Fish Skin and Exotic Leathers

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Abstract

Exotic leathers are favoured due to their appearance, unique natural pattern and strength properties. The use of exotic leathers from different animal sources has increased the demand in the leather market for various applications. Processing of these skins require a non-conventional approach in order to preserve the natural characteristics of the skin after converting into leather. The present work exploits a new raw material source for its utilization as exotic leather. An exceptional variety of star puffer fish was keyed out from Indian Ocean coast. Leather made out of this skin was unique from most of the exotic leathers. Unlike other fishes, skin surface morphology of this species is covered with calcified spines. These spines are aligned on dorsal and ventral sides. When they are converted into leather, spines get firmly anchored onto collagen matrix like caltrops. Scanning Electron Microscopy (SEM) served as a tool to relate structural morphology of the leather and spine composition was analyzed using Energy-Dispersive X-ray spectroscopy (EDX). The developed exotic leather exhibited better mechanical properties and finds application in making high grip articles, therapeutic footwear and other niche products.

Introduction

Exotic leathers are distinct from normally accessible leathers in terms of natural occurring marks, patterns and structure. In order to process skins for making exotic leather, an entirely different approach is required. It necessitates delicate handling to avoid any damage to the skin.[1]The skin has to be stabilized, dyed and finished without losing the natural characteristics. Some exotic leathers were made from ostrich, emu, alligator,[2] salmon, eel, shark,[3] python, lizard, frog,[4] and stingray fish[5] emphasizing on development of natural pattern.

Arothron stellatus, (common name star puffer) is an exotic fish with distinctive natural pattern on skin. International union for conservation of nature (IUCN)[6] classified it under species of least concern category. Bio diversity of this species spread across Indo-pacific region. *A. Stellatus* is a giant among puffer fishes reaching lengths in excess of one meter. Skin of this fish is covered with spines. Another interesting feature of puffer fish is that it expands its body volume greater than the actual size, this genetic trait of this specie helps to protect itself from external threats. Internal organs of these species are toxic (tetrodotoxin) making it unsuitable for human consumption. Extraction of collagen from puffer species such as *Takifugu rubripes*[7] and *A. Stellatus* [8] were reported. It is reported that acid soluble collagen extracted from *A. Stellatus* was about 73-77% making it potential alternative source of collagen for bio medical applications. In addition, type I collagen mainly forms structural component of the leather which is responsible for the structural integrity and strength of the leather.[9] Apart from this, fish skin leathers are usually used for making high end shoes, handbags and clothing, which can fetch high prices in global market.[3] Unique surface morphology of puffer fish makes it suitable for making leather.

In this study, *A. Stellatus* fish skin was converted into leather and used for making value added products. Different processing step for converting skins to leather were optimized. Morphological characteristics of leathers were studied using Scanning Electron Microscopy (SEM), and composition of spines was analyzed using Energy-dispersive X-ray spectroscopy (EDX). Final crust leathers were tested for strength and organoleptic properties. Leather was utilized for making gloves, which may find application in tea leaves harvesting, rock climbing, and motorbike riding.

Experimental Section

Materials

Fish skins were obtained from coastal region of southern Tamilnadu (India). All chemicals used for assays and estimations are of analytical grade and all leather chemicals are of commercial grade.

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Leather Processing

Flayed fish skins were stored at -20°C and used as per the requirement. Skins were processed in a rotary drum. Detailed leather processing from raw to tanning of fish skin is discussed in Table I and post tanning in Table II.

Hexosamine and Uronic Acid Estimation

For estimation of proteoglycans in the form of sugars, the known amount of tissue samples were hydrolyzed using 6N hydrochloric acid (HCl). The samples were sealed into the small test tubes and hydrolyzed for 6 h at 121°C. Then the tubes were decanted into a graduated cylinder and washed thoroughly with water and stored at 4°C. Hexosamine was estimated using Elson and Morgan[10] method and uronic acid was measured as described by Schiller *et al.*[11]

Analysis Wet Blue Leather

Hydrothermal stability of leather is determined using a Theis shrinkage tester. Leather samples were clamped and immersed in a mixture of water: glycerol (3:1). Gradual heat was provided and test sample was observed up to a point where it starts to shrink. Chrome content in leather was estimated using standard test method for chromic oxide in leather (perchloric acid oxidation).[12]

H & E Staining

Raw fish skin and de-limed fish skin were cut into 1x1 cm^2 and fixed in 4% formalin solution. Before staining, samples were de-hydrated using ethanol at different concentrations from low to high (up to 100%) and final cleaning using 100% xylene. Sections of skin were embedded into paraffin and cut into thin slices (7-10µm) using microtome. These slices were placed on glass slide and stained using H&E dyes. Hematoxylin is a dark blue or violet stain, Eosin is a red or pink stain that binds to proteins and images were acquired by means of digital camera attached to microscope.

SEM and EDX Analysis

Crusted fish skin was studied by means of a Hitachi S-3400 scanning electron microscope operating at 10-30 kV at room temperature of 25°C and relative humidity of 50%. Samples were cut into 2x2cm^2 sections and mounted on adhesive stub; fracture ends of the specimens were sputter coated with Hitachi E-1010 (ion sputter with a thin layer of gold) prior to examination. Samples were viewed for studying surface and cross section of the leather. EDX was analysed using Nano Analysis INCA Energy 250 Microanalysis system from Oxford instruments. EDX is an analytical technique used for elemental analysis of a specimen. This type of spectroscopic tool investigates samples interaction between electromagnetic radiation and matter.

2.7 Physical Testing and Organoleptic Properties

For physical testing of leather, sampling[13] was performed for analysing tensile[14] and tear[15] strength according to standard procedures. Values reported were average of four samples. Organoleptic properties were assessed for general appearance, colour, softness and flexibility and spine distortion. Experts rated the leathers on a scale of 0-10 points for each functional property, where higher points indicate better properties exhibited.

Results and Discussion

Surface Morphology of *A. Stellatus* Fish Skin

Fish skin is non-toxic and its bio compatibility is reported, hence it may serve as potential raw material source for leather making. Skin surface is pigmented (Figure 1), black or brown star shaped pattern is visible only on the dorsal side, while ventral side is non-pigmented. Spines were aligned on dorsal and ventral sides of fish, coverage of spines were dominant in these areas.

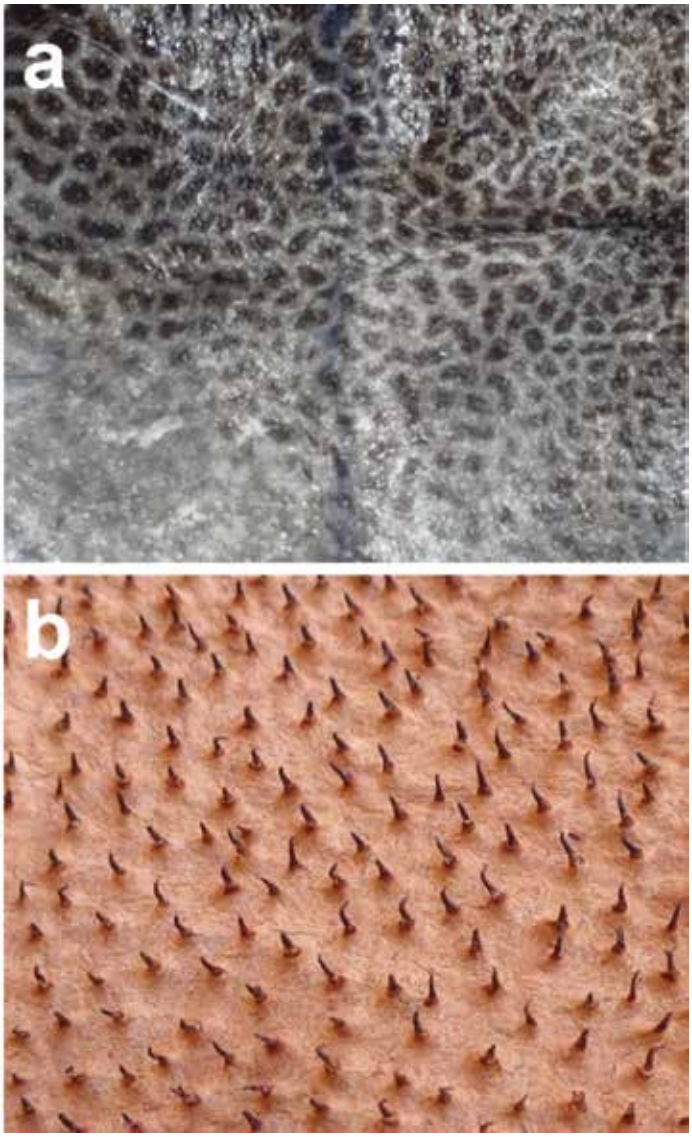

Figure 1. Surface morphology of a) raw skin b) crusted fish leather.

Table I
Raw to tanning process in detail.

	Chemical Name	Percentage (%)	Time (min)	Remarks
Washing	Water	100	0.2	Drain
Depigmentation	Water	10		
	Sodium sulfide	2	120	In 1: 10 dilutions with water. Use soft bristle brush to de-pigment. Wash and Drain
Liming	Water	100		
	Lime	5	720	Skins are immersed in lime liquor and handled twice every 300 min. Drain, wash and drain
De-liming	Water	100		
	Ammonium chloride	2	40	Check de-liming using phenolphthalein indicator.
				Drain liquor, wash and drain
De-greasing 1	Water	100		
	De-greasing agent	0.5	30	
	Wetting agent	0.2	0.2	Drain
De-greasing 2	Water	100		
	De-greasing agent	0.5	30	
	Wetting agent	0.2	0.2	Drain
Pickling	Water	80		
	Salt	8	0.2	
	Formic acid	0.5	0.20	In 1:10 dilution with water
	Sulphuric acid	0.2		In 1:10 dilution with water. Offer in 3 feeds for every 10 min, pH set to 3 and additional 30 min runtime.
Tanning	Pickle bath	50		
	Basic chromium sulphate (BCS)	4	30	Check cross section for penetration.
	BCS	4	30	
	Water	50	0.2	
	Sodium formate	0.5	0.20	
	Sodium Bi carbonate	1.5		In 1:10 dilution with water. Offer in 3 feeds for every 10 min, pH set to 4 and additional 30 min runtime.

Table II
Post tanning process in detail.

	Chemical Name	Percentage (%)	Time (min)	Remarks
Neutralization	Water	100		
	Sodium formate	0.5	10	
	Sodium Bi carbonate	0.5		In 1:10 dilution with water. Offer in 3 feeds for every 10 min, pH set to 5.5 and additional 30 min runtime.
				Drain, wash, drain
Retanning, dyeing and fatliquoring	Water	100		
	Acrylic syntan	2	30	In 1:10 dilution with water.
	Synthetic fatliquor	2	30	In 1:10 dilution with water.
	Dye	2	30	Check penetration.
	Synthetic fatliquor	4		
	Vegetable based fatliquor	4		
	Lecithin based fatliquor	4		
	Fish oil based fatliquor	2	60	In 1:10 dilution with water.
	Phenolic syntan	6		
	Melamine syntan	6	60	
Fixing	Formic acid	3		In 1:10 dilution with water. Offer in 3 feeds for every 10 min and additional 30 min runtime.

Spines are similar to caltrop arrangement, except one of the pointed ends elongate towards surface of skin, while the basal arrangement is aligned deeper into skin providing good anchorage.

Weight of spines accounts for 5-6% of total dry weight of skin. Flesh surface is clear without any marks. Thickness of raw skin was 1-2 mm with average area of 2-3 Sqft (medium to large fish).

Leather Processing

Skin surface pH was 6.5-7.5; appropriate care must be taken for processing leather. By applying conventional leather processing, hydrolysis of skin was observed. During de-pigmentation stage of processing, skins are treated with sodium sulfide solution in 1:10 dilution with water. Skin was de-pigmented using soft bristle brush after 5 h. Skins were immediately rinsed in fresh water, moderate swelling was noticed. Conventional paste application of lime and sodium sulfide required longer duration for de-pigmentation, this makes skin vulnerable for hydrolysis. Liming operation was essential for opening of fibers and removal of non-collagenous substances. Conventional processing of goat skin requires 36 hours of liming; for delicate skin like fish, this can be restricted to 12 h due to hydrolysing nature of skin in extreme alkaline conditions. Diluted lime liquor solution was used, skins were immersed and handled once every 3 h. Skin was thoroughly washed before de-liming. Conventional de-liming was followed using ammonium salt. After completion of de-liming skin was de-greased using mild de-greasing agent.

De-greasing step was repeated again and washed with non-ionic wetting agent. Conventional pickling and tanning was followed. Care should be taken while offering acid, leaving skins in pickle bath for longer durations may lead to hydrolysis. Hence it is advisable to immediately tan (wet blue leather) as the pH of skin was set to 3. Tanned leathers were converted to crust leathers after treating with retanning agents, dye and fatliquors.

Hexosamine and Uronic Acid Estimation

Glycosaminoglycans consists of long unbranched polysaccharides with repeating disaccharide units. These repeating units consists of an amino sugar along with uronic sugar, hence quantification of these units gives the measure of glycoproteins estimated in the form of sugars. Their affinity towards polar group makes a significant decrease in the levels of hexoamine and uronic acid. However these changes did not alter the properties of the leather. From Table III, hexosamine and uronic acid removal from raw skin and delimed skin were around 80 and 75% respectively.

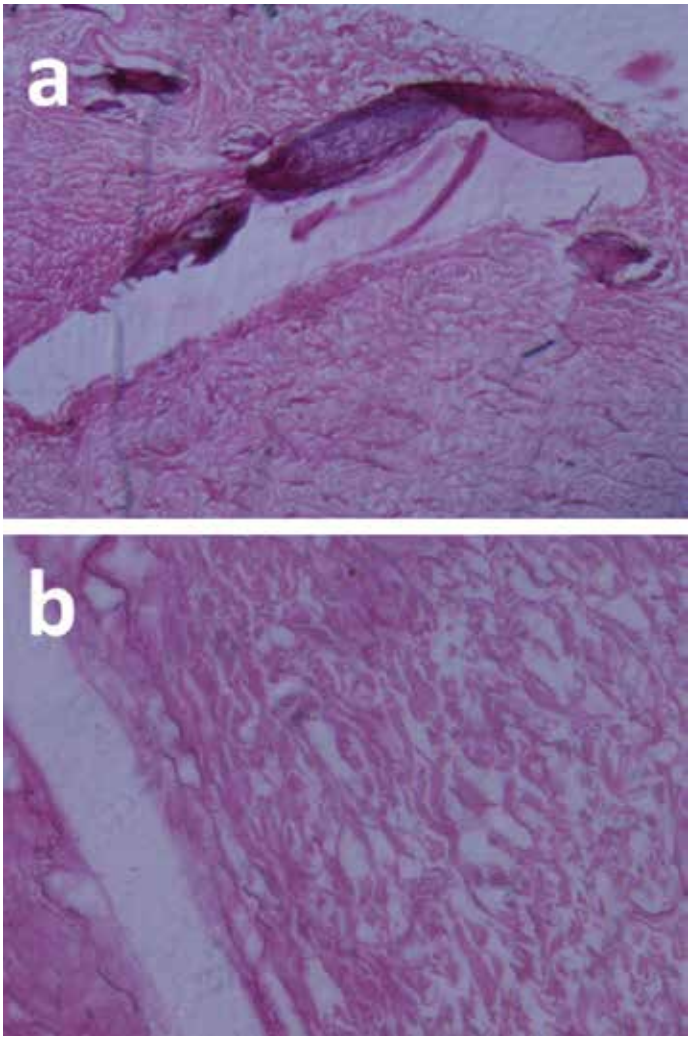

Figure 2. H&E staining images of a) raw fish skin and b) de-limed fish skin at 10X magnification.

Hematoxylin and Eosin Staining

Removal of non-collagenous material plays an integral part in leather tanning. Liming is essential for splitting of fiber bundles and removes proteoglycans, triglycerides and other conjugated proteins. Degree of splitting allows the penetration of chemicals in subsequent steps of leather processing. Presence of collagenous and non-collagenous proteins can be visualised by applying staining techniques. Staining images of raw skin showed the presence of violet colour indicating presence of proteoglycans. Fibers are closely oriented for raw skin. After de-liming, fibers were split apart and spacing between fibers is wider. This indicates removal of proteoglycans and other non proteinous substance. (Figure 2)

Chrome Tanning and Analysis of Wet Blue Leather

Similarly quantification of amino acid composition in acid solubilized collagen from *A. Stellatus* fish skin[8] (residues/1000) contains aspartic acid (25 residues) and glutamic acid (66 residues) along with asparagine and glutamine. It was reported earlier that the amount of aspartic acid and glutamic acid residues increase due to hydrolysis of aspartate and glutamate (a amino acids) after liming.[16] Hexaquo chromium complex reaction with fish skin may proceed similar to bovine skin, as generally summarized with plausible reaction. (Figure 2) Wet

Table III
Hexosamine and Uronic acid estimation.

	Hexosamines (µg/g of tissue)	Uronic acid (µg/g of tissue)
Raw skin	222±5	833±15
After de-liming	45±5	219±15

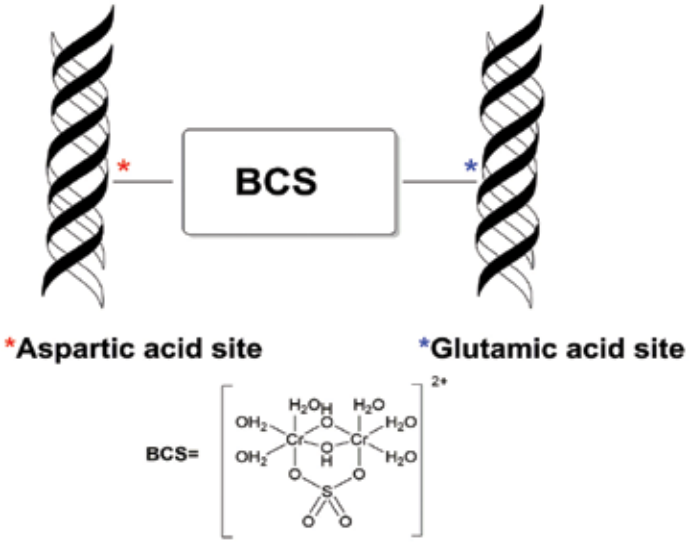

Figure 3. Plausible collagen interaction with BCS[16].

blue leathers were analysed for shrinkage temperature. Chrome tanned fish skin withstood temperature up to 106°C. Wet blue made from fish skin was analysed for chrome and it was found to be 3.1%. Colour was uniform with no chrome patches, penetration of chrome was through and through. Presence of chrome was evident from EDX analysis of leather surface (ESI).

SEM and EDX Analysis

Surface and cross section of leather was studied using SEM. Images were taken for analysing surface morphology of tanned fish leather. It was observed from surface of leather, collagen fibers were woven compactly one over another into a matrix. Spines are fixed firmly between the weave. Projected ends of spines and basal root can be viewed from Figure 3. EDX analysis indicated the presence of calcium and phosphorus deposits, elements which are predominant in bones and spines. (Figure 4)

Table IV
Organoleptic property score.

	Rating*
General appearance	9±0.5
Colour	9±0.5
Softness and flexibility	8±0.5
Spine distortion	7±0.5

*average of 4 samples evaluated

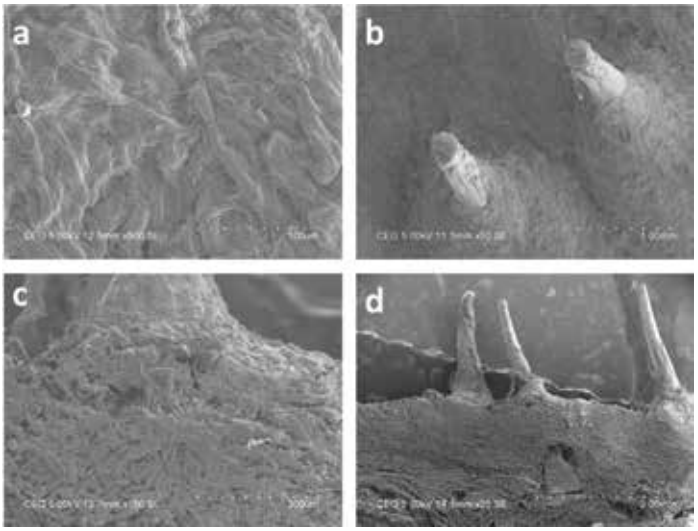

Figure 4. SEM images of crusted fish leather surface a) without spines at 500X magnification, b) spine at 50X magnification, cross section view c) from root of spine at 150X magnification and d) leather with spines at 25X magnification.

Characterisation revealed the presence of elements (%), N (16.85), O (50.58), Mg (0.27), P (9.46), Cl (1.09), Ca (21.76). Availability of calcium to phosphorus in ratio equivalents of 2.3± 0.03 gives the measure of basic calcium salt which might be freshly deposited on bone.[18] Hence it also indicates the transformation stage of fish from juvenile to adult stage. Though the skin was converted to leather, spines did not show any absorption of chromium. This shows the phobic nature of spines to react with metallic compounds used in leather processing.

Physical Properties and Organoleptic Properties

Tensile strength gives the measure of leathers ability to withstand load, while tearing load was measured to understand the materials resistance to break if it develops any cuts. For puffer leather, tensile strength value was measured to be 19±2 (N/mm^2) with elongation (%) of 45±5 and tear strength of 85±5 N/mm. Strength values are agreeable in comparison with upper leathers.[19] Organoleptic properties were analysed and rating

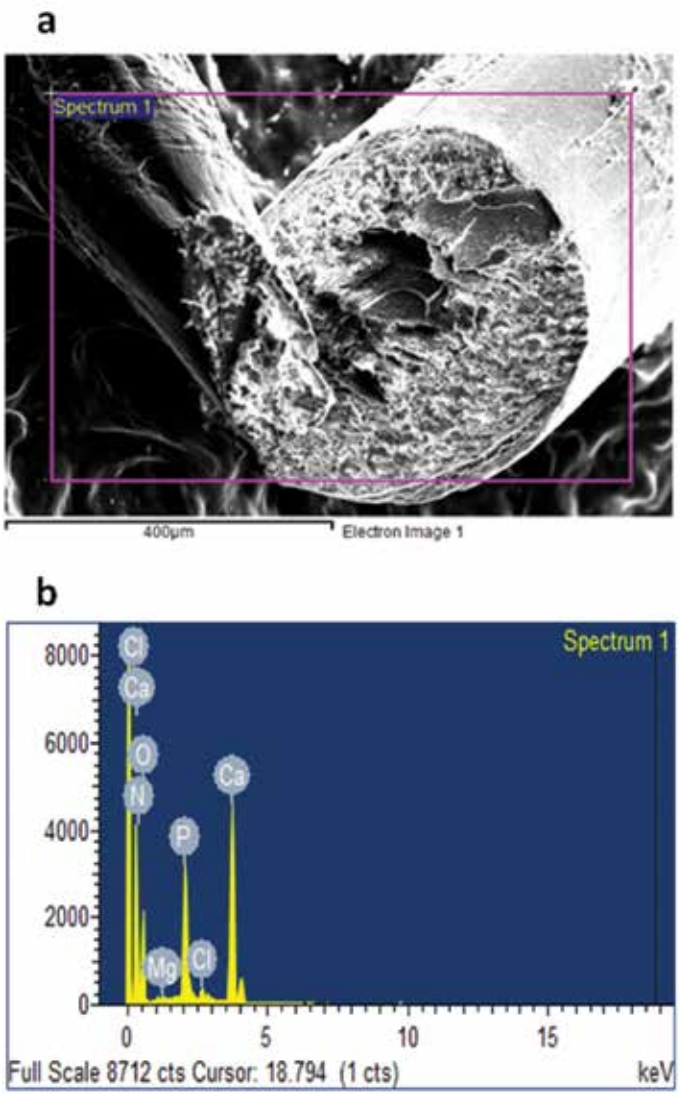

Figure 5. a) SEM image of surface and b) EDX data of fish spine.

were given in Table IV. After conversion of skin to leather, it was noticed that the sharpness of spines were deteriorating. This may be because of continual agitation of skin in drum during processing. Hence care should be taken to reduce the time and speed of the drum to the maximum extent for producing better quality leather.

Preparation of Gloves Using Fish Skin

Leathers made from fish skin were used for making gloves. Due to presence of spines on the surface of leather, entire skin was not used for making gloves. Small pieces were cut and embedded on to portions covering anterior parts of gloves. From Figure 5, it can be noticed that pieces of fish skin was used for covering fingers and palm region. These gloves find applications, *viz.* tea leaves harvesting, rock climbing and motorbike riding

Figure 6. Gloves made from fish skin.

Conclusions

In this study, puffer fish skins were tanned and converted to crust leathers. Standard recipe for processing fish skin of this origin was formulated for reproducing the leathers of same quality. Various experimental and analytical techniques such as H&E staining, SEM, EDX and shrinkage temperature and strength properties of leather were studied to understand the properties of puffer fish leather. These exotic leathers were used for making gloves. Future research may be focused for making different value added products such as therapeutic footwear and other high grip articles using fish skin leather.

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