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

Article *in* Journal of the American Leather Chemists Association · September 2023

CITATION
1

READS
114

1 author:

Joseph Ondari Nyakundi
University of Cincinnati
4 PUBLICATIONS **1** CITATION

SEE PROFILE

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

by

Joseph Ondari Nyakundi,[1]* Jackson Nyarongi Ombui,[2] Wycliffe Chisutia Wanyonyi[3] and Francis Jakim Mulaa[4]

[1]*Leather Research Laboratory, University of Cincinnati*, 5997 Center Hill Avenue Bldg. C, Cincinnati, Ohio 45224, USA

[2]*Department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi*, P.O. Box 30197 - 00100, Nairobi, Kenya

[3]*Department of Physical Sciences, School of Science & Technology, University of Kabianga,* P. O. Box 2030- 20200 Kericho, Kenya

[4]*Department of Biochemistry, College of Health Sciences, University of Nairobi,* P.O. Box 30197 - 00100, Nairobi, Kenya

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

*Corresponding author email: nyakunjo@ucmail.uc.edu or ondarijay@gmail.com

Manuscript received November 11, 2021, accepted for publication December 23, 2021.

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)

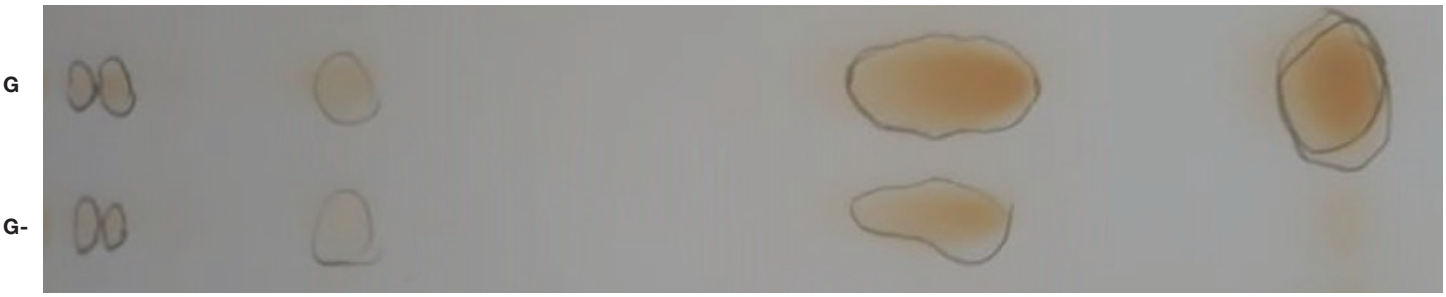

Figure 3. TLC Plate showing the movement of the sample (**G**) against a control (**G-**)

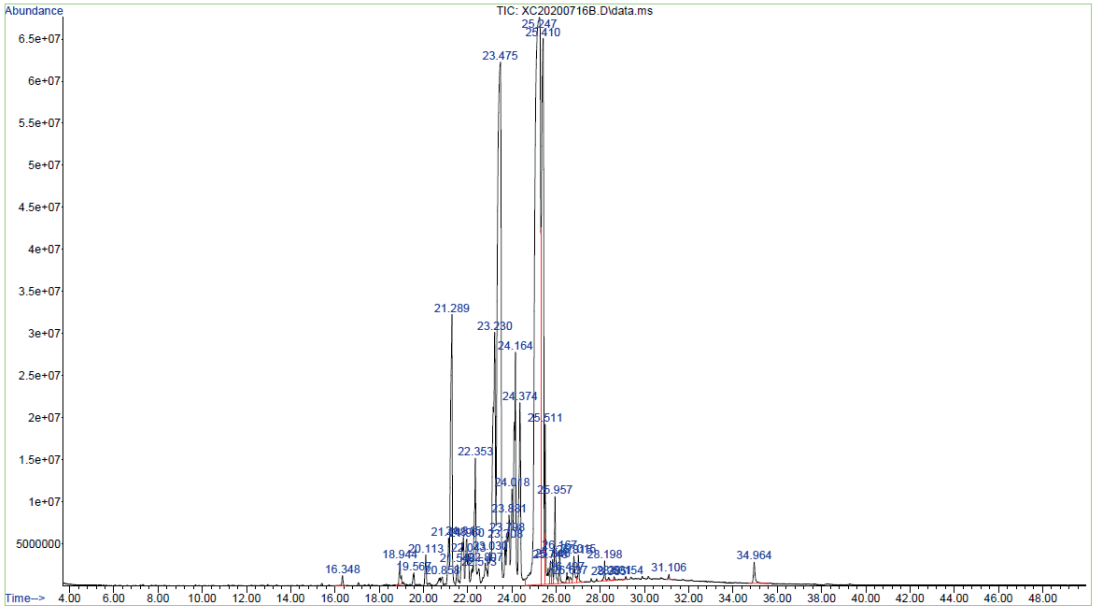

Figure 4. Total ion chromatogram with retention time

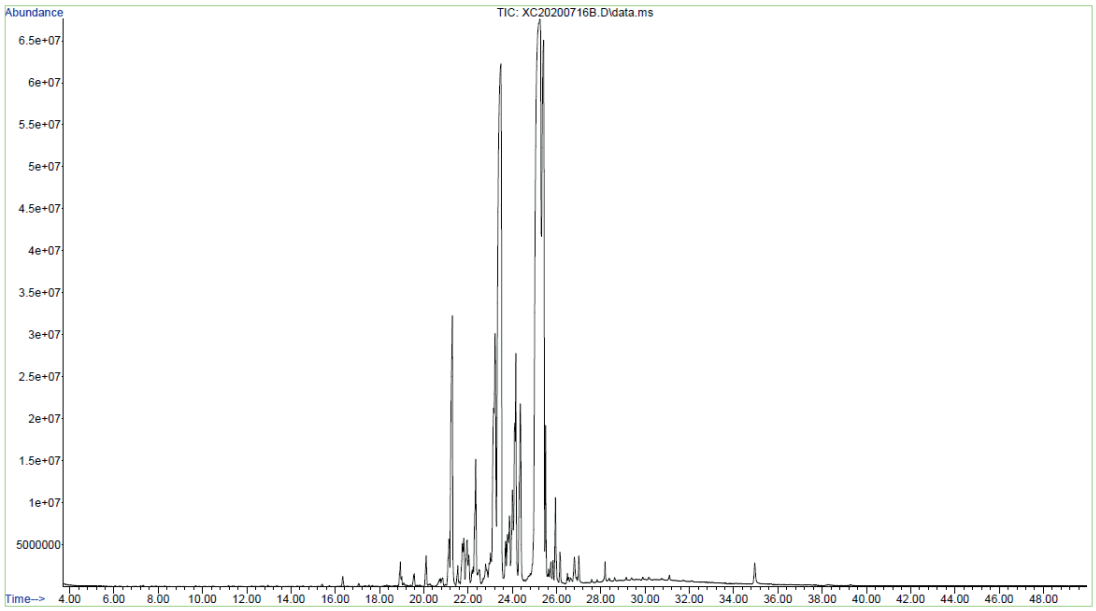

Figure 5. Total ion chromatogram without retention time

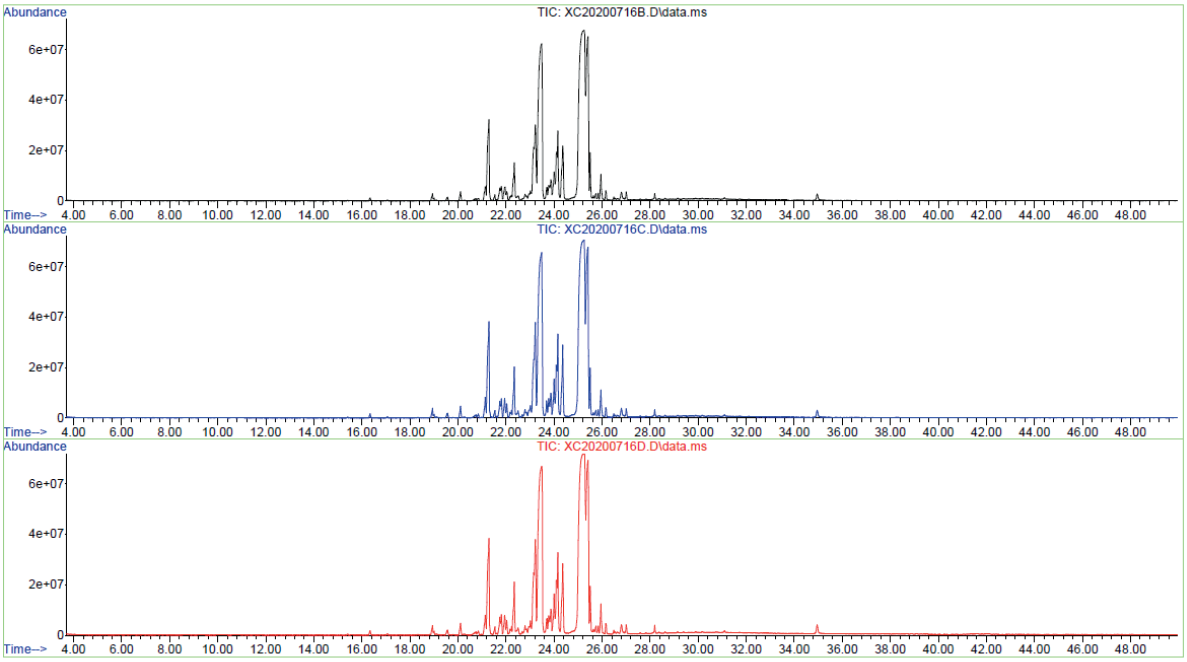

Figure 6. Overlay total ion chromatogram for the three samples

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.

Acknowledgments

The authors wish to thank Bio-Innovate Africa for their financial support of this study. The authors acknowledge the Leather Industries of Kenya for their kind collaboration in the processing of samples for the study. Special thanks to the University of Nairobi staff and colleagues from the Department of Public Health Pharmacology and Toxicology and the Department of Biochemistry.

References

1. Leather Manifesto. Leather Manifesto COP26: Call to the 2021 United Nations Climate Change Conference (COP26) by 31 international leather industry organizations, 2021.
2. Ozgunay, H., Çolak, S., Mutlu, M. M. & Akyuz, F. Characterization of Leather Industry Wastes. *Polish Journal of Environmental Studies* 16, 2007.
3. Meyer, M., Dietrich, S., Schulz, H. & Mondschein, A. Comparison of the technical performance of leather, artificial leather, and trendy alternatives. *Coatings* 11, 226, 2021.
4. Colak, S. Zengin, G., Ozgunay, H., Sarikahya, H., Sari, O. & Yuceer, L. Utilization of leather industry prefleshings in biodiesel production. *JALCA* 100, 137–141, 2005.
5. Cabeza, L. F., Taylor, M.M., DiMaio, G.L., Brown, E.M., Marmer, W.N., Carrio, R., Celma, P.J. & Cot, J. Processing of leather waste: pilot scale studies on chrome shavings. Isolation of potentially valuable protein products and chromium. *Waste management* 18, 211–218, 1998.
6. Hashem, M. A., Tomal, M. N. A. & Mondal, B. K. Generation of fleshings at beamhouse in tannery and its environmental impact assessment: Bangladesh perspective. *Bangladesh Journal of Scientific and Industrial Research* 50, 227–232, 2015.

7. Cuq, M. H., Benjelloun-Mlayah, B. & Delmas, M. Oil extracted from seal hides: Characterization and use as leather fat liquor. *Journal of the American Oil Chemists' Society* 75, 1015–1019, 1998.
8. Ongarora, B. G., Wainaina, P. N. & Tanui, P. Extraction of Oil from Tannery Fleshings for Chamois Leather Tanning, 2019.
9. Sivasubramanian, S., Murali Manohar, B., Rajaram, A. & Puvanakrishnan, R. Ecofriendly lime and sulfide free enzymatic dehairing of skins and hides using a bacterial alkaline protease. *Chemosphere* 70, 1015–1024, 2008.
10. Nyakundi, J. O., Ombui, J. N., Mulaa, F. J. & Wanyonyi, W. C. Evaluation of the Bacillus cereus Strain 1-p Protease for the Unhairing of Goatskins during Leather Production. *TLR* 4, 38–54, 2021.
11. Thanikaivelan, P., Rao, J. R., Nair, B. U. & Ramasami, T. Progress and recent trends in biotechnological methods for leather processing. *Trends in Biotechnology* 22, 181–188, 2004.
12. Balasubramanian, S. & Pugalenthi, V. A comparative study of the determination of sulfide in tannery waste water by ion selective electrode (ISE) and iodimetry. *Water Research* 34, 4201–4206, 2000.
13. Ranjithkumar, A., Durga, J., Ramesh, R., Rose, C. & Muralidharan, C. Cleaner processing: a sulfide—free approach for depilation of skins. *Environmental Science and Pollution Research* 24, 180–188, 2017.
14. Saravanabhavan, S., Thanikaivelan, P., Rao, J. R. & Nair, B. U. Silicate enhanced enzymatic dehairing: a new lime-sulfide-free process for cowhides. *Environmental science & technology* 39, 3776–3783, 2005.
15. Senthilvelan, T., Kanagaraj, J. & Mandal, A. B. Application of enzymes for dehairing of skins: cleaner leather processing. *Clean Techn Environ Policy* 14, 889–897, 2012.
16. Cheseto, X., Baleba, S., Tanga, C. M., Kelemu, S. & Torto, B. Chemistry and Sensory Characterization of a Bakery Product Prepared with Oils from African Edible Insects. *Foods* 9, 800, 2020.
17. Christie, W. W. Preparation of ester derivatives of fatty acids for chromatographic analysis. *Advances in lipid methodology* 2, e111, 1993.
18. Nyakundi, J. O., Ombui, J. N., Mulaa, F. J. & Wanyonyi, W. C. Enzymatic Unhairing of Goatskins During Leather Processing and Recovery of Valuable Products for Industrial Applications, University of Nairobi, 2021.
19. Khandelwal, H. B., More, S. V., Kalal, K. M. & Laxman, R. S. Eco-friendly enzymatic dehairing of skins and hides by C. brefeldianus protease. *Clean Technologies and Environmental Policy* 17, 393–405, 2015.
20. Wanyonyi, W. C., Shiundu, Paul M., Onyari, John Mmari, & Mulaa, Francis J. Isolation and characterization of biomass modifying enzymes for bioremediation and production of "green specialty products", 2015.
21. Rangel-Serrano, A., Kösters, K., Shelly, D. & Levy, J. Characterization of waste materials in tanneries for better ecological uses. Discussion. *JALCA* 98, 43–48, 2003.
22. Shaikh, M., Shafique, M., Naz, S. A., Jabeen, N. & Solangi, B. A. Streptomyces sp MM-3 from rhizosphere of psidium guajava: a potential candidate for protease with dehairing properties. *Pakistan Journal of Botany* 51, 735–742, 2018.
23. Robbins, C. R. Chemical Composition of Different Hair Types. in *Chemical and Physical Behavior of Human Hair*, 105–176, 2012. doi:10.1007/978-3-642-25611-0_2.
24. Rouse, J. G. & Van Dyke, M. E. A Review of Keratin-Based Biomaterials for Biomedical Applications. *Materials (Basel)* 3, 999–1014, 2010.
25. Shah, A., Tyagi, S., Bharagava, R.N., Belhaj, D., Kumar, A., Saxena, G., Saratale, G.D. & Mulla, S.I. Keratin Production and Its Applications: Current and Future Perspective. in *Keratin as a Protein Biopolymer*, 19–34, 2019. doi:10.1007/978-3-030-02901-2_2.
26. Alty, L. T. Analysis of Fatty Acid Methyl Esters in Egg Yolk Using GC–MS. *J. Chem. Educ.* 86, 962, 2009.
27. Karasek, F. W. & Clement, R. E. *Basic gas chromatography-mass spectrometry: principles and techniques*, 2012.
28. Bart, J. C. J., Palmeri, N. & Cavallaro, S. 6 - Emerging new energy crops for biodiesel production. in *Biodiesel Science and Technology*, 226–284, 2010. doi:10.1533/9781845697761.226.
29. Alcantara, R., Amores, J., Canoira, L.T., Fidalgo, E., Franco, M.J. & Navarro, A. Catalytic production of biodiesel from soy-bean oil, used frying oil and tallow. *Biomass and bioenergy* 18, 515–527, 2000.
30. Graboski, M. S. & McCormick, R. L. Combustion of fat and vegetable oil derived fuels in diesel engines. *Progress in energy and combustion science* 24, 125–164, 1998.
31. Ma, F. & Hanna, M. A. Biodiesel production: a review. *Bioresource technology* 70, 1–15, 1999.
32. Zheng, D. & Hanna, M. A. Preparation and properties of methyl esters of beef tallow. *Bioresource Technology* 57, 137–142, 1996.
33. Bondrea, D. A., Lazaroiu, G., Mocanu, R., Stan, A.N., Balanescu, L., Mihaescu, L. & Berbece, V. The energy method to protect the environment from waste in the leather industry. in *2017 International Conference on ENERGY and ENVIRONMENT (CIEM)* 105–109, 2017.
34. Dayanandan, A., Rani, S., Shanmugavel, M., Gnanamani, A. & Rajakumar, G. S. Enhanced production of Aspergillus tamarii lipase for recovery of fat from tannery fleshings. *Brazilian Journal of Microbiology* 44, 1089–1095, 2013.
35. Gaidau, C., Ghiga, M., Filipescu, L., Stepan, E., Lacatus, V. & Popescu, M. Materials obtained from leather–by products. in *CEEX conference proceedings Romania* 252–252, 2007.
36. Getahun, E. & Gabiyye, N. Experimental investigation and characterization of biodiesel production from leather industry fleshing wastes. *International Journal of Renewable and Sustainable Energy* 2, 120–129, 2013.
37. Kolomaznik, K., Barinova, M. & Fürst, T. Possibility of using tannery waste for biodiesel production. *JALCA* 104, 177–182, 2009.
38. Kubendran, D., Salma Aathika, A.R., Amudha, T., Thiruselvi, D., Yuvarani, M. & Sivanesan, S. Utilization of leather fleshing waste as a feedstock for sustainable biodiesel production. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects* 39, 1587–1593, 2017.
39. Lazaroiu, G., Pană, C., Mihaescu, L., Cernat, A., Negurescu, N., Mocanu, R. & Negreanu, G. Solutions for energy recovery of animal waste from leather industry. *Energy Conversion and Management* 149, 1085–1095, 2017.

40. Šánek, L., Pecha, J., Kolomazník, K. & Bařinová, M. Biodiesel production from tannery fleshings: Feedstock pretreatment and process modeling. *Fuel* 148, 16–24, 2015.
41. Stepan, E. S., Velea, S., Gaidau, C., Filipescu, L., Niculescu, D. & Radu, A.C. Surfactants and biofuels obtained from leather industry wastes. in *International Leather Engineering Symposium held in Turkey during April* 1–11, 2009.
42. Rahmawati, D. & Priatni, A. Characterization of Sulfited Fat from Limed Hides Fleshing as a Leather Fatliquor. in *IOP Conference Series: Earth and Environmental Science* vol. 355 012098, 2019.
43. Santos, L. M. & Gutterres, M. Reusing of a hide waste for leather fatliquoring. *Journal of Cleaner Production* 15, 12–16, 2007.
44. İşler, A., Sundu, S., Tüter, M. & Karaosmanoğlu, F. Transesterification reaction of the fat originated from solid waste of the leather industry. *Waste management* 30, 2631–2635, 2010.
45. Pathan, E., Ahmed, S. & Shakil, S. R. Utilization of Limed Flesh Through Fat Extraction and Soap Preparation. *European Journal of Engineering Research and Science* 4, 198–202, 2019.
46. Mittal, A., Sara, U., Ali, A. & Aqil, M. Status of Fatty Acids as Skin Penetration Enhancers-A Review. *Current drug delivery* 6, 274–9, 2009.
47. Ruiz, M. A., Arias, J. L. & Gallardo, V. Chapter 124 - Skin Creams Made with Olive Oil. in *Olives and Olive Oil in Health and Disease Prevention*, 1133–1141, 2010. doi:10.1016/B978-0-12-374420-3.00124-8.
48. Parveez, G. K. A., Rasid, O.A., Hashim, A.T., Ishak, Z., Rosli, S.K. & Sambanthamurthi, R. Tissue Culture and Genetic Engineering of Oil Palm. in *Palm Oil*, 87–135, 2012. doi:10.1016/B978-0-9818936-9-3.50007-1.
49. Lin, Y. & Peng, Q. *Stearic Acid: Synthesis, Properties and Applications*, 2014.
50. Zhen, Z., Xi, T. F. & Zheng, Y. F. 11 - Surface modification by natural biopolymer coatings on magnesium alloys for biomedical applications. in *Surface Modification of Magnesium and its Alloys for Biomedical Applications*, 301–333, 2015. doi:10.1016/B978-1-78242-078-1.00011-6.
51. Bajpai, P. Chapter 4 - Additives for Papermaking. in *Biermann's Handbook of Pulp and Paper (Third Edition)*, 77–94, 2018. doi:10.1016/B978-0-12-814238-7.00004-0.
52. Becker, L. C., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D. & Marks, J.G. Final report of the amended safety assessment of myristic acid and its salts and esters as used in cosmetics. *International journal of toxicology* 29, 162S-186S, 2010.