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A Mini Review
Role of Natural Binders in Leather Finishing- A Comparative Approach

by
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Abstract

Leather finishing has its own importance in leather making. A variety of chemicals are used in the finishing operation, to provide the aesthetic look of the leather products. The role of finishing chemicals such as pigments, dyes, plasticizers, wax emulsions, cross linkers, fillers, wetting, penetrating agents and binders have their own role to give finished look to the leather products. A binder plays significant role in the leather finishing. This review elaborated on the role of natural binders with respect to their properties, application and binding effects in leather finishing against synthetic binders. This review focuses on a greener approach to leather finishing.

Introduction

Leather is a natural material that has been used for versatile applications. The most common raw materials used for leather making, are domestic animals like cattle, goats, sheep, and to a lesser extent pig.[1] Multiple steps, such as pre-tanning, tanning, post tanning, and finishing are involved in leather processing.[2,3] The pre-tanning process consists of the removal of hair and flesh from the animal by trimming process, while tanning is an essential step which makes it stable and durable. The post tanning process involves the use of fat-liquoring, retanning agents and dyes for better appealing look of leather.[1,3] Leather finishing is the final step in the leather manufacturing process for the effective, natural, defect less, and regular appearance of leather.[4]

Role of finishing step

The finishing step of leather processing is the final and important step of leather making. The principle objective of leather finishing is to improve the aesthetics and surface properties of the leather by protecting the leather surface against dirt, stain, wetting problems, rubbing, scuffing, and flexing.[4,5] Leather finishing is an important phase to modify the leather surface, shade, gloss and physical strength.[5] The following practices and techniques are used for leather finishing.

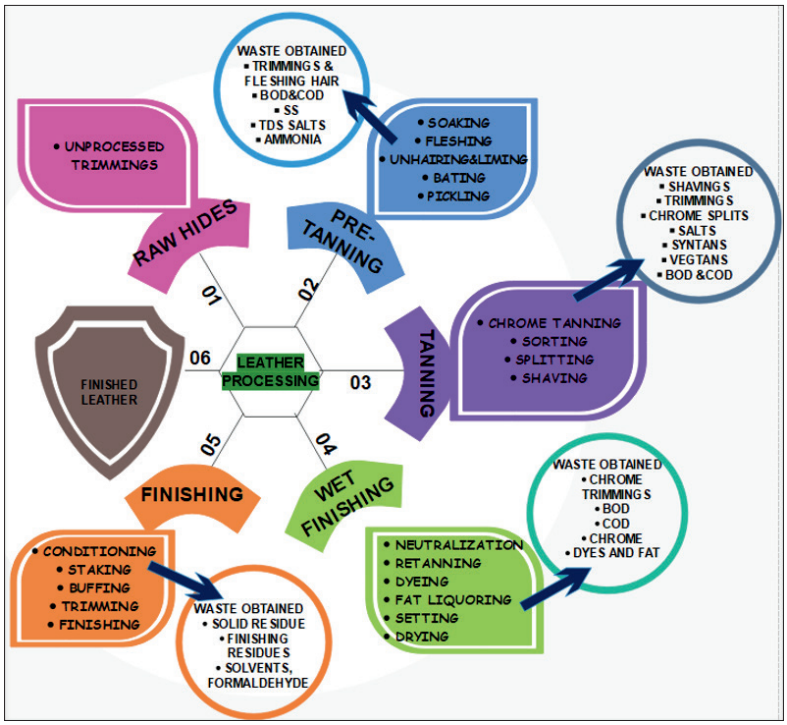

Figure 1. Processes involved in leather processing

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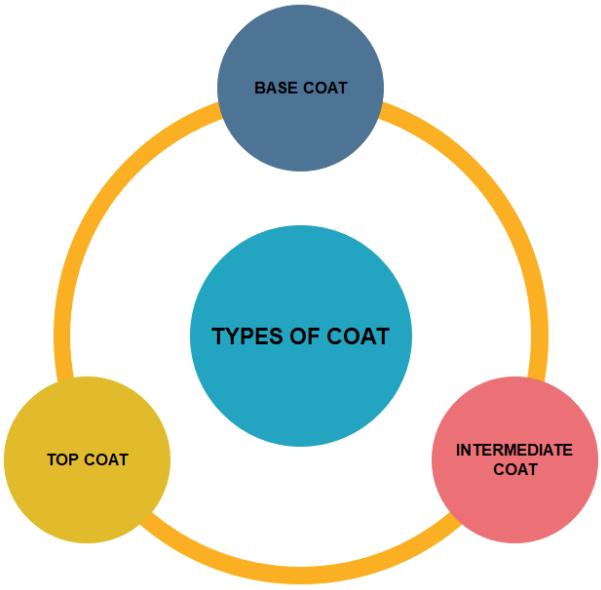

Figure 2. Different types of coating process in leather finishing

Different coats used in leather finishing

Leather finishing can be classified into three coats: base coat, intermediate coat and top coat

- Basecoat: It chemically modifies the surface property of the crust (post tanned) leathers to fix the finishing chemicals. The finishing formulation for base coat consists of solvents, resins and possibly pigments.[6,7]
- Intermediate coat: It majorly consists of binders for filling and enhancing the physical properties. It also acts as a carrier for pigments and dyes to be fixed on leather. In addition, depending on the choice of binders and fillers, the finishing properties can be controlled for the final application. Fillers in the finishing composition influence the uniform texture and levelling of dyes and pigments on the surface.[6,7]
- Topcoat: It is comprised of resins based on polyurethane and cellulose derivatives to protect the leather finish.[6,7]

Different types of finishing techniques used in leather finishing

Leather finishing is operated in many ways, which can produce finished leather with multifunctional properties depending upon the finishing chemicals employed. Following Table I and Figure 3 indicates the techniques used in leather finishing.

Different types of finishing chemicals consist of natural compounds

The application of versatile finishing chemicals on leather provides an aesthetic appearance with improved physical strength to the leather surface. Commonly used leather finishing chemicals are polymers, pigments, binders, wax, resin, dyes and auxiliaries. Various add-on properties such as anti-stickiness, resistance to abrasion, light/heat and water resistance can be implemented by the application of multiple finishing chemicals and processes.[8,9,10]

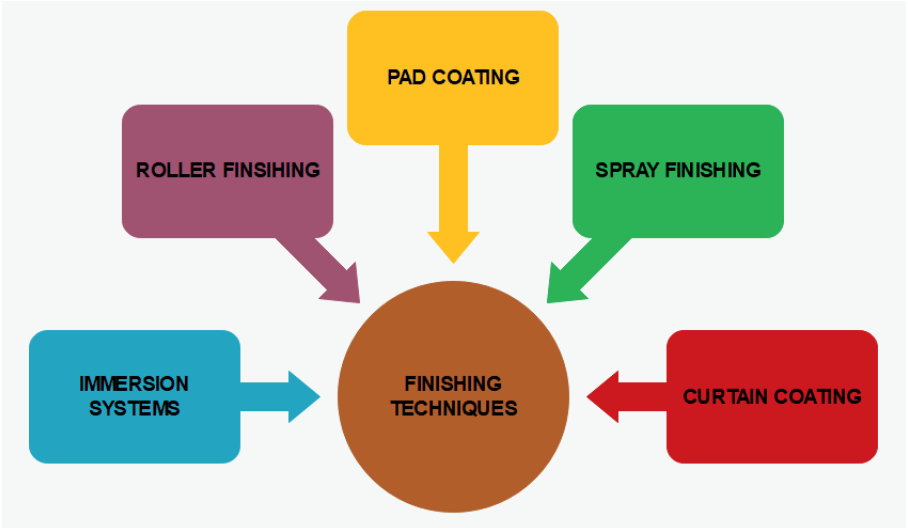

Figure 3. Different types of finishing Techniques for leather finishing

Table I

Represent the different types of leather finishing techniques

S. No	Techniques	Process	Reference
1	Pad coating	Pad coats are usually applied using a plush pad - a wooden board covered with a soft velvet-like cloth. It is done by hand and is, therefore, labor-intensive but it does ensure that the finish is worked well into the leather and evenly applied across the hide	6,7
2	Spray finishing	The most commonly used method. A finish, is supplied through a fine jet in the atomizing part of the spray gun.	6,7
3	Curtain coating	Curtain coating involves applying the finish to leather in the form of a liquid curtain of finish. Gap machines are particularly useful for applying heavy coats of finish, but their use in the industry is limited	6,7
4	Roller coating	In this technique, the finish is applied by passing the leather between two cylinders. Forward and reverse roller coating exists.	6,7
5	Immersion systems	Here crust leathers are passed through a dye solution as an alternative to traditional spray dyeing methods.	6,7

To provide color to the finished surface of the leather, pigment paste and dyes are applied. The pigment paste with natural compounds in the form of blending of oils is applied to enhance the resistance to ageing of coating.[11] The application of metal oxide with oil and wax emulsion also explored to increase the thermal stability.[12] The natural extract from Bixa orellana seeds can be used to finish the leather to improve fastness.[13] Wax emulsion from beeswax and biodegradable surfactants provide gloss, smoothness and touch to the leather surface.[12,37] The application of fillers in the form of nanocomposite latex were also tried and the resultant leather showed better mechanical and flame retardant properties.[14,15]

Since this review focuses on the use of natural compounds, specifically binders, in the leather finishing Table II represents the use of various finishing chemicals where such natural compounds are used.

Table II

Finishing materials consisting of natural compounds

Finishing materials	Natural compound used for finishing	Work	Details	Inference	Reference
Pigment pastes and plasticizers	Flax seed oil and poppy seed oil	Leather Finishing with New Pigment Paste	Pigment pastes in the form of Flax seed oil (brown color) and poppy seed oil (yellow color) as plasticizer	Plasticizers improved resistance to ageing of coating	11
	Castor oil	New Pigment Paste for Leather Finishing	New pigment paste composition made up of Black iron oxide, Polyacrylic binder, Ethoxylated lauric alcohol, castor oil and wax emulsion	Shows higher thermal stability	12
Dye	Extract from Bixa orellana seeds	Studies on the application of natural dye extract from Bixa orellana seeds for dyeing and finishing of leather	The extract from Bixa orellana seeds	Enhancement in color measurement and fastness properties	13
Wax emulsions	Beeswax, lanolin	Obtaining and Characterization of an Ecologic Wax Emulsions for Finishing Natural Leathers and Furs	The mixture obtained from the mixing of wax, lanoline and emulsifier triethanolamine monostearate	finishing films, a waxy feel and better resistance to scratches and water.	37
Fillers	Casein	Construction of hetero-structured fillers to significantly enhance the fire safety of bio-based nanocomposite coating	The spay of hetero-structured filler prepared by double hydroxide-reduced graphene oxide blended with waterborne casein-based nanocomposite latex	The mechanical properties of experimental leather were improved by 71.8% with increase in flame retardant property	14

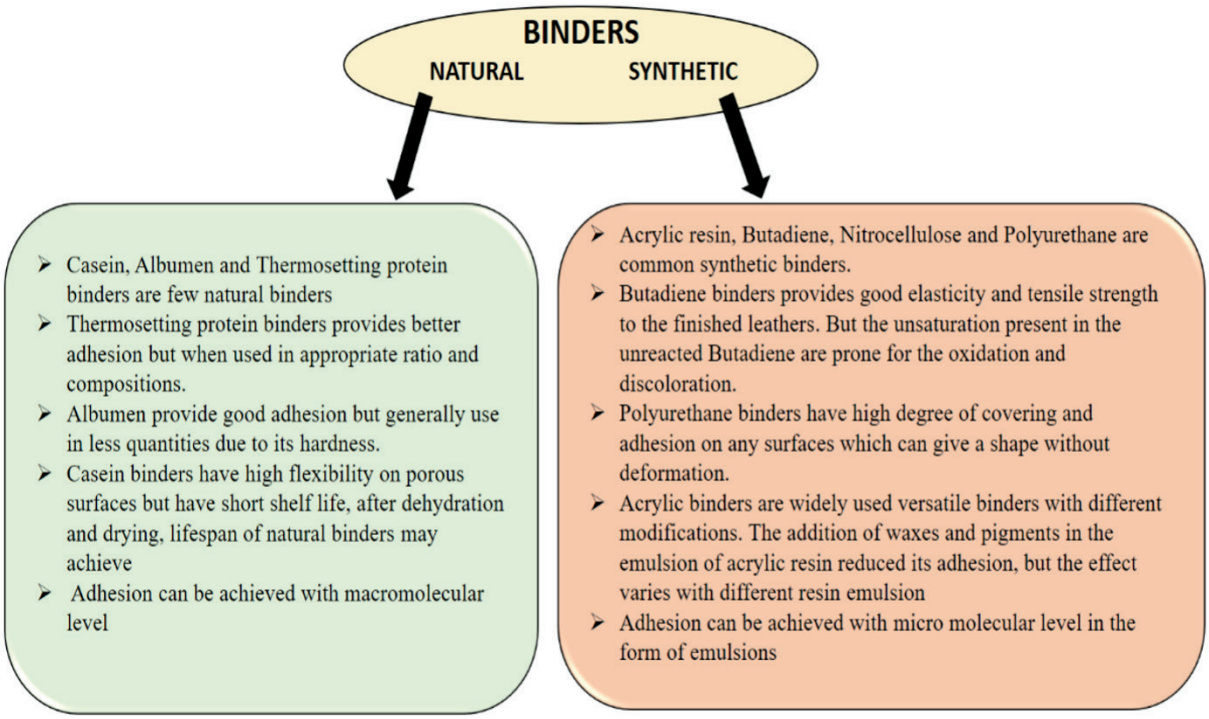

Figure 4. The comparative properties of natural and synthetic binders

Binders

Film-forming materials improve the properties of leather by holding the collagen fibers of leather together thereby creating a stronger leather structure. Binders are used for repairing the damages of the leather surface and to bind the pigments, their nanostructured derivatives and other finishing chemicals with leather.[16]

The role of Binder

The role of binders in leather finishing has its own importance. It forms a continuous film on the surface of the leather for the improvement of physical, stability and crack resistance. Leather finishing chemicals with additives and binders can hide leather surface defects.[16] Leather finishing describes the process for enhancing the physical and fastness properties such as resistance to abrasion, heat/light and water, and improving the surface coloring of leather. Commonly used binders for the leather industry are protein and resin-based binders. Generally, binders provide finish film with excellent properties like good flexibility, strong adhesion, light fastness, soft and elastic films.[16,17]

Types of binders used for leather finishing:

Natural binders

Protein-based binders contain casein, a natural protein, obtained from bovine milk which is biodegradable and non-toxic in nature and forms stable films with excellent adhesive force and strong heat resistance.[18,19] The formation of stable casein films is mainly due to its secondary protein structure and weak intermolecular interaction. Despite these properties, there are a few limitations for casein such as low flexibility, high water sensitivity, and susceptibility for the bacterial attack. To overcome these drawbacks casein, with its derivatives from biosynthetic polymers, inorganic nanoparticles, and plasticizers were explored.[15,19]

A biodegradable composite of caprolactam-modified casein and waterborne PU had been used for leather finishing. Experimental leathers were found to be superior in elongation at break (99.77%) and the fastness to wet rub 4/5 than control values 72.46% and 3 respectively.[20]

The chitosan- poly vinyl alcohol-based coating was explored as a thin transparent film on the leather surface. The prepared chitosan

product was applied on shoe lining leather, resulting in a low cost and environmentally friendly finished leather product with antimicrobial and antifungal properties.[21]

The application of gelatin, extracted from leather solid waste for leather finishing was explored. The chemically modified gelatin was blended with the commercial binders to produce a low-cost and eco-friendly film for leather finishing application.[22] The leather shaving scraps were also explored as protein binder for finishing application to set a better example of sustainability for the leather sector.[23]

Synthetic binders

Polymeric and resin-based binders are compatible and show versatile applications in leather finishing.[31] The combination of these synthetic binders with different polymers, nanoparticles, organic and inorganic composites is also a trend in leather finishing.[3] The commonly used synthetic binders used in leather finishing are acrylics, polyurethanes, butadiene and their derivatives. They are well known for their resistance to hydrolysis, hardness-softness, high blocking resistance, good adhesiveness, good film-forming properties, and are cost effective.[24,25]

Due to the capacity to form highly stable polymeric film, the polymeric binders are used to protect the leather from damage and provide desired properties such as better appearance, texture, color and surface feel.[8] Synthetic polymeric binders act as pigment carriers to form a homogeneous film on the leather surface. Blending biodegradable polymers with synthetic polymers to improve the biodegradability of leather has gained much interest recently.[16]

The application of acrylic-based resin binder and its effect on water vapor permeability of finished leather was also explored. The results indicated that the finished leather samples had good water vapor permeability 47.84 g/h m^2 and excellent wet rub fastness (3-4/5).[26]

The quality of flocked leathers coated with acrylic and polyurethane binders was tested and compared. Results proved that the polyurethane binder had better qualities and superior binding characteristics as compared to acrylic binders. The polyurethane binder shows better abrasion, fastness and durability characteristics than the solvent-based/ acrylic binders.[5]

The application of mixture of water-based polyurethane with silicon dioxide nanoparticles in leather finishing were tried. The experimental leather showed better water vapor permeability 0.3889 g/h m^2 than conventional leathers.[27]

The application of various blends in leather finishing has also been evaluated such as the cross-linking reaction between polyisocyanate with acrylic and polyurethane binders to improve the strength properties of finished leather.[38] Similarly, the crosslinking reactions of butadiene binders with different binders such as polyaziridine, polyisocyanate, epoxy compounds, polycarbodiimide, and polysilane improve the wet fastness properties.[28]

The application of silicon oxide nanoparticles modified acrylic resin as binder showed improved water and air permeability by 11.5% to 15.4%.[27,37]

The application of cellulose derivatives such as nitrocellulose and ethyl cellulose in the leather finishing has also been evaluated. These extracted cellulose derivatives from groundnut husk and sugarcane bagasse are used in leather finishing. The work indicates better upgrading of low-quality leathers, by the use of different concentrations of plasticizer and binders.[34]

The mixture of water-based acrylate/clay nanocomposites composed of terpolymer (butyl acrylate-methyl methacrylate-acrylamide) was explored as a base coat in leather finishing. The experimental leathers showed improvement in mechanical and thermal properties with better film-forming properties, elasticity and pigment binding efficiency than conventional binders.[29] Nanocoatings of acrylic resin are widely used in leather finishing because of their better penetration effect.[30] The protein binder mixed with PU finishing binder is also applied in leather finishing to achieve a natural binder effect with improved properties.[32]

The polymeric dispersion of polyurethane co-vinyl pyridine copolymer as a binder has its own importance in leather finishing. The presence of cationic charge in polymeric backbone provides good adhesion to the leather surface.[7] Polyvinyl alcohol blended with non-toxic chemicals, applied for leather finishing. Experimental leathers showed better wet and dry rub fastness, adhesion to finishing, tensile strength, elongation at break, and softness than control leathers.[43]

The following Table III provides details of the uses of natural and synthetic binders and the enhanced properties associated with experimental finished leather

Table III

List of binders for leather finishing

S. No	Title of paper	Binder used	Type of Binder	Property enhanced and application	Reference
1.	Evaluation and Application of Acrylic Based Binder for Leather Finishing	Acrylic-based resin binder	Synthetic	Finishing formulation containing a high amount of binder showed excellent rub fastness value (4-4/5) and water vapor permeability (47.84)	26
2	Enhancement of antimicrobial properties of shoe lining leather using chitosan in leather finishing	PVAc binder	Synthetic	The prepared chitosan with binder helped to minimize the microbial attack in shoe lining leather, employing a low-cost environmentally method to inhibit the bacterial and fungal attack on finished leather.	21
3.	A novel approach in leather finishing: Surface modification with flock fibers	Acrylic and polyurethane binders.	Synthetic	Polyurethane binders showed better results than the acrylic binders. PU binder can be a better replacement for solvent-based binders.	5
4	Preparation of polyurethane silicon oxide nanomaterials as a binder in leather finishing	Water-based polyurethane is used as a binder.	Synthetic	Improved mechanical properties with eco-friendly approach for leather finishing.	27
5	Use of water-based carbonyl-functional polymers on a cross-linker-free high performance leather finishing	Acrylic and polyurethane binders	Synthetic	The use of water-based acrylic polymers which contain a carbonyl-functional group shows high performance in upholstery leather without crosslinkers.	33
6	Study of Cross-linking Reactions on Butadiene Binders in Aqueous Finishing	Butadiene binders	Synthetic	The use of butadiene binders Improve wet fastness properties of finished leather. The applied butadiene binders improved the fastness properties (4/5) of finished leather.	28
7.	The acrylic resin leather coating agent modified by nano-sio$_2$	Acrylic resin binder	Synthetic	The application of SiO_2-acrylic resin in leather finishing showed an increase in air and water permeability.	37
8.	Extraction of cellulose from renewable resources and its application in leather finishing	Cellulose binder	Synthetic	This study provided better approach towards waste to best concept by utilization of cellulose to impart the fullness of finished leather.	34
9.	Preparation of stable acrylate/ montmorillonite nanocomposite latex via *in situ* batch emulsion polymerization: Effect of clay types	Acrylate binders	Synthetic	The application of water-based acrylate/clay nanocomposite provide better thermal properties to experimental leathers.	29
10	Aqueous dispersions of polyurethane–polyvinyl pyridine cationomers and their application as binder in base coat for leather finishing	PU binders	Synthetic	The performance of Aqueous dispersions of polyurethane–polyvinyl pyridine cationomers as binder exhibited good adhesion properties to the leather surface.	7
11	Preparation and evaluation of non-toxic top coatings for leather to minimize pollutants in leather finishing process	PVA binder	Synthetic	The effect of non-toxic top coatings of PVA binder brought eco-friendly approach for leather finishing.	43

S. No	Title of paper	Binder used	Type of Binder	Property enhanced and application	Reference
12	Application and evaluation of the performance of poly (vinyl alcohol) and its blend with nitrocellulose in leather top coating.	Nitrocellulose	Synthetic	The presence of surface-active agents on the derived binder showed smooth and continuous film with better dry and wet fastness properties.	35
13.	Preparation of acrylic silicon dioxide nanoparticles as a binder for leather finishing	Acrylic binders	Synthetic	The blending of acrylic compounds with silicon dioxide nanoparticles provides good flexibility, strong adhesion, light fastness, soft and elastic films to the leather surface	30
14	Blend composites of caprolactam-modified casein and waterborne polyurethane for film-forming binder: Miscibility, morphology, and properties	Biodegradable caprolactam-modified casein and waterborne polyurethane binder	Natural	Finished leather samples showed an increase in elongation at break from 72.46% to 99.77% and wet rub fastness from 3-4/5.	20
15	Application of Extracted and Modified Gelatin from the Leather Solid Waste in Commercial Finishing Agents	Modified gelatin	Natural	The binders blended with modified gelatin showed better biodegradability than commercially available binders.	22
16.	Effect of Finishing Auxiliaries on Permeability of Leathers	Protein and PU finishing binder	Natural and Synthetic	Leather coated with protein binder shows minimal permeability reduction, as compared with acrylic and polyurethane binders.	24
16.	Hydrolysis of leather shavings waste for protein binder	Protein binder	Natural	Protein binder obtained from leather shaving scraps explored for the alternate of synthetic binder and with sustainability approach.	23
18.	The use of protein binder from shaving waste for leather finishing: Judging from the physical, chemical, and morphological properties of lizard skin leather	Protein binder	Natural	The application of protein binder obtained from shaving waste showed better physical strength properties.	32

Environmental concern

Generally, leather finishing associates with application of natural compounds, synthetic chemicals and their derivatives such as polyamide–imide, polyethersulfone, polysulfone, polyphenylenesulfone, polyphenylenesulfide, polyimide, polyaryletherketones, polyetherimide and polyurethanes. The lack of purification and treatment of unused finished chemicals creates a negative impact on the aquatic environment which leads to the problems associated with high BOD, COD, TDS. The longer impact of these chemicals may develop possibilities of side reactions such as chlorination, amidation, oxidation and sulfonation due to presence of active groups in their structure.39 The polyurethane and its derivatives are preferred synthetic binders for leather finishing. Being a combustible material, they liberate high concentration of hydrogen cyanide, carbon dioxide, carbon monoxide and various oxides of nitrogen which contributes high to the carbon footprint of the leather industry.[40] Some of the solvents used in finishing with binders can liberate volatile organic compounds which are also toxic to the human health and the environment.[41] Chronic exposures to these chemicals may cause several diseases especially skin and lung cancer. The solvent free or water-based formulations and application of these binders may decrease the carbon footprint judicially.[42]

The application of natural binder alone or with the blending of synthetic binder may contribute to a smaller environmental footprint for leather finishing. Moreover, the influence of application of above blending depends upon the origin of the natural binder as well as surroundings of the chosen environment.[22]

Conclusion

The review focused on the detailed properties and importance of use of natural binders in leather finishing. The details of finishing processes and chemicals used are also discussed here. The uses of natural binders and its derivatives may overcome the dependency of uses of synthetic binders and contribute towards more sustainability and smaller carbon footprint. The eco-friendly and biodegradable binders may be considered for the leather finishing.

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Antibacterial Potency for Mimosa, Quebracho and Essential Oils of *Origanum* Species against *Acinetobacter pittii, Klebsiella pneumoniae* and *Bacillus cereus* from Diabetic Foot Patient

by

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Abstract

The diabetic patients may sometime suffer from foot lesions, foot ulcers and amputation that adversely affect their quality of life. In this respect, good footwear has a critical significance for diabetic foot patients. The aim of this study to evaluate the potential efficacy of mimosa, quebracho, and essential oils of *Origanum onites, Origanum onites oleum*, and *Origanum minutiflorum*, against *Acinetobacter pittii*, *Bacillus cereus*, and *Klebsiella pneumoniae*, which was isolated from diabetic foot patient. According to our results, the mimosa extracts were slightly more efficient when compared to quebracho extracts to control the bacterial growth of *A. pittii* (8.77±0.58-35.12±8.41% inhibition for three doses), and *K. pneumoniae* (21.40±0.48-47.04±0.51% inhibition) except *B. cereus* (71.1±0.31-23.51±1.66% inhibition). But these inhibition percentages remained at lower levels. On the other hand, essential oil samples of *O. onites, O. onites oleum,* and *O. minutiflorum* at tested doses have considerably high antibacterial effects against *A. pittii*, *B. cereus*, and *K. pneumoniae*. The tested essential oils almost completely inhibited *B. cereus* with percentages of inhibition ranging from 96.18±2.98-100±0.00. Also, the bacterial growth of *K. pneumoniae* and *A. pittii* was inhibited by 88.01±2.36 to 100±0.00% and 71.42±12.57 to 100±0.00%, respectively. Moreover, the essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum,* had bactericidal activity against *A. pittii,* and *K. pneumoniae* but bacteriostatic activity against *B. cereus*. This potency for essential oil of *Origanum* species may be evaluated for diabetic footwear. More detailed technical studies are required for the application of *Origanum* species to leather footwear.

Introduction

Diabetes is a disease associated with the endocrine system, with an increasing prevalence and affecting many people around the world. Therefore, it is evaluated as a global health problem nowadays. Considering the socioeconomic burden due to long-term drug use or prolonged hospital stay due to complications in diabetic patients, diabetes and diabetes-related complications have recently become an issue that needs to be emphasized.[1,2] The World Health Organization (WHO) reported 422 million patients suffering from diabetes mellitus are from low and middle-income countries.[3] Furthermore, the total cost of treating diabetic foot disease in the United States has been reported around 9-13 billion.[4] Taken into consideration the number of patients with diabetes, which is predicted to increase to 54 percent between 2015 and 2030, these costs will also be undoubtedly increased.[5] Moreover, increasing morbidity and mortality rates of these patient groups are associated with diabetic foot complications.[6] Diabetes is a major cause of foot infections, foot ulcerations, or impaired tissue integrity.[4] Unfortunately, it has been reported that at least 15% of diabetic patients may experience diabetic foot lesions at some point in their lives.[7] Foot ulceration leads to chronic foot infections and severe forms of gangrene that are responsible for 85% of amputation cases.[4] It has been suggested that one-third of these diabetic patients will probably experience amputation of the extremity. Furthermore, statistical data show that globally an amputation occurs every 30 seconds, usually due to secondary foot ulcers or lesions.[4,8,9] All these complications adversely affect the life quality of people.[10]

Some of the issues that should be especially considered in the diabetic foot are listed in the literature as choosing appropriate shoes, not cutting nails too short, not smoking, not using too many chemicals, etc.[2,11] The selection of good footwear is assessed as one of the most important parameters, especially in diabetic foot and it is stated that the prevention of foot infections or foot ulcers is possible with good foot care and screening of the risk of complications.[10] Following the guidelines of The Australian Diabetes Foot Network (2013) for footwear of patients with diabetes, advanced original experiments and guidelines have come into prominence.[12] The need for alternative footwear studies has been emphasized in primary and secondary prevention of foot ulcers.[11]

Various materials (leather, synthetic, fabric) with different properties are used in footwear production. However, it is highly

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important to select the appropriate material for diabetic shoes. In this view, leather appears to meet these criteria to be of a breathable, soft, and comfortable nature. In addition, leather has excellent moisture permeability by absorbing sweat and keeping the skin dry with its ability to take the shape of the feet of diabetics.[13] It is necessary to pay attention to these features in the production of shoes that will especially appeal to this patient group. At this point, as there is a close relationship between the skin surface of diabetics and the skin of the feet, an important point to be considered is bacterial colonization. Life-threatening foot infections result from bacterial colonization and sometimes biofilm formation on the leather/skin surface. The leather can provide suitable conditions for bacterial growth, such as temperature, humidity, and nutrients, and these bacterial populations can form a biofilm structure that is very difficult to eradicate from the environment. This scenario may potentially result in foot ulceration and amputation.[14]

Foot infections are sometimes caused by a mixed bacterial culture (aerobic bacteria and fungus) and sometimes by an individual bacterium. In the foot ulcer patients, possibly causative bacterial population may be Gram-positive (*Staphylococcus aureus, Enterococcus* species, etc.), or Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella* species, *Proteus* species, etc.), or anaerobes, with high potential for multi-drug resistance.[15, 16] Kramer et al. (2016) reported the percentages of bacteria isolated from patients with diabetic food infection as *Pseudomonas* spp. (29%), *Bacillus* spp. (3%), *Enterobacter* spp. (7%), *Staphylococcus* spp. (13%), *Acinetobacter* spp. (10%), *Enterococcus* spp. (9%) and *Klebsiella* spp. (8%). In other studies, *S. aureus, S. saprophyticus, S. epidermidis, Streptococcus pyogenes, S. mutans, P. aeruginosa, B. subtilis, Proteus sp., Escherichia coli* and *K. pneumoniae, Peptostreptococcus*, *Bacteroides fragilis,* and *Clostridium* species were reported from cases with foot infection.[17] In a study including 115 patients in 2021, the samples were taken from 94 patients with diabetic foot ulcers, and the most common bacteria were reported as *S. aureus* (30.1%), *K. pneumoniae* (21.9%), and *Acinetobacter* spp. (19.1%).[18] *Acinetobacter* spp. was also detected at a rate of 4.5% in samples taken from patients with diabetic foot ulcers. Similarly, in a study conducted in Egypt with 120 patients in 2021, 12.2% of microorganisms isolated from diabetic foot ulcer patients were found to be *Acinetobacter* spp.[19, 20] Cardosa et al. (2017), reported that the members of *Acinetobacter* spp. are major microorganisms that cause amputation in the ulcers of patients with diabetic foot.

The genus *Acinetobacter* is a Gram-negative bacterium with 68 species belonging to the *Moraxellaceae* family.[21] *Acinetobacter pittii,* belongs to the *A. calcoaceticus-A. baumannii* complex, which is responsible for hospital-acquired infections. Although *Acinetobacter pitti* is encountered less frequently than *A. baumannii*, clinical specimens of *A. pittii* have been increasingly reported in recent years.[22] Furthermore, the emergence of carbapenem-resistant strains of *A. pittii* with carbapenem-hydrolysis β-lactamases such as NDM-1 has become a major medical concern. Information on virulence factors belonging to the *A. pittii* species or their role in pathogenicity is limited.[23,24]

Klebsiella pneumoniae is a Gram-negative bacterium and a member of the *Enterobacteriaceae* family. Bacterial infections caused by the *K. pneumoniae* cannot be effectively treated due to the development of resistance of this bacterium to commonly prescribed antibiotics. This bacterium can cause serious healthcare-associated infections such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Furthermore, *K. pneumoniae* may cause serious infections in immunocompromised patients including diabetes.[24,25] Mukkunnath et al., (2015) reported approximately 21.7% of diabetic foot cases caused by *K. pneumoniae*.[27]

Bacillus cereus is an aerobic, spore-forming, Gram-positive bacterium that may potentially lead to infections in immunocompromised patients.[28] The cutaneous infections due to *B. cereus* may be observed in diabetic patients. *B. cereus* can be treated by several antibiotics but recently, its resistance to erythromycin and tetracycline antibiotics in Europe and in the United States was reported.[29] This bacterium can be seen in diabetic patients, albeit to a lesser extent. Michelotti and Jonathan Bodansky (2015) reported a case report for haemorrhagic superficial necrosis up to the knee due to *B. cereus* in 72-year-old man with Type 2 diabetes mellitus.[30]

In recent years, the potential bioactivities of plant-derived chemicals such as antioxidant, antibacterial, and antifungal activities have been reported.[31] There are numerous studies examining the antimicrobial efficacy of various plant extracts/the compounds/mixture of their compounds against a variety of bacteria. In the light of all this information, it is of great importance to have plant extracts or chemicals with antibacterial properties that can be used in the shoes of patients with diabetic foot. More recently, microencapsulated substances materials or components such as plant-based materials for their antimicrobial properties are tested in the footwear industry. These natural resources can help patients overcome unpleasant odors from foot complications thanks to their antibacterial properties and increase the durability of the leather. From this point, in this study, we aimed to investigate the potential efficacy of mimosa and quebracho, which are utilized as vegetable tanning agents, and also essential oils of *O. onites* (wild oregano), *O. onites oleum,* and *O. minutiflorum* (oregano) (endemic in Turkey), which have economic importance in the worldwide trade and have also various biological activities (antifungal, antimicrobial, etc.), against *A. pittii, B. cereus*, and *K. pneumoniae* which was isolated from diabetic foot patient.

Materials and Methods

Bacterial strain and test materials

A. pittii, K. pneumoniae and *B. cereus* were isolated from diabetic foot patients in Marmara University, Istanbul Pendik Training and Research Hospital. The test isolates were stored at -80°C until the experiments. Before experiments, the pure culture of the isolates were obtained on Tryptic Soy Agar at 37°C for 24 h. Mimosa and quebracho were purchased from Mimosa GS Powder Elephant Brand, UCL Company (PTY) LTD, and Unitan Atg Company, respectively. The essential from *O. onites, O. onites oleum*, and *O. minutiflorum* was purchased from Türer Bitkisel A.S., Botaniksan, Health and Sleep Company, respectively.

Antibacterial tests

Antibacterial tests were performed in sterile glass tubes containing Tryptic Soy Broth medium. Mimosa, quebracho, and also essential oils of *O. onites, O. onites oleum* and *O. minutiflorum* were added to each tube at a volume of 3.33%, 1.67, and 0.83% (v/v). Samples of mimosa and quebracho were resolved at maximum concentration. The final concentrations of mimosa extracts of stock solution were 69.2 mg/mL and quebracho extracts were 39.8 mg/mL. The applied concentrations for mimosa extract were 2.31, 1.15 and 0.58 mg/mL, and for quebracho extracts were 1.33, 0.66 and 0.33 mg/mL. The bacterial culture was added to the tubes by measuring the optical density (OD) at 600 nm and adjusting it to 0.5 Mc Farland. The tubes were incubated in a shaking incubator for 24 hours at 37°C. Optical density (OD) measurements were taken at 600 nm after the incubation period. The experiments included untreated, treated, and antibiotic-treated groups. The five antibiotics (gentamicin, apramycin, vancomycin, penicillin, and kanamycin) were tested in preliminary screening experiments. The gentamisin and apramycin were found to be effective against *A. pittii* and *K. pneumoniae,* respectively. For *B. cereus*, all antibiotics were successful except penicillin. From this respect, gentamisin and apramycin antibiotics were tested for the antibacterial efficacies against tested bacteria. The tests were performed in three replicates. The antibacterial efficacy was evaluated by comparing the test tubes to untreated groups.

Statistical analyses

The statistical analyses for the antibacterial activities of the extracts of mimosa, quebracho, and essential oil samples of *O. onites, O. onites oleum*, and *O. minutiflorum* against *A. pitti, K. pneumoniae,* and *B.cereus* were performed by One-Way Anova (Tukey) test via SPSS 16.0 program. The significance between the control (untreated) group, antibiotic treatment group, and three different doses of test materials was examined for each bacterial species. A p-value of 0.05 and below was considered significant. The statistically significant differences in analyses were given in the figures as (a,b,c,d) for *A. pittii*, (1,2,3,4) for *K. pneumoniae*, (□, ∆, Ω, ○, ⌓) for *B. cereus*.

Results and Discussion

In this study, the antibacterial potentials of the extracts of mimosa and quebracho, and also essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum* were investigated against *A. pittii*, *K. pneumoniae* and *B. cereus*. In the literature, there are numerous studies focusing on the potential bioactivities of plant extracts, essential oils and active compounds against many bacterial species. At the beginning of this study, it was prioritized that it would be more meaningful to select the bacteria to be studied directly from the foot of the diabetic foot patient. Because it is well known that many bacteria can develop antibiotic resistance against existing prescription antibiotics in the clinical course. In this respect, as emphasized in most studies, alternative agents to overcome this problem need to be discovered. Diabetic foot is of great importance because of the potential aggressive infections caused by various microorganisms, which can usually be caused by individuals themselves. Diabetic shoes have a pivotal role in the treatment of patients to ensure their quality of life. To our best of knowledge, there is no study in the literature examining the potential antibacterial efficacy of extracts or essential oils from tested plant materials against *A. pittii*, *K. pneumoniae* and *B. cereus* isolated from diabetic foot patient.

The assessment of the potency of antibiotics was performed. Then, gentamicin, and apramycin which are selected based on preliminary screening tests, were performed as positive controls. The gentamicin inhibited bacterial growth of *A. pittii* by the inhibition percentage of 94.77±0.07 whereas apramycin suppressed *K. pneumoniae* and *B. cereus* with inhibition rates of 96.10±*0*.3, and 84.44±0.21, respectively. These data were included as OD values in the figures below.

Antibacterial efficacy of mimosa extracts against tested bacteria

The extracts of mimosa had no remarkable inhibitory efficacy against *A. pittii.* The inhibition percentages of the extracts of mimosa were observed as 35.12±*8*.41, 11.76±*1*.0, and 8.77±*0*.58 for tested concentrations, respectively. Similar insufficient efficacy was observed against *K. pneumoniae*. The mimosa extracts inhibited the bacterial growth of *K. pneumoniae* by the inhibition ratios of 21.40±*0*.48%, 47.04±*0*.51%, and 21.62±1.08%, respectively. However, the antibacterial efficacy of mimosa extracts were obtained against *B. cereus* for the concentrations of 2.31 mg/mL and 1.15 mg/mL by the inhibition percentages of 70.36±0.29 and 71.10±*0*.31. In the 0.58 mg/mL treatment group, there was no notable suppressive effect against *B. cereus* (Figure 1).

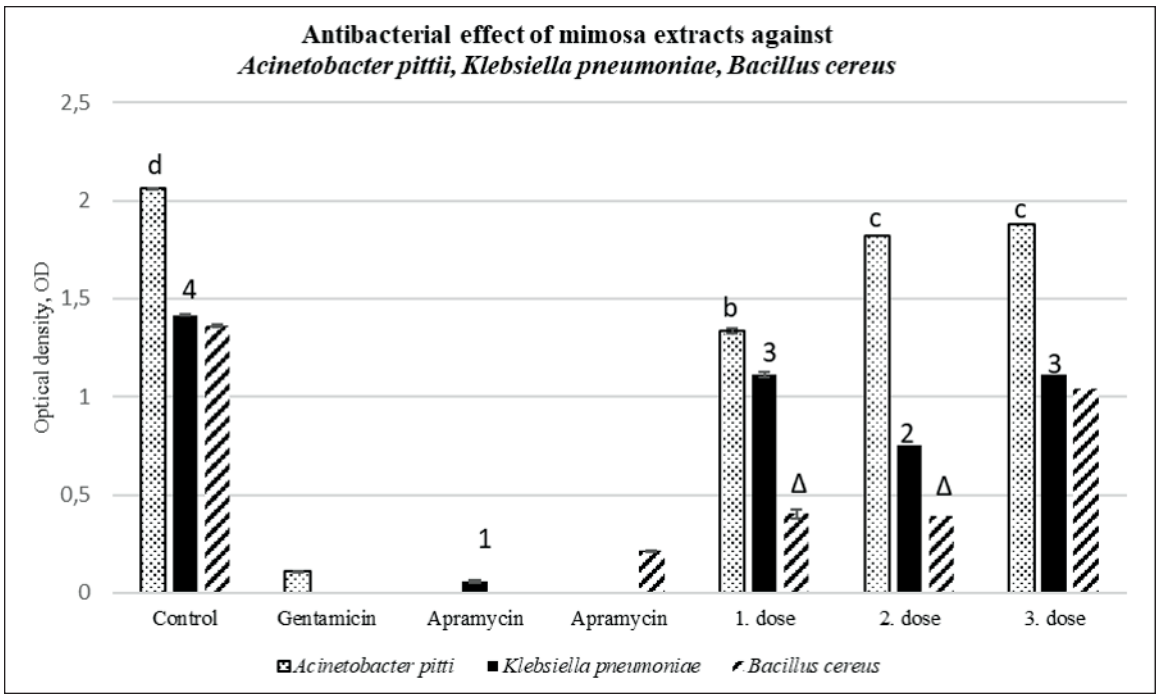

Figure 1. The antibacterial effects of mimosa extracts aganist *A. pittii, K. pneumoniae, B. cereus.* (1.dose 2.31 mg/mL, 2.dose 1.15 mg/mL and 3.dose 0.58 mg/mL). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○).

Antibacterial efficacy of quebracho extracts against tested bacteria

Similarly, as in mimosa extracts, quebracho extracts had no significant antibacterial efficiency against the bacterial growth of *A. pittii.* In comparison with untreated groups, the inhibition ratios were recorded as 16.73±0.37, 14.79±0.44, and 11.22±0.80% for tested concentrations, respectively. The inhibition percentages were detected as 49.05±0.53, 26.79±0.63 and 13.30±0.22 for the tested concentrations of the extracts of quebracho against *K. pneumoniae*, respectively. Just like in mimosa extracts, quebracho extract was found to be more effective on *B. cereus*, especially for the first dose with the inhibition rate of 62.40±1.28. The other lower test concentrations (0.66 mg/mL and 0.33 mg/mL) showed very low suppressive efficiency (29.68±0,35 and 16.03±0,92%, respectively) against this bacterium. (Figure 2).

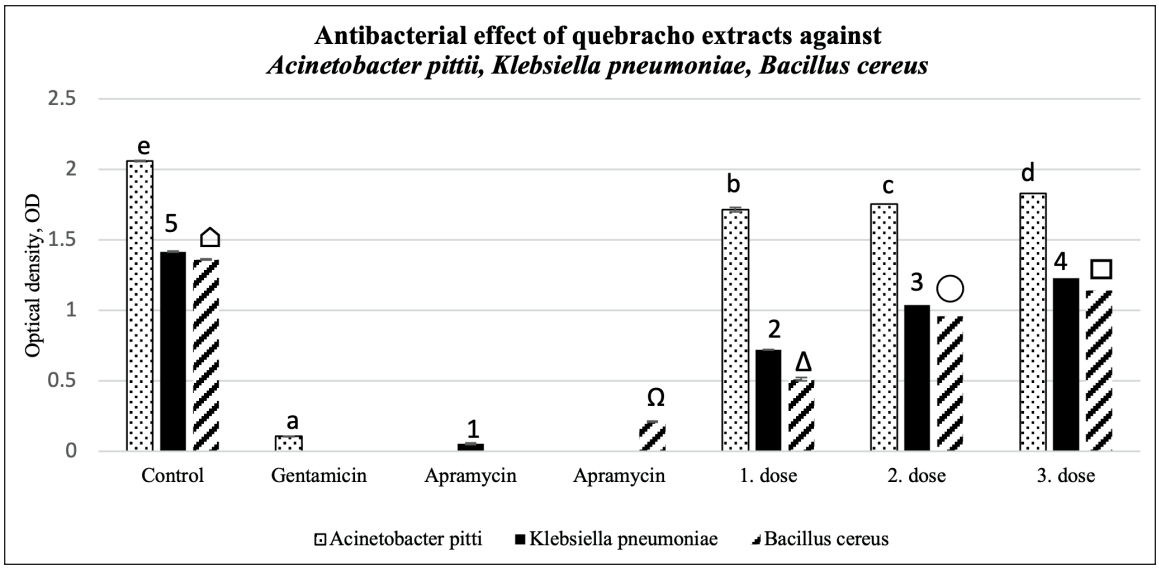

Figure 2. The antibacterial effects of quebracho extracts against *A. pittii, K. pneumoniae, B. cereus.* (1.dose 1.33 mg/mL, 2.dose 0.66 mg/mL and 3.dose 0.33 mg/mL). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○, ⌓).

Antibacterial efficacy of essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum*

We observed a considerable antibacterial effect for the essential oil of *O. onites* against *A. pittii* at the volumes of 3.33%, 1.67% and 0.83% (v/v). The inhibition percentages of the test material were recorded as 100±0.09, 86.69±1.62, and 71.42±12.57, respectively. The same pronounced efficacy was observed also against *K. pneumoniae* for tested essential oil samples (98.62± 2.07%, 100±0.00% and 100±0.00%, respectively), and *B. cereus* (100±0.00%, for each tested volumes) (Figure 3).

Similar outstanding results were also obtained for the essential oils of *O. onites oleum* and *O. minutiflorum*. The inhibitory ratios for *O. onites oleum* against *A. pittii* at the test volumes of 3.33%, 1.67% and 0.83% (v/v) were 100±0.00, 87.74±4.97, and 82.74±18.68, respectively. The potential antibacterial efficacy was also gathered by the essential oil of *O. minutiflorum* with the inhibition rates of 100±0.00, 80.04±4.97, and 78.52±2.47%, respectively (Figure 4 and 5). The notable suppressive effects of the essential oils of *O. onites oleum* and *O. minutiflorum* were observed also against *K. pneumoniae* and *B. cereus.* The inhibitory effects of essential oils of *O. onites oleum*

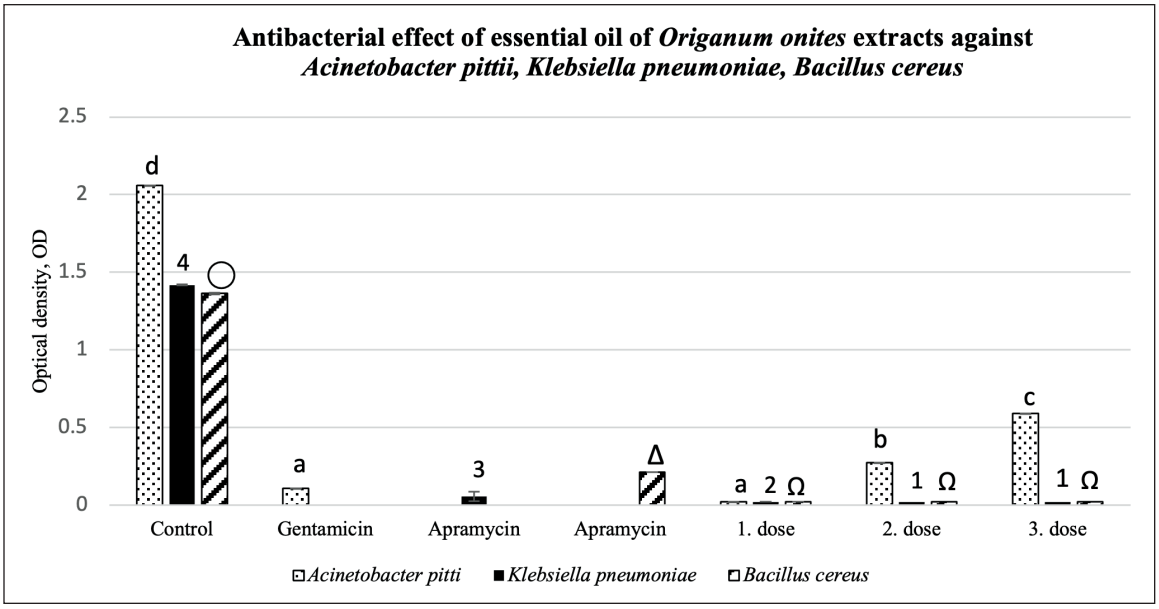

Figure 3. The antibacterial effects of the essential oil of *O. onites* against *A. pittii. K. pneumoniae, B. cereus.* (1.dose 3.33% (v/v), 2.dose 1.67% (v/v) and 3.dose 0.83% (v/v)). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○).

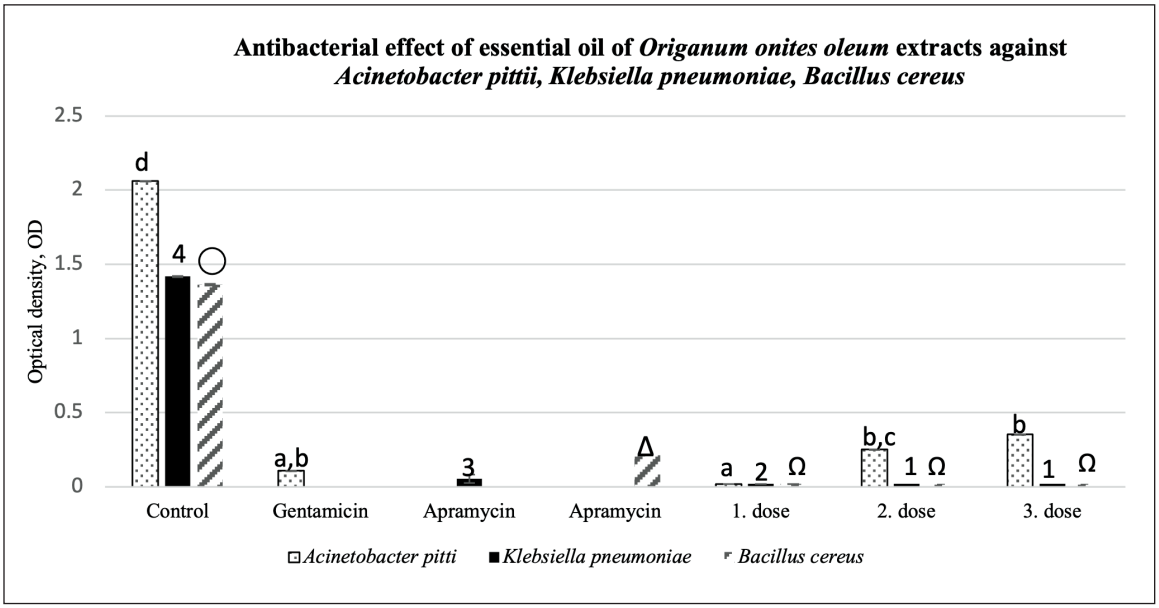

Figure 4. The antibacterial effects of essential oils of *O. onites oleum* against *A. pittii, K. pneumoniae, B. cereus.* (1.dose 3.33% (v/v), 2.dose 1.67% (v/v) and 3.dose 0.83% (v/v)). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, ∆, Ω, ○).

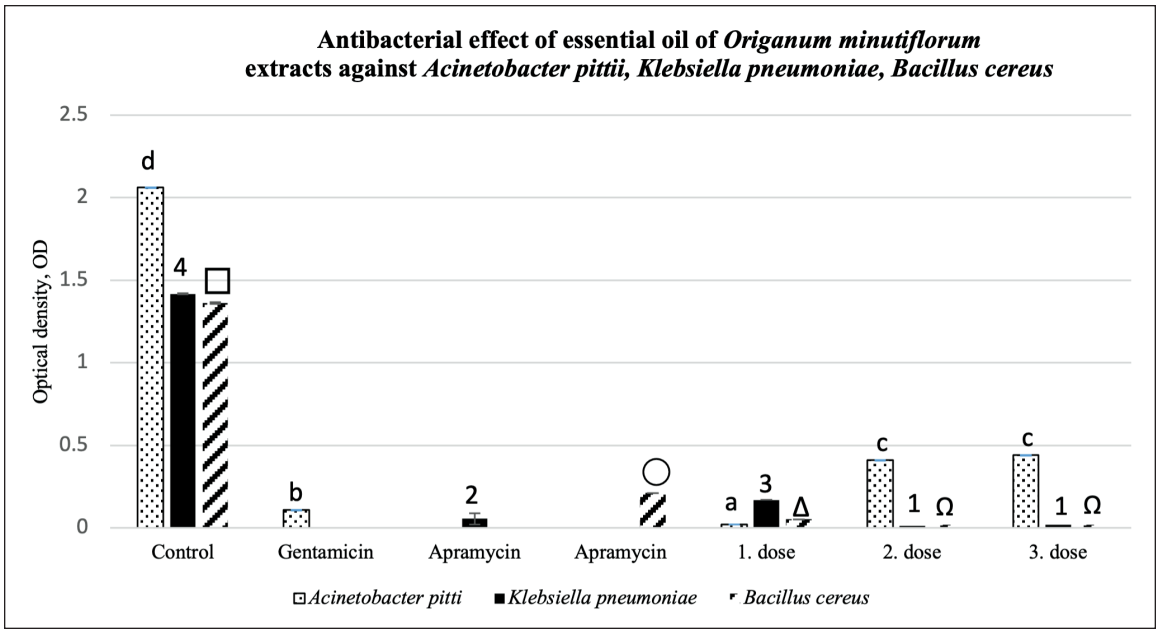

Figure 5. The antibacterial effects of the essential oil of *O. minutiflorum* against *A. pittii, K. pneumoniae, B. cereus.* (1.dose 3.33% (v/v), 2.dose 1.67% (v/v) and 3.dose 0.83% (v/v)). The statistically significant differences were given for *A. pittii* as (a,b,c,d,e), for *K. pneumoniae* as (1,2,3,4,5), for *B. cereus* as (□, Δ, Ω, ○).

were detected as, 98.62±2.07, 100±0.00 and 100±0.00% against *K. pneumoniae* and 100±0.00% (for all tested volumes) against *B. cereus*. Also, the essential oils of *O. minutiflorum* at the test volmes of 3.33%, 1.67%, 0.83% (v/v) inhibited bacterial growth of *K. pneumoniae* with the inhibition percentages of 88.01±2.36, 99.08±1.38, and 100±0.00, respectively. Similarly, essential oil samples of *O. minutiflorum* had antibacterial efficiency against *B. cereus* and inbition ratios were recorded as 96.18±2.98%, 100±0.00% and 100±0.00%, respectively.

Statistical analyses

For *A. pittii*, *K. pneumoniae* and *B. cereus,* it was observed that all three tested concentrations of extracts of mimosa and quebracho, and essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum* had significance compared to the control (untreated) groups (p=0.000). Also, the gentamicin treatment group for *A. pittii* and the apramycin treatment groups for *K. pneumoniae* and *B. cereus* was found to be statistically more significant compared to mimosa and quebracho extract treatment groups at all tested concentrations (p=0.000). It was observed that there was no statistically significant difference between the first and second doses of mimosa for *B. cereus*. There was a statistically significant difference (p=0.000) in quebracho extract treatment groups between the first dose with the 2nd and 3rd doses. According to the gentamicin group, the first dose of *O. onites* gave similar results, and no statistical significance was observed. On the other hand, all tested doses showed statistically significant difference when compared to apramycin treatment groups for *K. pneumoniae* and *B. cereus* (p=0.000). There was no significant difference in all three doses of *O. onites oleum* in comparison to gentamicin treatment group. The first dose was statistically significant according to the second dose (p=0.035) and the third dose of *O. onites oleum* (p=0.001). No statistically significant difference was found for *B. cereus* for all administered doses of *O. onites oleum*. According to the gentamicin group, a statistically significant difference was observed in *A. pittii* for the first dose (p=0.001) and the 2nd and 3rd doses (p=0.000) of *O. minutiflorum*. On the other had, there was no statistically significant difference between the 2nd and 3rd doses of *O. minutiflorum* for *K. pneumoniae* and *B. cereus.*

Overall, our results demonstrated that the essential oil samples from *O. onites, O. onites oleum,* and *O. minutiflorum* were more successful in suppressing the bacterial growth, where a 3% (v/v) volume of essential oil samples from three tested *Origanum* species totally killed *A. pittii*. In addition, 1.67% (v/v) and 0.83% (v/v) volumes of essential oil samples from three tested *Origanum* species were also highly effective in inhibiting the bacterial growth of *A. pittii*. On the other hand, the desired effect by the extracts of mimosa and quebracho was not detected at the test concentrations. As known, mimosa and quebracho has largely been used in the leather industry in the vegetable tanning process to give reddish color.[32] There are some studies investigating the antibacterial potential of these tanning agents. Digrak et al. (1999) examined the extracts of mimosa bark against some Gram-positive and Gram-negative bacteria including *Brevibacillus brevis, B. subtilis, B. cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium luteus, K. pneumoniae, M. smegmatis, Proteus vulgaris* and they reported antibacterial efficacy against tested bacteria.[32] However, Rosiati et al. (2020)highlighted the possibility of vegetable tanned leathers as a potential growth medium for bacteria.[33] Furthermore, they tested the antibacterial efficiency of vegetable tanned (mimosa tanning agent) goat skins against *S. aureus* and reported slight inhibition zones as 11.40 mm.

Sirvaityte et al. (2011) demonstrated that the high resistance of leather tanned with mimosa to *E. coli* in comparison to quebracho group.[34] Moreover, quebracho and mimosa (50:50) mix showed a great efficacy for the inhibition of *P. aeruginosa* and *S. aureus*. However, Colak (2006)tested some vegetable tannins in soaking process for their antibacterial potencies against total aerobic bacteria at the 8th and 24th hours.[35] The researcher recorded no notable results for mimosa and and quebracho when compared to control group. These studies show that the potential antibacterial efficiency for the extracts of mimosa and quebracho may vary depending on the type of bacteria or process.

In the literature, there are studies in the leather sector by using extracts/chemical components/essential oils of various plants for the different leather-making processes. For example, the extracts of *Coridothymus capitatus, Olea europaea, Corylus avellana,* and *Juglans regia*, were tested in retanning stage to reduce chromium (VI) and satisfying results were found.[36] Haibin et al. (2011) indicated eco-friendly fungicide potency of essential oils from cinnamon, garlic, clove, and star anise in the leather sector.[37] The antibacterial efficacy for essential oils of *Lavandula officinalis* and myrtle oil (1%) in soaking process was reported in previous studies.[36,38] Our promising results are consistent with several studies evaluating different *Origanum* species for their antibacterial properties.[39-42] Aligiannis et al. (2001) tested the antibacterial and antifungal properties of essential oils obtained from two *Origanum* species against *S. aureus*, *S. epidermidis*, *E. coli*, *Enterobacter cloacae*, *P. aeruginosa, Klebsiella pneumoniae*, *Candida albicans, C. tropicalis* and *C. glabrata* and they demonstrated considerably high efficacies on tested materials.[39] Bayramoglu (2007) evaluated the antibacterial efficiency of four test materials including 1% of essential oils from *O. onites*, two different *Origanum* species and *Foeniculum vulgare* on the growth of bacterial population in the soaking process at the 8th and 24th hours. The researcher tested commercial bactericide namely 7-25 % phenol and 4-chloro-3-methyl as a control group. The study results demonstrated that 1% of essential oils from three *Origanum* species had antibacterial activity at both time points when compared to the untreated group.[43] The essential oil of *O. onites* has been also found to be successful in the fatliquoring process to eliminate free formaldehyde.[44] Bayramoglu et al. (2006) also tested the potential antifungal efficiency for essential oils of *O. minutiflorum, Laurus nobilis, Foeniculum vulgare,* and *Schinus molle* against *Aspergillus niger, Alternaria alternata, Penicillium rubrum* and *Trichoderma viride,* which are easily grown during pickling stage of leather making process. They reported the strongest antifungal effect for *O. minutiflorum*.[45]

The bioactive properties for the species belonging to *Origanum* species are reported to be relevant to the content of major phenolic compounds, especially thymol and carvacrol.[46] In our study, considerably high efficacies may have been obtained due to this chemical compound content. Further studies are needed to be performed for testing the applicability of these natural resources on diabetic footwear. It should be noted that the chemical composition of essential oils may vary depending on the place and time of harvest and the way of processing and storage. In addition, their antibacterial potency may change based on the content or concentration of the plant or its essential oil, and also the type and density of the bacteria.[47] The essential oils of *Origanum* species, obtained at the right time, from the right place, and in the right way, can be applied to the leather, which is a soft and breathable material for diabetic foot patients. This application may be done after the fatliquoring process via microencapsulation or during finishing process by spraying method of nano/microencapsules. The microencapsulation method provides protection from reactions caused by moisture, light, oxygen, ensures the controlled release of natural extracts, essential oils, active compounds, and also longer-lasting antibacterial efficacy. There are studies evaluating microencapsulated essential oils as biocides in footwear.[48-50] Thus, complaints of many diabetic foot patients such as foot infections, lesions, or ulcers can be prevented, and also the risk of amputation can be eliminated.

Conclusion

The importance of a good footwear selection in the clinical course of diabetic foot patients is stated in the literature. Recently, due to the problem of antibiotic resistance, there has been a trend towards the search for new natural resources with antibacterial effect. Controlling the bacterial population in the diabetic foot is of paramount importance to avoid the patient's history of worsening foot infections, which may lead to amputation. Studies focusing on the microbial flora of the diabetic foot have demonstrated the presence of *A. pittii*. The results of this study showed that the essential oils of *O. onites, O. onites oleum,* and *O. minutiflorum* had remarkable inhibitory effects on the bacterial growth of *A. pittii*, *K. pneumoniae* and *B. cereus*. On the other hand, the extracts of mimosa demonstrated a very slight effect against *A. pittii* (35.12-8.77% inhibition range for three doses) and *K. pneumoniae* (47.04-21.40% inhibition range for three doses). However, the most efficacy of mimosa extracts was observed against *B. cereus*. Compared to quebracho extracts, the extracts of mimosa were found to be slightly more successful against tested bacteria. The greater than 50% inhibition was recorded at the concentration of 2.31 mg/mL of quebracho extracts against *B. cereus* whereas the same efficacy was not detected against other tested bacteria. The essential oils tested inhibited almost *B. cereus* with percentages of inhibition ranging from 96.18-100. The growth of *K. pneumoniae* and *A. pittii* was inhibited by 88.01 to 100% and 71.42 to 100%, respectively. From this point of view, these essential oils may be used in diabetic footwear by various application routes, such as microencapsulation or spraying on the leather. There is a need for studies investigating the effectiveness of these test materials against most common bacteria in diabetic foot patients when applied to the leather. The importance to study with mixed cultures should be kept in mind. Because in most cases, antibacterial agents can act on a single bacterium but sometimes this expected efficacy may not be seen on mixed cultures.

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Recovery of Industrially Useful Hair and Fat from Enzymatic Unhairing of Goatskins during Leather Processing

by

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Abstract

Leather processing not only serves social needs by putting into use the meat industry's by-products (hides and skins) but also makes a significant contribution to global economic growth through trade and job creation. In the wake of globalization, however, leather manufacturers are facing new challenges in meeting environmental imperatives and improving the utilization of wastes generated during leather processing. This study describes the recovery of hair and fat from fleshings obtained after enzymatic unhairing of goatskins using a protease from an isolate of *Bacillus cereus Strain 1-p*. The recovered hair and fats were further characterized to facilitate recommendations for different industrial applications. The following hair properties were visually examined and evaluated by hand; straight length, density and uniformity, hair strength and overall quality. The fats were analyzed by characterizing the fatty acid composition using the Gas Chromatography-Mass Spectrometry (GC-MS analysis). The recovered hair was intact and rated to be of average to good quality. The fat characterization indicated that methyl 9Z-octadecenoate (9Z-heptadecenoic acid; oleic acid) was the most abundant fatty acid with an abundance of 31.65%. The sulfide-free fats and intact hair, therefore, were recommended for use in various industrial applications such as manufacturing of poultry feedstuff, organic fertilizers, biodiesel and biofuels, fatliquoring agents, soaps and cosmetics after further purification where necessary. The hair and fats recovered from this study are particularly advantageous over those recovered from sulfide unhairing systems as they are free from any sulfides or lime contamination thus easier to purify and use. The study concluded that the use of the enzyme extract from *Bacillus cereus Strain 1-p* to unhair goatskins facilitated the recovery of valuable hair and fats that can be used for other industrial applications.

Introduction

In the wake of climate change and the increasing need to protect and restore nature, it is paramount that there is also a switch towards cleaner and low-carbon natural products. There is no practicable pathway geared towards net zero emissions that does not start with responsible and knowledgeable choices of products, production systems or even the choice of energy to be used. Choosing natural fibers such as leather, wool, cotton, mohair, silk and mycelium is a good starting point towards achieving the net-zero targets, protecting lives and livelihoods as well as saving the planet. These natural and readily available materials are made up of natural carbon that has been in the atmosphere for ages and thus are already part of the biogenic carbon cycle.[1] When these natural raw materials are produced and processed ethically, they are long-serving, recyclable, repairable and at the end of life, biodegradable, thus mitigating their impact and emissions.

Leather is not only an ethical and sustainable choice material for the global footwear, furniture, fashion and automotive and industries but also plays an important economic role globally. The leather industry utilizes by-products of the meat industry (hides and skins) as raw materials, which would otherwise be deemed as waste.[2] These unavoidable wastes (as long as people consume meat), if unused, will end up in the landfills and create another problem from the emissions thereafter. The leather industry, however, terms this "waste" as the meat industry's "by-product" which is now used as raw material for leather processing. The leather material is also a preferred choice for its unique properties which include strength, resistance to abrasion, durability and longevity, elasticity and water vapor permeability. A study conducted to compare leather's technical performance to that of artificial leather and other biogenic and synthetic alternatives showed that none of the alternatives perform as well as leather.[3] The findings further highlighted that the structure of leather could not be achieved by any bio-based or synthetic product.

Leather processing entails a series of stages that are aimed at converting the rawhide/skin to a stable product, leather. The initial stages involve cleansing steps that remove unwanted parts through chemical (soaking, unhairing, liming) and mechanical processes such as trimming, fleshing and shaving.[4] There is an estimated conversion rate of 20% whereby the processing of one tonne of

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hides produces approximately 200kg of quality leather while approximately 250 kg is yielded as tanned waste, 350 kg produced as untanned wastes and about 100 kg as effluent wastewater.[5] It is evident that this process generates large volumes of by-products, herein referred to as waste, from different stages as only the collagen section of the hide or skin is converted to leather. These include huge amounts of solid wastes such as raw trimmings, keratins (hair and wool), fleshings, wet-blue shavings and buffing dust, which pose disposal challenges.[6] Most of the tannery wastes are generated in the beamhouse, especially at the fleshing stage. Approximately 55% of the solid wastes from pre-tanning processes are fleshings while about 25% are hair and wool debris.[4,6] Fleshings and hair have caused major environmental concerns in the leather industry over the years characterized by troublesome and costly disposal techniques. Despite the significant fat content present in the fleshing waste, they have not found important use.[4]

Pre-tanning wastes mainly consist of natural fats, blood and proteins. The majority of these wastes have a great potential for reutilization. Disposal of these wastes through landfills has widely been practiced as a way of waste management which is quite costly and environmentally undesirable. Furthermore, the landfill sites continue to decrease making disposal costs higher coupled with costs incurred for transportation.[5] It is therefore prudent to find alternative uses for these materials to protect the environment and prevent loss of resources. Fleshing wastes have successfully been explored for use in the production of biodiesels through a transesterification process which can be used to replace fossil fuels and manufacture of fatliquors and oil tanning agents while hair has been explored for the production of animal feeds and fertilizers.[4,7–9]

While the use of sulfides for unhairing has been dependable over the years, it continues to raise concerns over their environmental impact. The unhairing process is known to be the dirtiest following the sulfide odor, broken down protein and hair, and the effluent load generated.[10,11] This unhairing technique is therefore associated with large wastewater volumes, huge levels of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) and Suspended Solids (SS).[12–14] The fleshings recovered after sulfide unhairing have very high sulfide content that limits their reutilization. The sulfide equally breaks down the hair structure into a pulped form reducing the chances of the utilization of this hair. The incorporation of proteolytic enzymes to replace the use of sulfides has been embraced as a viable alternative. The use of enzyme unhairing is a greener eco-friendly alternative that cuts approximately 50% of COD and 40% of BOD in leather processing.[10,15] Enzymatic unhairing eliminates sulfide contamination of the fleshings and does not solubilize the hair, thus good quality hair is recovered.[10,13]

Despite the extensive research studies carried out previously on the unhairing of hides and skins using protease extracts from various bacterial strains, little work is published on the use of the enzyme extract from the novel *Bacillus cereus strain 1-p,* obtained from a soda lake in the Rift Valley region of Kenya. Furthermore, no comprehensive work was dedicated to the analysis of the recovered by-products from the process. This study focuses on the characterization of hair and fat recovered from fleshings, all recovered from enzymatically unhaired goatskins using a crude protease extract from *Bacillus cereus strain 1-p*[10] to obtain more specific information on the characteristics of the wastes to determine their suitability for reutilization in other industrial applications.

Materials and Methods

Materials

The unhairing enzyme used for this work was extracted from the *Bacillus cereus strain 1-p bacteria* which was isolated and cultured at the University of Nairobi's Biochemistry laboratory. Fresh goatskins (often referred to as "green"), obtained from a local goat abattoir in Nairobi, Kenya, were used for this study. All the chemicals used were both of analytical and commercial grade.

Methods

Enzyme Preparation

An isolate of the *Bacillus cereus strain 1-p bacteria* was obtained and used to prepare more plates for enzyme production. To make the bacterial culture, the following ingredients were measured and a mixture prepared; agar, casein and distilled water. Sterile tips were used to transfer bacteria from the parent cultured plate onto the new plates which were incubated at 37°C for 72 hours. These plates were used for enzyme production. The enzyme was extracted at optimum parameters as described by Nyakundi *et al.* 2021.[10] A three-liter culture medium was prepared with sufficient proportions of yeast, casein and glucose to favor bacterial growth. This medium was thereafter set up in a Bioengineering RALF bioreactor and its pH adjusted to 11.5. The bacterial culture was then inoculated into the medium and was incubated for 72 hours at 150 rpm and 45˚C. The overnight bacterial culture was centrifuged at 12,000 rpm for 15 minutes and the supernatant was used as the crude enzyme that was applied to goatskins to facilitate unhairing.

Unhairing of Goatskins

Nine goatskins were washed thoroughly in a tannery experimental drum to remove the blood, dung and any dirt from the slaughterhouse. The skins were then treated with the enzyme to facilitate hair removal. Optimum unhairing conditions of pH, temperature and enzyme concentration were maintained as illustrated and described by Nyakundi *et al.* 2021 in their work comparing the quality of leather produced from enzyme unhairing to that of sulfide unhairing.[10] After 12 hours of exposure to the crude enzyme, the hair was gently scraped off the grain surface of the goatskins using a blunt blade. The hair was washed and rinsed using clean water to prevent any further enzyme activity, dried and weighed. More hair was recovered by filtration of the process liquor. The unhaired pelts were subsequently

fleshed using a goat fleshing machine for the recovery of flesh and fats (fleshings).

Characterization of the recovered products

Analysis of recovered hair

The recovered hair was assessed on the overall quality. The following hair properties were visually examined and evaluated by hand; straight length, density and uniformity, hair strength and overall quality. The hair was rated from 0 to 10 points for each parameter by an experienced hair and wool expert with the highest rating points denoting superior quality.

Analysis of recovered fat

The recovered fleshings were thoroughly washed with water to remove any enzyme and thus prevent chances of further enzyme activity. The fat was characterized using the Gas Chromatography-Mass Spectrometry (GCMS) method.[17] In order to identify the fatty acid composition of the sample using the GCMS, the sample had to be converted to fatty acid methyl esters through a transesterification process. This was done by taking 200g of the fleshings which were weighed and put in a conical flask. Hexane was then added to cover the fleshings in the flask and left overnight over a water bath maintained at 55^0C. The solution was then poured into a beaker and allowed to evaporate, leaving approximately 30g of fat.

A Thin Layer Chromatography (TLC) analysis of the sample was carried out to confirm the esterification process by spotting the sample on a silica gel plate. This was allowed to dry for approximately 3 minutes before being put in the mobile phase for development. The mobile phase contained hexane, diethyl ether and acetic acid in the ratio of 80:20:1. The visualization was subsequently done in an iodine chamber. A negative control test was also set up (without the lipase and Sodium Hydroxide catalysts) for comparison.

The fatty acid (FA) compositions of the goat fat sample (3.5g, split into three, 1.67g), were analyzed as fatty acid methyl esters (FAMEs) following previous methods.[16,17] A solution of sodium methoxide (15 mg/ml) was prepared in dry methanol and added (500 µl) to samples thawed at 50 °C for 30 min. The samples were vortexed for 1 min, sonicated for 5 min and incubated at 70 °C for 1 h, thereafter quenched by adding 100 µl deionized water followed by vortexing for another 1 min. The resulting methyl esters were extracted using GC-grade hexane (1000 µl) (Sigma–Aldrich, St. Louis, MO, USA) and centrifuged at 14,000 rpm for 5 min. The supernatant was dried over anhydrous Na_2SO_4 and analyzed (1.0 µl) by GC-MS on a 7890A gas chromatograph linked to a 5975 C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA). The GC was equipped with a HP-5 MS low bleed capillary column with a length of 30 m, a film thickness of 0.25 µm and an i.d. of 0.25 mm (J&W, Folsom, CA, USA). The GC oven temperature was programmed to increase from 35°C (5 min.) to 280°C at 10°C/min (24.5 min) then to 285°C at 50°C/min (20.5 min) and a run time of 50min. The split mode injector was used with an injection volume of 1µl. The flow rate was a constant flow mode of 1.25 ml/min, using Helium (He) as the carrier gas.

Results and Discussion

Recovered hair analysis

Figure 1 shows the hair recovered from the enzyme system (a) alongside hair from a sulfide unhairing system (b) for comparison. Approximately 543.6 g of dried hair was recovered from the 9 enzymatically unhaired goatskins. When examined visually, the hair structure was distinctive and intact as displayed in Figure 1.

The dried hair from the enzyme system was inspected for its quality by a hair and wool expert. The hair was assessed on the overall quality through rating from 0 to 10 points for each parameter with

(a) Hair from enzyme unhairing

(b) Hair from sulphide unhairing

Figure 1. Hair from an enzyme and a sulfide unhairing system

Table I

Assessment of recovered hair quality

Hair property	Straight length	Density and uniformity	Strength	Overall quality
Rating	9.0	5.0	8.5	6.5

higher points indicating a superior quality and the results displayed in Table I. The quality of enzyme recovered hair differed distinctly from that of the hair recovered from the conventional sulfide unhairing technique, which is usually broken down into a sludge-like mass (i.e pulped form) with no distinctive structure.[18]

The recovered hair was rated to be slightly above average on the overall quality (6.5), confirmed to be intact and without signs of fragileness when pulled apart. Its superior properties were straight length (9.0) and strength (8.5) while the density and uniformity were average (5.0). Out of 20 kg (raw weight) of goatskins processed, 0.5436 kg of dry intact hair was recovered. It can, therefore, be translated that the enzymatic unhairing system showed a hair recovery rate of approximately 2.72% of the total raw weight. Thus, processing one tonne of goatskins would yield approximately 27.2 kg of intact hair depending on the hair density on the skins. It was concluded that the recovery rate was quite significant and promising. This good quality hair is saleable and could be value-added to make other products. Previous research studies on enzyme unhairing have reported closely similar observations whereby the hair was recovered intact and undamaged.[9,19,20]

The recovered hair can be processed further or used as is depending on the intended end-use. The hair can find application in the processing of poultry feedstuff, felt and some organic fertilizers. Hair with closely similar properties recovered from the unhairing of hides and skins using a bacterial alkaline protease has previously been recommended for the manufacture of fertilizers as well as poultry feeds.[9] Sivasubramanian *et al.* (2008) went further to report that after subjection to chemical, biological and thermal hydrolysis, the recovered hair can be used in various ways including melanin recovery for suntan lotions preparation and cosmetic manufacturing, biogas generation, regeneration of keratins, manufacture of hair conditioners, pharmaceutics, synthetic products like nylon, retanning and chrome exhaustion agents to be used in leather processing.[9] Other studies have reported that hair and fleshings have been found to be protein and fat sources used in the manufacture of biological fertilizers for agricultural applications.[21]

This hair can also be used to make brushes or other textile products. Similar end uses have previously been recommended by other researchers.[20,22] Most of the hair cells are composed of the keratin protein.[23] The recovered hair is, therefore, a potential source of keratin that can be used in biomaterials for biomedical applications, polyvinyl alcohol fibers, manufacture of absorbents for toxic substances such as heavy metals ions and formaldehyde gases.[24,25]

Recovered fat analysis

Approximately 3.8 kg of wet fleshings (fat and flesh) were recovered after fleshing of the unhaired pelts (Figure 2). The fleshings were clean and free of any strong smell. Out of the 20 kg (raw weight) of goatskins processed, 3.8 kg of wet fleshings were recovered. This is an average recovery rate of 19%. This would translate to the recovery of approximately 190 kg of fleshings from every tonne of goatskins processed. This is a significant amount of a nutrient-rich by-product (as shall be indicated by the results below) that would otherwise be disposed of as waste.

The fat was extracted from the fleshings by dissolving in hexane and thereafter analyzed for the fatty acid composition after transesterification. The TLC analysis of the sample showed that transesterification took place due to the presence of the extra spot on the plate (labeled G with five spots) which is not present on the negative control run (labeled G- with four) as shown in Figure 3. This guided the decision to proceed with the GCMS analysis of the sample to determine the fatty acid composition.

Transesterification converts fatty acid esters to volatile fatty acid methyl esters (FAMEs).[26] These FAMEs were separated using gas chromatography (GC). The electron ionization mass spectrometry (MS) was used to detect the FAMES while a mass spectral library was used for identification. Figure 4 and Figure 5 (without retention time) display the total ion chromatogram for a single sample while Figure 6 shows an overlay total ion chromatogram for the three samples.

Figure 2. Recovered fleshings (fat and flesh)

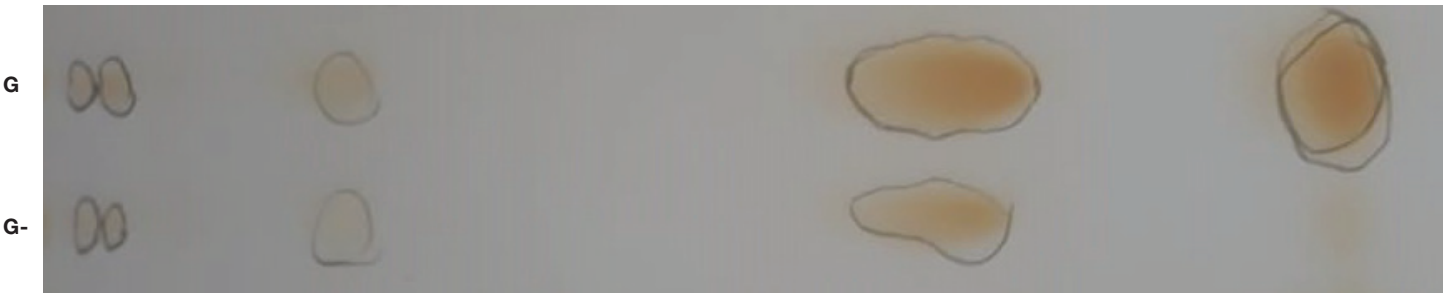

Figure 3. TLC Plate showing the movement of the sample (**G**) against a control (**G-**)

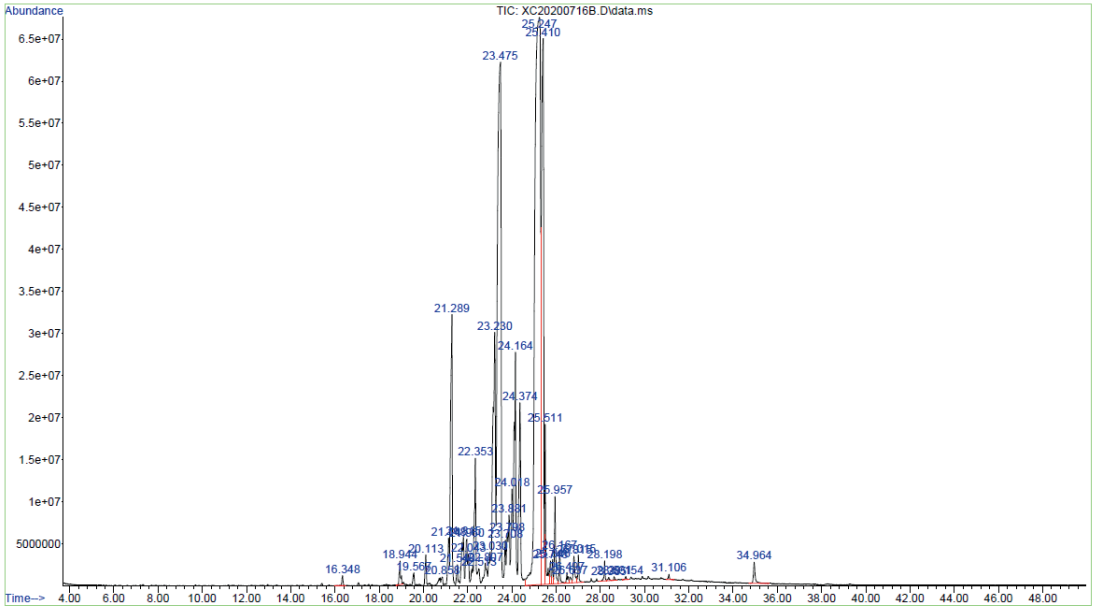

Figure 4. Total ion chromatogram with retention time

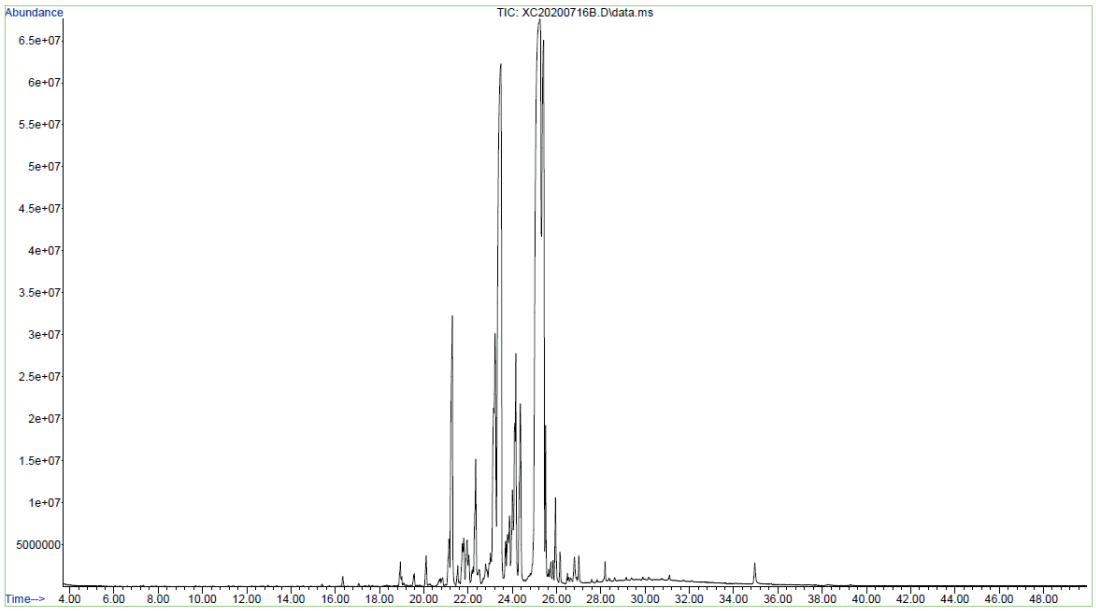

Figure 5. Total ion chromatogram without retention time

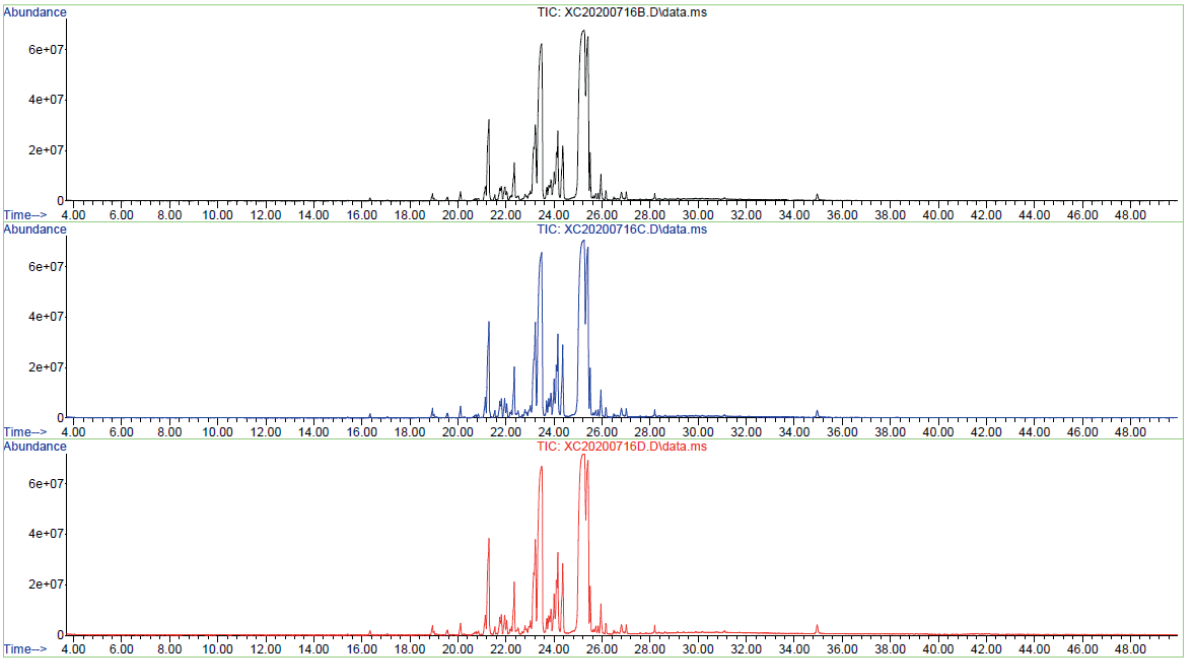

Figure 6. Overlay total ion chromatogram for the three samples

The X-axis of the chromatograms shows the retention time in minutes while the Y-axis (area of the peak) indicates the abundance of a compound. The retention indicates how much time a compound present in the sample was retained in the GC column between the sample injection time to when the sample finally elutes from the column.[27] This parameter can be used to differentiate between the different compounds in the sample or even for identification. However, the identification process might not be conclusive using the retention time alone as different compounds might have similar retention times.

The area of the peak, on the other hand, indicates the type of compounds in the sample as well as their concentration.[27] A higher concentration of a particular compound in the sample is displayed by a greater peak area than one with a lower concentration. The peak area is measured as the area under the curve. The identification process is facilitated by comparison to a standard mass spectral library. Table II outlines all the fatty acids whose peaks are displayed in the chromatogram, ranked from the one with the highest abundance in the analyzed samples. Alongside each fatty acid methyl ester, is the fatty acid observed, the mean concentration in mg/kg, the percentage abundance as well as the retention time in minutes.

The fatty acid composition indicates the methyl esters that make up the fat and determines the properties and uses of the fat.[28] The GCMS analysis report indicated that fifty-one (51) fatty acids were present in the sample goatskin fat that was recovered. Methyl 9Z-octadecenoate (9Z-heptadecenoic acid; oleic acid) was the most abundant fatty acid in the fat sample with an abundance of 31.65%. Other prominent fatty acids were Hexadecenoic acid (Palmitic acid) (20.04%), Octadecanoic acid (Stearic acid) (11.84%), 9-hexadecenoic acid (Palmitoleic acid) (6.23%), Tetradecanoic acid (Myristic acid) (4.19%), 8-heptadecenoic acid (3.97%) and Heptadecenoic acid (3.38%) as outlined in Table II. These results were used to suggest possible industrial applications of the recovered fat while comparing to and citing various previous studies.

The fatty acid composition recorded from the fat sample was closely similar to that reported from previous biodiesel studies on tallow and fat from fleshings.[4,29–32] Following the GCMS analysis of fat obtained from sheepskin fleshings, the following fatty acid composition was observed; Myristic acid (3.05%), Palmitic acid (20.59%), Palmitoleic acid (4.60%), Stearic acid (8.36%) and Oleic acid (41.08%).[4] These results, which had met the requirements of the international standards for biodiesels, are in agreement with the findings of this work making the obtained fat suitable for making biofuels and biodiesel. Various studies have reported the successful use of fat from fleshings in the production of environmentally friendly biodiesels and biogas to replace fossil fuels thus being rendered profitable.[4,33–41] The recovered fats, therefore, can be used in the production of biofuels, biodiesels and biogas thus increasing profitability and reducing disposal costs. The fats recovered from this study are particularly advantageous over those recovered from sulfide unhairing systems as it is free from any sulfide or lime contamination thus easier to purify and use.

Table II
Fatty acids test results

Retention time (minutes)	Fatty acid methyl ester	Fatty acid	Mean conc (mg/kg)	Abundance (%)
25.26	Methyl 9Z-octadecenoate	9Z-octadecenoic acid	143.06	31.65
23.49	Methyl hexadecanoate	Hexadecenoic acid	90.61	20.04
25.41	Methyl octadecanoate	Octadecanoic acid	53.50	11.84
23.24	Methyl 9-hexadecenoate	9-hexadecenoic acid	28.14	6.23
21.29	Methyl tetradecanoate	Tetradecanoic acid	18.94	4.19
24.16	Methyl 8-heptadecenoate	8-heptadecenoic acid	17.94	3.97
24.38	Methyl heptadecanoate	Heptadecenoic acid	15.26	3.38
22.34	Methyl pentadecanoate	Pentadecanoic acid	10.71	2.37
24.02	Methyl 15-methylhexadecanoate	15-methylhexadecanoic acid	6.84	1.51
25.50	Methyl (10E,12Z)-octadecadienoate	(10E,12Z)-octadecadienoic acid	6.74	1.49
25.95	Methyl 10-nonadecenoate	10-nonadecenoic acid	5.23	1.16
23.89	Methyl 5-methylhexadecanoate	5-methylhexadecanoic acid	4.66	1.03
21.16	Methyl 9Z-tetradecenoate	9Z-tetradecenoic acid	3.77	0.83
21.96	Methyl 13-methyltetradecanoate	13-methyltetradecanoic acid	3.41	0.75
23.80	Methyl 14-methylhexadecanoate	14-methylhexadecanoic acid	3.28	0.72
23.71	Methyl 2-methylhexadecanoate	2-methylhexadecanoic acid	3.16	0.70
26.82	Methyl 11Z-Eicosenoate	11Z-Eicosenoic acid	3.02	0.67
27.02	Methyl Eicosanoate	Eicosanoic acid	2.51	0.56
28.19	Methyl (7Z,10Z,13Z,16Z,19Z)-docosapentaenoate	(7Z,10Z,13Z,16Z,19Z)-docosapentaenoic acid	2.45	0.54
26.17	Methyl nonadecanoate	Nonadecanoic acid	2.21	0.49
22.52	Methyl 2,6,10-trimethyltridecanoate	2,6,10-trimethyltridecanoic acid	2.02	0.45
18.94	Methyl dodecanoate	Dodecanoic acid	2.02	0.45
22.05	Methyl 12-methyltetradecanoate	12-methyltetradecanoic acid	1.88	0.42
29.40	Methyl tricosanoate	Tricosanoic acid	1.85	0.41
30.18	Methyl tetracosanoate	Tetracosanoic acid	1.74	0.38
25.75	Methyl 11-methyloctadecanoate	11-methyloctadecanoic acid	1.73	0.38
32.11	Methyl hexacosanoate	Hexacosanoic acid	1.69	0.37
28.64	Methyl docosanoate	Docosanoic acid	1.63	0.36
26.49	Methyl (5Z,8Z,11Z,14Z)-Eicosatetraenoate	(5Z,8Z,11Z,14Z)-Eicosatetraenoic acid	1.37	0.30
25.84	Methyl (9E,12E)-octadecadienoate	(9E,12E)-octadecadienoic acid	1.29	0.29
20.75	Methyl 4,8,12-trimethyltridecanoate	4,8,12-trimethyltridecanoic acid	1.27	0.28
19.57	Methyl 8-methyldodecanoate	8-methyldodecanoic acid	1.15	0.26
27.83	Methyl heneicosanoate	Heneicosanoic acid	1.12	0.25
19.10	Methyl 2,4-dimethydodecanoate	2,4-dimethydodecanoic acid	1.01	0.22
26.64	Methyl 8,11,14-Eicosatrienoate	8,11,14-Eicosatrienoic acid	0.99	0.22
33.03	Methyl 24-methylhexacosanoate	24-methylhexacosanoic acid	0.98	0.22
20.87	Methyl 12-methyltridecanoate	12-methyltridecanoic acid	0.93	0.21
16.34	Methyl decanoate	Decanoic acid	0.67	0.15
26.31	Methyl 3-methoxyoctadecanoate	3-methoxyoctadecanoic acid	0.60	0.13
19.70	Methyl 11-methyldodecanoate	11-methyldodecanoic acid	0.26	0.06
17.53	Methyl 4,8-dimethylnonanoate	4,8-dimethylnonanoic acid	0.07	0.02
18.72	Methyl 4-methyldodecanoate	4-methyldodecanoic acid	0.05	0.01
13.36	Methyl octanoate	Octanoic acid	0.04	0.01
18.42	Methyl tridecanoate	Tridecanoic acid	0.04	0.01
14.91	Methyl Nonanoate	Nonanoic acid	0.03	0.01
17.21	Methyl undecanoate	Undecanoic acid	0.03	0.01
18.49	Methyl 10-methylundecanoate	10-methylundecanoic acid	0.03	0.01
14.30	Methyl 4-methylpentanoate	4-methylpentanoic acid	0.02	0.01
11.66	Methyl heptanoate	Heptanoic acid	0.02	0.01
9.69	Methyl hexanoate	Hexanoic acid	0.02	0.00
6.08	Methyl 2-methylbutanoate	Butanoic acid	0.01	0.00
			452.030	

This fat from the fleshings can also be used in the manufacturing of fatliquoring agents for leather processing. Its fatty-acid composition is comparable to the fats used for the processing of fatliquors.[2,7,42] Fatty acid analysis carried out on the fats extracted from fleshings by Rahmawati and Priatni[42] showed appreciable amounts of Tetradecanoic acid (2.87%), Pentadecanoic acid (18%) and Octadecenoic acid (9.48%). The fat was successfully used to make a quality fatliquoring agent. Another study on the application of fat obtained from seal skins indicated that the most abundant fatty esters were Oleic (27.34%) and palmitoleic (19.34%).[7] The fat displayed good characteristics when used to make a fat liquor. Fat samples recovered from goatskins in this study recorded closely similar fatty acid compositions coupled with low amounts of the stearic ester which is associated with the formation of fat spue on leather.[7] Oleic acid is suitable for the manufacturing of fatliquors due to its unsaturated nature and ability to react with sulfuric acid.[43] Successful trials have also been documented from the use of oils from goatskins to tan chamois leather.[8] The recovered fat, therefore, may be recommended for use in making fatliquors and oil tanning agents.

The recovered fat can also be used in the manufacture of cosmetics and lubricating agents. Previous studies on the analysis of raw fleshing oil indicated a composition of Oleic Acid (43.83%), Palmitic Acid (28.4%), Palmitoleic Acid (8.1%), Stearic Acid (10.67%) and Myristic Acid (4.2%).[44] The reported findings are in coherence with the results obtained in this study, with an added advantage of being sulfide-free as well as not having an acrid smell. İşler et al.[44] recommended the use of raw fleshing oil as a feedstock in the cosmetic industry and the production of lubricants. Goatskins equally yield substantial amounts of fat that can be processed into soaps.[45] Having recorded a significant amount of Stearic acid (11.84%), the fats recovered from goatskins in this study, hence, have a great potential for application in soap making.[43,45] Further analysis of the fat's iodine and acid values is however recommended to ascertain its suitability.

Industrial applications of fleshings have been studied widely and shown to have a high economic value. The significant amount of oleic acid present in the fat sample suggests that the fat can be used in the pharmaceutical industry. Oleic acid has the ability to soften and moisturize the skin to enhance the absorption of drugs applied as skin creams.[46,47] Palmitoleic acid, also present in the recovered fat, is used in pharmaceutical products.[48] The presence of Stearic acid (octadecanoic acid), a common saturated fatty acid used widely in the production of soaps, shampoos, detergents, pharmaceuticals, cosmetics and in the food industry gives another possible industrial application of the recovered fat.[49,50] Stearic acid is also used as an additive for papermaking where high whiteness is required.[51] An appreciable amount(4.19%) of myristic acid (tetradecanoic acid) was also present in the fat sample. This fatty acid has been reported to find wide usage in cosmetic pastes, creams and personal care products such as skin conditioning agents.[52] Albeit in smaller proportions, many other fatty acids were present in the recovered fat and can be explored for various applications.

Conclusion

The use of the enzyme extract from *Bacillus cereus Strain 1-p* to unhair goatskins demonstrated successful recovery of hair and fats. This novel technique facilitated the recovery of intact quality hair as well as sulfide-free fat that can not only find wide industrial applications but also greatly reduces the sludge concentration in the effluent stream and ultimately the pollution load and waste treatment costs when compared to the conventional unhairing technique. This study demonstrated that the recovered hair can find great application in the production of poultry feedstuff, organic fertilizers, cosmetics, pharmaceuticals, brushes, synthetic products and regeneration of keratins while the fat can be used for the production of soaps, shampoos, detergents, pharmaceuticals, cosmetics, environmentally friendly biodiesels and biogas, fatliquoring and oil tanning agents. This approach, therefore, is a viable option to make leather manufacturing cleaner and more sustainable as well as avail raw materials for various industries, applications to produce valuable end products.

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Study of the Biodegradability of Leather Tanned with Sodium Aluminosilicate

by

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Abstract

The leather industry transforms raw hides into leather by mechanical and chemical processes. The main chemical process is the tanning in which the fibers are stabilized and cannot putrefy. To this end, different chemicals can be used that are capable of forming cross-linkages between collagen molecules. In this sense, the most used products are: chromium salts, aluminum, vegetable tannins, synthetics, aldehydes, resins and silicates. In this work, a new biodegradable tanning process based on zeolites was studied in two steps. In the first one, a tanning process for sheepskins and cow hides was developed. In the second one, three types of retanning process to obtain shoe upper, vegetable and leather goods were studied. This new system allows to obtain leather that can decompose naturally in a relative short period of time. Leather tanned with this system shows similar values to the biodegradability of pure collagen. Specifically, with the retanning system used for shoe upper allows to obtain a 74.4% relative biodegradability compared to pure collagen.

Introduction

The leather industry transforms raw hides into leather by mechanical and chemical processes. The main chemical process is the tanning in which the fibers are stabilized and cannot putrefy. To this end, different chemicals can be used that are capable of forming cross-linkages between collagen molecules. In this sense, the most used products are: chromium salts, aluminum, vegetable tannins, synthetics, aldehydes, resins and silicates.[1]

About of 85% of the world's hides are chrome-tanned hides. The leather industry is making great efforts to apply cleaner processes and current chrome tanning processes make it possible to obtain wastewater with less than 3 ppm of chromium (III). However, it is difficult to eliminate solid wastes that contain chrome. Therefore, the leather industry has focused on the search for alternatives to chrome tanning.[2-4]

In this work, the use of zeolite (sodium aluminosilicate) is presented as a possible alternative to conventional tanning to obtain a biodegradable leather that allows solid waste to be safely disposed of and degraded in a short period of time.

Some previous studies have confirmed the reaction capacity of zeolites with collagen. The reaction between collagen and zeolite comes about by the activation of the dispersed tanning agent forming a sheath-like network around the fibers. Zeolites have specific binding with proteins due to their ion exchange capacity, surface properties, and controllable pore structure, which affects the performance and behavior of proteins.[5]

On the other hand, Zhang et al., studied if sodium silicates can affect collagen structure during tanning by SEM, SAXS and DSC. They stated that the introduction of silica into the leather matrix did not affect the axial periodicities of the collagen molecules, however an increase in collagen fibril diameter was observed during the main tanning step.[6]

Studies on the zeolites application can be found. Specifically, synthetic Na-zeolites were investigated as tanning agents in leather production from sheepskin and calfskin pelts. It was found that the combined use of zeolite and chrome sulphate results in both higher float exhaustion and higher shrinkage temperatures in shorter time than in conventional chrome tannage at lab scale.[7]

Constantini et al., published a study on the reactions involved in pretanning or tanning when using zeolite based masking agents. The hydrothermal stability of sodium aluminum silicate is considered to be too low for use in tanning solely by a zeolite. The role of pH and acidic solutions in aluminosilicate breakdown were emphasized and discussed in detail.[8]

Gürler and Gülümser determined the tanning possibilities of the combinations between alkali alumino silicate a kind of zeolite, vegetal tanning, vegetal-synthetic tanning and aluminum triformate and the utility of these combinations in garment leather production.[9]

Additionally, three patents related to the use of zeolite as tanning have been registered. GB2368346 discloses a pre-tannage system for leather using sodium aluminum silicate in a first pre-tannage step and thereafter treating the hide with one or more modified aldehyde

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tanning agents. US4264318 and US4264319 disclose a process of tanning for the production of dressed fur skin using a water-insoluble aluminosilicate containing bound water.[10]

Following the research using zeolites as tanning products, a new tanning process (called Wet Bright) was developed which produced perfectly white leather and meets all the requirements for automotive leather.[11]

Going one step further, a new biodegradable tanning process based on zeolites was studied in the present work. This new system consists in applying SERTAN WT (from QUIMSER), which is not classified as hazardous.

The process is based on a mineral tanning using organic and inorganic salts. SERTAN WT is a chemical free of phenol, naphthalene, sulfone and formaldehyde. It is also free of salts so this product is not directly contributing to the total dissolved solids level of the tan yard water discharge. It is compatible with other anionic synthetic, natural tanning and retanning agents. And it has the ability to decompose naturally and ecologically in a relatively short period of time, non-polluting the environment and becoming compost for the earth.

Materials and Methods

This study was divided in two steps. In the first one, the tanning formulations for sheepskins and cow hides were developed in order to assess the capability to obtain a biodegradable leather. In the second one, three types of retannage were developed to obtain different articles to validate the new system.

Zeolites tanning process

The aim of the first step of this study is to design a new tanning system based on zeolites for both, sheep skins and cow hides, in order to obtain a biodegradable material which could be decomposed naturally in a short period of time by the action of biological agents (i.e. fungi and/or bacteria).

In this sense, when the leather reaches its final use, it can be safely disposed of in a landfill and turned into compost for the earth. The new tanning system consists in applying SERTAN WT from Quimser. SERTAN WT is an anionic solid product, based on organic and inorganic salts, white, with a pH of 5.5 ± 0.5 and with a conductivity of 32.9 mS. It is free of phenol, naphthalene sulfone and formaldehyde. It gives excellent filling effect in the loose parts

Table I

Sheep skin tanning formulation

Operation	%	°C	Chemical	Time	Notes
Washing	80	20	Water		
	8.0		NaCl		°Be =6.8
	3.0		Tensoactive	90 minutes	pH = 3.0
			Drain / wash		
Washing	80	20	Water		
	8.0		NaCl		°Be =6.8
	0.5		Dispersing and fixing agent		
	3.0		Tensoactive	90 minutes	pH = 3.0
			Drain / wash		
Tanning	100	20	Water		
	8.0		NaCl	10 minutes	
	1.0		Dispersing and fixing agent		°Be = 7 pH=3.3
	2.5		Sertan WT	60 minutes	pH = 3.8
	0.5		Dispersing and fixing agent		
	2.5		Sertan WT		
	2.0		Synthetic oil	90 minutes	
	0.5		Dispersing and fixing agent		pH = 3.9
	3.0		Sertan WT		
			In the morning add temperature 35-42°C. And run 90'-120'		
			Drain / Wash		
			Pile up (minimum 24h.) - Shave		

Table II

Cow hide tanning formulation

Operation	%	°C	Chemical	Time	Notes
Tanning	80	20	Water		
	8.0		NaCl		°Be = 7
	1.5		Dispersing and fixing agent	15 minutes	pH=3.3
	3.5		Sertan WT	60 minutes	pH = 3.8
	3.5		Sertan WT		
	2.0		Synthetic oil		
	1.0		Dispersing and fixing agent	90 minutes	pH = 3.9
	2.0		Sertan WT		
	0.5		Dispersing and fixing agent	120 minutes	pH = 3.9
	0.6		Fungicide (thiocyanate)	25 minutes	
In the morning add temperature 35-45°C. And run 90'-120'					
Drain / Wash					
Pile up (minimum 24h.) - Shave					

of the hide, which can minimize the usage of polymers and resins. The dosages are between 6 and 10% in cow hides, and between 5 and 8% in sheep skins.

The tanning formulation for sheep skin can be seen in Table I. And in Table II, the formulation for cow hide is shown.

Once the tannages were performed, the sheep skin tanning (sample n°4) was compared with:

- Sample 1. Crust tanned with chrome and retanned with synthetics.
- Sample 2. Crust tanned with vegetable extract and aldehyde. Retanning with synthetics extracts.
- Sample 3. Crust tanned with SERTAN WT and retanned with vegetable extracts.

And the cow hide tanning (Sample 5) was compared with:

- Sample 1. Crust tanned with chrome and retanned with standard market products.
- Sample 2. Crust tanned and retanned with vegetable polymers.
- Sample 3. Crust tanned with aluminum salts and retanned with standard market products.
- Sample 4. Crust tanned with SERTAN WT and retanned with standard market products.

In order to check the natural biodegradation of leather from both tannages, the sheep skin samples were buried in compost substrate on July 8, 2020 and unearthed on August 25, 2020 (See Figure 1). The cow hide samples were buried in compost substrate on September 2, 2020 and unearthed on October 5, 2020 (See Figure 2).

Figure 1. Natural biodegradation test for sheep skin

Figure 2. Natural biodegradation test for cow hide

Study of three types of retanning for the new tanning system

The aim of the second step of the study was to design three types of retanning formulation in order to validate the new system at industrial level. In this way, three types of articles on cow hide tanned following the formulation shown in Table II were manufactured: vegetable (see Table III), leather goods (see Table IV) and shoe upper (see Table V).

Table III

Vegetable retanning formulation

Operation	%	°C	Chemical	Time	Notes
Soaking	300	35	Water		
	2.0		Dispersing and fixing agent	30 minutes	pH = 3.0
			Drain / wash		
Neutralizing	100	35	Water		
	2.0		Alkaline salts and masking		
	3.0		Sulphonic polymer	60 minutes	pH = 6.2
			Drain / wash		
Retanning	100	20	Water		
	3.0		Biopolymer	30 minutes	
	2.0		Sulphonic polymer		
	2.0		Sulphited oil		
	8.0		Synthetic agent		
	5.0		Mimosa extract	120 minutes	pH = 3.8
	2.0		Lecithin		
	4.0		Sulphated oil	30 minutes	
	4.0		Mimosa extract	60 minutes	
	3.0		Mimosa extract	60 minutes	
	1.5		Dispersing and fixing agent	60 minutes	pH = 3.6
			Drain / Wash		
Fatliquoring	100	40	Water		
	5.0		Sulphated oil		
	2.0		Synthetic waxy polymer		
	2.0		Sulphited oil		
	4.0		Lecithin	60 minutes	
	1.5		Dispersing and fixing agent	30 minutes	pH = 3.8
			Drain / Wash		
Sammying – Setting – Vacuum 70°C 45 seconds – Air drying – Wetting – Shaking –Toggling					

Table IV

Leather goods retanning formulation

Operation	%	°C	Chemical	Time	Notes
Soaking	100	20	Water		
	0.8		Surfactant	40 minutes	pH = 3.0
			Drain / wash		
Neutralizing	150	20	Water		
	2.0		Sertan WT	30 minutes	pH = 4.2
	2.0		Alkaline salts and masking	40 minutes	pH = 5.7
			Drain / wash		
Retanning	100	20	Water		
	4.0		Biopolymer		
	4.0		Synthetic agent		
	5.0		Sulphonic polymer		
	4.0		Dye		
	2.0		Sulphited oil	90 minutes	pH = 3.8
			Drain / Wash		
Fatliquoring	80	40	Water		
	3.0		Sulphated oil		
	5.0		Synthetic waxy polymer		
	3.0		Lecithin	90 minutes	pH = 4.9
	1.5		Dispersing and fixing agent	30 minutes	pH = 3.8
			Drain / Wash		
Sammying – Setting – Vacuum 70°C 45 seconds – Air drying – Wetting – Shaking –Toggling					

Table V

Shoe upper retanning formulation

Operation	%	°C	Chemical	Time	Notes
Soaking	100	20	Water		
	0.7		Surfactant	30 minutes	pH = 3.0
			Drain / wash		
Neutralizing	100	20	Water		
	2.0		Alkaline salts and masking	60 minutes	pH = 4.5
			Drain / wash		
Retanning	100	20	Water		
	2.0		Sertan WT		
	3.5		Synthetic agent		
	3.0		Sulphonic polymer		pH = 5.0
	3.5		Mimosa extract		
	3.0		Dye	120 minutes	pH = 5.0
			Drain / Wash		
Fatliquoring	80	40	Water		
	3.0		Sulphated oil		
	3.0		Synthetic waxy polymer		
	6.0		Lecithin	60 minutes	
	1.5		Dispersing and fixing agent	20 minutes	pH = 3.9
			Drain / Wash		
Sammying – Setting – Vacuum 70°C 45 seconds – Air drying – Wetting – Shaking –Toggling					

In order to determine the quality of the leathers the physical and chemical tests set up by the IULTCS were carried out, which allowed us to assess the capacity of the leathers to withhold the wear and tear of leather goods, shoe upper and vegetable.

The following official methods were used to this end:

- IUC 5 Determination of volatile matter (in accordance with ISO 4658).
- IUC 18-2 Determination of hexavalent chromium content (in accordance with ISO 17075-2 Chromatographic method).
- IUC 11 Determination of pH and difference figure (in accordance with ISO 4045).
- IUC 19-1 Formaldehyde (in accordance with ISO 17226-1).
- IUC 20 Chemical tests for the determination of certain azo colorants in dyed leathers (in accordance with ISO 17234).
- IUC 27-2 Chemical determination of metal content: chromium, aluminum, titanium, zirconium, iron and zinc (in accordance with ISO 17072-2).

Additionally, the amount of phthalates, dimethyl fumarate and clorofenols were determined.

- IUP 6 Measurement of tear load – Double edge tear (in accordance with ISO 3378-2).
- IUP 10-1 Water resistance of flexible leather. Part 1: linear (in accordance with ISO 5403-1).
- IUP 15 Measurement of water vapor permeability (in accordance with ISO 14268).
- IUF 402 Color fastness of leather to light: Xenon lamp (in accordance with ISO 105-B02).
- IUF 412 Change of color with accelerated ageing (in accordance with ISO 17228).

For the purpose of assessing the biodegradability of leathers, determination of relative biodegradability percent of leather using aerobic microorganisms following the standard UNE-EN ISO 20136 Leather - Determination of the degradability by microorganisms (ISO 20136: 2020) was performed.

Results

As mentioned in the previous section, the aim of this study was to develop a new tanning system that obtains a biodegradable leather which could be decomposed naturally in a short period of time and at the same time, fulfill all the required properties to be used as shoe upper and leather goods.

Zeolites tanning process

In Figure 3, the sheep skin samples unearthed after 47 days can be seen.

As shown in Figure 3, the skin tanned with the new biodegradable system disappears completely. It was fully biodegraded. In the contrary, crust tanned with chrome and retanned with synthetic remains with no damages. Crust tanned with vegetable extract and aldehyde and retanned with synthetics extracts shows a partial degradation. Sheep skin tanned with Sertan WT in optimal composting conditions is highly biodegradable. Therefore, the environmental impact of the disposal at its final use of that kind of leather is much lower than that of leather tanned with mineral salts or synthetic products based on petroleum chemistry.

In parallel form, Figure 4 shows the cow hide samples unearthed after 33 days.

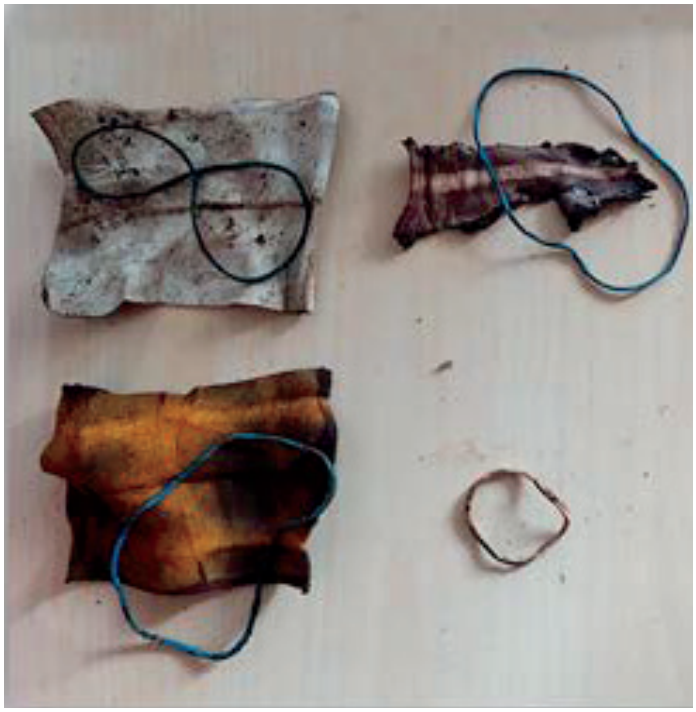

Figure 3. Unearthed sheep skin samples

Figure 4. Unearthed cow hide samples

As can be seen in Figure 4, the level of biodegradation of bovine cows is lower than that of sheep skins mainly due to the thickness difference between them. However, the cow hide tanned with the new biodegradable system show a higher level of biodegradation than those tanned with chrome and aluminum salts. In addition, the sample tanned with the new system and retanned with market products show a partial level of biodegradation. Therefore, adjusting the retanning formulation with more natural products a good level of biodegradation can be obtained.

Study of three types of retanning for the new tanning system

After the results obtained in the previous section, and in order to check if it is possible to obtain a biodegradable commercial leather fulfilling all the requirements to withhold the wear and tear of leather goods and shoe upper, the three formulations shown in Tables III, IV and V were performed.

Table VI

Physical and chemical tests results for vegetable

Analysis	Result	Requirements
Volatile matter	9.4	-
Hexavalent chromium	< 3 mg/kg	< 3 mg/kg
pH	4.5	3.5 < pH < 7.5
Formaldehyde	< 10 mg/kg	< 70 mg/kg
Certain azo colorants in dyed leather	< 30 mg/kg	< 30 mg/kg
Total chromium	13.3 mg/kg	< 1000 mg/kg
Total aluminum	12133 mg/kg	< 1000 mg/kg
Total titanium	<12 mg/kg	< 1000 mg/kg
Total zirconium	<12 mg/kg	< 1000 mg/kg
Total iron	38.0	
Total zinc	8.4	< 1000 mg/kg
Phthalates	< 25 mg/kg	< 1000 mg/kg
Dimethyl fumarate	< 0.5 mg/kg	-
Chlorophenols	< 5 mg/kg	-
COV and SCOV	Negative	-
Tensile strength	22.6 N/mm^2	30.0 N/mm^2
Elongation	59.6%	35%
Tear load	103.5 N/mm	
Water resistance	1 hour	
Water vapor	4.7 $mg/h.cm^2$	
Color fastness to light	1	
Color change with accelerated ageing	1	

Table VII

Physical and chemical tests results for shoe upper

Analysis	Result	Requirements
Volatile matter	10.7	-
Hexavalent chromium	< 3 mg/kg	< 3 mg/kg
pH	4.2	3.5 < pH < 7.5
Formaldehyde	< 10 mg/kg	< 70 mg/kg
Certain azo colorants in dyed leather	< 30 mg/kg	< 30 mg/kg
Total chromium	15.3 mg/kg	< 1000 mg/kg
Total aluminum	12499 mg/kg	< 1000 mg/kg
Total titanium	<12 mg/kg	< 1000 mg/kg
Total zirconium	<12 mg/kg	< 1000 mg/kg
Total iron	43.2	
Total zinc	4.5	< 1000 mg/kg
Phthalates	< 25 mg/kg	< 1000 mg/kg
Dimethyl fumarate	< 0.5 mg/kg	-
Chlorophenols	< 5 mg/kg	-
COV and SCOV	Negative	-
Tensile strength	16.0 N/mm^2	30.0 N/mm^2
Elongation	47.1%	35%
Tear load	64.9 N/mm	
Water resistance	1 hour	
Water vapor	10.4 $mg/h.cm^2$	
Color fastness to light	3	
Color change with accelerated ageing	3-4	

Table VIII

Physical and chemical tests results for leather goods

Analysis	Result	Requirements
Volatile matter	9.0	-
Hexavalent chromium	< 3 mg/kg	< 3 mg/kg
pH	4.2	3.5 < pH < 7.5
Formaldehyde	< 10 mg/kg	< 70 mg/kg
Certain azo colorants in dyed leather	< 30 mg/kg	< 30 mg/kg
Total chromium	6.4 mg/kg	< 1000 mg/kg
Total aluminum	12767 mg/kg	< 1000 mg/kg
Total titanium	<12 mg/kg	< 1000 mg/kg
Total zirconium	<12 mg/kg	< 1000 mg/kg
Total iron	75.7	
Total zinc	7.2	< 1000 mg/kg
Phthalates	< 25 mg/kg	< 1000 mg/kg
Dimethyl fumarate	< 0.5 mg/kg	-
Chlorophenols	< 5 mg/kg	-
COV and SCOV	Negative	-
Tensile strength	17.7 N/mm^2	30.0 N/mm^2
Elongation	45.1 %	35%
Tear load	86.5 N/mm	
Water resistance	1 hour	
Water vapor	7.6 $mg/h.cm^2$	
Color fastness to light	2-3	
Color change with accelerated ageing	1	

The results for the physical and chemical tests described in materials and methods section are shown in Table VI, VII and VII. All physical and chemical tests were carried out in triplicate, sampling as indicated by the IUP2 and IUC2 - Sampling location standards. The results have been presented as an absolute value, taking an average between the three replications.

As can be seen in Tables VI, VII and VIII, all the leathers are exempt of hazardous substances, which are restricted by REACH. All of them have also good physical resistance. However, all of them contain more than 12000 mg/kg of aluminum. Therefore, all of them can be marketed but cannot be considered as metal-free leather.

As the main goal of this study was to obtain biodegradable leather, to check if they can decompose naturally and ecologically in a relatively short period of time, determination of relative biodegradability percentage of the three types of leather using aerobic microorganisms was performed.

The test method to determine the degree and rate of aerobic biodegradation of leather is based on the indirect determination of the CO_2 produced by the degradation of collagen. Leather is exposed to an inoculum from activated tannery sewage sludge, in an aqueous medium.

Table IX

Carbon content

Materials	% Carbon ^{12}C
Pure collagen	50.98
Sertan WT on cow hide	28.18
Leather goods	44.27
Shoe upper	39.95
Vegetable	43.43
Chrome tanning	41.08
Oxazolidine (INESCOP Control)	44.76

The operative procedure consists of the quantification of the CO_2 produced during the degradation process of the polymerized amino acids that make up the collagen polymer through the action of the microorganisms present in the sludge of the tannery biological tanks. The CO_2 produced is stoichiometrically proportional to the amount of carbon present in said polymer. The CO_2 accumulated during the test is transformed into a percentage of biodegradation by means of mathematical equations. The test is considered valid when the degree of biodegradation of the positive control (pure collagen) is equal to or greater than 70%.

In order to assess the degree of biodegradability of the three type of studied leathers, the test was carried out according to UNE-EN ISO 20136 Leather - Determination of the degradability by microorganisms (ISO 20136: 2020) by INESCOP. The inoculum used was from the biological tank of Elda's municipal sewage treatment plant. The inoculum was obtained on 11/03/2021, stored in a clean plastic bottle and transported at + 4 °C. Arrival at the final destination on 12/03/2021. The water was decanted for solids removal.

The three leather samples were compared with pure collagen, cow hide tanned with Sertan WT, leather tanned with chromium and with oxazolidine (INESCOP control).

The carbon content of all tested materials can be seen in Table IX and the percentage of theoretical aerobic biodegradability of each sample tested, and the positive control material can be seen in Table X.

As mentioned above, the biodegradability analysis method consists of quantifying the CO_2 produced during the degradation process of the polymerized amino acids that make up the collagen polymer through the action of the microorganisms present in the biological tannery sludge. The CO_2 produced is stoichiometrically proportional to the amount of carbon present in the sample. The percentage of initial carbon present in the collagen under study is determined by the elemental analysis of each sample.

Table X

Percentage of theoretical aerobic biodegradability

Materials	Theoretical maximum of Carbon ^{12}C (g)	Theoretical maximum of CO_2 (g)
Pure collagen	0.3372	1.2365
Sertan WT on cow hide	0.1872	0.6865
Leather goods	0.2946	1.0803
Shoe upper	0.2662	0.9759
Vegetable	1.8800	6.8934
Chrome tanning	1.7783	6.5204
Oxazolidine (INESCOP Control)	1.9381	7.1063

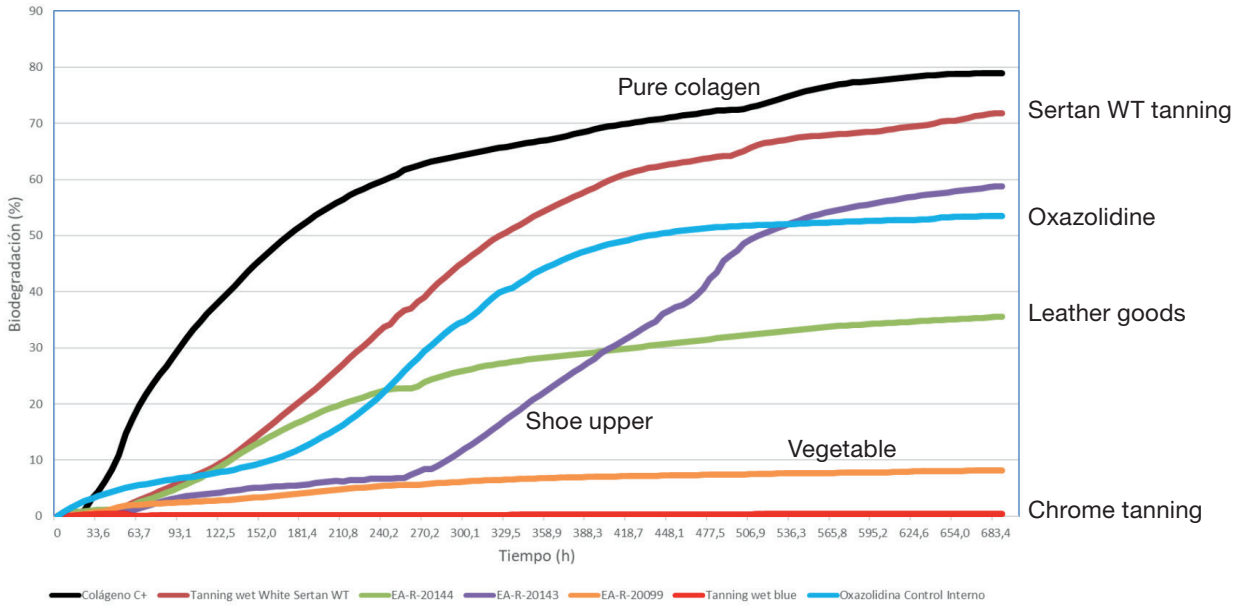

Figure 5. Accumulative average carbon dioxide evolution

Table XI

Percentage relative biodegradability

Materials	% absolute biodegradation	% relative biodegradability
Pure collagen	78.9	100.0
Sertan WT on cow hide	71.8	91.0
Leather goods	35.5	45.0
Shoe upper	58.7	74.4
Vegetable	8.1	10.3
Chrome tanning	0.4	0.5
Oxazolidine (INESCOP Control)	53.5	67.8

From the percentage of C presented in Table IX, previously determined by elemental analysis, it is possible to calculate the amount in mg of the total C present in the initial sample, and therefore determine the maximum CO_2 that this mass of C can produce. The maximum CO_2 that can be produced is shown in Table X.

In Figure 5 the accumulative average carbon dioxide evolution over time until plateau displayed graphically as lag-phase and slope (rate) can be seen. The test was performed during 28.5 days, that is 683.4 (horizontal axis).

In Table XI the percentage relative biodegradability of each sample is shown.

The percentage of degradation of the test material is determined from the CO_2 produced by the following formula:

$$B_{CO2} = [[m_{CO2} * 1000] / m_{TCO2}]] * 100$$

For the calculation of relative biodegradability percentage, the absolute biodegradation percentage of collagen obtained in the test was taken as 100%. The relative biodegradation percentage of each sample was calculated based on the value corresponding to 100% relative biodegradability of collagen.

As can be seen in the results obtained, the new biodegradable system using Sertan WT allows to obtain a similar percentage of absolute biodegradation as pure collagen. More specifically, the new system shows 9% less biodegradability than pure collagen, whereas a conventional tanning using chrome salts shows 99.5% less biodegradability than pure collagen.

When the leathers are retanned in order to obtain different leather articles, the biodegradability decrease considerably. However, with the retanning system used for shoe upper allows to obtain a 74.4% relative biodegradability compare to pure collagen. Therefore, adjusting the retanning formulation with more natural products a good level of biodegradation can be obtained.

Conclusions

We studied a new zeolites-based tanning process to make biodegradable leather. This new system gave leather that can decompose naturally in a relatively short period of time, with values like the biodegradability of pure collagen. This new system uses Sertan WT which is a product that is free of salts, phenol, naphthalene, sulfone and formaldehyde. In addition, it is compatible with other anionic synthetic agents, natural tanning and retanning agents. Therefore, this new leather can be retanned in order to obtain different leather articles, such as shoe upper and leather goods. However, the biodegradability can decrease considerably; depending on the type of retanning products used. The suggested retanning formulation gave a shoe upper leather with a value of 74.4% relative biodegradability to pure collagen. Therefore, a good level of biodegradation can be obtained by adjusting the retanning formulation with more natural products.

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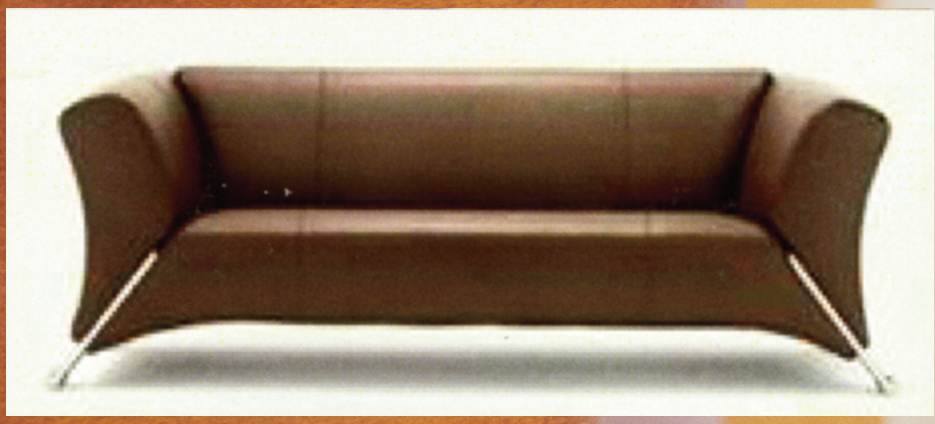

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Bindia Sahu, see *JALCA* 114, 359, 2019

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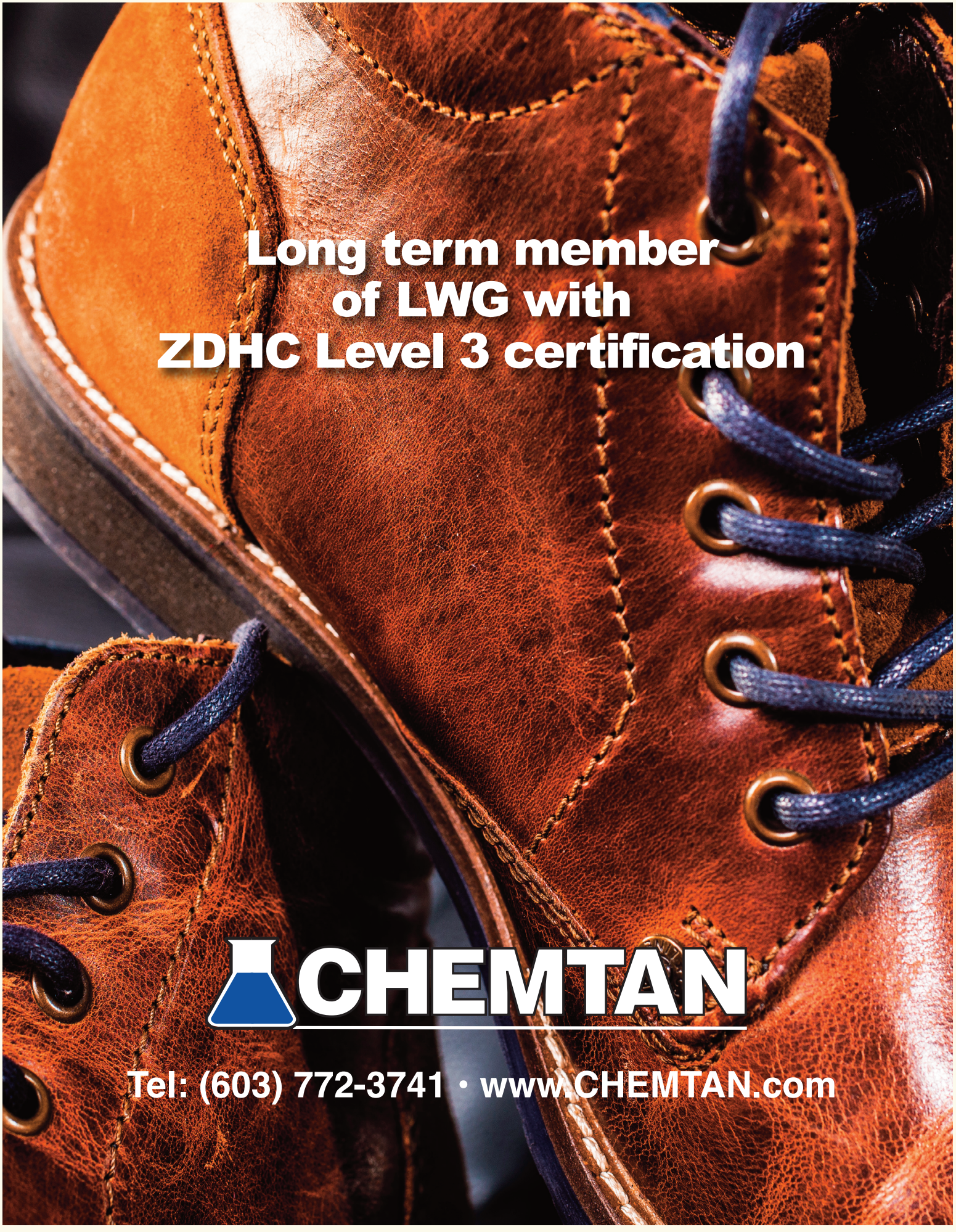
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