Study on the Treatment of Tannery Solid Wastes of Bangladesh for their Potential Utilisation.

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Dedicated

To

My Beloved Parents and Family Members

Acknowledgement

I start in the name of Almighty, for, He is the creator of the universe and He has flowered me with enough ability, knowledge, patients and strength to accomplish this research work successfully.

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Abstract

The tannery solid waste namely chrome shaving dust has been characterized and subjected to biochemical treatment using proteolytic enzyme and mild alkalis. In this study full chrome (chrome content 3.42 % and low chrome (chrome content 1.37 %) from cow wet blue leather have been used as raw materials for collagen protein extraction. MgO and CaO have been used for mild alkaline medium where trypsin, pepsin and proteinase K have been used as proteolytic enzymes. Several methods have been developed for the hydrolysis of chrome shaving dust after optimization of pH, types of alkalis and their dosages, variation of enzymes and their dosages and the amalgamation of enzymes. A three step hydrolysis process has been developed in all hydrolysis methods for the maximum recovery of collagen hydrolysate. The collagen hydrolysate has been extracted through vacuum filtration and preserved at 4 ºC in an incubator.

The prepared collagen hydrolysates by different methods have been analyzed for various physical and chemical parameters. MgO has been found to be better than CaO during hydrolysis since all the samples treated with MgO has given more protein yield. Trypsin enzyme has been found to be suitable for collagen hydrolysis compared to pepsin and proeinase K but the amalgamation of trypsin with proteinase K has given highest protein yield. The quality of collagen hydrolysate produced from the treatment of MgO is also better than that of CaO. Low chrome shaving dust has given more protein yield than that of full chrome shaving dust with the similar treatment. After complete hydrolysis, the chrome cake has been treated with sulfuric acid to separate the chromium present in chrome cake. The percentage of chrome recovery was 94.32% and 96.36% in samples 4 and 5 respectively.

All the samples have shown the characteristics protein peak during FTIR analysis. The collagen hydrolysates have been characterized by amide A and B bands, associated with NH stretching modes. The characteristics amide I, II and III bands have proved that the collagen was in hydrolyzed form. All collagen hydrolysates have been analyzed for the determination of chromium by atomic absorption spectrophotometer and trace amount of chromium (<4.5 ppm) was found in most of the samples while some samples contained below detection level of chromium (0.1 ppm). Ca and Mg content of collagen hydrolysate have also been determined by atomic absorption spectrophotometer and 1165 and 1033 ppm was found respectively. The protein content of the samples have been determined by BUCHI Kjeldhal instrument and found more protein yield in trypsin and MgO treated samples, amalgamated trypsin with proteinase K and MgO treated samples and low chrome shaving dust samples with similar treatment.

This biochemical method of producing collagen hydrolysate is environmentally friendly since there was no toxic chemical used in this method. Therefore, this approach of “waste to resource or wealth” will not only release the burden of disposal of tannery solid wastes but also produce valuable protein products which can be utilized in poultry feed, fertilizer and leather processing.

Contents

List of Tables

List of Figures

Chapter 1: Introduction and Literature Review

1.1. General

The principal aim of the leather industry, which plays a significant role in today's global economy, is to transform animal hides and skins into a physically and chemically stable material by subjecting them to chemical and mechanical sequential processes, and therefore to obtain products for meeting various needs of people. The leather industry generally uses hides and skins as raw materials, which are the by-products of meat and meat products industry. In this respect, the leather industry could have easily been distinguished as an environmentally friendly industry, since it processes waste products from meat production [01]. However, the leather industry has commonly been associated with high pollution due to the bad smell, organic wastes and high water consumption caused during traditional manufacturing processes [02]. Different forms of waste in quality and quantity, which emerge during the transformation of hides and skins into leather in thousands of leather factories, from primitive to modern all around the world, have negative impacts on the environment [03].

The tanneries of Bangladesh process more than 180 million sq. ft. or 74,000 tons of raw hides and skins annually. The peak production level is 400 tons a day which takes place during the Eid-Ul-Azha period [04]. The leather tanning industry has been identified as one of the main cause of environmental pollution in the capital city. According to the Department of Environment (DOE), each day 15000 cubic liters of liquid wastes in which 19 tones of solid wastes and 7.5 tones of Biological Oxygen Demand (BOD), go into Buriganga River [05]. About 12 sq. km. area of Hazaribagh and adjacent area are full of offensive odours of various toxic chemicals. An average of 19 cubic liter water containing more than 300 different chemical compounds is being discharged daily from these industries [06]. Most

of the solid wastes and effluents are subjected to natural decomposition in the environment, causing serious pollution problems affecting soil, water, air and human life [04]. The leather industry produces a significant amount of chromium bearing hazardous wastes. Solid waste disposal is increasingly becoming a huge challenge to tanners due to paucity of landfill sites and strict environmental legislations worldwide [07]. It has been estimated that about 0.8 million tons of chrome shavings could be generated per year globally. This waste is partly used in the manufacture of leather board, but most are normally disposed of in landfill sites, wasting all the contained resources [08-09].

The disposal of solid waste from leather manufacture is a significant issue in the tannery-environment relationship. With reference to the solids balance in the conversion of hides and skins into leather, out of every 1,000 kilos of salted bovine hides, only 260 kg are finally converted into leather [10]. Among the remaining solids, 230 kg are in the wet blue state, comprising 100 kg shavings, 1.10 kg unusable splits, and 20 kg trimmings. In terms of collagen, the yield as leather is 50%, with approximately 34% distributed among wet blue solid wastes. This shows the low efficiency in leather making, but also the potential for reclaiming this waste protein [11]. One of the major tanned solid wastes i.e., chrome shavings are simply thrown into street outside of tannery in Hazaribagh without any pre-treatments. It creates the blockage in drainage system of the tannery area and contamination of chromium is happened into the surface water, ground water, soil, plant and aquatic lives. It is known that only 20% of wet salted hides/skins are converted into commercial leather, while 25% becomes chromium-containing leather waste (CCLW), and the remainder becomes non-tanned waste or is lost in wastewater as fat, soluble protein and solid suspended pollutants [12]. The direct discharge of these wastes has contaminated the ground and surface water with dangerously high

concentrations of chromium, as well as cadmium, arsenic, and lead [13]. The contamination of rivers also allows these pollutants to accumulate in common fish and shellfish species, which are used as local food sources [14]. In addition, the chromium-laced solid wastes from tanneries are often converted into poultry feed as is the case in areas of Bangladesh and can thus impact livestock and humans [15].

Many small businessmen collect this chrome shavings and use for the production of poultry feed ingredient as a protein supplement in an unhygienic and unscientific way which not only creates severe environmental pollution but also the product is very dangerous as a poultry feed since it contains high concentration of chromium [15]. These wastes are converted to protein-concentrate to be used into poultry feed, fish feed, and in production of bio-fertilizers without any appropriate treatment. At present, several large and many small mills are converting the solid wastes into protein-concentrate for mixing into poultry feed. Each large mill produces 200-250 tons of protein-concentrate per day [15].

This process of producing protein supplement is also dangerous for the manufacturer as severe toxic fumes and hexavalent chromium, a well known carcinogen, are formed during the manufacturing process [14]. Research reveals that meat, liver, bones of poultry chicken feeded with this unhygienic protein product, contain very high content of chromium. Therefore poultry chicken meat is very dangerous for human body as the chromium accumulate into human body through food chain [15].

Solid wastes from the tanning industry are unavoidable [16]. The solid waste accounts for almost 25% of the weight of raw hide. During the manufacturing process of high grade leather, even 50% of the weight of raw hide becomes wastes [17]. Use of chromium in leather industry is being questioned owing to reports emerging on

the toxicity and disposal problems associated with it [18-20]. Meantime, the leather industry will be restricted by more and more environmental protection policies. It is hence obvious that worldwide research is being focused on chrome-free tanning systems. Many studies based on less chrome and chrome-free technologies have emerged in the recent past [21-24].

1.2. Solid Wastes

Solid wastes or residues are wastes in the solid physical state at the point at which they are utilized or disposed of, irrespective of their water content, the surrounding medium, and their consistency. Industrial solid waste is an important part of the total waste problem. The amount of industrial solid waste is growing in many countries; therefore, waste management and waste treatment methods are of utmost importance. Solid waste primarily falls into two categories: hazardous waste and nonhazardous waste [25]. Nonhazardous waste consists of building and demolition waste (glass, wood, tar, scrap, etc.), combustible waste (office waste, activated carbon etc.), noncombustible waste (shredder waste, salts, etc.), and coating used in the metal industry. Hazardous waste consists of contaminated soil, mud, sludge, residues from treated waste, and so on. Since the hazardous waste possesses a great potential environmental threat, it is managed more strictly than other categories. Similar to municipal waste, most hazardous waste is managed in accordance with national governmental requirements. Requirements are set for issues such as operating permits for the treatment, storage, or disposal of hazardous waste. There are several treatment methods or stages that are followed to dispose or treat wastes: (i) re-use of waste, (ii) treatment, (iii) storage, and (iv) disposal methods [25].

Many by-products from various industrial activities that have traditionally been treated as waste are now being viewed as “new raw

materials" [26]. The solid wastes produced by different industries are being categorized according to their composition. Although various treatment methods are being practiced as per norms of the legislation, the disposal costs continue to rise [25].

Solid wastes create a major problem for leather industry in terms of both their variety and quantity. A high amount of reusable waste is generated in the leather industry. It is possible to recycle these products and even use them as raw materials for different industries [27]. The variety and quantity of solid wastes depends on animal species, breeding conditions, slaughterhouse practices, conservation conditions, leather processing stages, mechanical operations, qualification of the personnel, and chemicals used in processes [03].

1.3. Tannery Solid Wastes

According to the data received from the studies of several researchers, approximately 200 kg of leather is manufactured from 1 tone of wet-salted hide [01-02, 28]. This amount constitutes about 20% of rawhide weight. More than 600 kg of solid waste is generated during the transformation of rawhide into leather. That is to say, solid wastes containing protein and fat that constitute more than 60% of rawhide weight are disposed to the environment by leather factories without turning them to good use (Table 1) [03]. In other words, besides the 30-35 m^3 waste water disposed to environment during the processing of every 1 ton of rawhide in world leather industry, the data from FAO reveals that approximately 8.5 million tons of solid waste is generated during the production of 11 million tons of rawhide processed in the world [29].

Table 1: Estimated amount of solid (protein, tanned and un-tanned) wastes during the processing of 1 ton of salted hides according to various authors [FAO 2004].

Types of wastes	Puntener	Alexander	Buljan
Untanned waste:			
shavings	530 kg	120 kg	100kg
Sub-epidermal Tissue trimmings	135 kg	70-230 kg	300kg
Tanned Waste:			
shavings	145 kg	100 kg	99 kg
split		115 kg	107 kg
Dyed and finished waste:			
shavings	10 kg	32 kg	10 kg
fluff		2 kg	1 kg
Total	820kg	529-599 kg	617 kg

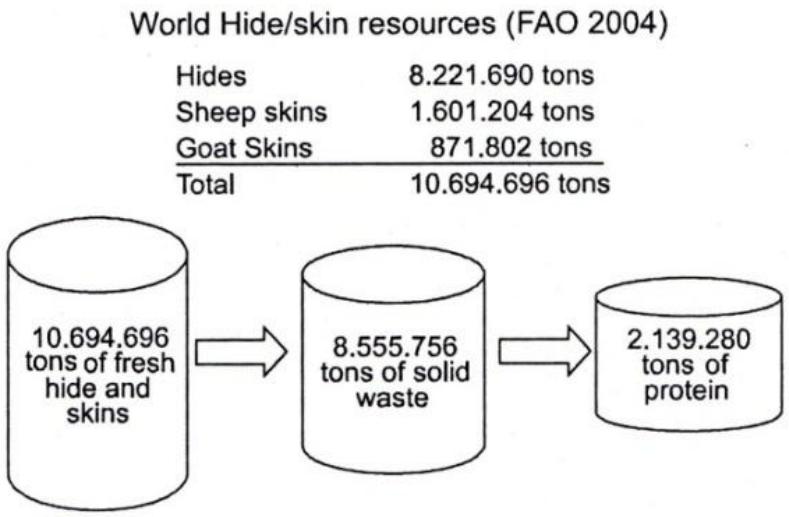


Figure1: Amounts of leather industry raw material input and solid waste output [29].

Wastes originate from all stages of leather making, such as fine leather particles, residues from various chemical discharges and reagents from different waste liquors comprising of large pieces of leather cuttings, trimmings and gross shavings, fleshing residues,

solid hair debris and remnants of paper bags [30]. Out of 1000 kg of raw hide, nearly 850 kg is generated as solid wastes in leather processing. Only 150 Kg of the raw material is converted in to leather [31]. A typical tannery generates huge amount of waste as follows [30-31]:

- Fleshing: 50-60%
- Chrome shaving, chrome splits and buffing dust: 35-40%
- Skin trimming: 5-7%
- Hair: 2-5%

Over 80 percent of the organic pollution load in BOD terms emanates from the beam house (pre-tanning); much of this comes from degraded hides & skins and hair matter [30]. During the tanning process at least 300 kg of chemicals (lime, salt etc.) are added per ton of hides. Excess of non-used salts will appear in the wastewater [32]. Because of the changing pH, these compounds can precipitate and contribute to the amount of solid waste or suspended solids. A large amount of waste produced by tanneries is discharged in natural water bodies directly or indirectly through the open drains without any treatment. The water in the low lying areas in developing countries, like India and Bangladesh, is polluted in such a degree that it has become unsuitable for public uses. In summer when the rate of decomposition of the waste is higher, serious air pollution is caused in residential areas by producing intolerable obnoxious odours [30].

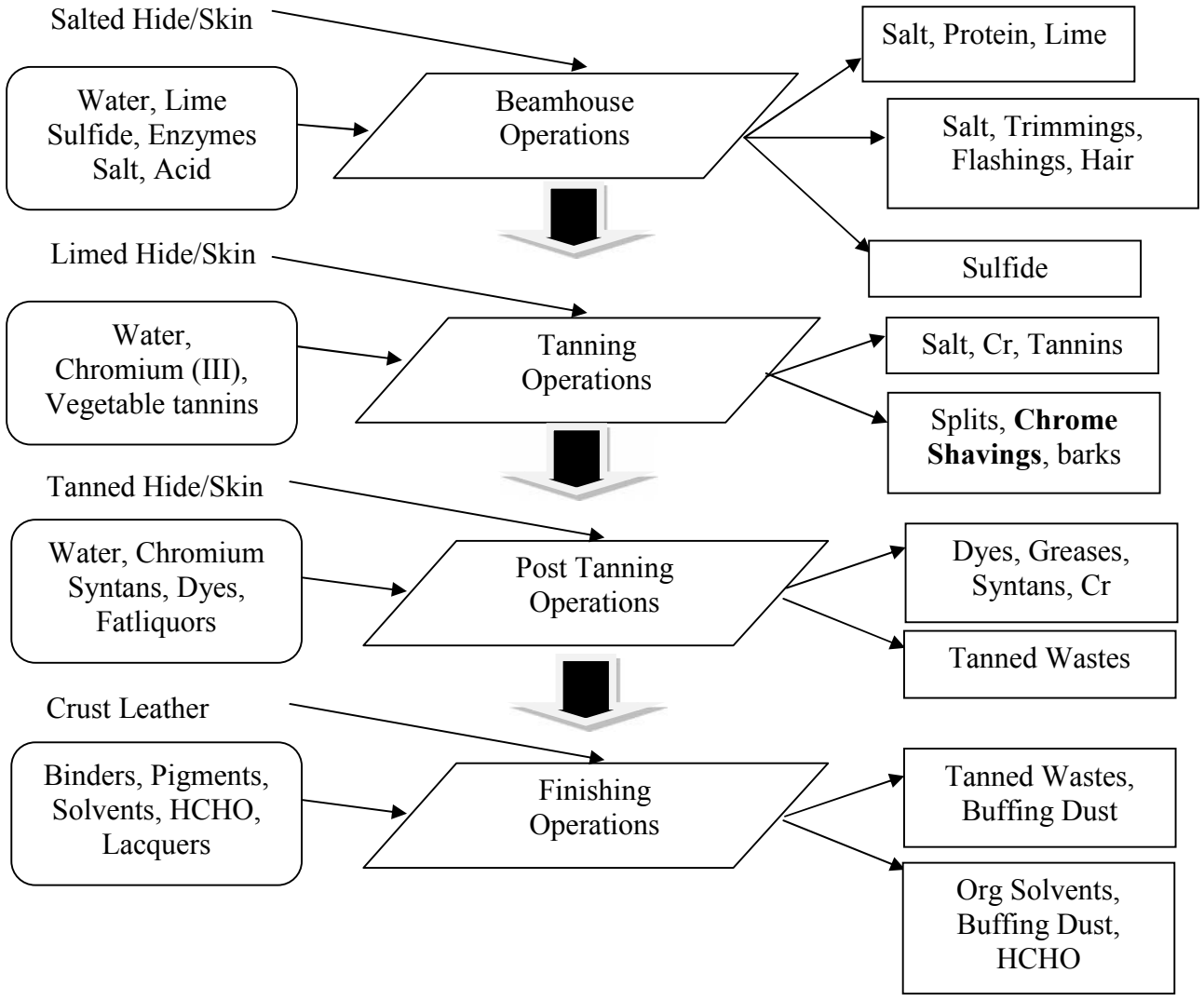


Figure 2: Input-Output profile of leather processing [25].

1.4. Types of Tannery Solid Wastes

Different types of tannery solid wastes in different forms have been generated at various operations of leather processing. The solid wastes generated from tannery have been depicted in the table 2. The wastes generated from leather industry can be broadly classified into tanned and un-tanned wastes [33]. The characteristics of these wastes are described next.

Table 2: Solid wastes generation from leather processing (for100 Tons Hides/Day Capacity) [25].

Types of waste	Quantity(tons/day)
Raw hide trimmings and waste	4.0–6.0
Dusted waste salt	2.5–4.0
Hair (for hides processing)	Negligible
Fleshing and trimmings	12.0–25.0
Sludge from lime pit	6.0–8.0
Bark/nuts from vegetables (heavy leathers)	80.0–120
Vegetable tanned trimmings	1.0–1.2
Chrome shavings	2.5–3.2
Chrome trimmings/finished leather pieces	1.0–1.2
Buffing dust	0.2–0.4
Sludge from pretreatment	20.0–24.0

1.4.1. Un-tanned Wastes

i. Raw Trimmings

One of the early operations in a tannery is trimming of neck and tail pieces from hides/skins to provide a shape to the final leather. Non removal of these pieces may decrease the efficiency of machine operations. The head and belly rounds and other trimmings are estimated to be around 6% [34]. The head/neck pieces can be processed into vegetable tanned leathers, and those leathers are subsequently converted into open-type sandals. The smaller raw trimmings are collected, stored, and sent to glue/gelatin and animal feed manufacturing units. The potential of hide collagen for human consumption as food supplement has been explored by substituting wet or dry fibrous collagen in meat-based products. The utilisation of the collagen rich hide and skin trimmings for preparation of cosmetic hair-care formulations, for example, shampoos, perming lotion, and bleaching aids, has been made possible [35].

ii. Green Fleshing

In some countries, the raw hides undergo fleshing operation yielding green fleshing and fatty tissues. This operation is carried out before washing and soaking. The yield of green fleshing is estimated to be 10% on the weight of the raw hide [34]. Since these fleshing are not contaminated with chemicals, they are invariably rendered along with the other inedible offals of the abattoir consisting of meat and bone. The end product obtained is used as meat-cum-bone meal. Green fleshing is also used to produce tallow, which is a raw material for the cosmetic and cleaning products industry and is also added to different food items such as cookies. If the market's price for those uses is low, tallow can be used as fuel for steamers [25].

iii. Limed Fleshing

Limed fleshing is obtained while scraping out the limed hides and skins either by hand or by machines. The fleshing are proteinaceous in nature comprising cutaneous muscle layers and sub cutaneous adhering tissues, which are undesirable in the subsequent operations of leather manufacture. The availability of limed fleshing is 35% on the wet weight of the raw hides (70% moisture). The limed fleshing is collected by the glue manufacturers and also by feed manufacturers [25]. The composition of the limed fleshing is: moisture content 78-80%, ash content 8.3% and total nitrogen 15.2% [36].

The fleshing obtained by employing machines in tanneries is potentially thermal denatured. The utilisation of the same fleshing for glue manufacture is not economically viable. Similarly, fleshing obtained from hides treated with a high percentage of sodium sulfide is found to be unfit for the production of glue. They are at best disposed through landfill. Disposal of such fleshing is currently a serious problem [25].

iv. Limed Trimmings

After the fleshing operation, the hides and skins are trimmed to remove the ragged and torn edges. These trimmings are proteinous in nature and found to be good raw material for glue, technical gelatin, and animal feed [35]. The limed trimmings are 2-7% on the wet weight of the hides [34]. Limed fleshing can also be used to produce tallow by pre-boiling to separate the fats from the other components. Then, the process to obtain tallow continues in the same way as for green fleshing. Notwithstanding the acidity index in general is higher than the index of the tallow obtained from green fleshing, the quality of the tallow is good enough for cosmetic purposes [25].

v. Hair and Wool

The hides and skins are treated with lime and sodium sulfide to remove hair/wool. Since hair and wool are valuable commodities, the goat and sheep skins are painted with lime and sulfide on the flesh side. After the treatment, which is known as hair saving process, hair and wool are removed mechanically or manually. Lime sulfide treatment of hides/skins leads to pulping of hair; and as such, pulping leads to environmental problems if the pulping is not screened by using suitable mechanical means and prevented from entering the wastewater stream. Hair constitutes 10-12% on the weight of the animal, which is also dependent on the season of the year; for instance, it is higher in winter [37]. Wool shares 15-20% of the body weight depending on the breed and climatic conditions [38]. The coarse hair and wool are collected, washed well, and used in carpet manufacturing. Hair could also be hydrolyzed to produce fertilizers [39].

1.4.2. Tanned Wastes

i. Wet Blue Trimmings

Excess water from chrome-tanned leather is removed before splitting and shaving. In this process, the hide develops pleats at the edges, which are trimmed out. If not, they may interfere in the forthcoming operations such as splitting and shaving operation. Sometimes, they may damage the hides and skins or sides in the machine operation. These trimmings (estimated at 5.5-6% of wet weight of hides) are collected from the tannery and utilized in the manufacture of leather boards and bricks production, using a low pressure and temperature process to avoid oxidation of Cr^{3+} to Cr^{6+} [25]. The use of chromed trimmings in fertilizer and leather meal applications is known [40]. Chrome trimmings may also be de-chromed and processed into glue and commercial gelatin [25].

ii. Wet Blue Splits

The splits obtained during the splitting operation are very thin in substance and cannot be utilized. As solid waste, chrome splits account for 4-6% of total waste. They are collected and used as raw material in the leather board industry. In most of the chrome split wastes, the chrome content as Cr_2O_3 is found to be 2.0-3.5%. Currently, these wastes are collected by feed manufacturers and are steam treated and converted into a leather meal. In many countries, cattle splits, which are 1-1.5 m^2 in area and 6-10 kg in weight, are re-tanned and dyed to obtain chamois leather and are also used to produce casual and sport shoes [25].

iii. Chrome Shavings

Chrome shavings are obtained as waste material when chrome-tanned leather undergoes the process of shaving operation. Chrome shavings in fibrous shredded form are available as 1-2% on the weight of the raw hide [16]. The chromium present in chrome

shavings is in trivalent state. The current purpose of collection of chrome shavings is mainly for utilizing them as leather meal, which is hydrolyzed in a similar manner to poultry feathers and used as a supplemental protein source for livestock [25].

iv. Crust Leather Trimmings

Generally, crust leathers (leathers obtained after re-tanning, dyeing, and fat-liquoring operations) are trimmed after staking, toggling, and nailing to remove the torn and ragged edges for aesthetic value and also for processing the leather in the subsequent machine without any damage. The crust leather trimmings are also collected and used for making leather boards and production of bricks [25].

v. Buffing Dust

The crust leathers are buffed on the flesh side and sometimes on the grain side. The buffing (done on flesh side) and snuffing operation (done on the grain side to produce specialty leathers such as nubuck) generates fine lightweight leather fluffy mass [25].

vi. Finished Leather Trimmings

After finishing, the leathers are trimmed at the edges uniformly. The purpose of trimming is aesthetic and also for easy measurement of the leather [25].

1.5. Waste Management

Waste management is the "generation, prevention, characterization, monitoring, treatment, handling, reuse and residual disposition of solid wastes". There are various types of solid wastes including municipal (residential, institutional, commercial), agricultural, and special (health care, household hazardous wastes, sewage sludge) [41].The term usually relates to materials produced by human

activity, and the process is generally undertaken to reduce their effect on health, the environment or aesthetics.

Waste management at present, primarily involves land filling, incineration with and without energy recovery, recycling and composting. Legislation, nature of wastes and market trends continue to redefine management operations and its responsibilities and impacts. Complexities are added to it by the nature of urban development as well. New studies and concepts like 3 Rs, cradle-to-cradle, industrial ecology, and integrated waste management are adding new dimensions for solving waste problems towards achieving sustainable resource use [42].

There is a wide array of issues relating to waste management and those areas include:

- Generation of waste
- Waste minimization
- Recycling and reuse
- Storage, collection, transport, and transfer
- Treatment
- Landfill disposal
- Environmental considerations
- Financial and marketing aspects
- Policy and regulations
- Education and training
- Planning and implementation.

1.5.1. Methods of Waste Disposal

i. Landfill

Disposal of waste in a landfill involves burying the waste and this remains a common practice in most countries. Landfills were often established in abandoned or unused quarries, mining voids or

borrow pits. A properly designed and well-managed landfill can be a hygienic and relatively inexpensive method of disposing of waste materials. Older, poorly designed or poorly managed landfills can create a number of adverse environmental impacts such as wind-blown litter, attraction of vermin, and generation of liquid leachate. Another common product of landfills is gas (mostly composed of methane and carbon dioxide), which is produced from anaerobic breakdown of organic waste. This gas can create odour problems, kill surface vegetation and is a greenhouse gas [43].

Design characteristics of a modern landfill include methods to contain leachate such as clay or plastic lining material. Deposited waste is normally compacted to increase its density and stability and covered to prevent attracting vermin (such as mice or rats). Many landfills also have landfill gas extraction systems installed to extract the landfill gas. Gas is pumped out of the landfill using perforated pipes and flared off or burnt in a gas engine to generate electricity [44].

ii. Incineration

Incineration is a disposal method in which solid organic wastes are subjected to combustion so as to convert them into residue and gaseous products. This method is useful for disposal of residue of both solid waste management and solid residue from waste water management. This process reduces the volumes of solid waste to 20 to 30 percent of the original volume. Incineration and other high temperature waste treatment systems are sometimes described as "thermal treatment". Incinerators convert waste materials into heat, gas, steam and ash [45].

Incineration is carried out both on a small scale by individuals and on a large scale by industry. It is used to dispose of solid, liquid and gaseous waste. It is recognized as a practical method of

disposing of certain hazardous waste materials (such as biological medical waste). Incineration is a controversial method of waste disposal, due to issues such as emission of gaseous pollutants [46].

Incineration is common in countries such as Japan where land is more scarce, as these facilities generally do not require as much area as landfills. Waste-to-energy (WtE) or energy-from-waste (EfW) is broad terms for facilities that burn waste in a furnace or boiler to generate heat, steam or electricity. Combustion in an incinerator is not always perfect and there have been concerns about pollutants in gaseous emissions from incinerator stacks. Particular concern has focused on some very persistent organic compounds such as dioxins, furans, and PAHs (Polycyclic Aromatic Hydrocarbons), which may be created and which may have serious environmental consequences [47].

iii. Recycling

Recycling is a resource recovery practice that refers to the collection and reuse of waste materials such as empty beverage containers. The materials from which the items are made can be reprocessed into new products. Material for recycling may be collected separately from general waste using dedicated bins and collection vehicles, a procedure called kerbside collection. In some communities, the owner of the waste is required to separate the materials into various different bins (e.g. for paper, plastics, metals) prior to its collection. In other communities, all recyclable materials are placed in a single bin for collection, and the sorting is handled later at a central facility. The latter method is known as "single-stream recycling [48].

The most common consumer products recycled include aluminium such as beverage cans, copper such as wire, steel from food and aerosol cans, old steel furnishings or equipment, polyethylene and PET bottles, glass bottles and jars, paperboard

cartons, newspapers, magazines and light paper, and corrugated fiberboard boxes [49].

1.5.2. Sustainability

The management of waste is a key component in a business ability to maintaining ISO14001 accreditation. Companies are encouraged to improve their environmental efficiencies each year by eliminating waste through resource recovery practices, which are sustainability-related activities. One way to do this is by shifting away from waste management to resource recovery practices like recycling materials such as glass, food scraps, paper and cardboard, plastic bottles and metal [49].

i. Biological Reprocessing

Recoverable materials that are organic in nature, such as plant material, food scraps, and paper products, can be recovered through composting and digestion processes to decompose the organic matter [50]. The resulting organic material is then recycled as mulch or compost for agricultural or landscaping purposes. In addition, waste gas from the process (such as methane) can be captured and used for generating electricity and heat (CHP/cogeneration) maximizing efficiencies. The intention of biological processing in waste management is to control and accelerate the natural process of decomposition of organic matter [50].

iii. Energy Recovery

Energy recovery from waste is the conversion of non-recyclable waste materials into usable heat, electricity, or fuel through a variety of processes, including combustion, gasification, pyrolyzation, anaerobic digestion, and landfill gas recovery [51] . This process is often called waste-to-energy. Energy recovery from waste is part of the non-hazardous waste management hierarchy. Using energy

recovery to convert non-recyclable waste materials into electricity and heat, generates a renewable energy source and can reduce carbon emissions by offsetting the need for energy from fossil sources as well as reduce methane generation from landfills. Globally, waste-to-energy accounts for 16% of waste management [52].

The energy content of waste products can be harnessed directly by using them as a direct combustion fuel, or indirectly by processing them into another type of fuel. Thermal treatment ranges from using waste as a fuel source for cooking or heating and the use of the gas fuel for boilers to generate steam and electricity in a turbine. Pyrolysis and gasification are two related forms of thermal treatment where waste materials are heated to high temperatures with limited oxygen availability. The process usually occurs in a sealed vessel under high pressure. Pyrolysis of solid waste converts the material into solid, liquid and gas products. The liquid and gas can be burnt to produce energy or refined into other chemical products (chemical refinery). The solid residue (char) can be further refined into products such as activated carbon. Gasification and advanced Plasma arc gasification are used to convert organic materials directly into a synthetic gas (syngas) composed of carbon monoxide and hydrogen. The gas is then burnt to produce electricity and steam. An alternative to pyrolysis is high temperature and pressure supercritical water decomposition (hydrothermal monophasic oxidation) [52].

iii. Resource Recovery

Resource recovery is the systematic diversion of waste, which was intended for disposal, for a specific next use [53]. It is the processing of recyclables to extract or recover materials and resources, or convert to energy. These activities are performed at a resource recovery facility. Resource recovery is not only environmentally

important, but it is also cost effective. It decreases the amount of waste for disposal, saves space in landfills, and conserves natural resources [54].

Resource recovery (as opposed to waste management) uses LCA (life cycle analysis) attempts to offer alternatives to waste management. For mixed MSW (Municipal Solid Waste) a number of broad studies have indicated that administration, source separation and collection followed by reuse and recycling of the non-organic fraction and energy and compost/fertilizer production of the organic material via anaerobic digestion to be the favoured path [55].

iv. Avoidance and Reduction Methods

An important method of waste management is the prevention of waste material being created, also known as waste reduction. Methods of avoidance include reuse of second-hand products, repairing broken items instead of buying new, designing products to be refillable or reusable (such as cotton instead of plastic shopping bags), encouraging consumers to avoid using disposable products (such as disposable cutlery), removing any food/liquid remains from cans and packaging, and designing products that use less material to achieve the same purpose (for example, light weighting of beverage cans) [56-57].

v. Waste Handling and Transport

Waste collection methods vary widely among different countries and regions. Domestic waste collection services are often provided by local government authorities, or by private companies in the industry. Some areas, especially those in less developed countries, do not have a formal waste collection system.

vi. Benefits

Waste is not something that should be discarded or disposed of with no regard for future use. It can be a valuable resource if addressed correctly, through policy and practice [58]. With rational and consistent waste management practices there is an opportunity to reap a range of benefits. Those benefits include:

- Economic: Improving economic efficiency through the means of resource use, treatment and disposal and creating markets for recycles can lead to efficient practices in the production and consumption of products and materials resulting in valuable materials being recovered for reuse and the potential for new jobs and new business opportunities [58].
- Social: By reducing adverse impacts on health by proper waste management practices, the resulting consequences are more appealing settlements. Better social advantages can lead to new sources of employment and potentially lifting communities out of poverty especially in some of the developing poorer countries and cities [58].
- Environmental: Reducing or eliminating has adverse impacts on the environmental through reducing, reusing and recycling, and minimizing resource extraction can provide improved air and water quality and help in the reduction of green house emissions [58].
- Inter-generational Equity: Following effective waste management practices can provide subsequent generations a more robust economy, a fairer and more inclusive society and a cleaner environment [58].

vii. Challenges in Developing Countries

Waste management in cities with developing countries and economies in transition experience exhausted waste collection

services, inadequately managed and uncontrolled dumpsites and the problems are worsening [58]. Problems with governance also complicate the situation. Waste management, in these countries and cities, is an ongoing challenge and many struggles due to weak institutions, chronic under-resourcing and rapid urbanization. All of these challenges along with the lack of understanding of different factors that contribute to the hierarchy of waste management affect the treatment of waste [59].

viii. Technologies

Traditionally the waste management industry has been slow to adopt new technologies such as RFID (Radio Frequency Identification) tags, GPS (Global Positioning System) and integrated software packages which enable better quality data to be collected without the use of estimation or manual data entry [59].

- Technologies like RFID tags are now being used to collect data on presentation rates for curb-side pick-ups.
- Benefits of GPS tracking is particularly evident when considering the efficiency of ad hoc pick-ups (like skip bins or dumpsters) where the collection is done on a consumer request basis.
- Integrated software packages are useful in aggregating this data for use in optimization of operations for waste collection operations.
- Rear vision cameras are commonly used for OHS (Occupational Health & Safety) reasons and video recording devices are becoming more widely used, particularly concerning residential services.

1.5.3. Central Principles of Waste Management

There are a number of concepts about waste management which vary in their usage between countries or regions. Some of the most general, widely used concepts include:

i. Waste Hierarchy

The waste hierarchy refers to the "3 Rs" reduce, reuse and recycle, which classify waste management strategies according to their desirability in terms of waste minimization. The waste hierarchy remains the cornerstone of most waste minimization strategies. The aim of the waste hierarchy is to extract the maximum practical benefits from products and to generate the minimum amount of waste [58].

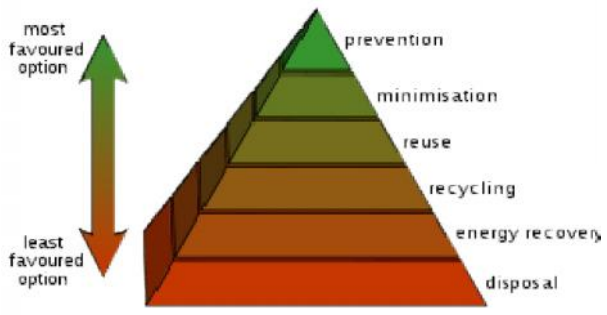


Figure 3: Diagram of the waste hierarchy.

The waste hierarchy is represented as a pyramid because the basic premise is for policy to take action first and prevent the generation of waste. The next step or preferred action is to reduce the generation of waste i.e. by re-use. The next is recycling which would include composting. Following this step is material recovery and waste-to-energy. Energy can be recovered from processes i.e. landfill and combustion, at this level of the hierarchy. The final action is disposal, in landfills or through incineration without energy recovery. This last step is the final resort for waste which has not been prevented, diverted or recovered. The waste hierarchy represents the

progression of a product or material through the sequential stages of the pyramid of waste management. The hierarchy represents the latter parts of the life-cycle for each product [58].

ii. Life-Cycle of a Product

The life-cycle begins with design, then proceeds through manufacture, distribution, use and then follows through the waste hierarchy's stages of reuse, recovery, recycling and disposal. Each of the above stages of the life-cycle offers opportunities for policy intervention, to rethink the need for the product, to redesign to minimize waste potential, to extend its use [58]. The key behind the life-cycle of a product is to optimize the use of the world's limited resources by avoiding the unnecessary generation of waste.

iii. Resource Efficiency

The current, global, economic growth and development cannot be sustained with the current production and consumption patterns. Globally, we are extracting more resources to produce goods than the planet can replenish. Resource efficiency is the reduction of the environmental impact from the production and consumption of these goods, from final raw material extraction to last use and disposal. This process of resource efficiency can address sustainability.

Polluter pays principle: the Polluter Pays Principle is a principle where the polluting party pays for the impact caused to the environment. With respect to waste management, this generally refers to the requirement for a waste generator to pay for appropriate disposal of the unrecoverable material [58].

1.6. Treatments of Tannery Solid Wastes

Research has been done and going on for the treatment and utilisation of tannery wastes throughout the world. In Bangladesh,

very few research had been carried out on the characterization, treatment and utilisation of tannery liquid wastes i.e., effluents but no research so far done on the characterization, treatment and utilisation of tannery solid wastes. Much study has been done for the treatment and utilisation of tannery solid wastes such as glue and gelatin making from raw trimmings, lime fleshing and chrome shavings. Production of energy from tannery solid wastes has been reported earlier [30].

1.6.1. Fleshing

Fleshing wastes from tanneries are characterized by a very high water (up to 870 g kg^{-1}) and protein content (40-60 g kg^{-1} of the dry mass), fat (10-20 g kg^{-1} of the dry mass) and carbohydrates [60-61]. Hydrolysis with alkaline proteinase results in fat (4-12% of the total mass of the initial material), collagen hydrolysate (5-10%), and protein concentrate (1-3% yield). Purification of the collagen hydrolysate fraction into edible gelatin has been carried out [62].

Fleshing is generally used in the manufacture of glue, adhesives, and gelatin [63]. Both de-limed fleshing and residual hair have been found to be important sources of protein with several applications as biological fertilizers in agriculture or horticulture [64]. Chicken feed supplement, plastics, surface-active agents, artificial leather, raw material for fungicides and bactericides, and pure amino acids have been among the proposed uses for fleshing not processed into glue and gelatin. Animal feed supplement has received the most attention, as it offers a potential for large scale utilisation. Accelerated digestion of fleshing using proteolytic bacteria and further processing to biomethanation has been studied [65] Liquefied tannery fleshing and sludge in combination with cow dung has been utilized for biogas production [65]. A heat-stable alkaline protease produced by *Paecilomuces lilacinus*, a fungal isolate, was used for hydrolysis [66]. Enzymatic hydrolysis of protein waste can be enhanced by power

ultrasound, which has been shown to give a better digestion yield of fleshing [67]. Combination of enzyme and ultrasound is a promising clean technology for the handling of un-tanned solid leather waste [68].

Experimental investigation and characterization of biogas and biodiesel production from leather industry fleshing wastes has also been published [07]. The possible use of fleshing fat in the production of biodiesel (fatty acid methyl esters) by trans-esterification for use as a replacement for fossil fuels was attempted [27]. The fat was released from the waste by boiling with water under high-speed grinding, dried, and used without further refining for the production of fatty acid methyl ethers. Thus, a contaminating waste can be transformed into an environmentally friendly fuel, providing economical and ecological profits [25]. Fleshing with a high fat content, fluidized by heating, was treated with sulfuric acid; and the fats were extracted along with biodiesel (biofuel based on fatty-acid methyl esters). The fats were converted by the catalytic trans-esterification process into fatty acid methyl esters, after suitable purifying, as biodiesel or heating biofuels [69-70].

Alkaline hydrolysates of fleshing have been used as fillers and syntans, and good exhaustion and leather properties have been reported [71]. Enzymatically modified leather waste has also been used as fillers in leather production [72]. These polymerized potential filler products were characterized for their physical properties and molecular weight distribution (degree of polymerization). These products were applied to wet blue and evaluated again using florescent labels to monitor their filling capability. It was shown that the proteins were evenly distributed throughout the hide and, more importantly, were not removed during the washing steps [73]. The blends composed of whey protein isolate, a by-product of the cheese industry; and small amounts of gelatin, a by-product of the leather

industry, could be effectively used as filling agents for both shoe upper and upholstery leather [74]

1.6.2. Trimmings

Un-tanned trimmings can be a good source for production of collagen [75]. Extraction of high-molecular-weight protein and enzymatic hydrolysis of the residue from pig and fish skin has been reported. The extract had been found to have properties for use as a cosmetic material due to its high water retention capacity, ability to repair rough skin, lack of any odour problem, and absence of harmful effects on skin [76].

Tanned trimmings have been used as polymer-waste composite materials for manufacturing soles. These composite materials contain 65%–75% of leather trimmings and 25%–35% of binder containing poly vinyl chloride (PVC). Leather scraps, metals, and thermosetting resins were mixed and molded by compression-polymerization, which resulted in composites for brake pads. Chromium-free collagen hydrolysate with properties useful as foliar growth enhancers and biostimulators for fruit and vegetable crops was obtained by using chemical and chemical-enzymatic hydrolysis [77]. The insulating properties of fibers were exploited for the preparation of collagen-polyisocyanate composite resistant to fire and collagen-synthetic rubber composite usable as phonic insulators. Various polymeric materials have been blended with leather wastes to prepare composites of different functional properties [78].

1.6.3. Chrome Shavings

Acidic and alkaline hydrolysis is widely used for treatment of chrome shavings, which results in chrome recovery and isolation of protein fractions. Mineral acids like HCl and H_2SO_4 are used in acidic hydrolysis while strong alkali like NaOH and KOH are used in alkaline hydrolysis. Several processes have been developed where

enzymes such as proteous alkalase have been applied to extract hydrolyzed and gelable protein products [79-89]. Organic volatile bases have also been used for dechromation of chrome shavings, which had resulted in increased yield of soluble protein with relatively low ash content [90]. The recovered chromium salts could be used to tan hides, as pigment for glass making, for manufacture of heat-resistant bricks [91]. The influence of surfactants on the gelatin isolated from chrome shavings was studied to avoid foam formation [88]. Biodegradation of chrome shavings using Aspergillus Carbonarius isolate in solid-state fermentation has been studied [92]. Approximately 97% liquefaction of the tannery waste was achieved, and the liquid obtained from long-term experiments was used to recover chromium. The resulting alkaline chromium sulfate solution was found to be useful in tanning procedures [25].

Alkaline process for recovery of proteins in the aqueous phase of treated tanning wastes and the metallic salts in the cake has been reported [93]. The recovered chrome has been used for synthesis of a pigment for paint industry and also for preparation of basic chromium sulfate (BCS) [94-96]. A re-tanning agent was synthesized from chrome shavings hydrolysate without dechroming and modified with vinyl monomers [97]. It has been shown that good stretch and filling properties can be obtained from leather re-tanned with this agent. Production of leather-like composites using chemically modified short leather fibers has been made using emulsion polymerization [98]. The grafting of acrylate monomers onto collagenous or soluble collagenous substrates, leather, and hide powder has been reported earlier [99-100].

The use of chrome shavings in the manufacture of leather boards, insulators, building materials, fibrous sheets and shoe soles has been established [101-103]. However, presently the market for these materials has been replaced by synthetics. Hence, there is no demand for leather boards and shoe soles made from leather waste

[104]. The separation of chromium and valuable protein from chrome shavings through enzymatic alkali process has also been reported [105-108]. **However, the extraction of protein has not been achieved completely [104]**. It has also been shown that chrome shavings can be used as a reductant in the manufacture of basic chromium sulfate (BCS) [109]. New parchment like material from chrome shavings has been developed and found useful in the manufacture of home furnishing products [110]. Chrome shavings can be used for the preparation of pigment through controlled incineration [16]. It has been reported that chrome shaving dusts can be used as adsorbent for the removal of toxic materials (heavy metals) in waste water [111].

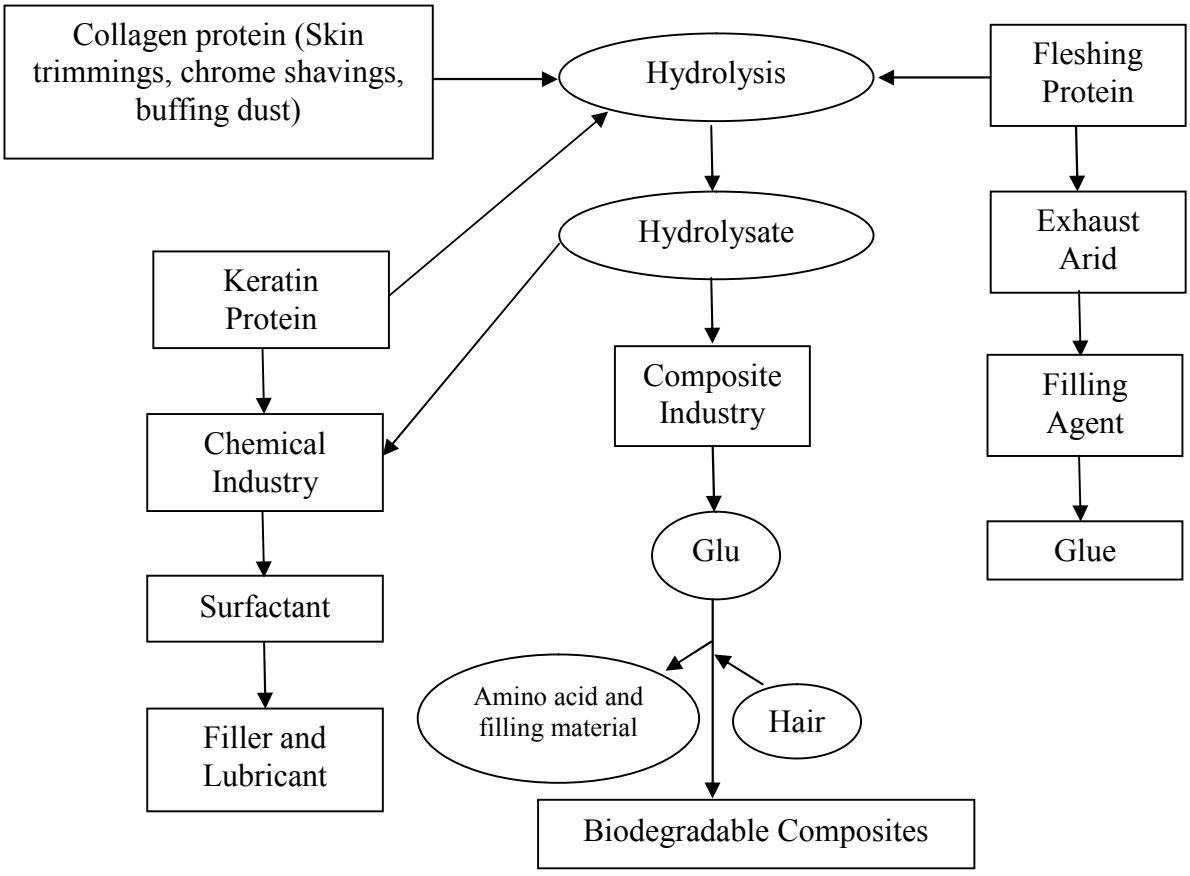


Figure 4: Treatment of tannery solid wastes.

1.7. Socio-Economic Importance

It has been reported that only about 20% of the large number of chemicals used in the tanning process is absorbed by leather, the rest is released as waste [112]. Hazaribagh which is the largest tannery region in Bangladesh consists of more than 200 tanneries generate 7.7 million liters of liquid waste and 88 million tons of solid wastes every day. These wastes are directly dumped into an open air and long term exposure of these wastes creates severe environmental pollution in the entire Hazaribagh and its adjacent area. Among the solid wastes, chromium containing leather wastes (CCLW) are found abundantly and staked outside the tannery.

Chromium is a naturally occurring heavy metal that can exist in air, water, soil, and food, and common exposure pathways include ingestion, inhalation, and dermal contact. The primary health impacts from chromium are damage to the gastrointestinal, respiratory, and immunological systems, as well as reproductive and developmental problems. Chromium is a well known human carcinogen [15]. In addition, the chromium-laced solid wastes from tanneries are often converted into poultry feed as is the case in areas of Bangladesh and can thus impact livestock and humans [15]. According to the WHO, over 8,000 workers in the tanneries of Hazaribagh suffer from gastrointestinal, dermatological, and other diseases, and 90% of this population dies before the age of 50 [113].

It has been estimated that nearly 100 kg of shavings are produced during the processing of one tone of raw hides and skins and annual generation of shaving is 85,000 tonnes in Bangladesh. Most of the chrome shaving dusts are usually dumped into the adjacent area of tannery in Hazaribagh and burned in open air [14]. Many small businessmen collect this chrome shaving dusts to produce protein concentrate without any pretreatment. This protein concentrate is used for manufacturing of poultry feed. In this process

some other materials like ground biscuit, fish oil and ground corns are mixed with this protein based materials. Then this is sent to the other feed industry located at Chittagong road and different part of the countries. The matter is that, in this manufacturing process of poultry feed, the chrome shaving dust is not subjected to any pretreatment and the high content of chromium is not removed. Much quantity of sulfuric acid is mixed with chrome shaving dust and boiled which leads to evolve acid fume and chrome VI may be formed at high temperature. Oxidation of Cr (III) into Cr (VI) normally occurs in presence of strong oxidation agent in acid environment [114]. Consequently the working environment is much polluted with noxious odour and the protein product contains high levels of chromium including hexavalent form, sand, mud and dust. The maximum chromium content of chrome shaving dust was found to be 3.2037 % [115]. Data analysis showed that boiling and drying treatments brought no significant change in chromium levels in protein concentrate samples. Protein concentrate sampled from a feed mill at Hazaribagh produced chromium concentration of 2.4901% as element [115].

Apart from chromium, solid wastes generated by the tannery industry contain appreciable amounts of other toxic metals such as Cd, As, Pb, which are converted to protein concentrate and used as poultry feed. Chickens consume contaminated feeds and the heavy metal contaminants are passed from these chickens to the body tissues of boilers and humans. Food chain contamination is the major pathway of heavy metal exposure for humans [116]. Chromium and other heavy metals come to the human body from poultry meat through food chain. Therefore, the current method of producing protein product for poultry feed at Hazaribagh is totally unscientific and hazardous. This product is dangerous for human health because research reveals that High content of chromium has been found to different organs like meat, lever, bones etc. of poultry

chicken after feeding this product. Research revealed that higher amounts of chromium in eggs and poultry meat than the tolerable limit have been found [115].

Due to cheap price, the broiler and fish grower are using the product ignoring the concern of public health issue. High price of the protein concentrates compelled the scrupulous trader to make money from it. Since the cost of feed ingredients is increasing at an alarming rate the growth of the poultry industries of the country will also be hindered eventually due to short supply of feed ingredients. Current practice of manufacturing poultry feed using chrome containing shaving dust is not only dangerous to the health of manufacturers but also awful to the human as the potential carcinogenic chromium is penetrating steadily from the chickens and fishes into the human body through the food chain [116]. Recently there is a stay order from the High Court on processing of chrome shavings for producing poultry feed and fish meal.

Chrome shaving dusts are one of the major solid wastes generated during the leather making process. The presence of chromium in this waste creates difficulty in disposing to landfill and incineration [117]. The cost effectiveness of solid wastes treatment and management in any industrial sector has for long remained the most important issue [118]. The approach of this study involves the pollution mitigation of tannery solid wastes i.e., chrome shaving dusts through cost effective treatment, technological up-gradation of wastes (chrome shaving dusts) treatment systems and new avenues for effective utilisation of chrome shavings to create wealth from waste.

Moreover substantial work has been done to study the tannery effluents. But the other way, which is probably not yet studied, is the entering of harmful chemicals into the food chain through the use of solid wastes as feed staff. This is a recent phenomenon happening at

large extent for the last several years [15]. Hence, this study is of utmost importance for the treatment and recovery of value added protein product from chrome shaving dusts for both Bangladesh and world perspective of tannery industries. Therefore, this research work deserves a scientific investigation in the context of public health, environment and concern industries.

Figure 5: Poultry feed production in Hazaribagh using chrome shaving dust.

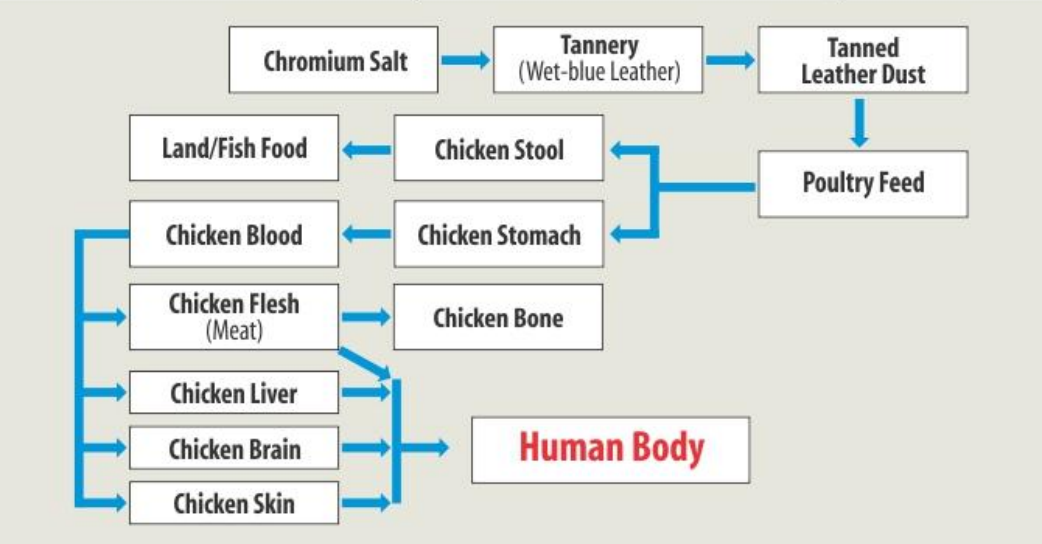


Figure 6: Transport mechanism of chromium from tannery waste to human body [116].

1.8. Aim and Objectives

The tannery solid wastes are simply thrown away without any pretreatment in Bangladesh with the exception is that some local businessmen produce protein concentrate from chrome shaving dusts in an unhygienic and unscientific way. From the literature review, it has been revealed that the process of producing protein concentrate using chrome shavings is a threat for the local people and environment and the product is not safe for poultry feed since it contains high chromium and other toxic chemicals. Even though, the acidic and alkaline hydrolysis of chrome shavings has been reported but there is a problem to purify the hydrolyzed protein products. The biochemical treatment of tannery solid wastes is a new approach to create "resource from waste". Therefore, the aim of this research work is to process tannery solid waste, namely chrome shaving dust generated by the leather industry and to transform them into valuable protein product i.e., protein or collagen hydrolysate that could be used as a protein supplement for the poultry feed industries

in Bangladesh. The other objectives of the research work can be summarized as follows:

- To explore the characteristic features, eco-friendly treatment and utilisation of discarded solid waste, namely chrome shaving dusts of leather industries in Bangladesh.

- To find, optimize and establish an effective and efficient bio-chemical method for the extraction of Protein or Collagen Hydrolysate from Chrome shaving dust.

- To analyze the prepared Collagen Hydrolysate for quality assessment i.e., to estimate the amount of protein content and available amino acids, level of remaining chromium, ash content; checking the presence of impurities, and other associated elements present in the extracted material; and finally,

- To separate and remove chromium from the final chrome containing residue after treatment and estimate the efficiency of chrome recovery and the ash content of chrome free residue.

1.9. Expected Outcome

Growing environmental concern about the toxicity and environmental impact of the chromium solid waste generated from the tannery has become key issue. In this study, collagen hydrolysate will be extracted from chrome shaving dusts through biochemical method. In this biochemical method, combination of chemical and enzymatic process will be employed to achieve the optimum extraction of protein. If the developed biochemical method could be implemented, the following outcomes would be visible:

- The slow and steady penetration of toxic heavy metals especially chromium including carcinogenic hexavalent

chromium in the human body through food chain by taking poultry eggs and meats could be stopped.

- The revealed result will help to introduce a new area of resourcing protein products i.e., collagen hydrolysate from a discarded tannery solid waste; namely chrome shaving dusts.

- Safe, scientific and eco-friendly manufacturing technique of protein hydrolysate from chrome containing toxic waste material will be available to the concern manufacturer.

- Curbing of feed price escalation would be possible; and improved quality of poultry feed without any impurities and toxic chemicals can be produced.

- The recovered basic chrome sulfate can be used in tanning and re-chroming operations of leather processing.

- Solid waste management at Hazaribagh tannery area in Bangladesh will find a new dimension.

1.10. Outline of this Thesis Paper

This thesis paper consists of five chapters namely chapter 1: Introduction and Literature Review, chapter 2: Leather Processing and Collagen Hydrolysate, chapter 3: Materials and Method, chapter 4: Results and Discussion and chapter 5: Summary and Conclusion.

- Chapter 1 describes the introduction of leather industries pollution, literature reviews of related works regarding the thesis topic, solid waste management, categories of tannery solid wastes, conventional poultry feed making in Hazaribagh, socioeconomic importance, aim and objectives, expected outcomes of the research work and structure of this thesis paper.

- Chapter 2 gives the description of leather making process, chemistry of hides & skins, pollution in tannery, environmental impacts of tannery wastes, utilisation of chrome shaving dust, structure of chrome shaving dust, collagen hudrolysate, mechanism of hydrolysis, characteristics of collagen hydrolysate, application of collagen hydrolysate, poultry feed and protein concentrate making in Hazaribagh.

- Chapter 3 briefs about the methodology of the research work. It includes materials & chemicals used, apparatus and instruments involved in the research work, chemical & biochemical methods of hydrolysis, recovery of chromium from chrome cake and process optimization.

- Chapter 4 describes the various analytical results of chrome shaving dust and prepared collagen hydrolysates. It depicts physical appearance of prepared collagen hydrolysate, solid content, protein content, pH, chromium, calcium and magnesium content, ash content and FTIR analysis of prepared collagen hydrolysate. This chapter also includes the optimization of pH, alkalis and enzymes concentrations and their amalgamation.

- Chapter 5 demonstrates the summary and findings of the research work. It also narrates the limitations and recommendations of this research work and finally concludes about the research work.

Chapter 2: Leather Processing & Collagen Hydrolysate

2.1. Leather Making Process

Animal hides and skins that have been processed to retain its flexibility, toughness, and waterproof nature are known as leather. The processes of leather industry for transforming raw hides and skins into leather can be divided into the following four main stages [03]:

i. Beam House Processes

The hides and skins in tannery are first subjected to a trimming process for removing the unwanted parts like tails, offal, ear etc. and raw trimmings are evolved as solid wastes in this stage. They are then subjected in soaking operation to rehydrate water and to remove substances like dirt, blood and conservation salt. After that the wetted hides and skins are fleshed to remove the excess flesh and fat adhering to the hide (hypodermis). Green fleshing is generated as solid wastes in this stage. In the liming operation they are treated with an intense alkali solution of lime [$Ca(OH)_2$] and sodium sulfide (Na_2S) to ensure hair and wool removal (un-hairing process). Later the hides/skins are swelled up in liming process by immersing them in a strong alkaline bath so as to open up the collagen structure. The hides/skins may be treated with a second fleshing process after liming in order to clean the flesh and lime fleshing is produced as solid wastes. At this stage, hides are treated with splitting process and split into two or three layers. The lime split is a solid waste in this stage. De-liming is then performed to decrease the pH level in order to remove the lime and to make hides/skins more receptive to the chemicals that will be used in further stages. Through bating process, hides/skins are exposed to an enzymatic effect for both opening up the structures, and the removal of unwanted proteins from the hide. Following the bating process, a degreasing process is applied to hides/skins for

removing the excess natural fat in their structure and providing a homogeneous distribution of fat in it [03].

ii. Tanning Processes

Tanning refers to the process by which collagen fibers in a hide/skin react with a chemical agent (tannin, alum or other chemicals). However, the term leather tanning also commonly refers to the entire leather-making process. Hides and skins have the ability to absorb tannic acid and other chemical substances that prevent them from decaying, make them resistant to wetting, and keep them supple and durable [119].

The hides and skins at this stage are first treated with pickle process in a solution composed of salt and acids so as to obtain a homogeneous distribution of tanning materials through the cross section of pelt. After the hides and skins are conditioned as above, the tanning process is applied with various tanning materials (materials able to form stable bonds with collagen) in order to provide the leather with a stable form and high thermal stability. Tanning materials such as vegetable tannins, mineral tanning materials and syntans (synthetic organic tanning materials) are used in tannage. Among mineral tanning materials, chrome is the most widely used in leather production due to its unique features that it gives to the leather. Aluminium and vegetable tanning materials are also widely used in leather production. Before the leathers are treated with further processes, the setting out and sammying process is applied, and shaving is done to obtain the desired thickness of the leather. Shaving dusts are generated as solid waste in shaving operation. Chrome shavings are produced during the shaving operation of chrome tanned (wet blue) leather.

iii. Post Tanning Processes

The next step for the leathers, which are tanned and standardized to a desired thickness, is re-tanning process with various re-tanning agents improving the required characteristics of leather. In this process, structural differences within leathers are compensated to obtain uniform structure. The fat-liquoring process is applied by using a combination of various fat-liquoring agents in order to allow the leather to be more supple and softer. In the dyeing process leathers are dyed to the desired color. After this stage, leathers are hanged and dried, and they are prepared for the finishing process through certain mechanical operations. The leather produced at this stage is called crust leather and the unwanted parts are trimmed and removed. Crust leather trimmings are evolved in this stage [119].

iv. Finishing Processes

After the leathers are fat-liquored and dyed following the tanning process, they are processed with a series of coatings on the surface in order to improve their resistance and produce appealing and uniform surface effects. After this process, leathers are trimmed for a final form and sent to confection.

In Bangladesh leather is made by processing the hides and skins of animals slaughtered in slaughter house, and hence the majority of hides and skins tanned are those of cattle, goats and sheep. Animal hides and skins are converted to leather in a nine step process as follows [120]:

Step 1 - Soaking

The hides & skins are dipped into water with liquid detergent to rehydrate, clean up and soften them.

Step 2 – Un-haring & **Liming**

The animal hides/skins are steeped in an alkali solution that breaks down the structure of the hair at its weakest point (the root) and so removes the hair. The hairless skin is immersed in a solution of alkali and sulfide to complete the removal of the hair and to alter the properties of the skin protein (collagen). The collagen becomes chemically modified and swells, leaving a more open structure.

Step 3 – De-liming

The limed pelt (after liming, the hides/skins are called pelt) after fleshing and washing with water is subjected to treat with chemicals to remove unbound lime from the pelt and to make it soft.

Step 4 – Bating

The hides/skins structure is then opened further by treatment with enzymes, and further unwanted materials are removed.

Step 5 – Pickling

The hides/skins are then treated with acid to make the condition for tanning and to preserve them for up to two years.

Step 6 – Tanning

This is the most chemically complex step. During tanning, the hides/skin structure is stabilized in its open form by replacing some of the collagen with complex ions of chromium. Depending on the compounds used the colour and texture of the leather changes. When leather has been tanned it is able to 'breathe' and to withstand 100 ºC boiling water, as being much more flexible than an untreated dead skin.

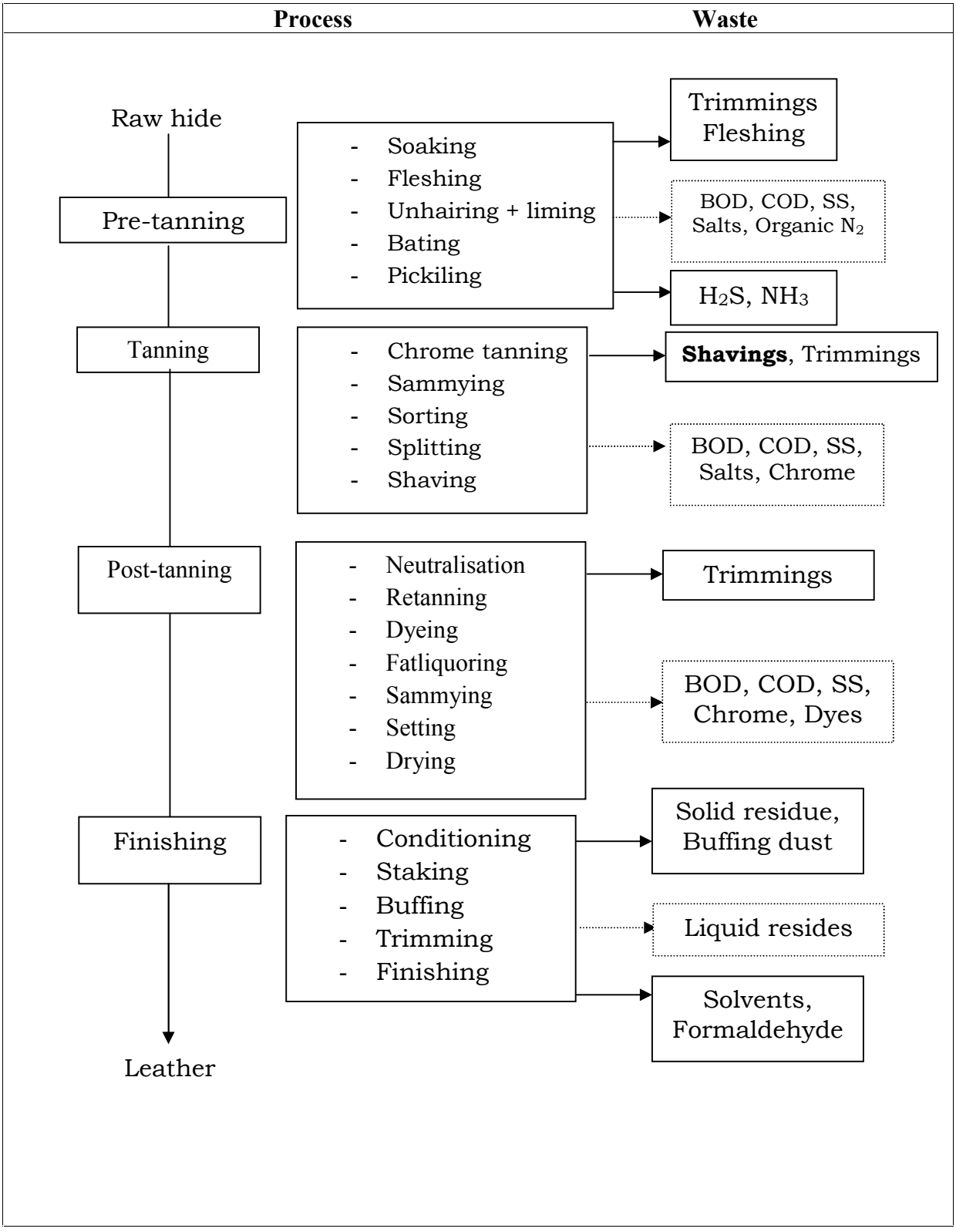


Figure 7: General flow diagram of Leather Making Process and waste generation.

Step 7 **– Neutralizing, Dyeing & Fat-liquoring**

The leather is then treated with alkali to neutralize it and so prevent deterioration, and then dyed. This involves fixing a variety of compounds onto the chromium, as that is the most reactive site present. Once the leather is dyed, it is treated with reactive oils that attach themselves to the fibrous structure, improving suppleness and flexibility.

Step 8 – Drying

Water is removed from the leather, and its chemical properties stabilized.

Step 9 – Finishing

A surface coating is applied to ensure an even colour and texture, and to improve its ability to wear. Suede leather is also buffed at this point to give it its distinctive finish.

2.2. Chemistry of Hides & Skins

When an animal is alive, its skin is soft, flexible, very tough and hard wearing, it has the ability to allow water vapour to pass out, but it will not allow water in. When the skin dies it loses these characteristics, if it is kept wet it rots, and if it is dried it goes hard and brittle. The process of tanning is to retain the skin's natural properties, to stabilize its structure and at the same time to chemically process it so it will no longer be subject to purification. Thus leather is animal skin or hide that has been treated such that its natural properties are retained [120]

Skin is made up of many bundles of interwoven protein fibres which are able to move in relation to one another when the skin is alive. When the skin dies, these fibres tend to shrivel and stick together. Essentially, the purpose of tanning is to permanently fix

the fibres apart by chemical treatment, and to lubricate them so they can move in relation to one another. Well tanned leather, therefore, retains the properties of flexibility, toughness and wear. It also continues to 'breathe', allowing water vapour to pass through but remaining reasonably waterproof. It is this characteristic which accounts for the comfort of genuine leather shoes and clothing. In addition, the process of tanning imparts the advantage of resistance to heat. This is an important 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 [120].

The basic component of the skin is collagen, a fibrous protein. The latest research indicates that the basic collagen structure consists of twined triple units of peptide chains of differing lengths. The amino acid residues are joined together by peptide links. The peptide chains within the triple helices are held together by hydrogen bonding [120].

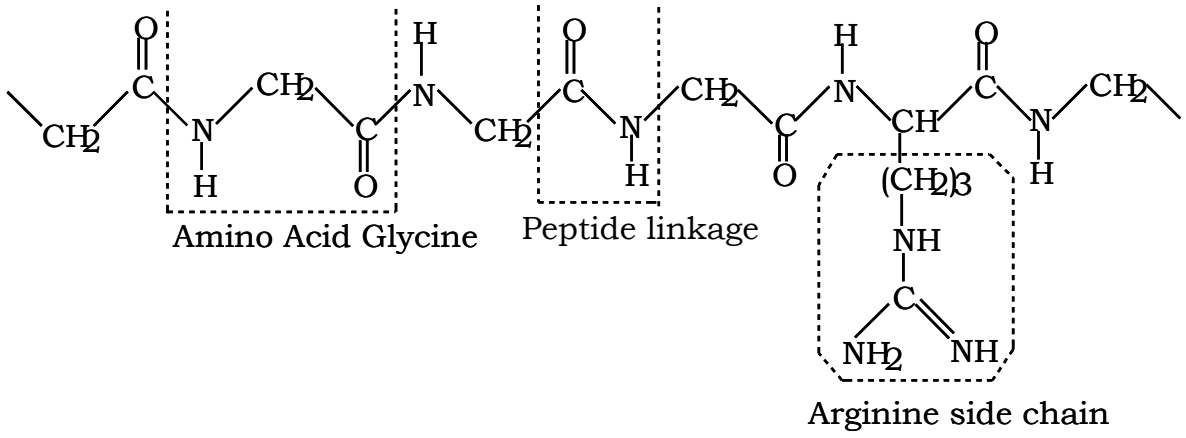
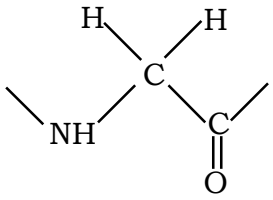
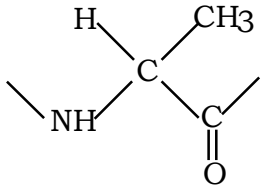
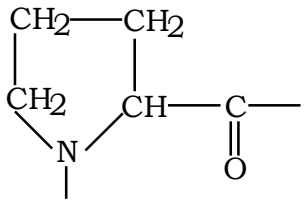
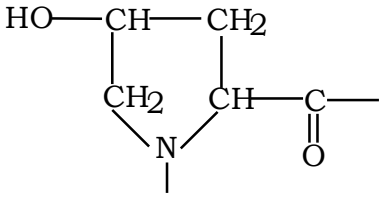
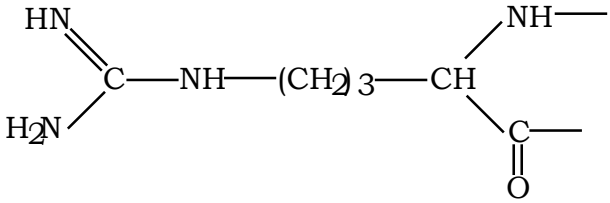


Figure 8: Fundamental structure of peptide [120].

The main amino acid contents of hides & skins are depicted in the table 3.

Table 3: Main Amino Acid content of hides and skins

Amino acid	Structure	Abundance
Glycine		26.8% of total Nitrogen
Alanine		8.0%
Proline		9.0%
Hydroxy proline		8.0%
Arganine		15.3%

2.3. Pollution in Tannery

Since leather processing is broadly divided into pre-tanning, tanning and post tanning operations, hence the pollutant generated from tannery is described under these three sections:

i. Pre-tanning Operations: These are the preparatory operations of raw hides and skins for tanning. They are also called beam house operations and are the main source of pollution in tannery. Operations involved in pre-tanning operations are curing, soaking, liming, de-liming, bating and pickling. The soaking and liming operations are responsible for the major part of Bio-chemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) load from a tannery. H_2S, NH_3 gases, lime sludge are generated from de-liming operation [50].

ii. Tanning Operation: The process of making raw hides and skins into leather is known as tanning operation. There is a number of tanning process that can be alone or in combination with each other. Chrome tanning is the most popular tanning generates unused chrome liquor. Vegetable Tanning is used for sole and some special leather and phenolic compounds are discharged from this tanning process. Synthetic tanning, aluminium, titanium, zirconium, formaldehyde, glutaraldehyde, oil tanning are also applied to some extent. They also produce toxic tan liquor which is discharged from the tannery [50].

iii. Post Tanning Operation: This is further processing of tanned leather for giving special properties, colour, and finishing effect. Re-tanning, dyeing and fat-liquoring are done for giving the leather versatile properties. Combined tan liquor, dye and fat liquor are generated as effluent. The

air emission of solvents in finishing is the major environmental problem. Shaving dust, buffing dust, crust and finished leather trimmings are generated as solid wastes.

Solid wastes generated by the leather industry in these stages of processes may be classified as follows [03]:

i. wastes from un-tanned hides/skins (trimmings, fleshing wastes)
ii. wastes from tanned leather (shaving wastes, buffing dust)
iii. wastes from dyed and finished leather (trimmings from leather)

Data obtained from research reveals that 80% of solid wastes are generated during pre-tanning processes, while 20% of the wastes are caused by post-tanning processes [121]. The wastes that are generated from this industry enable it to fall in the "red" category. Red means that the particular industry is in the category where the damage it causes to the environment is considered as high and not allowed to operate in the capital [25]. With the present pollution control and treatment technologies, it is possible to reduce a considerable amount of liquid waste that is generated without causing serious damage to the environment. However, the major problem of the industry presently facing is disposal of the solid wastes that are generated by the various operations right from tanning to finished products [122]. A part of these wastes such as trimmings, splits, and shavings are used in the manufacture of leather boards, whereas a major part of the wastes are dumped. The unavailability of dumping sites is now increasing the pressure on the tanning industries to come up with new ways to address these wastes [25].

Due to the bad smell they produce during their putrefaction and their harmful chemical content, un-tanned hide/skin wastes have

negative effects on the soil and/or water resources of the environment where they are discharged, in other words on the local plant flora and animal fauna. Therefore, uncontrolled discharge of such wastes should be prevented without taking adequate precautions. Legal arrangements gradually gaining speed all over the world, enforce the leather industry to apply innovations in terms of reusing solid wastes generated during leather production processes such as fleshing, shaving, trimming and splits [03].

The most significant approach in preventing environmental pollution is the idea that prevention is better than reuse, reuse is better than recycling, and recycling is better than disposing of the wastes [123], in other words, cleaner production. On this account, in order to provide cleaner production, the producers are supposed to prevent or reduce waste formation by using clean technology during production processes, and transform the inevitable small amounts of waste into environmentally friendly materials [25].

2.4. Environmental Impact of Tannery Wastes

The major public concern over tanneries is about odours, water and air pollutions from untreated discharges. The pollution situation in Hazaribagh and surrounding area is still serious. In addition to this pollution, occupational health and safety issues (OHS) and chemical poisoning in tannery are also crucial issues [05].

i. Effect on Surface Water

Untreated wastes in surface water give rise to noxious odours from the decomposition of organic matter. Their decomposition depletes the dissolved oxygen (DO) in water that is vital for aquatic life. The water becomes saline and hard. Aquatic plant growth is stimulated. Pathogenic micro-organisms such as B. Anthraxes may also occur in water. The primary link in the food chain is affected due to turbidity and colour [05].

ii. Effect on Buriganga

The water of Buriganga posed a serious threat to public life and is completely unfit for human use. Tannery waste exerts one tenth of total BOD load of Buriganga. The natural cleansing power of the river has almost been lost. The water of the river has become so polluted that its aquatic life has almost been extinguished. In fact, the river has become a dumping ground of all kinds of solid, liquid and chemical waste. During the lean season, the Buriganga River turns deadly for fish and other sub aquatic organisms. When solid waste and effluents run into the river, BOD in the water rises, creating oxygen is calamitous for the sub aqueous life. According to DoE and ammong others, effluents of tannery factories lower the DO content of the river water below the critical level of four milligrams per litre [05]. The DO in the river water was found to be nil during the dry season and no fish or other aquatic animals were found living, up to 500 m downstream of the sluice gate [124].

iii. Effect on Aquatic Plants

Various types of aquatic plants, particularly macrophytes, are present in the Buriganga River. The macrophytes absorb pollutants from the river water and store them in their cells. When biota further up the food-chain, such as humans, animals and fish, eat the macrophytes the biota is also affected by some of these undesirable substances. Macrophytes growing in the Buriganga are used as a source of animal fodder as well. So, the pollution that is occurring in the Buriganga River water is slowly entering the food chain and is likely to cause degradation or disruption of the natural ecosystem [125].

iv. Effect on Land

Of the heavy metals, tanneries have been found to discharge not only Cr but also significant amounts of Zn, Mn, Cu and Pb. High levels of

Cr (29402 mg L^{-1}) with Zn, Cu and Pb have been observed at the main waste disposal point, exceeding the toxic level range in soils [125]. The extractable fractions of heavy metals give some indication of their phytoavailability and mobility in soils of that area [125]. Soil structure is damaged due to severe toxic chemicals and accelerates erosion. Soil fertility has been lost which leads to loss agricultural production capacity. The sub-surface water is contaminated due to high salt content and toxic components. Continual discharge of tannery effluent, high in TDS and chloride, is reported to have affected soil fertility making the soil unfit for agriculture [126].Plant growth has been ceased and fruits and vegetable contain toxic heavy metals. Slow release of H_2S and high salt content in soil may erode building materials [13].

v. Effect on Ground Water

Ground water contamination occurs when waste water and chemicals seep through the soil from dumps and spills. Groundwater may take a long time to cleanse itself as it moves slowly and is out of contact with air. High level of N_2 in water is also deleterious to health. The vegetable, fruits are contaminated with toxic heavy metals like chromium, titanium, lead etc. due to leaching. The green coconut water also contains high level of chromium [127]. Continual discharge of tannery effluent, high in TDS and chloride, is reported to have contaminated the ground water, making the water unsuitable for drinking or other domestic purposes [125].

vi. Waste Dumps

Waste dumps are frequently highly noxious owing to odorous wastes. Re-use of containers may result in poisoning. Ground water contamination occurs from industrial waste dumps.

vii. Effect on Sewers and Sewage Treatment Works

The raw unsettled waste water can cause encrustation of calcium carbonate in sewers. Excessive sulfide content may accelerate corrosion and deterioration of concrete or cement. High sulfate levels are also deleterious to concrete. Very high pollution loads with toxic substance such as chromium can interfere with the biological processes of sewage treatment plant [06].

viii. Effect on Air Quality

Biological decomposition of organic materials, as well as sulfide emissions from waste water, is responsible for characteristics objectionable odours. Ammonia emissions occur from un-haired and de-limed liquor. The air is contaminated by H_2S, SO_2, during some tannery operations. Significant solvent emissions occur during finishing operations [06].

ix. Effect on Human Health

Direct contact with chemicals can cause disability, illness and death. Even relatively minor exposures, if they occur frequently, can eventually build up to toxic level. Leather dust has been listed as a potential carcinogen by EC [04]. Chemical accidents and spills can also be sources of harmful human and environmental exposure. Long term contamination of effluents cause various diseases like skin irritation, skin lesion , hazma, bronchitis, gangrion, and even cancer for the tannery worker as well as local dwellers. About half a million residents of Dhaka are at risk of serious illness due to chemical pollution from tanneries near their homes [04].

x. Other Effects

Gold ornaments at Hazaribagh get quick discolored. Home appliances and electronic items get easily damaged. Chromium in hexavalent from is very dangerous for human being. Hexavelent

chromium is carcinogenic [15]. It can cause allergic skin irritations, dermatitis, irritation to mucous membrane, gastro-intestinal ulcers, conjunctiva, bronchitis, lung cancer, Liver and kidney necrosis, nephritis. The excessive noise causes the loss of audibility of the workers. Air emissions from open burning of tannery wastes can cause cancer. Air emissions from other tannery operations make the tanning industry a bad neighbor [05].

2.5. Utilisation of Chrome Shaving Dust

The chrome shaving dust one of the major solid waste generated during the leather making process. The presence of chromium in waste creates difficulty in disposing to landfill and incineration [104].The chrome shaving dust can be treated in two ways [11]:

1. Disposal to landfill, with compliance to good practices

2. Up-gradation, by viewing the waste as potential raw material serving either existing or new markets.

With consideration to the wide spread, practice of land fill deposit for these wastes, the upgrade option requires a significant change in approach. Instead of "throwing" proteins "away" their reuse in agriculture provides one option, but they can also be seen as material for other industrial products [11]. Within this context, the processing of chrome containing leather wastes (CCLW) such as chrome shavings, unusable splits and trimmings by alkaline-enzymatic hydrolysis leads to a collagen hydrolysate and a chrome cake of impure chromium hydroxide. These products have potential in diverse industrial sectors. In recent years, collagen is widely used in various fields, including foods, cosmetics, medical material and cell culture technology [35]. As a kind of protein, collagen has a specific amino acid sequence, size and structure. Collagen is generally produced for industrial use from animal pelts. Tanning

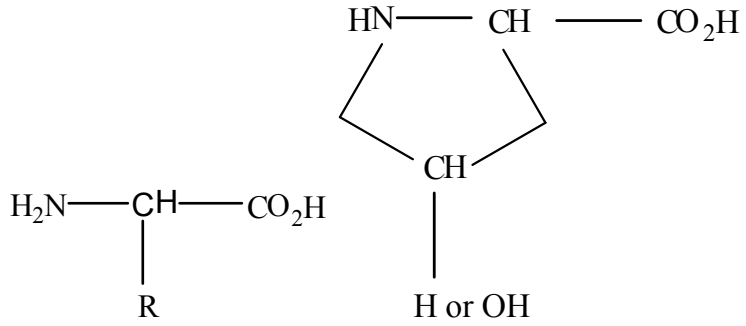
salts can be obtained from these cakes through acidification. These may be recycled to the tanning process [11].

Chromium has two important oxidation states: trivalent (CrIII) and hexavalent (CrIV). Trivalent chromium is of low toxicity and is an essential trace ion necessary for several biological activities, whereas hexavalent chromium is of high toxicity [15,17].Tannery wastes normally contain only trivalent chromium. The production of chromium-containing solid wastes (including chrome shavings) in a tannery has been recognized as a problem for many years but recently pressure from environmental authorities has given the problem increasing urgency. As a result, many scientific groups have oriented their research to find a process to recycle these wastes. The chemical composition of chrome shavings makes them suitable for processing to recover their constituents but the economics of the process is very important for industrial implementation [17]. On the other hand, about 90% of hides/skins in the world are still tanned with chrome, because other tanning materials fail to give the leather a high hydrothermal stability and other use properties that chrome provides [17,127].

2.6. Structure of Chrome Shaving Dust

Chrome shaving dust is evolved from the chrome tanned wet blue leather during the mechanical operation which is called shaving. Chrome shavings mainly consist of collagen and Cr (III) complexes, which could be treated to give the potential resources of collagen protein and chromium [128]. Hence the properties and chemical structure of chrome shaving dust is as like as wet blue leather. Leather is a portentous substance mainly collagenous protein. Collagen is a generic name for a family of at least 28 distinct collagen types, each serving different function in animals, importantly as connective tissue [129]. The major component of leather is type I collagen. Collagen is protein i.e., it is made up of amino acids. They

can be separated into α-amino acids and β-amino acids. Each one features a terminal amino group and a terminal carboxyl group, which become involved in the peptide link, and a side chain attached to the methylene group in the center of the molecule. When the amino acids are linked together to form proteins, they create an axis or "backbone" to the polymer, from which the side chains extended [129]. The total amino acids forming proteins is twenty one. The simplest amino acid is glycine [130].



α-amino acid β-amino acid

Figure 9: Amino Acid Structure [129].

Amino acids create macromolecules, proteins such as collagen, by reacting via a condensation process: the amide of peptide link has been shown in bold [129].

H_2N-CHR-CO_2H + H_2N-CHR-CO_2H ↔ H_2N-CHR-**CO-HN**- CHR-CO_2H + H_2O

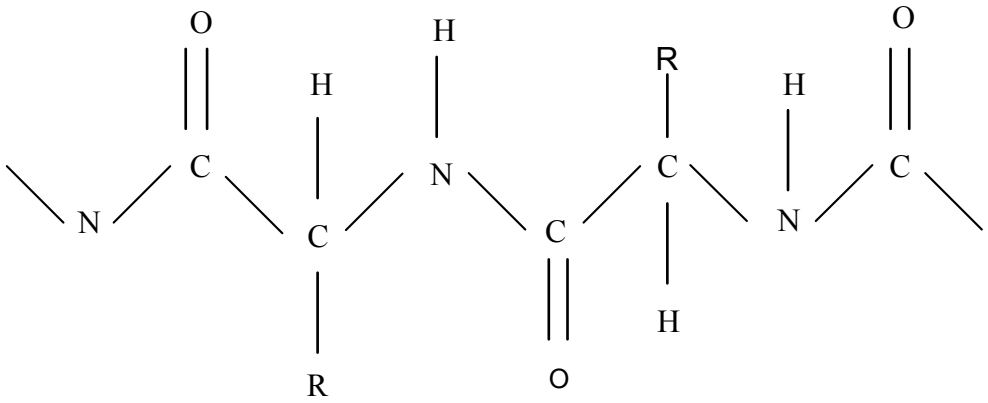


Figure 10: Peptide linkage or chain in Collagen.

The characteristics properties of proteins depend on [130]:

1. The number and types of amino acids compounding the chain, and the sequence in which they are arranged in the chain.
2. The type and amount of interaction between the chains and the number of chains per molecule of protein.
3. The spatial configuration of the chains, and the shapes of the protein molecules.

The collagen family of proteins is bio-macromolecules formed by poly peptide chains twisted together in helix. The side chains are placed outwards of the cylindrical helix. The side chains can finish with either non-polar or strongly basic or acidic groups and provide a charge profile along the chain, which spontaneously drives to the self-assembly of collagen molecules. One half of the total weight of the molecule is in the side chains [130].

Basic Chrome Sulphate (BCS), $Cr(OH)SO_4$ is used in chrome tanning and the basicity of chrome sulphate is 33 % . Trivalent chromium, Cr(III) of basic chrome sulphate reacts with the free carboxylic group of collagen protein by complex coordination bond during chrome tanning. Olation, oxolation and polymerization have also been occurred during the aging of chrome tanned leather and hence cross linking is developed prominently in the chrome tanned leather [131-132]. The following coordinate linkage is formed in chrome tanned leather and chrome shaving dust.

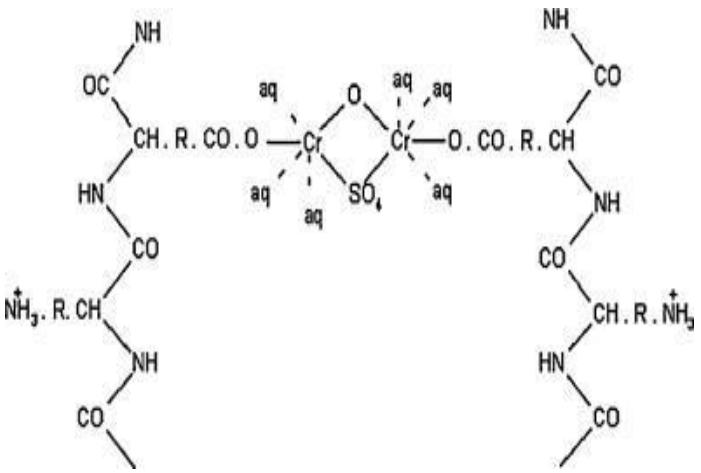


Figure 11: Molecular structure of Chrome Tanned Leather and Chrome Shavings Dust [07].

Collagen fibrils tanned with 33% BCS show a distinct cross-striation in SEM examination (X50,000) presumably due to distinct ordering of the collagen monomer. Though the exact way through which Cr(III) is incorporated into the collagen is not fully understood; it is understood that the initial operation should be diffusion controlled which is taken over by chemical reaction soon after. K.H. Gustavson first realized that it is the –COO- groups of the acidic amino acids that are the major reaction sites for Cr(III) fixation [133]. In the tanning process Cr(III) may be envisaged to reacting with free carboxylates of the collagen to give three distinct kinds of linkages (a) unipoint with a single carboxylate, (Fig.12a), (b) with cross-linking between strands via single chromium (Fig. 12b) and (c) with cross-linking between strands by a di- or oligo- nuclear chromium species polymerized either via –ol, oxo or –sulphato bridges (Fig. 12c). Bridging by –di or oligo-nuclear complexes is believed to be the most important interaction for tanning leather, from the stability of Cr-collagen complex point of view, which is manifested as the increase in hydrothermal stability of leather; although as much as 90% of the Cr species may be bound with collagen via unipoint linkage [127].

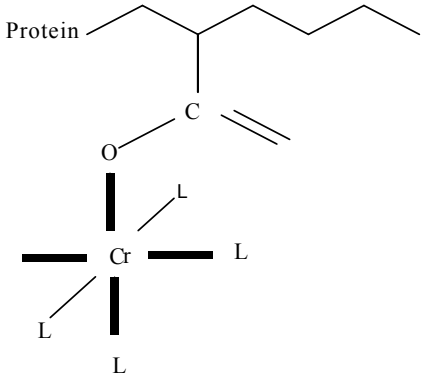


Fig. 12 a

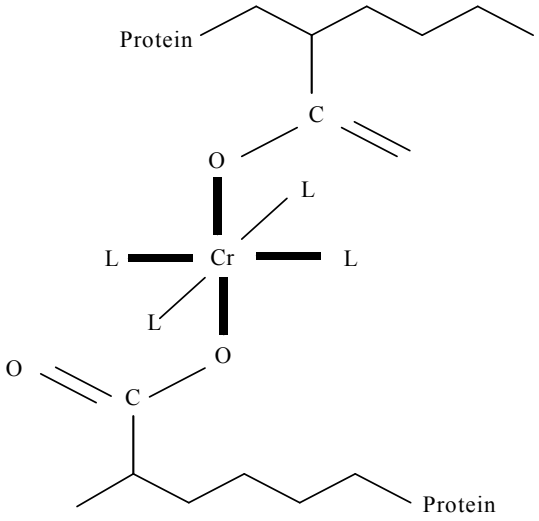


Fig. 12 b

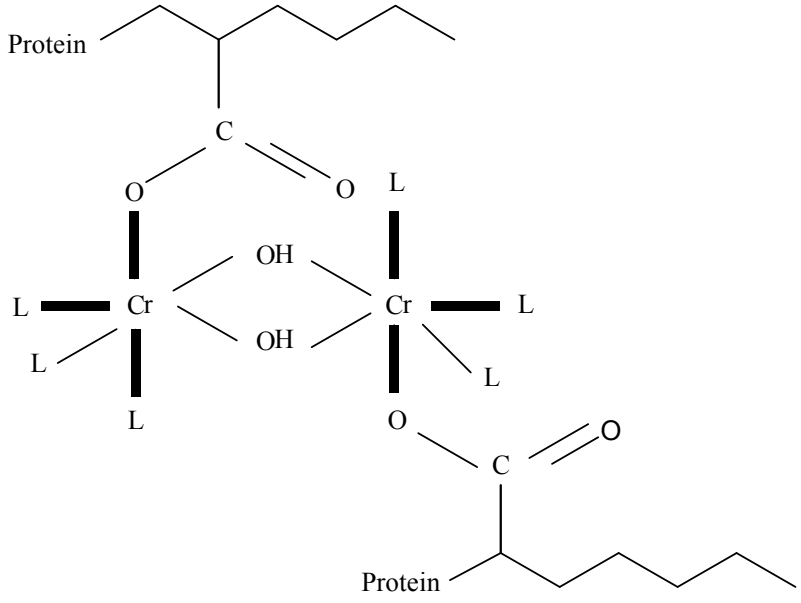


Fig. 12 c

Figure 12: Cr (III) reacting with free carboxylates of the collagen to give three distinct kinds of coordinate linkages [127].

2.7. Collagen Hydrolysate

Collagen hydrolysate a mixture of polypeptides where the spread of molecular weight is a function of the degree of digestion [11].The term collagen is derived from two Greek terms: “Kolla” means glue and “Genno” means producer [129]. Hence collagen can be dissolved

in warm water resulting in glue, gelatin and hydrolysate in various conditions. Hydrolysate refers to any product of hydrolysis. Protein hydrolysates are produced from purified protein sources by heating with acid or, preferably, addition of proteolytic enzymes, followed by purification procedures. Each protein hydrolysate is a complex mixture of peptides of different chain length together with free amino acids, which can be defined by a global value known as degree of hydrolysis (DH), which is the fraction of peptide bonds that have been cleaved in the starter protein [134]. However, even the exact information on DH cannot not tell us the whole story, as two protein hydrolysates made by different methods may have a similar degree of hydrolysis even though their absorption kinetics are likely quite different. Consequently, all protein hydrolysates are certainly not created equal [135].

Collagen hydrolysate is produced from collagen protein found in the bones, skin, and connective tissue of animals such as cattle, fish, horses, pigs, and rabbits. The process of hydrolysis involves breaking down the molecular bonds between individual collagen strands using combinations of heat, acids, alkalis, or enzymes. Typically, with skin-sourced collagen, hides are put in a lime slurry pit for up to 3 months, loosening collagen bonds; the hides are then washed to remove lime, and the collagen extracted in boiling water. The extracted collagen is concentrated by evaporation, desiccated with drum driers, and pulverized [136].

2.8. Mechanism of Hydrolysis

There are cold water soluble peptides or amino acid mixtures that can be obtained from collagen protein or gelatin. The manufacturing process involves treating the collagen with heat in an aqueous medium, and adding lyotropic substances, acids, alkalis or proteolytic enzymes [130]. The hydrolyzed collagen obtained is of higher purity when enzymes are used during hydrolysis. In alkali

treatments aminoacids are racemizated and the amides glutamine and asparagines are transformed into the alkali salt of glutamic and aspartic acids with detachment of ammonium. The salts can be removed using membranes that retain the amino acids and peptides. The acid hydrolysis preserves better the peptide and the amino acids but its neutralization is also necessary. In this case, this can be carried out with calcium carbonate and after, the soluble salts can be removed by means of membranes [130]. Enzymatic hydrolysis has the advantage that the resulting solutions are salt free and the size of the peptides is more homogenous [137]. Enzymatic proteolysis needs collagen denaturation, because the native collagen fibres only are attacked by specific collagenase. The selection of enzyme and hydrolysis conditions determines the properties of final product. Finally it is necessary to destroy the enzyme which can be done by acidification at pH 3 or lower or by heat. The solution is concentrated in vacuum and spray dried to yield a white or slightly yellow creamy powder [130].

The complete hydrolysis of chrome containing leather wastes (CCLW) is very difficult and partial hydrolysis is happened in most of the cases. There are possible three reasons for the incomplete hydrolysis of CCLW [105]:

1. Hydrophobic interaction: Non-polar amino acids and alkaline amino acids are all hydrophobic in alkaline conditions, and because they are common in the residue protein, they could form hydrophobic domains and tight clusters inaccessible to water, leading to the protein resisting hydrolysis [105].

2. Covalent bridges: The products in the residue protein might have covalent cross-linking formed between alkaline amino acids and hydroxy amino acids. These covalent bonds might break under acidic conditions leading to the complete hydrolysis of protein residues with acids to obtain chromium-

containing protein hydrolysates [105].

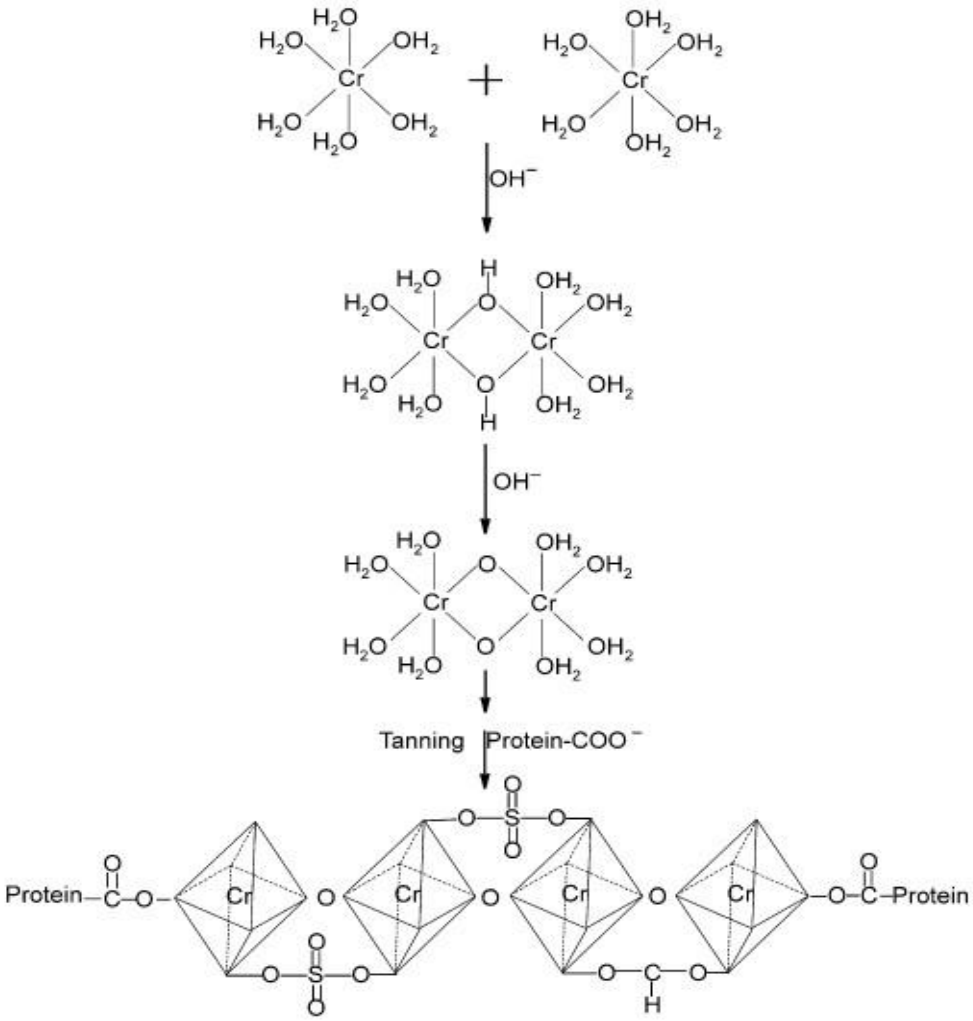


Figure 13: A schematic of oxolation chromium (III) complexes formed during basification and its complexation with protein carboxyl [105].

3. Cross-linking with chromium (III) complex: Under alkaline condition (pH<12), most of the coordinated chromium are divorced from the carboxyl groups on the side chain of aspartic acid (Asp) and glutamic acid (Glu) and precipitated as $Cr(OH)_3$. However, oxolation chromium (III) complexes formed during basification (as schematically shown in Fig 13) have strong resistance to alkali. It could crosslink with the carboxyl groups of a few Asp and Glu, or protein hydrolysates, and form insoluble macromolecular metal complexes. Using of peroxide to oxidize the Cr(III) to Cr(IV), dissolved most of the protein

residues soon even in alkaline solution, which supports this possibility. In addition, a certain amount of cystine (Cys) appeared in the hydrolysates, but not in chrome cake, which indicates that the S-S cross-linking in cystine has nothing to do with the residue protein [105].

2.9. Characteristics of Collagen Hydrolysate

The hydrolysis process results in reducing the collagen proteins of about 300,000 Dalton (Da.) into small peptides having an average molecular weight between 2000 and 5000 Da. collagen. The hydrolysate is white or light yellow colur, viscous gel [138].

i. Amino Acid Content

The amino acid content of hydrolyzed collagen is the same as collagen. Hydrolyzed collagen contains 20 amino acids, predominantly glycine, proline and hydroxyproline, which together represent around 50% of the total amino acid content [138].

Table 4: Amino acid content of collagen hydrolysate [138].

Amino acids	**Percentage**
Proline/Hydroxyproline	25%
Glycine	20%
Glutamic acid	11%
Arginine	8%
Alanine	8%
Other essential amino acids	16%
Other non-essential amino acids	12%

ii. Chemical Characteristics of Dry Collagen Hydrolysate

The chemical properties of collagen are mainly due to the carboxylic and amino groups. However, in the hydrolysate additional groups are produced by the alkaline/enzymatic breakdown of the peptide bonds

[11]. The chemical characteristics of collagen hydrolysate have been depicted in table 5 [139].

Table 5: Chemical characteristics of collagen hydrolysate [139].

Chemical Properties	Value
pH	7 - 8
Ash Content in dry substance	4.9 %
Chromium Content in dry substance	28.5 ppm
Ca content in dry substance	2746.62 ppm
Mg content in dry substance	4798.00 ppm
Dry substance	92.99 %
Nitrogen Content	14.85 %
Protein Content	92.81 %

2.10. Application of Collagen Hydrolysate

Collagen hydrolysate is potential resource for much industrial utilisation [35]. It can be effectively used in leather processing [11]. Collagen hydrolysate increases the chrome absorption capacity of leather during chrome tanning. Since leather is a proteinous substance and collagen hydrolysate has good compatibility with colagenous fibre after modification by acrylic monomer, it can act as protein filler in re-tanning operation of leather processing [97,140]. Modified Collagen hydrolysate with polyurethane, can be a very good finishing agent for leather finishing [141].

Hydrolyzed collagen of high purity grade is used for additions in food, cosmetics (creams, shampoos, etc.) or washing powder formulations. It has great buffer capacity and can stabilize technical solutions, avoiding settling of dispersions and therefore stabilizing the foam [35,130].

Protein is one of the principal nutrient of fish meal of poultry feed. Collagen hydrolysate can be effectively used as protein resource in fish meal or poultry feed preparation. But at present, poultry feed

manufacturer of the country directly use the chrome shaving dust as a protein ingredient of poultry feed without pretreatment or removing of high content of chromium from chrome shaving dust. Consequently the feed contain high level of chromium [15,116].

Collagen hydrolysate can also be used in preparation of organic fertilizer, bio gas preparation and enzyme production. Pure collagen hydrolysate may be used in tissue engineering, pharmaceutical and cosmetic industries [07,35].

2.11. Poultry Feed

Feed ingredients for poultry diets are selected for the nutrients they can provide, the absence of anti-nutritional or toxic factors, their palatability or effect on voluntary feed intake, and their cost. The key nutrients that need to be supplied by the dietary ingredients are amino acids contained in proteins, vitamins and minerals. All life functions also require energy, obtained from starches, lipids and proteins. Feed ingredients are broadly classified into cereal grains, protein meals, fats and oils, minerals, feed additives, and miscellaneous raw materials such as roots and tubers [142].

Protein is provided from both vegetable and animal sources, such as oilseed meals, legumes and abattoir and fish processing by-products. The main animal protein sources used in poultry diets are meat meal, meat and bone meal, fish meal, poultry by-product meal, blood meal and feather meal. Although the production of animal protein for human consumption has been under continual pressure and marred by much controversy, the world wide and domestic consumption of animal protein continues to grow and much of the future supply of the meat protein will come from poultry. With increased animal protein production there will be increased demand for feed, and in particular, a demand for ingredients high in protein and energy [142].

The animal industry evolved as a means of adding value (i.e. higher nutrient level and availability, flavour, variety etc.) to ingredients that were of marginal food value for humans. These ingredients include grains that are of poor quality or damaged by harvest or storage conditions; as well as a means of recycling by-products of brewing, vegetable oil, meat, milk and egg production. Approximately 50% of the live market weight of ruminants and 30% of poultry is by-product. These by-products are rendered, ground and available as a feed source [142].

Animal protein meals are usually defined by inputs. Those specifically used in poultry diets include meat (no bone) or meat and bone meal from ruminants and/or swine; blood meal; poultry by-product meal; feather meal and fish meal. There are specific limitations now assigned to these products with regards to inputs used and guarantees with respect to minimum nutrient levels. For example meat and bone meal may be specifically from ruminants and must be free from hair, wool and hide trimmings, except where it is naturally adhering to heads and hoofs. The products are rendered, which is a bio-secure process that evaporates water, extracts fat and yields a finished ground product high in protein (which has no resemblance to the raw product) and minerals. The products are marketed with guarantees as to minimum protein, phosphorus and calcium levels.

There are some challenges associated with the use of animal protein sources. First, food safety is the most important concern people have about the recycling of animal protein meals back through animals as feed ingredients. This is based on the links between the prion disease bovine spongiform encephalopathy (BSE-mad cow disease) and a variant Creutzfeldt-Jakob disease in humans [143]. In addition to BSE contamination, there are concerns that animal protein meals are responsible for food borne pathogen contamination, such as salmonella. Typically these bacteria are

destroyed by rendering and possible recontamination is often negated by pelleting of manufactured feeds. In most cases, if poultry acquire Salmonella it is likely to be from an environmental source other than feed. It is possible for animal protein meals to be contaminated with high levels of heavy metals, dioxins and PCBs (pesticides); however, meals are monitored and regulated to minimize this contamination.

Secondly, with respect to feeding the animal protein meals, the important practical issue is the variability in available nutrients (those that can be absorbed and retained by the bird) and limits to incorporation to maintain a diet balanced for all nutrients, particularly calcium and phosphorus.

Animal protein meals provide a good source of essential amino acids (e.g., lysine and methionine) and are also good sources of energy and minerals (particularly calcium and available phosphorus). However, there can be significant variation in availability (absorption and retention) of amino acids due to the day to day variation in inputs as well as processing conditions (temperature, moisture, pressure and time). The variation within processing plants can often be greater than variation between plants. It is important for users to establish strict criteria as to the quality of product and work with their suppliers to ensure these criteria are met. Quality should include measurements that indicate moisture; nutrient availability (particularly essential amino acids); levels of minerals (for example, calcium can vary from 8-12%; phosphorus from 4-6%); and stability of fat (all meals should be stabilized with an antioxidant) [142].

Animal protein meals have a long history in poultry nutrition. Utilisation of this valuable feed ingredient is important in minimizing loss (nutrient and economic value) in the production of safe, high quality poultry meat, eggs and bio-products. Collagen hydrolysate prepared from animal hides and skins, raw trimmings and shaving

dusts can be an effective protein resource for the preparation of poultry feed due to its high content of essential amino acids (particularly lysine and methionine) [116,138].

2.12. Protein Concentrate Making at Hazaribag.

Chrome shaving dust is put into a container (locally known as 'haandi') which is made by iron. Near about 800 Kg of chrome shaving dust is taken into the container at a time. The dimension of the container is 8"x4"x3.5". After filling the leather dust into the container is tightly covered and heated for 2-3 hrs at 100-150 °C. In this time, near about 720 liter normal water is supplied in the container. After 2-3 hours of heating, sulfuric acid is given into the container to melt the chrome shaving dust properly. If the leather is chrome based then 12 liter acid is mixed with 60 liter water and then added to the container. If the leather is vegetable based then 4 liter acid is mixed with 60 liter water and added to the container. If it is chrome shaving dust then 8 liter acid is mixed with the 60 liter water and added to the container. After the mixture of acid, the container is heated for more 3-4 hours for proper melting of material. When the materials colour is turned bluish colour and it forms wet cake heat treatment is stopped. Then the digested material is dried into open place under sunlight for 7-8 days depending on the weather condition.

Figure 14: Boiling of chrome shaving dust with H_2SO_4.

Figure 15: Grinding of dried protein concentrate.

Chapter – 3: Materials and Methods

3.1. General

Since Chrome Shaving Dust is one of the major tannery solid wastes [117] and it contains portentous substance mainly collagen protein to a reasonable content [128], it was treated to produce value added product "Protein Hydrolysate or Collagen Hydrolysate" by using chemical and biochemical methods. Strong alkaline or acidic treatment was avoided due to the intended application of protein hydrolysate for leather processing, poultry feed and fertilizer preparation. A biochemical method here refers to the chemical treatment first and then enzymatic treatment of chrome shaving dust. Mild alkaline treatment using CaO and MgO was carried out in the first step of chemical treatment and finally enzymatic treatment was followed for the biochemical treatment of chrome shaving dust. Proteolytic enzymes e.g., Trypsin, Pepsin and Proteinase K were used under mild alkaline condition. Hydrolysis was carried out under control temperature and pH for 3-5 hours.

3.2. Materials

The main raw material for the preparation of collagen hydrolysate is Chrome Shaving Dust which is generated as tannery solid waste during leather processing. After chrome tanning, the leather is called wet blue leather and this wet blue leather is subjected to undergo a mechanical operation called shaving to make the leather at uniform thickness and to smoothen the back side of the leather. In this operation the back or flesh side of wet blue leather is skiving or shaving by a cylindrical blade to adjust the required thickness of the finished leather. The narrow ribbon like or shredded waste of back side of wet blue leather after shaving is termed as "Chrome Shaving Dusts or Chrome Shavings". This is usually dumped without any pre-treatment into the road side of tannery area at Hazaribagh.

There are two types of chrome shaving dusts available in Hazaribagh namely full chrome and low chrome shaving dust. Full chrome shaving dust is generated form full chrome tanned wet blue leather which is used for making chrome re-tanned leather. On the other hand, low chrome shaving dust is generated from the partial chrome tanned wet blue leather which is used for making semi-chrome leather and contains less amount of chromium. The chrome shaving dust of cow wet blue leather was collected from the tannery of Hazaribagh, Dhaka for experimental purpose. Full chrome and low chrome shaving dusts were selected for experimental purposes.

Figure 16: Full Chrome Shavings.

Figure 17: Low Chrome Shavings.

Analytical grade of CaO and MgO (Merck Germany) were used as mild alkaline media. Proteolytic enzyme was used as bio-catalyst for biochemical treatment of chrome shaving dust and this was collected from Loba chemical co., India. Pepsin, Trypsin and Proteinase K were used as proteolytic enzymes under mild alkaline condition. Distilled water was used for all the experimental purposes.

3.3. Instruments

Muffle furnace was used to measure the ash content of chrome shaving dust. Analysis of oils and fats was performed by using Soxlet apparatus. Mammart Oven was deployed for the determination of

moisture content of chrome shaving dust. Hot plate (temperature range 25-150 ºC) and mechanical stirrer were used for the hydrolysis process. pH meter (Hanna) was used to measure the pH during hydrolysis of chrome shaving dust. Incubator was used to preserve the prepared collagen hydrolysate at 4 ºC. The protein analysis of collagen hydrolysate was done by using BUCHI Digest BUCHI AUTO Kjeldhal Unit K-370. Cr, Ca and Mg contents of the prepared hydrolysates were determined by a Perkin Elmer A Analyst-200 model Atomic Absorption Spectrophotometer. The FTIR analysis of the collagen hydrolysate was carried out by a Shimadzu FTIR.

3.4. Method

Hydrolysis of chrome shavings was carried out by using mild alkali and proteolytic enzyme. At the first phase, chemical hydrolysis was done using CaO/MgO, then it was followed by biochemical hydrolysis using pepsin, trypsin or proteinase K. Suitable chemical and biochemical methods were developed for the extraction of collagen hydrolysate from chrome shaving dust. A three step hydrolysis process was developed for both chemical and biochemical methods. MgO/or CaO can increase efficiency of solubilization and mastication of chrome shavings and at the same time reduce the amount of enzyme needed. The use of enzymes accelerated the cleavage of peptide linkage in presence of MgO/or CaO and hence the hydrolysis completed, and also deliverd superior product quality, of both hydrolysate and chrome cake i.e. the chrome residue, thus opening up enhanced possibilities for exploitation and making the treatment cost effective . The pH was kept at 8.5-9.5 at all cases. The cleavage of chrome complex occurred and chromium was precipitated as $Cr(OH)_3$ and some monmeric compounds of Mg were formed during hydrolysis.

$$Mg + H_2O = Mg^{2+} + (OH)^- \longrightarrow Mg(OH)_2$$

$$Cr(OH)SO_4 + Mg(OH)_2 = Cr(OH)_3 + MgSO_4$$

$$Ca + H_2O = Ca^{2+} + (OH)^- \longrightarrow Ca(OH)_2$$

$$Cr(OH)SO_4 + Ca(OH)_2 = Cr(OH)_3 + CaSO_4$$

Several process variations in the methods were carried out and the effective method was developed for the extraction of collagen hydrolysate from chrome shaving dust. The process was optimized by the variation of alkalis and enzymes and the various concentrations of alkalis and enzymes. After complete hydrolysis of chrome shavings, collagen hydrolysate was extracted through vacuum filtration and the remaining chrome residue or sludge which is termed as chrome cake, was collected from the filter dome. The prepared collagen hydrolysate was preserved at 4 ºC in an incubator. The chrome cake was treated with 100 % water and 10 % diluted sulfuric acid until the pH was reduced to 2.8-3.0 and left for overnight to separate the chromium as $Cr_2(SO_4)_3$ and treated with 10% Na_2CO_3 solution to prepare basic chrome sulfate (BCS) $Cr(OH)SO_4$.

$$2\,Cr(OH)_3 + 3H_2SO_4 = Cr_2(SO_4)_3 + 6H_2O$$

$$Cr_2(SO_4)_3 + Na_2CO_3 + H_2O = 2\,Cr(OH)SO_4 + Na_2SO_4 + CO_2$$

3.5. Hydrolysis Process

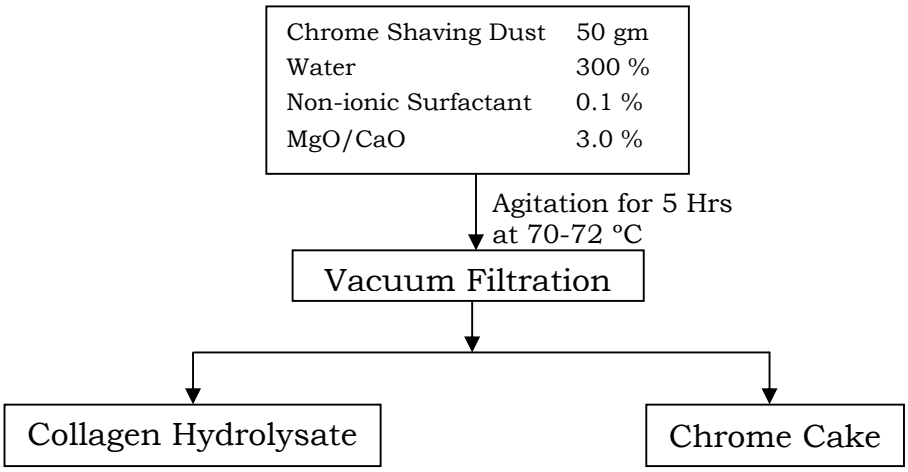
Hydrolysis of chrome shavings was carried out by several processes using different alkalis such as MgO and CaO and different proteolytic enzymes such as trypsin, pepsin and proteinase K. Amalgamation of any two of these enzymes was also experimented. The various experimental processes are described in the following sections.

3.5.1. Process 1: Hydrolysis by MgO

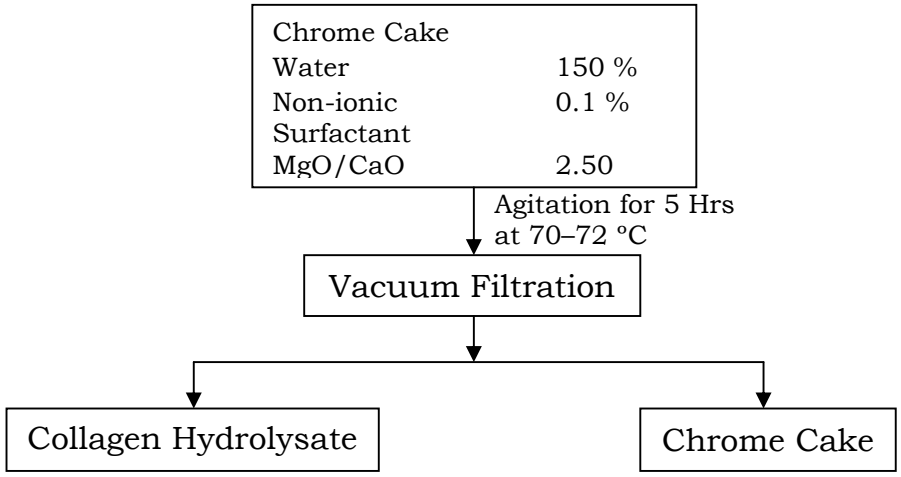
In the first phase of hydrolysis, chrome shaving dust was measured to 50 gm and taken into 1000 ml beaker. 150% of water and 0.1% of

non ionic surfactant based on the dry weight of chrome shaving dust were added to this and heated at 70-72 ºC for 15-20 minutes. Surfactant accelerated the wet back and soaking of chrome shaving dust. After complete soaking of chrome shaving dust, 3% of MgO was added and heated at 70-72 ºC for 5 hours. Stirring was continued during the whole hydrolysis process. The pH was adjusted at 8.5-9.5 using MgO. Water soluble collagen hydrolysate was extracted from the chrome shaving slurry during hydrolysis. Since the extraction was done in an open beaker instead of a reactor, the beaker was closed by using foil paper to protect the evaporation of water so that the water content of the slurry was not reduced. If the slurry would be dried, the chrome shavings might be burnt during hydrolysis and the separation of collagen hydrolysate from the slurry through vacuum filtration would be very difficult. After completion of hydrolysis, the slurry was cooled down to 40-45 ºC and collagen hydrolysate was separated from the slurry through vacuum filtration. The hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

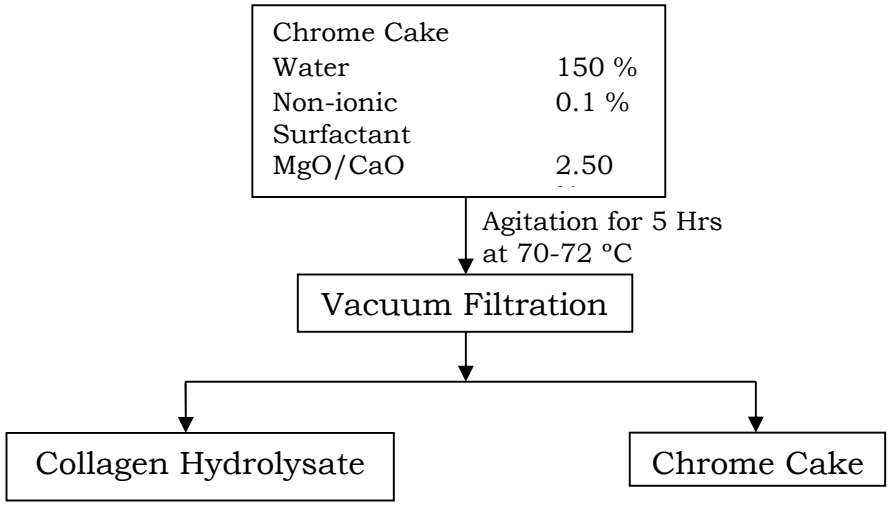
The sludge after vacuum filtration is called chrome cake and it was further hydrolyzed at the second step in the same procedure. In this step, less amount of MgO was required to adjust the pH at 8.5 to 9.5 and 2% was sufficient for this. The hydrolysis was continued up to 5 hours with constant stirring. After complete hydrolysis, the collagen hydrolysate was separated through vacuum filtration, collected in a volumetric flask and finally preserved at 4 ºC in an incubator. The chrome cake was also hydrolyzed at the third step in the same procedure and 1% of MgO was needed to keep the pH within the range. The collagen hydrolysate was separated, collected and preserved in the same procedure of first and second steps hydrolysis. The prepared collagen hydrolysate was in gel form during preservation in an incubator.



1st Step Hydrolysis



2nd Step Hydrolysis



3rd Step Hydrolysis

Figure 18: Flow Diagram of Chemical Treatment of Chrome Shavings.

3.5.2. Process 2: Hydrolysis by CaO

The procedure of this process was same as the procedure of process 1 except that CaO was used in this process instead of MgO. Step 1, 2 & 3 were followed in the same way of process 1 and all the percentages of CaO were the same as that of process 1. Collagen hydrolysate was separated through vacuum filtration after the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

3.5.3. Process 3: Hydrolysis by MgO & Trypsin

In the first step of hydrolysis, 50 gm of chrome shaving dust was measured and taken into 1000 ml beaker. 150% of water and 0.1% of non ionic surfactant were added to this and heated at 70-72 ºC for 15-20 minutes. Surfactant accelerated the wet back and soaking of chrome shaving dust. After complete soaking of chrome shaving dust, 3% of MgO was added and heated at 70-72 ºC for 30 minutes. Continuous stirring was maintained during the whole hydrolysis process. The pH was adjusted at 8.5-9.5 using MgO. After complete maceration, the temperature was reduced to 45-55 ºC and then added to this 0.1% of trypsin enzyme. The protein fibre of chrome shaving dust was broken down through mastication by the action of proteolytic enzyme and thus the hydrolysis process was accelerated. Hydrolysis was continued for 3 hours with frequent agitation of 120 rpm. Water soluble collagen hydrolysate was extracted from the chrome shaving slurry during hydrolysis. Since the extraction was done in an open beaker instead of a reactor, the beaker was closed by using foil paper to protect the evaporation of water so that the water content of the slurry was not reduced. If the slurry would be dried, the chrome shavings might be burnt during hydrolysis and the separation of collagen hydrolysate from the slurry through vacuum filtration would be very difficult. After completion of hydrolysis, the temperature was raised at 85 ºC and heated for 10 to 15 minutes to

completely deactivate the enzyme. After the deactivation of enzyme, the slurry was cooled down to 40-45 ºC and the collagen hydrolysate was separated from the slurry through vacuum filtration. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

The chrome cake after vacuum filtration was further hydrolyzed at the second step in the same procedure. The amount of MgO was less required to adjust the pH 8.5 to 9.5 and 2.5 % was sufficient in the second step but the same amount of enzyme was used. The hydrolysis was continued up to 3 hours. After complete hydrolysis, the collagen hydrolysate was separated through vacuum filtration, collected in a volumetric flask and preserved at 4 ºC in an incubator. The chrome cake was also hydrolyzed at the third step in the same procedure and 2% of MgO was added to keep the pH within the range and the same amount of enzyme was used. The collagen hydrolysate was separated, collected and preserved in the same way of first and second steps hydrolysis. The prepared collagen hydrolysate was in gel form during preservation in incubator.

3.5.4. Process 4: Hydrolysis by CaO & Trypsin

The procedure of this process was same as the procedure of process 3 except that CaO was used in this process instead of MgO. Step 1, 2 & 3 were followed in the same way of process 3 using similar percentage of CaO and collagen hydrolysate was separated through vacuum filtration at the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

3.5.5. Process 5: Hydrolysis by MgO & Pepsin

The procedure of this process was as like as the procedure of process 3 except that enzyme pepsin was used in this process instead of trypsin. Step 1, 2 & 3 of hydrolysis were followed in the same way of

process 3 using similar percentage of enzyme and collagen hydrolysate was separated through vacuum filtration at the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 °C in an incubator.

3.5.6. Process 6: Hydrolysis by CaO & Pepsin

The procedure of this process was similar to the procedure of process 5 except that CaO was used in this process instead of MgO. Step 1, 2 & 3 of hydrolysis were followed in the same way of process 5 using similar percentage of CaO and collagen hydrolysate was separated through vacuum filtration at the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 °C in an incubator.

3.5.7. Process 7: Hydrolysis by MgO, Trypsin & Pepsin

The procedure of this process was same as the procedure of process 3 except that enzyme pepsin was added along with enzyme trypsin in the process. 0.5% of each enzyme in combination was used during the all steps of hydrolysis. Step 1, 2 & 3 were followed in the same way of process 3 using similar percentage of MgO as used in earlier processes and collagen hydrolysate was separated through vacuum filtration at the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 °C in an incubator.

3.5.8. Process 8: Hydrolysis by CaO, Trypsin & Pepsin

The procedure of this process was similar to the procedure of process 7 except that CaO was used in this process instead of MgO. Step 1, 2 & 3 were followed in the same way of process 7 using similar percentage of CaO as used in earlier processes. Collagen hydrolysate was separated through vacuum filtration at the end of each step of

hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

3.5.9. Process 9: Hydrolysis by MgO, Trypsin & Proteinase K

The procedure of this process was same as the procedure of process 3 except that enzyme proteinase K was added along with enzyme trypsin in the process. 0.5% of each enzyme in combination was used during the all steps of hydrolysis. Proteinase K was a strong enzyme and speeded up the hydrolysis process. It also increased the percentage yield of collagen hydrolysate. After 10-15 minutes of adding trypsin, then proteinase K was added. Step 1, 2 & 3 were followed in the same way of process 3 and collagen hydrolysate was separated through vacuum filtration at the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

3.5.10. Process 10: Hydrolysis by MgO, Pepsin & Proteinase K

The procedure of this process was same as the procedure of process 4 except that enzyme proteinase K was added along with enzyme pepsin in the process. 0.5% of each enzyme in combination was used during the all steps of hydrolysis. Step 1, 2 & 3 were followed in the same way of process 4 and collagen hydrolysate was separated through vacuum filtration at the end of each step of hydrolysis. The prepared collagen hydrolysate was collected in a volumetric flask and preserved at 4 ºC in an incubator.

All the above processess were carried out with full chrome shaving dust and process 1, 3 and 7 were also carried out with low chrome shaving dust since the process 1, 3 and 9 gave better yields of protein hydrolysate using full chrome shaving dust.

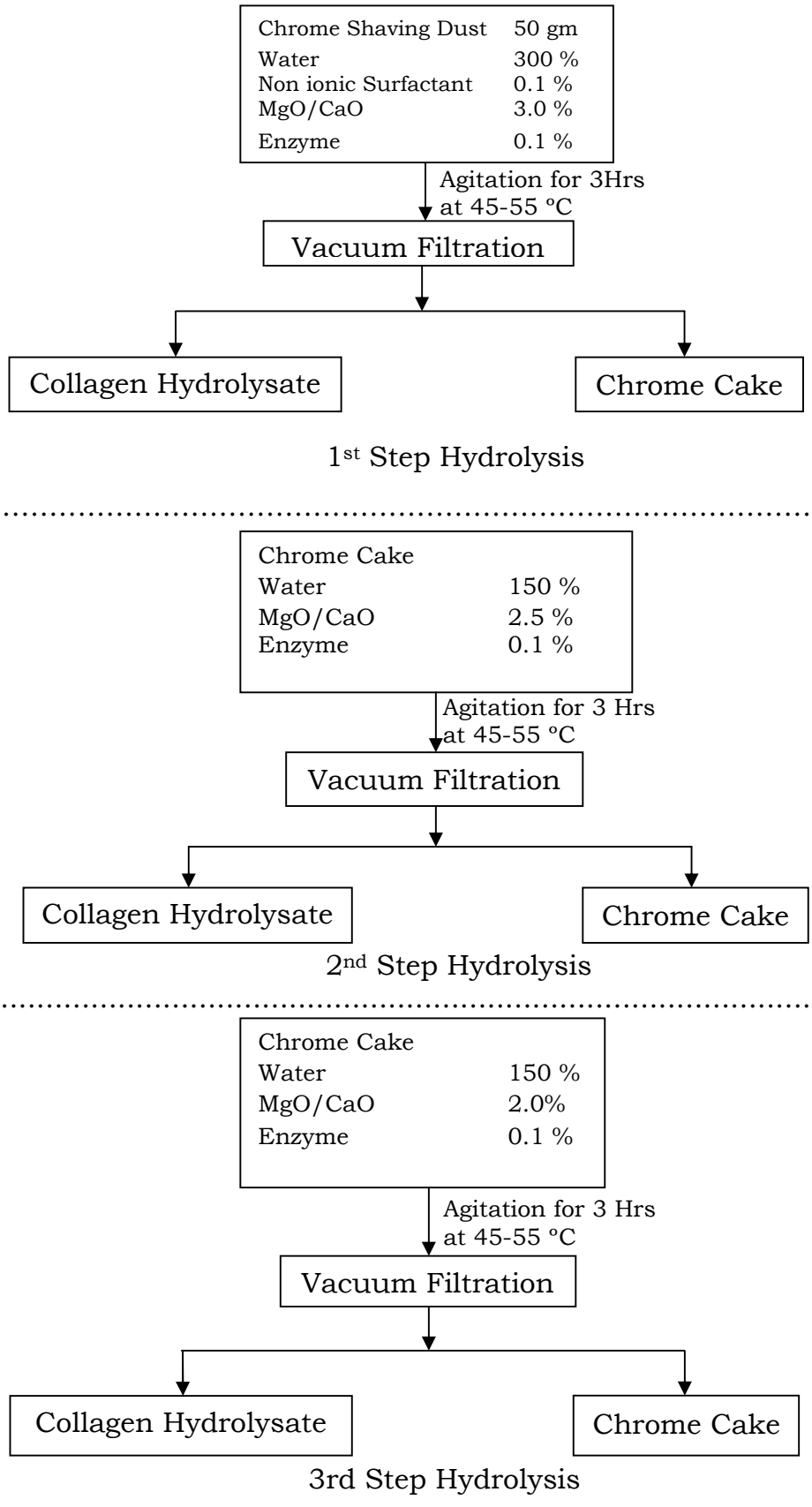


Figure 19: Flow Diagram of Biochemical Treatment of Chrome Shavings.

3.6. Chrome Recovery from Chrome Cake

The chromium was precipitated as $Cr(OH)_3$ in the chromium containing residue or chrome cake during hydrolysis of chrome shavings. 100 % water and 10 % diluted sulfuric acid were added to the chrome cake. The sulfuric acid was added untill the pH of the slurry was reduced to 2.5 to 2.8 and the slurry was left for overnight. The colour of the slurry was turned to blue which confirmed the colour of chromium (III). The sluury was filtrated with whatman number 100 filter paper and the slurry was washed with water and again filtrated. Then 10 % Na_2CO_3 was added to $Cr_2(SO_4)_3$ solution to increase the basicity of chrome sulfate at 33 %. The basic chrome sulfate (BCS) solution was evaporated and then dried in an oven at 70±2 °C until constant weight was reached. The basicity of the chrome sulfate has been determined by standard procedure.

$$2\,Cr(OH)_3 + 3H_2SO_4 = Cr_2(SO_4)_3 + 6H_2O$$

$$Cr_2(SO_4)_3 + Na_2CO_3 + H_2O = 2\,Cr(OH)SO_4 + Na_2SO_4 + CO_2$$

BCS

The filtrate sludge after chrome recovery was dried and analyzed for the determination of chromic oxide and ash content using standard method SLC-8 and SLC-6.

3.7. Process Optimization

The process of extraction of Collagen Hydrolysate was optimized by using different alkalis, enzymes, amalgamation of enzymes and the duration of hydrolysis time.

3.7.1. Optimization of Nature of Alkalis

Experiments were carried out by treating predetermined quantity of chrome shaving dust (50 gm on dry weight basis), different alkalis (2.0-3.0%) with and without different enzymes and water 150 % on dry weight basis of chrome shaving dust in a 1000 ml beaker with

optimum temperature, pH and time. The optimum temperature of chemical and biochemical hydrolysis were 70-72 ºC and 45-55 ºC where the optimum pH for both cases was 8.5-9.5. The optimum time duration for the complete chemical hydrolysis was 5 hours but for biochemical hydrolysis, less time was sufficient for complete hydrolysis of chrome shaving dust and it was found to complete the hydrolysis within 3 hours. The collagen hydrolysate was separated through sieves by vacuum filtration and the percentage yeild of protein was determined by BUCHI kjeldhal apparatus.

3.7.2. Optimization of Proteolytic Enzymes

The hydrolysis of chrome shavings was studied by using predetermined quantity of chrome shaving dust (50 gm on dry weight basis), with 150% water and 2-3% MgO/or CaO in a 1000 ml beaker at 70-72 ºC. The temperature was lowered to 45-55 ºC before adding enzyme. Proteolytic enzyme like trypsin or pepsin was added to the chrome shaving dust with a concentration range of 0.05-0.3% on dry weight of chrome shaving dust. The mixture was agitated with 120 rpm and the hydrolysis continued for 3 hours. The collagen hydrolysate was separated after the completion of hydrolysis through sieve by vacuum filtration and the protein content of the supernatant was determined by BUCHI Kjeldhal apparatus.

3.7.3. Optimization of Hydrolysis Time

Experiment was carried out by using predetermined quantity of chrome shaving dust (50 gm on dry weight basis), with 150 % water and 2-3% MgO or CaO in a 1000 ml beaker at 70-72 ºC. The temperature was lowered to 45-55 ºC before adding enzyme. Proteolytic enzyme like trypsin or pepsin was added to the chrome shaving dust with a concentration range of 0.1-0.2 % on dry weight of chrome shaving dust. The mixture was agitated with 120 rpm and the hydrolysis continued for 1, 2, 3, 4, and 5 hours respectively. The

collagen hydrolysate was separated after the completion of hydrolysis through sieve by vacuum filtration and the protein content of the supernatant was determined by BUCHI Kjeldhal apparatus.

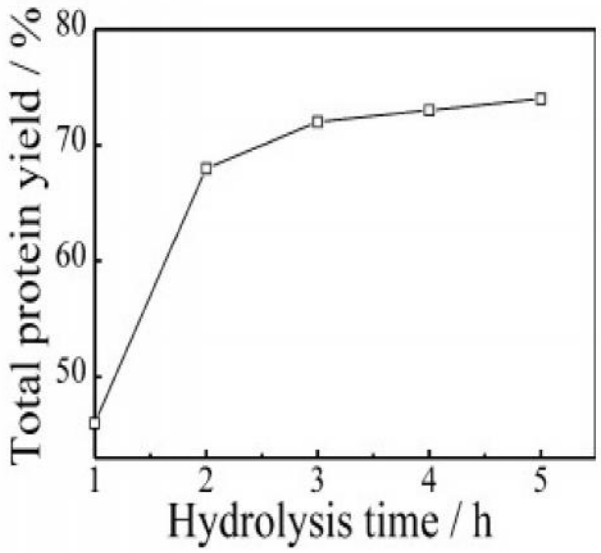


Figure 20: % of protein yield with respect to hydrolysis time.

3.7.4. Amalgamation of Proteolytic Enzemes

Extraction of collagen hydrolysate was studied by using predetermined quantity of chrome shaving dust (50 gm on dry weigh basis) with 150% water and 2-3% MgO or CaO in a 1000 ml beaker at 70-72 °C. The temperature was lowered to 45-55 °C before adding enzyme. Two types of proteolytic enzymes were amalgamated with equal ratio such as trypsin & pepsin, trypsin & proteinase K and pepsin & proteinase K. The amalgamated enzymes were added to the chrome shaving dust with a concentration range of 0.1-0.3 % on dry weight of chrome shaving dust. The mixture was agitated with 120 rpm and the hydrolysis continued for 3 hours. The collagen hydrolysate was separated after complete hydrolysis of chrome shavings through sieve by vacuum filtration and the protein content of the supernatant was determined by BUCHI Kjeldhal apparatus.

Chapter 4: Results and Discussion

4.1. Analysis of Chrome Shaving Dust

The collected Chrome Shaving Dust (both full chrome and low chrome) was characterized by both physical and chemical analysis using standard procedures [144]. The moisture content of chrome shaving dust was determined according to SLC-12, the pH according to SLC-13, Nitrogen content according to SLC-7, IUC/10, the amount of total ash, according to IUC/7, chromic oxide content according to SLC-8, oils & fat content according to SLC-4. The analytical measurements were obtained based on the dry weight of chrome shaving dust and triplicate measurements were done for each of the parameter. The physical and chemical parameters of chrome shavings are depicted in table 6 and 7. The analytical data obtained is nearly same as reported in the literature [07].

Table 6: Characteristics of Full Chrome Shaving Dust.

SL.No.	Parameters	Values
01.	Colour	Bluish
02.	Moisture Content	21.96%
03.	Apparent Density	0.89 g/ml
04.	Chrome Content	3.42%
05.	Ash Content	5.18%
06.	Oils & fats content	0.88%
07.	pH (10% aqueous solution)	3.72
08.	Nitrogen Content	14.53%
09.	Protein Content	90.81%

It can be noted that the chrome content was 3.42% and 1.37% shown in table 6 and 7 for full chrome and low chrome shaving dust respectively. The ash content of full chrome and low chrome shaving dust was 5.18% and 1.88% respectively while the protein content of

both chrome shavings was 90.81% and 91.56% respectively. This indicates the high chromium and ash contents of full chrome shaving dust whereas the protein content of both chrome shaving dusts was almost the same.

Table 7: Characteristics of Low Chrome Shaving Dust.

SL.No.	Parameters	Values
01.	Colour	Light Bluish
02.	Moisture Content	23.73%
03.	Apparent Density	0.85 g/ml
04.	Chrome Content	1.37%
05.	Ash Content	1.88%
06.	Oils & fats content	0.76%
07.	pH (10% aqueous solution)	3.82
08.	Nitrogen Content	14.65%
09.	Protein Content	91.56%

4.2. Physical Appearance of Collagen Hydrolysate

The physical appearance of the prepared different samples of collagen hydrolysate was almost the same. The colour of Collagen hydrolystae which was treated with MgO was light yellow or creamy colour while the CaO treated hydrolysate was light brownish colour. The hydrolysates were viscous gel, odourless and sticky. The sample of the prepared collagen hydrolysate treated by MgO and CaO are shown in figure 19 and 20 respectively. The freeze dried collagen hydrolysate sample is also shown in figure 21.

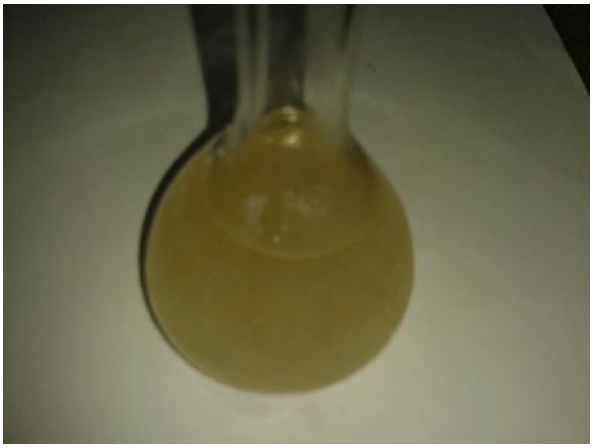


Figure 21: Collagen Hydrolysate prepared by MgO and trypsin.

Figure 22: Collagen Hydrolysate prepared by CaO and trypsin.

Figure 23: Freeze dried Collagen Hydrolysate prepared by MgO and trypsin.

4.3. Solid Content of Prepared Collagen Hydrolysate

The solid content of different samples of prepared collagen hydrolysate was determined by SLC-114. The results of solid content of different samples are shown in table 8.

Table 8: Solid content of prepared collagen hydrolysates.

SL. No.	Sample Description	Solid Content			% Recovery
		Extraction 1	Extraction 2	Extraction 3	
Sample 1	Full Chrome Shaving Dust + MgO	8.03%	7.87%	7.12%	46.04
Sample 2	Full Chrome Shaving Dust + CaO	6.53%	6.02%	5.89%	36.88
Sample 3	Low Chrome Shaving Dust + MgO	8.62%	7.91%	7.34%	47.74
Sample 4	Full Chrome Shaving Dust + MgO + Trypsin	12.45%	11.90%	11.01%	70.72
Sample 5	Full Chrome Shaving Dust + CaO + Trypsin	10.62%	9.64%	8.86%	58.24
Sample 6	Full Chrome Shaving Dust + MgO + Pepsin	11.27%	10.73%	10.23%	64.46
Sample 7	Full Chrome Shaving Dust + CaO + Pepsin	9.21%	8.67%	8.07%	51.9
Sample 8	Low Chrome Shaving Dust + MgO + Trypsin	13.02%	12.23%	11.88%	74.26
Sample 9	Full Chrome Shaving Dust + MgO + Trypsin + Pepsin	10.28%	9.43%	8.92%	57.26
Sample 10	Full Chrome Shaving Dust + MgO + Trypsin + Proteinase K	14.34%	13.12%	12.76%	80.44
Sample 11	Full Chrome Shaving Dust + MgO + Pepsin + Proteinase K	12.76%	11.89%	11.15%	71.60
Sample 12	Low Chrome Shaving Dust + MgO + Trypsin + Proteinase K	14.88%	14.20%	13.79%	85.74

From the above results, it can be seen that the efficiency of extraction of collagen hydrolysate by using MgO is more (46.04%) than that of CaO (36.88%). Most significant effect on hydrolysis of chrome shavings was found using MgO. It can be assumed that MgO can break down the cleavage of chrome complex more efficiently than CaO and the degree of maceration happened at maximum level with MgO [79]. Activity of trypsin was more than pepsin since trypsin gave

a better yield (70.72%) than pepsin (64.46%) [87] and the best result was found using MgO and trypsin (70.72%) with full chrome shaving dust. Amalgamation of trypsin and pepsin was not effective in the hydrolysis process due to less solid content (57.26%) of collagen hydrolysate was found by this method. The high solid content (80.44%) was found by the amalgamation of trypsin and proteinase K with MgO. It is notable that the hydrolysis of low chrome shaving dust was comparatively easy due to the fewer breakdowns of cleavage of chrome complex and faster maceration of low chrome shaving dust and gave more yield of protein (85.74%) by the same treatment.

4.4. pH of Hydrolysate

The pH of prepared collagen hydrolysate samples was determined using the standard method, SLC-13. The pH of 10 % aqueous solution of prepared collagen hydrolysate was measured and the results are shown in table 9.

Table 9: pH of prepared collagen hydrolysate samples.

SL. No.	Sample Description	pH	Standard pH
Sample 1	Full Chrome Shaving Dust + MgO	7.76	7.0 – 8.0 [139]
Sample 2	Full Chrome Shaving Dust + CaO	7.38	
Sample 3	Low Chrome Shaving Dust + MgO	7.57	
Sample 4	Full Chrome Shaving Dust + MgO + Trypsin	8.03	
Sample 5	Full Chrome Shaving Dust + CaO + Trypsin	7.78	
Sample 6	Full Chrome Shaving Dust + MgO + Pepsin	8.01	
Sample 7	Full Chrome Shaving Dust + CaO + Pepsin	7.88	
Sample 8	Low Chrome Shaving Dust + MgO + Trypsin	7.96	
Sample 9	Full Chrome Shaving Dust + MgO + Trypsin + Pepsin	8.30	
Sample 10	Full Chrome Shaving Dust + MgO + Trypsin + Proteinase K	7.91	
Sample 11	Full Chrome Shaving Dust + MgO + Pepsin + Proteinase K	7.89	
Sample 12	Low Chrome Shaving Dust + MgO + Trypsin + Proteinase K	7.67	

It may be mentioned here that the hydrolysis of chrome shaving dust was done at the pH range of 8.5-9.5. The pH of distilled water used in the hydrolysis process was 6.91. Since the collagen protein was hydrolyzed in water, the pH of the prepared collagen hydrolysates was found in between 7-8 which was also complied with the reference value [139].

4.5. Nitrogen and Protein Content

The nitrogen and protein contents were determined by the standard procedure using BUCHI Digest BUCHI AUTO Kjeldhal Unit K-370. The results are depicted below in table 10.

Table 10: Nitrogen and protein content of prepared collagen hydrolysates.

Sample No.	Sample Description	% of N_2	% of protein
Sample 1	Full Chrome Shaving Dust + MgO	6.84%	42.75%
Sample 2	Full Chrome Shaving Dust + CaO	5.67%	35.44%
Sample 3	Low Chrome Shaving Dust + MgO	7.72%	48.25%
Sample 4	Full Chrome Shaving Dust + MgO + Trypsin	10.34%	64.63%
Sample 5	Full Chrome Shaving Dust + CaO + Trypsin	8.89%	55.56%
Sample 6	Full Chrome Shaving Dust + MgO + Pepsin	9.36%	58.50%
Sample 7	Full Chrome Shaving Dust + CaO + Pepsin	7.73%	48.31%
Sample 8	Low Chrome Shaving Dust + MgO + Trypsin	11.08%	69.25%
Sample 9	Full Chrome Shaving Dust + MgO + Trypsin + Pepsin	9.25%	57.81%
Sample 10	Full Chrome Shaving Dust + MgO + Trypsin + Proteinase K	12.84%	80.25%
Sample 11	Full Chrome Shaving Dust + MgO + Pepsin + Proteinase K	11.77%	73.56%
Sample 12	Low Chrome Shaving Dust + MgO + Trypsin + Proteinase K	13.17%	82.31%

From the above table it has been revealed that sample 10 and 12 gave the highest yield of protein. It may be assumed that the action of proteinase K was very strong compared to other enzymes. The amalgamation of proteinase K with trypsin or pepsin completely broke down the amide linkage of protein and gave more yields of protein. But the cost of proteinase k is very high and hence the use of this enzyme will not be cost effective for the preparation of collagen hydrolysate. On the other hand, trypsin was also strong enzyme for collagen hydrolysis and is relatively cheaper than proteinase K. The percentage of protein yield with trypsin was also reasonably better. It is clearly understood that hydrolysis with MgO and trypsin enzyme was the best considering all the related factors.

4.6. Chromium Content

Total chromium content of the prepared collagen hydrolysates was determined by a Perkin Elmer A Analyst-200 model Atomic Absorption Spectrophotometer. Chromium (wavelength 357.9 nm) specific hollow cathode lamp was used to analyze the samples. The instrument had minimum detection limit of 0.10 mg/L for Cr in the flame method. Sample was aspirated through nebulizer and the absorbance was measured with a blank as reference. Calibration curve was obtained using standard samples (containing 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L of Cr). The correlation coefficient was found 0.991. The values given below are the results of collagen hydrolysate samples.

The chrome content (Cr_2O_3) of full chrome and low chrome shaving dusts was found 3.42% and 1.37% respectively [Table 6 & 7]. After extraction of collagen hydrolysate from chrome shaving dust by bio-chemical treatment, only trace amount of chromium was found in all prepared collagen hydrolysate samples. The results of chromium content have been tabulated in table 11.

Table 11: Chromium content of prepared collagen hydrolysate samples.

Sample No.	Sample Description	Cr Content in ppm	Standard in ppm
Sample 1	Full Chrome Shaving Dust + MgO	BDL**	< 4.5 ppm. [31]
Sample 2	Full Chrome Shaving Dust + CaO	0.73	
Sample 3	Low Chrome Shaving Dust + MgO	BDL	
Sample 4	Full Chrome Shaving Dust + MgO + Trypsin	1.47	
Sample 5	Full Chrome Shaving Dust + CaO + Trypsin	1.12	
Sample 6	Full Chrome Shaving Dust + MgO + Pepsin	0.39	
Sample 7	Full Chrome Shaving Dust + CaO + Pepsin	0.46	
Sample 8	Low Chrome Shaving Dust + MgO + Trypsin	2.81	
Sample 9	Full Chrome Shaving Dust + MgO + Trypsin + Pepsin	0.37	
Sample 10	Full Chrome Shaving Dust + MgO + Trypsin + Proteinase K	0.42	
Sample 11	Full Chrome Shaving Dust + MgO + Pepsin + Proteinase K	3.02	
Sample 12	Low Chrome Shaving Dust + MgO + Trypsin + Proteinase K	0.62	

Since the prepared collagen hydrolysates contain only trace amount of chromium and this is much less than the reference value (<4.5 ppm), hence, the developed biochemical method is effective for the recovery of collagen hydrolyste from chrome shavings. Moreover, the collagen hydrolysate prepared by this method is safe and potential for being used as poultry feed, fertilizer, or as an additive in the cosmetic industry which was reported in the literature [31].

**N.B. BDL = Below Detection Limit, 0.1 ppm.

4.7. Calcium and Magnesium Content

Ca and Mg content of the prepared collagen hydrolysates were determined by a Perkin Elmer A Analyst-200 model Atomic Absorption Spectrophotometer. Calcium (wavelength 422.7 nm) and Magnesium (wavelength 285.2 nm) specific hollow cathode lamps were used to analyze the samples. The instrument had minimum detection limit of 0.01 mg/L for both Ca and Mg in the flame method. Samples were aspirated through nebulizer and the absorbance was measured with a blank as reference. Calibration curves were obtained using standard samples (containing 0.10, 0.15, 0.25, 0.30, 0.60, 1.0 and 2.0 mg/L of both Ca and Mg). The correlation coefficient was found for Ca 0.997 and for Mg 0.998. The samples 1, 2 and 4 were analyzed for the determination of Ca and Mg and other samples were not analyzed due to the high contents of Ca and Mg in samples 1, 2 and 4 already found. Since 2-3% CaO and MgO were used in the hydrolysis process and hence prepared collagen hydrolysate contained high amount of Ca and Mg. Ca and Mg are essential mineral for poultry feed and fertilizer and thus, the collagen hydrolysate prepared by this method can be used as a poultry feed or fertilizer as reported in the literature [142]. The values given in table 12 are the results of prepared collagen hydrolysates.

Table 12: Ca and Mg Contents of prepared Collagen Hydrolystate.

SL. No.	Sample Description	Ca Content	Mg Content	Reference Value
01.	Sample 1: Full Chrome Shaving Dust + MgO.	-	1030 ppm	Ca2746.62ppm, Mg4798.00ppm [140]
02.	Sample 2: Full Chrome Shaving Dust + CaO.	1165 ppm	-	
03.	Sample 4: Full Chrome Shaving Dust + MgO + Trypsin.	-	1042 ppm	

4.8. Ash Content

The ash content of the prepared collagen hydrolysate samples was determined by SLC-7 and the results are given in the table13. The ash contents of full chrome and low chrome shaving dusts were found 5.18% and 1.88% respectively (Table 6 & 7). It has been noticed that all the prepared collagen hydrolysate samples showed less amount of ash content compared to untreated full chrome and low chrome shaving dusts.

Table 13: Ash content of prepared Collagen Hydrolysate samples.

SL. No.	Sample Description	Ash Content	Reference Value
Sample 1	Full Chrome Shaving Dust + MgO	2.09	4.9 % [139]
Sample 2	Full Chrome Shaving Dust + CaO	2.21	
Sample 3	Low Chrome Shaving Dust + MgO	1.56	
Sample 4	Full Chrome Shaving Dust + MgO + Trypsin	2.10	
Sample 5	Full Chrome Shaving Dust + CaO + Trypsin	2.32	
Sample 6	Full Chrome Shaving Dust + MgO + Pepsin	2.43	
Sample 7	Full Chrome Shaving Dust + CaO + Pepsin	3.03	
Sample 8	Low Chrome Shaving Dust + MgO + Trypsin	2.36	
Sample 9	Full Chrome Shaving Dust + MgO + Trypsin + Pepsin	2.98	
Sample 10	Full Chrome Shaving Dust + MgO + Trypsin + Proteinase K	2.96	
Sample 11	Full Chrome Shaving Dust + MgO + Pepsin + Proteinase K	2.24	
Sample 12	Low Chrome Shaving Dust + MgO + Trypsin + Proteinase K	2.31	

The reduction of ash content is significant after the treatment of chrome shaving dust. Since all samples contained very trace amount of chromium, the ash content of prepared collagen hydrolysates was also reduced to a large extent. The ash content of collagen

hydrolysate was not found to be very low due to high content of Ca and Mg in the samples. Other elements like iron might also be present in the samples due to the contamination of shaving dust while shaving the leather using metal blade. Iron might also come into the wet blue leather from water during the processing of leather. In this respect, all samples can be considered as good since the ash content of the samples was much less than that of reference value (4.9%) [139].

4.9. FTIR Analysis

The FTIR analysis of the collagen hydrolysate was carried out by a Shimadzu FTIR. The FTIR spectra for collagen hydrolysate were recorded using a FT-IR 6000 spectrophotometer with ATR reflection system. The spectra were scanned in transmission mode at 4 cm^{-1} resolution.

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 [145-146]. The polypeptide and protein repeat units give rise to nine characteristic IR absorption bands, namely, amide A, B, and I–VII. Of these, the amide I and II bands are the two most prominent vibrational bands of the protein backbone [146]. The most sensitive spectral region to the protein secondary structural components is the amide I band (1700–1600 cm^{-1}), which is due almost entirely to the C=O stretch vibrations of the peptide linkages (approximately 80%). The frequencies of the amide I band components are found to be correlated closely to the each secondary structural element of the proteins. The amide II band, in contrast, derives mainly from in-plane NH bending (40–60% of the potential energy) and from the CN stretching vibration (18–40%), showing much less protein conformational sensitivity than its amide I counterpart. Other amide vibrational bands are very complex

depending on the details of the force field, the nature of side chains and hydrogen bonding, which therefore are of little practical use in the protein conformational studies [146]. The characteristic IR bands of the proteins and peptides are listed in table 14.

Table 14: Characteristic infrared bands of peptide linkage [145-146].

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

The FTIR spectra of the prepared collagen hydrolysate have been shown in the following figures 24-35.

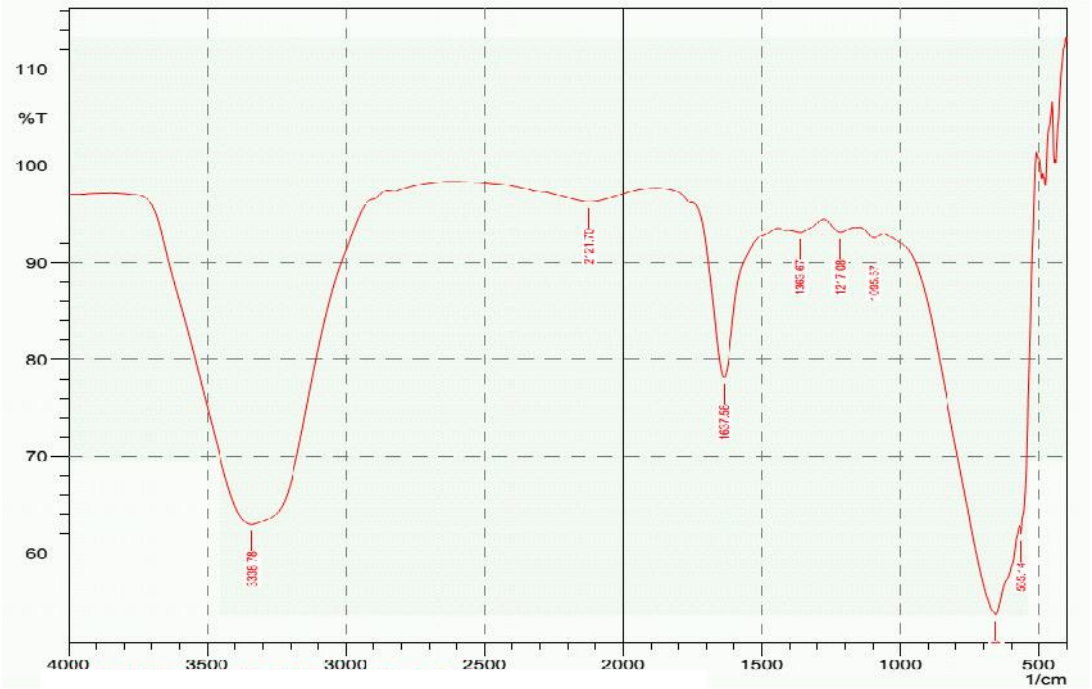


Figure 24: IR Spectra of Sample 1

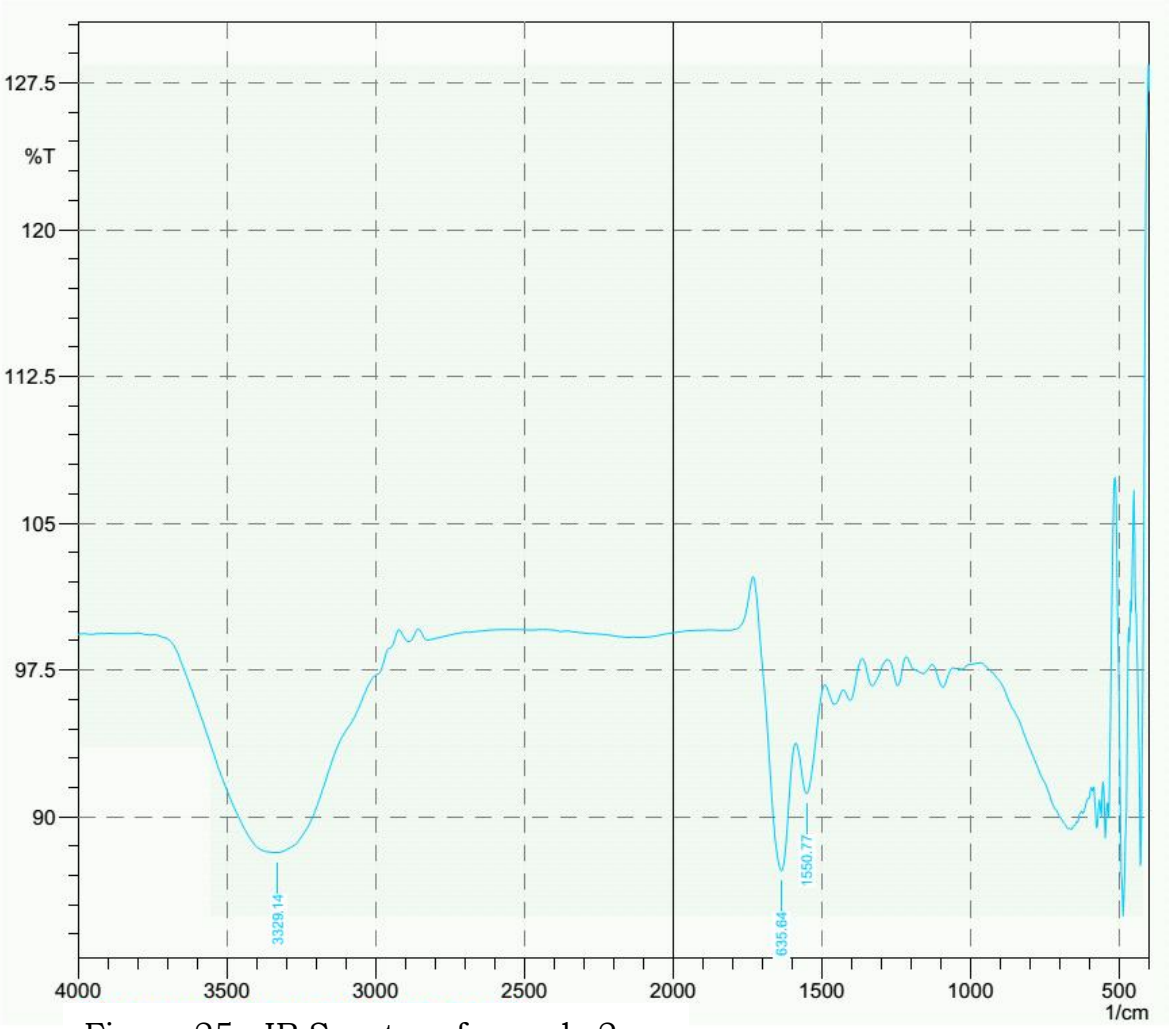


Figure 25: IR Spectra of sample 2

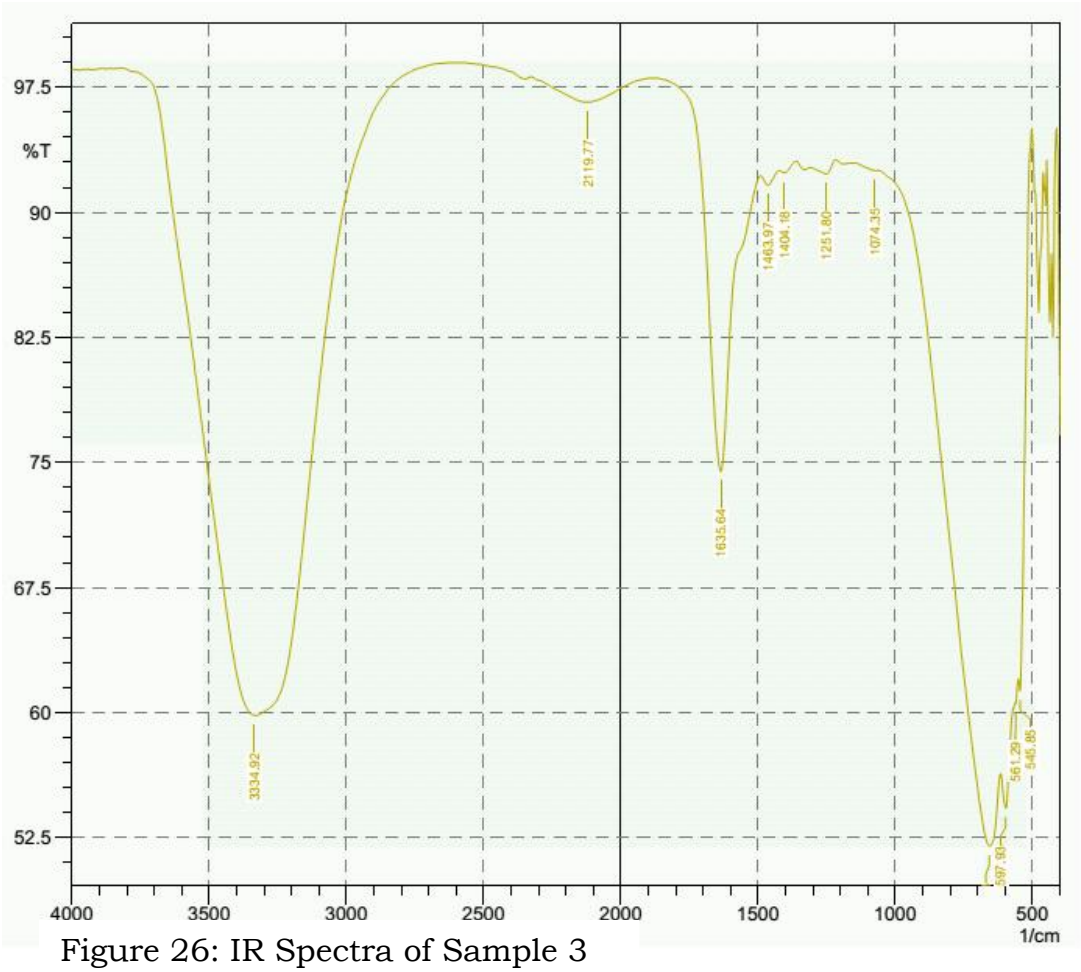


Figure 26: IR Spectra of Sample 3

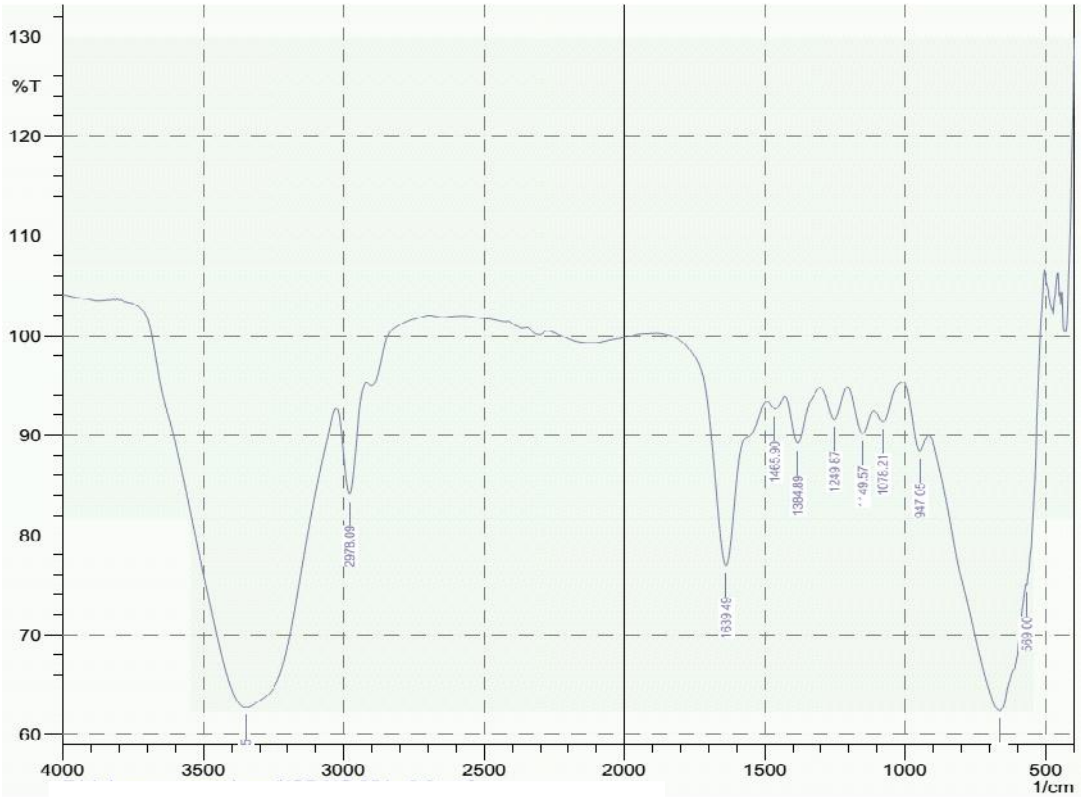


Figure 27: IR Spectra of Sample 4

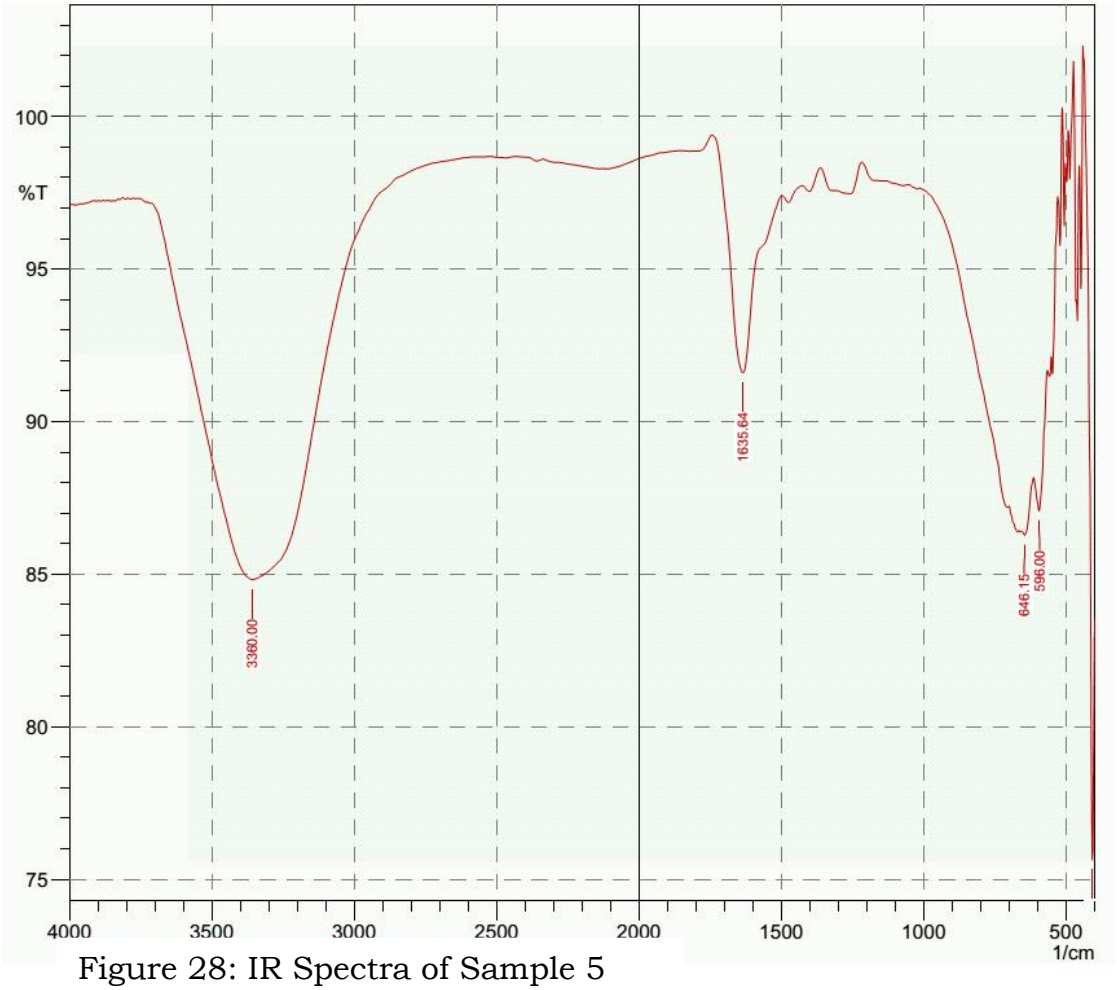


Figure 28: IR Spectra of Sample 5

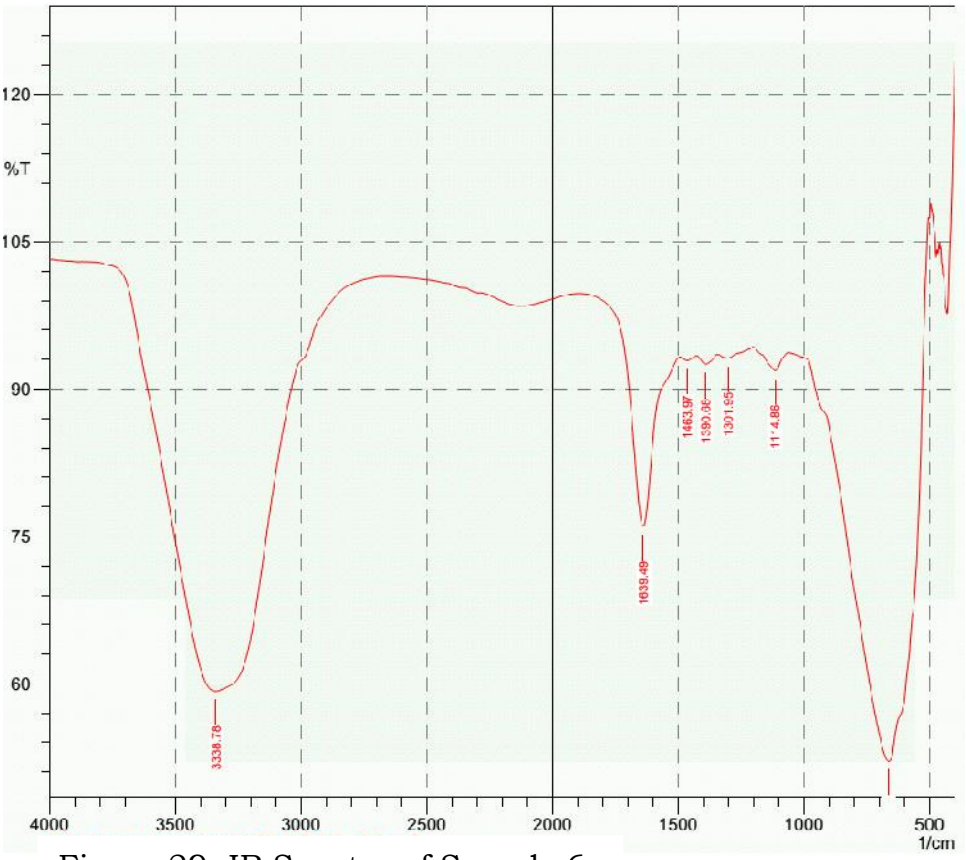


Figure 29: IR Spectra of Sample 6

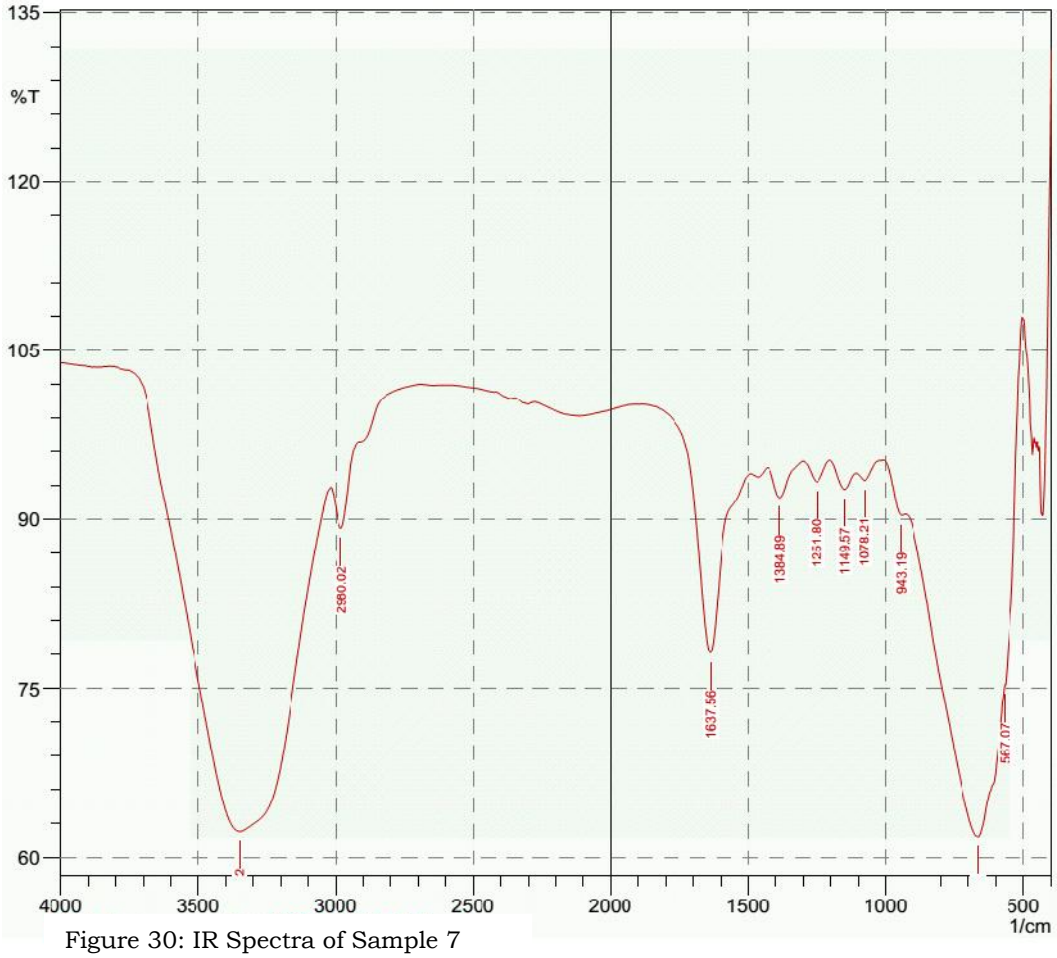


Figure 30: IR Spectra of Sample 7

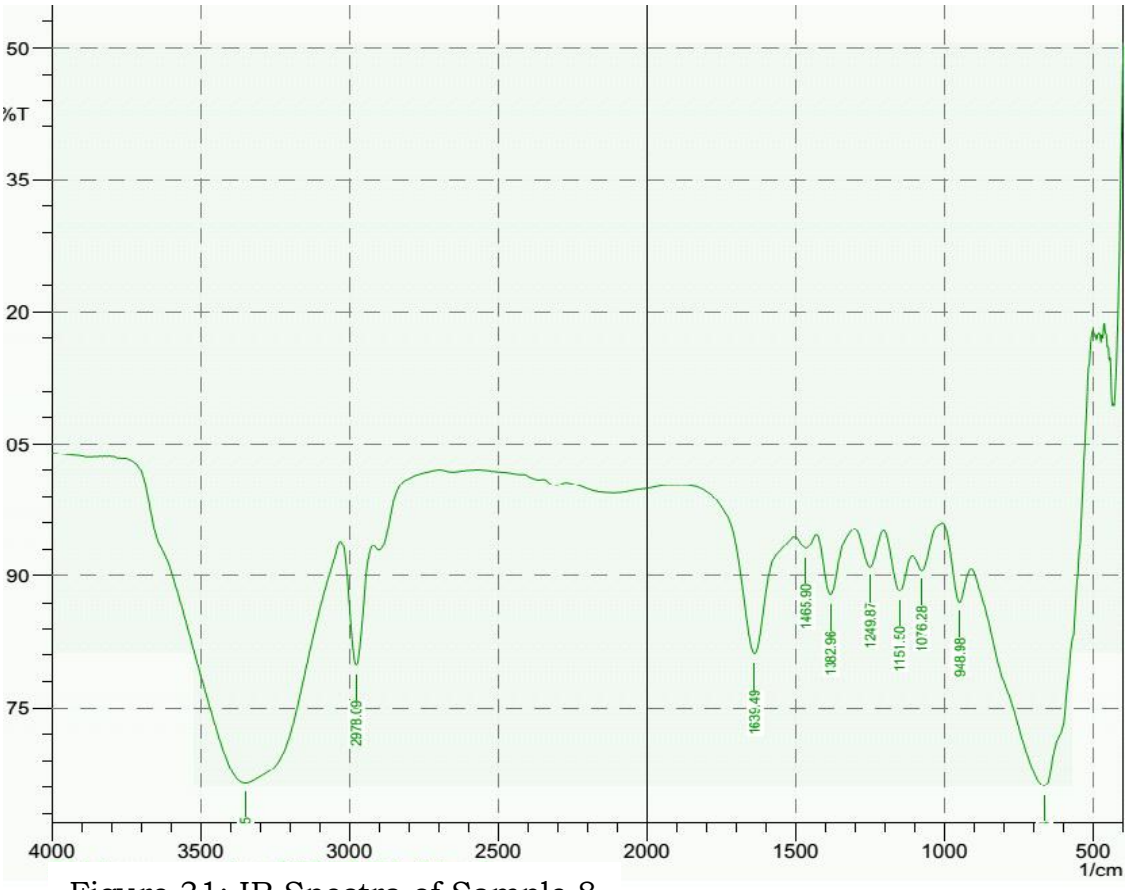


Figure 31: IR Spectra of Sample 8

Figure 32: IR Spectra of Sample 9

Figure 33: IR Spectra of Sample 10

Figure 34: IR Spectra of Sample 11

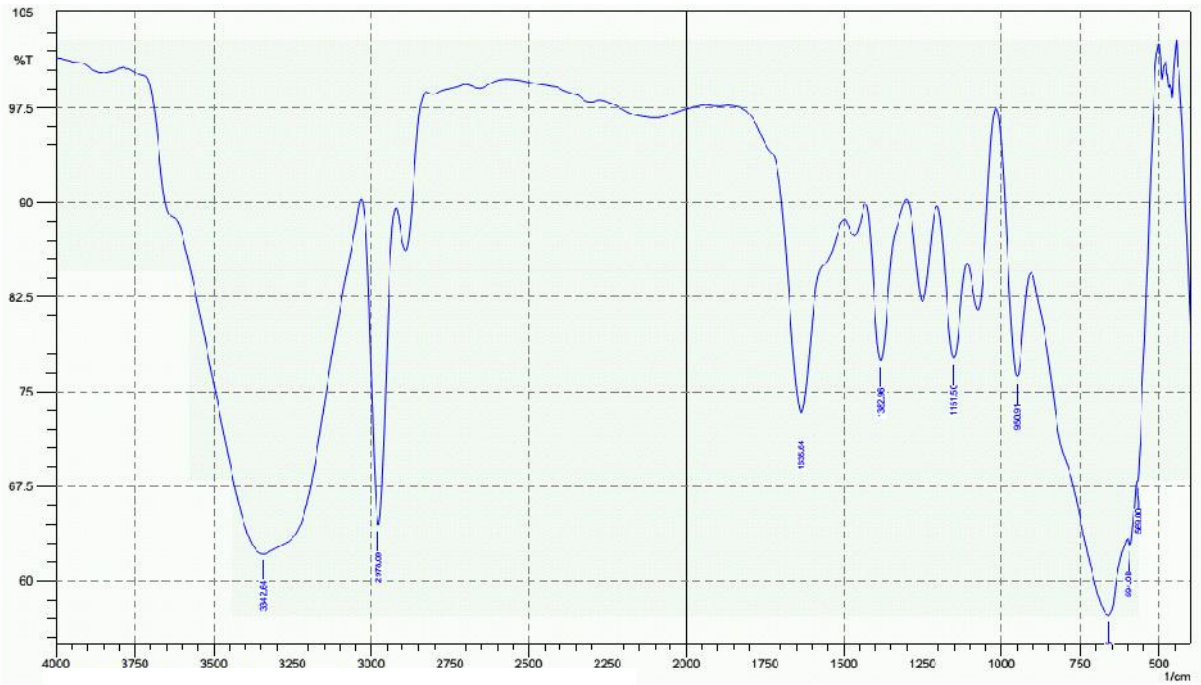


Figure 35: IR Spectra of Sample 12

It can be recognized from the IR spectra of samples 1-12 in figures 23-34, the collagen hydrolysate (CH) is characterized by amide A (3329 cm^{-1} –3360 cm^{-1}), amide B (2978 cm^{-1} –3285 cm^{-1})

bands, associated with NH stretching modes. Amide I (1633 cm^{-1} – 1640 cm^{-1}), amide II (1384 cm^{-1} –1550 cm^{-1}) and amide III (1149 cm^{-1} –1260 cm^{-1}) are characteristic for collagen in random coil protein which proved that the collagen was in hydrolyzed form [147].

4.10. Optimization of pH

The following figure 35 represents the protein extraction as a function of pH over the pH range 6-10. The influence of pH on the activity of the enzyme is given in figure 35. It is seen that the activity was very low at pH 6 and increased significantly upon increased in the pH, especially in the alkaline region (pH 8-9). Amalgamated trypsin and proteinase K with MgO gave more than 80% protein yield at the pH range 8-9. The protein yield extracted from full chrome and low chrome shavings was found 80.25% and 82.31% respectively at pH 8.5. Chromium present in the chrome shavings tends to precipitate as chromium (III) hydroxide at pH values above 8 [104]. The chrome complex of chrome shavings was cleaved and chromium was precipitated as $Cr(OH)_3$ during hydrolysis and hence, collagen was extracted efficiently.

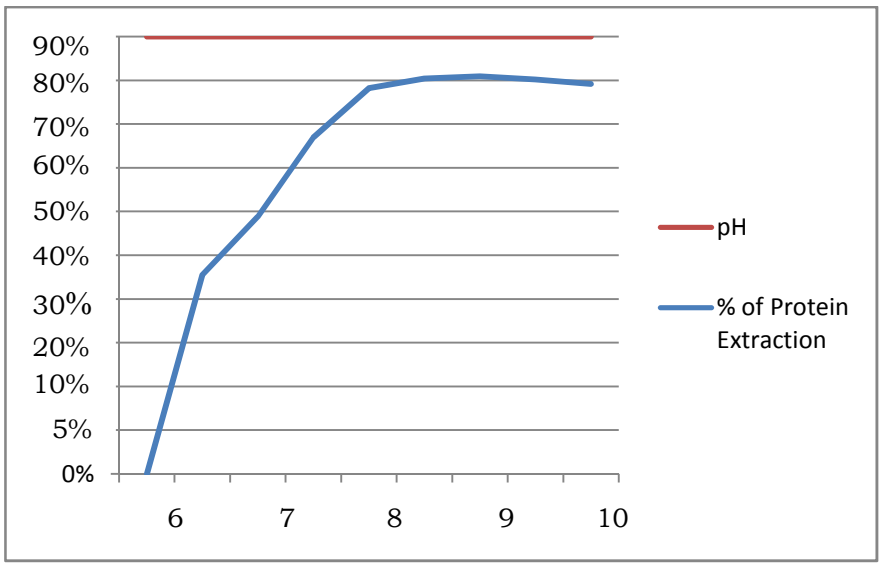


Figure 36: % of Protein yield at different pH.

4.11. Optimization of Alkali and its Dosages

The extraction of collagen hydrolysate from chrome shaving dust by different alkalis like MgO and CaO with different concentrations with and without enzymes was studied to find out suitable hydrolysis process. The collagen hydrolysate extracted by different alkalis is given in table 15. It was observed that the presence of trypsin and pepsin gave more protein yield. There was no significant difference in protein yield above 3 % alkali concentration in all the cases. MgO seemed to be good for hydrolysis since more protein yield was found with this. Therefore, MgO was selected as a suitable alkali with the optimum concentration of 3 % for the first extraction. The percentage of MgO/CaO was reduced to 2.5% and 2.0% for second and third extraction respectively, because the pH of the chrome sludge was already adjusted at 8-9 in the first hydrolysis.

Table15: Effect of different alkalis and their dosages on quantity of protein extraction.

Alkali	Concentration	Protein yield (%)		
		Without enzyme	Trypsin (0.1%)	Pepsin (0.1%)
MgO	2.0 %	34.27	43.75	38.89
	3.0 %	42.75	64.63	58.50
	4.0 %	43.84	66.13	60.32
CaO	2.0 %	27.50	41.54	33.12
	3.0 %	35.44	55.56	48.31
	4.0 %	36.62	56.88	48.98

4.12. Optimization and Amalgamation of Enzymes and their Dosages

In order to find the effect of different enzymes and their various concentrations on the extraction of collagen hydrolysate, 0.05, 0.10, 0.20 and 0.30% of different enzymes alone and in combination were used. The protein yield obtained by the amalgamation of trypsin and proteinase K with MgO was the highest (80.25%), then pepsin and proteinase K with MgO (73.56%). The protein yield by the treatment of trypsin and MgO was higher than that of pepsin and MgO and that was 64.63% and 58.50% respectively. The amalgamation of trypsin and pepsin with MgO was not effective because the protein yield by this treatment was not higher than the individual treatment with trypsin and pepsin and it was only 57.81%.**

Different concentrations of trypsin, pepsin, trypsin+proteinase K, pepsin+proteinase K and trypsin+pepsin were used in the hydrolysis process and 0.1% concentration was found to be optimum. The concentration of MgO used in all the experiments was 3.0%. Equal percentage of both enzymes was amalgamated and used during hydrolysis. It is seen from the table 16 and figures 37 and 38 that the activity of trypsin and pepsin increased when amalgamated them with proteinase K. The activity of enzymes on the extraction of collagen hydrolysate during the biochemical treatment of chrome shaving dust can be expressed in the following order:
trypsin+proteinase K > pepsin+proteinase K > trypsin > pepsin > trypsin+pepsin.

**N.B. Quantity of protein extraction is expressed in grams. Chrome shaving dust dosage: 50 g on dry basis.

Table 16: Effect of different enzymes and their dosages on quantity of protein extraction.

Concentration of enzyme	Protein yield (%)				
	Trypsin	Pepsin	Trypsin +Proteinase K	Pepsin +Proteinase K	Trypsin +pepsin
0.05%	55.04%	46.57%	72.65%	64.82%	49.38%
0.10%	64.63%	58.50%	80.25%	73.56%	57.81%
0.20%	65.37%	58.86%	81.58%	74.75%	58.25%
0.30%	65.79%	59.12%	81.67%	74.45%	58.42%

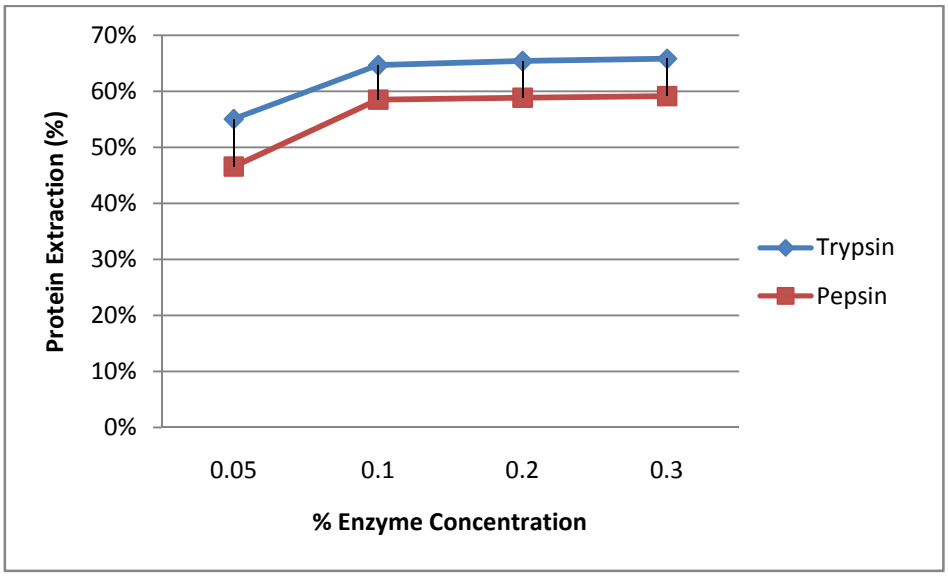


Figure 37: % of Protein yield at different concentrations of enzyme.

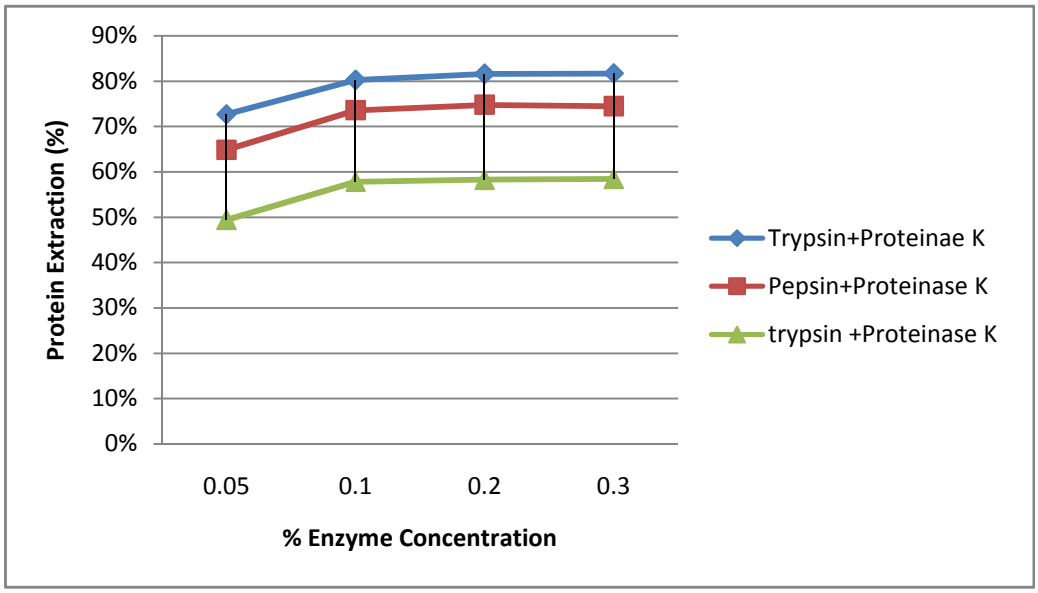


Figure 38: % of Protein yield at different concentration of amalgamated enzymes.

4.13. Analysis of Final Residue

The final residue after chrome recovery was analyzed for the determination of chrome and ash contents. The efficiency of chrome recovery was also calculated. The results are depicted in table 17 and 18.

Table 17: Efficiency of chrome recovery from chrome cake.

Sl. No.	Description	% Cr_2O_3	Efficiency of Cr Removal
01.	Full Chrome Shaving Dust	3.42 %	-
02.	Chrome cake of Sample 4 (Full Chrome Shaving Dust + MgO + Trypsin)	0.1244%	96.36 %
03.	Chrome cake of Sample 5 (Full Chrome Shaving Dust + CaO + Trypsin)	0.1942 %	94.32 %

The chrome content of full chrome shaving dust was 3.42% (Table 6) and after recovery of chromium from the chrome cake, the chrome content was found only o.1244 and 0.1942% for samples 4

and 5 respectively. Therefore, the percentage of chromium recovery was 96.36 and 94.32% respectively for both samples. It can be considered that satisfactory amount of chromium was recovered from the chrome cake and hence, the final residue was almost free of chromium.

Table 18: Result of Ash content of final residue.

Sl. No.	Description	Ash Content	% of Reduction
01.	Full Chrome Shaving Dust	5.18 %	-
02.	Residue of Sample 4 after chromium removal.	2.10 %	59.46 %
03.	Residue of Sample 5 after chromium removal.	2.32 %	55.21 %

The ash content of full chrome shaving dust was 5.18% (Table 6) and after chrome recovery it was found 2.10 and 2.32% for sample 4 and 5 respectively. It is seen that the ash content of final residue after removal of chromium was significantly reduced and it was 59.46 % and 55.21 % for sample 4 and 5 respectively.

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Chapter 5: Summary and Conclusion

5.1. Summary

This study has explored the efficient way of separating protein and chromium from chrome shavings through biochemical method. From the different analytical data, it can be said that the quality of prepared protein product i.e., collagen hydrolysate was superior. The product was totally odourless and was in gel form. The protein content of the product was also satisfactory and it was 60-82% in most of the prepared samples. The efficiency of protein extraction was between 60-85% in most of the cases. The most important and crucial parameter of this product was the chromium content since the product was extracted from the high chromium containing leather waste i.e., chrome shavings. The chromium content of all the prepared collagen hydrolysates did not exceed the reference value (<4.5 ppm) [31]. In some samples it was less than the detection limit (1 ppm). Therefore, the prepared collagen hydrolysate is completely safe for being used as protein ingredient for the preparation of poultry feed and fertilizer.

The key findings of this research work are outlined below:

i. A safe and scientific method has been developed for the preparation of collagen hydrolysate from the tannery solid wastes, namely chrome shavings. This method is eco-friendly and can be referred as biochemical method.
ii. The prepared collagen hydrolysate is almost free of chromium except trace amounts found in all samples (<4.5 ppm).
iii. The prepared collagen hydrolysate also contained appreciable amount of calcium and magnesium (1165 ppm and 1030 ppm respectively) which are essential minerals for poultry feed and fertilizer and thus, the product will be very potential for use as poultry feed and fertilizer.

iv. The ash content of the prepared collagen hydrolysate was found to be minimum and it was much less than the reference value (4.9%) [139].
v. The protein content of the prepared collagen hydrolysate was also reasonable and it was found 60-82% in most of the prepared samples.
vi. The chromium from chrome cake has been effectively separated and can be used as chrome tanning agent. 96.36% of chromium was recovered from the chrome cake which proved the efficiency of the developed method.
vii. The final residue after chrome recovery is almost free of chromium and the ash content of the final residue was also found much less (2.10%) than the untreated full chrome shaving dust (5.18%) and thus, the final residue will be safe for land filling and composting without creating any environmental pollution.

5.2. Limitations

This research work is innovative and totally new in Bangladesh and, therefore, laboratory facilities were not sufficient for hydrolysis of tannery solid waste and the complete analysis of prepared collagen hydrolysate.

The following limitations have been identified during the research work:

i. Batch reactor was necessary for the hydrolysis process of chrome shaving dust but the reactor was not available during the practical work. Therefore, process control could not be maintained accurately.
ii. Proteolytic enzyme was not available in the market and especially proteous alkalase was not found and it was

the main enzyme for hydrolysis of chrome shaving dust. Trypsin and pepsin have been used as alternative of proteous alkalase in hydrolysis process and hence, extraction of protein was not much more than 80 %.

iii. Collagen hydrolysate is sticky in nature and after hydrolysis, it should be separated through vacuum filtration but vacuum filtration unit was not available during the practical work. Force filtration was used to separate the collagen hydrolysate from hydrolyzed chrome shavings. For this reason, recovery of protein yield was little less than the expectation.

iv. The protein or collagen hydrolysate after extraction should be preserved at 4 ºC in an incubator but incubator was not available in the laboratory and hence, the degradation of hydrolysate might happen during the preservation in a normal refrigerator at 8-10 ºC.

v. Determination of Amino Acid content of prepared collagen hydrolysate was not possible at any laboratory in the country due to the restriction of analysis of leather sample.

vi. Gel strength and viscosity which are also important parameter of collagen hydrolysate for quality assessment, could not be measured due to the unavailability of the instruments.

vii. The whole practical work was carried out at the laboratories of Leather Research Institute (LRI), Noyarhat, Savar and all instrumental analysis was done at Center for Advanced Research on Science (CARS), University of Dhaka. Therefore, long time transportation at hot environment might cause the degradation of samples and gave poor results in some cases.

5.3. Recommendation

Since the treatment of tannery solid wastes is a new approach in Bangladesh and it is a "waste to resource or wealth" approach, the research work will have a tremendous future prospect. This approach will find out a complete solution of tannery solid wastes to create valuable protein products which could be potential raw materials for various industries including leather processing, poultry feed and fertilizer production. New industrialization of producing protein products from discarded tannery solid wastes may create more employment and business opportunities in Bangladesh.

There is some recommendations mentioned below for the integrated solid waste management of Bangladesh tannery industries to find a new dimension for solid wastes treatment and to reveal new market for extracted protein products from tannery solid wastes.

i. The traditional unscientific and hazardous method of producing protein products from chrome shavings in Hazaribag should be immediately stopped, since the product contains much chromium and other toxic elements. Protein concentrate produced in Hazaribagh contained chromium concentration of 2.49% as element [115]. Therefore poultry chicken meat is very dangerous for human body as the chromium accumulate into human body through food chain [15].

ii. The prepared collagen hydrolysate can be used in poultry feed manufacture, since it is almost free of chromium and thus, safe. Moreover, it contains appreciable amount of Ca (1165 ppm) and Mg (1030 ppm) which are essential minerals for poultry feed [142].

iii. The prepared collagen hydrolysate after chemically modified may also be used in tanning, re-tanning and finishing of leather processing.

iv. This may be a potential raw material for fertilizer manufacturing, bio-fuel preparation, tissue engineering, pharmaceutical and cosmetic industries.

v. The recycled basic chrome sulfate can be used alone or in combination with fresh basic chrome sulfate in chrome tanning and re-chroming operation of leather processing.

vi. The final residue, after protein extraction and chrome recovery, may be used for composting or land filling purpose since the chrome and ash contents of the residue was found much less (0.12 and 2.10% respectively) than the untreated chrome shavings (3.42 and 5.18% respectively).

vii. Several methods were developed to extract collagen hydrolysate from chrome shavings. The efficient and cost effective method may be implemented at pilot plant basis to proceed for industrial application of the developed method.

viii. Further research may be carried out on this research topic to reveal and evaluate the multitudinous applications of collagen hydrolysate especially in chrome tanning, re-tanning and finishing operations of leather processing, bio-gas generation, cosmetic and pharmaceutical industries.

5.4. Conclusion

In this study, solid wastes generated from tannery namely, chrome shaving dust was treated by bio-chemical method using mild alkali and laboratory grade proteolytic enzymes to separate protein and chromium. Several methods were developed for bio-chemical hydrolysis of chrome shaving dust to extract collagen hydrolysate. All the methods consisted of a three-step process in which collagen hydrolysate was extracted and separated at the end of each step. The prepared collagen hydrolysate contained very trace amount of chromium (<3.5 ppm) and reasonable amount of essential minerals such as Ca (1165 ppm) and Mg (1033 ppm). Hence, it will be potential as protein ingredient for poultry feed and fertilizer, and as proteinic re-tanning and finishing agents after chemical modification. Chromium was also recovered from the chrome cake after hydrolysis and the recovery rate was 96.36% and 94.32% for samples 4 and 5 respectively. The ash content of the final residue after chrome removal was found to be reduced significantly and thus, the final residue can be safely disposed of or composted without any pollution. Therefore, the developed method will be a very much potential for the leather industry in solving the difficulties of disposal problem of chrome containing leather wastes and may obtain economically beneficial.

The FTIR study of the protein product proved that the prepared collagen protein was in hydrolyzed form. From the analytical results, it is seen that the collagen hydrolysate extracted by the treatment of trypsin enzyme and MgO, gave high content of protein. On the other hand, trypsin enzyme amalgamted with proteinase K with MgO, extracted the highest protein yield (82.31%). Collagen hydrolysate extracted by other processes also gave good protein yield. All physical and chemical parameters of prepared collagen hydrolysate were found to be good and complied with the reference values. It is notable

here that all the prepared collagen hydrolysate samples were almost free of chromium except the presence of very trace amount which did not exceed the reference value (<4.5 ppm) [31].

Waste management approaches are now a days gaining importance in all the industries for sustainability. In this study an attempt was made to extract and reuse protein and chromium from chrome shaving dust through bio-chemical hydrolysis method. It was demonstrated in this research work that enzyme technology had great potential for recycling solid wastes, namely chrome shaving dust. The developed method required a few chemicals such as proteolytic enzymes (trypsin, pepsin and proteinase K) and mild alkalis (MgO and CaO) and no hazardous chemicals were used in the hydrolysis process. Thus the developed method can be claimed as totally environmentally friendly, cost effective and efficient in extraction of value added products from chrome containing leather wastes (CCLW), avoiding the generation of any other waste. In other words, this method can completely utilize CCLW with zero discharge.

There is a need to evaluate the viability of the developed method for commercial applications. Therefore, extracting collagen from tannery solid wastes not only decreases environmental contamination, but also can give economical benefits to tanners and can create new era for industrialization and business.

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Costing

The collagen hydrolysate is very potential to be used as poultry feed, organic fertilizers and re-tanning & finishing agents in leather processing. The estimated cost of the prepared collagen hydrolysate is projected according to the standard costing procedure and format of Bangladesh Council of Science & Industrial Research (BCSIR). A pattent Entitled "A Process for High Value of Protein from Chrome Shavings" has been submitted to BCSIR for approval.

1. Production capacity per year: 300 M.T

   i) No. of working days/year : 300 days/year

   ii) No. of working shifts/day : 1(one) shift/day

2. FIXED COST

2.a. Land: Mill will be set up in a rented house (area approx. 1500 sft.).

2.b. Plant and machinery including laboratory equipments:

i) Local (as per annexure-I)	: Tk. 24,50,000.00
ii) Erection and commissioning of plant & machineries (Civil/Electrical/Mechanical 7.5% of fixed machinery cost)	: Tk. 1,83,750.00
	Total: Tk. **26,33,750.00**

2.c. Utility system & other service equipments:

i) Power supply installation /connection cost + security money	:Tk. 30,000.00
ii) Water supply installation /connection cost + security money (Hand Tube-well)	:Tk. 6,000.00
iv) Furniture /Fixture and Office/Safety equipments	:Tk 40,000.00
v) Transport, if any (Rickshaw van 2 Nos.)	:Tk. 40,000.00
vi) Effluent disposal system	:Tk 10,000.00
vii) Others, if any, specify	:Tk. 10,000.00
	Total:Tk. **1, 36,000.00**

2.d. Technical know-how, consultancy & others:

i) Technical (process) know-how fee (premium/Royalties etc)	:Tk. **3,00,000.00**

Total fixed cost: Tk. = **30, 69,750.00**

3. Running cost/year

3.a. Raw materials (annexure-II)

i) Local (including stock) :Tk. **4,00,80,000.00**
(delivery at site, including transportation)

3.b. Manpower (annexure III)

i) Wages for technical/skilled manpower :Tk. 9,80,,000.00
ii) Salaries for Supervisory manpower :Tk. 2,10,000.00
iii) Salaries for Marketing/Sales :Tk. 2,80,000.00
iv) Wages for unskilled labour :Tk. 3,22,000.00

Total: Tk. = **17, 92,000.00**

3.c. Utilites/year (with supporting estimates)

i) Cost of water consumption :Tk. 36,000.00
ii) Cost of electricity Tk. 100/- per day :Tk. 2,16,000.00
iii) Cost of other fuels/lub. oil. etc. :Tk. 10,000.00
v) House rent @ 3,000/- per month :Tk. 3,60,000.00

Total: Tk. **6, 22,000/-**

3.d. Depreciation & other indirect expenditure /year:

i) Depreciation on plant & machineries :Tk. 2,63,375.00
(considering salvage value and life period) 10% of machinery cost .
ii) Repair & maintenance cost :Tk. 50,000.00
iii) Insurance & Taxes 1% of fixed cost :Tk. 30,697.50
iv) Contingency and other unforeseen expenses :Tk. 30,000.00
v) Marketing & distribution expenditure :Tk. 50,000.00
(packing/labeling/advertisement & sales commission etc.)

Total: Tk. **4, 24,072.50**

Total cost of production :Tk.= **4,29,18,072.50**

4. Other financial expenses/year

Loan : Tk. 4,50,00,000.00

i) Bank interest on fixed cost Installment : Tk. 2,04,650.00

(15 years) + Installment Interest : Tk. 399,067.50

(13%)

ii) Bank interest on running cost(13%) Loan : Tk. 4,50,00,000.00

(15 years) + Installment Installment: Tk. 30,00,000.00

Interest: Tk. 58,50,000.00

iii) Miscellaneous expenses :Tk.63,79,5,00.00
(if any, please specify)

Total Tk. = **1, 58,33,217.50**

5. Revenue from sales/year

i) From finished product (300 M.T @ Tk.250.00 per kg) :Tk. =7,50,00,000.00

From by-product (500 M.T @ 30.00 per :Tk. =1,50,00,000.00

Total: Tk.= **9,00,00,000.00**

6. Gross profit/year = (9,00,00,000.00 – 4,29,18,072.50)
= **4, 70,81,927.50**
(Total revenue from sale – total cost of production)

7. Net profit = (4,70,81,927.50 – 1, 58, 33,217.50)
(Sl. No. 6 - Sl. No. 4) = **3,12,48,710.00**

Profitability analysis

8. Return on investment (%)

$$= \frac{Net\ \text{Pr}ofit}{Totalcapitalinvestment} \times 100 = \frac{3,12,48,710.00}{4,55,63,750.00} \times 100 = 68.58\%$$

Here, Total capital investment = Total fixed cost + Working capital
= 30,69,750.00 + 4,24,94,000.00
= 4,55,63,750.00

Working capital = Raw materials = 4,00,80,000.00
+ Man power = 17,92,000.00
+ Utilities = 6,22,000.00
= 4,24,94,000.00

9. P/V Ratio = $\frac{\text{Revenue from sales/year}}{\text{Total cost of production/year}} = \frac{9,00,00,000.00}{4,29,18,072.50} = 2.10$

10. Breakeven point (sales)

$$= \frac{\text{Total Fixed cost}}{\text{Revenue from Sales - Running Cost}} = \frac{30,69,750.00}{9,00,00,000.00 - 4,29,18,072.50}$$

$$= \frac{30,69,750.00}{4,70,81,927.50} = 0.07$$

11. Pay back period $= \frac{\text{Total capital investment}}{\text{Net profit/year}} \frac{4,55,63,750.00}{3,12,48,710.00} = 1.46$ years

Annexure i

List of machineries (local) with cost

Sl. No.	Name and specification of machineries/plant	Quantity /unit	Unit Price (Tk)	Total price (Tk)
1.	Reaction Vessel 500 kg capacity	3 No.	5,00,000.00	15,00,000.00
2.	Balance (top load) 100 kg	2 Nos.	25,000.00	50,000.00
3.	Vacuum filtration unit	1 No.	8,00,000.00	800,000.00
4.	Analytical Balance	2 Nos.	25,000.00	50,000.00
5.	Other tools and accessories	-	-	50,000.00
			TotalTk=	24,50,000.00

Annexure ii

DETAILS OF RAW MATERIALS

A. IMPORTED RAW MATERIALS – Not applicable

B. LOCAL RAW MATERIALS

Sl. No.	Description of raw materials	Probable source	Quantity required	Unit price / M.T. (Tk)	Total price (Tk)
1.	Chrome shaving dust	Local Markets	900 M.T	500.00	4,50,000.00
2.	CaO	Local Markets	18 M.T	5,00,000.00	90,00,000.00
3.	MgO	Local Markets	18 M.T	5,00,000.00	90,00,000.00
4.	Enzymes	Local Markets	1.8 M.T	1,20,00,000.00	2,16,00,000.00
5.	Sodium benzoate	Local Markets	0.3 M.T	1,00,000.00	30,000.00
					Total Tk.=4,00,80,000.00

Annexure iii

DETAILS OF MANPOWER

Sl. No.	Category of manpower	Nos. required	Monthly wages/ salary (Tk)	Annual involvement (including two bonus) (Tk)
A.	Technical (skilled)			
	1. Manager	1 No.	40,000.00	5,60,000.00
	2. Plant Engineer/chemist	1 No.	30,000.00	4,20,000.00
	3. Supervisor	1 No.	15,000.00	2,10,000.00
	4.			
B.	Unskilled labour			
	1. Labour	2 Nos.	8,000.00	1,12,000.00
	2. Peon	1 No.	8,000.00	1,12,000.00
	3. Night guard	2 Nos.	7,000.00	98,000.00
C.	Marketing/Sales Promotion Officer	1 Nos.	20,000.00	2,80,000.00

Total Tk. 17,92,000.00