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**Review Article**

# The Economic Significance of Animal Products and Methods Used in Leather Protection

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## Abstract

Since ancient times, humans have used animal skin in their daily lives to create shelters and clothing. They have also developed special techniques to preserve animal hides for a long time after slaughter, such as salting and cooling. Skin is an ideal environment for microorganisms to inhabit due to its temperature and moisture content. According to our knowledge, many factors, including pH, temperature, salt concentration, nutrition, etc., influence bacterial growth. Therefore, many bacteria can thrive in a wide range of NaCl concentrations, from 1% to 20%, and are classified accordingly. Despite the high concentration of NaCl in the soak liquor used in the preparation of leather, numerous bacterial species can readily grow and produce lipolytic and proteolytic enzymes as a form of metabolic activity. Lipolytic and proteolytic enzymes have negative effects on leather and leather products, including spotting, disagreeable odor, discoloration of the resultant leather, and consequentially significant economic losses. To overcome this obstacle, a variety of preventative measures have been implemented, including salting, the use of antibiotics, plant extracts (essential oils), and, more recently, electric currents.

**Keywords**: *Antibiotics, Antifungal,* Electric Current, *Economic Losses, Collagen, and Leather*.



## 1. INTRODUCTION

### 1.1. Skin

The hide, or skin, is a vital organ that protects animal bodies from external threats and serves as the initial non-specific defense mechanism. Additionally, it assists regulate the animal's body temperature. Due to its significant influence on leather production, it is commonly referred to as the core of the leather industry. The skin has been completely examined by numerous researchers and is naturally made up of three layers. The outer layer, known as the epidermis, consists primarily of soft keratin and serves as the main structural unit of this layer. The second layer, called the dermis, is primarily composed of collagen and is responsible for the flexibility and strength of the skin. The third layer is made up of adipose tissue, also known as subcutaneous tissue, which is crucial to the overall structure of the skin as it serves as the control center for skin functions [1, 2]. Figure (1). Animal raw skins contain approximately 25-30% protein, primarily collagen, which is a polymer. They also consist of 60-65% water, making them a favorable environment for bacterial and fungal growth. Additionally, they contain 2% lipids and other residual components such as minerals and carbohydrates. Due to the composition of the skin, it provides an appropriate environment for microbial reproduction. Typically, the skin undergoes bacterial decomposition within 8-12 hours after an animal is slaughtered [3]. The chemical compositions of raw animal hides are explained by [4], and can be seen in Figure (2). The thickness and morphology of animal skins vary depending on factors such as age, gender, race, species, diet, nutritional composition, and environmental conditions, and region. The hairy dorsal animal has thicker skin anatomically compared to the hairy skin in the central region [5]. The epidermis layer is generally thinner than the dermis and hypodermis, but it can be thicker in certain areas that serve a protective function [5]. Hides contribute for around 4-12% of the total weight of living animals [6]. Other researchers have provided data on the proportion of skin in proportion to the total body mass of various animals. For instance, the skin percentage of total body mass in cow, pig, and sheep ranges from 11.0% to 11.7%, 3.0% to 8.0%, and 5.1% to 8.5% respectively (wt/wt) [7]. The thickness of animal skin was approximately 9.0 (wt/wt) in goats [8], and ranged from 8.0 to 20.0 (wt/wt) in chickens [9]. Animal skin has a higher nutritional content compared to plants, making it a preferred source of nutrition for humans [10, 11]. The structure of animal skins is a unique composition that is susceptible to both internal and external environmental influences, as revealed by earlier research [12-14]. The thickness and morphology of animal skins vary depending on factors such as age, gender, race, species, and region. The hairy dorsal animals have thicker skin anatomically compared to the hairy skin in the central region [5]. The epidermis layer is generally thinner than the dermis and hypodermis, but it can be thicker in certain areas that serve a protective function [5]. Hides contribute for around 4-12% of the total weight of living animals [6].

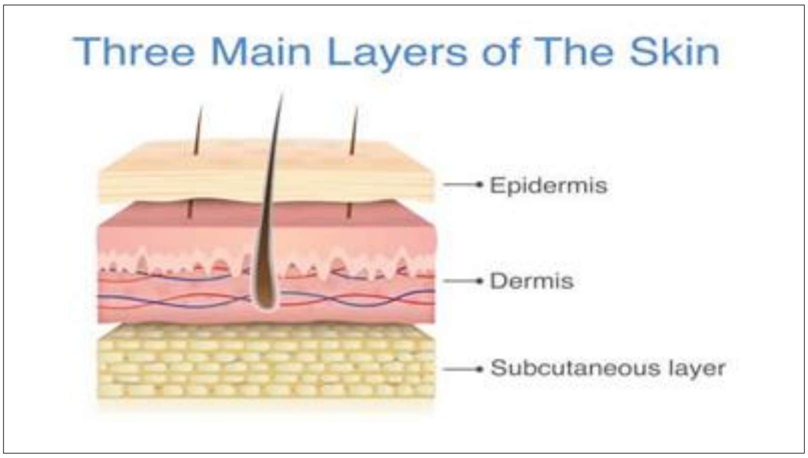



**Figure 1: The layers of animal skin [15].**

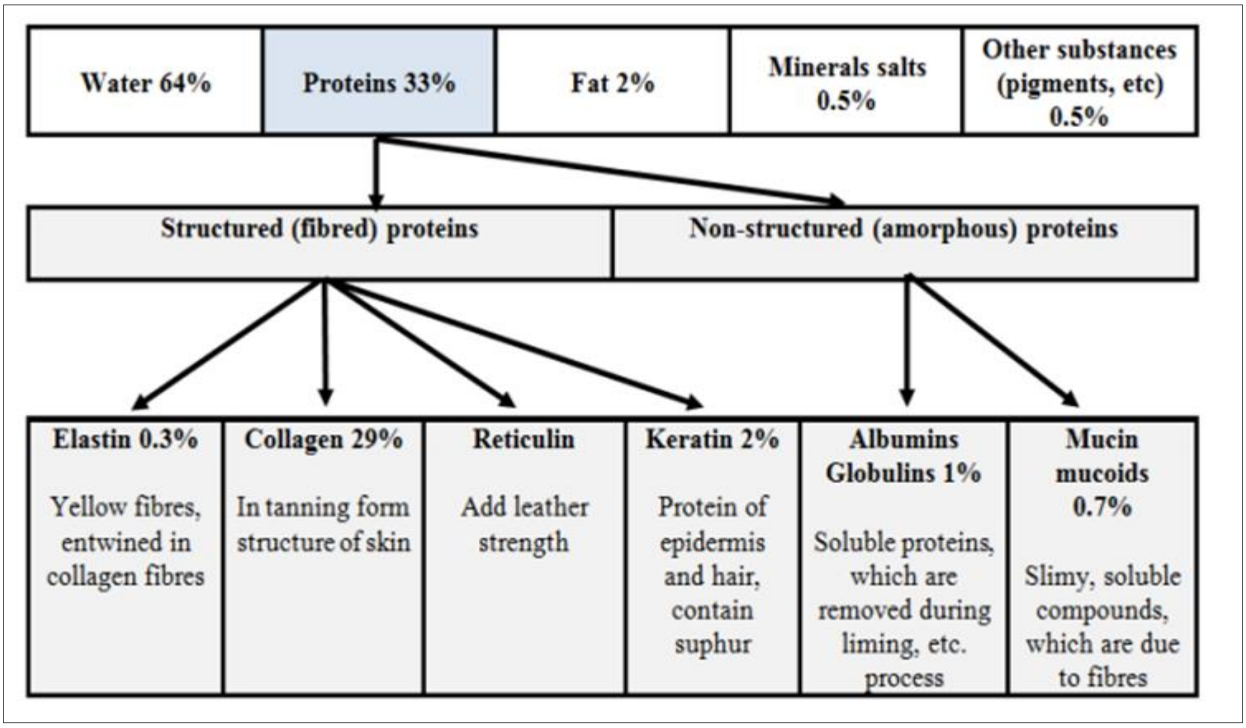



**Figure 2: Illustration of the chemical compositions of Animal raw Skin/Hides [16], cited by [17].**

The embryological origin of the components of animal skins is varied. Dermal fibroblasts are derived from the neural crest and mesoderm, whereas epidermal keratinocytes are derived from the ectoderm [18]. On the other hand, melanocytes are derived from the neural crest [19]. The main animal products encompass hide, dairy, and flesh [20]. Traditionally, animal products are commonly used as a main component of the human diet (National Research Council). Animal products account for more than 60% of the protein consumption in the United States. From an immunological perspective, the skin is regarded as a durable barrier due to its abundance of blood vessels, immune cells (such as macrophages, T cells, monocytes, neutrophils, mast cells, and dendritic cells), [21, 22] immune receptors (specifically Toll-like receptors) produced by keratinocytes, and skin microbiomes (normal flora) [23]. Therefore, the skin offers protection against external environmental influences, such as invading germs including bacteria, viruses, parasites, and fungi. The dermis layer typically has a higher concentration of immune cells compared to the epidermis layer, which is the location of Langerhans cells, a kind of antigen presentation cell (APC). The skin has a role in directing the immune response by generating chemokines, cytokines, TLR, and growth factors through the action of Keratinocytes in the dermis layers [23, 24]. The skin is a complex organ that contains antimicrobial peptides, such as ß-defensins, which serve as a protective barrier against pathogens. The skin functions as an immune regulatory organ by detecting invasive microorganisms and triggering an immune response or creating an environment that is tolerant to foreign antigens. Additionally, it can establish a connection between the particular and non-specific immune systems [23]. In addition, the skin acts several functions, such as acting as a barrier against the penetration of chemical, biological, and physical agents. It also protects DNA from UV damage and functions as an excretory system and thermoregulation organ, because of its sweat glands, blood vessels, and hair. Furthermore, the skin plays a role in the sensory system and contributes to communication [25]. The skin microbiome refers to the collection of bacteria that inhabit the surface of healthy animals' skin. The skin microbiome of humans, dogs, and chickens has been studied independently by [26-28]. The findings by Chen *et al*., (2018) indicate that there are distinct differences between the skin microbiome and the gut microbiome. Furthermore, the lack of the gut microbiome has detrimental consequences on gut development, but the removal of the skin microbiome does not have any impact on skin development [29].

**2. Leather Industry**

According to data from the FAO (Food and Agriculture Organization) in 2001, the global production of leather was approximately 1.67 billion square meters [30]. Typically, animal skins and hides, particularly those from vertebrates such as sheep, goat, cow, buffalo, and pig, are commonly utilized in the production of leather [30]. Furthermore, fish skins are utilized in the manufacturing of leather [31]. Leather is formed using a series of interrelated steps that transform skins and hides into a hard and flexible product, which serves as the major structural unit of leather. Throughout history, animal skins, particularly those of cattle, have been widely utilized in the production of leather. The quality of employed materials and their validity can be determined by many indicators, such as high permeability and the presence of natural grain in leather [32]. The primary and crucial initial stage in leather production is salting. After the skin is taken from a slaughtered animal, it is referred to as fresh green at this stage. The aim of this approach is to maintain and preserve the skin to the greatest extent feasible. Various chemical curing agents have been utilized to achieve this objective, including 50% w/w of sodium chloride (NaCl) for skin preservation [33, 34], a combination of NaCl and EDTA [33; 34], NaCl with silica gel [35], NaCl with sodium meta-bisulphite (SMBS) [36], NaCl with boric acid (BA) [37], potassium chloride [38-40], sodium sulphate ($Na_2SO_4$) [41-43], silicate [44], peracetic acid [45]. Due to the potential of the aforementioned materials to cause skin dehydration, it is necessary to proceed to the next step, which involves rehydrating the skin. This can be achieved by washing the skin and immersing it in a solution containing suitable chemicals and NaCl, or by utilizing protease enzymes derived from microorganisms. The purpose of this step is to remove unwanted non-protein substances, hair, dirt, and grease. Additionally, this process serves to facilitate the opening of the skin layers, enhancing the permeation of chemicals into the skin and preparing it for subsequent procedures [46]. The first phase showed above consists of several secondary steps, namely salting, dowsing, fat, and hair removal, liming, deliming, and tanning methods. Furthermore, the primary subsequent stage, known as the "pickling" stage, involves treating the skin with alkaline chemicals and a solution containing NaCl + $H_2SO_4$. This treatment assists to protect the skin against degradation caused by lipolytic and proteolytic bacteria and fungi, as well as to regulate the pH value of the skin. This procedure confers long-lasting antibacterial protection to the epidermis over a period of one to two years [46]. The third stage involves the tanning process, when the skin is treated with metal salts like chromium. Alternatively, other methods such as employing biomaterials derived from plants or microbial enzymes can be employed to achieve this stage [31-47]. Due to the negative environmental impacts associated with the extensive use of chemicals in the tanning process, researchers have investigated the using of eco-friendly and readily biodegradable materials as alternatives. These materials include plant extracts, animal byproducts such as animal brains, and microbial enzymes. Indeed, these substances enhance the flexibility of leather and its ability to withstand high temperatures. The leather produced from the tanning process is referred to as wet blue [31-48]. In order to enhance the elasticity of the leather for subsequent procedures, re-tanning techniques have been employed through the utilization of the vegetable tanning method. Additionally, the re-tanning stage is intended to render the leather more supple and facilitate the application of paint. Ultimately, the leather has been colored using specifically chosen dyes and a liquid solution [48]. Several aspects connected to animals influence leather manufacturing, such as the age of the animal. Researchers have discovered that the skins of older animals are stronger than those of younger animals, and they also exhibit reduced distension. The physical characteristics of leather are influenced by factors such as sex, slaughter weight, and nutrition. Leather obtained from male animals tends to be strong, whereas leather obtained from female animals tends to be soft. Therefore, the physical properties of animal leather, particularly skin thickness, are affected. These characteristics demonstrate the impact on leather products.

## 1. Tanning

Tanning is a process that alters the structure of the skin to transform it into leather and leather products. This is achieved through the use of chemical and natural substances, which help mitigate the negative impact of environmental pollutants. Chemical and biological methods are used in order to complete tanning process, among these approaches are chromium tanning methods, bio-tanning methods, calgon method, tungsten tanning method, iron and silica processes [6]. Typically, Chromium salts are widely utilized in the tanning process, with approximately 90% of leather industries worldwide employing metal salts such as chromium III and VI. These metals are known for their non-toxic properties [6-17]. However, it is important to note that they also have detrimental effects on the environment [49]. Leather industries employ two tanning procedures based on the specific requirements of leather manufacturing:

### 1.1. Chemical Tanning

Tanning is the process by which raw skin and hide convert into leather. In this stage is reliance on using various chemicals such as Chromium (Sodium dichromate ($Na_2Cr_2O_7$)) which is used in percentage to the skin at 1.5%-3% that cause changing in skins' pH. The objective of using chrome and other tanning agents is to increase the cross linking between free amino and carboxyl groups in collagen structure and tanning factors in order to increase the flexibility and the strength of leather as show in figure (3a). Also, $ZrO_2$ (Zirconium) + Silica, $AlK(SO_4)_2$ (aluminum potassium sulfate), formalin solution (formaldehyde), 5% sodium hexametaphosphate and aluminum use in leather manufacturing as tanning factors [6]. By diffusion process the chrome enters skin through the pores and causes physical, biological and chemical in collagen structure by cross linking binding to carboxyl free groups as reveal in figure (3b). Depending on information obtain from article posted by Andualem *et al*., (2014) the chromium salts cause environmental pollution due to the amounts of chrome that uptake by skin are 60-65% and the rest discharged with effluents [50]; also chrome is a carcinogenic substance that affects workers' skin, respiratory system, mucous membrane and kidney [17]. Tanning stage takes four to six hours.

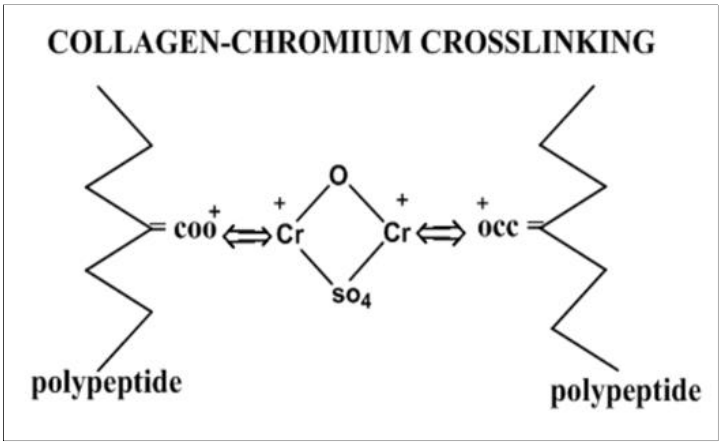



**Figure 3a: Explain the connection between collagen and chromium salts [51].**

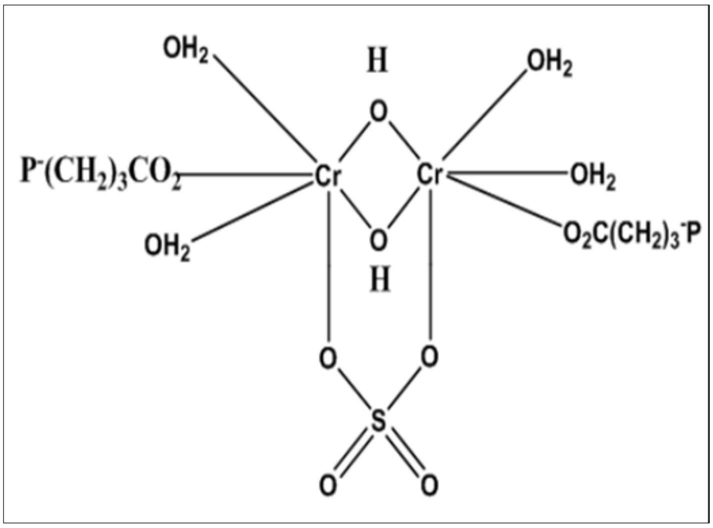


**Figure 3b: Explain the connection between collagen and chromium salts [52]**

**1.2. Bio-Tanning**

The bio-tanning or green tanning process involves the use of natural materials derived from plants (such as leaves, bark, roots, pods, cups, fruits, and woods) in a method known as vegetable tanning (Rolland, 2014). It also utilizes animal oils (such as fish oil from whales, sharks, cod, and seals), animal brains, and enzymes produced by microorganisms. These materials possess biodegradable properties, are environmentally friendly, and can be easily applied. The leathers that are tanned using this procedure are commonly utilized in the production of various things such as shoes, belts, wallets, pouches, saddles, holsters, and more [31]. Tannic acid and phenolic compounds derived from plants are utilized as tanning agents. These substances immediately bind to collagen in the skin, altering its structure and rendering it resistant to the detrimental effects of bacteria and fungi. Additionally, they reduce the solubility of collagen in water (figure 4). Several parameters influence the leaching process in vegetable tanning, including extraction temperature, extraction time, agitator speed, and the ratio of solvent to solid.

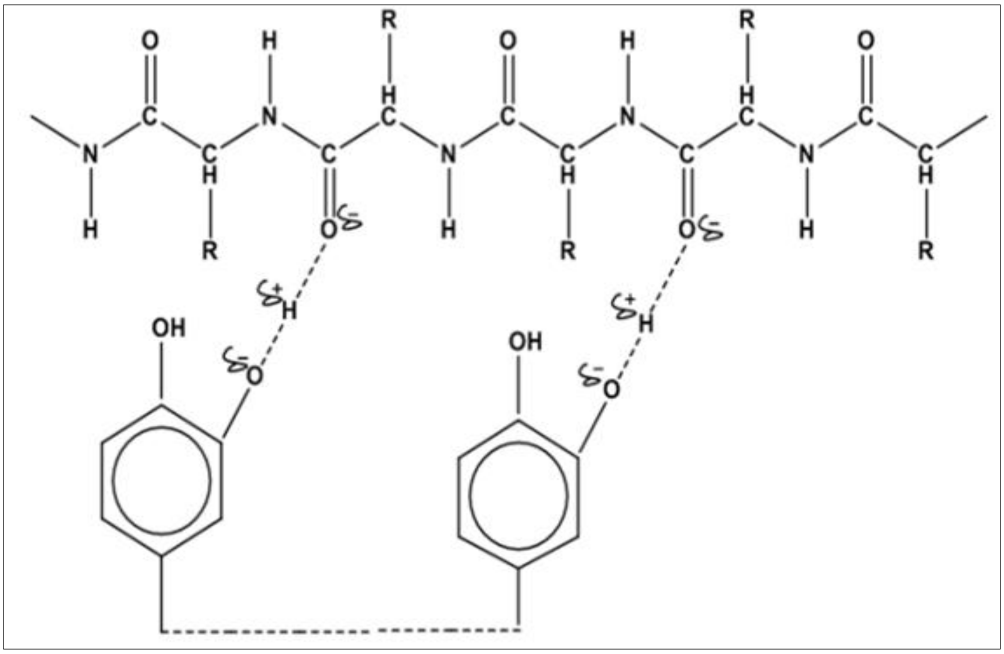


**Figure 4: Describes the interaction between plants extracts and collagen [53].**

**2. Economic Importance of Animal Skins**

Since a long time human has utilized animal hides for own requirements. Especially nomadic people nowadays uses the animal skin in purpose of produce their weapons, shelters, food containers and clothes. Even though the development of new techniques and produce new synthetic materials the animal skins has been still demanded because they have many distinguish characteristics such as cheap, high easy to handle, and abundant in a large amount [6]. In developing countries the leather industries have a clear economic effect [54]. According to published data by [55], nearly US 80 billion have been traded from the global leather trade annually. Brazil contributes in leather trade globally because it has more than 300 leather industries and it exports a huge amount of raw skin/hide to other countries due to it has approximately 213.5 million cattle heads. According to data in 2019 and 2020 by IBGE and CICD respectively Brazil has the biggest commercial cattle herd around world [56, 57]. The sustainable management of animal by products has been noticed as an important effect on the global economic situation. Altogether, the information that published about this sector is insufficient [58]. There are many reasons that caused losing in the animal by-products economic trade especially in India including the grow up of livestock population, increasing demand of meat and meat products, increasing slaughter housing and improper utilization of animal byproducts. Depending on the statistical data just in India approximately 8000 million Rs. (Indian rupees) every year has lost due to the misuse of animal byproducts [58]. On the contrary, each part of livestock's body use in order to make a lot of goods and use it in different aspects for example animal blood have used as a fertilizer (soil pH stabilizer, seed coating), animal feed, in medicine (to do some tests such as Agglutinin test), in the lab for make a media that used for bacteria growth and make tissue culture media), also as adhesive material, in plastic producing (industry), and produce cosmetic equipments [58]. The Interest in animal bones increased in the last years and used in the synthesis of Gelatin capsule and syrup, food industry as well. So far many or industries were established depending on animal bones because bones are rich with phosphor and calcium (15.2% and 32.6% respectively), in addition to small amounts of other mineral such as Na, K and mg and traces of manganese, zinc, copper, cobalt and iron [58, 59]. Sheep skin and goat skin have been used in parchment and paper synthesis for the first time in 1450s. Moreover, animal cadaver by-products such as bones, skin, and ligaments are used in hydrolysis collagen production, this type of collagen is a digestible collagen, proper foam stabilizer and can use it in produce useful material such as adhesive, cream fillings, etc. [59]. Intriguingly, animal carcasses are used extensively in the medical aspects such as using of animals' heart valves as an alternative solution in some patients, also the animals' sexual organs used as a source for hyaluronic acid; similarly, the animals' endocrine glands are demonstrated as a proper source for insulin and thyroxin production [59]. According to the published paper by Gutterres *et al*., in (2015) about tannery processes clarified leather process categorized into three major stages including; integrated tannery, wet blue tannery and posttanning process or finished tannery [60]. The Turkish leather sector has an important role in the global leather trade because it is a combination between historical and modern designs. Leather trading has always been one of the most important export sectors in Türkiye. The leather and leather products have been increased remarkably in the end of 2021 and approached to 1.9 billion US dollars. Footwear is among the most important export leather products. Roughly 21.7% from the total leather exports are leather goods, while 13.3% is raw skin. The registered information by Ministry of Trade- Republic of Türkiye elucidates the numbers of raw leathers that export during 2019 were 234.076.143 similarly the numbers of leather goods, furs and footwear were 351.082.990, 161.764.054 and 958.237.938 respectively in the same year. Whereas, there were fluctuated decreasing in numbers of leathers and leather products exports in 2020 in contrast to 2019 and they were 155.983.870, 284.759.307, 101.784.750 and 829.418.182 respectively for raw skin, leather goods, furs and footwear. On the contrary, in the last of 2021 leathers and leather products exports registered dramatically increased and they were 253.123.288 raw skin, 413.286.541 leather goods, 152.955.824 furs and 1.081.908.288 footwear, this data was revealed the effects of COVID-19 on the economical status around word. The total numbers of leather and leather products were 1.705.161.125 in 2019, 1.371.946.109 in 2020 and 1.901.273.941 in 2021. Obviously, there is a gap between the numbers that products exported in 2020 and 2021; also the footwear was the most exported product and represented 56.9% among the total exported leather products. Based on statistical data in 2019, 2020 and 2021 obtained of Republic of Türkiye – Ministry of trade explained there are many of leather goods and leather products have been exported to different countries including Russian Federation, France, America and German (9.8%, 10.3%, 11.7% and 13.2% respectively). The leather products which have been exported from Turkey to America in 2022 were 141.6 US$ [61]. According to ministry of trade statistical data annually reported there are noticeable progress in export of leather products and leather industries Germany, USA, France, Russian federation, Kazakhstan, Holland, England, Italy, Sweden, Tajikistan, Iraq, Denmark, Belgium and China were respectively 20.639.613, 14.446.582, 16.645.844, 9.458.373, 728.124, 13.362.791, 4.054.476, 9.145.847, 3.981.200, 7.622, 3.840.907, 1.648.463, 1.771.803 and 920.960 in 2019 and these values were affected by COVID-19 distributed in 2020 and registered a fluctuation 15.268.065, 10.964.108, 15.354.981, 9.329.969, 3.121.692, 12.160.606, 6.012.453, 5.766.954, 2.595.086, 486.273, 4.184.600, 1.370.889, 1.031.877 and 1.565.812 respectively. Whereas, these values were increased to the same countries in the end of 2021 and registered 18.640.344 to Germany, 16.542.158 USA, 14.598.923 France, 13.942.403 Russian federation, 10.441.466 Kazakhstan, 10.143.791 Holland, 8.852.074 England, 6.581.929 Italy, 3.806.857 Sweden, 3.228.177 Tajikistan, 2.214.474 Iraq, 1.676.224 Denmark, 1.581.322 Belgium, 1.444.612 Chinese. Based on one estimate, there are 6.5 million, 15.2 million and 22 million sheep, goats and cattle respectively in Tanzania, therefore it is considered the second country in Africa after Ethiopia worth with livestock population [62]. The abundance of animals in Tanzania has a significant impact on the country's economy. They are recognized as a valuable source of skin/hide, meat, and milk, as indicated by Ndaro and China in (2016) [63]. Unfortunately, due to the absence of innovative methods in domestic animal slaughtering and inadequate instruction, there is a significant amount of untapped potential. Also improper utilization and wasteful lose of animal byproducts affect the productivity of leathers; so that the tanning and leather production is too poor in Tanzania. The leather industry has traditionally been seen as a vital economic structure for several nations, since it has been regarded as a significant economic producer that remains resilient to external influences. Furthermore, the present-day manufacturers of leather encompass economically, and industrially advanced nations like as China, the United States, India, and other countries. Turkey provides a significant role in the international leather products market, with a stable position as an exporter. Since 1970, the leather industry has played a vital role in the Turkish economy and has emerged as a major sector. In recent decades, the leather sector in Türkiye has seen substantial economic expansion, which is closely linked to the ample availability of rawhide resources. The leather industry has experienced significant export volumes, with a big portion of the necessary raw materials being sourced through imports. Based on the data from the Social Security Institution for 2021, Turkey has a total of 71,685 workers employed in 7,086 industries involved in the production of leather and leather-related goods. According to the Leather and Leather Products Sector (2022), the primary Turkish cities engaged in leather production include Istanbul, Izmir, Bursa, Bolu, Hatay, Manisa, Isparta, Balikesir, UĢak, Tekirdağ, and Ordu. The leather industry has experienced a transition towards environmentally sustainable methods in response to its significant adverse ecological effects on a global scale. The establishment of an environmental protocol, sanctioned by the ministry of climate change, has facilitated this development. In 2021, there was a significant rise in the exports of leather and leather-related products, with a huge growth of 38.6% and reaching a total value of $1.9 billion. Shoes constitute the majority of exports in this business, at 56.9% of the overall total. Consequently, shoes have been identified as the predominant export item in the Leather and Leather Products Sector for the year 2022. The percentages of Turkish total exports attributed to leather goods, raw and processed leather goods, leather, and furs are 21%, 7%, 13%, and 8%, respectively. The Turkey's shoe industry dominates the sub-sector of leather and leather goods exports, accounting for a significant part of 1.1 million dollars, equivalent to around 56.9% of total exports in this category. The footwear industry in Turkey is a substantial contributor to the country's export sector and plays a significant role in job the next. Leather is the primary commodity in the shoe export industry. In 2021, the exports of leather shoes experienced a significant the rise of 31.1% compared to the previous year, reaching a total value of 466.8 million dollars. Russia is the primary destination for the leather shoe company's exports, representing a significant portion of the overall export volume, particularly 14.9%. Germany, Romania, Spain, England, and Italy are notable economic regions.

**3. Nonfood Using of Animal Products**

For a considerable duration, humans have utilized animal raw hides to create clothing, shelters, footwear, parchment, blankets, and furniture. Keratin proteins are derived from the skin, wool, hair, and hooves of animals and are utilized in the production of clothing, shampoo, hair conditioners, and other products [59]. Primarily, keratin, the primary component of the major structure, is sourced from higher vertebrates including reptiles, birds, and mammals. Keratin is composed of 90% fiber protein, which possesses a strong structure and contains nitrogen, sulfur, fat, and mineral components in proportions of 15-18%, 2-55%, 1.27%, and 3.20% correspondingly. Every year, around 40 million tons of animal keratins are produced and exported for use in both the food and non-food industries. The primary manufacturers of keratin are China, the USA, and Brazil [64]. Keratin is an organic material that is derived from animal skin appendages, such as hair, wool, nails, and feathers. It is primarily obtained from animals like sheep, goats, buffalos, and cattle either after or before they are slaughtered. Among the various components of animal skin, the stratum corneum and scales have the highest concentration of keratin [65]. The organic biomass derived from animals, such as keratin, is non-toxic to both humans and the environment since it quickly breaks down. As a result, it can be utilized in the production of medicinal and cosmetic products [64]. Keratin has been extensively researched and utilized in several applications, particularly in the medical field. These applications include cell seeding, drug transportation, diffusion, tissue regeneration, and wound healing [66]. Despite the considerable economic significance of leather industries, they are recognized as major contributors to water and air pollution due to the discharge of large quantities of toxic materials, which in turn impact the ratio of BOD (biological oxygen demand) and COD (chemical oxygen demand). Sodium sulfide and lime are utilized in the leather production process to remove hair from the epidermis and hides. Sodium sulfide has a crucial function in the breakdown of disulfide connections between amino acids in the structure of keratin found in the skin and its appendages, such as hair. This leads to the removal of hair without causing any adverse effects on the surface of the skin [67].

**4. Bacterial Influents**

A bacterium produces a lot of side effects on leather and leather products. Owing to the high proteinaceous content of skin/ hides the worst bacterial effects on skin is putrefaction that happens by activity of bacterial proteolytic enzymes which cause negative influences such as unpleasant odor [68]. In spite the harsh environmental condition in leather industries that may be prevents bacteria and fungi from growth and withstand, many of bacterial species have ability to grow and produce primary and secondary metabolic products such enzyme and pigments which are responsible for leather deterioration. Similarly, the chemical materials in leather tanneries' effluents endow appropriate environments to bacterial growth. Among bacteria that isolate from raw skin are *Pseudomonas, Micrococcus, Staphylococcus* and *Bacillus* sp. [30]. The study by Anderson *et al*., (1945) indicated the vast majority of bacterial species which are isolated from animal skin have the ability to grow and replicate in high NaCl content ranging from 1.5-9% w/v. Many of bacteria was isolated from animal fresh calf hides such as *Bacillus coli, Bacillus proteus, Bacillus megatherium, Bacillus mycoides, Bacillus subtilis, Staphylococcus albus, Staphylococcus aureus, Sarcina lutea and Micrococcus rose us. Bacillus subtilis* and *Bacillus mycoides*, all these isolates can survive and grow in a suitable sodium chloride contents but stay in potential state in high concentration of NaCl for instance 20% w/w [69]. Also, among the bacterial harmful influences on leathers are devastating leather, unacceptable odors, decrease the flexibility and elasticity of skin, skin discoloration, spots with different colors such as red, violet, grey, white and black and decline in collagen molecular weight [70]. In a recent study conducted by Ozbay and Caglayan (2022), it was discovered that the combination of proteolytic and lipolytic haloversatile bacteria has detrimental effects on cow hides after a continued incubation period of 40 days [71].

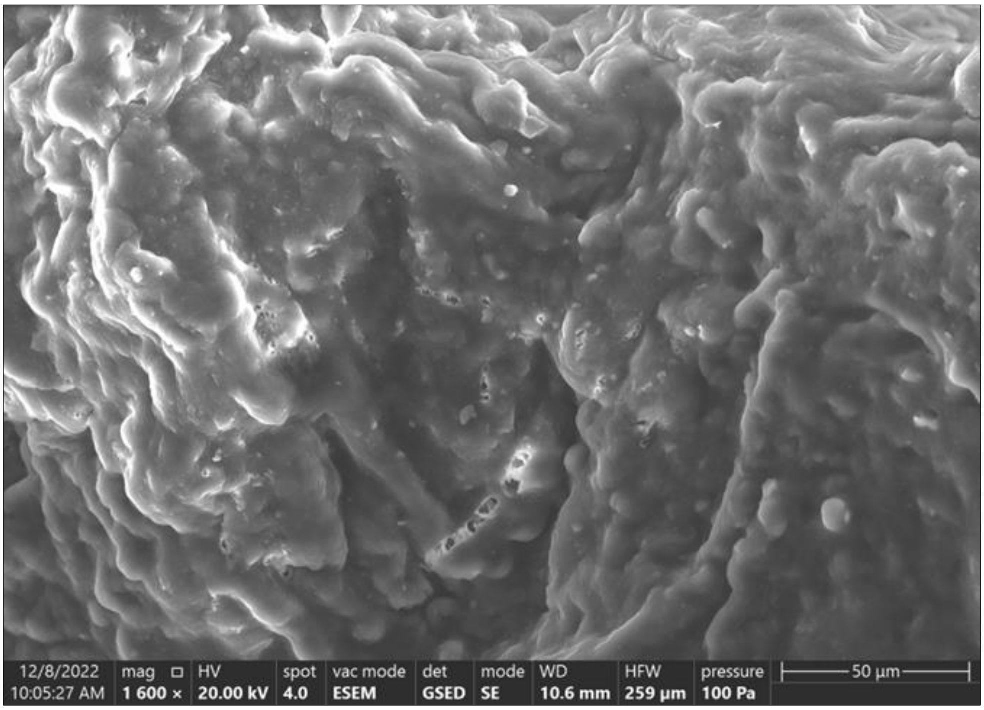



**Figure 5: Sheep skin treated with mixed culture of Lipolytic and proteolytic bacteria isolated from leather industries (20 µm) [72]**

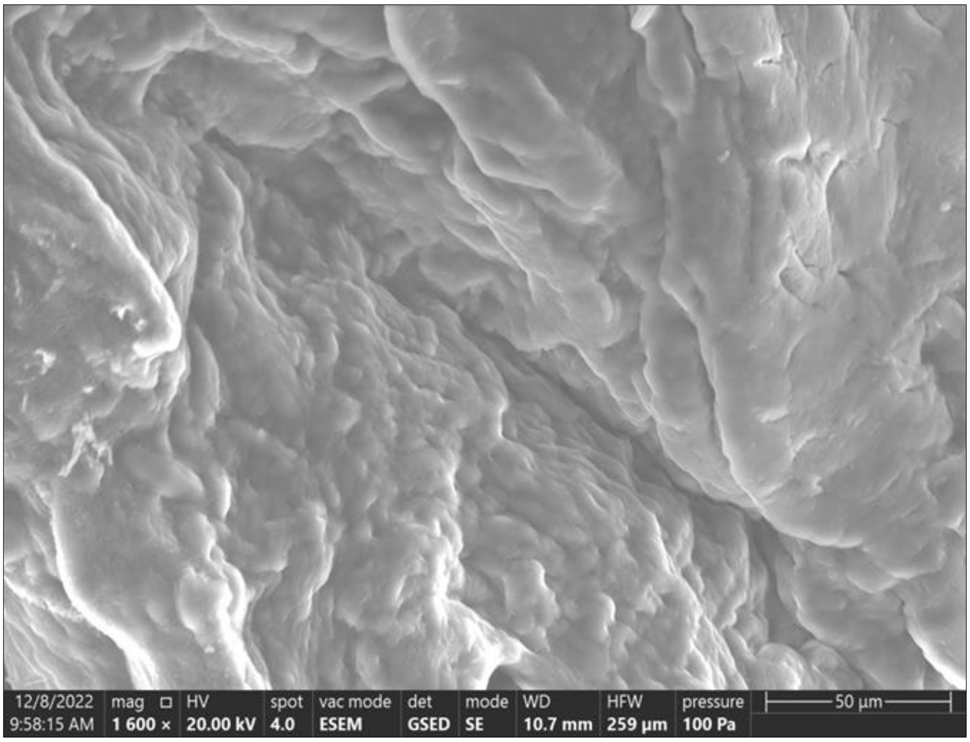



**Figure 6: Goat skin treated with mixed culture of Lipolytic and proteolytic bacteria isolated from leather industries [72]**

## 5. Fungal Influents

In view of high water and nutrients contents of animal raw skin/hides many of proteolytic and lipolytic fungus have grew and replicated in the leathers and cause a lot of side effects such as unacceptable smell, rot and decolorization. Fungi has ability to grow in low pH value therefore it easily to grow and replicate in pickling stage because during this step the pH value it is low [73, 74]. Approximately 33 fungal species were isolated from TAFCO (tannery and footwear) in India (Kanpur) and diagnosed by Nigam (1997) in samples obtained from liming stage. *Alternaria spp.*, *Mucor spp.*, *Trichoderma spp.*, *Fusarium* spp., *Phoma* spp., *CUNularia* spp., *Rhizopus* spp., *Cunningham ella* spp., *Drechslera* spp., *Cephalosporium* spp., *Cladosporium* spp. *Penicillium* spp., *Aspergillus* spp. *and Chaetomium* spp. were among the isolated species. The environmental circumstances in the tannery are perfect and encourage the bacterial and fungal growth, so that the skins' normal flora takes advantage from high nutrients and typical temperature and oxygen particularly the species with short generation time [73-76]. *Aspergillus* spp., *Penicillium* spp., *Scopulariopsis* spp., *Cladosporium* spp. and *Alternaria* spp. were isolated from 14 leather industries in Türkiye (Istanbul), among these species *Penicillium* spp. and *Aspergillus* spp. were found to be the most frequently isolated fungus species [77]. Also found the likelihood of respiratory infections among tannery workers with fungi is high. The previous studies concluded the fungal growth is happen not only during pickling stage but also through tanning stage especially on chrome, vegetable tanned and finishing leather processes. As aforementioned, among the harmful impacts by fungi on leather is discoloration which occurred by some types of fungi having ability to produce pigments on leather such as *Paecilomyces* spp., *Penicillium* spp. and *Aspergillus* spp. According to study Bitlisli *et al*., (2004) *Aspergillus fumigates, Aspergillus niger, Penicillium restrictum, Altemia spp., Cladosporium spp., Penicillium citrinum* and *Aspergillus terreus* were the most commonly species that isolated from salted skin of sheep. The salts, antibiotics, acidic solutions and in some cases essential oils have been used to prevent microbial growth, notwithstanding some bacteria and a lot of fungi have ability to form spores that can be lastly grow and convert into vegetative cells whenever proper conditions are a valuable. Hence, fungal spores are volatilized in the tanneries' ambient and resist harsh conditions such as NaCl (20-30%) [78-30].

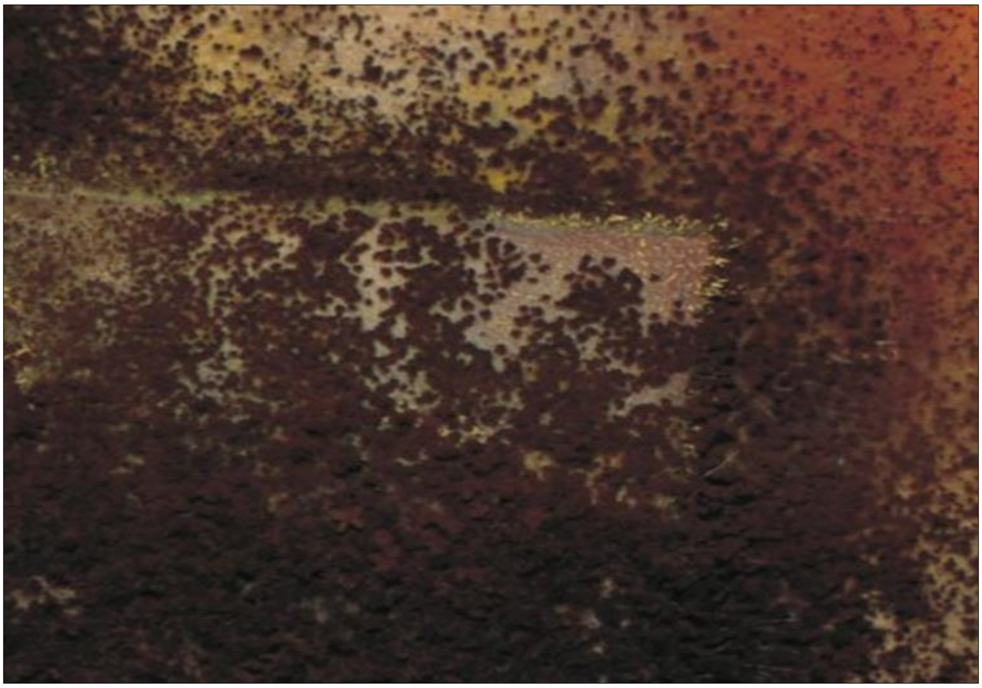
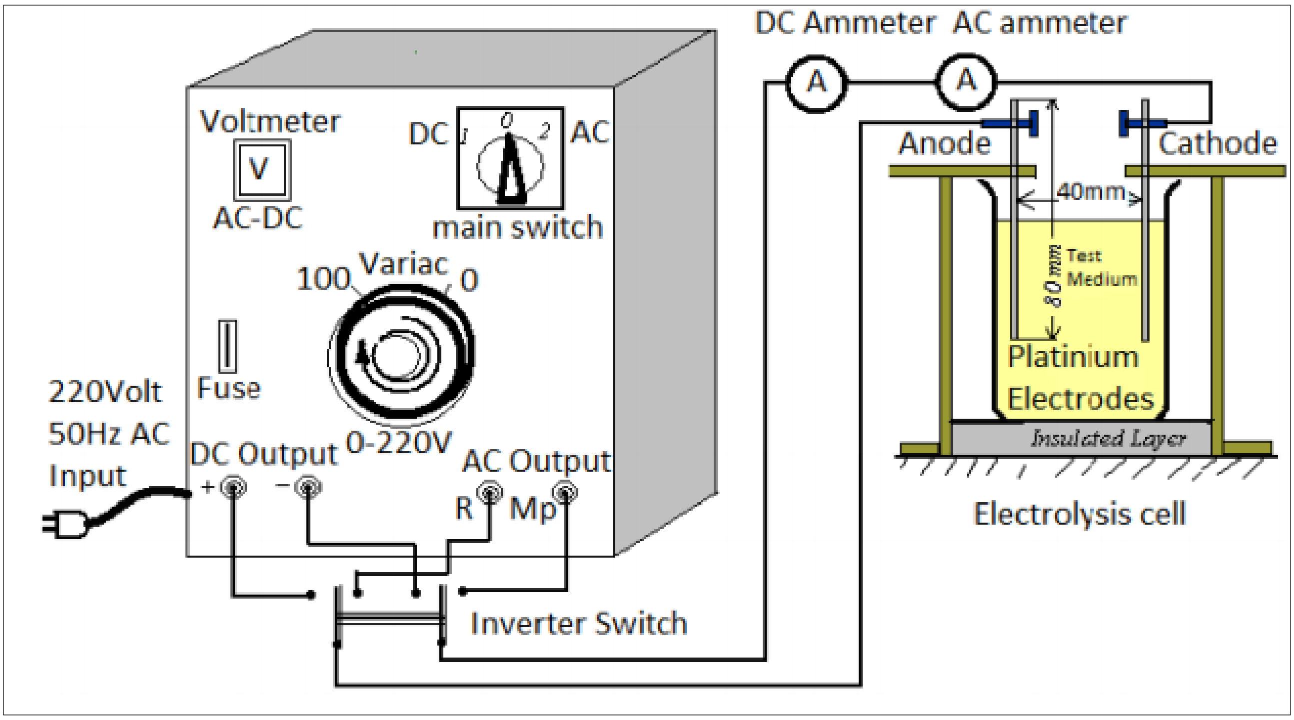


**Figure 7. The growth of Fungi (*Aspergillus niger* and *Aspergillus flavus*) on the leather [79]**

**6. Leather Preservation Methods**

As previously declared, the leather manufacturing facility utilizes untreated animal skin that is inhabited with several microorganisms. While many of these microorganisms are harmless, they can release extracellular enzymes that can have negative effects on the skin after the animal has been slaughtered. Within a time, period of approximately 5-6 hours following the slaughtering of an animal, it is necessary to apply salt to the raw animal skin as a temporary preservation technique. This procedure effectively reduces the potential for bacterial growth. Due to the warm in raw skin, high content water and proteins various types of potential pathogens replicate and produce enzymes and pigments on skin as a result devastating hides with unpleasant odor. Consequently, numerous techniques have been devised to manage this challenge, such as the standard preservation approach including the use of NaCl and Khari salts in appropriate proportions [43]. Microorganisms can have detrimental effects on skin and hides; hence they must be treated with preservatives such as NaCl and antibiotics. Common salts are commonly employed in the leather manufacturing process, particularly during soaking, pickling, and post-tanning, to effectively disintegrate bacterial and fungus cells [47]. Based on numerous published data, the use of NaCl in treating raw skin leads to an increase in the levels of chlorides and total dissolved solids (TDS) in the waste generated by the leather industry [80, 81]. Consequently, researchers have directed their attention towards finding alternative curing methods that can effectively eliminate bacteria from raw skin using lower concentrations of NaCl. These methods are commonly referred to as salt-less and salt-free methods [47]. Up until now, the overall majority of leather enterprises worldwide continue to employ conventional preservation methods due to their simplicity, ease of application, and cost-effectiveness compared to alternative approaches. Russell *et al*., (1997; 1998) utilized a mixture of NaCl+ EDTA (Tetra-sodium ethylene diamine tetra-acetic acid) on animal skin to reduce the quantity of salt required for hide preservation [33, 34]. The mixture consisted of 25% EDTA, 35% pine sawdust, and 40% ordinary salt. This technique offers storage duration of six weeks and was firstly documented as Liricure, with its first recorded use in South Africa. The mixture of 5% silica gel and 5% NaCl, along with 0.1% P-Chlorometacresol, was sprayed onto animal skin to reduce its moisture content. The function of silica gel is to adsorb moisture from the skin. Interestingly, this technique reduces the levels of total dissolved solids (TDS) by 70-75% and chlorides by 80-85% [35]. Another effective salt used in this method is potassium chloride (KCl), which shares similar chemical and physical properties with sodium chloride (NaCl) and can be utilized as a preservative in salt-free methods. Typically, KCl does not have a detrimental impact on the environment as it promotes plant growth, unlike sodium chloride which has harmful effects on plants and soil [38-40]. A combination of NaCl and SMBS (sodium meta-bisulphite) was used on hide. The results revealed the application of both a technique without salts and a technique completely free of salt. This novel preservation method involved the use of 0.5% sodium meta bisulphate and 5% sodium for the salt-less method, and only 1% SMBS for the salt-free method. The temperature range for this method was between 30 and 40 degrees Celsius. The results demonstrated a high level of effectiveness [36]. Peracetic acid, a potent oxidizing agent, is commonly employed as a preservative when combined with water, hydrogen peroxide, and sodium hydroxide at a temperature of 15-20 degrees Celsius for duration of 2 hours. This treatment can effectively preserve fresh hide for a period of 20 days at ambient temperature (Valeika *et al*., 2016). Boric acid typically exhibits bactericidal properties and possesses the capacity to decrease moisture levels in the inner layer of the skin. Boric acid is specifically optimized for use in salt-free and salt-less methods. It is employed in two different formulations: one containing 5% boric acid and 5% NaCl, and another containing 2% boric acid and 5% NaCl. This approach demonstrated antimicrobial effectiveness against bacterial proliferation on goat skin [37]. Depending on the information published by Vankar, *et al*., (2006, 2009a, 2009b) using of sodium sulphate alone as a preservation factor showed such a perfect result to keep buffalo and goad skin for 21 days at 30-35 ºC in contrast to NaCl, 20: 80 of NaCl: Na2SO4 and 10:90 of NaCl: Na2SO4. Moreover, this method contributes to reduce the TDS and $Cl^-$ ions [41-43]. Another prior study utilized silicate as either a powder or a spray to directly treat animal skins, effectively preserving them for an extended period of many months [44]. Halophilic bacteria, moderately halophilic bacteria, extreme halophilic bacteria, archaea, and salt-tolerant bacteria are capable of inhabiting the skin and producing enzymes that can cause harmful effects. These effects include skin discoloration, reduced flexibility of leather, degradation of collagen fibers, and the formation of cavities on the surface of the skin [82]. The deterioration and damage of skins due to bacterial proliferation during various phases of the leather manufacturing process pose a significant problem, leading to evident economic losses in leather production [83]. Notwithstanding many of antimicrobial agents (bacteriostatic and bactericidal) have been applied (including NaCl and Boric acid) throughout skin processes in order to eliminate or kill microbes which found in soak liquor and skins' surface, many of bacteria have been developing antibiotic resistance mechanisms and this type of resistance have transported [84] among species vertically and horizontally. Furthermore, the excessive utilization of antimicrobial agents has been identified as the primary cause of environmental contamination. Consequently, researchers tried to identify novel ways that are both environmentally benign and possess potent antibacterial properties, while also being non-toxic. A many of novel alternative methods have been identified and carried out to reduce bacterial populations or eradicate them in various domains, including food preservation. These technologies include PEF (pulsed electric fields), electrolyzed water, UV decontamination, high power ultrasound, oscillating magnetic fields, radiation, high intensity laser, ionization, and high-pressure homogenization [85]. Additional methods employed include cold plasma, high hydrostatic pressure, and pulsed white light [86]. Electric current was widely employed to destroy germs isolated from leather industry. For several decades, researchers have been studying the effects of pulsed electric field (PEF) on microorganisms. The impact of a high-voltage electric field on the transportation, metabolic activity, and survival of bacteria has been investigated [87, 88]. The application of pulsed electric field has a remarkable impact on eliminating extremely halophilic archaea with proteolytic and lipolytic activities that have been isolated from a salty lake [84]. Two distinct forms of electric current have been widely employed to inhibit or destroy halophilic bacteria and very halophilic archea that are found in salt, brine, and salted animal hides used in leather tanning procedures. Furthermore, both alternate and direct electric currents have been utilized to eliminate bacterial populations and decontaminate food and other liquid items. There are two processes by which electric current can deactivate bacterial development and destroy germs. Firstly, electroporation involves subjecting bacteria to high voltage, which temporarily alters the components of the bacterial membrane, including the lipid bilayer and proteins. Another process is referred to as electric obliteration. Both procedures include the perforation of the bacterium cell wall, causing the release of the inner bacteria material and ultimately leading to bacterial death [89, 90]. Several parameters influence the process of electric current in bacterial inhibition, such as the pH level of the medium used, temperature, conductivity of the medium, species of microorganisms being studied, and the duration and type of electric current. Electric current exerts deadly effects on moulds, yeasts, and the form of vegetative bacteria. However, there has been evidence of resistance against bacterial spores. Bacteria have greater resilience compared to yeast cells. In addition, Gram-negative bacteria exhibit greater susceptibility to pulse electric field compared to Gram-positive bacteria. This method has been employed in the food industry to eliminate *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus brevis*, and *Saccharomyces cerevisiae* from pea soup, milk, skim milk, liquid egg, yoghurt, and apple juice, respectively [91]. Both alternating and direct electric currents were utilized synergistically to combat six isolates of gram-positive and gram-negative bacteria in a liquid medium containing 2% NaCl. The outcomes demonstrated a significant reduction in the number of bacterial colonies within short treatment duration. [92]. While electric current technologies are highly efficient in combating resistant germs without the need for toxic chemicals, their application in the leather industry remains complex. Electric current can be utilized in various areas, not just limited to food production. In addition, several physical methods such as irradiation, electron beam therapy, and chilling techniques have been used to safeguard skins following salting and washing procedures. The cooling process can be classified into two categories: cooling by ice and cooling in a vacuum. Researchers really favor utilizing chemical approaches over physical ways for short-term preservation of leather due to the absence of any requirement for specialized equipment.




**Scheme 1: Illustrate the electrification apparatus that used to kill haloversatile bacteria and extreme halophilic archaea isolated from leather industries by using Alternating and Direct electric currents [92, 93]**

**7. Preservation By Using Plants Essential Oils**

Chemical and physical preservation methods, whether used on leather or in other contexts, can have negative effects on both the material itself and the environment. Consequently, there has been significant research conducted on this topic. Fortunately, many researchers have focused on finding alternative preservation methods that utilize natural materials derived from plants. For example, essential oils such as 1,8-cineol, α-pinene, limonene, linalool, myrtenyl acetate, α-terpineol, carvacrol, γ-terpinen, thymol, terpinen-4-ol, para-cymene, and styrene, extracted from plants involving Myrtus communis, Oregano, and Siğla tree, have been investigated as potential preservation agents. These oils can be used during the soaking stage of the preservation process, as demonstrated previously [94-96]. The powdered form of plants produced from *Salicornia brachiata*, *Tamarindus indica*, and *sesuvium portulacastrum* was used as a paste to cure skin and hides [97-102]. Vegetable tannins and tannic acid are used as a soaking solution to maintain the skin from microorganisms during the tanning procedure and to prepare it for subsequent stages [103-105]. In addition, certain plant extracts, such as *Citrus sinensis* peel [106], and *Semecarpus anacardium* [107], have been utilized for skin preservation in the leather industry. An investigation was conducted on the aqueous extract of Henna (*Lawsonia inermis*) to determine its antibacterial properties in the leather industry [108].

**8.4. Antibiotic**

Biocides are substances, either synthetic or derived from nature, that have the ability to inhibit the growth or kill microorganisms. Short-term leather protection can be achieved by using bactericidal and bacteriostatic chemicals, which can provide protection for a period ranging from days to weeks. Historically, chlorinated chemicals and mercury phenoles have been commonly utilized as bactericidal agents. Although both of them have utility, their detrimental environmental effects have led to their prohibition [109]. Biocides are commonly used in combination with dyes and surfactants during the storage and processing of leather to protect the salty skin from bacterial and fungus growth [109]. In previous study, Shakeel *et al*., (2019) synthesized a novel type of formazan dyes composed of a combination of (2-phenyl 2-4-sulfophenyl-hydrazono methyl diazenyl) benzoic acid and (2-aminobenzoic acid) [110]. These dyes were then reacted with 4-[(2Z)-2-benzylidenehydrazinyl] benzene sulfonic acid. The synthesized dyes were tested against both Gram-positive and Gram-negative bacteria, including *Bacillus subtilis*, *Klebsiella*, *Escherichia coli*, and *Staphylococcus aureus*. Additionally, they were tested against various fungi commonly found in leather industries, such as *Trichoderma harzianum*, *Aspergillus chevalieri*, *Aspergillus candidus*, *Aspergillus flavus*, *Penicillium stipitatum*, and *Aspergillus niger*. The leather's antibacterial properties have been developed using pre-tanning procedures [111]. In a recent study conducted by Ding *et al*., (2022), a novel antibacterial agent was developed by combining Polyurethane and ciprofloxacin. The resulting compound was then tested on both Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). The findings revealed that the compound exhibited a significant bacteriostatic effect on both types of bacteria by inhibiting the activity of DNA gyrase in these isolates. The findings from studies conducted by Bayramoglu (2007) and Bayramoglu *et al*., (2009) demonstrated that Oregano essential oils had stronger bactericidal properties compared to the commercial antibiotic agents commonly used in leather processing [95, 96]. In the fatliquoring process conducted by Bielak *et al*., (2017), the antimicrobial properties of three types of plant extracts (specifically, essential oils derived from thyme, cinnamon, and oregano) were tested. These extracts were mixed with leather at a concentration of 5% of the weight of the skin [112]. The study aimed to evaluate the effectiveness of these essential oils against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. The results clearly demonstrated the strong antibacterial and antifungal activity of the aforementioned essential oils. In previous investigation by Veyselova *et al*., (2013) examined the effectiveness of a combination of QAC (quaternary ammonium compound), specifically 12.5% benzyl dimethyl ammonium chloride and 12.5% didecyl dimethyl ammonium chloride, when applied to soaking water in leather industries [113]. The researchers tested this combination against various Gram- positive bacteria (including *Bacillus licheniformis*, *Staphylococcus intermedius*, and *Bacillus pumilus*) as well as Gram-negative bacteria (including *Enterococcus faecium*, *Vibrio fluvialis*, *Enterobacter cloacae*, and *Pseudomonas luteola*). The results showed that this combination had antimicrobial effects against all tested isolates, with the effectiveness depending on the concentration used. Furthermore, several articles examined and assessed different antibacterial agents used in the leather industry. For instance, Methylisothiazolinone was examined as an antibacterial factor, and its minimum inhibitor concentration (MIC) was evaluated against Gram-negative bacteria like Pseudomonas aeruginosa and Escherichia coli, as well as Gram-positive bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, and *Enterococcus faecalis* [114]. Also, the study examined the minimum concentration of Sodium Dimethyl-dithio carbamate, an ingredient with antibacterial properties, against antibiotic resistant bacteria that were resistant to penicillin, streptomycin, meropenem, ampicillin, and spectinomycin. The concentrations used were 10 µg, 25 µg, 10 µg, 10 µg, and 25 µg respectively. The results showed that Sodium Dimethyl-dithiocarbamate had antimicrobial effects against a mixed bacterial culture at a concentration of 1000 µg/l [115]. The antibacterial properties of acetone extracts of four lichen species (Usnea sp., *Hypogymnia physodes*, *Pseudevernia furfuracea*, and *Evernia divaricata*) were investigated against six Gram-positive *Bacillus* sp. isolates. All isolates exhibited positive catalase, protease, and oxidase activity. The results revealed that these extracts were capable of killing certain isolates at a specific concentration, while inhibiting others at the same concentration [82]. Lichens, such as *Cetraria* sp. and *Lobaria* sp., have been utilized in the leather industry as natural and alternative antibacterial agents for a long period of time [116]. In the textile industry, a combination of synthetic and natural materials has been used to create and apply new antibacterial properties. The efficacy of sodium pentaborane pentahydrate and triclosan has been evaluated against *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Klebsiella pneumonia* [117]. The antimicrobial activity of lanthanide-doped strontium aluminium oxide was investigated against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* by Khattab *et al*., in 2019. The study examined the antibacterial effects of Chitosan and Dielectric barrier discharge (DBD) plasma-treated materials that were impregnated with alkyl dimethyl benzyl ammonium chloride [118]. The materials tested included Polyester, nonwoven cellulose/polyester, woven cotton, and Jute-cotton blended denim. The antibacterial activity was assessed against *Staphylococcus aureus* and *Escherichia coli* bacteria, as reported previously [119, 120]. The antimicrobial efficacy of Rose bengal against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* was evaluated on wool and acrylic blended fabric [29]. The antimicrobial efficacy of Chitosan–silver nanoparticles [121], and *Lycium ruthenicum* Murray extract [122], was evaluated against *Escherichia coli* and *Staphylococcus aureus* on wool fabrics. The researchers in the study conducted by Yıldız *et al*., (2018) utilised Quat silane, an antibacterial agent, in cotton and elastane fabrics to combat *Staphylococcus aureus* [123]. The study conducted by Sadeghi-Kiakhani *et al*., (2020) utilized chitosan-silver nanoparticles as an antibacterial agent to combat *Escherichia coli* and *Staphylococcus aureus* in the linen industry [124]. Morever, in silk industry 6mer-HNP1 [125], silane quaternary ammonium salt and silica nanoparticles [126], were employed to inhibit bacterial development.

## CONCLUSİON

According to the importance of animals products in humen lives, a lot of methods and techniques have been used in indestry sector in order to protect leather or skin from the damage that can be cause by the microorganisims. It is importent to note that the skin animals is consederd to be as a suitable enviroment for the microorganisims growth so using diffrent and conveniaent methods are needed.

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