ICMIEE22-031

ECO-FRIENDLY TANNING PROCESS BASED ON AMINO ACID AND DIALDEHYDE

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ABSTRACT

Animal skin is basically a protein structure inside that can be stable through some crosslinking agent that can make it durable, and imputrescible with excellent properties and transform it into leather. In recent studies, tanning with unnatural amino acids like D-Lysine or D-Arginine combined with some aldehyde such as glutaraldehyde gives a direction towards implementing protein crosslinking reactions in leather tanning with dialdehyde. Usually, Maillard reaction happens in human body between collagen and three carbon methylglyoxal that generates from six carbon sugar. From that thought, we tried to carry out the Maillard reaction of protein crosslinking between the amino group of amino acids and carbonyl groups of sugars that leads to the crosslinking agent methylglyoxal. We designed the recipe for tanning from previous acquisitions and faced some complications in pH range for penetrating and fixing the chemicals to the collagen. Several re-tests were performed to validate the measurements of thermal stability change of the pelt. Finding the optimum activity condition for the collagen and chemicals might lead us to an eco-friendly method to leather tanning process. In that case, we were trying to find the optimum condition and interactive behavior of amino acids and methylglyoxal with collagen. The crosslinking activity of the methylglyoxal with amino acids and collagen in various pH ranges have been investigated to check the physiochemical properties of leather with respect to hydrothermal stability. The shrinkage temperature was determined after each re tests with shrinkage tester, which indicates the variation of outcomes with the variation of pH. The approach to do the crosslinking between dialdehyde and amino acid first and penetrating to leather also gave fluctuation in result with pH. In all the cases, we could not find our desired shrinkage temperature. In one case, to tan at pH 3.5 and fixation at 4.5, the shrinkage temperature was found above 70 degrees Celsius, but in other pH ranges, shrinkage temperature was below 70 degrees Celsius. The concept to make crosslink between chemical first and then adding it to a tanned bath, the shrinkage temperature was still below 70 degrees Celsius. The whole work gives the insight of two statements. Either the crosslinking with methyglyoxal like Maillard reaction is not possible in leather, as methylglyoxal doesn't have much tanning power on its own, or there might be some intertwining required to get the expected result. The activity of amino acid was checked by ninhydrin test and the activity of carbonyl group of methylglyoxal also checked. The outcome of our work indicate that the prospective shrinkage temperature is not possible with methylglyoxal and amino acid tanning, but there might be a good chance of getting positive output using glyoxal as a cross linker dialdehyde. As a result, shoe-upper leather cannot be produced if we use tanning chemicals methylglyoxal and amino acid. Methylgyoxal solo tanning produced shrinkage temperature up to 50 degrees Celsius, but combining with amino acid showed result up to 70 degrees before fixation process. There is still an immense possibility to find shrinkage in higher extent in final leather if glyoxal is used instead methylglyoxal. There might be some barrier or twisting holding us from finding a new era of leather science.

Keywords: Amino acids, tanning, greener approach, Maillard reaction, methylglyoxal

1. Introduction

Tanning is a technique that transforms raw hide into long-lasting leather by reacting tanning ingredients with the collagen protein in the hide. By improving mechanical qualities and storage capacity, as well as lowering putrefaction and encouraging chemically, thermally, and microbial resistance, these forms and structures the leather's added value [1]. A chrome tanning agent is now the most extensively utilized tanning chemical in the leather-making business, for performance, economic, and environmental reasons [2]. Cr (VI) poisoning is harmful to the human body and causes a variety of symptoms because Cr (III) is quickly oxidized to Cr (VI) under certain chemical circumstances. The chrome-tanning effluent, on the other hand, is more difficult to treat, putting a greater strain on the environment. Above all, this is a detriment to clean manufacturing and is in violation of REACH regulations (Registration, Evaluation, Authorization and Restriction of Chemicals). Chrome tanning agent is not considered an environmentally sustainable tanning agent in green chemical or cleaner manufacture [3]. As a result, scientists have conducted a series of studies on ecologically friendly materials and tanning procedures [4].

An inquiry on an environmentally acceptable way of employing unnatural amino acids without chrome tanning, which resulted in outstanding tanned leather tanning qualities, non-toxic tanning effluent, and zero solid waste discharge [4], where they have claimed shrinkage temperature >115°C. The consequence of crosslinking two collagen lysine side chains through amino groups are immediately connected via an amide bond and play a vital role in collagen stability in the presence of GTA [4].

The outcome of crosslinking is a direct amide connection between the amino group of collagen lysine and the side chain of arginine, which helps to stabilize collagen in the presence of GTA. The creation of a further Schiff base between one of the nearby -aldehyde groups and the second amino group of arginine might lead up to the arginine side [4].

In order to boost the physico-chemical stability, organoleptic properties and resistance to proteolytic

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activity, herein we've studied tanning process using the unnatural amino acids [5]. Protein crosslinking via the Maillard reaction with a-dicarbonyl compounds has been the topic of intense literature scrutiny. Glyoxal alone and together with wattle extract has been tried to ascertain a brand-new tanning system. Glyoxal because the solo-tanning agent, optimized at the extent of fifty, can produce shrinkage temperature up to level of 72 °C [6]. In some research they had reported a scientific study of three previously-neglected aspects of the reaction. Firstly, structural requirements were probed. An arginine-free peptide that contains two lysine residues, and a lysine-free peptide that contains arginine, were reacted with glyoxal, methylglyoxal and biacetyl. Methylglyoxal was ready to crosslink within the absence of arginine residues, but glyoxal and biacetyl weren't.

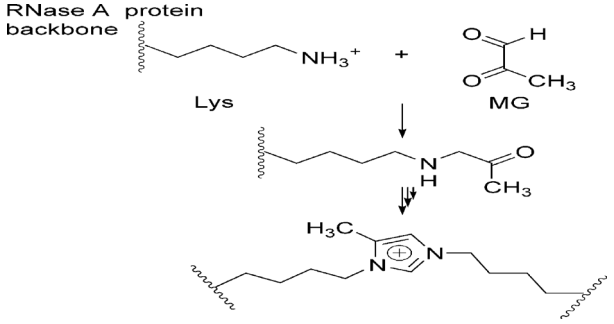


Fig. 1 Possible reaction pathways for lysine modification by methylglyoxal reaction with lysine in vitro [7]

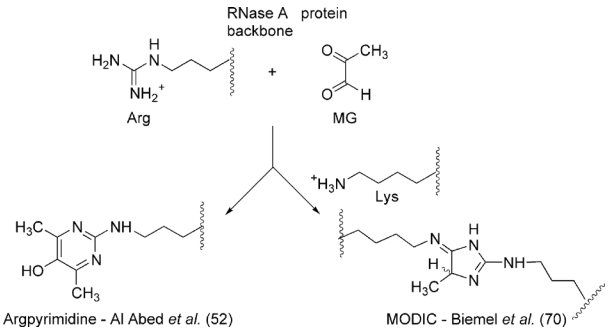


Fig. 2 Possible reaction pathways for arginine modification by methylglyoxal in vitro [7].

Glyoxal crosslinked the lysine-free peptide via the N-terminus, but methylglyoxal and biacetyl couldn't. During this study, crosslinking failed to require the presence of arginine but did require a free group, from a lysine residue, or the N-terminus [7]. That clearly gives us the pathway or idea to induce a crosslink formation using combination of:

- Methylglyoxal and Lysine
- Methylglyoxal first with Arginine then Lysine
- Methylglyoxal solo tanning

An amino group has a positive electrical charge in an acidic medium (low pH), whereas it has an opposite charge in a basic solution (high pH). There must be an intermediate pH where the amino acid is uniformly distributed across the two forms as dipolar zwitterions have a net charge of zero.

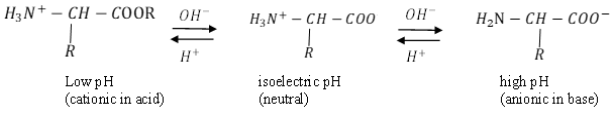


Fig. 3 IEP shifting of collagen

The isoelectric pH, also known as the isoelectric point, is the pH at which the isoelectric point exists.
Below are the isoelectric points of several common amino acids. It's worth noting that the isoelectric pH varies predictably depending on the amino acid structure.
Acidic amino acids:
Aspartic acid (2.8), Glutamic acid (3.2)
Neutral amino acids:
(5.0 to 6.3)
Basic amino acids:
Lysine (9.7), Arginine (10.8), Histidine (7.6)
Aspartic and glutamic acid side chains include acidic carboxyl groups. These amino acids have acidic isoelectric values of around pH 3. An acidic solution is essential to avoid de protonation of the second carboxylic acid group in order to keep the amino acid in its neutral isoelectric state.
Basic amino acids (histidine, lysine, and arginine) have isoelectric values of 7.6, 9.7, and 10.8, respectively. These figures represent the weak basicity of the imidazole ring, the intermediate basicity of an amino group, and the strong basicity of the guanidino group. To avoid protonation of the basic side chain and retain the amino acid electrically neutral, a basic solution is necessary in each situation. The remaining amino acids are termed neutral since their side chains are neither significantly acidic nor basic. Because the NH3+ group is somewhat more acidic than the COO^{-} group is basic, their isoelectric points are slightly acidic (from around 5 to 6).
To react with the collagen lysine or arginine of the COO^{-} of Lys-MG in solution, we must make the collagen negative (at high pH) for penetration and positive (at low pH) for fixation.
From that perspective have proposed some thoughts on a new process development based on unnatural amino acids and dialdehyde Methylglyoxal. We tried to find some optimum condition and environment for the methods we have developed. The book represents the obstacle and complication we have followed along.

2. Materials and methods

2.1 Materials

Table 1 Required chemicals for this thesis work

List of Chemicals	Use
L-Lysine	Tanning agent.
L-Arginine	Tanning agent.
Methylglyoxal	Tanning agent.
Sodium Formate	For fixation.
Sodium Bicarbonate	For depickling and fixation.
Formic Acid	For fixation.

Methylglyoxal, commercial grade (purity above 40%) collected from Creative scientific company, Dhaka, Bangladesh. Goat skin, purchased from Khulna, Bangladesh. L-Lysine Monohydrochloride and L-Arginine (purity 99%) were collected from Creative scientific company, Dhaka, Bangladesh that's chemical was used for Tanning. Sodium formate, sodium bicarbonate, wetting agent, sodium ash, Busan 40L, sodium sulphide, lime, ammonium sulphate, ammonium chloride, sodium metabisulphite, alkaline bating enzyme, NaCl, Sodium perchlorate, Formic acid, sulphuric acid for soaking, unhairing anywhere collected from our lab.

3. Methodology

3.1 Leather tanning with methylglyoxal

The pickled goat pelt was made by conventional process in our laboratory Khulna, Bangladesh. The tanning process was controlled by pickled goat pelt. The single pelt was divided into 4 parts and one part of pickled pelt with bath was treated with 1% sodium formate and 1% sodium biacarbinate for depickling in all cases.

Tanning was carried out by 4% methylglyoxal and agitated in a mug for 3 hours until penetration at pH 4.5 and then fixation was carried out by 1% sodium bicarbonate and in 4 installments at an interval of 15 minutes and checked pH 5.5.

Then the tanned leather was piled. After completed tanning,24h and 48h the shrinkage temperature was checked. Then the leather was washed with water and again we were trying to tanned the leather with 100% of water and 4% methylglyoxal and agitated in a mug for 3h at pH 4.5. And fixation was carried out by 1% formic acid at; pH 3.8. Then the leather was piled and after completed tanning, 24h and 48h check the shrinkage temperature.

3.2 Leather tanning with methylglyoxal and lysine

After depickling tanning was carried out by 4% methylglyoxal and 2%lysine and agitated in a mug for 3 hours until penetration at pH 4.5 and then fixation was carried out by 1% sodium bicarbonate and in 4 installments at an interval of 15 minutes and checked pH 5.5. Then the tanned leather was piled.

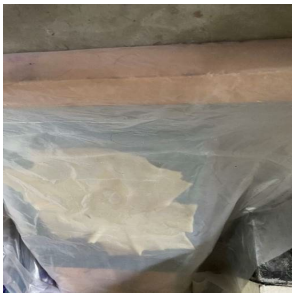
After completed tanning, 24h and 48h the shrinkage temperature was checked. Then the leather was washed with water and again we were trying to tanned the leather with 100% of water and 4% methylglyoxal and 2% of lysine and agitated in a mug for 3h at pH 4.5 and fixation was carried out by 1% formic acid at pH 3.8. Then the leather was piled and after completed tanning,24h and 48h check the shrinkage temperature. Then we were again trying to tan the leather. Firstly, we set and piled the Goat skin at pH 3.0 then next morning we washed the goat skin and remove unfixed acid from goat skin.

Then 100% water was taken and sodium bicarbonate was added and checked pH at 6.0. And then tanned the leather was 4% methylglyoxal and 2% lysine and agitated in laboratory shaker for 3h and fixation was carried out by formic acid and checked pH 4.0. The leather was piled and after 24h and 48h checked shrinkage temperature. Then we again try to tanned the leather at pH 3.5 and fixation was carried out at pH 5.5. And after completed tanning, 24h and 48h we checked shrinkage temperature.

3.3 Leather tanning with methylglyoxal, lysine and arginine

Divided the pickled pelt into three parts

Tanning was done in mug



Tanned leathers piled in inclined surface

Fig. 4 Process operations

After depickling tanning was carried out by 4% methylglyoxal and 2%lysine agitated in a mug for 2 hours and also added 2% arginine and run 1h and then until penetration at pH 4.5 and then fixation was carried out by 1% sodium bicarbonate and in 4 installments at an interval of 15 minutes and checked pH 5.5. Then the tanned leather was piled. After completed tanning, 24h and 48h the shrinkage temperature was checked.

Then the leather was washed with water and again we were trying to tanned the leather with 100% of water

and 4% methylglyoxal and 2% of lysine and agitated in a mug for 2h and 2% arginine was added and run at pH 4.5. And fixation was carried out by 1% formic acid at pH 3.8. Then the leather was piled and after completed tanning, 24h and 48h check the shrinkage temperature. Then we were again trying for tanned the leather. Firstly, we were set the the bath pH at 3.8. Then tanned the leather was 4% methylglyoxal and 2% lysine and agitated in a mug for 2h and also added 2% arginine and run 1h and fixation was carried out by sodium formate and checked pH 7.2. Then checked shrinkage temperature.

4. Results

4.1 Shrinkage temperature

At several pH ranges, we checked shrinkage temperature of tanned leather. We tried to determine the what ranges of pH for tanning the leather and the tanning chemical to fix into the leather. At pH shift to 4.0 before tanning then tanned the leather perfectly and we got shrinkage temperature of sample 71 degree Celsius. This result we got that was most exciting. But when we fixed the tanned leather at pH 5 then after 24 hours, we got the shrinkage temperature 61 degree Celsius and after 48 hours we got shrinkage temperature 54 degree Celsius. So, our result was decreased gradually. The optimum pH range for fixation of chemicals after crosslinking couldn't be measured.

Table 2 T_s test of Methylglyoxal solo tanning

Step 1:			
	pH	Time	T_s
		After Tanning	70°C
Tanning	4.5	24h	57°C
Fixation	5.5	48h	55°C
Step 2 (in the same bath):			
	pH	Time	T_s
		After Tanning	54°C
Tanning	4.5	24h	54°C
Fixation	3.8	48h	50°C

Table 3 T_s test of Methylglyoxal + Lysine tanning

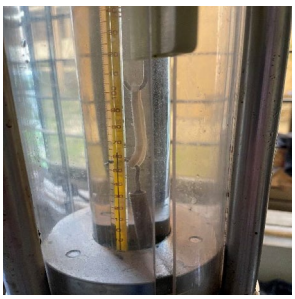
Step 1:			
	pH	Time	T_s
		After Tanning	70°C
Tanning	4.5	24h	55°C
Fixation	5.5	48h	51°C
Step 2 (in the same bath):			
	pH	Time	T_s
		After Tanning	54°C
Tanning	4.5	24h	54°C
Fixation	3.8	48h	54°C
Step 3 (in the same bath):			
	pH	Time	T_s
Tanning	3.5	24h	57°C
Fixation	5.0	48h	51°C
Step 4 (in a new bath with 200% water solution)			
	pH	Time	T_s
Tanning	6.0	24h	45°C
Fixation	4.0		

Table 4 T_s test of tanning with Methylglyoxal + Lysine + Arginine

Step 1:			
	pH	Time	T_s
		After Tanning	71°C
Tanning	4.5	24h	61°C
Fixation	5.5	48h	57°C
Step 2 (in the same bath):			
	pH	Time	T_s
		After Tanning	54°C
Tanning	4.5	24h	54°C
Fixation	3.8	48h	54°C
Step 3 (in the same bath):			
	pH	Time	T_s
		After Tanning	58°C
Tanning	5.0	24h	57°C
Fixation	7.2	48h	51°C

Acid swelling at step 4 for MG + Lys tanning

Ts found at step 1 for MG + Arg + Lys



Ts found after 24 hours with Arg+MG+Lys tanning

Ts during fixation at different pH ranges

Fig. 5 Test for shrinkage temperature

4.3 Ninhydrin test

We used some chemicals for carried out tanned the leather. So, we checked the effectiveness of chemicals (L-lysine, L-arginine). So, we checked effectiveness of chemicals by ninhydrin test. In this test we seen that Lysine give the positive result. But arginine gave negative result.

Table 5 Test results for ninhydrin test

Chemicals Name	Observation	Comments
L-Lysine	Purple colored complex formed	Positive
L-Arginine	No color / no complex formed	Negative

4.4 Biuret test

Biuret test was carried out in solution for peptide bond present in solution. We were carried out this test for when we got shrinkage temperature 45 degree Celsius that was very less. So, we did not understand why the shrinkage decreases so much. So, we were thought that some peptide bond was came into solution from collagen. But when we done the test, we found negative result that means collagen structure was not rupture.

Table 6 Test result for Biuret test of solution

Sample collection	Observation	Comments
Solution of new sample bath of methylglyoxal + lysine tanning at last pH 4	Blue color formed	Negative

4. Conclusion

Ts findings of our studies gave us some insights about work plan and chemical natures. Shrinkage temperature shifted up at initial tanning, but couldn't hold the outcome for long, as suspected some problem in fixing. It might happen due to penetration of methylglyoxal at first what couldn't be fixed.

Major findings:

• Due to shifting, the pH in the same bath several times might change the reactive condition of the bath for the agents.

• During planning the recipe, collagen's reactivity

As a result, the reactivity of the unnatural amino acids used in tanning is a significant influence in chemical crosslinking, which was not shown in the earlier study on amino acid tanning.

• From recent studies, the recipe designs the crosslinking of the chemicals was formed in the tanning bath, but it could be tried to do it initially and then apply it to the tanning.

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NOMENCLATURE

GTA : Glutaraldehyde
Lys : Lysine
Arg : Arginine
IEP : Isoelectric Point
Ts : Shrinkage Temperature, ^{o}C
Cr : Chromium