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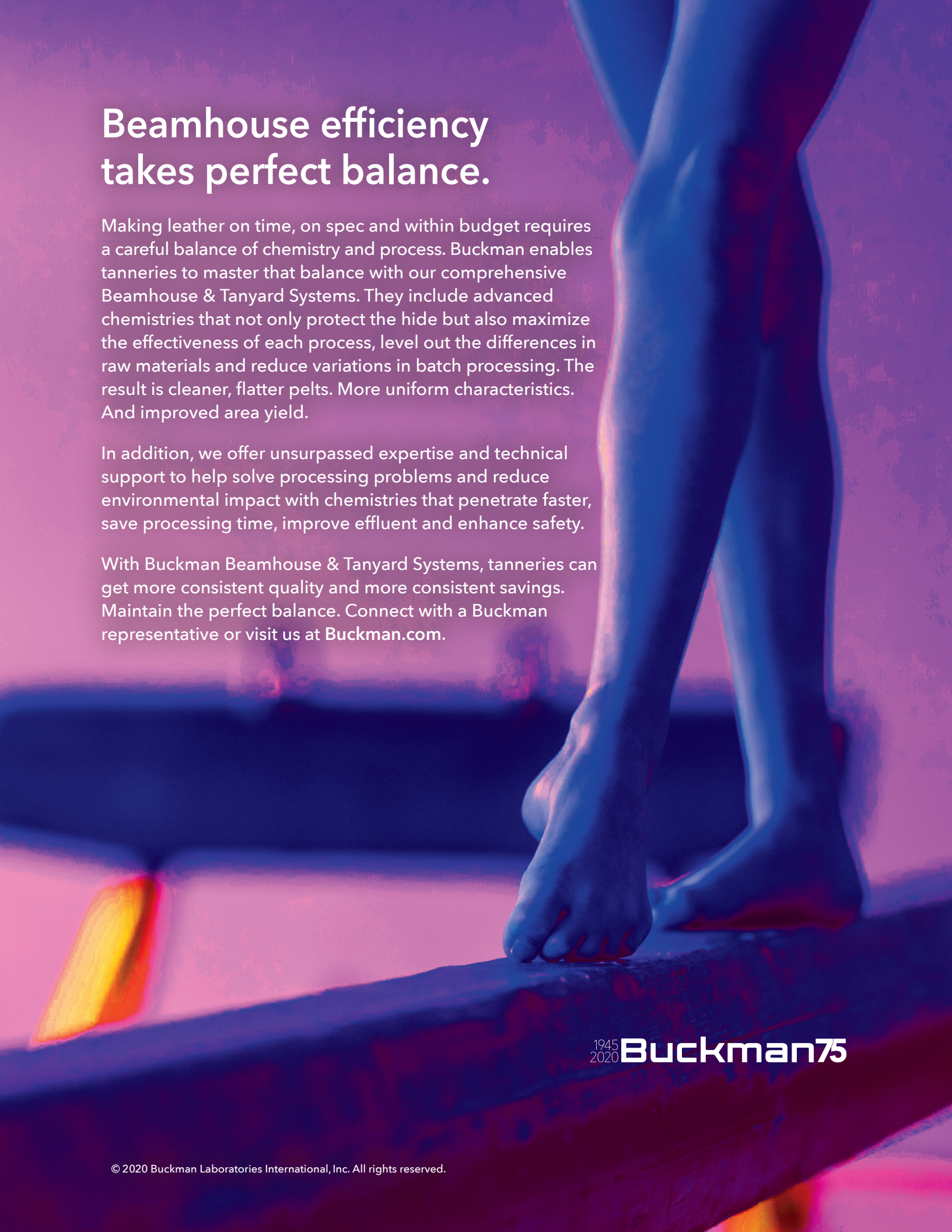

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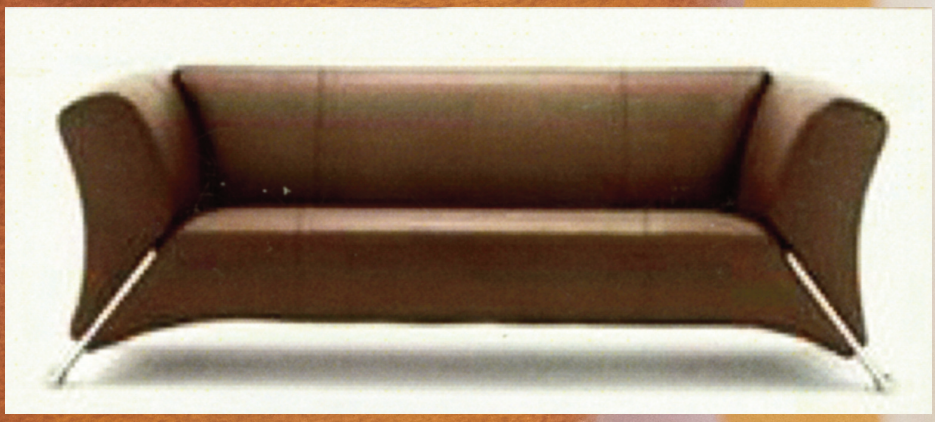

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Ethiopian *Ankelba*: An Attempt to Modernize the Cultural Leather Artifact

by

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Abstract

Ethiopia has many cultural artifacts made of traditionally processed leathers. *Ankelba* is one of them and utilized in most part of Ethiopia to carry baby. Here, we report salient features of *ankelba* and its value in Ethiopia region, demerits of the product and strategies to enhance the design feature of cultural *ankelba* while maintaining its cultural value. Data were collected through interview guided questionaries' to analyze the causes for the reduced usage of the product. We identified that the traditionally processed cow leathers are heavy with poor color fastness. Further, we recognized that cultural *ankelba* has flawed design features such as fixed size, improperly placed *zagols* (sea shell) and uncomfortable strap construction to hold the baby. Here, we present an efficiently designed *ankelba* overcoming the key deficiencies while preserving the cultural value of local people. This study would pave way for restoration of several cultural artifacts available across the globe.

Introduction

Ethiopia is an ancient country with a remarkable cultural diversity. This diversity includes tangible and intangible heritage with both traditional and modern cultural expressions, language, and practice in artifact production.[1] Artifact is an individual's expression of identity, typically an object, which people assign meaning to express their aspirations and present to the world. Based on these shared meanings, individuals impress their appearance to express their desired identities in social contexts.[2] *Ankelba* is one of the artifacts produced by Ethiopian rural society at home. In Ethiopia, rural area mothers used *ankelba* often to carry their babies.[3] Baby wearing is the culturally borrowed practice of carrying an infant on the body using a sling or cloth carrier.[4] Prior to the 19th Century, worldwide parents used a variety of long cloths, shawls, scarves and even bed sheets to snuggle up their babies and get the chores done.[5-7] Cultural baby wearing is one of the historic artifacts practiced by most civilizations around the world. The main reason for popularity of baby wearing is due to better attachment of baby to parent.[5] Even today, traditional types of baby carriers are still used in developing countries, where each region has its own traditional design to meet their particular needs such as climate, type of work of mothers and cultural baby wearing positions.[6]

In Mexico, *rebozo* has been used to cover, carry and transport infants with different colors, materials, patterns and usage from region to region.[8] In some parts of Africa, mothers use a short piece of cloth tied around the chest called *khanga* to carry baby. Similarly, Ethiopia has long history of using good cultural tradition of carrying baby using *ankelba*. *Ankelba* is baby wearing product made up of traditionally processed cow hides tied over both shoulder with baby usually on the back. *Ankelba* allow mothers for continuous holding, carrying of baby and walking long distance or working. Leather used for *ankelba* production processed by household traditional tanners. They utilize natural plants locally available for dyeing of *ankelba*.[9] There are more than 6722 household traditional leather processing tanners in Ethiopia. They process hides to generate leather that can be used for bags, wallets, belts, musical instruments, traditional kitchen goods as well as *ankelba*.[10] Even though a*nkelba* is used as a traditional leather product to carry baby, it has its own problems related to design, material and manufacturing methods. *Ankelba* lost its popularity in modern generations due to certain design and material related drawbacks. However, rural people still use *ankelba* in spite of its drawbacks. Not much work has been done to understand the techniques used for either traditional Ethiopian hides and skin processing, its drawbacks and methods to improve it or *ankelba* production, its drawbacks and methods to sustain.

The purpose of this study is to explore the history, significance, and meaning of traditional Ethiopian *ankelba* and answer the following questions: What factors contributed to the decline in percentage utilization of *ankelba* in Ethiopian rural areas? How leather used for *ankelba* was processed? What are the techniques and procedures adopted for manufacturing *ankelba*? What are the ways to increase the use of *ankelba* in Ethiopia and how to sustain it? The present study also aims to investigate the Ethiopian cultural *ankelba* design, materials and the key drawbacks that contribute to the reduction in utilization of the precious cultural article. Additionally, we made an attempt to substitute the traditionally processed heavy cow leather with a suitable alternative leather and improve the design of *Ankelba*.

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Materials and Methods

Methods

Research about historical cultural artifacts aims to create an account of people assets, values and events of the past in an effort to create a foundation of knowledge for the future. Cultural artifacts research has customarily made use of interpretive approaches that rely upon the analysis of qualitative data.[11] However, in recent years, historic cultural artifact researchers have begun to apply statistical approaches to analyze qualitative data, and/or to collect and analyze quantitative data.[12] Here, we used both qualitative as well as quantitative data for collecting facts related to Ethiopian traditional *ankelba*.

Data Collection and Sampling

Data were collected through interview guided questionaries from regions where *ankelba* was highly utilized in the past and present in order to identify the value of utilizing this product and the main problems existing with traditional *ankelba*. Interviews were conducted face-to-face in Ethiopia in the homes or working place of the interviewees. The duration of the interview differed from interviewee to interviewee. All interviews were conducted in Amharic, translated into English, and reviewed by three readers fluent in Amharic and English. Extra attention was given to the cultural tones of these personal conversations in order to make the translations as precise as possible.

Data were collected from 25 traditional leather processing artisan, 6 *ankelba* producers and 372 *ankelba* user family members. Furthermore, picture and measurements of traditional *ankelba* were taken to understand the design related problems.

Geographical Domain for the Research

Data were collected from different geographical locations of Ethiopia where *ankelba* was highly utilized such as *Adet*, *Merawi*, *Qoqa* and *Hamusit* districts of Amhara region. These districts were selected based on the time required for data collection, expenditure involved for travel and data collection and also the utilization rate of *ankelba* products. Data were collected from three focus groups namely the user, producer and traditional leather processing artisans that process cow hides as shown in Table I.

Data Analysis

Fishbone diagram was used to identify the causes for un-comfortable cultural *ankelba* problems. Anonymous rating was used to identify the causes that need priority to tackle and ranked by experienced expert by interviewing the local users. The problems associated with the material and design of *ankelba* were listed in the questionnaire with the scores of 1 to 3, where low score means the least important problem while high score means the most important problem. The material related problems were bad smell, huge weight, color fading, oil removal and unwashability, while design related problems were fixed size, many *zagols* (sea shell) per *ankelba*, many button per *ankelba*, narrow strap width, un-comfortable neck construction and absence of lining and pad.

Following explanation by experienced expert to all members individually, the expert ranked the problems with the scores of 1 to 3. The expert raised single question at a time about the problems. After they understood the respondent's view, they gave the scores. Finally, the total score for each problem was calculated by multiplying each score with the respective number of total respondents followed by summing up all the scores.

Characterization

Tensile strength of the traditionally tanned cow leathers and modern tanned sheep garment leathers was evaluated according to ISO 3376:2011/IUP 6/ SATRA TM 43 method.[13] The test specimens were conditioned at 20±2°C and 65±5% relative humidity in accordance with ISO 2419:2012 method. Tearing strength of leather was measured according to SATRA TM 162 /ISO 3377-2:2011 test method after conditioning the specimens as described above.[14] Both traditionally and modern tanned leathers were also analyzed for bulk properties such as softness, grain smoothness, surface color uniformity and general appearance by an experienced tanner by hand and visual evaluation in a scale of 0 to 10 points. Higher numbers indicate better property. Rubbing fastness (dry and wet) of the samples was carried out according to ISO 11640:2012 test method.[15] Samples were obtained according to ISO 2418:2002 procedure[16] and conditioned at 20±2°C and 65±5% relative humidity as per ISO 2419:2012.[17]

Table I

Area of data collection and number of participants for data collection

	Total number of populations		Number of participants in the study		
Area of data collection	Total number of families	Number of families having *ankelba*	Cow hide processors	*Ankelba* producers	Users
Adet	20117	5113	8	2	127
Merawi	16405	3096	4	2	73
Qoqa	5671	1171	6	1	97
Hamusit	4325	2043	7	1	75

Results and Discussion

Traditional Leather Processing in Ethiopia

As seen in Figure 1a, traditional leather processing in Ethiopia starts by soaking cow hides in small pond or in part of rivers that have dip height. Small-dammed river helps traditional leather processors to soak large amount of hides in single process. The soaking operation normally takes 3 to 5 days. The purpose of soaking operation is to soften the hides and make ready for the fleshing operations. During soaking solid and liquid wastes such as hide dust, blood, fleshing and hair may be released to local rivers. This has its own impact on people living in downstream that utilize the river. Then fleshing process carried out by local artesian using a knife and small axe to remove the flesh and waste from the hides as shown in Figure 1b. Small amount of water is sprayed all over the hides for softening the hides. After fleshing operation, unhairing process proceeds using mixture of cow urine, grinded *Solanum incanum* fruit (*Embuay*) and water. This solution was prepared by mixing approximately 1 kg of *Solanum incanum* in 10 liters of cow urine. Hides are immersed in this solution for 24 h up to 3 days and after removal from solution the hides are piled and kept in a place where it gets some heat and is ready for manual unhairing operation as seen in Figure 1c. The solution acts as surfactant and helps to clean the hides and make easy to remove the hair. After unhairing, oiling process is carried out using grinded castor bean (*kachma*, Figure 1e) for softening the processed cow hides as shown in Figure 1d. The amount of oil applied on the leather depends on the size of the hides. The oil was spread on the surface of leather and treated leathers were kept for a day in sun light. Extra oil was swept by brush. "*Faki*" is the name commonly given for the artisan processing such traditional leathers.

Traditionally processed leathers have been used for different purposes such as *kurbet* (sleeping mat), musical instruments, *mecagna* (rope), *ankelba* (baby wearing), *doniya* (bags for grain transportation), package for praying book pocket and traditional leather goods production.[10]

Traditional *Ankelba* Production

Ethiopia has different artifacts that have been used for a long time, which express Ethiopian society, culture, history and traditions. Among various artifacts that differentiate Ethiopian society from other parts of the world, baby carrying methods and materials used for preparing *ankelba* is one of them. In most parts of Amhara region, some parts of Tegria region, some parts of Oromia and Southern Nations Nationalities and People (SNNPR) regions have long history of using leather *ankelba* for carrying babies. The design of *ankelba* in each region has its own unique features. Leather *ankelba* used in different regions have different designs especially in the placement of accessories although the function is the same.

In the Amhara region, *ankelba* has especial value among society and expresses the wealth of individuals in addition to its basic function. High number of *zagol* attached at the end of *ankelba* expresses high wealth of *ankelba* owners. *Ankelba* is designed and manufactured by local artisans and sold to rural society by themselves or through local traders (local community). The art and technology are transferred through family legacy.

Figure 1. Traditional leather processing in Ethiopia. **(a)** Hide soaking in damped river, **(b)** fleshing, **(c)** cow hides preparation for unhairing, **(d)** oiling and inset **(e)** oil used for softening leather.

Table II

***Ankelba* size measurements**

Components	In arm measurement	Converted measurement (average)
Main Body		
Length	3 cubit	150 cm
Width	2 cubit	100 cm
Straps		
Length	Guess	150 cm
Width	Guess	3 cm
Strap insertion		
Length	Guess	30 cm
Width	Guess	3 cm

The leathers used for *ankelba* production are processed cowhides, which have better area utilization with less stitching requirements. The manufacturing of *ankelba* starts by purchasing of processed hides from household traditional leather processor in dyed or undyed form. The price of the leathers is agreed between the seller and purchaser depending on the guesstimate area of the leather. There is no scientific measurement of the surface area of the leather like modern practices. Leathers were sold only in full thickness form and split leathers are not possible to purchase. Undyed leather was colored by *ankelba* producers as shown in Figure 2a after cut (Table II) into 3×2 cubit (ancient unit of length based on the length of the forearm from the elbow to the tip of the middle finger). Waste engine oil and locally available '*Alela kelem*' dye were used for coloring the leathers. Mill oil may be used for further softening of purchased leather. After dyeing, the leathers were cut into straps that hold *zagols* as shown in Figure 2b.

Button attaching to body of *ankelba* is carried out as shown in the Figure 2c. Local producers believe buttons are accessories, which enhance the aesthetic value of the product. Buttons used for *ankelba* are white in color and the number of buttons varies from product to product. To reduce complication during stitching they prepare holes before attaching buttons to *ankelba*. They use an awl called '*wesfa*' and thread called '*chinga*' for attaching the buttons. *Zagols* (inset Figure 2e) are other accessories, which enhances aesthetic value of the product and inserted to all straps at the end of *ankelba* as seen in Figure 2d. *Zagol* provides sound during movement and the people believe that it entertains the baby. More number of *zagols* in single *ankelba* also indicates the wealth of that *ankelba* user. *Ankelba* can also be made without *zagol* attachments. As shown in Figure 2f, the edge is folded approximately by 5 cm and stitched. Finally, strap and other decorative neck terry leathers were also attached to *ankelba*.

Major Causes for the Declined Use of Traditional *Ankelba*

Various causes for un-comfortability of cultural *ankelba* are categorized in fishbone diagram as shown in Figure 3.

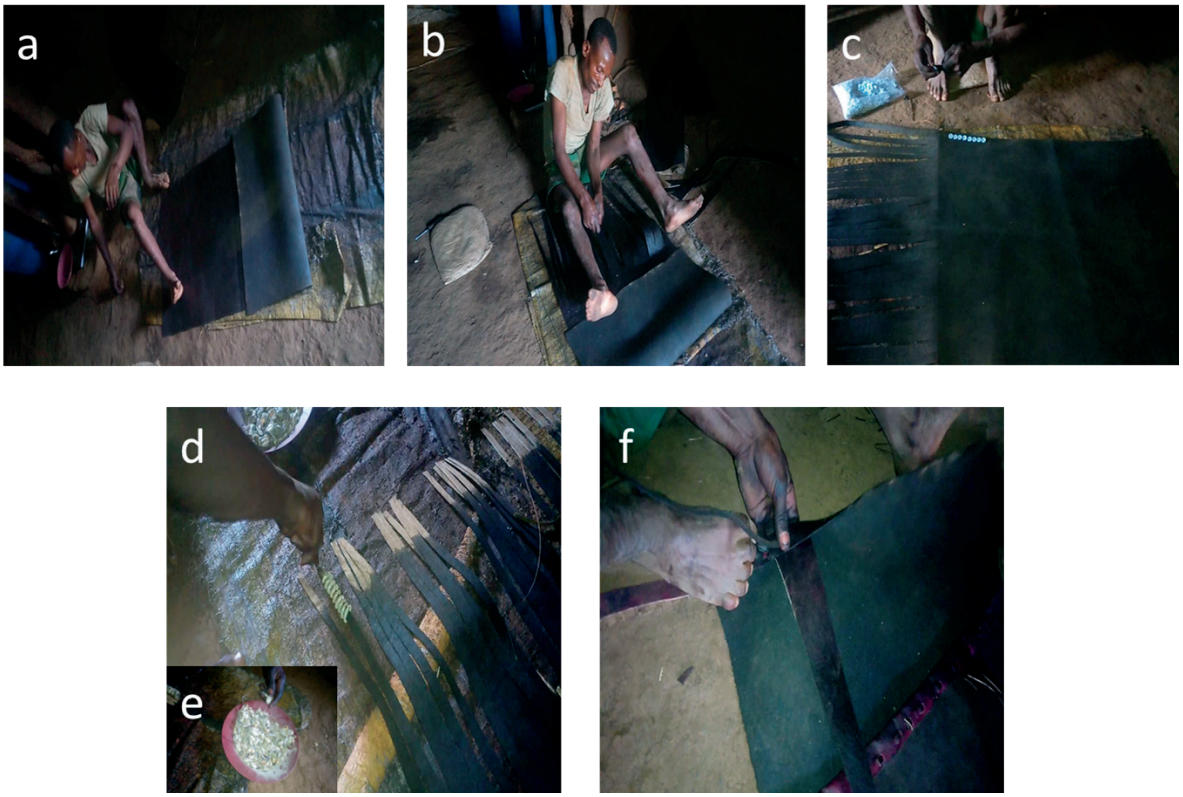

Figure 2. *Ankelba* leather preparation steps. **(a)** Leather cutting and dyeing, **(b)** leather strap making, **(c)** button attaching, **(d)** *zagol* inserting and **(f)** strap stitching. Inset in **(e)** shows *zagols*.

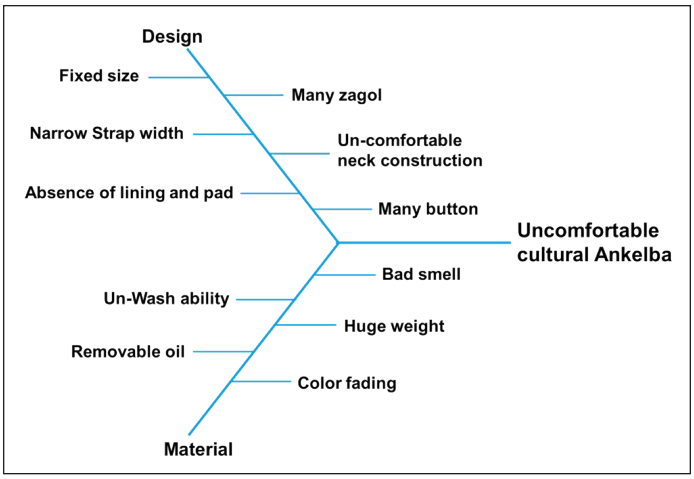

Figure 3. Major causes for the declined use of traditional *ankelba*

As seen from diagram, un-comfortability of cultural *ankelba* is mainly caused by poor design and material selection. Further, these two major causes are sub-divided into different small problems. Design related problems are caused due to fixed size of *ankelba*, many *zagols*, narrow strap width, un-comfortable neck construction, absence of lining and pad and many buttons. On the other hand, material related problems are caused by bad smell, un-washability, huge weight, oil removal and color fading of traditionally processed leathers.

Problems Associated with the Use of Traditional Leathers for *Ankelba* Production

Table III shows a compilation of the scores for the material related problems associated with the *ankelba* through communication with 372 users of *ankelba* and ranked by experienced experts. As seen in Table III, 21% of respondents said *ankelba* leather has bad smell. The smell is the result of the use of oil that used to treat leathers and also due to the urination by the baby on *ankelba* where cultural *ankelba* design did not have material that can absorb the urine. Moreover, user of *ankelba* applies butter, oil and suet to soften the *ankelba* during utilization that inherits additional smell to the leather. Traditional leather is not washable and lining was not included in cultural *ankelba*, which can absorb baby urine. Baby's urine also causes puckering of *ankelba* that makes *ankelba* uncomfortable for baby to sit. The respondents almost equally ranked all the other problems. According to about 20% of the respondents, un-washability of *ankelba* leather is one of the material problems that need improvements. In rural areas, babies do not use underwear or any diapers that can absorb baby urine and protect *ankelba* from spoiling. Additionally, this increases the friction between baby and *ankelba*, which causes physical and other problems on babies. Cultural *ankelba* is heavy in weight owing to the thickness of the material used for *ankelba* production (> 1.4 mm), which was ranked by 1/5th of the respondents. Furthermore, accessories attached to *ankelba* such as *zagol* and buttons increase the weight of *ankelba*. This causes health problems on mothers. Further, *ankelba* leather fades after rubbing according to 19.5% of the respondents as seen in Table III. Almost equal respondents also indicate oil removal as other issue hindering the continued utilization of *ankelba*.

Table III

Leather related problems and their ranking in cultural *ankelba*

Material problems	Score=3	Score=2	Score=1	Total score	Percentage (%)
Bad smell	202	134	36	910	21.0
Huge weight	187	97	88	843	19.4
Color fading	178	118	76	846	19.5
Oil removal	183	134	55	872	20.0
Un-washability	197	112	63	878	20.1

Where a score of 3 means the most important problem, 2 means moderate important problem and 1 means least important problem.

Problems Associated with the Traditional Design Features of *Ankelba*

Sizing plays a big role in every fitting requirement. There are large number of standard sizing systems for various garments such as dress, tops, skirts, and trousers.[18] Hence, appropriate sizing and fitting of *ankelba* to the body of baby and mother is important. Traditional *ankelba* is one of the products used in rural area by most of the families. *Ankelba* product measurements were passed through generation to generation without changing. Measurements carried out by cubit even though forearms are different from one person to the other. As seen in Table IV, about 20% of the respondents said size of *ankelba* is fixed and un-adjustable and not suitable to carry baby by other members of the family. *Ankelba* is culturally produced by ready-to-wear sizing styles and there is no grading system. The size of *ankelba* is fixed to 3×2 cubit (length by width) and this not comfortable to carry all age babies.

Another major drawback of *ankelba* design is *zagol* number and placement. As seen in Table IV, 18.4% of the respondents indicate *zagol* number makes the product heavier, even though the number of *zagol* used per *ankelba* is an indicator of the wealth level of the user. *Ankelba* has 0 to 20 *zagols* per strap. On average, one *ankelba* has about 39 straps with 14 zagols per strap. In other words, an *ankelba* has about 546 *zagols*. Because of these reasons, *ankelba* is not suitable for movement and long walk and causes calf problems.

The strap is used to tight the baby on mother's back. However, the width of the strap is not sufficient to hold the weight of the baby and distribute the baby load. This causes irritation and sometimes wound on mother's shoulder and 19% of respondents believe this is one of the main issues hindering *ankelba* usage. Another major problem highlighted by the respondents (17.7%) was the absence of

Table IV

Design related problems and their ranking in cultural *ankelba*

Ankelba design problems	Score=3	Score=2	Score=1	Total score	Percentage (%)
Fixed size of *ankelba*	228	132	12	960	19.7
Many *zagols* per *ankelba*	207	112	53	898	18.4
Many buttons per *ankelba*	52	83	237	559	11.5
Narrow Strap width	234	92	46	932	19.1
Un-comfortable neck construction	63	167	142	665	13.6
Absence of lining and pad	167	158	47	864	17.7

Where a score of 3 means the most important problem, 2 means moderate important problem and 1 means least important problem.

lining and pad. The presence of pad is expected to absorb the urine of the baby thereby not only maintaining the hygienics but also reducing the bad smell. Lining may help in avoiding the transfer of urine to the leather thereby improving the washability as well as maintaining the properties of the leather.

About 25% of respondents replied cultural *ankelba* has many buttons per *ankelba* and un-comfortable neck construction, although these problems were recognized by fewer number of respondents. Neck construction did not support baby head and caused scratches on baby neck due to improper stitching as seen in Figure 4. As seen, there are many *zagols* and buttons per *ankelba*, narrow strap width and un-comfortable neck construction and other problems associated with the design of traditional *ankelba*. To support and protect a baby's developing spine and hips, it is important that the carrier can support the baby's back, hips and back of the head. However, as seen in Figure 4, Ethiopian cultural *ankelba* did not fulfill baby wearing safety features.

Strategy for Improving the Materials and Design of *Ankelba*

All problems associated with the material are predominantly due to the choice of heavy cow hides and the traditional way of processing them. This can be solved by selecting lighter sheep skins as raw material and utilizing vegetable or chrome tanning based modern techniques. Here, we have developed a sample *ankelba* for babies aged 6 to 24 months old by solving the design and material oriented problems observed in cultural *ankelba*. Sheep garment leather of Ethiopian origin with 0.8 mm thickness processed through modern chrome tanning based technique was selected to solve material related problems. As shown in Figure 5, the size of *ankelba* for selected age group was fixed at 40×22 inch length by width according to the standard sizing system followed for modern baby wearing products.[18-20] It is possible to employ a standard grading system for other age groups. Adjustable straps are designed to ensure all members of the family can utilize one *ankelba*. The weight of the newly designed *ankelba* is just about 50% of the cultural *ankelba*. The lower weight was due to low thickness of leather (0.8 mm) and

Figure 4. Drawbacks of Ethiopian cultural *ankelba*. **(a)** Inappropriate size and many *zagols* and **(b)** un-comfortable neck construction and many buttons.

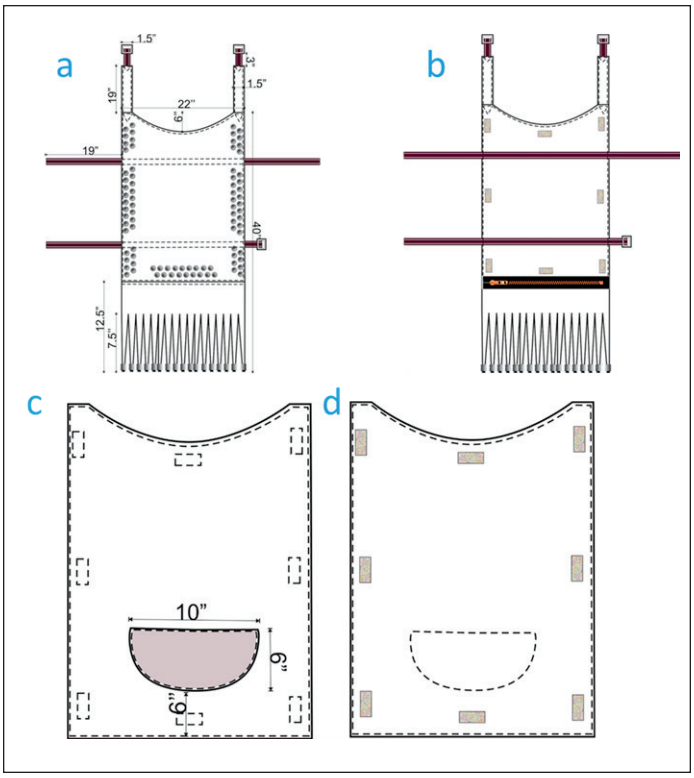

Figure 5. New strategic design for *ankelba* overcoming the key deficiencies. **(a)** Leather body exterior view, **(b)** leather body interior view, **(c)** lining exterior view and **(d)** lining interior view.

reduced number of *zagols* and buttons. Number of *zagols* can be reduced without affecting cultural values. Society did not count the number of *zagols* on *ankelba* but they see the aesthetic values, the sound *zagols* produce during walking and the fit.

Hence, the sound can be achieved by changing the design of *zagol* placement and by reducing the number. Newly designed *ankelba* has 17 straps and 5 *zagols* per strap, which mean the total number of *zagols* used is 85. Number of *zagols* per strap reduced almost by 3 folds and total number of *zagols* per *ankelba* reduced by more than 6 folds. Similarly, number of buttons reduced by half.

Lining made up of cotton and foam inserted into newly designed *ankelba* can easily prevent baby urine passing to leather and enhance the comfort, as seen in Figure 5. Further, it is possible to diminish the bad smell by providing an economically viable waterproof post-tanning and finishing system during the manufacture of sheep garment leather, which can reduce the absorption of urine. Adjustable shoulder straps and waist straps, having 19 inch length and 1.5 inch width, ending with Velcro attachment were selected to prevent problems related to tightness of cultural *ankelba*. The size of the straps was based on the standard measurements of shoulder straps and waist straps and it would help to balance the weight distribution. The actual *ankelba* product produced using modern sheep garment leather with improvised design is shown in Figure 6.

Physical and Bulk properties of Leathers Processed Using Traditional and Modern Techniques

The physical and bulk properties of leathers vary significantly depending on the chemical treatments and processing strategy. Physical properties such as tensile strength, tear strength and percentage elongation and bulk properties such as softness, grain smoothness and general appearance, for example, can be influenced by the choice of tanning agent, re-tanning, fatliquoring and finishing chemicals. Hence, we tested tensile strength, tear strength and percentage elongation of traditionally tanned and modern tanned leathers and the results are shown in Table V. As can be seen, both the tensile and tearing strength of traditionally tanned and modern tanned leathers are not altered significantly. The variations seen in data are mostly due to the nature of leather in which properties change from leather to leather and within different parts of the same leather. Nevertheless, the percentage elongation of modern sheep

Figure 6. Newly designed sheep garment leather based *ankelba* product. **(a)** Exterior view and **(b)** interior view.

Table V

Physical properties of traditionally tanned and modern tanned leathers

Sample	Tear strength (N/mm)	Tensile strength (N/mm^2)	Elongation at break (%)
Traditionally tanned cow leather	177.2	29.0	58.5
Modern sheep garment leather	182.1	29.4	68.0

garment leathers is significantly higher than the traditionally tanned cow leathers. This could be due to the choice of the raw material as well as the modern processing technique.

The bulk properties of the traditionally tanned and modern tanned leathers are shown in Figure 7. Softness and color uniformity of the traditionally tanned leathers are significantly lower compared to the modern tanned leather indicating traditional tanned leathers are hard with uneven coloration. Further, the grain smoothness of the traditional tanned leather is not good, which indicates the leather is rough. Considering all the bulk properties including general appearance, the modern tanned sheep garment leather has better properties in comparison to traditionally tanned cow leathers.

Dry and Wet Rub Color Fastness of Traditionally Tanned and Modern Tanned Leathers

Dyestuffs employed for coloring leathers should not be leached out easily on usage or when the leather is washed. The use of leather products with low color fastness to rubbing is not acceptable especially for casual products. Hence, color fastness of traditionally tanned and modern tanned leathers were analyzed and compared against standards. As seen in Table VI, the dry and wet rubbing fastness is below standard requirement for the traditionally tanned leather, which indicates that the traditionally tanned leather lose its color when it is rubbed.[21] This can cause fading of product in its color

Table VI

Dry and wet rubbing fastness of traditionally tanned and modern tanned leathers

Samples	Rub fastness		Standard requirement[21]	
	Dry	Wet	Dry	Wet
Traditionally tanned cow leather	1	1	3	3
Modern sheep garment leather	5	4/5	3	3

appearance and may also cause damage on other wearable clothes. On the other hand, modern tanned sheep nappa leather has good dry and wet rub fastness comparable to the standard requirements. This is primarily due to the proper processing conditions, which included a step for fixing the dyestuffs with the chromium-collagen fibers present in the leather.

Conclusion

Ethiopian cultural *ankelba* has been made of traditionally processed cow leathers and used for carrying baby. This traditional product has material related problems such as bad smell, heavy weight, un-washability and color fading as well as design related problems such as fixed size, number and placement of *zagol*, neck and strap construction, which resulted in the gradual disappearance of this prestigious product. In this study, the material and design related problems of cultural *ankelba* were overcome to revive the *ankelba* product usage. Traditionally processed cow leathers were replaced by sheep garment leather having less weight and thickness to solve material related problems. New and efficiently designed *ankelba* has lesser number of straps and *zagols*. Number of *zagols* per strap was reduced almost by 3 folds and total number of *zagols* per *ankelba* was reduced by more than 6 folds to make the new design appeal to the user. The adjustable strap provides overall size adjustments in the

Figure 7. Bulk properties of traditional tanned and modern tanned leathers.

newly designed *ankelba* such that it can be used for babies aged from 6 to 24 months. Further, the new design allows for grading, which will help to design and manufacture *ankelba* that can be used for toddlers aged more than 24 months. The results of this study would help to continue the usage of *ankelba* not only in rural areas but also amongst urban society. The strategy proposed in this study can be applied to the revival of any such traditional products around the world for their continued and effective usage.

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Enzymatic Bating Technology for Wet Blue: II. The Basic Properties and Application Effectiveness of Typical Acidic Proteases

by

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Abstract

Most of the reported bating technologies for wet blue are based on the usage of acidic protease, which takes a long time and needs large enzyme dosage. A thorough understanding of the basic characteristics of typical acidic proteases and the interaction between enzyme proteins and wet blue fibers will help to improve bating technology for wet blue by selecting the suitable proteases. In this paper, the enzymatic characteristics, molecular weight (M_r) and isoelectric point (pI) of several proteases and their bating effectiveness were investigated. The results indicated that there are two main factors which may affect the wet blue bating effectiveness of acidic proteases. First, the common acidic proteases exhibited low activity towards chrome-tanned collagen fiber which lead to inefficient bating effect through normal dosage. Nonetheless, when the dosages of chrome-tanned collagen fiber activity reached up to 50 U/mL, these acidic proteases also can achieve a good bating effect, the caseinolytic activity has been reached up to 1000 U/mL-4000 U/mL. Second, because of the large molecular weight and the charge repulsion between enzyme proteins and wet blue fibers, the enzymatic hydrolysis process, the penetration and distribution of acidic protease proteins, into wet blue is very difficult. Additionally, neutral proteases have more prospects in wet blue bating process due to the higher chrome-tanned collagen fiber activity and less charge repulsion than acidic proteases.

Introduction

Currently, to reduce pollution and control tannery beamhouse waste disposal costs, more and more tanneries are tending to purchase wet blue as raw materials. However, purchased wet blue generally has some obvious defects, such as insufficient opening up of fiber structure, vein marks, scars, wrinkles, hardened fibers from long-term transportation and storage, especially, evident quality difference from different tanneries, and so on, which seriously affects the unification of the subsequent processing technology and the performance and use value of the leather.[1-4] Hence, enzymatic bating operation is usually the first stage of the post-tanning process to resolve the problems and improve the quality of purchased wet blue in most tanneries. In conventional enzymatic wet blue bating process, protein fiber structure is further opened, grease and inter-fibrillary substances are removed, defects are alleviated, complimentary, by using enzyme preparations, especially proteases.[5-9] Consequently, wet blue quality difference from different tanneries is reduced and the subsequent processing can be standardized. Hence, the evenness, softness, physical and mechanical properties and organoleptic performances of finished leather are improved.

The main components of most of the commercial enzyme preparations for wet blue bating are acidic proteases. This is restricted by the traditional thinking pattern that post-tanning processing is carried on acidic conditions. It is well accepted that through properly selecting bating enzymes and bating conditions according to wet blue situations and the property requirement to finished leather, the use of acidic protease preparations in the post-tanning process is useful in improving the quality of finished leather.[10-12] However, it takes a long time and needs a large dosage of enzyme to hit the spot of wet blue bating in the traditional acidic enzymatic bating technology, which does not only defect the production efficiency but increases the cost of production. Further, most of the enzymatic bating technologies only involved the bating effectiveness of some specific enzyme preparations to wet blue and the optimization of the using conditions, but rarely mentioned the mechanism of enzymes in wet blue bating process, especially the interaction between enzyme proteins and wet blue fiber substrates. This makes it difficult to select suitable and efficient protease for wet blue bating process.

Proteolytic activity is the main parameter during the application of proteases. Many methods were used to assay the activity of protease in tanneries, and the commonest one is the Folin method by using casein as substrate,[13,14] which is distinctively different

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from the chrome-tanned protein fibers in wet blue. Therefore, the protease selected based on the proteolytic activity by these traditional methods cannot reflect the actual proteolytic ability to chrome-tanned protein fibers. Previously, we have established a quantitative method for characterizing the proteolytic activities towards chrome-tanned elastin and collagen fibers through measuring the produced amount of the unique amino acid Desmosine (DES) and Hydroxyproline (Hypro) in the reaction liquor, respectively.[4] This method can be used as an available tool to correctly select proteases and optimize process parameters for wet blue bating.

Furthermore, wet blue is a porous material woven from chrome-tanned collagen fibers, the enzymatic bating of wet blue is a solid-liquid heterogeneous reaction. The collagen fibers would not be opened up evenly if the proteases cannot penetrate into the inner layers of the wet blue. The mass transfer characters of enzyme proteins would be related to the molecular weight of enzyme protein molecules and charge interactions between the enzyme proteins and hide/leather protein fibers, for both collagen fibers and proteases are amphiprotic substances. Many studies such as fluorescent tracer technology,[15-18] protease protein purification technology[19] and enzymatic hydrolysis properties regulation technology[20] have been conducted to investigate the mass transfer and reaction character of proteases in the leather manufacturing. But the influence role of enzyme molecular weight (M_r) and isoelectric point (pI) on bating effectiveness is still indistinct and should be further investigated.

In this paper, the basic properties of several typical proteases, including molecular weight (M_r), isoelectric point (pI) and hydrolyzing ability to chrome-tanned collagen fiber, were analyzed. The bating effectiveness of these proteases on wet blue was studied comparatively. Based on this we can provide some useful scientific methods and enzyme information to guide the choosing of highly efficient proteases and optimizing process parameters for wet blue bating process.

Materials and Methods

Materials

All protease preparations (Table I) were purchased from the market. Hydroxyproline (Hypro) standard was obtained from MembraPure GmbH (Germany). Shaved cowhide wet blue (1.2 mm) was supplied by Tongtianxing Group Co.Ltd., China. Chrome-tanned collagen fiber powder was prepared by our laboratory from the reticular layer of bovine hide.[4] All the other chemicals used for the analysis were of analytical grade and other chemicals used for leather processing were of commercial grade.

Thermal stability of proteases

Protease preparations were diluted into a certain concentration by 0.1 mol/L of Britton-Robinson buffer (B-R buffer, pH 3.5 for acidic proteases and pH 6.5 for neutral protease) and incubated at 40° for 12 h. The caseinolytic activity was determined according to the modified Folin method[13,14] at intervals of 2 h at pH 3.5 or pH 6.5 and 40°.

Assay of proteolytic activity on casein substrate

The caseinolytic activity was determined by the Folin method under certain conditions. First, the proteolysis was performed by incubating 1mL of diluted enzyme solution with 1 mL of 1 % (m/v) casein in 0.1 mol/L B-R buffer (pH 3.5 for acidic proteases and pH 6.5 for neutral proteases) at 40° for 10 min. Then, the reaction was quenched by adding 2 mL of trichloroacetic acid (0.4 mol/L) and allowed to centrifuged at 3500 r/min for 10 min. Finally, 1 mL of the supernatant was transferred into a 15 mL test tube and reacted with 5 mL of Na_2CO_3 solution (0.4 mol/L) and 1 mL of Folin-Phenol reagent at 40° for 20 min. After the reaction, the absorbance of the reaction mixture was measured at 680 nm to determine the amount of tyrosine released during the proteolysis. One unit of caseinolytic activity is defined as the amount of enzyme capable of digesting the casein substrate to produce 1 μg of tyrosine in 1 min under certain conditions.

Table I

Selected wet blue bating protease preparations

Proteases	Characterization	Company
YNU-A	Acidic bacteria protease	Qactive Bio-technology Co. Ltd.
LKT-A	Acidic bacteria protease	Longda Bio-products Co. Ltd.
ABG	Acidic bacteria protease	Novozymes Investment Co. Ltd.
TP	Acidic bacteria protease	Denykem Co. Ltd.
EW01	Complex neutral bacteria protease	Longda Bio-products Co. Ltd.

Assay of proteolytic activity on chrome-tanned collagen fiber substrate

Chrome-tanned collagen fiber activity was determined according to the established method by our laboratory, the chrome-tanned collagen fiber with 2.11% of chrome content was chosen to be the substrate.[4] The amount of Hypro in the digested reaction liquor was tested to represent the performance of proteases hydrolyzing chrome-tanned collagen fiber. In detail, 100 mg ± 1 mg of chrome-tanned collagen fiber was accurately weighed in a test tube, followed by adding 5 mL of B-R buffer (0.1 mol/L) and stirred in an incubator for 10 min. Then, 1 mL of enzyme solution was added and stirred for another 4 h at 150 r/min, then the concentration of Hypro in the digested reaction liquor was tested. One unit of chrome-tanned collagen fiber activity is defined as the amount of enzyme capable of digesting the chrome-tanned collagen fiber substrate to produce 1 μg of Hypro in 1 hour under certain conditions.

Determination the molecular weight and isoelectric point of acidic proteases

The molecular weight (M_r) and isoelectric point (pI) of these proteases were tested through SDS-PAGE and IEF-PAGE method, respectively, and stained by Coomassie Brilliant Blue R-250.[21]

Bating wet blue with typical protease preparations

Shaved cowhide wet blue (1.2 mm) from a supplier in China was chosen as the raw materials for this study. Pieces of wet blue (50 cm ×50 cm) were symmetrically taken along the backbone in a piece of shaved cowhide wet blue. Samples were wetted, bleached and adjusted the pH to 3.5 (for acidic proteases) or 6.5 (for neutral protease) by using sodium bicarbonate solution (1:10, w/v). Samples were treated by different protease preparations with a certain dosage of proteolytic activity at 40°, pH 3.5 or 6.5, run for 4 h then left overnight. After bating, the concentration of soluble protein and Hypro in the reaction liquors were tested according to the steps described in the following measuring method section. The opening-up of collagen fiber was observed with an optical microscope after staining per the Weigert-Van Gieson method. Then, samples were neutralized, retanned, fatliquored, squeeze-spread, toggle-dried and milled as per the same standard post-tanning procedures. The softness and main physical properties of the crust leathers from the adjacent and symmetrical parts of the same wet blue were evaluated. The organoleptic properties of these crust leathers were evaluated by 10 professional skilled tanners.

Determination of the concentration of soluble protein in the reaction liquor

After the end of the reaction, the concentration of soluble protein was determined according to the modified Lowry method.[22] First, the reaction liquor was filtered with a qualitative filter paper and diluted into a certain concentration by ultrapure water. 1 mL of filtrate was mixed with 5 mL of Folin-phenol reagent-A at room temperature for 10 min, then 0.5 mL of Folin-phenol reagent-B was added and incubated at 30° for 30 min. After the reaction, the absorbance of the mixture was measured at 660 nm to determine the amount of soluble protein.

Determination of the concentration of Hypro in the reaction liquor

After the end of the reaction, the reaction liquor was filtered with a qualitative filter paper and 2 mL of filtrate was mixed with 2 mL of concentrated hydrochloric acid (12 mol/L) in a 10 mL COD digestion tube (HACH, America). The mixture was hydrolyzed at 120° for 12 h and 2 mL of EDTA-Na_2 (20 g/L) solution was added to avoid the interference of chromium.[23] Then the acid hydrolysate was evaporated to dryness with a Vacuum Concentrator (TC-8F, TAITEC, Japan). A certain amount of amino acid analysis sample dilution buffer was added to dissolve the dry sample. The dissolved liquor was filtered with an aqueous filter head (D=0.22 um). The concentration of Hypro was tested with an Amino Acid Analyzer (A300, MembraPure GmbH, Germany).

Histological analysis of collagen fiber

Samples of 1 cm^2 were cut from identical official sampling portions of the corresponding bated wet blue. Sections of 12 μm thicknesses were obtained using CM1950 freezing microtome (Leica, Germany) and stained with the Weigert-Van Gieson staining method. The opening-up of collagen fiber was observed with an optical microscope (CX41, Olympus, Japan).

Test of softness and physical properties of crust leather

Dried crust leather samples of each enzyme bating group were taken out in the adjacent and symmetrical parts of the same leather for testing softness and physical properties. The softness of the crust leather was determined with a Leather Softness Tester (GT-303, Gotech Testing Machines Inc., China). The physical properties such as tensile strength, elongation at break, tear strength and bursting strength were examined as per the standard procedures.[24]

Table II

Enzymatic characteristics of proteases

Protease (40°)	Caseinolytic activity (F, U/g)	Chrome-tanned collagen fiber activity (H_{Cr}, U/g)	H_{Cr}/F
ABG (pH 3.5)	4426	18	0.004
LKT-A (pH 3.5)	2091506	25758	0.012
TP (pH 3.5)	694716	10745	0.015
YNU-A (pH 3.5)	49605	2064	0.042
EW01 (pH 6.5)	6768	3749	0.554

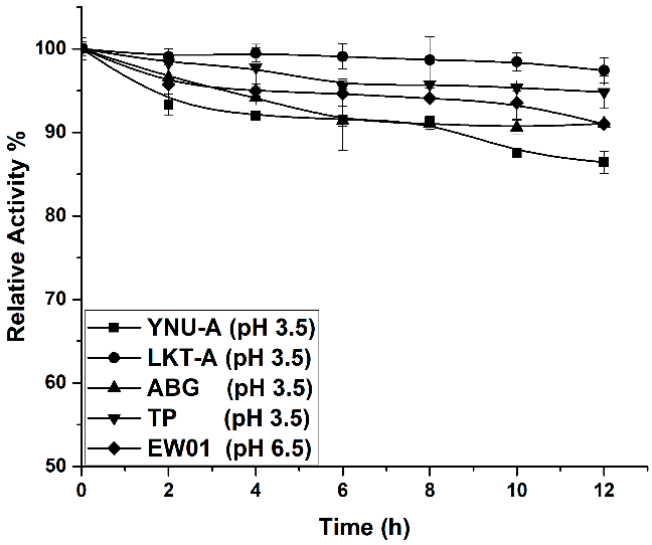

Figure 1. Thermal stability of proteases

Results

Characteristics of typical proteases

Caseinolytic and chrome-tanned collagen fiber activities of proteases

Wet blue bating is usually conducted on the pH is around 3.5 to improve the opening up of chrome-tanned collagen fibers by using acidic proteases. To evaluate the viabilities of the typical acidic proteases, the caseinolytic and chrome-tanned collagen fiber activities of several protease preparations were assayed at pH 3.5 (for acidic proteases) or pH 6.5 (for neutral proteases) and 40°. The results in Table II shows that the proteolytic activities determined by different methods were found to be significantly different. All of these acidic proteases exhibit much higher activity to casein substrate than that of chrome-tanned collagen fiber substrate. The relative activity of the acidic protease defined as the chrome-tanned collagen fiber activity to 1 unit of casein hydrolysis activity (H_{Cr}/F)[4] is at a very low level. As a comparison, the activity of a complex neutral protease EW01 was also evaluated. The results indicate that EW01 exhibit significantly higher H_{Cr}/F than all of the acidic proteases.

The thermal stability of proteases

As mentioned above, it usually takes a long time to bate the wet blue with proteases. Therefore, the thermal stability of the proteases was measured at the conditions of pH 3.5 for acidic protease (pH 6.5 for neutral protease) and 40°. The results in Fig. 1 illustrated that protease LKT-A has the most stable activity for there was almost no activity lost after incubation at 40° for 12 hours. The thermal stability of protease YNU-A is relatively weaker than the other proteases, its activity kept dropping for 12 hours. The activities of proteases ABG, TP and EW01 tended to be stable after incubation for 12 hours.

Analyzation of the M_r and pI of acidic proteases

As mentioned above, wet blue is a porous material woven from chrome-tanned collagen fibers and the collagen fibers would not be opened up evenly if the proteases cannot penetrate into the inner layers of the wet blue. The mass transfer characteristics of enzyme proteins would be related to the molecular weight of the enzyme molecules and charge interactions between the enzyme proteins and leather protein fibers. To further analyze the characters of acidic proteases in wet blue bating process, the molecular information, such as molecular weight (M_r) and isoelectric point (pI), of these acidic proteases were investigated through SDS-PAGE and IEF-PAGE methods, respectively. Fig. 2A shows that all of these acidic proteases contain a variety of protein molecules, the M_r of the major components is approximately 45 kDa. Fig. 2B shows that all of these acidic proteases contain at least two different electrophoretic bands,

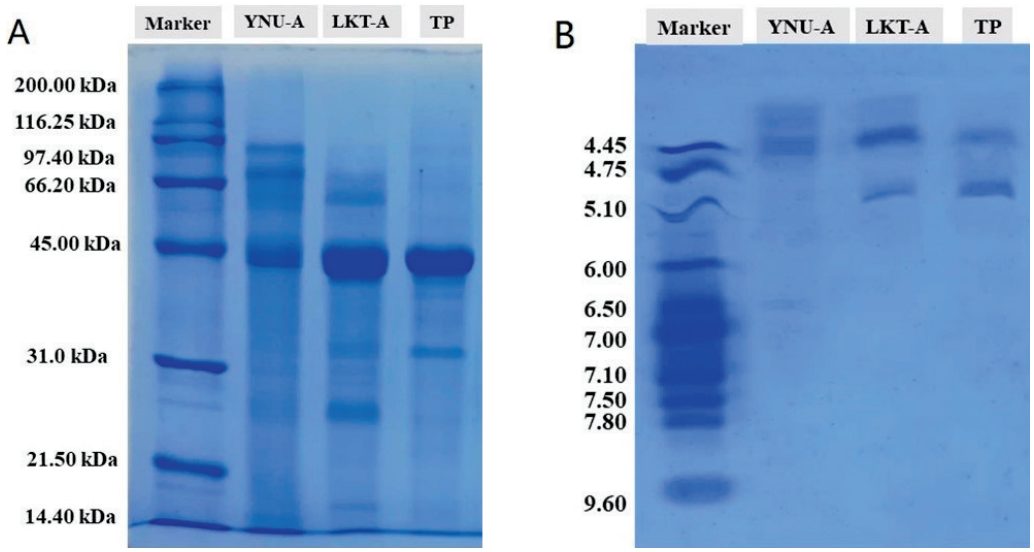

Figure 2. SDS-PAGE **(A)** and IEF-PAGE **(B)** electrophoresis diagrams of acidic proteases

and all of the pI values are higher than 4.0, even higher than 4.5, which is approximately 4.45-5.10. Hence, at acidic wet blue bating conditions (the pH is approximately 3.5), acidic proteases and wet blue protein fibers (pI is approximately 6.5-7.5[25-28]) are all carrying in large amounts of positive charge. Additionally, the M_r and pI values of commercial complex neutral protease EW01 is different than all of the acidic proteases, which is approximately 25 kDa-35 kDa and 7.5-8.5, respectively. It is supposed that the charge interaction between enzymes and collagen fibers may have some effect on the penetration, distribution and reaction of proteases and thus influence the bating process, that like charges repel each other but opposite charges attract.

Effects of activities of acidic proteases on wet blue bating

Bating effectiveness of wet blue with same caseinolytic activity concentration

Typical acidic protease preparations YNU-A, LKT-A, ABG and TP were chosen for bating wet blue as the following conditions: 40°, pH 3.5, run for 4 h then left overnight. The dosages of the acidic proteases were the same based on the caseinolytic activity (cF) was 90 U/mL reaction liquor, which is equal to the normal dosage of commercial acidic protease preparations. Table III shows that the concentration of chrome-tanned collagen fiber activity (cH_{Cr}) in the bating liquor for each protease was significantly different. After bating, the concentrations of soluble protein (SP) and hydroxyproline (Hyp) in the bating liquors were measured. Although some differences of the produced amounts of SP and Hyp have existed between different enzymes, the total amount of SP and Hyp is small. With the same caseinolytic activity concentration, the content of soluble protein hydrolyzed by 1 unit of caseinolytic activity (SP/cF) is approximately 4.5 μg; the content of hydroxyproline hydrolyzed by 1 unit of chrome-tanned collagen fiber activity (Hyp/cH_{Cr}) is approximately 1.7 μg.

Table III

Amounts of proteases, soluble protein and hydroxyproline in wet blue bating liquor treated by same dosage of caseinolytic activity

Proteases	cF*1 (U/mL)	cH_{Cr}*2 (U/mL)	SP*3 (μg/mL)	Hyp*4 (μg/mL)	SP/cF* (μg/U)	Hyp/cH_{Cr}*6 (μg/U)
YNU-A	90.0	3.75	454	3.72	5.03	0.99
LKT-A	90.0	1.11	356	1.86	3.94	1.67
ABG	90.0	0.36	463	1.21	5.13	3.37
TP	90.0	1.40	363	2.17	4.02	1.56

*1: cF represents the concentration of caseinolytic activity in the bating liquor.

*2: cH_{Cr} represents the concentration of chrome-tanned collagen fiber activity in the bating liquor.

*3: SP represents the concentration of produced soluble protein in the bating liquor.

*4: Hyp: represents the concentration of produced hydroxyproline in the bating liquor.

*5: SP/cF represents the amount of soluble protein hydrolyzed by 1 unit of caseinolytic activity.

*6: Hyp/cH_{Cr} presents the amount of hydroxyproline hydrolyzed by 1 unit of chrome-tanned collagen fiber activity.

The opening-up of the collagen fiber in the vertical-section of bated wet blue was observed through Weigert-Van Gieson staining method. Fig. 3 shows that after treatment with these acidic protease preparations, the collagen fibers have a higher degree of opening-up than the control. Compared with the analysis data in Table III, it is obvious that the higher the chrome-tanned collagen fiber activity, the larger the concentration of hydroxyproline produced in the reaction liquor and, further, the higher the degree of collagen fibers opening-up.

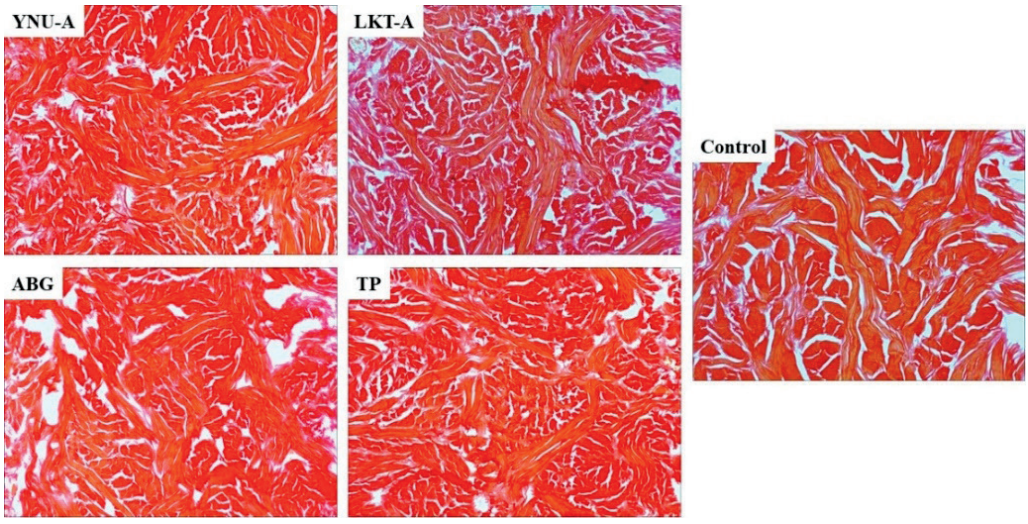

Figure 3. Staining results of collagen fiber in wet blue treated by same dosage of caseinolytic activity (100× whole ver. sec.)

Table IV

Softness and physical properties of crust leather treated by same dosage of caseinolytic activity

Proteases	YNU-A	LKT-A	ABG	TP	Control
Softness (mm)	8.57	8.65	7.43	7.70	7.43
Tear strength (N/mm)	143	139	149	143	145
Tensile strength (N/mm^2)	75	85	81	84	86
Elongation at break (%)	122	121	105	114	129
Bursting strength (N/mm)	443	479	496	486	458
Bursting height (mm)	11.8	12.3	11.2	11.7	12.3

The softness and main physical properties of the crust leathers from the adjacent and symmetrical parts of a same wet blue were evaluated. Table IV shows that acidic proteases YNU-A and LKT-A gives the crust leather the highest softness, but ABG and TP enzyme scarcely improving the softness of the crust leathers. Besides, the tear strength, tensile strength, bursting strength and elongation at break of these protease preparations treated wet blue are higher or comparable to the control.

Further, the shaved wet blue bated with different protease preparations were neutralized, retanned, fatliquored, squeeze-spread, toggle-dried and milled as per the same standard procedures. The organoleptic properties of these crust leathers were evaluated by 10 professional skilled tanners. They thought that the crust leather treated by YUN-A and LKT-A enzyme is a little better than ABG and TP enzyme treated in the respect of softness, hand feeling, and so on. However, the whole quality of crust leathers treated by these acidic proteases has no obvious improvement over the control.

Bating effectiveness of wet blue with same chrome-tanned collagen fiber activity concentration

For improving the bating effect of acidic proteases, a larger dosage of acidic proteases is needed, in other words, the concentration of chrome-tanned collagen fiber activity in the bating liquor should be increased. YNU-A, LKT-A and TP enzyme were chosen for bating wet blue as the following conditions with larger enzyme dosages: 40°, pH 3.5, run for 4 h then left overnight. The dosages of the acidic proteases were the same based on the chrome-tanned collagen fiber activity (cH_{Cr}) was 50 U/mL reaction liquor, which is based on the experiences of wet blue bating technology in our laboratory. Also, the produced amounts of soluble protein and Hyp in the bating liquors were measured.

Table V

Amounts of proteases, soluble protein and hydroxyproline in wet blue bating liquor treated by same dosage of chrome-tanned collagen fiber activity

Proteases	cF (U/mL)	cH_{Cr} (U/mL)	SP (μg/mL)	Hyp (μg/mL)	SP/cF (μg/U)	Hyp/cH_{Cr} (μg/U)
YNU-A	1171	50	5690	23.33	4.86	0.47
LKT-A	4044	50	9307	34.56	2.30	0.69
TP	3242	50	6861	29.63	2.12	0.59

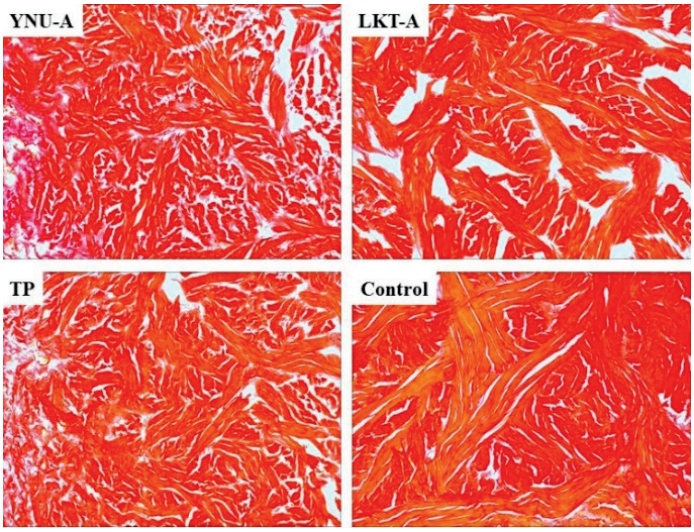

Figure 4. Staining results of collagen fiber in wet blue treated by same dosage of chrome-tanned collagen fiber activity (100× whole ver. sec.)

Table V shows that with same chrome-tanned collagen fiber activity concentration, the content of soluble protein hydrolyzed by 1 unit of caseinolytic activity is approximately 3.1 μg; the content of hydroxyproline hydrolyzed by 1 unit of chrome-tanned collagen fiber activity is approximately 0.58 μg. Compared with Table III, the dosages of the acidic protease preparations in the bating liquors are approximately 13-45 times higher, the produced amount of soluble protein is approximately 13-26 times higher and the produced amount of hydroxyproline is approximately 6-19 times higher than above bating operation.

Fig. 4 shows that all of these protease preparations opening-up most of the collagen fibers in the wet blue. Meanwhile, the opening-up degree of collagen fiber is higher than the results shown in Fig. 3.

Table VI

Softness and physical properties of crust leather treated by same dosages of chrome-tanned collagen fiber activity

Proteases	YNU-A	LKT-A	TP	Control
Softness (mm)	8.38	8.40	8.37	7.74
Tear strength (N/mm)	145	128	167	162
Tensile strength (N/mm^2)	56	52	60	49
Elongation at break (%)	133	124	103	117
Bursting strength (N/mm)	421	457	462	454
Bursting height (mm)	12.5	13.4	12.1	12.2

Table VI shows that all of these protease preparations give the crust leathers better softness than control. Besides, the tear strength, tensile strength, bursting strength and elongation at break of these proteases treated wet blue are higher or comparable than control. Also, all of these wet blues were carried out the same standard post-tanning procedures, and the organoleptic properties of these crust leathers were evaluated. The result shows that the crust leather treated by these acidic proteases is better than control. Besides, the organoleptic properties and the whole quality of the finished leathers are significantly better than above-bated crust leathers.

In summary, with larger acidic proteases dosage in wet blue bating liquors can significantly improve the softness, organoleptic properties and the whole quality of the finished leather without affecting the physical properties. However, the dosage of acidic protease preparations is 13-45 times higher than normal dosage, as a result, the costs of the post-tanning process increased a lot, which is unacceptable by the tanneries.

Comparison of the bating effectiveness of acidic and neutral proteases

As mentioned above, caused by low chrome-tanned collagen fiber activity and large charge repulsion between enzyme proteins and wet blue fiber substrates at acidic bating conditions, the enzymatic hydrolysis process and the penetration of acidic protease proteins in wet blue are difficult. All of this makes an inefficient bating effect of acidic proteases even under a large dosage. Whereas, neutral protease EW01 have higher chrome-tanned collagen fiber activity than all of the acidic proteases. In addition, wet blue protein fibers are close to electric neutrality at neutral conditions, which means that less charge repulsion exists between neutral proteases and wet blue fiber substrates. For comparing the wet blue bating effectiveness of neutral and acidic proteases, neutral protease EW01 and acidic protease YNU-A were chosen for bating wet blue as the following conditions: 40° and pH 6.5 or pH 3.5, run for 4 h then left overnight. The dosages were the same based on the chrome-tanned collagen fiber activity was 50 U/mL reaction liquor. The produced amount of soluble protein and Hyp in the bating liquors were measured after bated, the softness and main physical properties of the crust leathers from the adjacent and symmetrical parts were also evaluated.

Table VII shows that with same concentration of chrome-tanned collagen fiber activity, the caseinolytic activity of acidic protease YNU-A is 13 times higher than neutral protease EW01. However, the produced amount of soluble protein and Hyp by EW01 enzyme was 1.1 and 2.9 times higher than YNU-A, respectively. Besides, the content of soluble protein hydrolyzed by 1 unit of caseinolytic activity of YNU-A and EW01 enzyme is 4.7 μg and 67.8 μg, respectively; the content of hydroxyproline hydrolyzed by 1 unit of chrome-tanned collagen fiber activity of YNU-A and EW01 enzyme is 0.46 μg and 1.33 μg, respectively.

Table VII

Amounts of proteases, soluble protein and hydroxyproline in wet blue bating liquor

Proteases	cF (U/mL)	cH_{Cr} (U/mL)	SP (μg/mL)	Hyp (μg/mL)	SP/cF (μg/U)	Hyp/cH_{Cr} (μg/U)
YNU-A	1171	50	5533	23.08	4.73	0.46
EW01	90	50	6117	66.60	67.78	1.33

Table VIII

Softness and physical properties of crust leather

Proteases	Softness (mm)	Tear strength (N/mm)	Tensile strength (N/mm^2)	Elongation at break (%)	Bursting strength (N/mm)	Bursting height (mm)
YNU-A	8.20	154	56	143	424	12.5
EW01	8.56	151	50	117	483	12.8

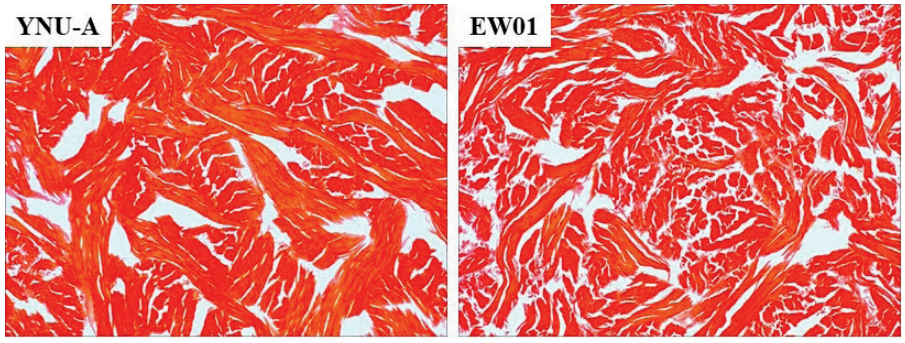

Figure 5. Staining results of collagen fiber in wet blue treated by acidic (YNU-A) or neutral (EW01) protease (100× whole ver. sec.)

Table VIII shows that the softness of EW01 enzyme treated crust leather is better than that of the YNU-A enzyme. The tear strength, tensile strength, bursting strength and elongation at break is comparable than that of the YNU-A enzyme treated crust leather. Furthermore, Fig. 5 shows that EW01 enzyme opening-up more collagen fibers than YNU-A enzyme, which makes the softness of the finished leather better. Additionally, all of the professional skilled tanners also thought that the whole quality of the finished leather treated by neutral protease EW01 is better than that of acidic protease YNU-A.

Discussion

Generally, the use of acidic proteases in wet blue bating processing required a long time and large enzyme dosage. To find the causes and solve this problem, studies have been conducted by us for a long time. Firstly, an accurate method for the quantitative characterization of proteases activity towards chrome-tanned elastin and collagen fibers by determining the unique amino acid, namely desmosine and hydroxyproline, in the reaction liquors was successfully established.[4] Our research found that acidic proteases almost have no effect on chrome-tanned elastin fiber, therefore, the proteolysis abilities of acidic proteases against chrome-tanned collagen fiber may have large influences on the bating effectiveness of wet blue. Hence, a thorough understanding of the basic characteristics of acidic proteases in wet blue bating process is necessary, especially the interaction mechanism between protease proteins and chrome-tanned collagen fiber substrates.

Previously, we proved that well-tanned protein fibers exhibit highly protease-resistance ability,[4] therefore, the enzymatic wet blue bating process is usually sustained over long periods. Fig. 1 shows that all of the selected typical proteases have excellent thermal stability under 40° and pH 3.5 or pH 6.5, which is preliminarily adapted to the wet blue bating conditions. Then, the enzymatic characteristics of these proteases towards casein and chrome-tanned collagen fiber substrates were investigated. The results in Table II shows that acidic proteases exhibit rather higher activity to casein substrate than chrome-tanned collagen fiber substrate. The chrome-tanned collagen fiber activity per 1 unit of caseinolytic activity is approximately 0.018 U, however, it is extremely lower than neutral protease EW01, which is 0.554 U.

One of the main factors affecting the acceptability of a wet blue bating enzyme preparation by tannery is whether it can improve the softness, the whole quality and keep the original physical properties of the finished leather. The highly cross-linked collagen fiber structure can be further opened up by using proteases, thus the inter-fibrillary substances hidden in collagen fiber bundles can be removed effectively. The bating effectiveness of proteases on wet blue was positively related to their chrome-tanned collagen fiber activity, proteases with highly chrome-tanned collagen fiber activity could significantly improve the softness of crust leather. However, excessive proteolysis of collagen structure is harmful to the leather matrix and significantly affects the properties of the final leather product. Nevertheless, the proteolysis degree of collagen fiber at mentioned enzyme dosage and wet blue bating conditions have a negligible effect on the mechanical properties

but a positive effect on the softness of the crust leather, as shown in Table IV, Table VI and Table VIII. Therefore, the deficiency of acidic proteases in the wet blue bating process is most probably related to its insufficient to chrome-tanned collagen fiber. Table III, Table IV and Fig. 3 shows that the bating effectiveness and fiber opening-up degree of the crust leathers treated by acidic proteases are not obvious under the dosage of caseinolytic activity is 90 U/mL. Although, acidic proteases can obviously improve the softness, fiber opening-up degree and hand feelings of the crust leather under the caseinolytic activity is 1000 U/mL-4000 U/mL, the chrome-tanned collagen fiber activity is 50 U/mL, (Table V, Table VI and Fig. 4), but the cost of the post-tanning process increased a lot as well due to the large dosage of enzymes, which is unacceptable by the tannery.

Apart from the enzymatic characteristics of acidic proteases, Fig. 2(A) shows that the M_r of acidic proteases is approximately 45 kDa, which is much larger than the reported commercial leather making neutral and alkaline proteases.[19,29] The molecular weight of the main component of EW01 enzyme is approximately 25 kDa-35 kDa. Therefore, it can be speculated that the penetration and distribution of acidic protease molecules into wet blue are harder than neutral and alkaline proteases. Furthermore, as mentioned above, at normal acidic wet blue bating conditions (the pH is approximately 3.5), acidic protease molecules (pI is approximately 4.45-5.10) and wet blue protein fibers (pI is approximately 6.5-7.0) are all carrying a large amount of positive charge. Caused by large charge repulsion between protease proteins and wet blue fibers, the penetration and distribution of acidic protease molecules in wet blue are difficult. Hence, the bating effect of acidic proteases is insufficient even at large enzyme dosage. Additionally, although acidic proteases may have less charge repulsions at pH 4.5-5.0, the caseinolytic and chrome-tanned collagenolytic activity of acidic proteases is extremely low, which is also unacceptable.

As mentioned above, the isoelectric point of chrome-tanned collagen fibers in wet blue is approximately 6.5-7.5,[25-28] which is close to electric neutrality at neutral wet blue bating conditions. The isoelectric point of the main component of EW01 enzyme is approximately 7.5-8.5, which is carrying a trace amount of positive charge at neutral condition. Considering less charge repulsion exists between enzyme proteins and wet blue fiber substrates, it can be speculated that the penetration and distribution of neutral proteases may be easier than acidic proteases at neutral wet blue bating conditions.

Finally, the bating effectiveness of neutral protease EW01 was investigated and compared with acidic protease YNU-A. Table VII shows that the chrome-tanned collagen fiber activity per 1 unit of caseinolytic activity of the EW01 enzyme (0.556 U) is 13 times higher than the YNU-A enzyme (0.042 U). The produced amount of hydroxyproline per 1 unit of chrome-tanned collagen fiber activity by EW01 (1.33 μg) is 2.9 times higher than YNU-A (0.46 μg). Moreover, the results in Table VIII and Fig. 5 shows that the wet blue bating effectiveness of neutral protease EW01 is better than that of acidic protease YNU-A. These results suggest that neutral proteases may have more prospects in wet blue bating process.

Conclusions

Based on the investigation of the thermal stability, enzymatic characteristics and molecular information of several proteases, the bating effectiveness of acidic and neutral proteases on wet blue was studied comparatively. The mechanism of enzyme penetration and distribution in wet blue was also preliminarily investigated. The results showed that there are two main factors may affect the wet blue bating effectiveness of acidic proteases. First, the common acidic proteases exhibited low activity towards chrome-tanned collagen fiber leads to inefficient bating effect through normal dosage. Second, caused by large molecular weight and charge repulsion between enzyme proteins and wet blue fibers, the enzymatic hydrolysis process, the penetration and distribution of acidic protease proteins into wet blue are very difficult. Additionally, the results suggested that neutral proteases have more prospects in wet blue bating process due to the higher chrome-tanned collagen fiber activity and less charge repulsion than acidic proteases. The results can provide some useful scientific methods and enzyme information to guide the choosing of high-efficient proteases and optimizing process parameters for wet blue bating process.

Of course, the mechanism of enzyme in acidic and neutral wet blue bating conditions should be further investigated. As mentioned above, the enzymatic hydrolysis process, the penetration and distribution of acidic protease molecules into wet blue are harder than neutral protease molecules. Therefore, correctly application of neutral proteases, even alkaline proteases, should be detailly studied. We speculated that these problems can be solved by investigating the mass transfer and reaction mechanism of enzyme proteins in wet blue bating process, and this work is undertaken.

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Development of Sustainable Strategies for Leather Waste Management through Bacterial Remediation

by

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Abstract

A new *Bacillus* species has been isolated and used for treating chrome leather wastes. The activity of *Bacillus* species is evaluated for the degradability of the Chrome Leather Waste (CLW). An initial CLW substrate concentration at 0.5 and 1%, along with the bacterial strain has been studied against the control sample without bacterial strain. The higher proteolytic enzyme production and hydroxyproline release in the CLW containing medium confirms the degradation process, whereas it is significantly less in control samples. The degradation profile of CLW shows higher in 1% CLW as compared to 0.5% CLW. In 1% CLW, the protease activity of the isolated strain has been increased from 1.615 to 5.625 U/mL. In addition to protease activity, the isolated strain also expressed chromate reductase activity. Furthermore, FTIR, TGA, and SEM studies confirm the degradation of leather wastes.

Introduction

In the leather manufacture, tanning plays a major role in converting hides or skins into leather, which makes the leather more resistant to microbial attack and increases its thermal stability properties.[1-2] Among the different methods of tanning, chrome tanning is commonly used in leather manufacture as it produces leathers with high temperature tolerance and excellent washable capability.[3-7] Chrome tanning employs the trivalent heavy metal salt chromium, which generates wastes as chrome shaving, splitting, and trimming waste resulting in heavy metal pollution in the environment. The traditional way of Chrome Leather Waste (CLW) disposal is landfilling.

Biodegradation of chrome tanned leathers is of much interest to the leather fraternity. Biological degradation involves two aspects, one is remediation of chromium, and another is nutrient source utilization for bacterial growth, which offers a sustainable approach for biodegradation of CLW. Microbial management of industrial wastes for the removal of hazardous compounds can be achieved through bio-leaching, bio-mineralization, bio-accumulation, enzyme-catalyzed redox reactions, biosorption, or bioconversion to less toxic forms.[8-9] Microbial degradation is, therefore, the best alternative for processing waste materials containing heavy metal, which facilitates the recycling of CLW in an eco-benign manner. A general mechanism of chromium absorption internally occurs through the membrane sulfate transport channel present in the cells, and it spontaneously reacts with intracellular reductants to generate unstable intermediate products.[10-11] This mode of action of the bioremediation process occurs in chrome tolerant bacterial species.[12-13] Among different class of organisms bacteria is found to have a high degrading ability.[14]

In the present study, a new chromium tolerant bacterium is isolated from the tannery effluent which is used for the degradation of CLW.

Experimental

Characterization of bacterial species

Effluent from pilot tannery was used for the bacterial isolation and screened using chrome tolerance assay, most responsive strain was chosen and identified by rDNA sequencing. The resulted sequence was deposited to GenBank, NCBI and obtained the accession number, MG995009.

Evaluation of bacterial strain on chrome leather wastes

Chrome leather waste obtained from the tannery was used as a substrate for the biodegradation study. The microbial degradation of CLW was studied with the isolated bacterial culture (10^7 CFU/mL) incubated in the liquid medium for 15 days at 37°C. Samples collected every 5 days were subjected to protease assay and hydroxyproline assay.[15-16] The efficiency of Cr (VI) conversion was assessed based on the colorimetric assay, diphenyl carbazide method.[17]

ATR-FTIR of the chrome shavings

Vibrational spectroscopic study was carried out for the control and experimental CLW samples using ATR-FTIR spectrophotometer (JASCO 4200).

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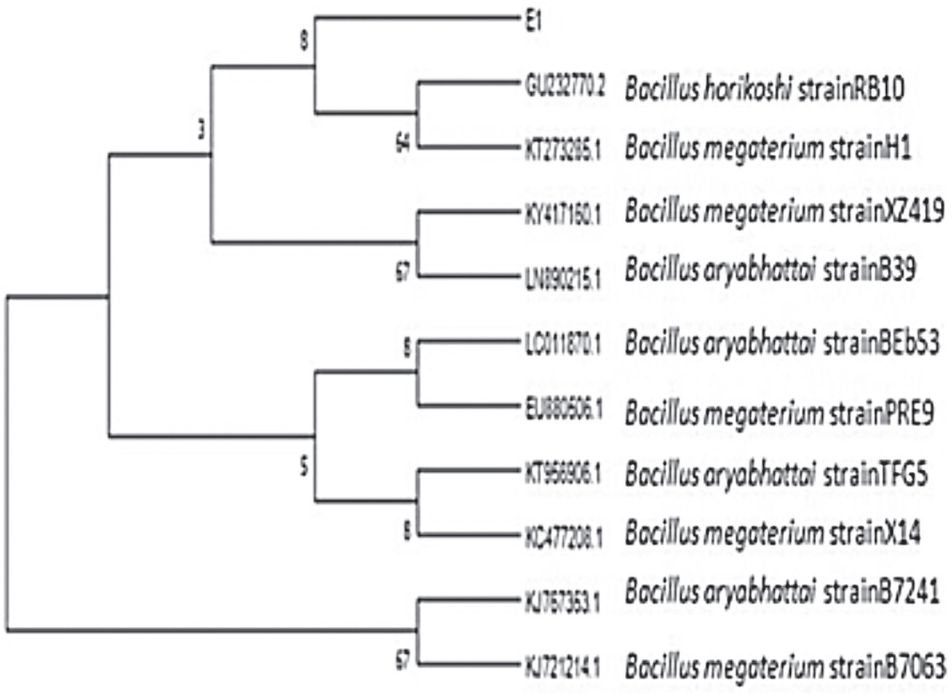

Figure 1. Phylogenetic tree of strain MG995009

TGA of the chrome shavings

TGA study was performed for control and experimental CLW using Q50 thermogravimetric analyzer in a N_2 atmosphere with a heating range from 25° to 800°C at 10°C/min.

Microscope Evaluation

Samples of 5 × 2mm sizes were cut and mounted on aluminum stubs. Then coated with gold using an Edwards E-306 sputter coater and introduced into the chamber of PHENOM ProX Scanning Electron Microscope. This was done to understand the structural morphology of the control and *Bacillus aryabhattai* (MG995009) treated CLW.

Results and Discussion

The present research unravels the biodegradation efficiency of the newly isolated bacterial species *Bacillus aryabhattai* (MG995009) on CLW which possess both hydrolytic and chrome reductase activities (Figure 1)

The newly isolated strain sequence was submitted in NCBI and obtained the accession number, MG995009. Based on the phylogenetic information, the newly isolated strain belongs to the *Bacillus* genus which is commonly present in the skins and hides.

The isolated bacterial strain was tested for its proteolytic activity to understand the biodegradation profile of CLW, and the results are given in Table I. It is observed that 0.5% of the substrate showed 3.12 U/mL, and 1% of the substrate showed 5.63 U/mL on 15th day. At higher substrate concentration, the proteolytic activity has found to be a little higher, which ascertains the effective utilization of substrate by the new bacterial isolate. From the results, it can be ascertained that 1% of CLW was chosen to understand the biodegradation in further studies.

Table I

Release of protease during the degradation process of CLW

Time intervals (Days)	Protease activity (U/mL)		
	Control	0.5%	1%
0	1.25±0.002	1.27±0.002	1.65±0.003
5	2.45±0.004	2.86±0.005	3.11±0.006
10	2.48±0.004	2.85±0.005	3.96±0.007
15	2.58±0.005	3.12±0.006	5.63±0.011

Hydroxyproline release is quantified, to understand the biodegradability of leather, and the results are given in Table II. Hydroxyproline in 1% CLW liquor increased from 18 to 84 µg/mL during the degradation period of 0 to 15 days. From the results, it can be inferred that *Bacillus sp.* utilized the CLW, which ensures chrome tolerance ability of the newly isolated species.

Table II

Release of hydroxyproline during the degradation process of CLW

	Hydroxyproline release (µg/mL)
Days	**1%**
0	18±0.036
5	33±0.040
10	40±0.080
15	84±0.126

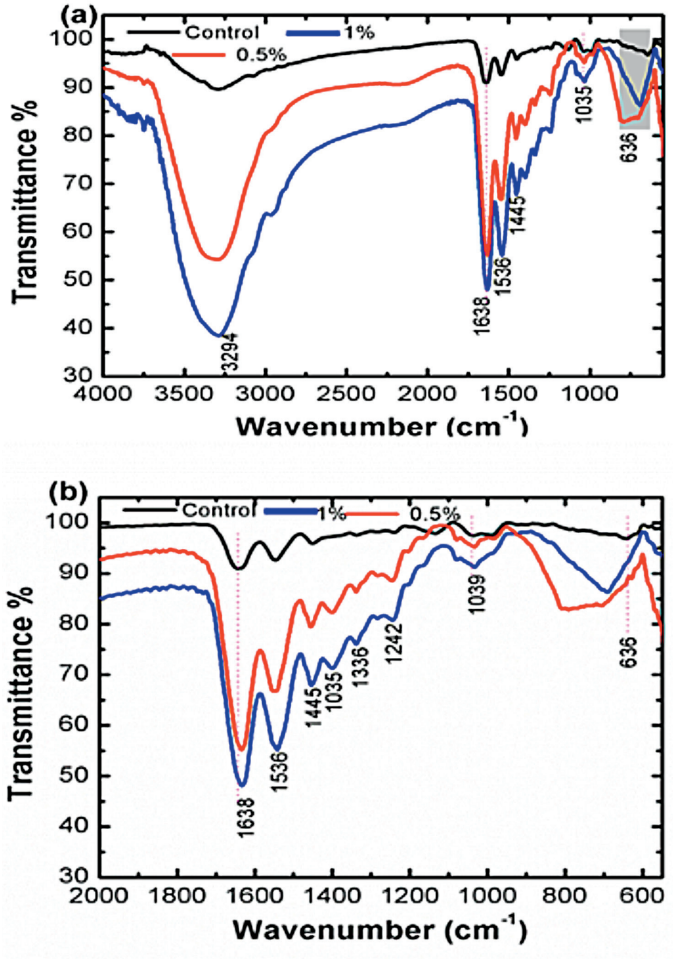

Figure 2. (a) FT-IR spectra for treated and untreated samples, **(b)** Magnified image of changes in wave numbers of treated and untreated samples

Chrome tolerant bacterial strain *Bacillus aryabhattai* isolated in this study have been evaluated for its chromate reductase activity, which would help in reducing toxic chrome species.[18] Isolated bacterial strain reduced the 100 µM Cr (VI) to 64.88 µM Cr (VI) within 20 min at the volume of 250 µg of the crude enzyme. However, at a lower concentration of 50 µg crude enzyme, the reduction is significantly less.

Vibrational spectrums of the control and experimental samples are represented in Figure 2. From the spectrum, it can be observed that the signatory peaks of amide I, II, and III are prominent in control, and the amide peaks are less intense in experimental samples (0.5 and 1.0%), which is due to the biodegradation process. These peaks represent the protein material, which was reduced in wave numbers after degradation due to the blue shift. In 0.5 and 1% CLW samples, peaks exerted at 1445, 1035, and 1331 cm^{-1} with reduced intensities and these peaks have corresponded to methylene (CH_2), ester, and OH groups.

Thermal analysis of control and experimental CLW showed distinct mass losses have been inferred from the thermograms as seen in Figure 3.

As seen in Figure 3, the mass loss percentage of the experimental samples 0.5 and 1% CLW are found to be 76.96 and 89.61%, respectively, whereas control shows about 68.83%.[19] The reduction in thermal stability and increase in mass loss indirectly indicate the degradation of CLW samples.

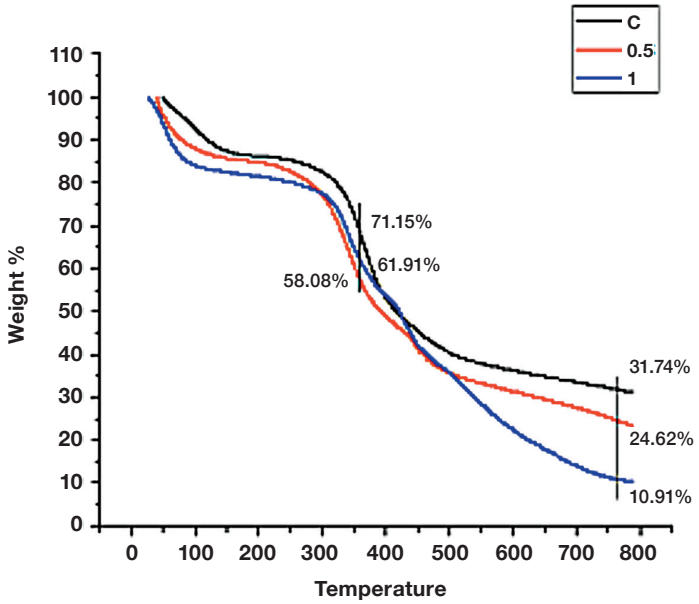

Figure 3. Thermogravimetric Analysis of treated and untreated CLW

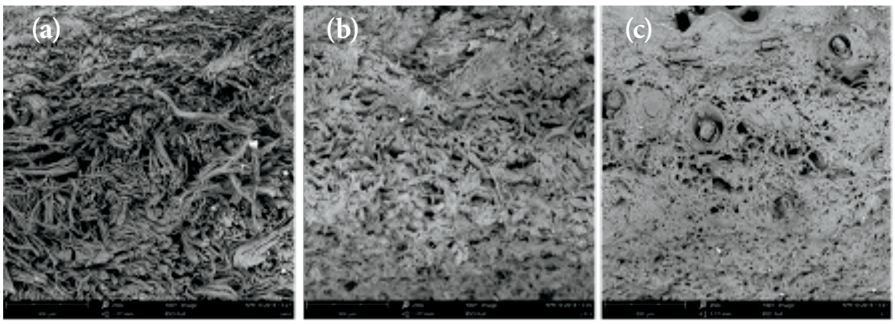

Figure 4. SEM images of treated and untreated CLW of **(a)** Control **(b)** 0.5% **(c)** 1%

The morphological study helps in the visual understanding of the biodegradation process. As seen in Figure 4a, the control CLW has defined and ordered fibrous structure.

Moreover, the compactness of fibers is well maintained in the control samples, which confirm that the substrate has not undergone degradation. From Figure 4b, the effect of bacterial strains on the CLW (0.5%) has been well pronounced, and it can be observed from the swollen and coalesced fibrous network. Moreover, fiber compactness and orientation are changed owing to the effect due bacterial enzyme. Whereas, as seen from Figure 4c in 1% CLW sample, the degradation is faster with the broken fragments and formation of grooves. The definite fiber orientation and compactness are disappeared in 1% CLW completely as compared to 0.5% CLW.

Conclusion

Biodegradation of tanned leather is of a great need to achieve eco-sustainability. In the current research, an attempt has been successfully addressed in identifying new bacterial *Bacillus* sp., which possesses dual characteristics viz., degradation of chrome leathers, and the ability to reduce the chromium. From the collagen content measurement, it is observed that the hydroxyproline released in the 1% CLW has gradually increased towards 15th day. Furthermore, thermal, vibrational and morphological studies confirm the degradation of CLW by *Bacillus aryabhattai*. The study holistically addresses the major challenge towards the disposal of CLW.

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Authors Contribution

All the authors have shared their knowledge and contributed to frame the manuscript. AS and CK performed the experiments, GCJ contributed towards framing the manuscript and structured the methodology of the work, AY contributed towards the analytical assay and SVK framed the conceptual idea and structured the final version of the manuscript.

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Thermal Sensitive Agents for Making Stimuli Responsive Leathers

by
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Abstract

Herein, we report the synthesis and application of a smart polymeric acrylic syntan (synthetic tanning material), which can respond to temperature and pH. Behavior of polymer at different pH (1-10) at 28°C was studied. Also interactions with collagen were ascertained in order to understand the polymers' response towards secondary structures of collagen. Leather made using the experimental syntan demonstrated higher temperature resistance of 3±0.5°C compared to control syntan treated leathers (1±0.5°C) when exposed to heat under artificial simulation. Experimental leathers exhibited better strength and organoleptic properties. These smart leathers find its application in extreme climatic conditions of heat or cold.

Introduction

Skin is very sensitive towards stimuli and plays an important role in protection from extremes of environment, its sensitisation to multitude of senses such as light, temperature, pain and perspiration making it a smart material.[1-3] Skin from a flayed animal is susceptible to autolysis hence skin needs to be tanned to leather in order to prevent from putrefaction.[4] Skin is a thermoregulatory machine of own; when converted into leather, its thermal sensing capability is lost as it cannot maintain homeostasis. In order to regain some functionality of responsiveness, skin needs to be treated with certain smart chemicals during leather processing.

The conventional tanning method limits the physical properties of the final leather. This limited functionality is due to the type of processes employed and nature of chemicals used in leather making. Conventionally used acrylic syntans play a major role in modern leather processing. Skins or hides with loose grain structure were treated with acrylic syntans to achieve grain tightening and also have greater affinity towards other post tanning chemicals, which in turn increases the chemical uptake by leathers.[5,6] Acrylic syntans were formulated by combination of different acrylic monomers[7,8] to achieve better properties such as grain tightening,[9] filling, retanning,[10] light fastness, and waterproofing[11] on final leathers. The main limitation of conventional acrylic polymer is that they do not respond to thermal changes at specific temperature range. This necessitates for developing tailor made syntans, which can be functionalised to respond to thermal changes by co-polymerising with specific monomers, which in turn can sense heat or cold.

Poly N-isopropylacrylamide (NIPAM) is one of the thermoresponsive polymers being extensively studied since last few decades. The ability to undergo reversible phase separations at temperatures above its lower critical solution temperature (LCST) and its ability to respond to pH when copolymerized with other monomers, led to development of smart chemicals,[12-14] which are capable of responding to both temperature and pH.

In this study thermo responsive acrylic syntan was prepared by radical polymerization using three different acrylic monomers and treated at retanning stage of leather processing. The prepared syntan exhibit the properties of conventional acrylic syntan, alongside it possesses thermoresponsive properties. Post preparation, leathers were analysed for thermoresponsive character. Final leathers were evaluated for strength and organoleptic properties.

Experimental section

Materials

Methyl methacrylate (MMA, ≥99% pure), methacrylic acid (MA, ≥99% pure) was obtained from Loba Chemie Pvt. Ltd., potassium persulfate (PPS, ≥99% pure), sodium lauryl sulphate (SLS, ≥99% pure), sodium formate (≥99% pure), sodium bicarbonate (≥99% pure), sodium metabisulfite (≥99% pure) were purchased from MERCK Specialities Pvt. Ltd., sodium hydroxide (NaOH, ≥99% pure) from RANBAXY Laboratories and *N*-isopropyl acrylamide (NIPAM, ≥99% pure) from Sigma-Aldrich. All other leather chemicals were of commercial grade.

Preparation of thermoresponsive acrylic Syntan

Polymeric syntan was prepared by Radical polymerisation method. NIPAM 5g (0.044 mol) in 10 mL H_2O, MMA 1.5g (0.015 mol) in 20 mL H_2O with 0.7g SLS, MA 0.5g (0.006 mol), 0.02g sodium metabisulfite was added to a reactor and heated to 75°C, PPS (0.22g PPS in 10 mL H_2O) as initiator was added drop wise to the reactor vessel, reaction was continued in nitrogen atmosphere for 3h at 75°C, final pH of the experimental syntan was adjusted to 4.5 using NaOH and labelled

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as MTP02. Control syntan was prepared without using NIPAM. Demineralised water was used in syntan preparation. Small sample of the polymeric syntan was dried and used for analysis.

Collagen- polymer interaction studies

Collagen was extracted from teased tail fibers of *Wistar albino* rat (six months old) and washed with 0.9% NaCl at 4°C. Acid extraction method[15] was followed and 5% NaCl solution was used for purification. The precipitate was collected by centrifugation followed by dialysis against phosphate buffer. The concentration of collagen was determined from hydroxyproline content according to Woessner method.[16] The molar concentration of collagen was determined considering the average molecular weight of collagen as 300 kDa.[17] Structural conformations were studied using circular dichroism[18] and the extracted collagen was used for studying polymer interactions. Different concentrations of polymer containing solid content from 0.1 to 1% on weight basis was mixed with 1ml of collagen (8 μM) solution under continuous stirring for 3h at 4˚C. Then the reacted collagen- polymer (CP) solutions were analysed for stability.

Characterization of thermoresponsive Syntan

Solid content for syntans were measured according to standard procedure.[19] Particle size analysis was done using particle analyser (Zetasizer Nano series- ZS, Malvern) at 25˚C, scattering angle of 173°C and wavelength of 633 nm. The functional groups present in the syntan were analysed using FTIR spectrum (Jasco spectrometer, FT/IR-4000 Series). Polymeric syntan was made into pellet using Potassium Bromide (KBr) and analysis was carried out.

Clouding behaviour was observed by exposing the MTP02 sample to room temperature and photographs were taken using digital camera (Sony, Cyber-shot, 16.1 Megapixels with 8X optical zoom)

Leather processing and syntan application

Entire leather processing was done in a rotary drum, rotating at 4-6 revolutions per minute (rpm). All percentages were based on wet blue (chrome tanned leather) weight. Re-chromed wet-blue leathers of 1-1.1mm thickness were neutralized using 0.5% sodium formate and 0.5% sodium bicarbonate, pH of the leather was adjusted to 5.5-6, with drum running time of 45 min. After that leathers were rinsed in 100% H_2O. The pH adjusted leathers were further processed with 100% H_2O, 5% acrylic syntan (control and experimental syntan) with drum running time of 40 min. Then 2% synthetic fatliquor was offered with drum running time of 30 min and 2% dye was added and drum was rotated for 30 min. Fatliquoring was done in the same bath with 7% synthetic, 4% natural, 3% lecithin and 2% fish oil based fatliquors and were emulsified in 10% H_2O (weight basis). Three percentage of Phenolic syntan was offered with drum running time of 30 min. Leathers were fixed using 3% formic acid in 10% dilute H_2O and was offered in three feeds at 10 min interval each. Leathers were rotated in drum for another 30 min and liquor was drained. Finally, leathers were rinsed in 100% H_2O and piled on horse (Stand for drying leather).

Scanning Electron Microscopy (SEM) study of polymer treated leathers

SEM micrographs of the syntans and the leathers were studied using Hitachi S-3400 SEM microscope operating at 10-30 kV at room temperature of 25°C and relative humidity of 50%. The fracture ends of the specimens were sputter coated (Hitachi E-1010, Ion sputter) with a thin layer of gold prior to examination.

Thermal analysis

Thermoresponsive function analysis for leather was done according to the procedure mentioned,[20] leather surface images were taken by thermal imaging camera (E60, from FLIR), which has IR and visible systems with thermal sensitivity of less than 0.05°C temperature, measurement range of -20°C to +650°C. The samples were analyzed at room temperature of 25°C and relative humidity of 50%. Samples were strapped to the heating/cooling unit. It was ensured that the heating/cooling unit achieves equilibrium after setting the required temperature and then the images were taken focusing the leather samples. Samples were dismounted only after finishing the range of measurement.

Physical strength and organoleptic properties of Leather

Physical Testing of sampling was done according to standard procedure.[21] Tensile strength[22], tear strength[23] and grain crack index[24] were measured as per the standard procedures and compared with UNIDO norms.[25] Prior to testing, specimens were conditioned at 20±2 °C and RH 65±2% for 48 h. Values reported were average of four samples. Strength properties of the crust leathers were measured using an INSTRON universal testing machine. Organoleptic properties were assessed for softness, fullness and grain smoothness by standard hand evaluation technique. Four experienced leather technologists evaluated the samples and ratings were given to each sample. Experts rated the leathers on a scale of 0-10 points for each functional property, where higher points indicate better properties exhibited. Organoleptic properties were evaluated based on individual perception and is not a precise measurement of the particular property. Water-vapour permeability tests were performed to determine the ability of leather to permeate the amount of water vapours in terms of mg/unit area and for a specified period of time using Croydon England test method (SATRA 06121). Test samples are subjected to expose from grain surface to silica gel beads, which were placed in a container. This container was subjected to cyclic rotations for 12 h duration and differential weight of the container was noted and water vapour permeability was calculated. Softness analysis[26] of leather was done using ST-300 Leather softness tester. Porosity and air permeability testing of leathers was done using advance

automated humid air porometer (HCFP-1100AE, Porous materials Inc).

Effluent parameters

Wastewater obtained after leather processing using control and experimental syntan were collected and analysed for COD, BOD and TS according to the standard procedures.[19]

Results and Discussion

Characterization of thermoresponsive syntan

Total solid content of control and experimental (MTP02) syntan were analysed and it was observed around 31 and 30±2%, as leather processing was carried out by offering chemicals on the weight basis hence it was necessary to estimate the weight content of polymer present in the syntan. Number average molecular weight was estimated using dynamic light scattering (DLS) method and it was found to be 542±6.4 and 322±3.8 kDa for the control and MTP02 syntan. The low molecular weight of the MTP02 syntan may be due to higher solubility of NIPAM. Particle size of control and experimental syntan were observed to be 950±20 and 350±20 nm (Fig. 1), which was also analysed by DLS method, particle size of the experimental syntan was much lower compared to the control syntan. A lower particle size material penetrates the leather matrix in lesser time and with minimal effort, penetration throughout cross section of leather can be achieved easily. As the acrylic type of polymers were well known for their ability to absorb other post tanning chemicals, MTP02 syntan treated leathers showed higher affinities towards leather. This was well indicated by physical strength and organoleptic property values.

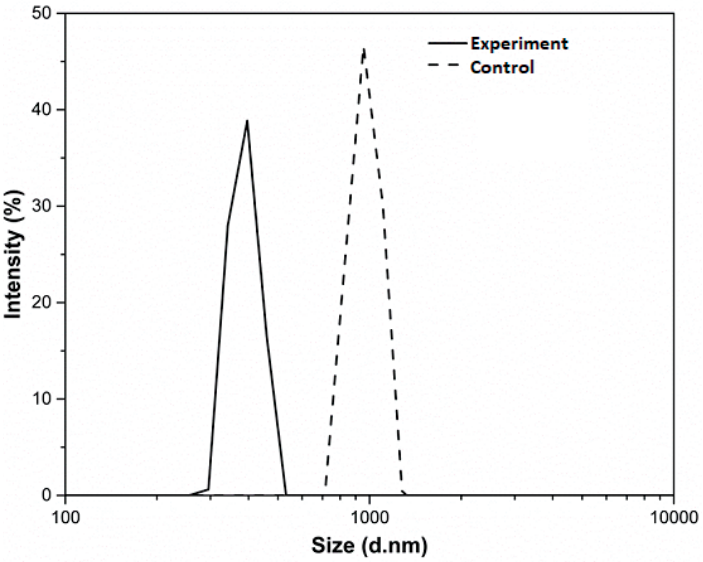

Figure 1. Particle size distribution of control and experiment (MTP02)

Clouding behaviour of thermoresponsive polymer

Clouding behaviour exhibited by thermoresponsive polymer (MTP02) was analysed from pH 1-10 at room temperature, photographs were taken (Fig. 2). This observation was made at room temperature in order to check the feasibility for leather application. Polymer responded differently at different pH under constant temperature condition. Immediate coagulation of polymer was observed at pH 1 and 2, phase change occurred at much less than room temperature and also settling of polymer was observed indicating complete phase separation. From pH 3 to 5, clouding was observed indicating the phase changing in the range of normal room temperature and it exhibited colloidal nature without any phase separations. From pH 6-10 clouding was observed at temperatures more than 37°C, this observation also implies the polymers is tuneable towards temperature and pH. Clouding temperature of the polymer was studied visually by raising the temperature of the polymer. From table I, it can be observed that the thermal sensitivity of the polymer can be varied based on change in pH. Also, starting point of the clouding was mentioned with an error of 2°C for clarifying the range at which the actual clouding started.

Table I
Clouding behaviour of the polymer at different pH

pH	Clouding Temperature (°C)
1	6±2
2	14±2
3	22±2
4	30±2
5	37±2
6	42±2
7	48±2
8	56±2
9	65±2
10	72±2

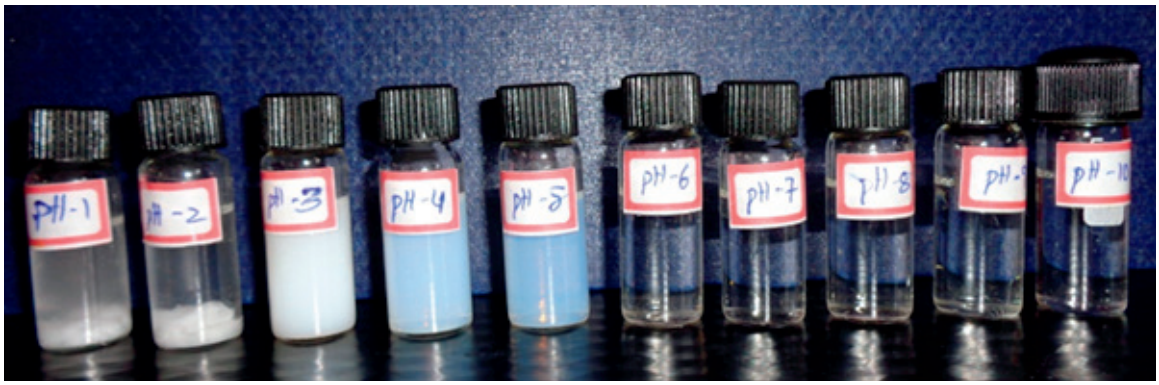

Figure 2. Clouding behaviour of polymer at room temperature

Polymer interaction with collagen

Polymer and its interactions with collagen were studied by identifying the functional groups using FTIR technique. (Fig. 3) Spectral conformation of collagen by vibration and stretching occurring at amide I (C=O stretch vibrations), amide II (N-H bending with C-N stretch vibration) and amide III (C-N stretching with N-H bending of amide linkages) occurring at 1659, 1550, and 1240 cm^{-1} provide information about secondary structure of collagen. The peaks at 1730 and 1250 cm^{-1} show associated non-ionized carboxyl groups and N–H stretching of amide II.[27]

FTIR spectra of collagen (untreated) in our study showed peaks at 1640, 1552 and 1244 cm^{-1} concurring with above reported values. Peaks representing amide I give information about the secondary structure hence the peaks arising at the intervals between 1600-1700 cm^{-1} are more important to confirm the structure. Collagen-polymer (CP) showed amide I, amide II and amide III occurring at 1634, 1537, and 1237 cm^{-1}. Occurrence of this blue shift might be due to the interaction of polymer with collagen molecule.

Circular dichroism spectroscopic studies have been employed to understand the stability of collagen when treated with polymer, as collagen is the main constituent of leather. Interaction with polymer were monitored by the change in molar ellipticity value of at 222 and 197 nm, from (Fig. 4) it can be observed that the collagen as standard showed negative and positive peaks at 197 and 222 nm. Collagen when treated with different concentrations of polymer there is a change in stability (Molar ellepticity plot shown in Fig. 4 inset). It can also be observed that the stability of collagen increased with addition of 0.1 (CP-10) and 0.5 % (CP-50) polymer (weight %). This conformation supports the FTIR analysis and gives the proof of collagen stabilization by treatment of polymer.

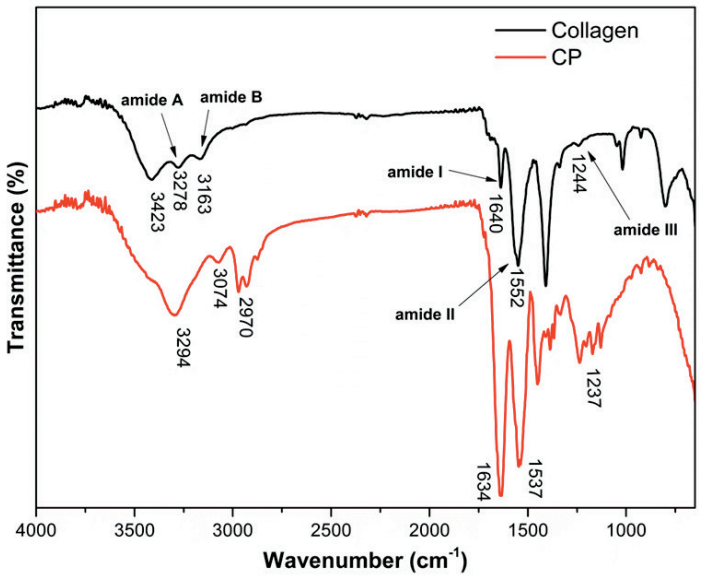

Figure 3. Fourier transform infrared spectroscopy analysis of collagen and collagen-polymer (CP) samples

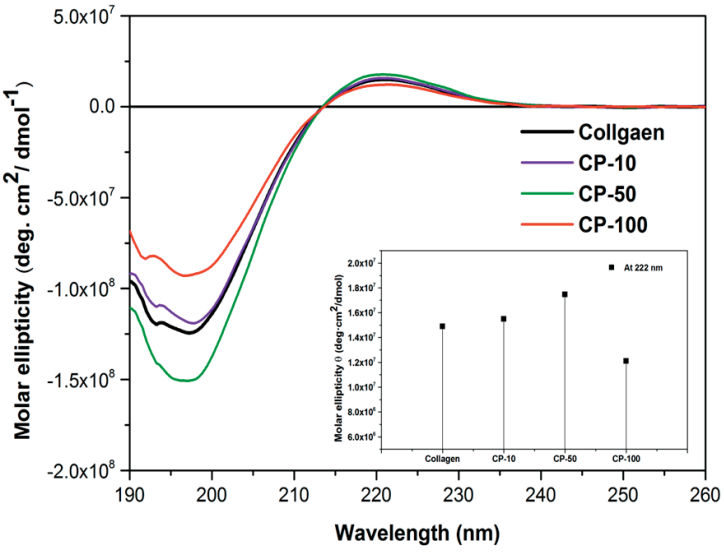

Figure 4. Circular dichroism spectra for understanding stability of collagen-polymer interactions

Application of syntan in leather making

Detailed study of the polymeric syntan was also carried out in order to understand its behaviour towards leather. Application of syntan at post tanning stage of leather processing and performance of leather was evaluated by various testing methods.

Infrared thermal imaging of syntan treated leathers

Thermal imaging of leather was intended to understand the comfort of the wearer. Human body responds to heat or cold by internal mechanism of thermo regulation. With change in temperature, physiological changes also occur in the body.[28] In order to maintain thermal comfort, temperature of the surrounding also needs to be maintained. Thermal comfort provided by the leathers was assessed by infrared imaging technique. Images of control and experimental leathers were taken at different temperature starting from 31 to 42°C in order to simulate the comfort of the wearer as the temperature fluctuation below 35 (Hypothermia) and above 37.5°C (Hyperthermia) and may lead to discomfort. Leathers were strapped to a chilling or heating unit and temperature measurements were carried out. Current studies showed control

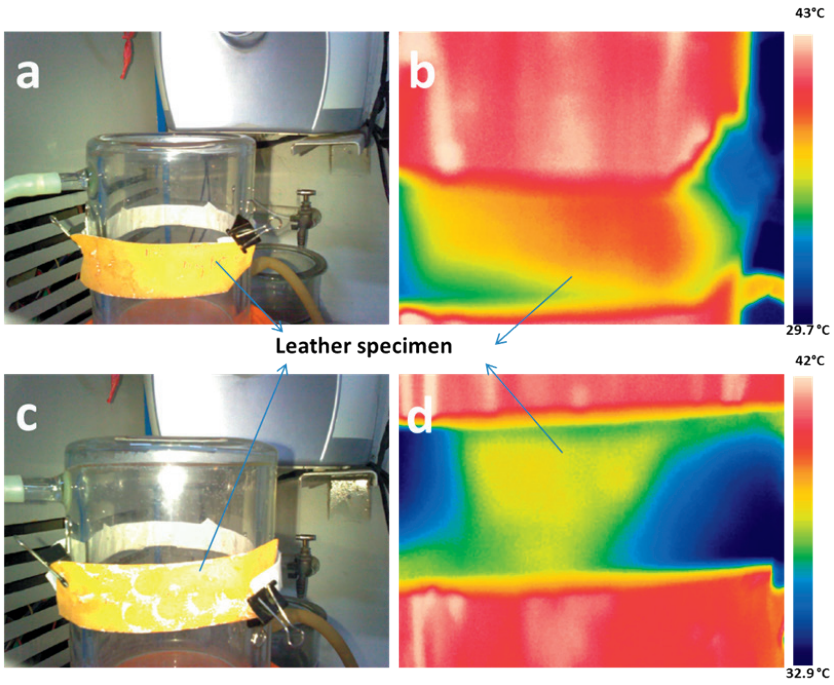

Figure 5. Digital images of **a)** control **c)** experimental, infra-red images of **b)** control **d)** experimental syntan treated leather

Table II

Temperature difference exhibited by control and experimental leathers

	Control		Experiment		
	Grain surface temperature, °C	Flesh surface temperature, °C	Grain surface temperature, °C	Flesh surface temperature, °C	
Comfort Zone	32.5	32.2	32.7	31.7	Comfort Zone
	34.6	34.5	34.2	32.3	
	35.4	34.6	35.6	33.4	
	36.5	35.7	36.8	34.4	
	37.8	37.4	37.4	35.2	
	38.6	37.9	38.6	35.8	
	39.5	38.7	39.1	36.2	
	40.4	39.6	40.6	37.5	
	41.5	40.6	41.7	38.4	
	42.3	41.4	42.5	39.1	
Average Change		1±0.5		3±0.5	

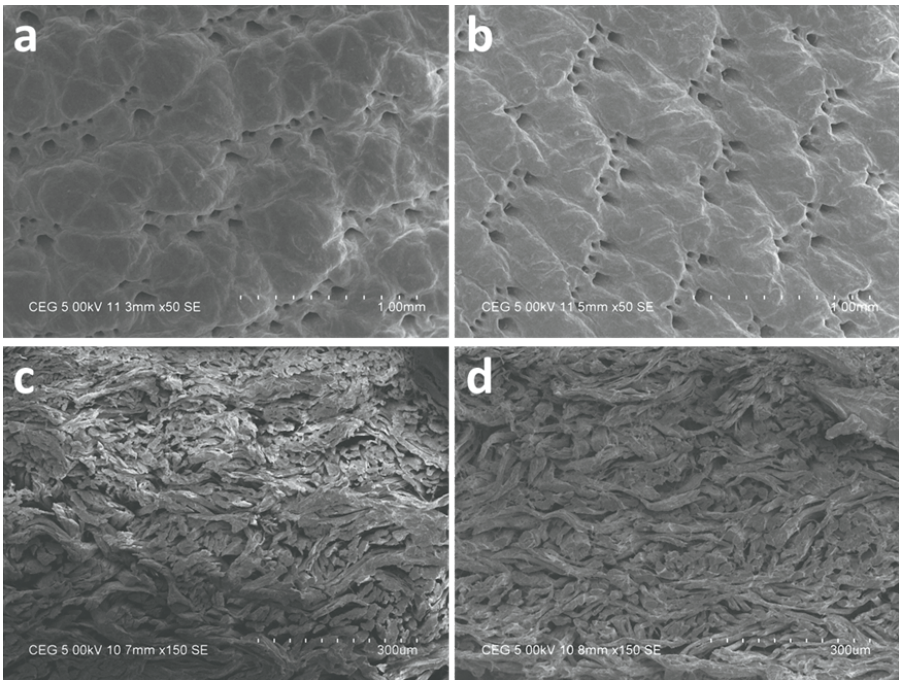

Figure 6. Scanning electron microscopy images showing grain surface of **a)** control **b)** experimental leathers at 50X, cross section images of **c)** control and **d)** experiment at 150× magnification

leathers comfort range (grain surface temperature) was limited to 37.8 and 40.6°C for control and experimental leathers. (Table II)(Fig. 5) Experimental leathers exhibited a higher comfort temperature range of 3 ± 0.5°C compared to control syntan treated leathers.

Scanning electron microscopy of leathers

Scanning electron microscopy images of experimental and control syntan treated leathers were analysed to understand the morphology of the leather surface and compactness of fibers by observing the cross section. Experimental leathers were compared to control leathers (Fig. 6) and it was observed that both leathers were flat with no surface deformities. Cross section view of experimental leathers indicated more compact structure than control leathers. This might be due to higher reactivity of experimental syntan, which may have enhanced uptake of other post tanning chemicals.

Physical testing of leather

Tensile strength property of MTP02 syntan treated leather was comparable to that of control, with no major difference on percentage elongation at break values indicating that both experiment and control leathers have similar stretching property (Table III). Experimental leathers exhibited higher tear strength property, which might be due to higher cross linking of the experimental syntan with collagen fibers. Grain crack index values were almost similar to that of control leathers. Water vapour permeability gives the measure of leathers ability to allow water vapour to permeate through and through over a period of time, experimental leathers exhibited the value slightly lesser than control syntan treated leathers, which indicated that the experimental leathers retained the moisture without permeating, which in turn increased the leather capability to retain heat. Water absorption values were 0.8072 and 1.0726 mg/cm^2 for control and experiment leathers. This clearly shows that the experimental

Table III

Physical strength properties of control and experimental syntan treated leather

				Grain crack index		
	Tensile strength (N mm^{-2})	Elongation at break (%)	Tear strength (N mm^{-1})	Mean load (kg)	Mean distension (mm)	Water vapour permeability (mg cm^{-2} hr^{-1})
Control	12±5	60±5	34±2	26	9.30	13.60
Experiment	14±3	61±5	50±2	25	9.25	12.50

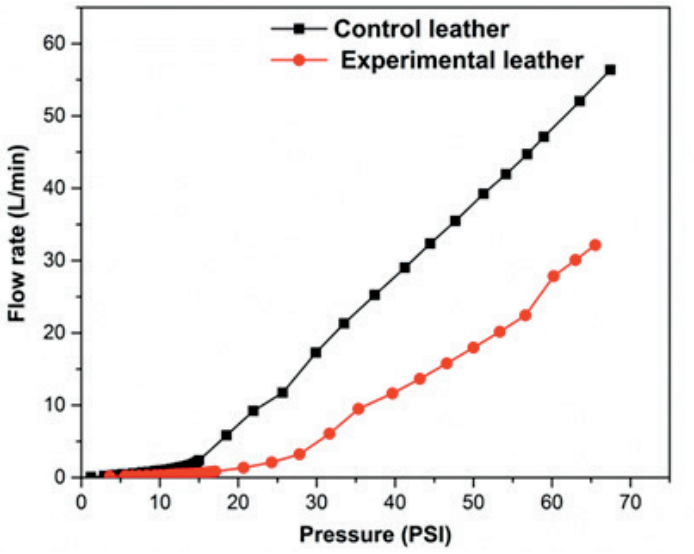

Figure 7. Permeability analysis of leather

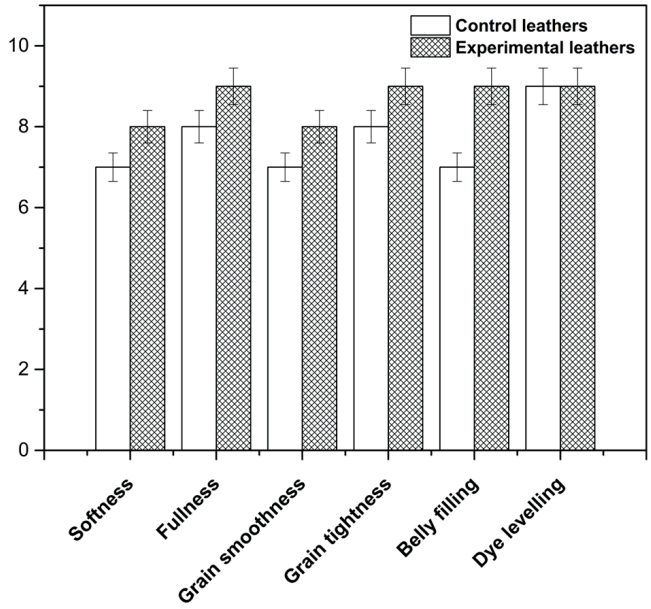

Figure 8. Organoleptic properties analysis of leather

leathers have higher water absorption and high water retention capability.

Softness analysis of leather helps to understand the ability of fatliquor absorbed, control and experimental leathers showed value of 5.4771 and 6.1328. This indicates that the experimental leathers absorbed higher amounts of post tanning chemicals than compared to control. Higher softness means more sliding of fiber bundles in leather matrix, which also mean higher absorption of fatliquor.

Porosity analysis of leather

Air permeability and porosity of leather were analysed to understand the breathability of leather, which is one of the essential characteristics of leather. The air permeability of experimental leather is lower than the control leather (Fig. 7), which indicate no void spaces present in the leather matrix. Lower air permeability can also influence the heat flow as indicated by the infra-red image of experimental leathers. Experimental syntan might be filling the leather and absorbing water vapour due to its hygroscopic nature. It was found that the largest pore present in control and experimental leathers is 5.3258 and 1.8185 µm.

Color measurement of leather

Color values confirmed that the experimental syntan treated leathers showed increase in color strength compared to control leathers. (Table IV) This was confirmed by the increase in ΔE value by 3.91, this gives the measure of just noticeable difference (JND),[29] it can be concluded that experimental syntan treated leathers exhibited an increase in intensity of color.

Organoleptic properties

Organoleptic properties of leathers made from control and experimental syntan are shown in Fig. 8. The ratings were plotted by taking an average of four samples evaluated with an error margin of 5%. Properties such as softness, fullness, grain smoothness, grain tightness, belly filling and dye levelling were compared with control syntan treated leathers. Syntan was uniformly distributed, as there was no evidence of looseness in belly regions of the experimental syntan treated leathers, dye uniformity and levelling properties were excellent, dye intensity was slightly enhanced in case of experimental leathers. Overall, leathers made using experimental syntan exhibited better properties than the control counterpart.

Table IV

Color values of control and experimental leathers

Sample	L*	a*	b*	ΔE
Control	72.543	9.151	24.352	-
Experiment	73.048	10.315	28.051	3.91

L* represents lightness, a* represents redness-greenness and b* represents blueness-yellowness of the color

Table V

Analysis of waste water

	COD (mg/L)	BOD (mg/L)	TS (mg/L)
Control	1800±50	175±5	5760±50
Experiment	1350±50	168±5	3750±50

Wastewater analysis

Effluent from leather processing is analysed for COD, BOD and Total solids to understand the pollution characteristics. From Table V, there is a reduction in COD, BOD and TS content. There was reduction of COD and TS values by 25 and 35%. This might be due to the higher uptake of leather chemicals making the experimental syntan not only efficient but also eco-friendly in nature.

Conclusions

It can be observed from the present study that the prepared polymer MTP02 exhibited better pH and temperature response. From the clouding behavior analysis, it can be observed that the temperature response of polymer can be altered by changing pH. Collagen- polymer interaction studies provided insights about the stability and functional groups were analyzed using FTIR. After treating leather with syntan, thermoresponsive behaviour was analysed and a temperature difference of 3±0.5 compared to 1±0.5 °C of control leathers. Improved tensile and tear strength values was observed and also organoleptic properties such as softness, fullness, grain tightness and belly filling were better than control counter parts. Scanning electron microscopy analysis provided information about leather surface and fiber alignment. Water vapor and air permeability of leathers were tested to understand the filling behaviour of syntan. Color values were measured to understand the dye uptake and covering. Synthesized acrylic syntan can be used for production of stimuli responsive leathers, which can respond to temperature and pH. Thus experimental leathers find its applications in developing environment and pH adjustable leathers.

Acknowledgements

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Xiang Zhong see *JALCA* **115**, 270-273, 2020

Mengchu Gao see *JALCA* **115**, 309-312, 2020

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THE AMERICAN LEATHER CHEMISTS ASSOCIATION

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Carol Adcock, Executive Secretary

December 1, 2020

Dear ALCA Member or Subscriber,

This year has certainly been a challenging one for everyone and the ALCA has been challenged as well. I am happy to report that our offices have stayed open during this time and our Executive Secretary, Carol Adcock, has been working from home since March. As needed she is able to go to the office and handle anything that can't be handled from her home.

Our first challenge was the Annual ALCA Convention. It was originally set for June of 2020. We had already started soliciting sponsorships for the convention and many folks had already committed and paid for their sponsorships. We felt there was just no way to gather folks at that time so we changed the date to September. As September approached, it became obvious that we still would not be able to gather our group so we postponed it once again until May 4 – 7, 2021. It will be at the same location that hosted us in 2018, namely Eaglewood Resort and Spa in Itasca, Illinois just outside of Chicago. Randy Johnson, our 2020 Wilson Lectureship speaker, has generously agreed to postpone his presentation until May, 2021. We are excited about his presentation and what he has to say to the leather industry. Since we already had committed sponsors for a convention this year, we asked each of them to rollover their sponsorship to the 2021 convention, and they did. We have a wonderful support group of sponsors, and it really helped the Association financially.

Another thing the Association did to keep stability was to ask each Officer, Councilor and Committee member to extend their term of office by one year. All unanimously agreed.

In August we applied for and received a PPP loan in the amount of $10,343. To date there have been no instructions on how to apply for forgiveness of the loan, but we are hopeful it will be forgiven as the monies were used for the intended purpose.

Still, we are struggling financially since we realized no income from the convention. If you would like to help us overcome our financial difficulties, we would appreciate any contribution to the Association you are able to give. We are a non-profit organization and are able to give you a statement for any donations to the Association that will help you deduct it as a charitable contribution from your income tax. If you have any questions about this, please contact our Executive Secretary, Carol Adcock, at (806)744-1798 or by emailing to carol.adcock@ttu.edu.

I look forward to next year and hope we are able to have our convention in May.

Mike Bley
President, ALCA

CALL FOR PAPERS

FOR THE 116th ANNUAL CONVENTION OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION

Eaglewood Resort & Spa, Itasca, Illinois
May 4-7, 2021

If you have recently completed or will shortly be completing research studies relevant to hide preservation, hide and leather defects, leather manufacturing technology, new product development, tannery equipment development, leather properties and specifications, tannery environmental management, or other related subjects, you are encouraged to present the results of this research at the next annual convention of the Association to be held at the Eaglewood Resort & Spa, Itasca, Illinois, May 4-7, 2021.

Abstracts are due by February 1, 2021
Full Presentations are due by May 1, 2021

They are to be submitted by e-mail to the
ALCA Vice-President and Chair of the Technical Program:

Joseph Hoefler
The Dow Chemical Company
400 Arcola Rd.
Collegeville, PA 19426
E-mail: jhoefler@dow.com

The **Abstract** should begin with the title in capital letters, followed by the authors' names. An asterisk should denote the name of the speaker, and contact information should be provided that includes an e-mail address. The abstract should be no longer than 300 English words, and in the Microsoft Word format.

Full Presentations at the convention will be limited to 25 minutes. In accordance with the Association Bylaws, all presentations are considered for publication by *The Journal of the American Leather Chemists Association.* They are not to be published elsewhere, other than in abstract form, without permission of the *Journal* Editor. For further paper preparation guidelines please refer to the *JALCA* Publication Policy on our website: leatherchemists.org

Full Presentations are to be submitted by e-mail to the *JALCA* editor:

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116th ALCA ANNUAL CONVENTION Change of Date: May 4-7, 2021 Eaglewood Resort & Spa Itasca, IL

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By Randy Johnson, President and CEO
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Title: Road Ahead

Tentative Schedule

Tuesday, May 4
Golf Tournament, Opening Reception and Dinner

Wednesday, May 5
John Arthur Wilson Memorial Lecture
All Day Technical Sessions, Fun Run
Reception and Dinner, Activities - Bowling, Pool,
Darts and an Open Bar

Thursday, May 6
All Day Technical Sessions, Annual Business Meeting
Activities Awards Luncheon
Social Hour, ALCA Awards Banquet

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