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Green chemistry approaches to leather tanning process for making chrome-free leather by unnatural amino acids

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ABSTRACT

In the present study, green and sustainable method or eco-friendly approaches to tanning process based on unnatural D-amino acids (D-AA)-aldehyde (Ald) as a substitute for chrome-free tanning has been attempted. The distribution of optically active D-AA in tanned leather, the hydrothermal stability, the mechanical properties and resistance to collagenolytic activity of tanned leather, the evaluation of eco-friendly characteristics were investigated. Scanning electron microscopic (SEM) and Atomic force microscopic (AFM) analyses indicate the surface morphology and roughness, respectively, of the tanned leather collagen matrix. Shrinkage and Differential scanning calorimetric (DSC) analyses shows that the shrinkage temperature (T_s) and denaturation temperature (T_d) of tanned leather are related to the content of D-AA+Ald present in the leather matrix. It has been found that the T_s of D-AA tanned leather is more than that of Ald tanned leather and also more or less equal to chrome tanned leather. Environmental impact assessment (EIA) shows that the developed process results in significant reduction in total solids content (TSC) and improves better biodegradability of organic compound present in the effluent compared to chrome tanning.

1. Introduction

Originally, leather production is governed by an eco-friendly industrial process, because slaughter house waste such as the hides/skins discarded as waste material, is processed into useful materials such as leather goods, footwear and garments. During the leather making process, tanning is one of the most important operations, which improves the durability and practicability of leather products and prevent putrefaction, in which the tanning agents react with the collagen molecule, stabilizing the triple helical structure of collagen matrix; thereby the leather acquiring resistance towards chemical, thermal and microbiological degradation [1,2].

Usually, the tanning process primarily employs inorganic tannins such as chromium, aluminum, titanium, iron, zirconium and organic tannins such as aldehyde and high molecular weight vegetable tannins (hydrolyzable and condensed), synthetic tannins and above mentioned combinations. However, each of the above tannins has some advantages and disadvantages [3,4]. The disadvantages of the known tanning agents include their limited availability and their minimal recoverability or reusability from leather waste. Wastewaters are difficult to be treated with conventional treatment systems.

Conventional tanning processes include the use of chrome and other inorganic agents, which transform the perishable raw hides into durable leather, but at the expense of the natural resources. Chrome tanning is the most widely used tanning process in the industry, for performance, economic and ecological reasons. In the state-of-the-art, although tanneries do succeed in reducing the chromium contents of their effluents to below the stipulated maximum values, this is only possible at enormous expense. On the other hand, the quality and variety of leathers obtained by chrome tanning must be considered extremely high, so that no other tanning processes have yet been found that provide the same universality, quality and variety of styles. The chrome and other inorganic pollutants contaminate air and water. The chrome tanned leather sometimes generates certain hazardous substances and they are cytotoxic in human health and pollute environment. Hexavalent chromium in leather waste is potentially hazardous to human health and a new technology of its treatment has been reported [5,6].

Vegetable tannins are natural materials which have been considered as a suitable eco-friendly option to replace chromium. Vegetable tannins are also being employed for making some kinds of leathers. However, this tanning leads to excessive loading in the leathers, which reduces its versatility to make different end products and also has low resource availability. These vegetable tanned

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leathers are also known to be poorly biodegradable, which results in high Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD). Hence, the combination tanning systems were considered as suitable tanning method to overcome the problems arise from single tanning system. Various combination tanning exist, presently, the combination tanning systems are mainly based on chrome-less and chrome-free tanning processes. For example, less chrome combination tanning systems are chromium and silica, chromium and iron, etc. The chrome-free combination tanning based on organic such as vegetable with synthetic tannin and inorganic such as metal ions other than chromium has been reported [7–13]. However, all the above combination tanning systems do not have commercial importance in the global leather industry due to processing difficulty, toxicity, availability, cost, etc. The elimination/reduction of chrome and other inorganic substances assures importance in the tanning process in present conditions. The 21st century has embraced the appearing of green chemistry era, which is an up-coming area that has attracted the attention and participation of many researchers in resulting in many new reactions, new processes, and new commercial possibilities and processes that minimize the use and generation of hazardous substances. The importance of green chemistry process in research and development for industrial application in minimizing the use and generation of hazardous substances has been reviewed [14–16]. The effects of various small molecules viz. surfactants, urea and salt additive environments on the stability, conformation and geometry of the collagen triple helix for stabilization were discussed in detail in the past using hydrodynamic and thermodynamic studies [17–19].

In the present study, the development of eco-friendly tanning based on optically active unnatural D-amino acids (D-AA) (Table 1) with aldehyde (Ald) has been made as an alternative chrome-free tanning. The tanning conditions of D-AA, physico-chemical characteristics of leathers and the environmental impact of these tanning methods have been compared with conventional tanning processes.

2. Experimental

2.1. Materials

Raw material used for leather processing was pickled goat skins processed from wet salted goat skins. The chemicals employed in subsequent operations were those normally used in leather industry. All the other chemicals were commercial grade products.

2.2. Methods

2.2.1. Preparation of unnatural D-AA

L-Alanine (L-Ala) racemization was performed using a 30% solution of L-Ala hydrochloride with 25% strength NH_3 solution for 5 h at 70 °C in an autoclave. The discharged mixture was brought to pH 6.1 with Conc. HCl and salts were removed by electrodialysis. Subsequent crystallization by evaporating down the aqueous solution gave 70% of pure D-Alanine (D-Ala).

L-Glutamic acid (L-Glu) racemization was performed using a 30% solution of L-Glu hydrochloride. This solution was stirred at 135 °C for 105 h and added 10% of aniline. To the resulting solution, 800 mL ethanol and allowed to stand overnight in the refrigerator. The crystalline D-Glutamic acid (D-Glu) is filtered off and washed with 95% ethanol until free of chloride. The yield is 95%.

L-Lysine (L-Lys) racemization was performed using a 30% solution of L-Lys hydrochloride. A 200 mL solution in a 300 mL autoclave was stirred at 135 °C for 105 h. To the resulting solution, 800 mL ethanol was added to crystallize-out D-Lysine (D-Lys). The yield is 90%.

2.2.2. Tanning and shrinkage temperature determination

4 pickled goat skins were tanned by 5% (based on pickled weight) unnatural D-AA (Control, D-Ala, D-Glu and D-Lys) with 1% glutaraldehyde (GA), for 3 h and then the basification was carried out by the addition of 1% sodium formate and 1% sodium bicarbonate in four instalments at an interval of 15 min. Finally, the tanning drum was run for 1 h and shrinkage temperature (T_s) of each tanned leathers was determined by shrinkage tester.

2.2.3. Characterization

The mechanical and thermal properties, and morphological changes of unnatural D-AA tanned leathers were analyzed using Thermal mechanical analysis (TMA, Instron series II automated materials testing system), Differential scanning calorimetric (DSC, TA-DSC Q 200), Scanning electron microscopic (SEM, FEI QUANTA 200) and Atomic force microscopic (AFM, Park Systems XE-70) techniques.

The % of degradation of D-AA tanned leathers against collagenase activity was calculated by our earlier method [20].

The D-AA tanned leathers liquor from experimental processing were collected and analyzed for BOD and COD. The total solids content (TSC), dissolved solid (DS) and suspended solids (SS) were calculated by the standard procedure. The values reported are average of 3 experiments along with their standard errors (SE).

3. Results

Interest in stereoisomer of optically active unnatural D-AA has increased attention in recent decades with the development of new analytical methods. Incorporation of unnatural D-AA improves the stability to protein, from a functional point of view; their unnatural stereochemistry often means that compounds containing D-residues are much more resistance to enzyme-catalyzed breakdown than natural peptides, a property of considerable pharmaceutical, food and microbiological importance [21,22]. These are of profound interest to establish how the optically active D-AA assisted tanning process improves the stability and resistance to collagenolytic activity.

3.1. The hydrothermal stability

The denaturation temperatures (T_d) of unnatural D-AA tanned leathers were studied using DSC analysis. The position, width, height and symmetry of the thermogram peak provide information about the thermal denaturation of leather over a defined temperature range. The T_d of leather is normally related to the onset temperature of its peak in a DSC pattern and can be used as a measure of the hydrothermal stability of tanned leather subjected to different D-AA treatments shown in Fig. 1. An increase in the T_d is usually an indication of an increase in the stability of wet tanned leather. Fortunately, hide treated with D-Lys+Ald, the endothermic peaks are similar to chrome (BCS) tanned leather and become narrow and sharp. Moreover, the shrinkage temperature (T_s) of final leather reaches $104 \pm 3, 83 \pm 2, 90 \pm 5, 96 \pm 3, 92 \pm 4$ and 120 ± 3, for BCS, Ald, Veg, D-Ala+Ald, D-Glu+Ald and D-Lys+Ald tanned leather, respectively (Table 2). Obviously, the combination tanning can efficiently improve the hydrothermal stability of leathers and the tanning effect of D-Lys+Ald is superior to Ald and Veg single tannin. The D-Lys+Ald tanned leather was found to be softness, fullness and possessed tight grain; these leathers, having better hydrothermal

Table 1
Schematic representation of stereochemistry of unnatural D-AA.

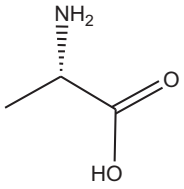
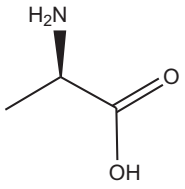
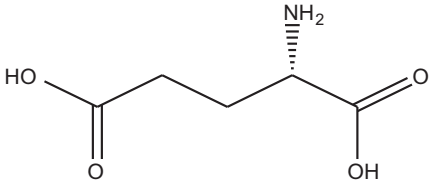
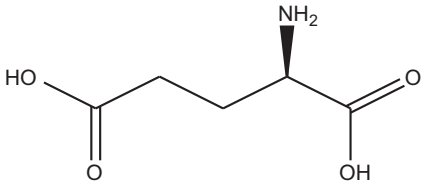
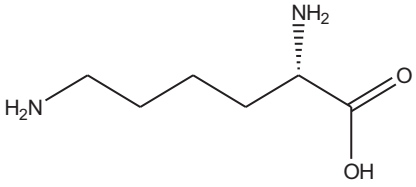
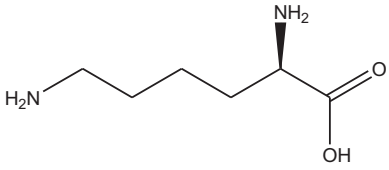
Amino acids	L-Configuration	D-Configuration
Alanine		
Glutamic acid		
Lysine		

Table 2
Denaturation temperature (T_d), shrinkage temperature (T_s), tensile strength (TS) and % elongation (%E) of unnatural D-AA tanned leathers.

Processes	T_d (°C)	T_s (°C)	TS (MPa)	%E
BCS	120 ± 3	104 ± 3	18	56 ± 3
Veg	93 ± 5	83 ± 2	15	67 ± 5
Ald	110 ± 5	90 ± 5	15	72 ± 6
D-Ala+Ald	105 ± 3	96 ± 3	14	67 ± 3
D-Glu+Ald	101 ± 7	92 ± 4	15	52 ± 6
D-Lys+Ald	120 ± 7	120 ± 3	18	56 ± 6

stability. This could be possibly due to the decrease in the availability of the active sites in the leather matrix for interaction of D-Lys after treatment with Ald. The T_s and T_d of fibrillar collagens depend on stable intermolecular cross-links formed through the Lys+Ald.

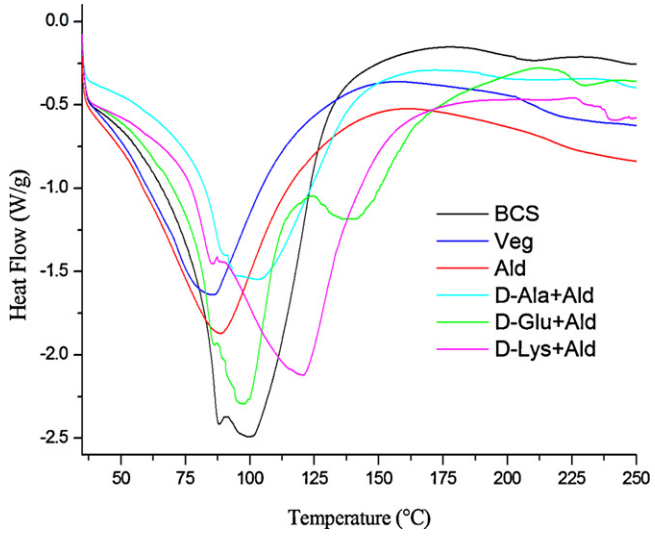


Fig. 1. Differential scanning calorimetric analysis of BCS, Veg, Ald, D-Ala+Ald, D-Glu+Ald and D-Lys+Ald tanned leathers .

3.2. Mechanical properties

Tensile strength (TS) and % Elongation (%E) of D-AA tanned leathers were determined and compared to BCS and Veg tanned leather (Table 2). The mean values corresponding to each experiment are given in Table 2. The D-Lys+Ald tanned leather is close to the BCS tanned leather. D-Lys+Ald tanned exhibited high TS and low %E, whereas D-Ala+Ald and D-Glu+Ald tanned leather exhibited low TS with high %E. This is due to the fact that the fiber bundles are well separated in the case of D-Ala+Ald and D-Glu+Ald tanned leather, while D-Lys+Ald tanned leather shows cemented fiber bundles, as revealed by SEM studies.

3.3. AFM analysis of grain surface roughness

Fig. 2 shows the plane and three-dimensional profiles of the tanned leather grain surfaces. Lighter areas of the images correspond to higher topography and darker areas correspond to lower topography. D-AA assisted tanned leathers have been attempted for the first time in the present study. The Ald tanned leather shows a rougher texture with higher peaks than those of BCS and Veg tanned leather. Tanning by D-AA is thought to be the reason for the increased stability of the tanned leather that is manifested by a higher T_s. Tanned leather clearly formed fibrils in the presence of D-AA as shown by the AFM images where the typical collagen banding pattern of grooves and ridges (Fig. 2). The width of the D-periodicity of collagen fibrils from tanned leather did not appear to be significantly different. Also, there is no difference between the fibrils formed in the absence and presence of D-AA. Measurements of

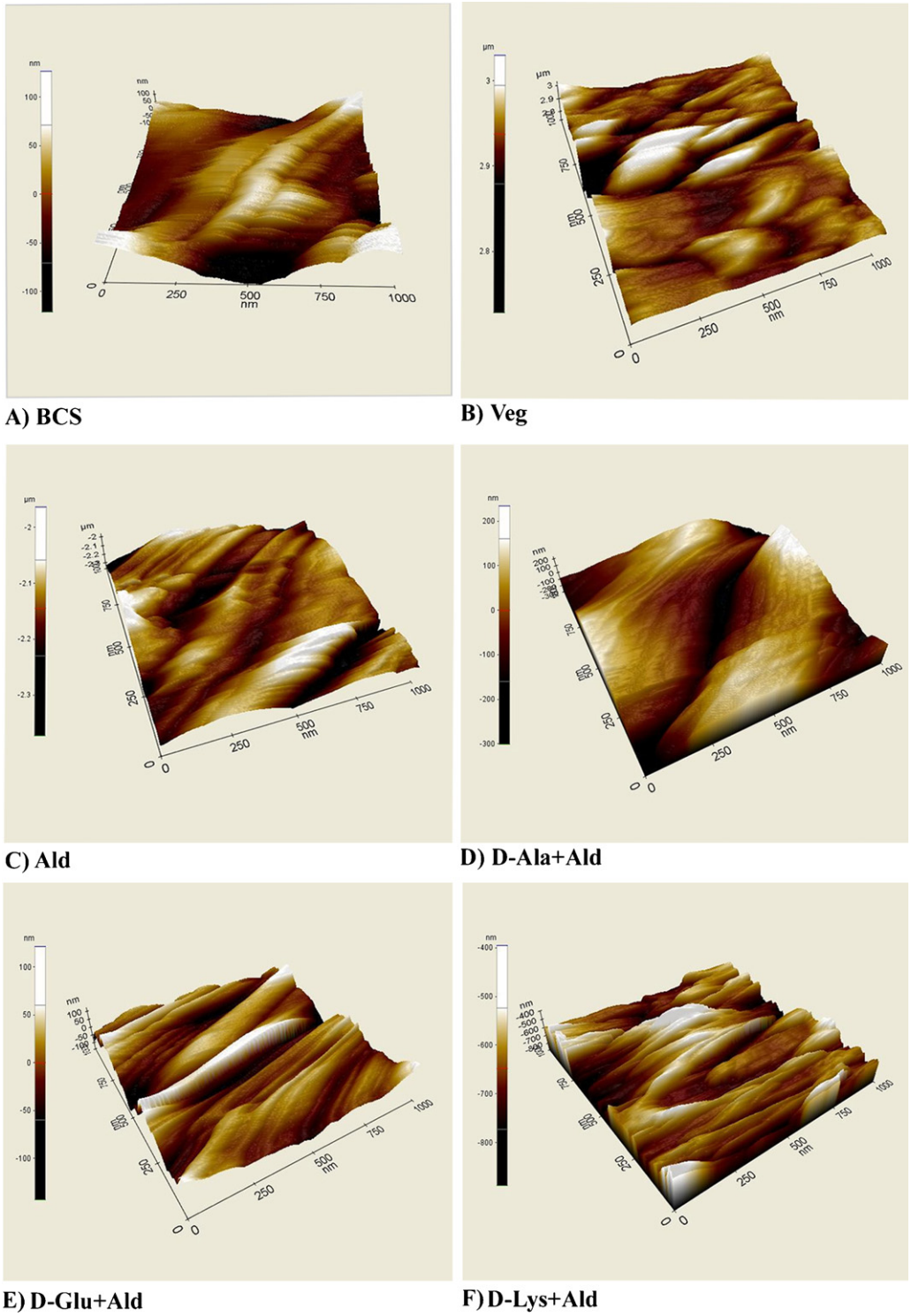


Fig. 2. Atomic force microscopic analysis of (A) BCS, (B) Veg, (C) Ald, (D) D-Ala+Ald, (E) D-Glu+Ald and (F) D-Lys+Ald tanned leathers. Vertical scale bar lighter areas correspond to higher topography and darker areas correspond to lower topography of tanned leather grain surface of the images. No significant differences between the typical tanned leather banding pattern of grooves and ridges.

collagen fibrils in BCS tanned leather (Fig. 2A) showed them to have a mean D-periodicity of 32 nm with a standard deviation of 9; measurements of Veg tanned leather (Fig. 2B) had a mean D-periodicity of 165 nm; Ald tanned leather (Fig. 2C) had a mean D-periodicity of 65 nm; presence of D-Ala (Fig. 2D) had a mean D-periodicity of 65 nm; presence of D-Glu (Fig. 2E) had a mean D-periodicity of 165 nm; and presence of D-Lys (Fig. 2F) had a mean D-periodicity of 166 nm. These distances are not significantly different and are characteristic of the axial periodicity of collagen fibrils.

While the D-periodicity did, neither vary nor there was a statistically significant difference between the widths of the fibrils. Measuring widths of fibrils are more difficult than measuring the D-periodicity and are also more subjective as the fibrils form overlapping networks where the sides of a fibril may not always be

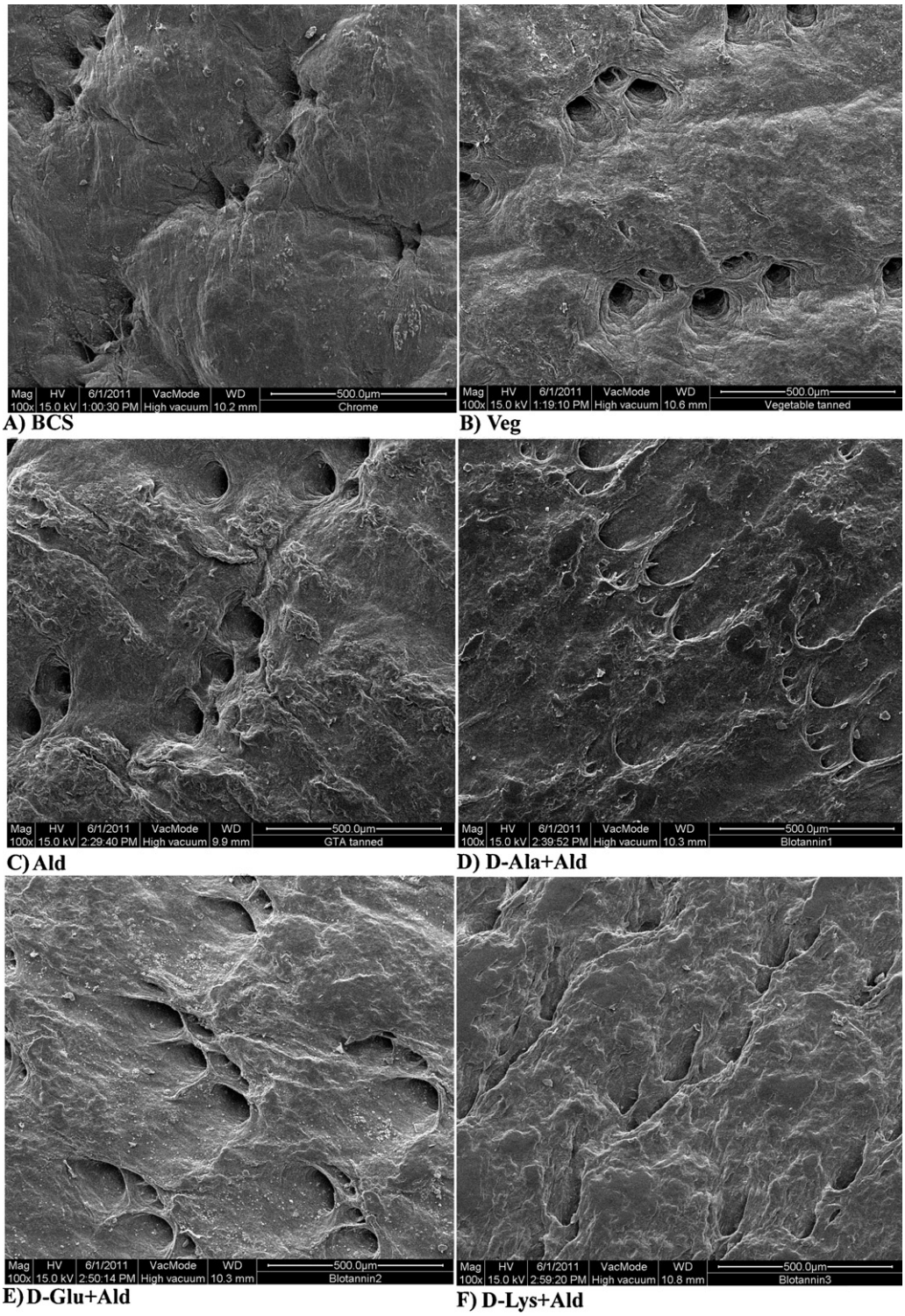


Fig. 3. Scanning electron microscopic image of grain surface of (A) BCS, (B) Veg, (C) Ald, (D) D-Ala+Ald, (E) D-Glu+Ald and (F) D-Lys+Ald tanned leathers.

clearly determined. The fibril widths measured can therefore only be considered as approximate. Measurements of collagen fibrils in BCS tanned leather (Fig. 2A) showed that they had a mean width of 123 nm with a standard deviation of 20; Veg tanned leather (Fig. 2B) had a mean width of 82 nm; Ald tanned leather (Fig. 2C) had a mean width of 72 nm; D-Ala+Ald tanned leather (Fig. 2D) had a mean width 92 nm; D-Glu+Ald tanned leather (Fig. 2E) had a mean width 92 nm; and D-Lys+Ald tanned leather (Fig. 2F) had a mean width 92 nm.

The ability of the collagen monomers to align in a quarter-stagger arrangement in the presence of D-Ala+Ald (Fig. 2D) shows that the size of these molecules does not hinder the formation of collagen fibrils. Although it has been shown that D-Glu+Ald results in the formation of reactive adducts of some AA residues found in collagen, which in turn may result in the formation of inter or intramolecular cross-links between collagen molecules, it does not appear to affect the structure or the arrangement of the collagen fibrils.

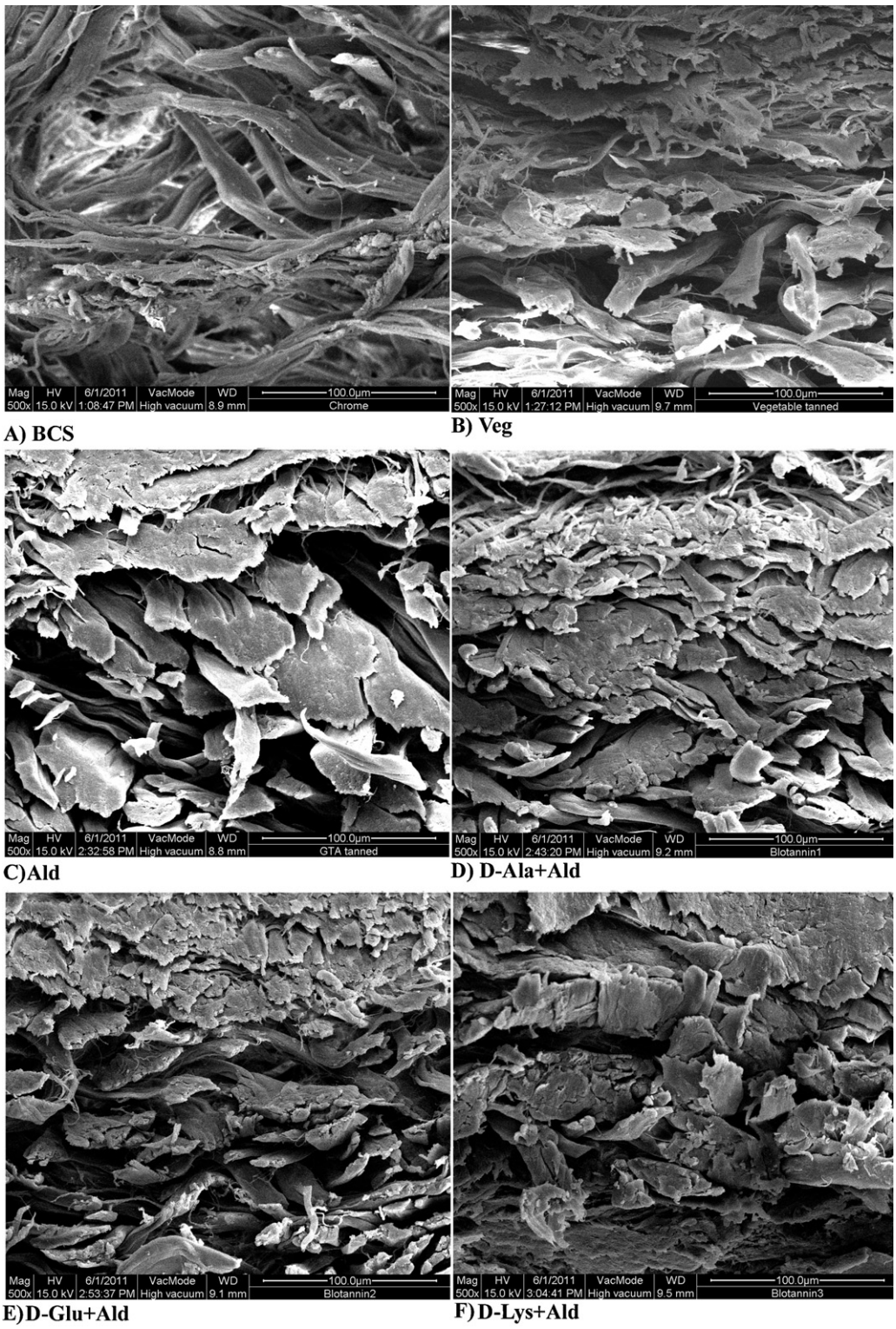


Fig. 4. Scanning electron microscopic image of cross section of (A) BCS, (B) Veg, (C) Ald, (D) D-Ala+Ald, (E) D-Glu+Ald and (F) D-Lys+Ald tanned leathers.

A similar study performed with D-Lys+Ald complexes showed (Fig. 2F) that though D-Lys helped in forming the quarter-stagger structure and induced a fibrous network of aggregated collagen molecules that lacked the D-periodicity, possibly because of size constraints. The presence of D-Lys with Ald during lateral assembly of collagen filaments decreased the rate of lateral disassembly thus helping a faster net rate of stable fibril formation. In our study with D-Lys+Ald, we do not observe an increase in collagen molecules lacking D-periodicity or a fibrous aggregated network. On the contrary, AFM analysis of collagen fibrils cross-linked with D-Lys+Ald revealed a well-ordered structure with the fibrils properly oriented and well aligned compared to collagen fibrils in the absence of D-Lys+Ald (Fig. 2F).

3.4. SEM analysis

The crust leathers can be assessed by viewing the grain surface and cross section of leather samples using SEM. The SEM analysis

of BCS and D-AA tanned leather samples showing grain surface are given in Fig. 3A–F. All the D-AA+Ald tanned leathers exhibit a clear grain surface, which indicates that there is no physical deposition. Higher magnification (1000×) SEM [not shown] confirms the above observation, where the hair follicles look clean without any foreign materials in all cases. The grain surface of the leather tanned using D-AA+Ald seems to be flat without any wrinkles compared to the leather tanned with the BCS. This could be due to the rapid reaction of the D-AA+Ald with the grain surface as against a mild and gradual fixation of chromium in chrome tanning. The SEM analyses of D-AA tanned leather samples showing cross section in a magnification of 500 are depicted and are given in Fig. 4A–F. The D-AA tanned leather shows the compactness in the fibers structure throughout the cross section indicating the uniform structure. In specific, D-Lys+Ald tanned leathers show more compact fiber structure compared to BCS. The fiber bundles seem to be less dispersed (separation of fibers) in the D-Lys+Ald tanned sample compared to a BCS tanned sample. Since the D-Lys+Ald tanned sample exhibits slightly dispersed (opened up) fiber structure, it would, in principle, exhibit an increased fullness and softness in the final leather.

3.5. Biodegradation

%Leather degradation (based on hydroxyproline released) for unnatural D-AA tanned leather by collagenase with various concentration of D-AA has been determined (Fig. 5A and B). Significant reduction in the degradation of leather is observed for D-AA tanned leather compared to chrome tanned leather. The D-Lys+Ald tanned leather exhibited 9% degradation of leather as against 99% degradation in the case of untanned (pickled skin) native at 96 h period of incubation. The stability of D-AA tanned leather against collagenase would have been brought about by protecting the active sites in collagen through interaction with D-AA recognized by collagenase. The significant differences in the enzymatic stability offered by D-Lys+Ald could be due to the effectiveness of the latter in exhibiting better interaction with collagen through multiple inter and intramolecular crosslinks. Lysine and hydroxylysine Ald-derived crosslinking between and among collagen molecules are major elements in stabilizing fibrillar collagens.

3.6. Environmental impact assessments

The impact of pickle less unnatural D-AA assisted tanning system on the environment was assessed by the spent tan liquor analysis for TSC, DS, SS, BOD and COD. It can be seen from Table 3 that the COD value in combination tanning is higher than in BCS tanning due to the applications of D-AA. While the TSC load value is lower than that of the BCS tanning process. The residues of D-AA assisted tanning in the effluent would become the part of soil. It is known that the pickling process increases the TSC of the effluent. In D-AA+Ald tanning, the pollution of neutral salt is significantly decreased. In comparison with BCS tanning, the BOD/COD value is greater for D-AA tanning than for BCS tanning, which demonstrates that the effluent of D-AA assisted tanning process is easily biodegradable than that of BCS tanning process.

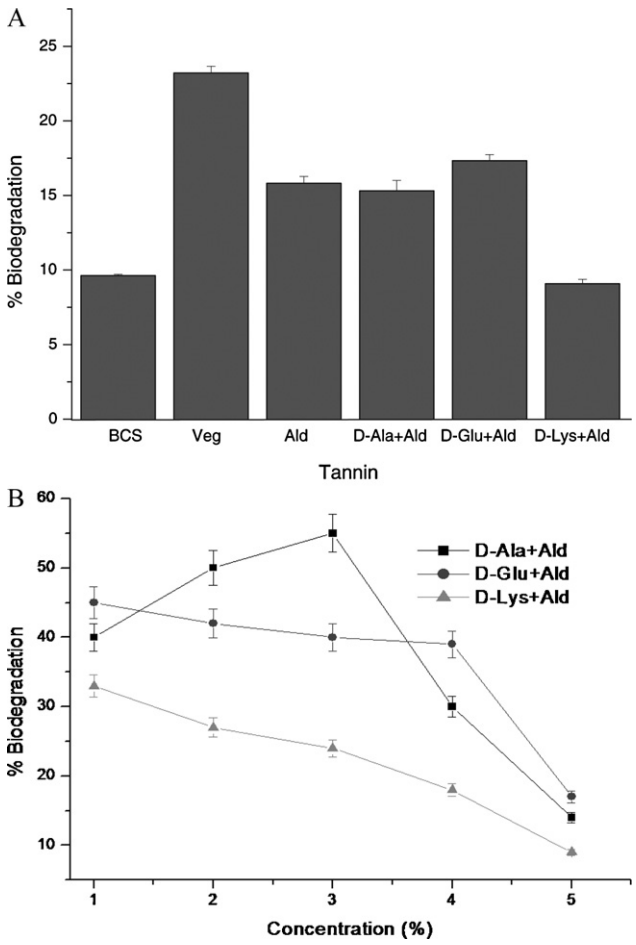


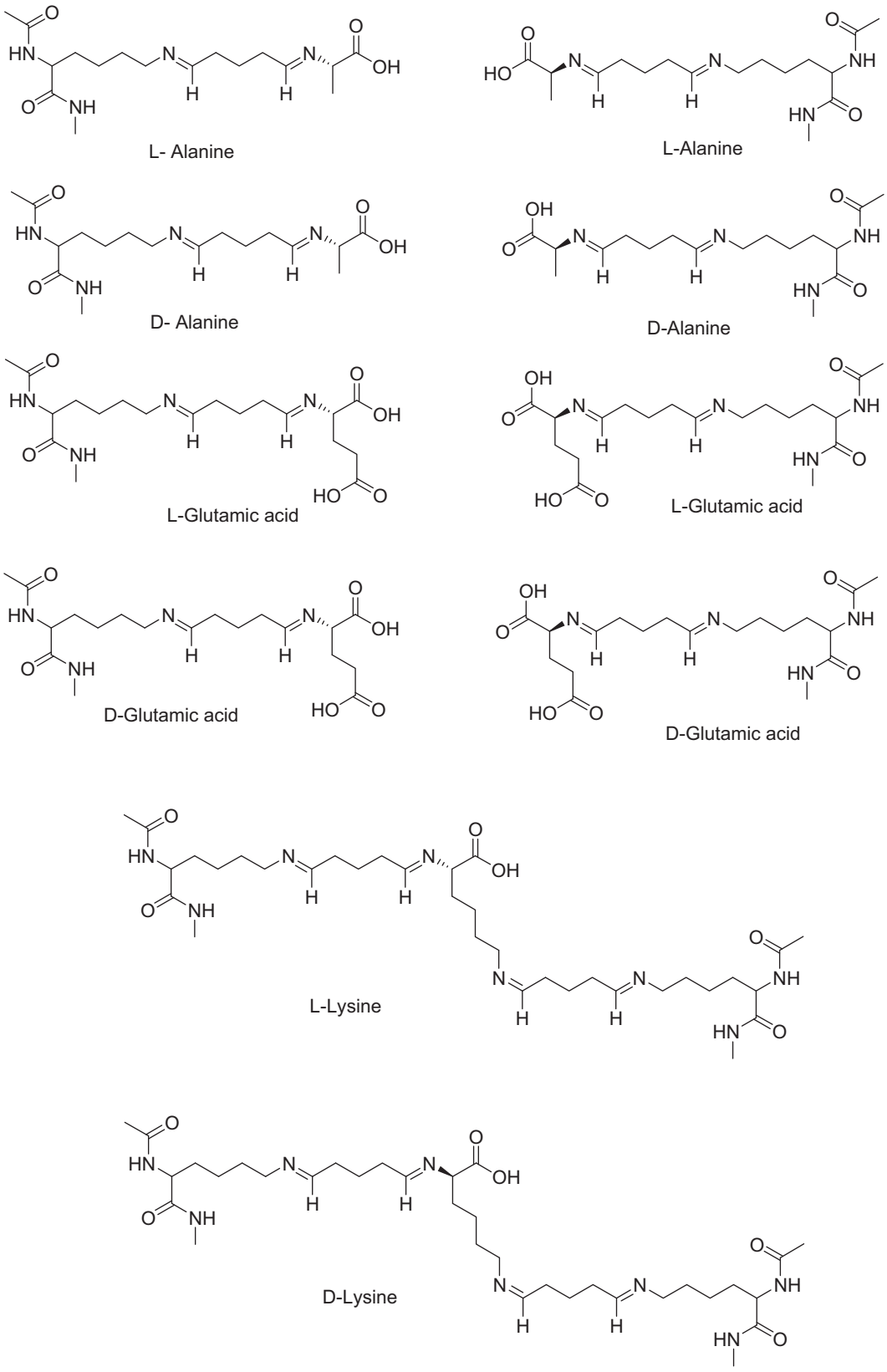
Fig. 5. The degradation of D-AA tanned leather by collagenase. (A) BCS, Veg, Ald, D-Ala+Ald, D-Glu+Ald and D-Lys+Ald tanned leathers; (B) various concentrations of D-Ala, D-Glu and D-Lys with constant Ald. The values reported are average of 3 experiments along with their standard errors (SE).

4. Discussion

Unnatural D-AA are non-genetically coded AA that either occur naturally or are chemically synthesized and becoming very important tools for chiral building block, conformational constraints, molecular scaffolds and also modern drug discovery and represent a nearly infinite array of diverse structural elements for the development of new leads in peptidic and non-peptidic compounds. Though natural and synthetic peptides formed of L-AA dominate applications in a wide variety of fields, it is largely felt that sooner the trend will favor peptides formed of D-AA [23]. The introduction of D-AA can significantly increase stability and resistance to proteolytic activity. Non-canonical AA in protein polymer design has been reviewed for development of biosensors, novel surfaces and materials [24]. To improve the structural consequences of D-AA in collagen triple-helical peptides have been studied. Also, recent

Table 3
Biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids content (TSC), dissolved solids (DS) and suspended solids (SS) analyses of unnatural D-AA tanned leathers.

Process	pH	COD (ppm)	BOD (ppm)	TS (ppm)	DS (ppm)	SS (ppm)
BCS	2.5–3	1000–2500	400–1000	30,000–60,000	29,000–57,500	1000–2500
Veg	4.0–5.0	1200–3000	500–1200	35,000–65,000	30,000–59,500	1300–2800
Ald	3.0–4.0	1200–3000	400–1000	20,000–40,000	25,000–48,500	800–1800
D-Ala+Ald	3.0–4.0	1200–3100	500–7000	16,000–37,000	24,000–45,000	500–1500
D-Glu+Ald	2.5–3.5	1100–3200	600–9000	18,000–38,000	22,000–42,500	700–1600
D-Lys+Ald	3.0–4.0	1300–3300	300–700	15,000–32,000	20,000–40,000	400–1400



Scheme 1. Schematic representation of proposed structure of side chain crosslinking of collagen with D-Ala, D-Glu and D-Lys. The results of crosslinking via amino groups of two collagen like peptides are directly joined via an amide bond also play the major role in stabilization of collagen in presence of Ald.

computational study suggested that replacement AA residues L to D-conformation would stabilize the collagen triple helix [25].

In the present study, skin/hide was tanned by Ald in the presence of three kinds of unnatural D-AA. The D-AA, i.e. D-Asp/Glu, D-Ala and D-Lys, that contain 1/2, 1/1 and 2/1 of NH_2/COOH groups, respectively, were selected as bridging agents to enhance the stability to leather. As expected, enhanced stability and resistance to collagenase activity for the leather can be surely gained, when the cross-linking is conducted in the presence of basic D-Lys. However, surprising results were obtained in the presence of D-Ala or D-Asp/Glu. The former has less efficiency to improve the stability of the leather, and the latter even results in a worse stability. In addition, D-AA can be better than L-AA as glycine surrogates, retaining protein function without perturbing protein structure have been studied [26,27]. The possible reason is that the addition of D-AA changed the ratio of NH_2/COOH in the crosslinking. There are two competing reactions in the cross-linking system: the bridging linking between leather collagen molecules under the assistance of the added D-AA, and the blocking of the functional groups of the collagen matrix by the D-AA reagents. The ratio of collagen NH_2/COOH will determine which kind of reaction is dominant (Scheme 1).

In general, the ratio of NH_2/COOH is about 2:3 in the goat skin. This is understandable that over the existence of one kind of group shall decrease the general cross-linking efficiency, leading to poor stability. For example, addition of Asp/Glu shall introduce more COOH groups than NH_2 groups, yielding far excess of COOH groups. One Glu molecule can just react with one amino group, so that the Glu not only loses the function of cross-linking bridge but also blocks a large amount of functional groups in the collagen molecules. As a result, the cross-linking efficiency is significantly decreased. In contrast to D-Glu, the addition of D-Lys can amend this unbalance of NH_2/COOH, resulting in higher tanning efficiency. Green methods for the self-aldol condensation of Ald have been conveniently accomplished by the catalytic action of Lys [28]. This can explain that D-Lys can enhance the stability and resistance to collagenases activity in a wide range of concentrations. D-Lys+Ald might also be involved in the process of polymerization of collagen networks. The permanent, D-AA+Ald crosslinking is associated with tough tanned leather that bears high loads and is subjected to minimal turnover and improves the mechanical strength of a particular quality, for example preventing lateral slippage between sub-elements of fibrils or offer a mechanism for interfibrillar bonding. D-AA along a peptide chain and protein fragments may be less digestible than the L-forms. D–D, D–L and L–D forms of peptide bonds partly or fully inaccessible to proteolytic activities have been studied.

Furthermore, the optically active D-AA, accompanied by the improvement of the mechanical, thermal and biological stability of leather can be expected. AFM and SEM were used for revealing the influence of D-AA+Ald treatment on the microstructure. The results indicate that the leather could preserve the structure during the cross-linking treatment in the presence of D-AA. Also D-Lys improves the hydrophobicity of the leather/collagen molecules. The introduction of D-Lys into collagen represents important advances in the chemistry of protein-based biomaterials. Isomerization improves the hydrophobicity of peptides containing D-AA has been studied [29]. These D-AA proteins and peptides are longer acting anti-microbial, antioxidant, anti-mutagenic and anti-carcinogenic activity than their L-enantiomeric analogues, because D-Ala, D-Ser and D-Glu/Asp are essential building blocks for peptidoglycan in bacterial cell walls. These unnatural D-AA, not usually found in natural proteins, have conformational attributes that are useful for the imposition of conformational stability, as structural probes and D-AA substitution to yield more stable protein.

5. Conclusions

Implementation of green chemistry approaches to cleaner production and pollution prevention measures can provide both economic and environmental benefits. A green chemistry or eco-friendly approaches to chrome-free tanning based on unnatural D-AA assisted tanning process has been developed. The properties of the tanned leathers are improved by D-AA with regard to texture, thermal stability, mechanical strength and resistance to collagenase activity. A further advantage is that the tanning wastewater is completely nontoxic and presents no disposal problems, either with regard to liquid waste or with regard to solid waste. In general, cleaner chrome-free tanning practice involves implementation of the following strategies: (1) able to paradigm shift from chemical to greener chemistry; (2) improvement of conventional tanning processes which helps to reduce the total solids contents (TS) and other toxic chemical outputs; (3) use of greener chemistry systems where the uptake of the tanning agent is greater and improve the structural hydrophobicity; (4) increase the stability of triple helical structure of collagen via intra and inter molecular crosslinks; (5) modify potential sites for physical, chemical and biological degradation; and (6) significant reduction in tannery waste of pollution loads, toxic chemicals and controls the environmental problems. These D-AA assisted tanning technologies are regarded as eco-friendly and hence, contribute to the development of cleaner process.

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