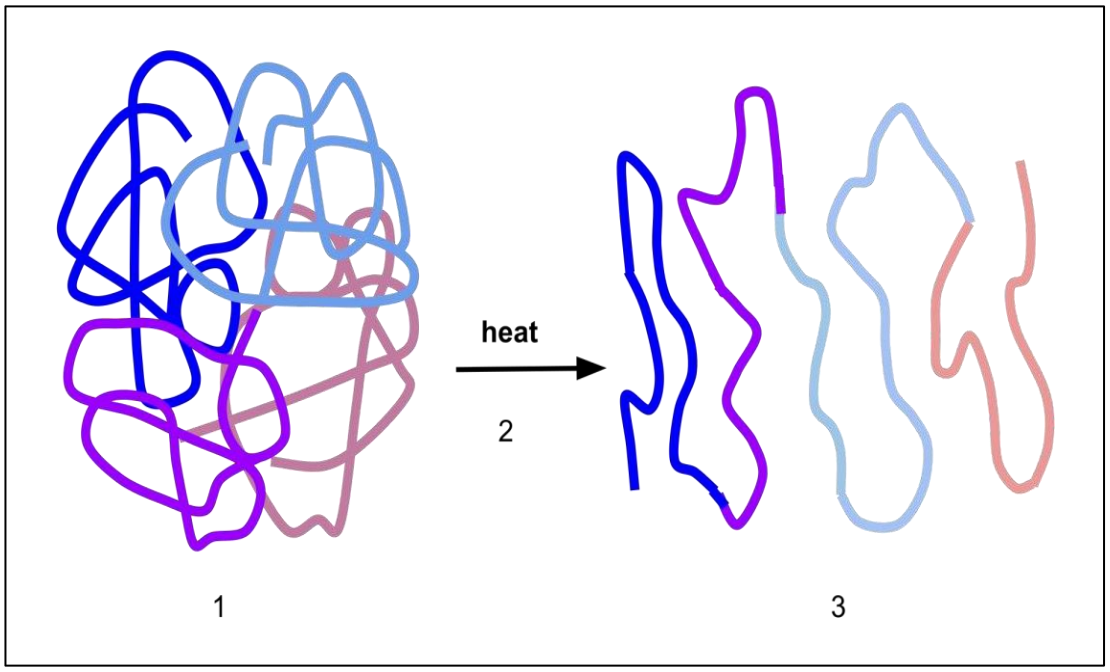


Figure 2.3: Protein denaturation process

(1) Functional protein showing a quaternary structure

2) When heat is applied it alters the intramolecular bonds of the protein

3) Unfolding of the polypeptides (amino acids) (Wikipedia, Denaturation, 2021)

2.6 Protein denaturants

When a protein is exposed to a denaturant, the hydrogen bonds that help form the 3D structure of the protein are broken. This results in the disruption of the protein structure (Virtual Chembook, 2021). In a solution with such high denaturant concentrations, both the structure of the protein and the structure of the solvent around the protein can be altered. (Dunbar et. al., 1997). The “active site” is also lost. Thus, the substrates that originally can bind to the active site of the proteins will no longer be able to bind to it following denaturation. As a result, the biochemical process that is instigated by the binding of the Protein and the substrate will also be disrupted (Virtual Chembook, 2021). This explains how extreme conditions, such as exposure to prolonged radiation, heat, and strong chemicals could be lethal to organisms. The denaturing agents can break the hydrogen bonds that help form the 3D configuration of the biomolecules. Apparently, hydrogen bonds are a weak type of chemical bond; they break easily by exposure to heat, radiation, and other stressors (Virtual Chembook, 2021). Denaturants also act as cross-linking agents of the protein (Dunbar et. al., 1997).

Acid base denaturants: Salt Bridges Are Disrupted by Acids and Bases. Salt bridges are formed when an acid and an amine are neutralized on side chains. The positive ammonium group and the negative acid group have an ionic connection in the end. This action can be produced by any mix of acidic or amine amino acid side chains (Virtual Chembook, 2021). Acids and bases break down salt bridges that are kept together by ionic charges. The positive and negative ions in the salt change partners with the positive and negative ions in the new acid or base added in a form of double replacement reaction (Virtual Chembook, 2021; Wikipedia, Denaturation, 2021) . It has been found that if the temperature is kept low, some proteins keep their native conformations at very low pH, whilst others unfold. In the second category of proteins, a reduction in pH/low pH can trigger conformational changes (Acharya and Chaudhuri, 2021). Acidic protein denaturants include: Acetic acid, Oxalic acid, Lactic acid, Trichloroacetic acid 12% in water, Sulfosalicylic acid. Acidic nucleic acid denaturants include: Acetic acid, HCl, Nitric Acid (Wikipedia, 2021). Other nucleic acid denaturants include: Formamide, Guanidine, Sodium salicylate, Propylene glycol, Urea.

Bases work similarly to acids in denaturation. Some proteins' alkaline denaturation follows the same pattern as their acid denaturation, while others' alkaline denaturation follows a distinct pattern. Apart from chemical modification, the products of alkaline denaturation are

undoubtedly as diverse as the products of acid denaturation (Acharya and Chaudhuri, 2021). They include: Sodium bicarbonate. Basic nucleic acid denaturants include: Sodium Hydroxide (Wikipedia, Denaturation, 2021).

Organic solvants denaturants: Most organic solvents are denaturing, including: Ethanol. Alcohols and glycol can be good denaturant. The branching of hydrocarbon in alcohol tends to decrease their denaturing properties. As denaturants, glycols are less efficient than the corresponding alcohols. It suggests that increased polarity or hydrogen-bonding capacity is of secondary importance when compared with the effects of increasing hydrocarbon content (Acharya and Chaudhuri, 2021).

Heavy metal salts: Heavy metal salts disrupt disulfide bonds. Heavy metals may also disrupt disulfide bonds because of their high affinity and attraction for sulfur and will also lead to the denaturation of proteins. Heavy metal salts act to denature proteins in much the same manner as acids and bases. Heavy metal salts usually contain Hg^{+2}, Pb^{+2}, Ag^{+1} Ti^{+1}, Cd^{+2} and other metals with high atomic weights. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt (Virtual Chembook, 2021).

Cross linking agents: Protein cross-linking reagents usually contain two or more chemically reactive ends that attach themselves to the functional groups found in proteins and other molecules (G-Biosciences, 2021). Cross-linking agents for proteins include: Formaldehyde, Glutaraldehyde Chaotropic agents (Wikipedia, Denaturation, 2021)

Chaotropic agents include: Urea 6 – 8 mol/l, Guanidinium chloride 6 mol/l, Lithium perchlorate 4.5 mol/l, Sodium dodecyl sulfate (Wikipedia, Denaturation, 2021). Denaturing agents mostly used in the folding and unfolding experiments of protein are urea and guanidinium chloride. In research studies, it was found that urea helps in denaturation either by directly interacting with protein initiating solvation of polypeptide chain by water and urea or indirectly by modifying the water molecule structure resulting in changes in the behavior of solvent which weakens or reduces the hydrophobic effect (Acharya and Chaudhuri, 2021).

Disulfide bond reducers: Disulfide bonds are formed by oxidation of the sulfhydryl groups on cysteine. Reducing Agents Disrupt Disulfide Bonds. Different protein chains or loops within a single chain are held together by the strong covalent disulfide bonds. If oxidizing agents cause the formation of a disulfide bond, then reducing agents, of course, act on any disulfide bonds to split it apart. Reducing agents add hydrogen atoms to make the thiol group,

-SH (Virtual Chembook, 2021). Agents that break disulfide bonds by reduction include: 2-Mercaptoethanol, Dithiothreitol, TCEP (tris (2-carboxyethyl) phosphine) (Wikipedia, Denaturation, 2021).

Chemically reactive agents: Agents such as hydrogen peroxide, elemental chlorine, hypochlorous acid (chlorine water), bromine, bromine water, iodine, nitric and oxidizing acids, and ozone react with sensitive moieties such as sulfide/thiol, activated aromatic rings (phenylalanine) in effect damage the protein and render it useless (Wikipedia, Denaturation, 2021).

2.7 Hydrolysis of collagen

The majority of the leather wastes are dumped on the ground and burned. Landfill costs are rising and incineration in the atmosphere produces other pollutants (gas and discharges). As a result, it is critical to devise practical methods for converting solid leather wastes into more value products (Artoni, 2001). Some techniques of recycling these wastes have been proposed in recent decades. For the separation and recovery of chromium and collagen material from wastes, acid hydrolysis, alkali hydrolysis, oxidation hydrolysis, and enzymatic hydrolysis have all been frequently used. (Artoni, 2001; Ding et al., 2015). During the oxidation processes, collagen chain is split into shorter fragments consisting of acid amides or keto acid derivatives. Oxidation causes weakening of physical stability of leather. The rate of this process increases at higher temperatures and lower relative humidity, light and presence of air pollutants. The oxidation process is effective to dechrome leather but toxic Cr^{6+} is formed during the procedure (Losi et. al., 1994). Being affordable, chemical hydrolysis is the most commonly used method in industrial practices. Enzymatic hydrolysis is fast and produce waste in minimal amounts, but they are more expensive to carry out (Noor et. al., 2021). The researchers utilized organic inorganic acids to study the dechroming effect of solid leather waste. Liquid water contains H_3O^+ and OH^- ions that can act as both nucleophilic and electrophilic reagents that can start alkali or acidic hydrolysis of collagen. Peptide bonds are broken and cause the formation of N-terminal and C-terminal residues and thus the collagen molecular weight decreases at the same time. The total hydrolysis can even lead to the formation of single amino acids. Reaction rate rises at relative humidity > 70% and pH < 3 (for acid hydrolysis) (Vyskočilová et. al., 2019). Both organic and inorganic acid hydrolysis dechroming have been proven to be beneficial. It has been proven that the greater

the amount of dechroming, the more significant the collagen hydrolysis and the destruction of the collagen's natural structure. Sulfuric acid, hydrochloric acid, oxalic acid, lactic acid etc are used for dechroming process (Jiang et al., 2016).

Dechroming chrome tanned leather with alkaline hydrolysis is also a common procedure. To dissolve Cr^{3+}, CaO, MgO, NaOH, and Na_2CO_3 were utilized. In this situation, alkali will precipitate the Cr^{3+}, resulting in the insoluble $Cr(OH)_3$ sediment. Because no carcinogenic Cr^{6+} is created during the hydrolysis of chrome tanned solid leather wastes in mild acidic or alkaline conditions, it is considered to be more environmentally friendly (Kolomaznik, 2008). Per oxide oxidation is more effective technique yet hazardous for health. Clearly, they have an advantage over the oxidation dechroming method. Dechroming of chrome tanned solid leather wastes, using a single dechroming technique, has various demerits and can hardly meet the requirement of industry. To increase the dechroming efficiency, the combination of two or three dechroming methods is proposed. Currently, the combination of the acid-alkali method and acid/alkali-enzymes treatment has become attractive. Xie (as cited in Jiang et. al., 2016) treated the chrome tanned solid leather wastes alternately, using H_2SO_4, NaOH and H_2SO_4 process steps in a tanning factory in Chengdu (China). This suggests that the combination of acid and alkali methods has an advantage over the individual applications.

In recent years research indicates that hydrolysis must be addressed in a complex way, in several stages, with different mechanisms of development, initiated by different chemical agents, which complement each other (Hrncirik et al., 2005; Niculescu et. al., 2012). Our study highlights at the stepwise low hydrolysis method with alkali/acid alternately to dechrome the chrome tanned shavings and trimmings. It has an advantage of less affecting the collagen structure of the leather wastes to preserve a good quality for the gelatin extraction stage.

2.8 Denaturation of collagen

Collagen, the most abundant protein in mammals, is a fibrous structural protein and provides an intriguing example of a hierarchical biological nanomaterial. The collagen molecule consists of triple helical tropocollagen molecules that have highly conserved lengths of L-300 µm, roughly 1.5 µm in diameter. Staggered arrays of collagen molecules form fibrils, which arrange to form collagen fibers. Collagen plays an important structural role in many biological tissues, such as, for example, in tendon, bone, teeth, cartilage, and the

cardiovascular system (Leikina et. al., 2002). Collagen is also the main component of parchment which in the form of archival material bears the testimony of recorded cultural history and so necessitates its preservation. This relies on knowledge of the hydrothermal stability of the fibers of historical parchments. Studies in previous projects (Gatta et. al., 2007) have shown that fibers of some historical parchments with low hydrothermal stability are transformed into a gelatin structure by immediate contact with water at room temperature. Thus, studies for characterizing the extent of gelatinization of parchment fibers, used on collagen in its dry state, will prevent irreversible damage which can occur during storage and conservation treatment (Gatta et. al., 2007).

The structural properties of collagen have been the subject of numerous studies over past decades. Thermal transitions can be related to the process of gelatinization of the collagen fibrils, whereas at higher temperatures, both the gelatin and collagen samples underwent two-stage transitions with a common initial degradation temperature at (300 ± 10)°C and a secondary degradation temperature of (340 ± 10)°C for the collagen and of (420 ± 10)°C for the gelatin, respectively (Leikina et. al., 2002). There has been a recent report suggesting that collagen may be unstable at room temperature, leading to the conclusion that this protein, the main constituent of the human body and skeleton, may appear in a denatured state under normal physiological conditions (Leikina et. al., 2002). Upon denaturation, collagen fibrils undergo several conformational changes caused by the breaking of different cross-links present at the intermolecular level such as the nonenzymatic glycosylation of lysine and hydroxylysine residues and at the intramolecular level such as the disulphide bridges (Bozecn et. al., 2011; Virtual chembook, 2021). Furthermore, the H-bonded water used to stabilize the collagen molecule is released, leading to the collapse of the triple helix structure of the molecules. The end result of the thermal denaturation of collagen is random fragmentation of the collagen fibril and molecule, due to the loss of those cross-links that are necessary to stabilize the collagen fibril ultra-structure. It is, however, possible to observe some fibrillar structure in one studied area of the gelatin sample (Bozecn et. al., 2011).

2.9 Gelatin from collagen hydrolysis

Gelatin is a protein obtained by partial hydrolysis of collagen, the main protein component in skin, bones and white connective tissues of the animal body (Sebastian et.al., 2014; Yorgancioglu, 2020). It is obtained by thermal denaturation of collagen (Paul et. al., 1998).

Because it is obtained from collagen by a controlled partial hydrolysis and does not exist in nature, gelatin is classified as a derived protein. Animal glue and gelatin hydrolysate, sometimes referred to as liquid protein, are products obtained by a more complete hydrolysis (Sebastian et. al., 2014). Gelatin and collagen are polypeptide chains that are linked together by hydrogen bonds between the adjacent amino acids chains. The secondary structure of a protein is the structural conformation (shape) that results from these interactions. Collagen is made up of three polypeptide chains that closely tangle to form a highly stable triple-helix structure. These triple-helical molecules are subsequently arranged into collagen fibril bundles. Collagen's outstanding mechanical qualities are due to its high degree of organization (Fegan, 2021). When collagen is hydrolyzed under heat, the triple-helix unwinds and the secondary structure is partially lost. This process is referred to as the denaturation of collagen; gelatin is simply denatured collagen. Collagen may be considered an anhydride of gelatin (Thakur, 2015). The hydrolytic conversion of collagen to gelatin yields molecules of varying mass: each is a fragment of the collagen chain from which it was cleaved. Therefore, gelatin is not a single chemical entity, but a mixture of fractions composed entirely of amino acids joined by peptide linkages to form polymers varying in molecular mass from 15,000 to 400,000 (Thakur, 2015). Denaturation of collagen produces thermo reversible gelatin. At the sol-gel transition temperature (about 35°C), water molecules in the gelatin solution become trapped within the gelatin network, forming a semi-rigid gel. The polypeptide chains agglomerate and seek to restore their secondary structure when the gelatin solution is cooled below the sol-gel transition temperature (Fegan, 2021). In terms of collagen, however, this structure is only partially repaired. Junction zones are locations where the triple-helices renature (reform), whereas amorphous regions are those where the chains randomly coil (Fegan, 2021). The process of gelatin manufacturing may take up to several weeks, and differences in the processes have a great effect on the properties of the final gelatin product. High molecular weight gelatin material, formed by processing collagenous material, results in characteristic ‘collagen gels’ (Silock, 2010). Here, gelatin forms a solution of high viscosity in water, which sets to a gel on cooling, and its chemical composition, in many respects, closely resembles that of its parent collagen. If the acid or alkaline treatments are continued until complete hydrolysis occurs, this results in a low molecular weight fraction of protein that is termed gelatin hydrolysate (Silock 2010). The Figure 2.4 (Fegan, 2021) represents thermoreversible gelatin formation by collagen denaturation where water molecules in the gelatin solution become trapped within the gelatin network at the sol-gel transition temperature, generating semi-rigid gel.

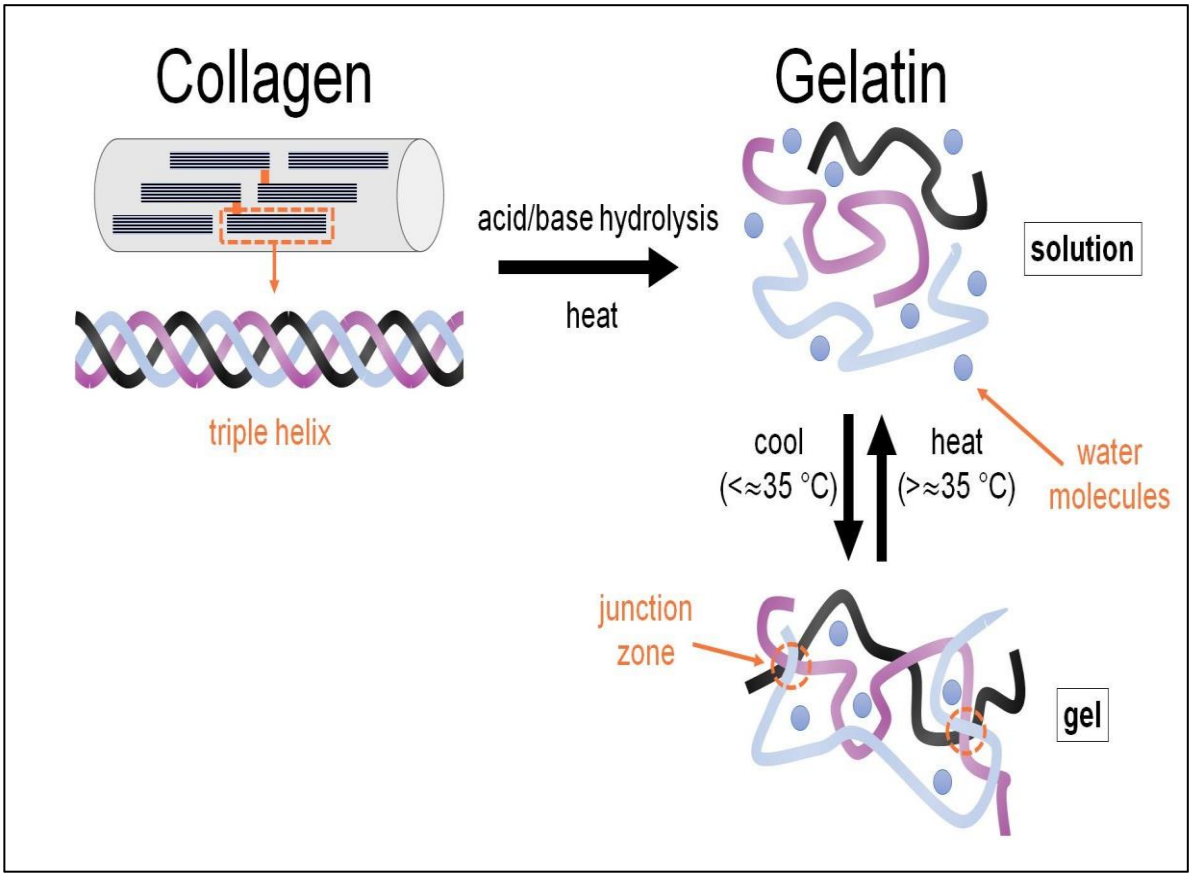


Figure 2.4: Hydrolysis of collagen in acid/alkali medium (Fegan, 2021)

Reaction involved in gelatin production is not very complex rather simple hydrolysis reaction at elevated temperature (Shakil et. al., 2019):

$$C_{102}H_{149}N_{31}O_{38} + H_2O = C_{102}H_{151}N_{31}O_{39} \quad (2.1)$$

Collagen Gelatin

Further hydrolysis of gelatin for prolong period of time produces degradation of collagen into glutenous protein:

$$C_{102}H_{151}N_{31}O_{39} + 2H_2O = C_{55}H_{85}N_{17}O_{22} + C_4H_{70}N_{14}O_{19} \quad (2.2)$$

Gelatin Semiglutin Hemikline

2.10 Gelatin extraction and types of gelatins depending onmanufacturing

The conventional method of obtaining gelatin is based on a two-step process (maturation or ripening, also called maceration and extraction). The aim of the maceration, an alkali-acid pre-treatment, is to weaken the collagen structure, solubilizing the non-collagenic proteins and hydrolyzing part of the peptide bonds, but keeping the consistency of the collagen fibers. The second step involves using hot water extraction (Catalina et. al., 2007). This is the simplest way where collagen is transformed into gelatin to denature soluble collagen. It involves hydrolysis catalyzed by enzymes, acid or alkali. Thermal denaturation takes place in mild conditions by heating the collagen in neutral or slightly acidic conditions to about 40^{o}C. The transition is sharp and complete within a few minutes over a small temperature interval. The activation energy for denaturation is approximately 81 Kcal. At that point only the hydrogen bonds and hydrophobic bonds that help to stabilize the collagen helix are broken causing the fibers and fibrils of collagen to dissociate into tropocollagen units. The next step, in the hydrolysis of collagen consist in breaking the intramolecular bonds between the three chains of the helix (Ofori, 1999).

The most influential parameter on the final quality of the gelatin is hydrolytic agent (alkali/acid). Although new methods for processing gelatin, including ion exchange and cross-flow membrane filtration have been introduced since 1960, the basic technology for modern gelatin manufacture was developed in the early 1920s. Acid and lime processes have separate facilities and are not interchangeable. In the past bones and ossein,ie, decalcified bone, have been supplied by India and South America. In the 1990s, slaughterhouses and meat-packing houses are an important source of bones (Sebastian, 2014). The supply of bone has been greatly increased since the meat-packing industry introduced packaged and fabricated meats, assisted by the growth of fast-food restaurants. Dried and rendered bones yield about 14-18% gelatin, whereas pork skins yield about 18-22%. Commercially, gelatin is made from by-products of the meat and leather industry, mainly pork skins, pork and cattle bones, or split cattle hides (Sebastian, 2014). The bovine hide is composed of approximately 30% protein, and the inner corium layer of the hide is rich in collagen. This collagen has a high denaturation temperature in comparison to collagen from other sources (Yorgancioglu, et. al., 2020). However, by-products of the fishery industry have also been considered as raw materials for gelatin production. The process of gelatin manufacture has been improved

considerably over the years. Today there are two commercial grades of gelatin available. Type A: which is produced through acid treatment of collagenous raw materials, primarily porcine skin. Type B: which results from alkaline treatment of bovine hide and bones (Silock, 2010). It was also reported that type A gelatin is produced by acid hydrolysis of bones, skins, connective tissue of pork and type B gelatin is produced by alkaline hydrolysis of bones, skins and tissues of animals (Shakil et. al., 2019).

The dissociation of collagen polypeptide chains by thermal or chemical methods results in a variety of gelatin derivatives. Gelatin can theoretically be made by simply heating collagen with water. However, this results in a very low-grade product. To create a high gel strength and viscosity material, the collagen is treated with an alkaline or acid procedure (Thakur, 1979). By alkaline hydrolysis process many impurities are removed such as noncollagenous proteins, mucopolysaccharides and salts, the amount of carboxyl groups increases as the amide groups are hydrolyzed, some terminal amino groups are freed, indicating a small degree of hydrolysis of peptide chains; and there is a swelling of the collagen which increases with the duration of the liming treatment (Veis and Cohen, 1955). It was assumed that the process of extracting gelatin dissociates the collagen structure by separating the polypeptide chains and breaking them into shorter segments. Collagen shrinkage temperature is greatly influenced by interactions with tiny molecules such as electrolytes and non-electrolytes, acids and bases, tanning agents, and so on. If collagen hydrolysis is extended, the polypeptide chains are broken into shorter and shorter lengths and the characteristic properties of gelatin are lost. Gelatin is also extracted by an acid extraction process which involves soaking skins in dilute acid, followed by extraction with warm water at an acid pH (Thakur, 1979).

Veis and Cohen concluded that the acid dissolution data suggest that collagen fibers break apart into more or less discrete units, depending on the pH of the extraction (Veis and Cohen, 1955). Also, the behavior of the subsidized collagen obtained by mild treatment is not that of a typical gelatin. The proteins extracted during more drastic acid degradations are distinguished from the first products of mild degradation by their higher iso-electric points, overall acid-base titration curves, lower average molecular weights and their behavior in mixed solvents (alcohol-water and acetonewater) (Thakur, 1979). Feng (Feng et. al., 2022) suggested a combined acid alkali hydrolysis process for gelatin extraction by microwave-rapid freezing-thawing coupling method. Chrome tanned leather needs to be treated and dechromed before gelatin extraction. Jiang (Jiang et. al., 2016) described various popular methods of dechroming process including acid-alkali combined method. The triple-helix

unwinds and the secondary structure is partially destroyed when collagen is hydrolyzed under heat. Denaturation of collagen is the term for this process; gelatin is just denatured collagen. The hydrolysis reaction is catalyzed in acidic or basic conditions because collagen is insoluble in water. Type A (acid hydrolysis) and Type B (acid hydrolysis) gelatin are produced (base hydrolysis) (Fegan, 2021; Mariod and Adam, 2013).

Most type A gelatin is made from pork skins, yielding grease as a marketable by-product. The process includes macerating of skins, washing to remove extraneous matter, and swelling for 10-30 h in 1-5% solution of hydrochloric acid, phosphoric acid, or sulfuric acid. The four to five extractions are made at temperatures increasing for 55^{o}-65^{o}C for the first extract to 95^{o}-100^{o}C for the last extract. Each extraction lasts about 4-8 h (Sebastian, 2014). Grease is then removed, the gelatin solution filtered, and, for most applications, deionized. Concentration to 20-40% solids is carried out, in several stages by continuous vacuum evaporation. The viscous solution is chilled, extruded into thin noodles, and dried at 30-60^{o}C on a continuous wire-mesh belt (Sebastian, 2014). Drying is completed by passing the noodles through zones of successive temperature changes wherein conditioned air blows across the surface and through the noodle mass. The dry gelatin is then ground and blended to specification.

Type B gelatin is made mostly from bones, but also from bovine hides and pork skins (Shakil et. al., 2019). The bones for type B gelatin are crushed and degreased at the rendering facilities, which are usually located at a meat-packing plant. Rendered bone pieces, 0.5-4 cm, with less than 3% fat, are treated with cool, 4-7% HCl acid from 4 to 14 days to remove the mineral content (Sebastian, 2014). During the liming process, some deamination of the collagen occurs with evolution of ammonia. This is the primary process that results in low isoelectric ranges for type B gelatin. After washing for 15-30 h to remove the lime, the ossein is acidified to pH 5-7 with an appropriate acid. Then the extraction processing for type A gelatin is followed (Sebastian, 2014). Throughout the manufacturing process, cleanliness is important.

2.11 Use of industrial/technical grade gelatin

The gelatin produced from chrome-tanned solid leather waste can be used as an industrial grade gelatin making itself a useful value added product from a hazardous waste. Technical gelatin differs from edible and medicinal gelatin in that it is not required that they meet the stringent human consumption criteria established by local, state, and federal governments to protect the public's health. In many respects these gelatins are similar to edible gelatins. The test methods are generally the same as those used for edible gelatins. Both Type A and Type B gelatin may be used in the various applications that follow, but in certain applications one may be preferred over the other.

Coating and Sizing : Technical gelatins are used in the warp sizing of rayon and acetate yarns. The gelatin size adds strength to the warp and resistance to abrasion so that breakage of the warp is minimized. Gelatin is particularly well suited for this application because of its excellent solubility and film strength. It is applied in aqueous solution along with penetrating oils, plasticizers and anti-foaming agents before weaving, and later removed during finishing by washing with warm water. Panama hats are sized with gelatin. Sizing helps preserve the shape of the hat while imparting resistance to water and dirt. An emerging technology involves the use of gelatin to size quartz fibers for space-age fabrics (GMIA, 1986).

Paper Manufacture : Gelatin is used for surface sizing and for coating purposes. Either used alone or with other adhesive materials, the gelatin coating creates a smooth surface by filling up the small surface imperfections thereby ensuring improved printing reproduction. Examples include posters, playing cards, wallpaper, and glossy magazine pages. High quality rag-based papers, such as those used for blueprints and currency, also feature a gelatin size coating. The result is a paper which has good moisture and abrasion resistance as well as good adhesion to printing inks. During manufacture the gelatin coatings are rendered insoluble by treatment with cross linking agents. The permanent crinkle in crepe paper is the result of gelatin sizing (GMIA, 1986).

Printing Processes: For over a century, gelatin compositions have been used in printers' rollers and plate wiping rollers for multicolor presses and offset lithography. Several photo-printing methods depend on the effect of light on a gelatin film which has been sensitized by treatment with potassium or ammonium dichromate. Examples of printing applications include carbon printing, collotype printing, silk screen printing, and photoengraver.

Some high-quality ink formulations include a small amount of gelatin as a suspension agent, athickener or a protective colloid (GMIA, 1986).

Protective Colloidal Applications: Much like edible gelatin is used for clarification of wine, beer and juices, technical gelatin is employed in the removal of extremely fine particles that cannot be settled out or filtered out of chemical solutions. Here gelatin is absorbed onto the surface of the particles effecting a coagulate that may be removed by settling or filtration. Approximately one pound of gelatin per ton of ore is used as a filtration aid during the extraction of uranium ore (GMIA, 1986; Mariod and Adam, 2013).

Stabilizer: Gelatin is used to stabilize emulsions for water-proofing fabrics. In suspension polymerization gelatin functions to control particle size as well as to prevent coalescence of the particles. About one pound of gelatin is sufficient to control particle size in a 10,000 pound batch of polyvinyl chloride (GMIA, 1986; Mariod and Adam, 2013).

Chemical: Gelatin is added to electroplating baths to control the deposition rate. Similarly, gelatin functions as a zinc brightener by controlling the crystallization of zinc during deposit (GMIA, 1986).

Matches: Gelatin is used almost universally as the binder for the complex mixture of chemicals are used to form the head of a match. The surface activity properties of gelatin are important since the foam characteristics of the match head influence the performance of the match on ignition (GMIA,1986).

Coated abrasives : Gelatin is used as the binder between the paper substrate and the abrasive particles of sandpaper. During manufacture the paper backing is first coated with a concentrated gelatin solution and then dusted with abrasive grit of the required particle size. Abrasive wheels, disks and belts are similarly prepared. Oven drying and a cross linking treatment complete the process printing (GMIA, 1986).

Adhesives: Over the past few decades gelatin-based adhesives have slowly been replaced by a variety of synthetics. Recently, however, the natural biodegradability of gelatin adhesives is being realized. Today, gelatin is the adhesive of choice in telephone book binding and corrugated cardboard sealing (GMIA,1986; Mariod and Adam, 2013).

Packaging: The tackifying power of gelatin is used to advantage in the manufacture of packaging ribbons, decals, gummed tapes, glass laminates and composition cork gaskets. Hard cover book bindings typically utilize gelatin-based adhesives (GMIA, 1986).

Cosmetics: Although gelatin is used in cosmetic applications such as creams and wave-set lotions, the use of gelatin hydrolysate is more widespread in personal care products, especially in hair preparations (GMIA, 1986).

Microencapsulation: Microencapsulation was first introduced commercially in the mid-1950's as the now well-known carbon less paper wherein a leuco dye material is enclosed in microcapsules coated onto a sheet of paper. When pressure from handwriting or typing breaks the microcapsules, the dye reacts with the coating on the next sheet to form a perfect image of the top sheet. The applications for microencapsulation are literally limitless: dyes, drugs, flavors & fragrances and spray drying are just a few examples (GMIA, 1986).

Gelatin films and light filters: Films of various colors are produced with gelatin for use in photographic lighting and theatrical spotlights. Some bottle caps utilize gelatin films for shrunk-on seals (GMIA, 1986).

Other Applications: Analytics is another area of interest where gelatin is used in ballistics testing in the Forensic Science laboratory. Gelatin is also used in Detergents and Cleansing Agents to minimize the accumulation of residues. Gelatin also has many uses in the Environmental protection area (GMIA,1986).

2.12 Hazardous waste management

A hazardous waste, simply defined, is a waste that has features that make it dangerous or capable of harming human health or the environment. Hazardous waste can come in a variety of forms, including liquids, solids, gases, and sludges (EPA, 2021). Leather industry has long been regarded as one of the most polluting and constant sources of creating hazardous waste, mainly solid waste. The unfavorable nature of most of the chemicals employed during the process makes large-scale leather production a significant issue for the industry. A foul odor is also released as the proteinous content of the skin degrades.

Because of the many mechanical and chemical procedures used on the hides and skins, the tannery solid wastes contain a variety of compounds. Environmental issues could arise if they are accumulated or improperly disposed of. Chromium salt is widely utilized in tanning companies that generate chromium-containing solid waste. As a result, all of these wastes must be examined and managed. Nur Mohammad and Kanagamani (as cited in John, 2021) stated that hazardous wastes must be handled and stored with care to minimize the danger of

exposure, as they offer a significant or potential threat to public health and the environment. Waste management refers to the entire process of identifying, storing, transporting, treating, recycling, and disposing of hazardous materials.

2.12.1 Classification of hazardous waste

It can be difficult and complex to identify hazardous waste, but according to (EPA, 2005), a waste can be classified as hazardous if it fits any of the following criteria:

If the waste contains any of the following chemicals, as stated by the Environmental Protection Agency (EPA): the F List (non-specific source wastes), the K List (non-specific source wastes) (source-specific wastes), the P and U lists (discarded commercial chemical products). If the waste has one or more of the following four characteristics: corrosivity, reactivity, toxicity, flammability/ignitability (John, 2021). The F list identifies wastes from common manufacturing and industrial processes as hazardous. Because the processes generating these wastes can occur in different sectors of industry, the F list wastes are known as wastes from non-specific sources. They can be divided into seven groups depending on the type of manufacturing or industrial operation that creates them: spent solvent wastes, electroplating and other metal finishing wastes, dioxin-bearing wastes, chlorinated aliphatic hydrocarbons production, wood preserving wastes, petroleum refinery wastewater treatment sludges, and multisource leachate (EPA, Hazardous Waste, 2021). Tannery wastes dumped in the environment can form leachate which is hazardous because of Chromium content. The K-list identifies hazardous wastes from specific sectors of industry and manufacturing and are considered source-specific wastes. To qualify as a K-listed hazardous waste, a waste must fit into one of the 13 categories on the list and the waste must match one of the detailed K list waste descriptions. The P and U lists designate as hazardous waste pure and commercial grade formulations of certain unused chemicals that are being disposed. For a waste to be considered a P or U listed waste it must meeting the three criteria: the waste must contain one of the chemicals listed on the P or U list, the chemical in the waste must be unused; and the chemical in the waste must be in the form of a commercial chemical product (EPA, Hazardous Waste, 2021).

2.12.2 Characteristics of hazardous waste

A hazardous waste characteristic is a property that, when present in a waste, indicates that the waste provides a significant enough concern to warrant hazardous waste regulation.

Ignitability, corrosivity, reactivity, and toxicity are the four hazardous waste characteristics determined by the EPA (EPA, 2005).

Ignitability: Wastes that are hazardous due to the ignitability characteristic include liquids with flash points below 60°C, non-liquids that cause fire through specific conditions, ignitable compressed gases and oxidizers. The waste code for ignitable hazardous wastes is D001, according to the EPA. (EPA, Hazardous waste, 2021). This property aids in the identification of flammable wastes that can quickly catch fire and burn for a long time. When a chemical is exposed to a flame, the flush point test establishes the lowest temperature at which it ignites. Non-liquid trash is only dangerous because of its ignitability if it may catch fire spontaneously under normal handling conditions and burn so hotly that it causes a hazard (EPA, 2005). oxidizing wastes, which are certain compressed gases and chemicals that may produce oxygen, causing or contributing to the combustion of other materials; and volatile liquids, which are solvents with vapors ignited at temperatures of 60^{o} C or less, are two examples (John, 2021).

Corrosivity

This characteristic distinguishes acidic or alkaline (or basic) wastes that can readily dissolve flesh, metal (under certain conditions), or other materials, causing severe acute damage when in contact with them, or, in the case of leakage, may destroy other goods or modes of transportation. Aqueous wastes with a pH of 2.0 or lower, or 12.5 or higher, are corrosive, according to a pH test (EPA, 2005). D002 is the waste code for corrosive hazardous wastes, according to the EPA (EPA, Hazardous Waste, 2021).

Toxicity

Hazardous wastes have a toxicity characteristic that makes them harmful when consumed or absorbed. Toxic wastes are a threat because they can seep from garbage and damage groundwater. The Toxicity Characteristic Leaching Procedure is used to determine a waste's toxicity (TCLP). EPA assigned wastes codes D004 through D043 that correspond to a contaminant and its associated TCLP concentration (EPA, Hazardous Waste, 2021). Toxicity Characteristic Leaching Procedure (TCLP), a lab procedure used on a waste sample, is used to determine whether a waste contains any of the 39 toxic chemicals with concentration levels above specified regulatory levels or the threshold, according to the Environmental Protection Agency (EPA, 2014). Two main phases are involved in establishing whether a sample show

the toxicity characteristic: (a) using TCLP to create a leachate sample; and (b) comparing the concentrations of 39 compounds in that sample to regulatory levels (John, 2021).

Reactivity

Hazardous wastes, by virtue of their reactivity, may be unstable under normal settings, react with water, emit toxic fumes, and be capable of detonation or explosion under normal conditions or when heated. D003 is the waste code for reactive hazardous wastes, according to the EPA. There are no reactivity tests available (EPA, Hazardous Waste, 2021). A waste is considered reactive if it can explode or violently react when exposed to water, when heated, or under normal handling, create toxic fumes or gases when exposed to water under normal handling conditions, or generate toxic levels of sulfide or cyanide gas when exposed to a pH range of 2 through 12.5 (John, 2021).

2.12.3 Hazardous waste treatment, recycling, storage and disposal

Hazardous waste management can be done in a variety of ways. The most desirable option is to decrease waste at the source or recycle the materials for another beneficial purpose. Despite the fact that waste reduction and recycling are desirable solutions, they are not considered the final solution to the problem of hazardous waste disposal. There will always be a need for hazardous waste treatment, storage, or disposal in some form (Britanica, Hazardous Waste Management, 2021). EPA attempted to design hazardous waste laws that strike a balance between resource conservation and human health and environmental protection. Many hazardous wastes can be safely recycled, but others will be handled and disposed of in landfills or incinerators (EPA, Hazardous Waste, 2021).

Treatment of hazardous waste

Chemical, thermal, biological, and physical methods can all be used to treat hazardous waste. Ion exchange, precipitation, oxidation and reduction, and neutralization are all chemical methods. High-temperature incineration is one of the thermal technologies that may not only cleanse but also destroy some organic wastes. Thermal equipment is used to burn trash that is solid, liquid, or sludge. Fluidized-bed incinerators, multiple-hearth furnaces, rotary kilns, and liquid-injection incinerators are among them. The possibility for air pollution is one issue that hazardous-waste incineration poses. The chemical, thermal, and biological treatment methods change the molecular form of the waste material. Physical treatment, on the other hand, concentrates, solidifies, or reduces the volume of the waste. Physical processes

include evaporation, sedimentation, flotation, and filtration (Britanica, Hazardous Waste Management, 2021).

Recycling hazardous waste

Recycling hazardous waste has a number of advantages, including lowering raw material consumption and the volume of waste that must be treated and disposed of. However, poor storage of such materials could result in spills, leaks, fires, and soil and drinking water contamination. To encourage hazardous waste recycling while safeguarding human health and the environment, the Environmental Protection Agency (EPA) set laws to ensure that recycling was done safely (EPA, Hazardous Waste, 2021).

Surface storage and land disposal of hazardous waste

Hazardous waste that cannot be eliminated through incineration or other chemical processes must be appropriately disposed of. Land disposal is the final destination for most of these wastes, despite the fact that it is not an appealing practice due to the inherent environmental dangers. Landfilling and subterranean injection are the two most common ways of land disposal. Surface storage or containment systems are frequently used as a temporary measure prior to land disposal (Britanica, Hazardous Waste Management, 2021). Hazardous wastes are temporarily stored and then treated or disposed of at Treatment Storage and Disposal Facilities (TSDFs). TSDFs are heavily regulated since they handle significant amounts of waste and engage in activities that may provide a higher level of danger. The TSDF mandates general facility management standards, as well as particular measures governing hazardous waste management units and other safeguards to protect soil, groundwater, and air resources (EPA, Hazardous Waste, 2021).

2.12.4 Delisting hazardous waste

Fortunately, the Environmental Protection Agency (EPA) has a method for removing a waste stream from its list of hazardous wastes if it can be demonstrated to be free of corrosivity, ignitability, reactivity, and/or toxicity. A delisting petition is what it's called. The Resource Conservation and Recovery Act (RCRA) provides a process to remove, or “delist,” a waste generated at a facility from the list of hazardous wastes (EPA, Hazardous Waste, 2021). If a facility's waste does not possess the harmful qualities, RCRA regulations provide a petition method to exclude or "delist" the waste from the list of hazardous wastes. Only listed wastes

can be delisted, despite the fact that there are two types of hazardous waste: characteristic and listed (EPA, Hazardous Waste, 2021).

2.12.5 The RCRA 8 Metals

The RCRA 8 is a set of eight heavy metals that are listed and monitored under the Resource Recovery and Conservation Act (RCRA). The reason for this is that each of these eight metals is exceedingly hazardous in even minute amount. As a result, the amount of each metal present in waste is strictly regulated, and waste can consist of anything from light bulbs to batteries. As a result, it's critical to understand each metal and the maximum amounts that can be used (Hazardous Waste Experts, 2021). The RCRA 8 metals are: Arsenic (As), Barium (Ba), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg), Selenium (Se), and Silver (Ag). The EPA allowable limit for RCRA 8 metals is listed below in Table 2.1.

Table 2.1: EPA allowable limit for RCRA 8 metals

Heavy metal	Hazardous waste code	EPA allowable limits ppm (mg/L) (Hazardous Waste Experts, 2021)
Arsenic (As)	D004	5
Barium (Ba)	D005	100
Cadmium (Cd)	D006	1
Chromium (Cr)	D007	5
Lead (Pb)	D008	5
Mercury (Hg)	D009	0.2
Selenium (Se)	D0010	1
Silver (Ag)	D0011	5

Chapter 3
METHODOLOGY

3.1 Introduction

The chapter methodology explains the material and methods used during this study. The sample preparation, dechroming process and gelatin extraction processes are elaborated with specific measurement and explained with flow diagram in this chapter. The study was conducted at the Environmental Engineering Laboratory in department of Civil Engineering, BUET, Dhaka, Bangladesh. Some analysis of the chrome tanned, dechromed and gelatin sample were conducted in Bangladesh Council of Scientific and Industrial Research (BCSIR) and Glass and Ceramic Engineering Department, BUET, Dhaka, Bangladesh.

3.2 Sample collection and pre-treatment

Chrome tanned trimmings and shavings were collected in dry form from a tannery in Savar, Bangladesh. The chromed trimmings were washed thoroughly with tap water, then with distilled water to remove dirt. Trimmings were air dried at room temperature. Shavings samples were not washed. Chrome tanned leather trimmings and shavings were heated at 60^0C separately in an oven overnight. The shavings were stored in a container and trimmings were cut into reduced size (5mm-2cm) (Poulopoulou et. al., 1998) and stored in a container for further treatment.

Figure 3.1: a) chrome-tanned trimmings b) leather cutting tool c) chrome-tanned shavings

3.3 Physico-chemical characterization of sample

Characterization of chrome tanned trimmings and shavings samples were done by determining the pH, chromium concentration, moisture content, ash content, SEM (Scanning Electron Microscope) analysis and FTIR (Fourier Transform Infra-Red) spectroscopy analysis.

3.3.1 Chromium measurement

The aqua regia extractable chromium of the samples was determined following ISO standard procedure: ISO 12914:2012 which requires a minimum of 0.5g of sample mixed with 8mL of aqua regia and digested in a microwave digestion vessel at a temperature of 175^0C for 20 min. The extracts were then filtered through ashless paper filters and diluted to need. After dilution the chromium content of the sample was measured. The APHA standard method SM 3111B was used to determine chromium levels (APHA, 2012). 9 ml of diluted solution from each sample was placed in a vial, along with 1 ml of 65 percent nitric acid, and the bottle was forcefully shaken. The mixture was then cooked for 1-2 hrs on a digester at 150°C until a clear solution was achieved. The vial was taken to a Flame Atomic Absorption Spectrophotometer (AA-7000, GFA-7000; Shimadzu) to estimate the chromium conc. after cooling to room temperature and suitable dilution. Total chromium was obtained in all of the studies because the laboratory machine could not detect individual chromium components.

3.3.2 pH measurement

Initial pH values of chrome tanned leather wastes were accomplished at the laboratory environment as the following way: 5 g of leather wastes sample were placed in 100 ml distilled water at room temperature for two hours with agitation in shaker machine. After decantation without filtration of soluble matter (aqueous fraction) we proceed to determine pH of the filtrated solution using the pH-meter (Elsayed et. al., 2021).

3.3.3 Ash content measurement

To determine the ash content of chrome tanned trimmings and shavings sample following steps from literature (Elsayed et. al., 2021) were followed at laboratory environment. In a burnt crucible about 10g of sample was accurately weighed, the sample was carefully ignited in a muffle furnace at about 600°C for 2 hrs. Finally, the crucible with its contents was cooled in a desiccator and weighed. The ignition, cooling and weighting were repeated till constant weight. The ash content of the samples was measured according to following equation:

$$\text{Ash \%} = \frac{W_1}{W_2} \times 100 \quad \text{----------(3.1)}$$

W_1= Weight of residue

W_2= Weight of original gelatin

3.3.4 Moisture content measurement

10 g of sample were accurately weighted in a beaker, then heated at 105ºC for 3 hrs in oven at which the temperature was as uniform as possible; the beaker was allowed to cool in a desiccator, and then weighed. The process of heating, cooling and weighting was repeated till constant weight, the moisture content is defined as the percentage loss in weight of the sample (Elsayed et. al., 2021).

$$\text{Moisture \%} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad \text{----------(3.2)}$$

Where;

W_1 = weight of the sample before dryness.

W_2 = weight of the sample after dryness

3.3.5 FTIR analysis

FTIR spectroscopy is a measurement of wavelength and intensity of the absorption of IR radiation by a sample. The IR spectral data of high polymers are usually interpreted in terms of the vibrations of a structural repeat unit. Any product's bond structure can be understood via FTIR analysis. The FTIR of chrome tanned trimmings was evaluated utilizing 2g of dry sample by the PerkinElmer Spectrum machine, version 10.4.4. at Bangladesh Council of Scientific and Industrial Research.

3.4 Stage selection of dechroming process

In general, gelatin processing consists of three stages: pre-treatment, extraction, and recovery. Samples are usually treated with acid or an alkali to remove non collagenous protein and fat (Noor et. al., 2021). From the literature review (Jiang et. al., 2016). Combined alkali-acid hydrolysis was selected to dechrome the chrome tanned trimmings and shavings sample. Alkali-acid dechroming hydrolysis process is considered environmentally friendly because hazardous Cr^{6+} is not formed during this process. The chromium oxide formed in this process were in Cr^{3+} form, which is non carcinogenic (Matyasovsky et. Al., 2011). Also, alkali and acid are common chemicals and low-cost material. Multistage acid alkali operation is very effective in removing chromium concentration and denaturing leather comparative to single stage acid or alkali hydrolysis operation (Jiang et. al., 2016). Overall, four stages of combined alkali-acid treatment were designed to dechrome the trimmings and shavings. To get better result, forward and reverse stage performances were investigated. We divided our study of combined dechroming operation into two pathways to find out suitable material as the starting chemical. One operation was started with alkali hydrolysis (Forward stage operation) and another starting with acidic hydrolysis (Reverse stage operation). The aim of this operation was to compare the dechroming rates and yield between dechroming operation started with alkali vs dechroming operation started with acid. Depending on the acid or alkali used in the last stage of dechroming operation, gelatin is classified as type A or type B gelatin (Shakil et. al., 2019).

Figure 3.2 shows the experimental procedure selection flow chart of current study. Indirect dechroming treatment of chrome tanned leather waste is of three types: oxidation dechroming, hydrolysis dechroming and combination dechroming (Jiang et. al., 2016). Comparing hydrolysis dechroming and combination dechroming methods with oxidation dechroming, the major advantage of them is that the valence state of $Cr\ 3^{+}$ can be kept intact. Thus, the pollution caused by toxic $Cr\ 6^{+}$ will be highly decreased (Jiang et al., 2016). Among these three, combination hydrolysis dechroming process was found favorable for our objective. It was selected as our dechroming process for its simple mechanism and material availability. Then, as previously stated, forward and reverse stage operations were implemented.

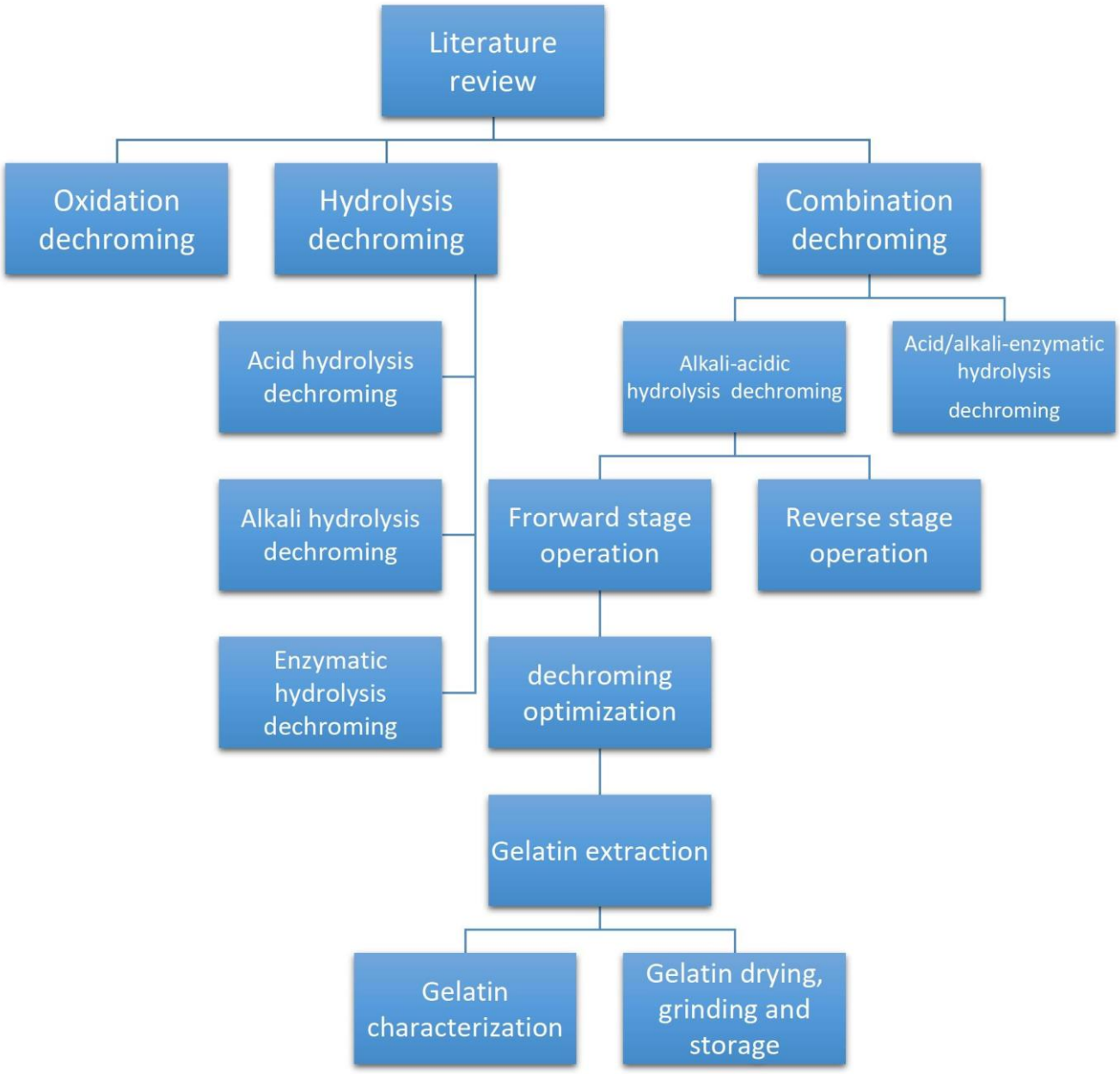


Figure 3.2: Experimental procedure selection

3.4.1 Forward stage operation

Forward stage operation was designed to see the effect alkali as starting chemical in multistage dechroming operation. Forward stage operation starts with alkali treatment of the tanned leather samples. In the 2nd stage, acidic operation was conducted. These two stages were repeated with modification in the following 3rd and 4th stages. In forward stage

operation, 1st step of dechroming started with soaking the sample in CaO solution for 4 days. In the 2nd stage the washed residue from first stage went through acidic hydrolysis for 4 hours of shaking where Oxalic acid (organic acid), HCl (inorganic acid), NaCl (hydrolysis assistant) was used. The 3rd stage was basic hydrolysis where MgO (alkali), NaCl (hydrolysis assistant) was used to stir (with low heating) the washed residue from second stage for 1hr. In 4th stage is acid wash stage where inorganic H_2SO_4 acid was used to shake the washed residue for 2hrs from the third stage. Chromium content and yield of leather from each step were calculated to determine the efficiency of forward stage operation. Filtrates from each step of hydrolysis were used to measure chromium of the residual sample. The residual samples were air dried to determine the yield from each step.

3.4.2 Reverse stage operation

A reverse stage operational path was designed to see the effect of acid as starting chemical for multistage dechroming. All the four stages were operated in reverse order at same conditions where the acidic hydrolysis was its 1st step. H_2SO_4 was used to shake the tanned leather sample for 2hrs. Alkali hydrolysis by MgO, NaCl induced shaking with low heating was applied in the 2nd step. Then 4 hrs of shaking with Oxalic acid (organic acid), HCl (inorganic acid) and NaCl (hydrolysis assistant) was applied in the 3rd step. In the 4th step of reverse stage operation alkali hydrolysis was used with slide modification where CaO was applied to shake the washed residue from 3rd step for 2hrs. At the 4th step 4hrs rigorous shaking with CaO (alkali) was applied for the residual sample. Filtrates from each step were used to measure chromium of the residual sample. The final residue sample was air dried to determine the final yield from step. After determining final chromium level and yield of the dechromed sample from this study, forward process was selected as suitable process to dechrome both chrome tanned trimmings and shavings.

Figure 3.3 shows the steps and differences of forward stage and reverse stage operation process. The number of steps is same for both operations, but the conditions are in reverse order. Forward stage operation was started with alkali hydrolysis and reverse stage operation was started with acid hydrolysis. After analyzing data from this study, we proceeded with forward stage operation to dechrome the chrome tanned solid leather waste.

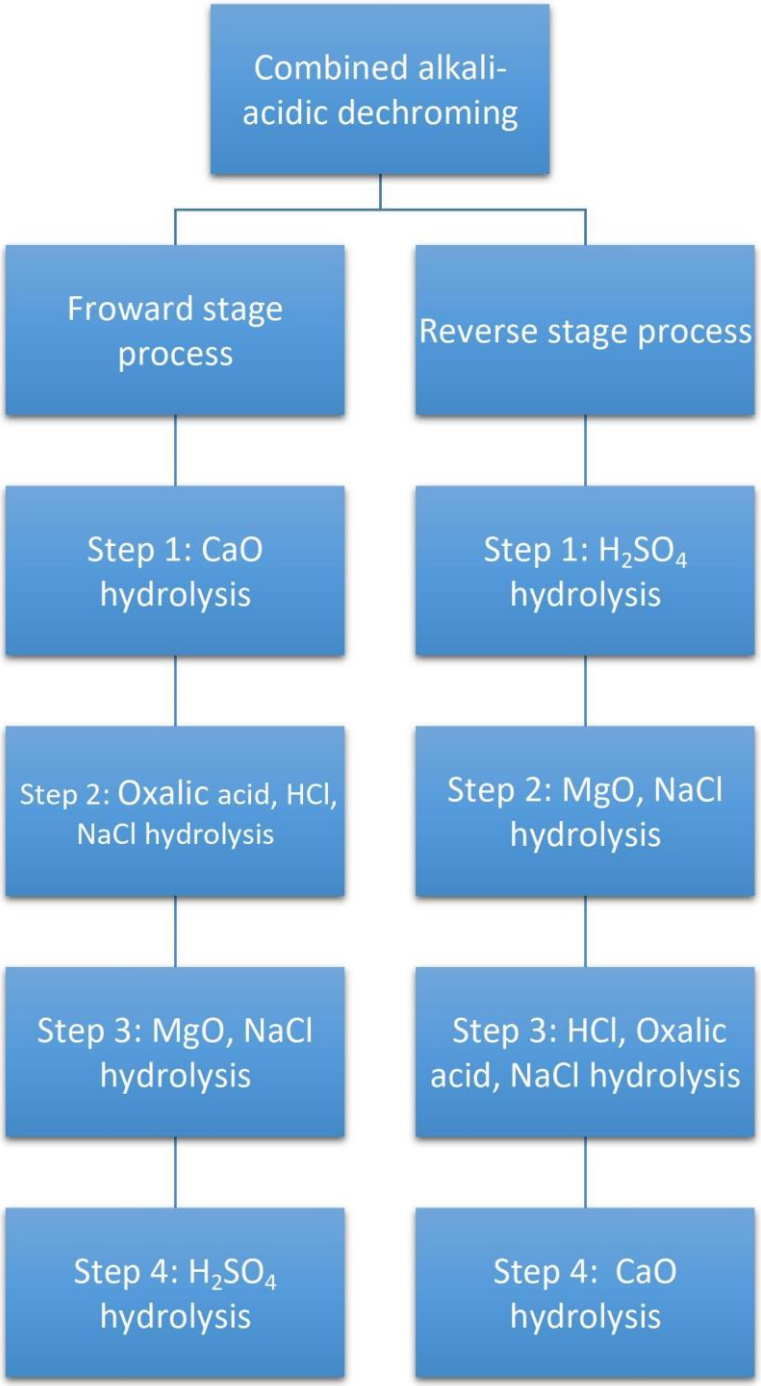


Figure 3.3: Forward and Reverse stage operation dechroming

3.5 Dechroming optimization

Forward stage dechroming operation of chrome tanned leather trimmings and shavings were carried out by using combined alkali-acid hydrolysis treatment in four stages. The operation

was optimized by changing dosage of the chemicals of each step, the soaking time of the 1st step of dechroming and by changing the hydrolysis time of the 3rd step. Both HCl and H_2SO_4 was used in acidic hydrolysis and the effect of utilizing each acid in different steps were investigated by running a trial operation. To determine the optimization effect of a single step, all the four steps were conducted and the effect was determined by using the final dechroming values and yields.

5g of chrome tanned leather sample from each initial sample was used per operation for optimization. After optimization, finally 50g from each initial sample was used to dehrome into optimized condition. Dechromed leather from this operation was used to extract gelatin and for characterization. The yields from dechroming optimization operation was based on 5g of dry initial sample and other yields from optimized dechromed leather was based on 50g of dry initial sample.

3.5.1 Inorganic acid selection for 2nd and 4th step

Two types of inorganic acids were used in the dechroming process. One was used in the 2nd step and other was used in 4th step. In the 2nd step inorganic acid was mixed with organic acid and salt, in the 4th step only inorganic acid was used to wash and reduce the chromium concentration. To get better dechroming result combined and single acidic performances for two different types of inorganic acids were observed for 2nd and 4th steps. H_2SO_4 and HCl were used to determine the dechroming effects by four interchanged trial operations. The organic acid and the salt concentration and all the conditions were kept fixed for all other steps. In 1st trial, HCl was used in both 2nd and 4th step as inorganic acid. In 2nd trial, HCl acid was used in 2nd step and H_2SO_4 was in the 4th step. In 3rd trial, H_2SO_4 was used in both 2nd and 4th steps. In the 4th trial, H_2SO_4 was used in both 2nd and 4th steps. The trial experiment was applied on both trimmings and shavings sample. The dechroming rate and gelatin formation time was observed to predict the suitability of the acids.

3.5.2 Dechroming dose optimization

Six samples (A,B,C,D,E and F) of each 5g trimming/shavings were kept in six 250ml beakers and were treated with six portions of chemical dosages for a fixed time, temperature and fixed amount of solution in dosage optimization operation. All the four steps were continued with six different portions of chemicals for dosage optimization.

Step 1 (soaking) : The soaking optimization was carried out for step 1 where alkali was used. Among the reagents, CaO is the most easily gained and the cheapest one (Jiang et. al., 2016). In step 1 dechroming, sample A was soaked with 0.5g CaO in 40ml distilled water. Sample B was soaked with 1g CaO in 40ml distilled water. Sample C was soaked with 1.5g CaO in 40ml distilled water. Sample D was soaked with 2g CaO in 40ml distilled water. Sample E was soaked with 2.5g CaO in 40ml distilled water. Sample F was soaked with 3g CaO in 40ml distilled water. All the samples were soaked for 4days. Samples were occasionally shaken in shaker machine at 250 RPM. Soaking is generally carried out at pH values between 9-10 (Beghetto et. al., 2013); The pH of the solutions was measured with pH paper. pH of sample A was 9, sample B was 10, sample C was around 10, sample D and sample E was around 11 and sample F was 12. This stage was conducted at room temperature. After soaking, all the samples were filtered using a thin porous cloth and washed in tap water then in distilled water and pressed to reduce the excess water. After washing the samples were ready for step 2.

Step 2 (Acidic hydrolysis): In step 2 the shaking speed for all the sample was 250 RPM and operation was conducted at room temperature for 4hrs. It was found from literature that higher degree of acidic hydrolysis could be obtained in room temperature (Jiang et. al., 2016). Sample A was shaken with 0.5g NaCl, 0.5g Oxalic acid and 1ml HCl acid in 25ml distilled water. Sample B was shaken with 1g NaCl, 1g Oxalic acid and 2 ml HCl acid in 25ml distilled water. Sample C was shaken with 1.5g NaCl, 1.5g Oxalic acid and 3ml HCl acid in 25ml distilled water. Sample D was shaken with 2g NaCl, 2g Oxalic acid and 4 ml HCl acid in 25ml distilled water. Sample E was shaken with 2.5g NaCl, 2.5g Oxalic acid and 4 ml HCl acid in 25ml distilled water. Sample F was shaken with 3g NaCl, 3g Oxalic acid and 6 ml HCl acid in 25 ml distilled water. All the solutions were highly acidic. pH of all solutions was found 1 by using pH paper. After acidic hydrolysis, all the samples were filtered using a thin porous cloth and washed in tap water then in distilled water and pressed to reduce the excess water. After washing the samples were ready for step 3.

Step 3 (Alkali hydrolysis): In step 3 alkali hydrolysis, alkali and salt as hydrolysis assistant was used. Residual amount of NaCl helps diffusion of water down hierarchical structure of skin fibers (Beghetto et. al., 2013). Dosage optimization of step 3 was carried out at 40^0-45^0C temperature in a heating plate magnetic stirrer for 1hr. The sample beaker was covered while heating. The temperature of the slurry was monitored and adjusted by a thermometer time to time. For step 3 optimization, 3 sample A was stirred with 0.5g MgO, 0.5g NaCl in 50ml

distilled water. Sample B was stirred with 1g MgO, 1g NaCl in 50ml distilled water. Sample C was stirred with 1.5g MgO, 1.5g NaCl in 50ml distilled water. Sample D was stirred with 2g MgO, 2g NaCl in 50 ml distilled water. Sample E was stirred with 2.5g MgO, 2.5g NaCl in 50ml distilled water. Sample F was stirred with 3g MgO, 3g NaCl in 50ml distilled water. The pH of the solutions was measured with pH paper. pH of sample A was 9, sample B was 10, sample C was around 10, sample D and sample E was around 11 and sample F was 12. After alkali hydrolysis, all the samples were filtered using a thin porous cloth and washed in tap water then in distilled water and pressed to reduce the excess water. After washing the samples were ready for step 4

Step 4 (Acid Wash): Dosage optimization of step 4 was conducted at room temperature for 2 hrs at 250 RPM. In this step sample A was shaken with 1ml H_2SO_4 and 25 ml distilled water. Sample B` was shaken with 2ml H_2SO_4 and 25ml distilled water. Sample C was shaken with 3 ml H_2SO_4 and 25 ml distilled water. Sample D was shaken with 4ml H_2SO_4 and 25ml distilled water. Sample E was shaken with 5ml H_2SO_4 and 25ml distilled water. Sample F was shaken with 6ml distilled water for 2 hrs. After alkali hydrolysis, all the samples were filtered using a thin porous cloth and washed in tap water then in distilled water and pressed to reduce the excess water.

3.5.3 Soaking time optimization

Six samples each of 5g were soaked for six different soaking times 1, 2, 3, 4, 5 and 6 days for step 1 soaking optimization. Each sample was soaked in same solution in 250ml different open beakers for different period of times. The leather with solution was shaken occasionally at 250 RPM to prevent the alkali clogging into the leather sample. The experiment was conducted at room temperature at laboratory condition. During soaking, chromium precipitation was seen to form with the alkali. After multiple days of soaking all the samples were washed using a thin porous cloth under tap water then with distilled water to remove the chromium precipitation and to neutralize the pH and pressed to reduce the excess water. Then After washing all the sample (in wet condition) of different soaking times went through further steps 2,3 and 4. After final dechroming of each sample of different soaking time the dechroming effect and yield was determined to choose the optimized soaking time. Same procedure was applied for both trimmings and shavings sample.

3.5.4 Heating temperature optimization for 3rd step

Three temperature ranges 40^0C-45^0C, 60^0C-65^0C, 80^0C-85^0C for the third step were applied to observe the temperature effect on hydrolysis. The other conditions and chemicals were kept fixed for the respected and rest of the steps. This optimization operation was conducted by using heating plate magnetic stirrer. The slurry of leather sample and chemical was kept on a beaker. Beaker was covered during operation. Heating temperature was monitored time to time by using a thermometer. This optimization operation was conducted at 250 RPM for 1hrs. After heating temperature optimization of step 3, the hot slurry was filtered through a thin cloth and pressed to reduce the excess water. After step 3, step 4 was continued and the temperature effect was determined by calculating the final dechroming rate and yield of the leather samples.

3.6 Image figures from different experiments of current study

The figures from dechroming operation are presented in this section. Figure 3.4 and 3.5 is from dosage operation. Changes on chromium conc. from sample A to sample F can be observed from Figure 3.4. and Figure 3.5. Figure 3.5 also shows good dechroming condition as the color of final dechromed leathers are much lighter than that of leathers shown on Figure

3.4. Figure 3.6 and 3.7 shows the images of washed samples after step 1 dechroming process. These figures shows the instant reaction of chromium with alkali as it turned into yellowish as soon as it was dissolved in alkali solution. The alkali hydrolysis opens up the hydrogen bonds to remove chromium. It also hinders the bacterial growth of the leather (Shupack 1991). Figure 3.8 and 3.9 shows the final dechromed trimmings and shavings samples at optimized condition determined from previous section 3.7. The final samples are much lighter in color. The optimized dechromed leathers are ready for gelatin extraction stage. Figure 3.10 shows some laboratory facilities from Environmental Engineering Laboratory of Civil Engineering Department used in this study. Figure 3.11 shows gelatin solutions from dechromed trimmings and shavings. 3.12, 3.13 are the figures of aqueous gelatin extracted from dechromed trimmings and shavings. These samples were colorless, odorless, thick enough to resist flow after cooling and showed gelatinous property. The characteristics of extracted gelatin solutions from current study were compared with the standard gelatin properties to check the quality of gelatin. The detailed gelatin characteristic analyses are described on section 4.7 in next chapter.

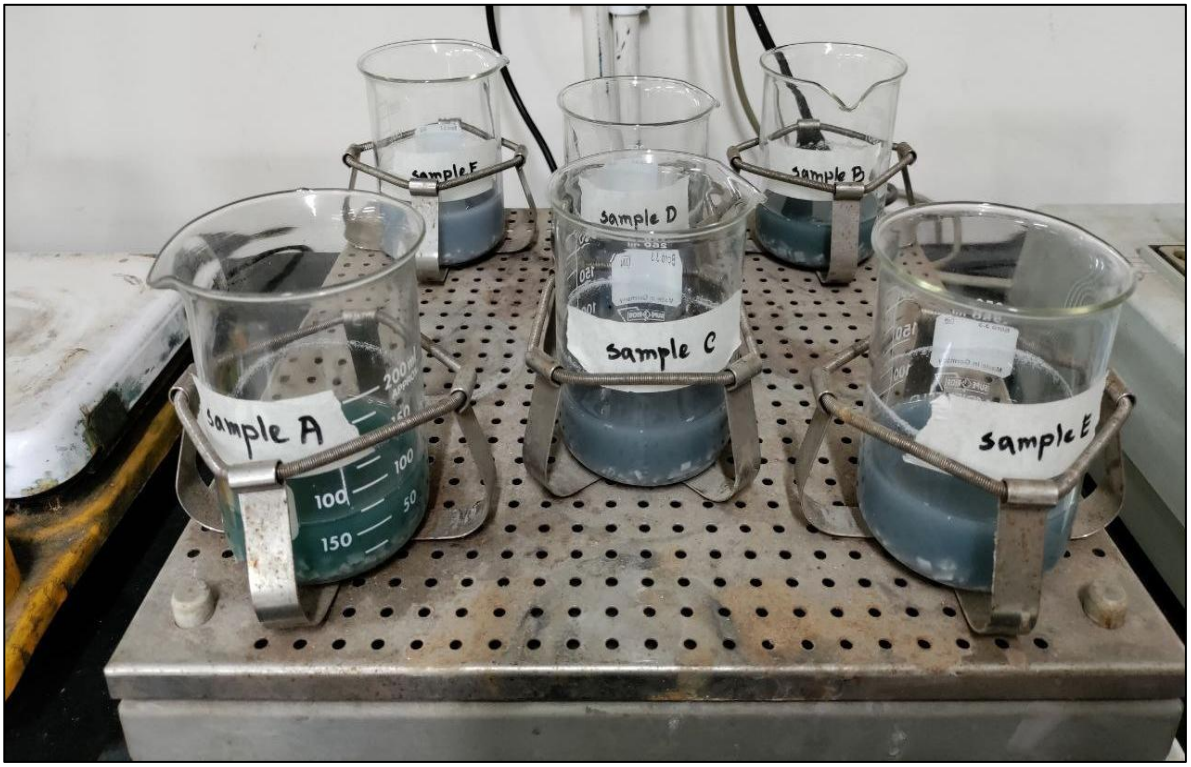


Figure 3.4: Dosage optimization in step 2 dechroming

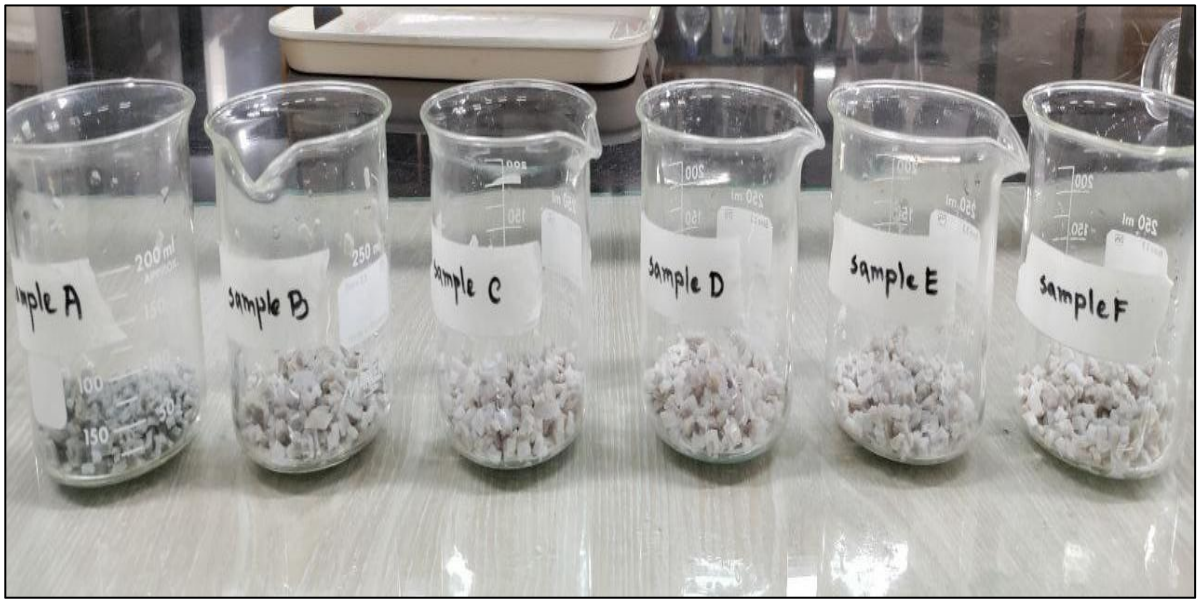


Figure 3.5: Dechromed leather after 4 steps of dosage optimization

Figure 3.6: Dechromed trimmings after step 1

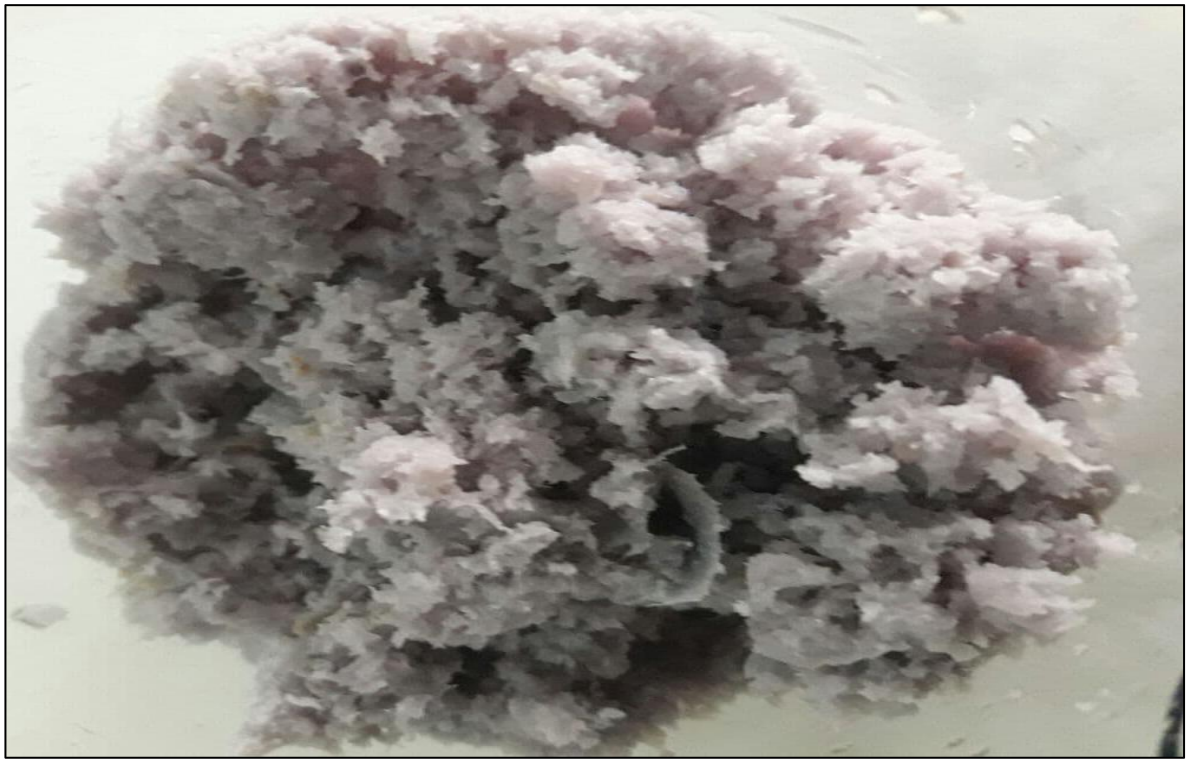


Figure 3.7: Dechromed shavings after step 1

Figure3.8: Dechromed trimmings after step 4

Figure 3.9: Dechromed shavings after step 4

Figure 3.10: Lab facilities used to dry and ash leather sample, determine chromium and extract gelatin

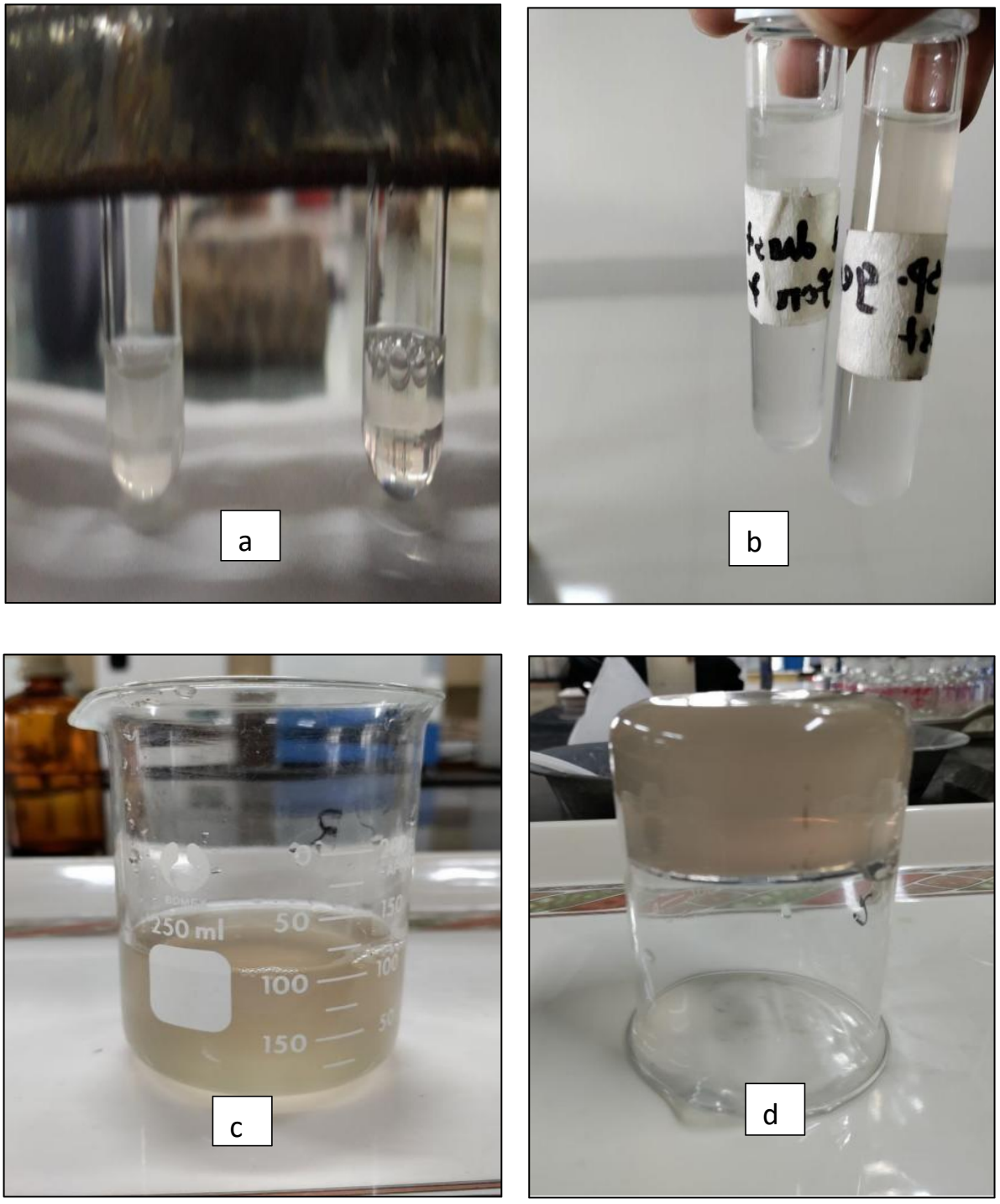


Figure 3.11: a), b) c) Gelatin in solution state d) Gelatin after cooling

Figure 3.12: Aqueous gelatin from dechromed trimmings (1hr extraction)

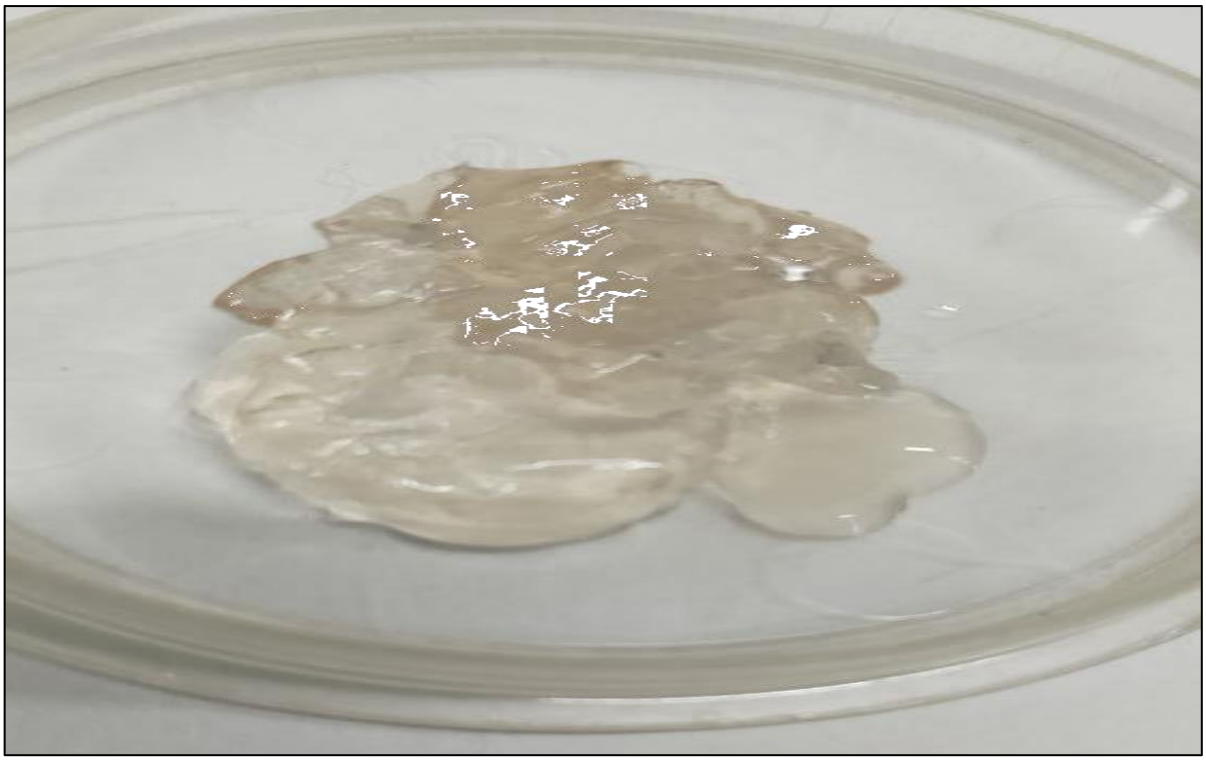


Figure 3.13: aqueous gelatin from dechromed shavings (2hrs extraction)

3.7 Data analysis and interpretation

The primary data generated under the study were analyzed with a view to assess the gelatin making capacity of the dechromed trimmings and shavings. All the samples went through gelatin extraction process after dechroming and the extracts from optimized dechromed samples were tested for chromium measurement, other heavy metal measurement, and yield analysis.

3.8 Physico-chemical characterization dechromed trimmings and shavings

The pH, dechroming rate, yield, SEM (Scanning Electron Microscope) analysis, FTIR (Fourier Transform Infrared) spectroscopy, and TCLP analysis were used to characterize dechromed trimmings and shavings samples (after washing).

3.8.1 Chromium measurement

The aqua regia extractable chromium of the samples was determined following ISO standard procedure: ISO 12914:2012 in which we used of 0.5g of dried dechromed leather sample from trimmings and shavings to mix with 8mL of aqua regia and digested in a microwave digestion vessel at a temperature of 175^0C for 20 min. The digested solutions were then filtered through ashless paper filters and diluted to need. After dilution, Cr conc. of the filtrate was determined. APHA standard method SM 3111B was used to determine chromium levels (APHA, 2012). 9 ml of filtrate sample from each trimmings and shavings was placed in a vial, along with 1mL of 65 percent nitric acid, and the bottle was forcefully shaken. The mixture was then cooked for 1-2 hrs on a digester at 150°C until a clear solution was achieved. The vial was taken to a Flame Atomic Absorption Spectrophotometer (AA-7000, GFA-7000; Shimadzu) to estimate the Chromium conc. after cooling to room temperature and suitable dilution. Total chromium was obtained in all of the studies because the laboratory machine could not detect individual chromium components.

3.8.2 The Dechroming rate

The chromium content (calculated as total Chromium) in trimmings, shavings and from the dechroming steps were determined by AAS with the following equation:

$$\text{Dechroming rate (\%)} = \frac{(Cr_{initial} - Cr_{dechromed})}{Cr_{initial}} \times 100\% \text{ --------(3.3)}$$

$Cr_{initial}$ = Initial sample Chromium conc.

$Cr_{dechromed}$ = Dechromed sample Chromium conc.

3.8.3 Yield of dechromed leather

The treated leather sample after 4stage of dechroming was air dried at room temperature under laboratory condition. Air dried dechromed samples were then weighted accurately. Yield of dechromed leather was calculated by dividing the dry weight of dechromed leather sample by the dry weight of the initial sample.

$$\text{Yield} = \frac{\text{dry weight of dechromed leather}}{\text{dry weight of initial sample}} \times 100\% \text{ -------- (3.4)}$$

3.8.4 pH measurement

After four steps of dechroming the dechromed samples were washed thoroughly with tap water than with distilled water. The pH of the wet and washed dechromed samples were measured using pH paper.

3.8.5 SEM analysis

SEM analysis of dechromed samples were done for better understanding of the morphology of the samples. This analysis also aids in determining the status of the treated leather's dechroming condition. The dechromed trimmings and shavings were dried at room temperature for few days. After drying the analysis was conducted by taking 1g correctly weighted sample of dried dechromed trimming and shavings. SEM analysis was instrumented at model: EVO18, manufacturer: Carl Zeiss AG. CoO:UK. SEM analysis of dechromed samples were conducted at Bangladesh Council of Scientific and Industrial Research.

3.8.6 FTIR analysis

The functional groups were measured with a PerkinElmer Spectrum machine, version 10.4.4 utilizing 2g of each sample at Bangladesh Council of Scientific and Industrial Research over a spectral range of 4000-650cm^{-1} to understand the structural conformation of dechromed samples. FTIR spectroscopy is a measurement of wavelength and intensity of the absorption of IR radiation by a sample. The IR spectral data of high polymers are usually interpreted in

terms of the vibrations of a structural repeat unit. Any product's bond structure can be understood via FTIR analysis.

3.9 Gelatin Extraction

By careful thermal control the gelatin is gradually and gently released in the form of liquor (Barbooti et. al., 2008). The dechromed sample was ready for the gelatin extraction process after washing. The suitable pH for gelatin extraction found from literature was 3-4 (Cabeza. et. al., 1998). The dechromed trimmings and shavings were washed with tap water then with distilled water and pH was randomly measured by pH paper to get the required pH. The gelatin extraction temperature was chosen to be 65^0-67^0C which is close to the shrinkage temperature of raw hide (Leather Dictionary, 2021). Above this temperature the dechromed sample began to melt. In a temperature-controlled oven, gelatin was extracted in a water bath from both dechromed trimmings and shavings leather. The extraction process was observed for 1 hr, 2hrs and 3hrs. Dechromed gelatin was mixed with distilled water at 1:2 (w/v) initial leather/water ratio (Shakil et. al., 2019). The hot gelatin solution was pressed and filtered through a thin cloth then through filter paper. Figure 3.10 and Figure 3.11 shows images of gelatin extracted in the current study. The extracted gelatin solution and dry gelatin was used to characterize detailed physico-chemical properties of the product and determine the chromium value.

3.9.1 Flash heating

The extracted gelatin solution was sterilized by flash heating it in 140^0C for four seconds. The sterilizing effect of flash heating hinders bacterial growth of the gelatin. For better gelatin quality, flash heating is really essential (Shakil et. al., 2019).

3.9.2 Gelatin drying grinding and storage

Sun drying (Shakil et al., 2019), lyophilization (Cabeza et al., 1998), and dryer drying (Barbooti et. al., 2008) are all options for drying gelatin. Gelatin extracted from dechromed trimmings and shavings in this study was dried using available laboratory facilities: refrigerator and oven. The gelatin solution was placed in refrigerator at 19^0C for 48hr. After 48 hrs the gelatin solution became thick and resisted to flow. After cooling it was placed in an oven at 75^0C (Barbooti et. al., 2008) for another 48hr until it lost moisture completely. After drying the dry gelatin was accurately weighted in a weight machine then grinned in a mortar and stored in a zip-lock bag for further study.

3.10 Physico-chemical characterization of gelatin

3.10.1 pH measurement

1g dried gelatin was mixed with distilled water and heated at 55^0C and then further diluted up to 100ml distilled water. pH of the solution at this temperature was measured according to the procedure of recently published WHO draft paper (WHO, 2021).

3.10.2 Chromium concentration measurement

The APHA standard method SM 3111B was used to determine chromium levels (APHA, 2012). 9mL of each gelatin solution sample was placed in a vial, along with 1mL of 65 percent nitric acid, and the bottle was forcefully shaken. The mixture was then cooked for 1-2 hrs on a digester at 150°C until a clear solution was achieved. The vial was taken to a Flame Atomic Absorption Spectrophotometer (AA-7000, GFA-7000; Shimadzu) to estimate the Cr. conc. after cooling to room temperature and suitable dilution. Total chromium was obtained in all of the studies because the laboratory machine could not detect individual chromium components.

3.10.3 Other heavy metal concentration measurement

Other heavy metal conc. of gelatin was measured by following the same standard SM 3111B used for Chromium conc. determination. Cd, Pb and Zn was measured from trimmings gelatin solution.

3.10.4 Yield calculation

The gelatin extract was used to calculated the yield of gelatin from the optimization process. The weight of dry and aqueous gelatin was used to calculate the final yield of gelatin produced from dechromed trimmings and shavings. The aqueous weight of the gelatin was calculated after chilling it in refrigerator at 3^0C for 48hrs. Then aqueous yield of gelatin was calculated following previous study (Kim et. al., 2020):

$$\text{Aqueous Yield} = \frac{\text{Weight of extract before drying}}{\text{Weight of raw material}} \times 100\% \quad (3.3)$$

The yield of dry gelatin was calculated as follows:

$$\text{Dry Yield} = \frac{\text{Dry weight of gelatin}}{\text{Weight of raw material}} \times 100\% \quad (3.4)$$

3.10.5 Visual property observation

The extracted gelatin was a transparent, colorless or yellowish substance with a gelling capacity that was tasteless and odorless. Figures 3.11, 3.12 and 3.13 indicate that extracted gelatin has a good gelling property. After chilling at 3°C, the gelatin solution was thick enough to resist flow. When dry gelatin was dissolved in warm water, it regained its gelling ability.

3.10.6 Viscosity test

Kinematic viscosity of the gelatin solution was measured at 60^0C by measuring the flow time of 10ml of the solution of each sample and was carried out by using ASTM D2170 method in a viscometer. Viscosity test was done at Bangladesh Council for Scientific and Industrial Research.

3.10.7 Gel forming ability of dry gelatin

Gel forming ability of gelatin is an important property. Gelatin with better gel forming ability indicates better property development. The gel forming ability of gelatin solution was examined by adding warm water with it. After drying at 77^0C for 48hrs, 3g of the dry gelatin was mixed with 15ml warm distilled water to observe the gel forming ability of the dry gelatin.

3.10.8 FTIR analysis

The FTIR analysis of the dried gelatin was measured to identify the functional bonds in it by using Perkin Elmer Spectrum, version 10.4.4.1g sample of each gelatin was used to measure FTIR analysis. The FTIR analysis was conducted at Bangladesh Council for Scientific and Industrial Research.

3.10.9 XRF analysis of gelatin from trimmings sample

XRF is a state-of-the-art analytical method to determine the chemical composition of all kind of materials in solid, liquid and powder form. The XRF analysis was done in the Glass and Ceramics Department of BUET. For XRF analysis 2g dried gelatin powder from trimmings was taken to measure by using Lab Center XRF-1800, Shimadzu Japan Spectrum machine.

3.10.10 TCLP for the residual chrome cakes

Toxicity Characteristic Leaching Procedure (TCLP) is a method for extracting soil samples for chemical analysis that is used as an analytical approach to simulate leaching via a landfill.

The testing process is used to assess if a waste is characteristically dangerous, that is, if it is categorized as one of the EPA's de-listed wastes. The extract is tested for the chemicals required by the methodology. Water and other liquids seep through landfills over time. Because of the toxins it collects, percolating liquids frequently react with solid trash in landfills, posing a public and environmental health danger. The TCLP model is used to model landfill conditions. Chrome cakes from gelatin extraction process are considered as secondary wastes of current study and it is important to determine the toxicity level of Cr content of the chrome cakes. Because most solid wastes are land filled in open spaces, there is a risk of leaching, TCLP is the appropriate test for this purpose. The Toxicity Characteristics Leaching Procedure (TCLP) test of chrome cakes were performed in the Environmental Engineering Laboratory of Civil Engineering Department, BUET, to investigate the leachate behavior and characteristics generated by chrome cakes from the optimized dechroming process. After gelatin extraction, 30.1g chrome cake from trims and 18.2g chrome cake from shavings samples were obtained from a 50g original sample. These are the secondary wastes identified in the current research. The TCLP analysis of the secondary waste, chrome cake of trimmings and shavings were conducted to determine the chromium conc. in leaching solution of the chrome cakes. The chrome cakes were air dried at room temperature for several days and the samples were accurately weighted until constant weight was obtained. Mixture of TCLP reagent was made by adding 5L distilled water with a 8.5ml acetic acid. The mixture was mixed with the chrome cakes according to 1:2 (w/v) ratio. TCLP reagent and the chrome cakes were tumbled in a closed container for 18hrs. The whole procedure was carried out following TCLP Method 1311. After TCLP test both the leachate solutions were filtered through a thin cloth then through a fiber glass funnel. The leachates were separated for Chromium determination. The APHA standard method SM 3111B was used to determine chromium levels (APHA, 2012). 9 ml of leachate sample from each chrome cake was placed in a vial, along with 1mL of 65 percent nitric acid, and the bottle was forcefully shaken. The mixture was then cooked for 1-2 hrs on a digester at 150°C until a clear solution was achieved. The vial was taken to a Flame Atomic Absorption Spectrophotometer (AA-7000, GFA-7000; Shimadzu) to estimate the Chromium conc. after cooling to room temperature and suitable dilution. Total chromium was obtained in all of the studies because the laboratory machine could not detect individual chromium components. Figure 3.14 shows the leachate filtrates of from TCLP analysis. Filtration of the entire solution took a long time. When compared to the filtrates from the dechroming process, the filtrates display a significantly lighter hue of the leachates, suggesting a much lower Chromium level.

Figure 3.14: Filtrates from TCLP of chrome cakes

3.11 Flow diagram of trimmings and shavings dechroming and gelatin extraction process

Figure 3.15 shows the entire dechroming and gelatin extraction operation in a flow diagram. The diagram shows the optimal operating conditions in a step-by-step way and the final product yields. The detailed analysis of selection of optimum conditions are described before in section 3.5. After dechroming optimization 50g of each sample was further dechromed at optimized condition. These dechromed samples were used to extract gelatin using 1:2 (w/v) water. From 50g chrome-tanned trimmings yielded 32g of aqueous gelatin and 1.5g of dry gelatin, while 50g chrome-tanned shavings yielded 33g of aqueous gelatin and 0.67g of dry gelatin. Some weight loss of the samples was observed during dechroming and washing stage.

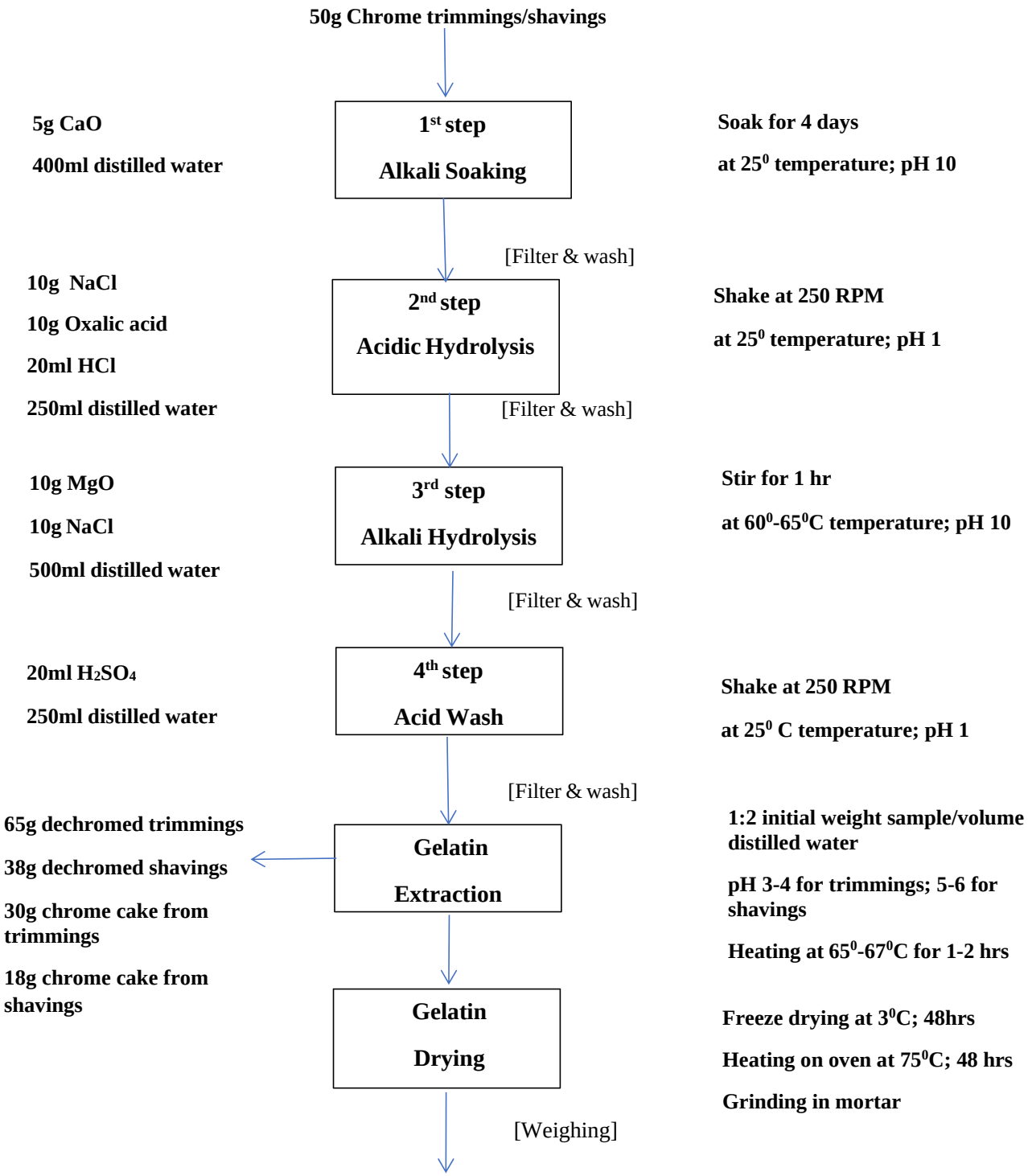


Figure 3.15: Flow diagram of trimmings and shavings dechroming and gelatin extraction process

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Introduction

The results of this study started with the analysis of solid leather waste samples for dechroming efficiency and gelatin extraction possibility. This study was carried out to analyze the variation of dechroming and gelatin extraction routs. This section highlights the detail analysis of the experimental results and findings. This includes the analysis of the total chromium content of the waste samples, performance evaluation of dechroming methods and gelatin extraction. Based on the test results suitable dechroming ratio was selected for chrome shavings and trimmings and gelatin extraction was conducted for the ratio.

4.2 Results of preliminary analyses of solid leather waste samples

The initial characterization of raw samples was conducted using the facilities of the Environmental Laboratory of BUET. The analyses helped to characterize the raw materials. Different physical and chemical properties of the raw materials distinguish it from other type of leathers. The images of chrome-tanned trimmings and shavings are given in Figure 3.1. The chrome-tanned leather trimmings and shavings used in this study were from cow hides. The chromium content, pH, moisture content and ash content of the tanned trimmings and shavings sample were measured.

The chromium content found in the samples were much high making both the samples hazardous for us. Trimmings chromium content was higher than shavings. pH of both samples was acidic. The results from this study of these experiments were compared with other study (Paul et.al., 2013). The chromium conc., pH and ash content of leather sample of this work was found lower than the sample in previous study. The moisture content of the samples of this study was found higher than the sample from previous study. The ash and moisture content were determined in dry basis. These physical properties found in the trimmings and shavings are presented in Table 4.1. All of the tests were performed in a trial setting, and the mean value was used to pick the values. Leather properties from our study shows difference from other study (Paul et. al., 2013). The material quality and features differ

depending on where the finished leather comes from in the hide. Many other elements can have an impact on leather quality. These factors can include the animal's breed, the climate in which it resided, the food it was fed, and the quantity of exercise it received (Liberty Leather Goods, 2021). Hides are a natural product that is heavily influenced by the lives of the animals from whom they are derived. The meatpacking, tanning, and finishing methods used during manufacture can also have an impact on leather quality (Liberty Leather Goods, 2021). Table 4.1 lists all the characteristics of raw materials from current study. Data from previous study are also listed on the table to compare data from this study.

Table 4.1: Characteristics of raw materials

Property	Unit	This study		Previous study (Paul et.al. 2013)	
		Trimmings	Shavings	Trimmings	Shavings
Chromium conc.	mg/kg	14417	11067	41200	37200
pH	-	3.8	3.4	4.77	4.10
Moisture content	%	53.57	51.63	38.89	36.09
Ash content	%	5.40	8.00	10.67	9.82

4.3 Effect of forward and reverse operation

The effect of forward and reverse operations was investigated in order to assess the efficiency of alkali and acid as dechroming starting chemicals. Figure 4.1 and Figure 4.2 demonstrates the dechroming percentages of 1 to 4 steps of combined alkali-acid hydrolysis in forward and reverse stages. The chromium removal chart shows that the reverse process starting with acidic hydrolysis resulted lower chromium removal rate than the forward process starting with alkali soaking up to step 4. Therefore, the dechroming of solid leather trimmings and shavings starting with alkali hydrolysis seems favorable condition. Because of its high rate of Cr reduction. Figure 4.1 and 4.2 shows the step-by-step removal rate of forward process and reverse process. In Figure 4.1 the forward process started with alkali hydrolysis and ended with acid hydrolysis shows above 90% final Cr removal efficiency which is 92% and 94% from trimmings and shavings respectably. This value is greater than dechroming efficiency of reverse process. Figure 4.2 shows that reverse process started with acid hydrolysis and ended with alkali hydrolysis had 76% and 78% final dechroming efficiency from trimmings and shavings respectably. This study clearly helped to select alkali as the starting chemical for combined dechroming process.

Dechroming process starting with alkali also reduces energy consumption as alkali was used to soak the leather and acid was used to shake the leather in in step 1. These figures also show that multiple stages of dechroming is more effective than single stage dechroming. Although the increased yield was found for decreased stages, dechroming increased with increased steps. Up to four steps were effective to achieve above 90% dechroming rate. Other researchers also conducted dechroming operation starting with alkali dechroming found better results (Cabeza et. al., 1998). Higher chromium conc. of the trimmings and shavings leather wastes leads the dechroming process to operate on multi steps.

Figure 4.3 shows the % yield of the dechromed leather wastes of 3rd and 4th steps. Yields of these steps were calculated to determine the difference of yields with respect to the dechroming rate. The yields of the dechromed leathers were calculated on dry basis. The 3rd step of both processes shows bigger yields. 4th steps had lower yields than 3rd steps. The initial Chromium conc. value is very large 14417 mg/kg and 11067 mg/kg and it is difficult to reduce it in a good amount by operating only one step of dechroming. The Chromium conc. and yield of the leather wastes were reduced successively during four steps of dechroming. Shavings sample had lower yields because of loss of shavings during dechroming process and

during washing stage. The reverse process (Figure 4.3) exhibits 85% and 82% final yield and the forward process exhibits 70% and 79% final yield from trimmings and shavings leather waste. When compared to the yield from the forward process, the yield from the reverse process dechroming exhibits a better outcome. However, the primary goal of this work was to dechrome leather waste in order to create a value-added product. As a result, a higher dechroming rate was deemed more beneficial than a higher yield. The dechroming efficiency of the selected process was more than 90% but the chromium level of the dechromed leathers were still high and out of safe range; 10mg/kg (WHO, 2021). The dechromed trimmings sample had 1154 mg/kg Cr and the dechromed shavings sample had 665 mg/kg Chromium on it. As a result, it was necessary to improve all four steps of the process in order to reduce more Chromium. The physical properties of the both leather samples were similar and all the operating conditions of the dechroming operations of trimmings and shavings sample were kept same for forward and reverse process. As the Chromium level of both the sample was much higher than the required level mentioned above, it was necessary to optimize the forward process for both sample.

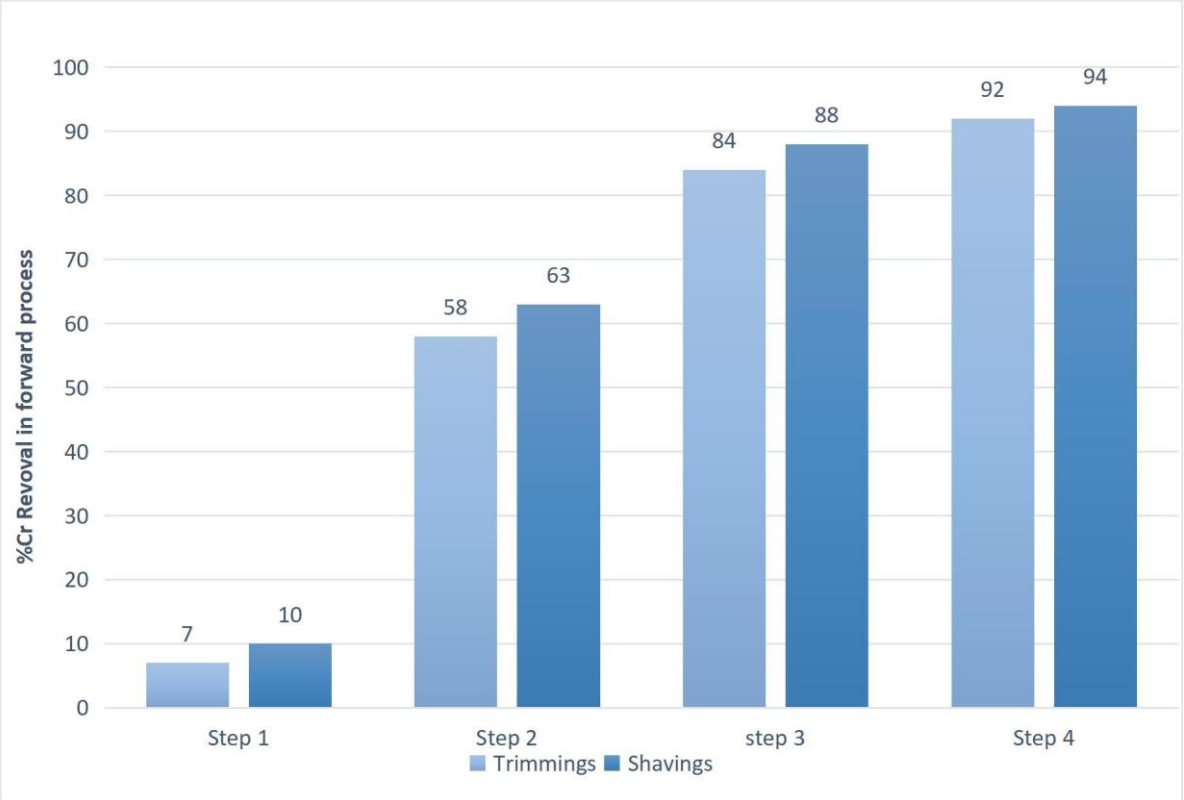


Figure 4.1: Cr removal of dechromed leather trimmings and shavings in forward process

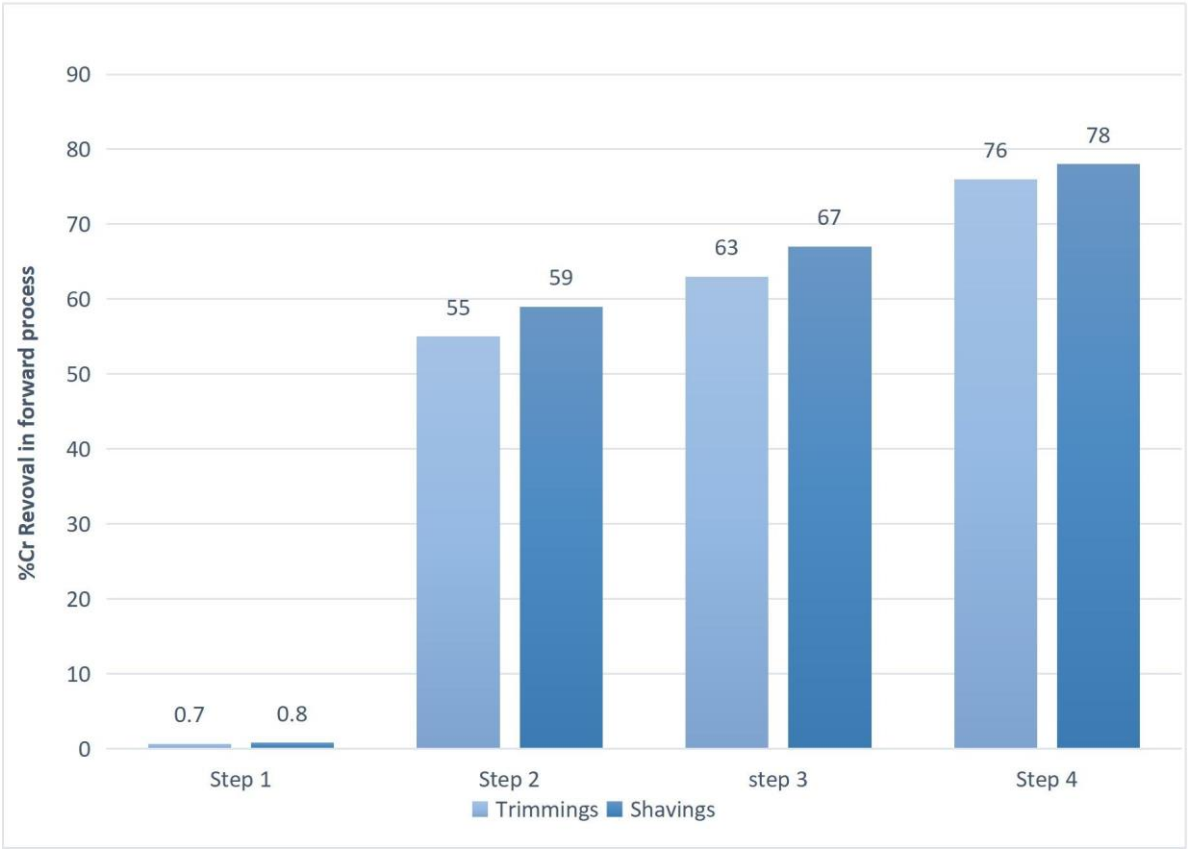


Figure 4.2: Cr removal of dechromed leather trimmings and shavings in reverse process

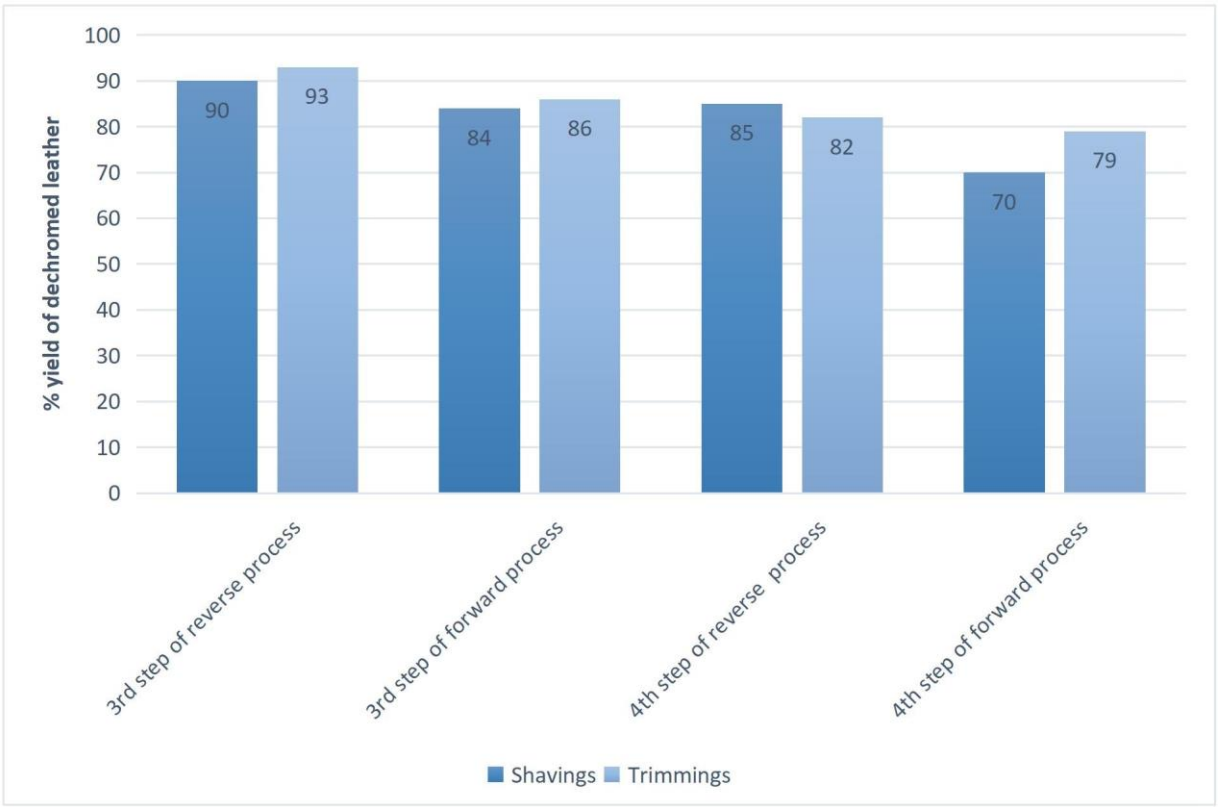


Figure 4.3: Yield (%) of dechromed leather trimmings and shavings

4.4 Inorganic acid selection for 2nd and 4th stages

A trial operation was performed to evaluate the order of inorganic acid hydrolysis, and the outcome was obtained based on observation. The combination of HCl in the second stage and H_2SO_4 in the fourth stage was observed to be the most suited use for our dechroming optimization during the trial. In the gelatin extraction stage, dechroming with the same inorganic acids resulted in a hydrolyzed sample. Between two samples of HCl: H_2SO_4 and H_2SO_4: HCl, HCl in the second step and H_2SO_4 in the fourth step produced a superior result because this sequence produced a better dechroming effect and the sample did not hydrolyze as quickly.

4.5 Effect of dosage in 4 steps dechroming process

For the effect of chemical dosage on each step of dechroming, the process was controlled to a set of dosage ratios. The effect of dosage for all the 4 steps are listed in Table 4.2. The chemical ratios applied on each step of dechroming are described in the previous chapter section 3.5.2. The dosage was increased from sample A to sample F. Other experimental conditions were kept fixed in each step. It was found that for same operating time and conditions in four steps, the dechroming effect increased with increased dosage from sample A to F for trimmings and shavings. But in the gelatin extraction stage, increased chemical ratios showed decreased yield of gelatin. Also, there is a chance of acid consumption to neutralize the leftover alkali from previous step of dechroming. Which will cause loss of acid and the dechroming degree will be lower due to insufficient acid to remove Chromium. The dosage of the samples was increased from 0.5g to 3g in sample A to sample F. Increased dosage of alkali and acids decreased the yield of the residue after washing. Collagen loss due to excess chemicals was also observed. For little increase in chemical ratios much lower yield of gelatin solution was obtained. From this operation, chemical ratio from sample B was suitable for both trimmings and shavings samples. This operation indicates that with higher chemical ratio, lower yield and lower gelatin forming ability is a common phenomenon of leather dechroming process. Excess alkali can cause formation of carcinogenic Cr^{6+} in the solution which is totally undesirable condition. Excess acid concentration also can swell the leather sample (Beghetto et. al., 2013). And the shavings were hydrolyzed in addition of excess alkali and acid concentration. Sample B has a moderate ratio of chemical dosage and does not affect on the both trimming and shaving sample. The capacity of the gelatin

solutions to form gels was also investigated, and the solution from sample B demonstrated superior gel forming ability after chilling it compared to other samples. Sample C,D,E's gelatin solution demonstrated poor gel forming ability on cooling the samples at 3^0C temperature. The solution from C,D,E of trimming sample began to form gel on cooling but started to melt instantly at room temperature. D,E,F trimmings gelatin showed good dechroming effect but yield was much lower. Gelatin sample from A,B did not melt instantly at room temperature. No gelatin solution was extracted from E,F of shavings samples, as the leather hydrolyzed during the dechroming operation. The gelatin solution extracted from sample B was considered better compared to sample A gelatin as the dechroming rate was better. Chemical ratio of sample B was considered optimized dosage for alkali-acid dechroming process. As described before, melting condition of the aqueous gelatin sample was also considered to select optimized dosage for this process. After determining the optimum dosage this dose was kept fixed for rest of the experiments of dechroming trimmings and shavings.

Table 4.2: Effect of dosage in 4 steps of dechroming process

Sample	Trimmings		Shavings	
	Dechroming %	Yield %	Dechroming %	Yield %
A	96.70	89.00	98.86	91.70
B	99.55	70.20	99.90	78.00
C	99.97	54.67	99.98	59.30
D	99.98	37.40	99.99	43.00
E	99.99	25.90	Hydrolyzed	Null
F	Hydrolyzed	Null	Hydrolyzed	Null

4.6 Effect of soaking time on gelatin formation extent

To determine the effect of soaking time on trimmings and shavings dechroming, each step of dechroming process was controlled to a set of soaking time in days. To observe the soaking impact, soaking conditions were varied from 1 to 6 days. Table 4.3 displays the results. Of this operation. The operating conditions of this process are described in section 3.5.3 of previous chapter. For the purpose of observation, the yield of gelatin was determined. For even 1 day of soaking time, the trimming and shavings samples both started to exhibit a dechroming rate of more than 80%. Which is a good scenario for soaking in CaO solution in step 1. The dechroming effect for trimmings and shavings increased with increased days of soaking from 1 to 4 days for the same amount of chemicals applied in other steps. Trimmings sample showed better dechroming effect but decreased yield after 5 days of soaking. The shavings sample was totally hydrolyzed after 5 and 6 days of immersion. Trimmings sample was also totally hydrolyzed during gelatin extraction stage for 6 days of soaking, which is an undesirable situation for chromium collagen separation. Because separating collagen from a hydrolyzed sample is difficult, time-consuming, and expensive.

Chromium conc. of hydrolyzed samples were undetermined. Excess soaking time can also cause clogging of CaO into the leather trimmings and shavings. This phenomenon was observed in 5 and 6 days of soaking, which is more common in shaving sample. This may hinder the aim of soaking process by reducing the active alkali conc. to react with the Chromium. The shavings sample showed better dechroming rate then trimmings sample because it had less initial Chromium conc. (11067 mg/kg). Same dosage of chemical was applied to dechrome the shavings and trimmings sample. The yield of leather after washing also decreased with increasing days. This operation yielded a favorable soaking condition for step 1 at room temperature after 4 days of soaking for trimmings and shavings. For 4 days of soaking time, the dechroming rate of trimmings and shavings was 99.55% and 99.90% respectively. The aqueous yield was 70.2% and 78%. although the dechroming percentage of this operation was good but the Chromium conc. of the samples were still high (65 mg/kg and 11 mg/kg).

Table 4.3: Effect of soaking time of step 1 on gelatin forming extent

\Soaking time (day)	Trimmings		Shavings	
	Dechroming %	Yield %	Dechroming %	Yield %
1	87.00	88.80	89.00	91.60
2	93.95	84.50	95.95	87.00
3	97.90	79.00	98.85	83.10
4	99.55	70.20	99.90	78.00
5	99.87	63.00	Hydrolyzed	Null
6	Hydrolyzed	Null	Hydrolyzed	Null

4.7 Effect of temperature in dechroming extant

As Chromium conc. from the above optimization processes were not satisfactory (65 mg/kg and 11 mg/kg), further optimization of the leather waste samples were needed to remove more Chromium from the trimmings and shavings. After soaking time optimization and dosage optimization we intended to get an optimum temperature for the step 3 hydrolysis. To optimize the operation temperature in step 3, three different temperature ranges were selected to measure the effects. Other conditions and chemicals were fixed for the whole process. Temperature of other step dechroming operation was also fixed. The overall operating conditions of this operation are described in section 3.5.4 of previous chapter. The higher temperature ranges resulted no gelatin from both trimmings and shavings in the final stage of gelatin extraction. Dechromed trimmings and shavings went through 80^0C-85^0C temperature range showed no extract and the dechromed leather was completely hydrolyzed on extraction step. To avoid excess collagen loss 80^0C-85^0C temperature range was avoided. 40^0C-45^0C temperature range could not fulfill the required Chromium level; 10mg/kg (WHO, 2021), which was applied on both soaking time and dosage optimization process stated above. Therefore, 60^0C-65^0C was selected as optimum temperature range of step 3 for both of the samples. The chromium level (7.2 mg/kg and 1.1 mg/kg for trimmings and shavings respectively) found at this temperature range was below the required level. The Chromium level decreased significantly at this temperature but the yield decreased to 64% and 68% for

trimmings and shavings. The effect of temperature in dechroming extant of step 3 is listed in table 4.4 showed below.

Table 4.4: Effect of temperature in dechroming extant of step 3

Temperature (^{0}C)	Trimmings		Shavings	
	Dechroming %	Yield %	Dechroming %	Yield %
40-45	99.55	70.20	99.90	78.00
60-65	99.95	64.00	99.99	68.00
80-85	Hydrolyzed	Null	Hydrolyzed	Null

4.8 Gelatin extraction

The process described in section 3.8 of the preceding chapter was used to extract gelatin from dechromed leather samples. The appropriate pH (3-4) for gelatin extraction could be obtained for the trimmings sample during the washing stage. During washing, however, the shavings sample immediately increased its pH to 5-6. 1 hr, 2 hrs, and 3 hrs of gelatin extraction periods at 65^{0}C-67^{0}C were observed. In 1 hr, the trimmings sample extracted gelatin. Dechromed trimmings leather sample began to hydrolyze after 1 hr. Gelatin was recovered in 2 hrs from shavings samples. Dechromed shavings leather sample did not extract any gelatin after 2 hrs and eventually began to melt. After 3 hrs, both the dechromed trimmings and shavings samples began to melt. As a result, the extraction duration for the trimmings sample was set to 1 hr, and the extraction period for the shavings sample was set to 2 hrs.

4.9 Hydrolysis degree of collagen

Based on the results of above operations, the optimum conditions to dechrome 50g chrome tanned leather wastes are summarized in Table 4.5 and Table 4.6. The hydrolysis degree of waste collagen under the optimum conditions were estimated according to the chromium content and yield of the gelatin extracted. Under the individual optimum experimental conditions, the dechroming extant exceeded 99% and the optimum aqueous yield was more

than 60%. The optimum conditions to dechrome chrome-tanned leather trimmings are listed in the Table 4.5. 4 days soaking time was found suitable for both trimming and shavings samples. In second step of dechroming both inorganic and organic acids were used to get better result. After 4 hrs of shaking with acid and salt, MgO and NaCl were used at 60^0C-65^0C in 3rd step to stir the residue for 1 hr. MgO clogged with the leather sample was not completely washed away after washing the samples in this step. To reduce more Chromium and wash MgO residue, H_2SO_4 solution was used in 4th step. The dechromed leathers showed good physical condition for gelatin extraction, as they did no hydrolyzed during dechroming or gelatin extraction period. The final dechromed trimmings and shavings were lighter in color. Which indicates good dechroming effect after four steps of dechroming hydrolysis. It was softer than the chrome-tanned initial samples. But the dry yield of the gelatin extracted from savings was too low for commercial use. These results are described on section 4.7.

Table 4.5: The optimum conditions for dechroming and gelatin extraction of leather trimmings

Step 1	5g CaO in 400ml distilled water, 25^0C, 4 days soaking
Step 2	10g NaCl, 10g Oxalic acid, 20ml HCl in 250ml distilled water, 25^0C, 4 hr shaking
Step 3	10g MgO, 10g NaCl in 500ml distilled water, 60^0C-65^0C, 1hr stirring
Step 4	20ml H_2SO_4 in 250ml distilled water, 25^0C, 2 hr shaking
Gelatin extraction	1: 2 (w/v) initial weight of sample/distilled water, 65^0-67^0C, 1hr, pH 3-4

Table 4.6: The optimum conditions for dechroming and gelatin extraction of leather shavings

Step 1	5g CaO in 400ml distilled water, 25^0C, 4 days soaking
Step 2	10g NaCl, 10g Oxalic acid, 20ml HCl in 250ml distilled water, 25^0C, 4 hr shaking
Step 3	10g MgO, 10g NaCl in 500ml distilled water, 60^0C-65^0C, 1hr stirring
Step 4	20ml H_2SO_4 in 250ml distilled water, 25^0C, 2 hr shaking
Gelatin extraction	1: 2 (w/v) initial weight of sample/distilled water, 65^0-67^0C, 2hr, pH 5-6

4.10 Physico-chemical characteristics of gelatin

The physico-chemical characteristics of gelatin from dechromed trimmings and shavings are as listed below in Table 4.7. The properties found from this study are comparable with standard properties of edible gelatin given in a draft proposal paper of WHO published recently in 2021. Both data are presented in the following table (Table 4.7) for comparison.

4.10.1 Cr concentration

Both trimmings and shavings gelatin had Chromium concentrations (7.2 mg/kg and 1.1 mg/kg) less than the regulatory limit 10mg/kg (WHO, 2021). Gelatin from this study is intended to be utilized in industrial purpose. Commercial/technical gelatin does not have a specified chrome level. Although the Chromium limit for edible gelatin is 10 mg/kg, we compared this range to the Chromium content of our gelatin. In comparison to the normal conc., the Chromium of both of our gelatin extracts yielded good results.

4.10.2 Type of gelatin

Type of gelatin depends on the pretreatment procedure of the leather sample. Gelatin type A is produced by acid pretreatment, whereas gelatin type B is produced by alkaline pretreatment. In this study, shavings gelatin displayed a neutral pH (pH 6-7), while the pH values of trimmings gelatin was slightly acidic (pH 4-5). Both of the gelatin extracted from trimmings and shavings are type A gelatin because gelatin in this work, was extracted from dechromed leathers processed by acid solution in the last step. This condition determines the type of the gelatin extracted. For commercial use, the gelatin is sold at pH 5.2–5.5 (Muyonga et. al., 2004). However, higher pH is also acceptable.

4.10.3 Viscosity

Viscosity of the gelatin solution from trimmings fulfilled the requirement. But the viscosity of the gelatin solution from shavings was very poor. However, there was no specific viscosity required for gelatin in WHO draft paper (WHO, 2021).

4.10.4 Clarity and color

The changes of gelatin color depend on the reaction and duration time of the resultants. The color of gelatin has a great impact on its usage. It is regarded as a gelatin characterization feature. Figure 3.10, 3.11, 3.12 show trimmings and shavings extracts exhibit translucent gel

solution. After a longer hydrolysis process, the gelatin had a slightly brown color. Clear color of gelatin is appreciable for any kind of use. Both gelatin extracts were odorless as well.

4.10.5 Thermal stability

For the gelling and melting temperatures, the thermal stability of the gelatin solution was investigated. Within 30 minutes at 3°C, gelatin solution began to create thick gelatin. To minimize moisture, it was kept at this temperature for 48 hours. Gelatin did not melt immediately after being removed from the freezer. The temperature was gradually raised to 30^0C. Gelatin began to melt at this temperature.

4.10.6 Gel forming ability of dry gelatin

The gel forming ability of dry gelatin was observed by soaking dry gelatin in distilled water. The experimental steps were described in previous chapter in section 3.9.7. After soaking and chilling the solution at refrigerator, the dry gelatin instantly retained gelatinous solution which was thick.

4.10.7 Yield of gelatin

At 3°C, the extracted solution formed gel and was maintained there for 48 hrs in refrigerator. The aqueous yields of the gelatin samples were measured. The aqueous yield of the shavings gelatin was slightly better than the trimmings gelatin. Gelatin solution was cooled into refrigerator at 3^0C which is below the sol-gel transition temperature (35^0C). The peptide chains of gelatin solution aggregated at this temperature and retaining their secondary structure turned into aqueous gelatin. The gel formation is caused by the development of collagen triple helices in solution as it cools. When a gelatin solution is chilled below gelation temperature, the protein coils begin to form triple helices, eventually forming a three-dimensional network. The triple-helices affect the phrasing of collagen's original structure, albeit only slightly. When the temperature is raised, the helix to coil transition occurs, and the gel becomes liquid (Catalina et. al., 2007).

Table 4.7: Properties of gelatin solution from trimmings and shavings

Properties	Trimmings	Shavings	Standard
Chromium (mg/kg)	7.2	1.1	10 (WHO, 2021)
pH	4-5	6-7	3.8-7.6 (WHO, 2021)
Viscosity (e)	1.2	0.65	1.2-3.4 (Global sources, 2021) Not specified from WHO
Type	A	A	A/B (WHO 2021)
Color and odor	Colorless Odorless	Colorless Odorless	Colorless to yellow; odorless (WHO, 2021)
Solubility	Insoluble in cold water Soluble in 55^0C warm water	Insoluble in cold water Soluble in 55^0C warm water	Insoluble in cold water. Soluble in 55^0C warm water (WHO, 2021)
Yield % (aqueous)	64	68	

4.11 SEM images of dechromed trimmings and shavings

The dechromed trimmings and shavings, as well as raw samples, were subjected to SEM examination to obtain a clear picture of the morphological changes before and after the dechroming procedure. The results of the dechroming procedure were also better understood due to the SEM analysis of the samples. From Figure 4.4 to Figure 4.7, the findings of the SEM study are displayed. Every image from the SEM study is magnified to 100 µm and 2µm. The dechroming of the trimmings and shavings is confirmed by SEM images (Figure 4.6 and Figure 4.7). They differ from tanned leather sample images (Figure 4.4 and Figure 4.5). The sample's fibrillar structure is seen in the chrome-tanned images. After the dechroming process, it was slightly deformed. This confirms the dechroming of the leathers. The morphology of dechromable leather can also be observed through the SEM analysis from this work.

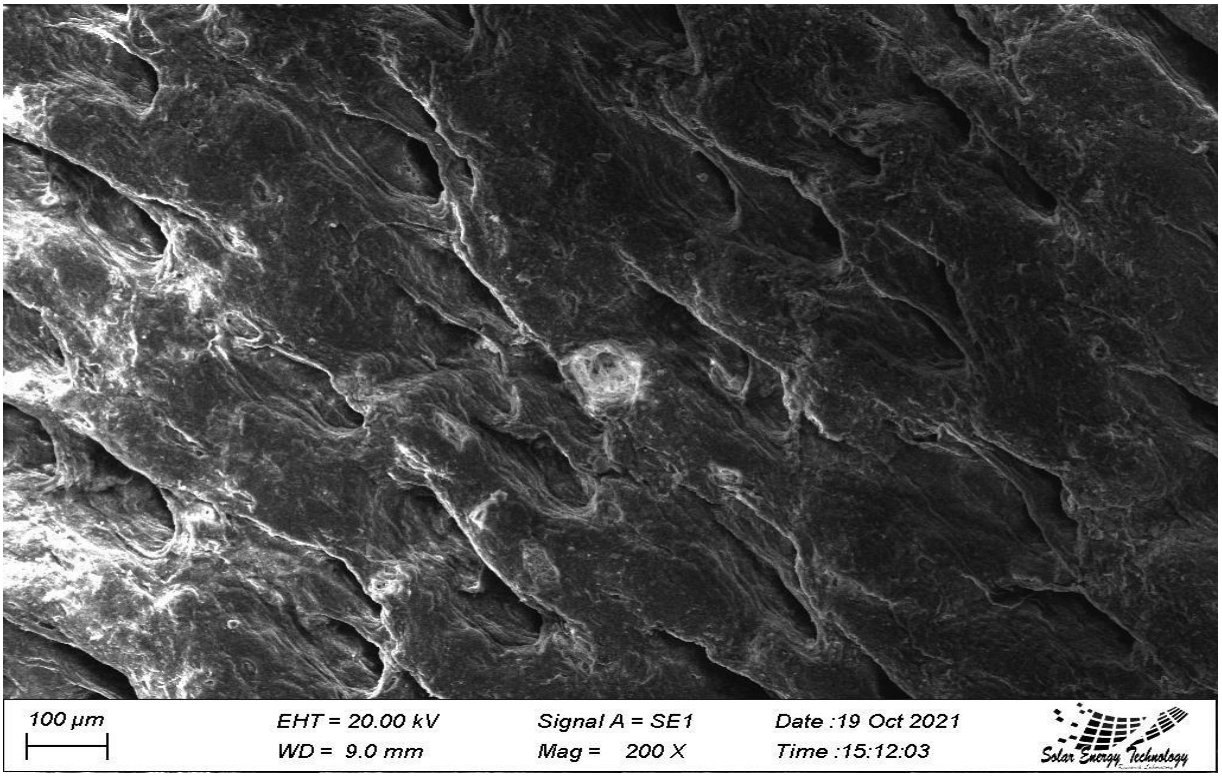


Figure 4.4: SEM images of chrome-tanned leather trimmings

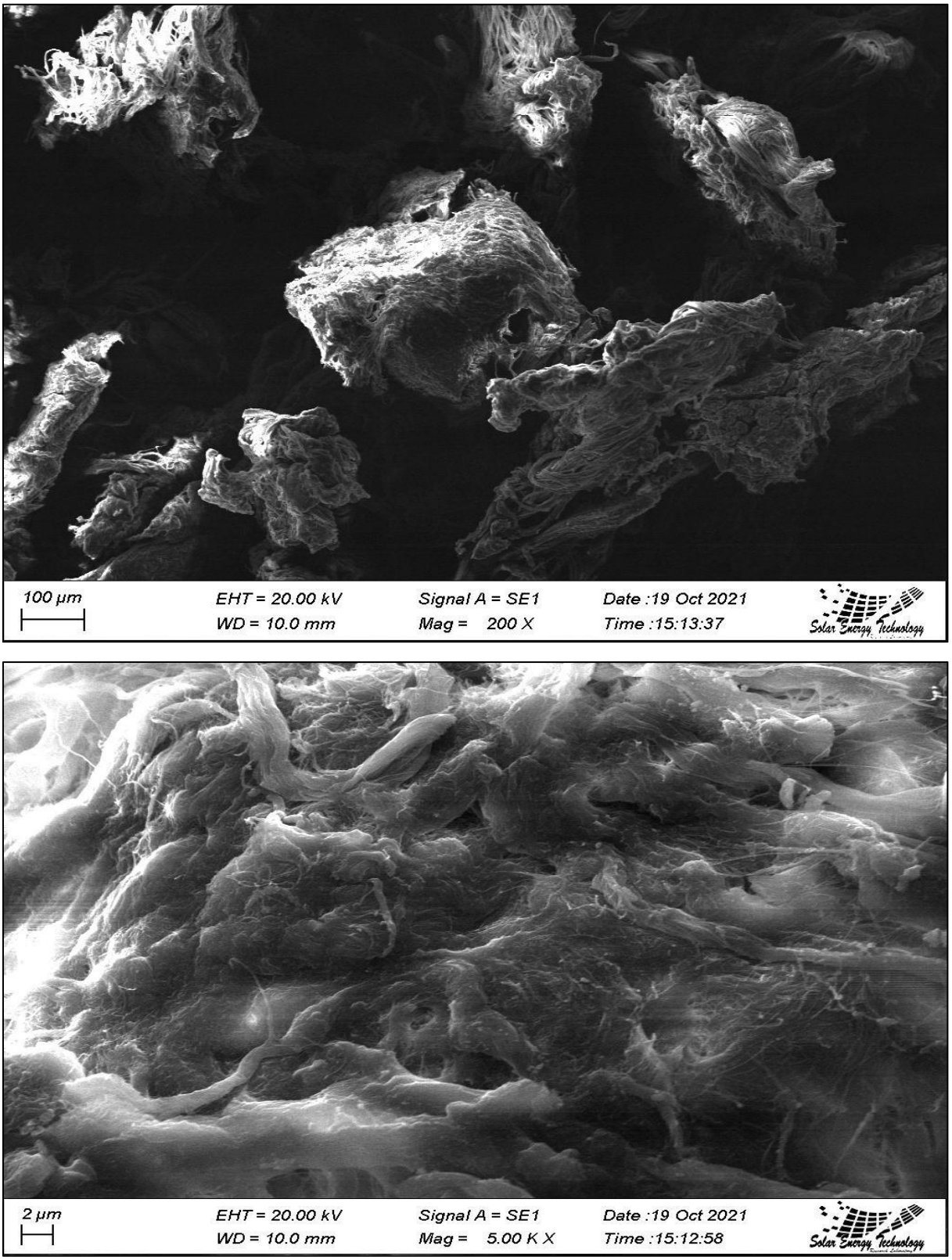
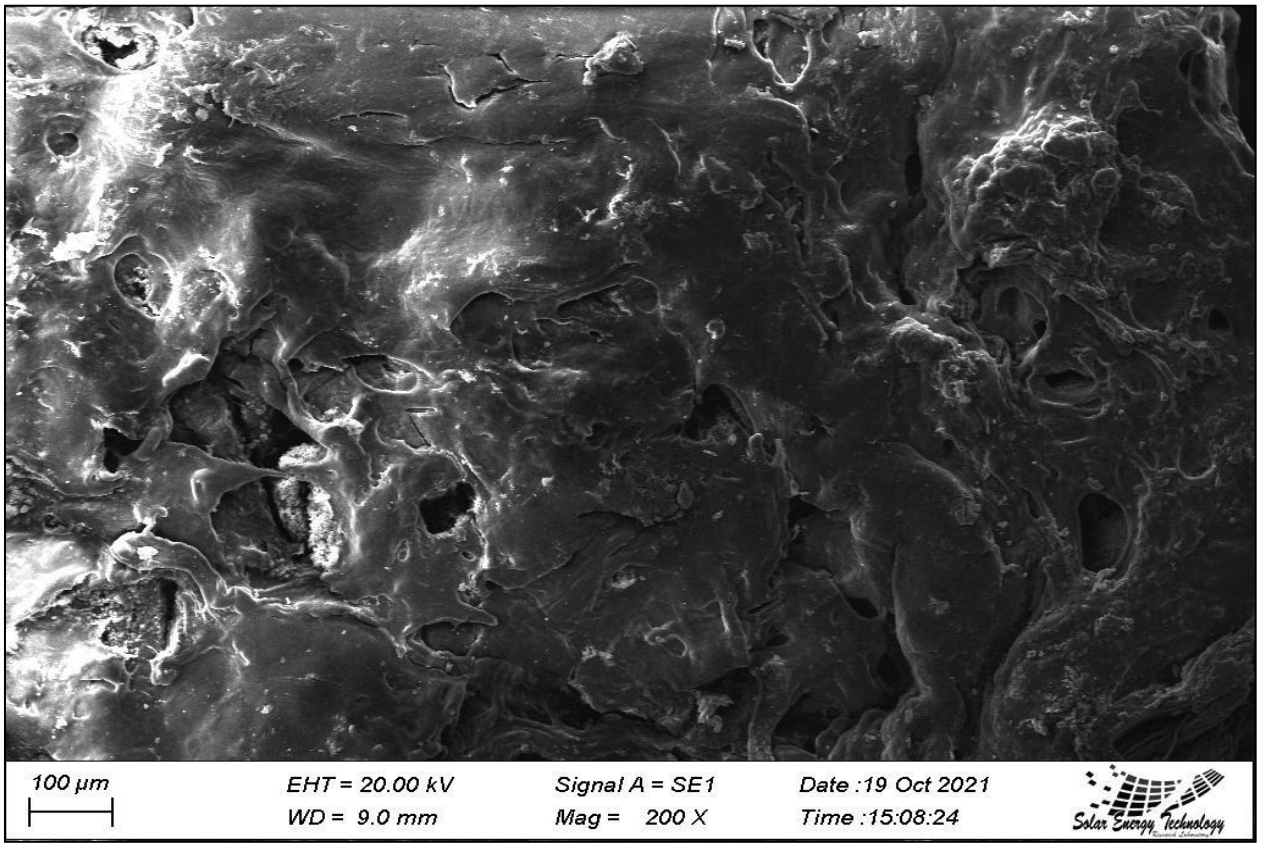


Figure 4.5: SEM images of chrome-tanned leather shavings



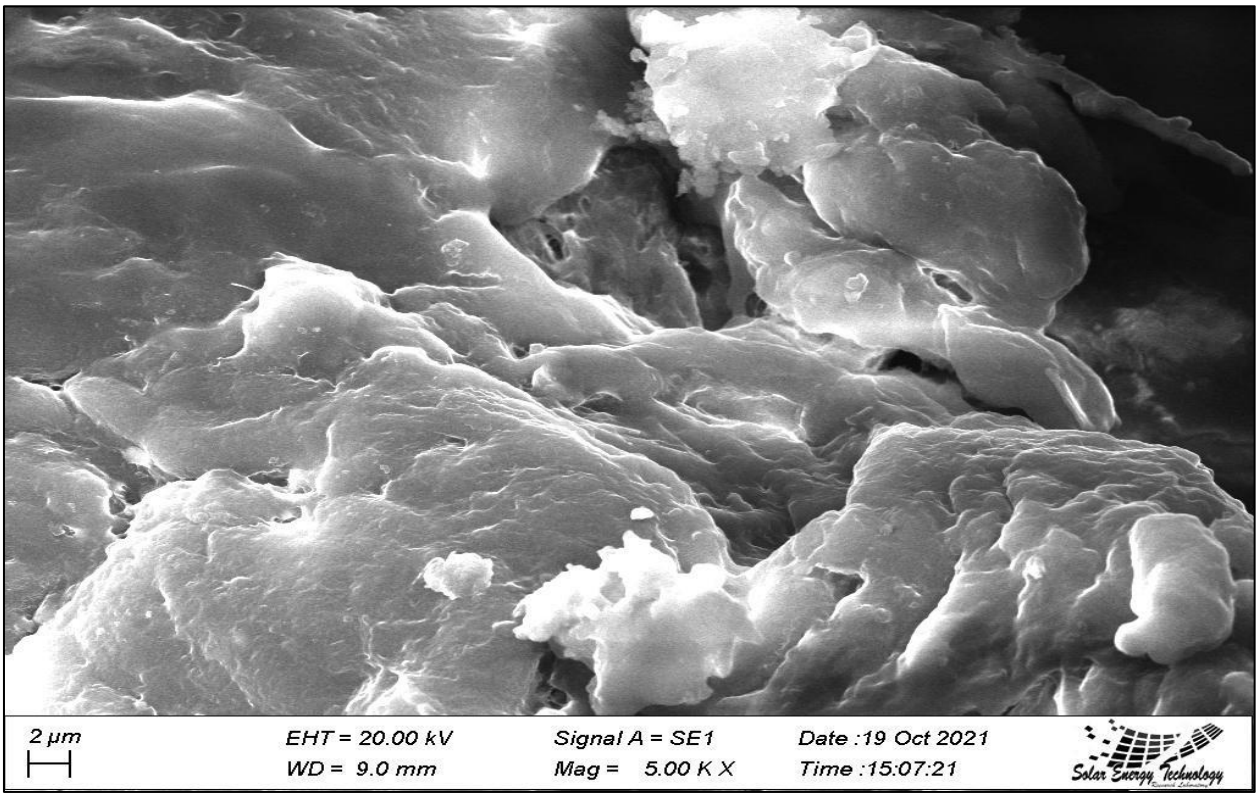
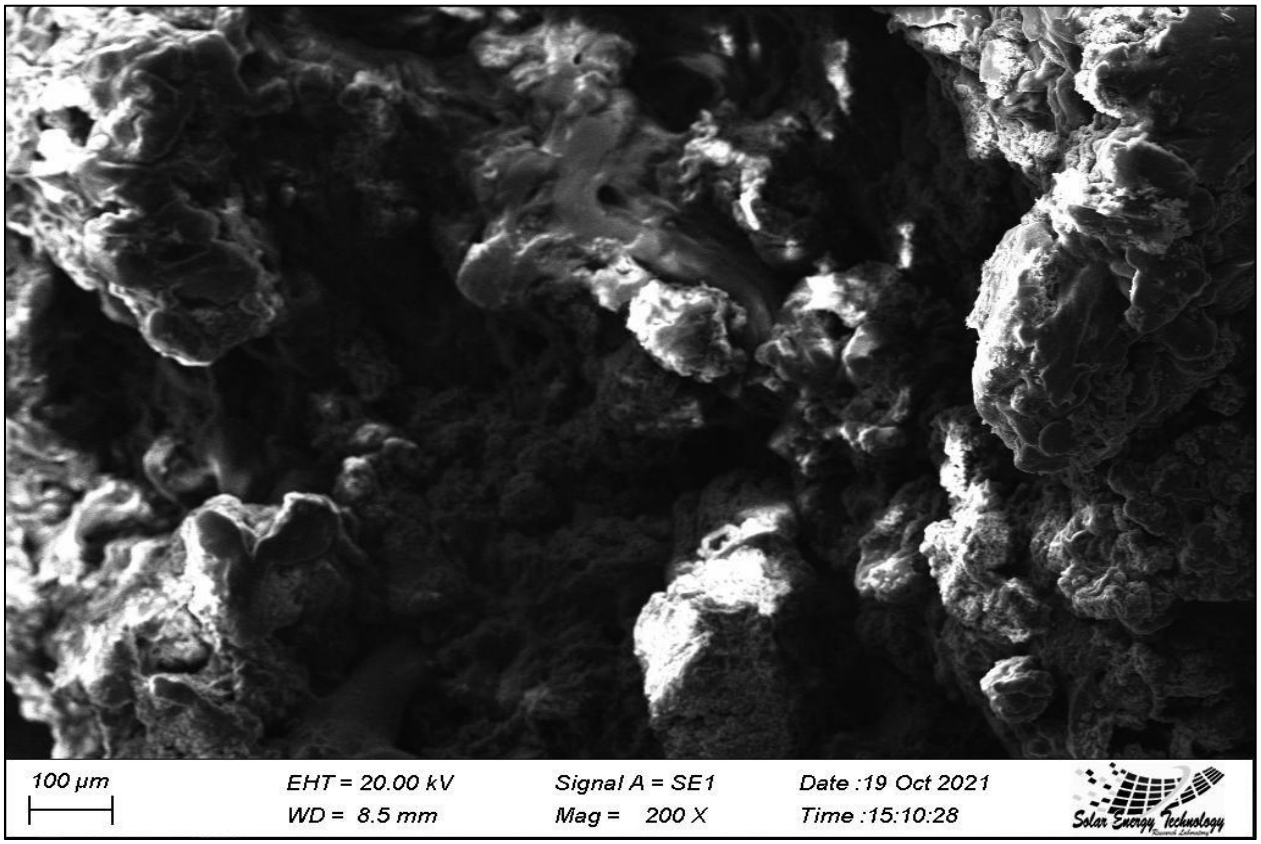


Figure 4.6: SEM images of trimmings after dechroming



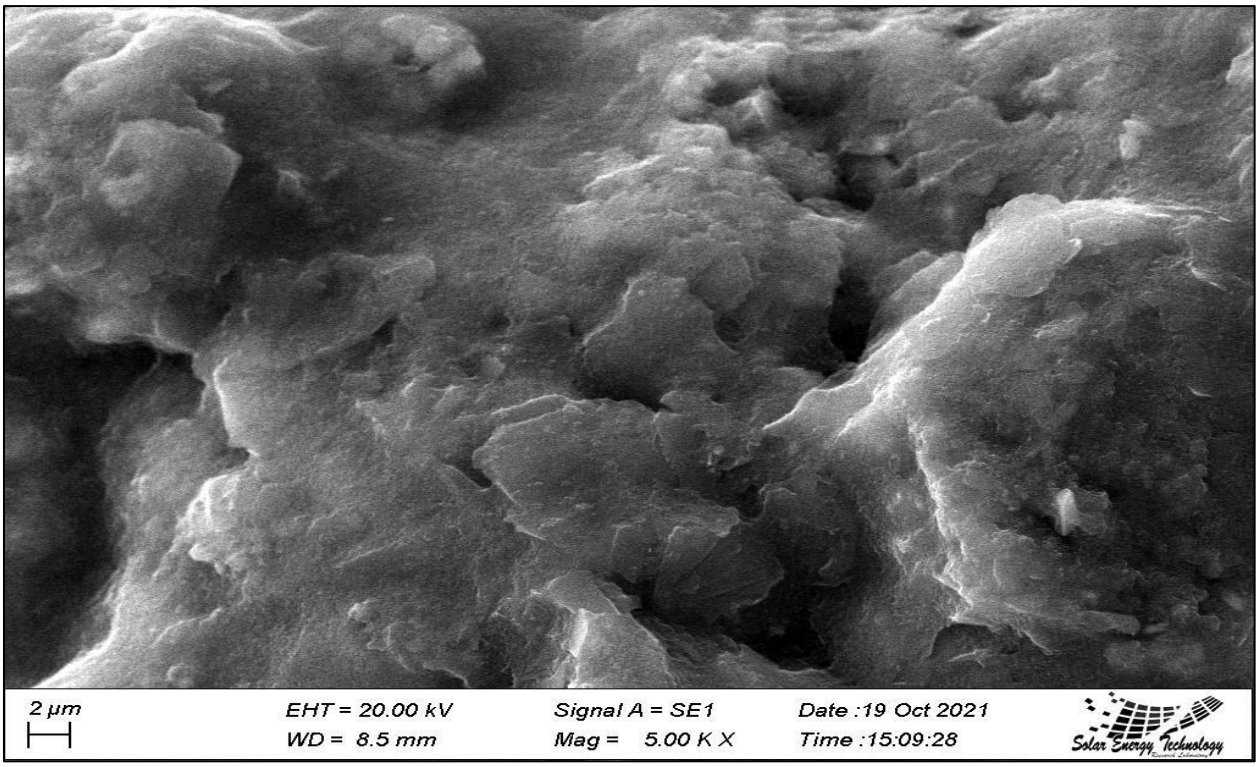


Figure 4.7: SEM images of shavings after dechroming

4.12 FTIR results of chrome-tanned, dechromed and gelatin samples

The FTIR study of the chrome tanned, dechromed and gelatin samples were accomplished to identify the compositional bonds and bendings of the samples. All the bonds were described with table and the bonds found in gelatin sample were compared with bonds of pure gelatin. The gelatin samples extracted in this study had similar bonds to the reference gelatin.

Figure 4.8 to Figure 4.13 demonstrate the FTIR results of the samples. Range from 650cm^{-1} to 4000cm^{-1} was used to identify the peaks of the samples. All the peaks and bends found from this analysis are described with assignments in Table 4.8. Figure 4.18 to Figure 4.12 represents the individual FTIR curves of the samples and Figure 4.13 shows the combined FTIR curve of all the samples.

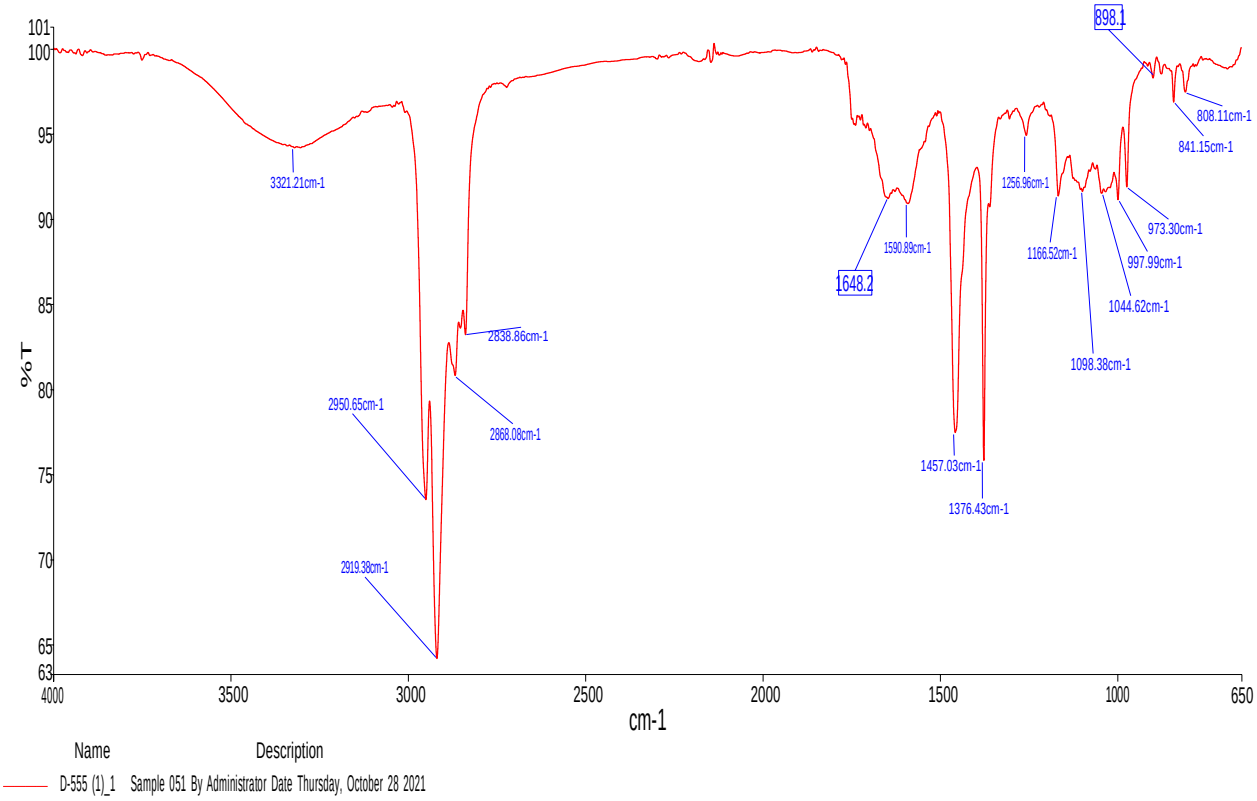


Figure 4.8: FTIR of chrome-tanned trimmings (before dechroming)

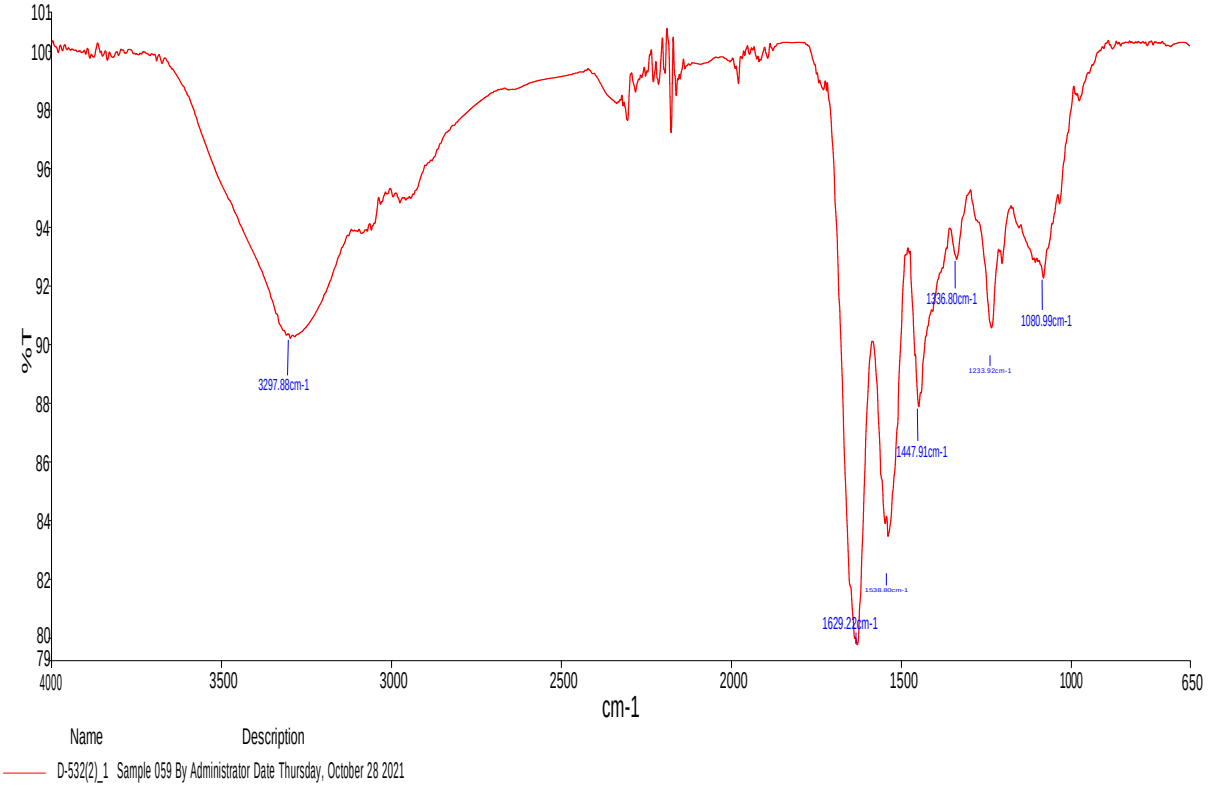


Figure 4.9 FTIR of shavings after dechroming

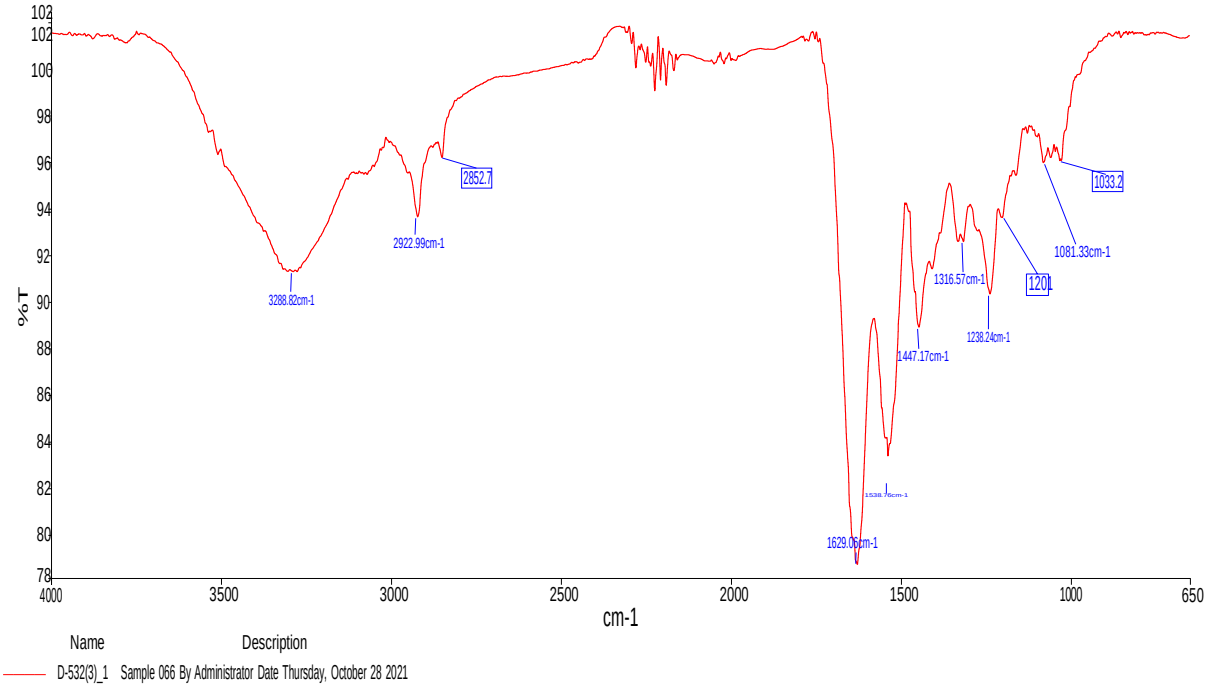


Figure 4.10: FTIR of trimmings after dechroming

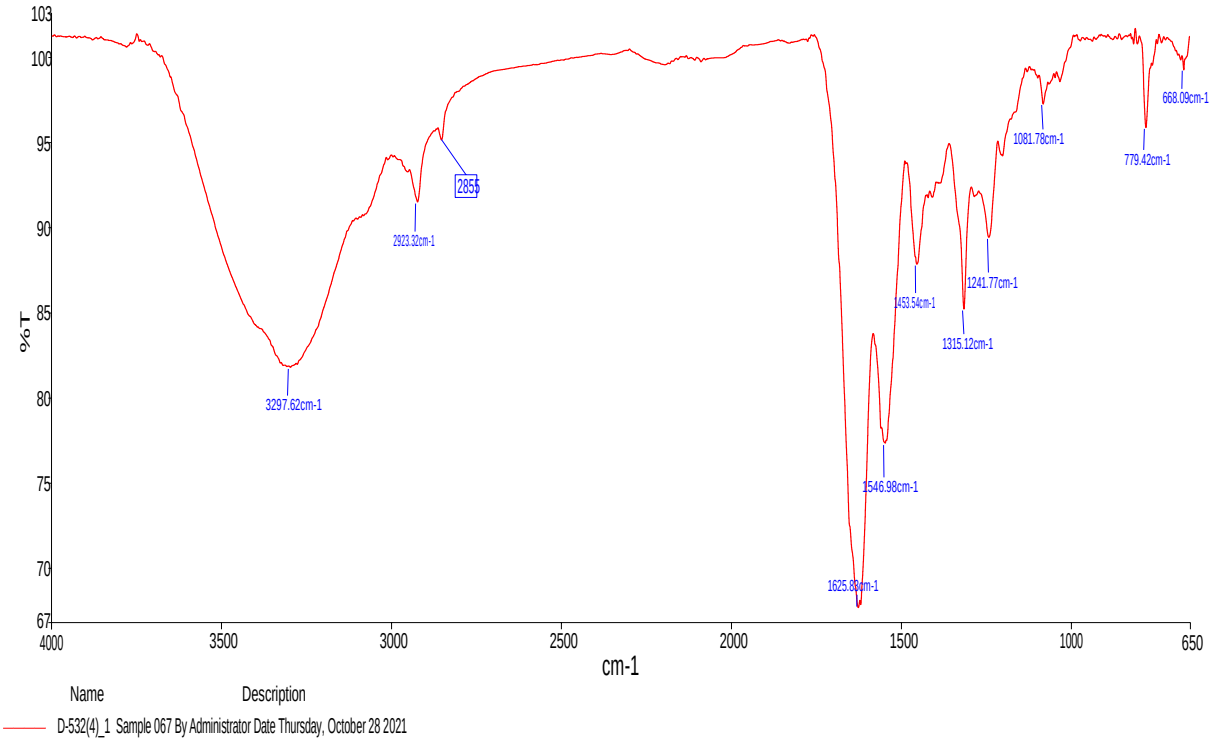


Figure 4.11: FTIR of Gelatin extracted from shavings

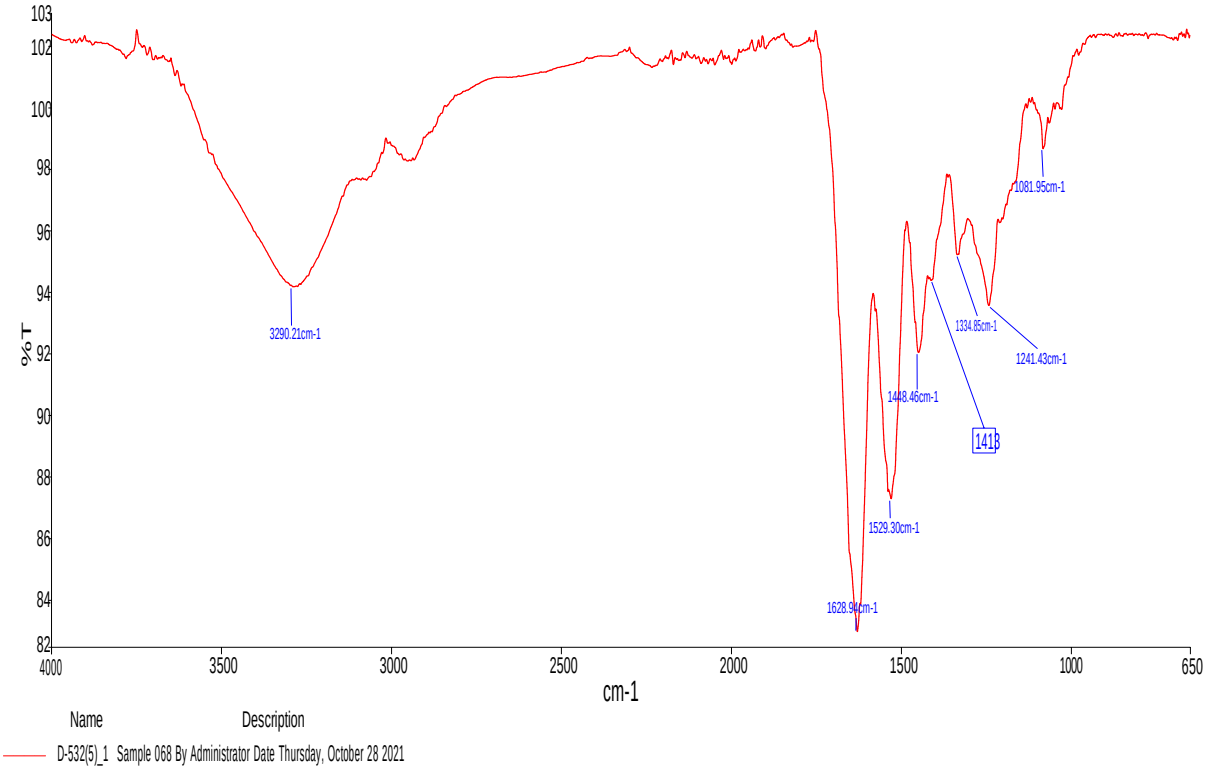


Figure 4.12: FTIR of Gelatin extracted from trimmings

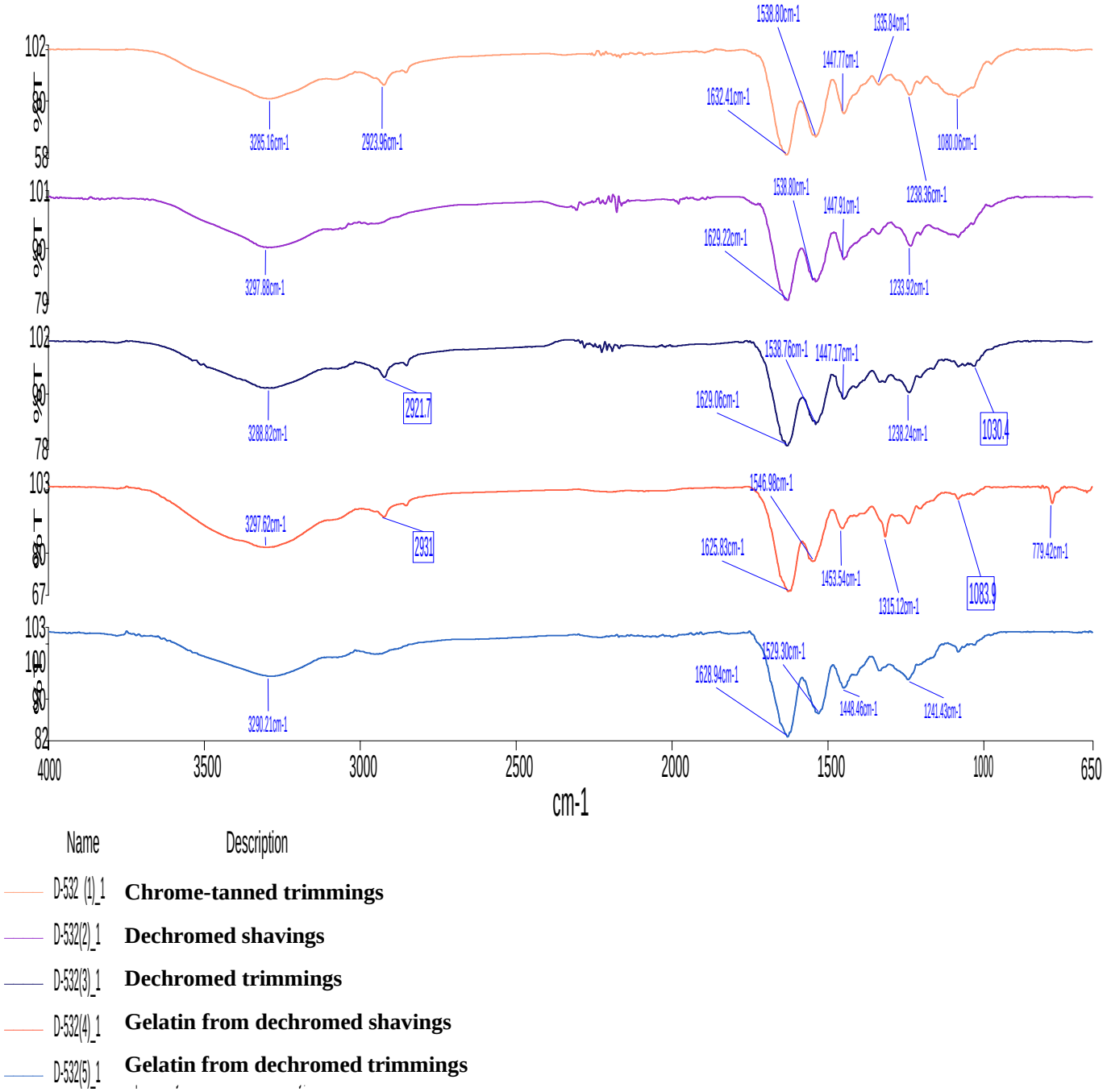


Figure 4.13: Combined FTIR curve of chrome-tanned trimmings, dechromed shavings, dechromed trimmings, shavings gelatin and trimmings gelatin

The characteristic of dechromed samples, trimmings and shavings gelatin were identified using Fourier Transform Infrared (FTIR) Spectroscopy. This characterization identified the functional groups and bonds of gelatin, dechromed samples and initial sample. Similar results also observed in other work (Hamed et. al., 2018). The spectra obtained was compared to that of the pure gelatin. In Appendix B there is a picture of pure gelatin. Elliott, Ambrose and Krimm, Bandekar (as cited in Deb, 2014) described the characteristic IR bands of the proteins and peptides which are listed in table 4.8. these frequencies will help to determine the functional groups present in samples of current study.

The FTIR spectrum for extracted gelatin, dechromed trimmings, shavings and chrome tanned trimmings has two primary peak regions: the first is 3300-2900 cm^{-1} and the second is 1700-1000 cm^{-1}. Each sample of FTIR spectra has at least five peaks meaning the samples are complex organic molecules (Nandiyanto et. al., 2019). The OH- bond in the carboxylic group of the protein was assigned to a strong and broad overlapping band in the range of 3400-3200 cm^{-1}.

Table 4.8: Characteristic infrared bands of peptide linkage

Designation	Approximate frequency (cm^{-1}) (Deb, 2014)	Description (Deb, 2014)
Amide A	3300	NH stretching
Amide B	3100	NH stretching
Amide I	1690-1600	C=O stretching
Amide II	1575-1480	CN stretching, NH bending
Amide III	1301-1229	CN stretching, NH bending
Amide IV	767-625	OCN bending
Amide V	800-640	Out-of-plane NH bending
Amide VI	606-537	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

The FTIR spectrum of every sample has more than 5 absorption bands, indicating all the samples are complex molecules (Nandiyanto et. al., 2019). The polymeric OH- stretching was observed in all the samples. Gelatin is a complex organic bio-polymer. Trimmings gelatin and dechromed shavings sample have weaker presence of CH- stretching. The presence of functional groups (primarily OH, COOH, and NH) in water soluble polymers gives them their solubility qualities, which can be exploited to make hydro gels (Byrne et. al., 2002). The reaction of functional groups with complementary reactivity, such as an amine-carboxylic acid or isocynate-OH/NH_2 reaction, can be used to create covalent connections between polymer chains (Byrne et. al., 2002). Absorption band for long-chain linear aliphatic compounds is identified at 2935 and 2860 cm^{-1} (Nandiyanto et. al., 2019). Peaks in this ranges were observed in each sample. There are spectra below 1700 cm^{-1}, replying amides or carboxylates functional group for all samples (Nandiyanto et. al., 2019).

Nine distinct IR absorption bands, amide A, B, and I to VII, are produced by polypeptide and protein repeat units. The two most significant vibrational bands of the protein backbone are the amide I and II bands. Inan said amide I, amide II and amide III are characteristic for collagen in random coil protein which proved that the collagen was in hydrolyzed form (as cited in Deb, 2014). The primary amine, C=O stretching is responsible for the strong bend at 1628 cm^{-1} in trimmings gelatin and 1625 cm^{-1} in shavings gelatin. Pure gelatin shows a 1626 cm^{-1} for this bending (Institute of Chemistry, 2021). All the tanned and dechromed samples also show similar bendings in this region. But the shifting in the peaks from dechromed sample to gelatin samples indicate change in chemical composition. All these peaks fall in the range of 1670 cm^{-1} to a 1620 cm^{-1} which is a region for unsaturated bonds (Nandiyanto et. al., 2019). It indicates that there are some double and triple bonds in this region.

As can be followed from Figures 4.8 to 4.13 as well as from Table 4.9, the intense major Amide I peak at 1629 cm^{-1} is consistent in the spectra of both dechromed trimmings and dechromed shavings, 1628 cm^{-1} in trimmings gelatin and 1625 cm^{-1} in shavings gelatin indicate strong helical conformation of the collagen structure (Menderes, 2002). 1500-2000 cm^{-1} is called double bond region. Each sample has absorbance in this region. N-H bend and C-N stretches are observed by 1529 cm^{-1} and 1546 cm^{-1} peaks in trimmings gelatin and shavings gelatin. Dechromed trimmings and shavings showed 1538 cm^{-1} wavelength. Rahman (as cited in Amertaningtyas et. al., 2019) explains that compounds with same functional groups tends to undergo the same chemical reactions. These peaks indicate presence of Amide II in sample (Deb, 2014). 1413cm^{-1} band in gelatin from trimmings

represents OH bond (Nandiyanto et. al., 2019). It was also weakly present in the shavings gelatin. The shavings gelatin sample weakly shows absorbance peak in this region. Bending vibrations of CH bending and tertiary amine were recognized for the bands at 1334, 1315, 1448 and 1453 cm^{-1}. These wavelengths were also found in tanned leather dechromed samples. There are no significant frequency changes at 1448, 1453, 1447, 1447, 1447 and 1444 cm^{-1}. All the samples displayed carbonate ion bands around 1445 cm^{-1}. The presence of carbonate is due to the traditional procedure of leather manufacturing which includes soaking of skins in a lime bath (Carsote et. al., 2014). The band at 1241, 1256, 1238, 1233 cm^{-1} was assigned to -CN stretching, NH bending (Amide III) vibration of the amide group (Deb, 2014) which was found in trimmings and shavings gelatin sample too.

The absorption bands seen between 1285cm^{-1} and 1200cm^{-1} are specific and predominant absorption bands of collagen molecule and changes to wave numbers indicate structural rearrangements in the helical structure or disruption of the helical structure by denaturing effects. Compared to dechromed leather there are no significant absorption band shifts between 1285 to 1200 cm^{-1} in collagen. As these bands are representative of collagen, constancy in the bands indicates no conformational changes at the molecular level (Menderes, 2002). The band at 1082 cm^{-1} and 1083 cm^{-1} are attributed to the strong primary amine, -CN stretching vibrations in the gelatin samples (Nandiyanto et. al., 2019). The tanned and dechromed samples also have similar vibrations. The absorbance bands around 1080 cm^{-1} and 1030 cm^{-1} are for C-O stretching vibrations of carbohydrate residues present in the collagen structure (Menderes, 2002).

The bending of gelatin from shavings (Figure 4.11) at 780 cm^{-1} is for skeletal C-C vibration (Nandiyanto et. al., 2019). The FTIR data demonstrates that the gelatin bonds in this investigation are quite similar to pure gelatin bonds. The chemical components of pure gelatin (Appendix B) are very similar to the components detected in the FTIR result of gelatin from current study. According to literature (Carsote et. al., 2019), presence of carbonyl groups band around 1740 cm^{-1} can be found due to oxidation hydrolysis. The carbonyl band around 1740 cm^{-1} was not found in any sample of current study suggesting that oxidative degradation was insignificant (Carsote et. al., 2019). This is a promising outcome, indicating that the current investigation was successful in avoiding oxidation.

Table 4.9: FTIR spectrum and assignments of extracted gelatin, dechromed samples and chrome sample

Peak wave of gelatin cm^{-1}		Peak wave of chrome- tanned and dechromed samples cm^{-1}			Peak wave of pure gelatin	Wavelengths cm^{-1}	Assignments
Trimming	Shavings	Chrome-Tanned Trimming	De-chromed trimming	De-chromed shaving	(Institute of Chemistry, University of Tartu, Estonia, 2021)	(Nandiyanto et. al., 2019); (Deb, 2014); (Vyskocilova et a., 2014)	(Nandiyanto et. al., 2019); (Deb, 2014); (Vyskocilova et a., 2014)
3290	3297	3321	3288	3297	3275	3570-3200 (broad)	Hydroxy group, H-bonded OH stretching
1628	1625	1632	1629	1629	1626	1690−1600	C=O stretching (Amide I)
Weakly present	2931	2923	2921	Weakly present	2934	2935-2915	Methylene CH-sym/asym stretch
1529	1546	1538	1538	1538	1521	1575-1480	-CN stretching, NH bending (Amide II)
1448	1453	1447	1447	1447	1444	1485-1445	CH-bending
1413	Weakly present	–	–	–	1400	1410-1310	-OH bend
1334	1315	1335	1316	1336	1334	1360-1310	Aromatic tertiary amine,-CH stretch
1241	1241	1256	1238	1233	1235	1301-1229	-CN stretching, NH bending (Amide III)
1082	1083	1080	1030	1080	1080	1090-1020	Primary amine, -CN stretch

4.13 Other Heavy metals concentration in gelatin

The gelatin solution from trimmings and shavings was tested for other heavy metal detection. Cd, Pb, Zn was tested to observe the toxicity level by AAS analysis. The results were compared with the residue limit for edible gelatin set by European Union (EU, 2016). The results are listed in the Table 4.9. The gelatin solution contained no lead, and all heavy metal concentrations were below the European Union's (EU, 2016) concentration level. This makes the gelatin extracted in this study less toxic and favorable to use in industrial purpose.

Table 4.10: Heavy metals concentration found in gelatin extracted

Heavy metal	Conc. Detected (mg/kg)		Conc. Limit (mg/kg)
	Trimmings gelatin	Shavings gelatin	(EU, 2016)
Pb	0.00	0.00	5.0
Cd	0.31	0.03	0.5
Zn	3.93	2.41	50

4.14 TCLP results of trimming chrome cake and shavings chrome cake

From 50g sample of trimmings and shavings, 30.1g and 18.2g dry chrome cake were obtained after a combined dechroming and extraction operation. The Cr conc. of the leaching solution of the remaining chrome cakes were determined using TCLP of these samples. Table 4.10 shows the Cr conc. we found from the chrome cake leachates which is 19.5 mg/kg from trimmings and 34.6 mg/kg from shavings. Although trace levels of the metal are required for human health, excessive exposure to the material in the body can cause harm to the liver, kidneys, and bloodstream. The Environmental Protection Agency (EPA) allows for only a certain concentration of each metal in waste. Chromium is regulated by the EPA at a level of 5 mg/kg. TCLP conc. of chromium from our chrome cakes are higher than the EPA TCLP limit (TCLP-Toxicity Characteristic Leaching Procedure,). But this result is obviously much lower than the initial conc. of the trimmings and shavings raw material which was meant to land-filled or reuse directly without any treatment. Although it is a positive side, both the chrome cakes are still considered hazardous and should be treated before dumping in the environment or reused.

The chrome cake chromium conc. from trimmings is less than the chrome cake conc. from shavings. It is likely because the Chromium conc. in gelatin from trimmings are higher than the Chromium conc. in shavings.

Table 4.11: TCLP results of trimming chrome cake and shavings chrome cake

Gealtin source	Trimmings	Shavings
Cr conc. (mg/kg)	19.50	34.60

4.15 XRF result of dry gelatin from leather trimmings

The gelatin extracted from trimmings sample shows better performance than gelatin extracted from shavings. The trimmings gelatin was further subjected to XRF study to revise a detailed compositional analysis of the trimmings gelatin sample. Table 4.12 shows the XRF results of the dry gelatin sample. The XRF study shows a significant decrease of Chromium in the dry gelatin sample. It also confirms trivalent format of chromium in the final product.

The result of XRF study shows a list of large number of chemical compositions present on the gelatin sample from dechromed trimmings. The dry gelatin sample had a higher percentage of SO3 sample, according to the XRF analysis. The percentages of all other components in the gelatin sample were lower, and the hazardous elements (TiO_2, P_2O_5, Cr_2O_3, SrO, CuO, ZrO_2) were insignificant.

The Chromium oxide in the gelatin sample was present in the form of Cr_2O_3 which justifies the FTIR result that no harmful oxidation to Cr^{6+} happened during dechroming operation. The presence of Mg, Ca, Cl, Na in the dry gelatin may had occurred during the dechroming process. These elements are not harmful and does not hamper the quality of gelatin. As the leather tanning process contains numerous chemicals and other components found in XRF, were not used in the current study, it can be assumed that K, Ti, P, Fe oxides were already present in the tanned leather sample. All these elements are present in negligible amount in the dry trimming gelatin sample (Table 4.12).

Table 4.12: XRF results of trimmings gelatin sample

Compositions (% w/w)	Trimmings gelatin (%)
SO_3	70. 09
CaO	7.99
SiO_2	7.55
Al_2O_3	6.67
MgO	2.09
Fe_2O_3	1.49
Cl	1.48
Na_2O	1.22
K_2O	0.78
TiO_2	0.38
P_2O_5	0.18
Cr_2O_3	0.03
SrO	0.02
CuO	0.02
ZrO_2	0.01
Total	100

4.16 Hazardous waste handling by waste to energy (WTE) technology

The goal of waste to energy technologies is to generate energy from solid waste. In order to convert waste into energy, a wide range of technologies and techniques can be used. Thermo chemical conversion and bio chemical conversion are two of the most common methods for extracting energy from the organic part of waste (Mohapatra, 2014) which can be utilized to handle hazardous chrome cakes from this study.

4.16.1 Thermo chemical conversion

The thermal decomposition of organic materials to generate heat energy, gas, or fuel oil is known as thermo-chemical conversion. These methods work well with wastes that contain high amount of organic non-biodegradable matter and have a low moisture/water content. The chrome cake from current study can be dried after all operation to put into further thermo chemical conversion. Incineration and pyrolysis/gasification are the two primary processes considered in this group of waste management (Tsunatu et. al., 2015).

4.16.2 Bio chemical conversion

By enzymatic degradation of organic matter carried out by microbial action, the bio-chemical conversion process produces methane gas or alcohol. These procedures are suited for wastes with a high moisture content and a large amount of organic biodegradable matter in this scenario. Microbial activity thrives in these settings. Anaerobic digestion (Bio-methanation) and Composting are the two most common processes in this group (Ghaly, 2008). Following this conversion method, the wet chrome cakes can be processed to decrease the risk.

Chapter 5
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Chrome-tanned solid leather waste is a major environmental issue, and several countries are working to find a solution. Making appropriate reuse is an excellent answer to this issue. This research shows that solid leather waste can be dechromed and from that industrial-grade gelatin may be recovered as a value-added product. In this work there was presented and experimentally tested a combined alkali-acid hydrolysis method for dechroming chrome-tanned leather wastes without oxidation to carcinogenic Cr^{6+} and a gelatin forming procedure. In order to prepare collagen or gelatin with high purity and low cost, two or more hydrolysis pathways were used in combination is an alternative way. The study's findings emphasize the use of extracted gelatin in industrial applications, as consumable and pharmaceutical gelatin require complex processing and sensitivity. The following are the findings of this research:

1. The optimized conditions to treat 50g of sample with alkali/acid dechroming procedure were: optimized soaking time for step 1 was 4 days. Optimized dose for step 1 was 5g CaO in 400ml distilled water, for step 2 was 10g Oxalic acid, 10g NaCl and 20ml HCl acid in 250ml distilled water, for step 3 was 10g MgO, 10g NaCl in 500ml distilled water and for step 4 was 20ml H_2SO_4 in 250ml distilled water. Optimized temperature for step 3 was 60^0-65^0C. The optimum conditions helped to decrease collagen loss during dechroming operation and retain better viscosity and gel forming ability in the final gelatin extraction stage.

2. Before optimization the efficiency of four-step alkali-acid dechroming rate of chrome-tanned trimmings and shavings were 92% and 94%. After dechroming optimization, the extracted gelatin solution of trimmings and shavings were 99.94% and 99.99% Chromium free. The shavings and trimmings gelatin samples had 1.1 mg/kg and 7.2 mg/kg Chromium conc. respectively that were below the standard limit 10 mg/kg with good gelatinous properties. From the obtained results it follows that the optimization enables to speed up the operation and favorable condition for gelatin formation. This obviously supports the approach of this research to extract gelatin from tanned leather waste for industrial usage.

3. Another important parameter for measuring the efficacy of the dechroming procedure is the production of aqueous gelatin. Trimming and shavings gelatins exhibited aqueous yields of 64% and 68%, respectively, making them appropriate for industrial use.

4. The study's benefits included a reduced chromium concentration and gelatin that was easy to deal with. The gelatin recovered from shavings had a lower ultimate chromium content than the gelatin recovered from trims, but the trimming gelatin had a higher viscosity 1.2e and dry yield 1.5g/50g than the shavings gelatin, which had a viscosity 0.65e and dry yield 0.67g/50g. The shavings sample had more mass loss than the trimmings sample. WHO's standard levels were met by all of the properties (WHO, 2021).

5. The pH of the gelatin samples were between the range (4-5 and 6-7) required by WHO (WHO, 2021). As the final dechroming step was operated in acidic condition both the gelatin extracted can be characterized as type A gelatin.

6. The dechromed leather and gelatin samples' SEM data show that the suggested dechroming procedure is effective. The chemical makeup from FTIR measurement of the tanned leather and dechromed leather showed the different bond states of the product in different stages. The FTIR results of extracted gelatin revealed bonds and bendings that were similar to pure gelatin.

7. The TCLP result of chrome cakes after dechroming process demonstrates that chrome cakes are slightly hazardous but much lower than the initial concentration of raw samples and should be handled before being reused or dumped. AAS analysis of gelatin from trimmings and shavings samples exhibited no lead. Cd and Zn were found to be below the European Union Commission's residue limit (EU, 2016).

8. The XRF results of the gelatin from trimmings reveal that it contains a substantial amount of SO_3. CaO and SiO_2 were identified in the sample at 7.99% and 7.55% respectively. 6.67% Al_2O_3 and 2.09% MgO were discovered, respectively. Other oxides of Fe, Cl, Na, K, Ti, P, Sr, Cu, and Zr were detected in the sample in trace amounts.

5.2 Recommendations for future study

1. The extracted gelatin characteristics were compared to edible gelatin property standards. All of the properties met the standards. These gelatins are favorable as industrial gelatin, but more research should be carried out for those can be used as edible gelatin.

2. The chromium that was removed from the leather was not recovered. The filtrates chromium from chrome-tanned leather trimmings and shavings, can be used in tanning agent solutions if properly recovered.

3. The gelatin from the shavings waste sample had a reduced viscosity and dry yield. It is advised to assess effective approaches to reduce protein loss during dechroming from the shavings sample.

4. The residue chrome cake can be further used to recover more chromium from it. Hydrolysis of the chrome cake can be useful to recover the chromium. Chromium recovery from the cake can be little complicated but it is possible.

5. After being enough dechromed, the chrome cake can also be a good source of poultry feed if it satisfies the lower Cr limit. High chromium solid leather waste is already being used in our country for poultry feed. The dechromed solid leather residue can be a good alternative to this and also can be a good commercial product.

6. The dechroming procedures were carried out at the natural pH of the solution. However, Cr optimization can also be a good way to reduce it faster. A series of complicated studies will be needed to optimize the whole process.

7. The extracted gelatin samples were not subjected to some standard procedures, such as UV analysis to assess protein properties and a bloom test to determine gelatin quality. It is recommended to carry out these tests on further studies.

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Appendix A

Tanning

Tanning is the process of treating skins of animals to produce leather, which is more durable and less susceptible to decomposition. Traditionally, tanning used tannin, an acidic chemical compound from which the tanning process draws its name (tannin is in turn named after an old German word for oak or fir trees, from which the compound was derived). Coloring may occur during tanning. A tannery is the term for a place where the skins are processed.

Tanning leather involves a process which permanently alters the protein structure of skin. Making "rawhide" (untanned but worked hide) does not require the use of tannin. Rawhide is made by removing the flesh and fat and then the hair by use of an aqueous solution (this process is often called "liming" when using lime and water or "bucking" when using wood ash (lye) and water), then scraping over a beam with a somewhat dull knife, then drying.

The two aforementioned solutions for removing the hair also act to clean the fiber network of the skin and allow penetration and action of the tanning agent, so that all the steps in preparation of rawhide except drying are often preludes to the more complex process of tanning and production of leather.

Tanning can be performed with either vegetable or mineral methods. Before tanning, the skins are unhaired, degreased, desalted and soaked in water over a period of 6 hours to 2 days. To prevent damage of the skin by bacterial growth during the soaking period, biocides, typically dithiocarbamates, are used. Fungicides such as TCMBT, 2-(Thiocyanomethylthio) benzothiazole, are added later in the process to protect wet leathers from mould growth. After 1980 the use of pentachlorophenol and quicksilver based biocides and their derivatives was forbidden.

Preparatory steps prior to tanning

Skinning

The actual tanning process begins with the obtaining of an animal skin. When an animal skin is to be tanned, the beast is killed and skinned before the body heat leaves the tissues. This can be done by the tanner, or by obtaining a skin at a slaughterhouse or farm.

Curing

Preparing hides begins by curing them with salt. Curing is employed to prevent putrefaction of the protein substance (collagen) from bacterial growth during the time lag that might occur from procuring the hide to when it is processed. Curing removes excess water from the hides and skins using a difference in osmotic pressure. The moisture content of hides and skins gets greatly reduced. In wet salting, the hides are heavily salted, then pressed into packs for about 30 days. In brine-curing the hides are agitated in a salt water bath for about 16 hours. Generally speaking, curing substantially reduces the chance of spoilage by bacteria. Curing can also be done by preserving the hides and skins at a very low temperature.

Beamhouse operations

The steps in the production of leather between curing and tanning are collectively referred to as beamhouse operations. They include, in order, soaking, liming, removal of extraneous tissues (unhairing, scudding, and fleshing), deliming, bating (including puering), drenching, and pickling.

Soaking

In the process known as *soaking*, the hides are soaked in clean water to remove the salt left over from curing and increase the moisture so that the hide or skin can be further treated

Liming

After soaking, the hides and skins are taken for *liming*: treatment with milk of lime (a basic agent) that may involve the addition of "sharpening agents" (disulfide reducing agents) like sodium sulfide, cyanides, amines etc. The objectives of this operation are mainly to:

Remove the hairs, nails and other keratinous matter.
Remove some of the interfibrillary soluble proteins like mucins.
Swell up and split up the fibres to the desired extent.
Remove the natural grease and fats to some extent.
Bring the collagen in the hide to a proper condition for satisfactory tannage.

The weakening of hair is dependent on the breakdown of the disulfide link of the amino acid called cystine, which is the characteristic of the keratin class of protein that gives strength to hair and wool (keratin typically makes up 90% of the dry weight of hair). The hydrogen

atoms supplied by the sharpening agent weaken the cystine molecular link whereby the covalent disulfide bond links are ultimately ruptured, weakening the keratin. To some extent, sharpening also contributes to unhairing, as it tends to break down the hair proteins. The isoelectric point of the collagen in the hide (this is a tissue strengthening protein unrelated to keratin) is also shifted to around 4.7 due to liming.

Unhairing and scudding

Unhairing agents used at this time are: Sodium sulfide, sodium hydroxide, sodium hydrosulfite, calcium hydrosulfide, dimethyl amine, and Sodium sulfhydrate. The majority of hair is then removed mechanically, initially with a machine and then by hand using a dull knife, a process known as scudding.

Deliming and bating

The pH of the collagen is brought down to a lower level so that enzymes may act on it, in a process known as deliming. Depending on the end use of the leather, hides may be treated with enzymes to soften them, a process called bating.

Pickling

Once bating is complete, the hides and skins are treated with a mixture of common table salt and sulfuric acid, in case a mineral tanning is to be done. This is done to bring down the pH of collagen to a very low level so as to facilitate the penetration of mineral tanning agent into the substance. This process is known as pickling. The common salt (sodium chloride) penetrates the hide twice as fast as the acid and checks the ill effect of sudden drop of pH.Peeling bark for the tannery in Prattsville, New York, during the 1840s, when it was the largest in the world.

Type of tanning

1. Vegetable tanning

Vegetable tanning uses tannin. The tannins (a class of polyphenol astringent chemical) occur naturally in the bark and leaves of many plants. Tannins bind to the collagen proteins in the hide and coat them causing them to become less watersoluble, and more resistant to bacterial attack. The process also causes the hide to become more flexible. The primary barks, processed in bark mills and used in modern times are chestnut, oak, redoul, tanoak, hemlock, quebracho, mangrove, wattle (acacia; see catechu), and myrobalan[disambiguation needed].

Hides are stretched on frames and immersed for several weeks in vats of increasing concentrations of tannin. Vegetable tanned hide is flexible and is used for luggage and furniture.

2. Chrome tanning

Prior to the introduction of the basic chromium species in tanning, several steps are required to produce a tannable hide. These steps include: Scudding (removing the hair), Liming (the introduction of alkali agents such as sodium hydroxide), Deliming (restoring neutral pH), Bating (softening the skin with enzymes), and Pickling (lowering pH of the hide with salt and sulfuric acid). The pH is very acidic when the chromium is introduced to ensure that the chromium complexes are small enough to fit in between the fibers and residues of the collagen. Once the desired level of penetration of chrome into the substance is achieved,the pH of the material is raised again to facilitate the process. This step is known as "basification". In the raw state chrome tanned skins are blue and therefore referred to as "wet blue." Chrome tanning is faster than vegetable tanning (less than a day for this part of the process) and produces a stretchable leather which is excellent for use in handbags and garments.

Appendix B

FTIR of pure gelatin

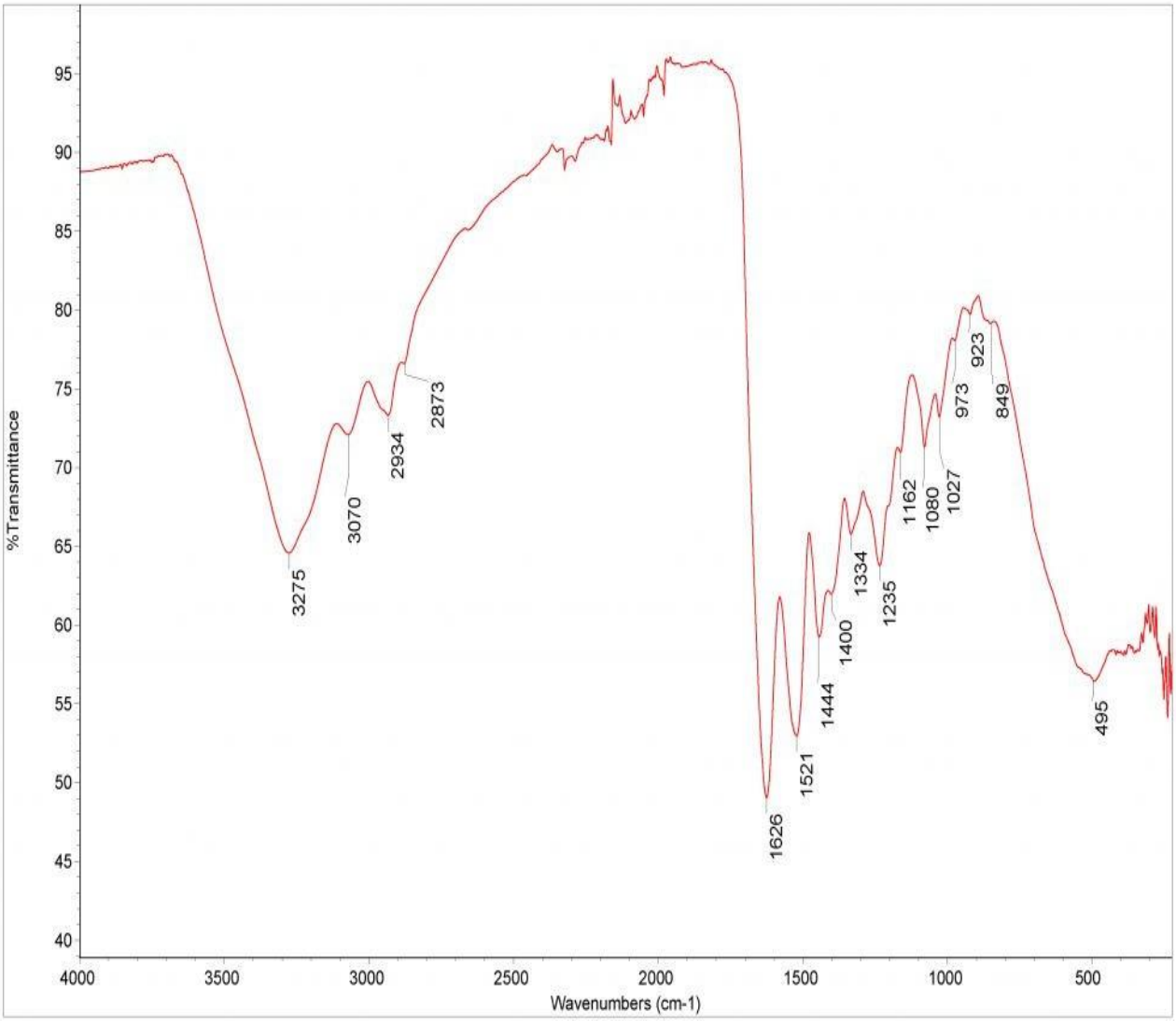

Figure: FTIR curve for pure gelatin (Institute of Chemistry University of Tartu, Estonia, 2021)