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Amino Acids Extraction from Hair Dissolving Liming Waste to Reduce Pollution in Tannery

Sadia Mim, Anika Tabassum, and Md. Abul Hashem[*]
Department of Leather Engineering, Khulna University of Engineering & Technology, Khulna-9203, BANGLADESH

ABSTRACT
In leather processing, hair/wool and epidermis are removed in liming operation and discharged as waste. These liming wastes consist of amino acids also yield to wastes. In this study, an investigation was made to extract an amino acids reductive-oxidative method from hair dissolving liming waste to reduce environmental pollution. The hair dissolving liming waste was collected and treated with sodium sulphide (Na_2S) and hydrogen peroxide (H_2O_2). The decanted solution was subjected for the presence of different amino acids was confirmed by different tests e.g., Sakaguchi test, xanthoproteic test, lead sulphide test etc. The tests have confirmed the presence of amino acids namely phenylalanine, tyrosine, tryptophan, cysteine and arginine. The extraction of amino acids from the hair dissolving liming wastes could be a solution to reduce pollution in tannery especially hair dissolving liming operation.

Keywords: Keratin, Hair dissolving liming, Protein, Pollution, Extraction

1. INTRODUCTION

Leather industry plays an important role in Bangladesh Economy due to its importance as a labour-based export-oriented industry [1]. Leather industry, one of the most polluting industries, generates a huge amount of solid and liquid wastes with the emission of obnoxious smell for the degradation of the proteinous material of the animal skin [2]. Solid wastes including raw trimmings, fleshings, chrome shavings, buffing dust and keratin wastes are being produced from the industries [3]. Hence, the resultants of all those wastes are responsible for pollutions as they are thrown to landfills or other wetlands. Also, treatment of solid wastes is not cost effective, posing an economic burden to the tanners [2]. Tannery wastes contain protein, which could be extracted for proper utilization; otherwise, it will pollute the environment that will be a threat for the human beings [4].

Keratin, a fibrous protein forming a main structural constituent of the feather, hair, wool, horn, hoof etc., is abundantly available as a by-product from the poultry, slaughterhouse, tanning and fur processing industry [5]. It is a ubiquitous biological material representing a group of insoluble, usually high-sulfur content and filament-forming proteins, constituting the bulk of epidermal appendages [6]. Extensive quantities of keratinase by-products are disposed of annually by animal-processing industry, causing a mounting ecological problem due to the extreme resilience of these materials to enzymatic breakdown [7]. Though keratins have applications in food, pharmaceutical, cosmetic and fertilizer industry, a considerable amount of these products is being wasted repeatedly. It's difficult to perform the degradation of keratin and their disposal causes environmental pollutions [5]. Research is being performed globally for utilization of these wastes and to apply cheap as well as environment-friendly methods to recycle keratinase wastes [7]. Keratin having high immunity to physical and chemical factors, searching for new methods of keratin waste conversion is usually found. It reduces a problem with storage of solid wastes [8]. Keratin is a biopolymer that contains about 18 amino acids especially in the helical regions of their structure [9]. The main amino acids are cysteine, cystine, arginine, serine, glycine and very little histidine, tryptophan and methionine where the presence of a high portion of cystine disulphide linkages is noticed [9].

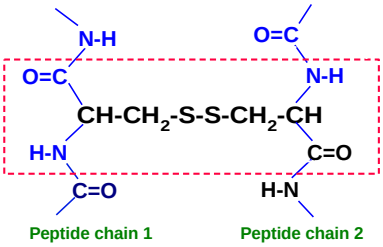

Fig.1 Representation of the cysteine disulfide crosslinks between polypeptides

The pretanning and tanning processes contribute 80–90% of the total pollution load like biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS), chromium (Cr), sulphur (S), sludge etc. and toxic gases like ammonia (NH_3) and hydrogen sulfide (H_2S) are also emitted [10]. Nonetheless, most of the pollutions and contaminations generally derive from the unhairing or liming operations. Liming and unhairing wastes contain mainly solid wastes like limed hair/wool and chemicals. The main reasons work behind pollutions is simply all the chemicals used in liming or unhairing processes. The liming of waste is supposed to stop biodegradation and reduce the impacts of landfilling activity [11].

Hair and wool contain a huge amount of amino acids. When these limed hair/ wool are thrown to earth it causes environmental pollution as liming of hair is

* Corresponding author. Tel.: +88-01674590373
E-mail addresses: mahashem96@yahoo.com

supposed to stop biodegradation. Others the valuable amino acids in the limed hairs are also being wasted. To reduce the pollutions is a huge challenge as it takes quite tough and longterm processes by using several different chemicals. So utilizing these pollutants as bi-products, minimization of environmental pollutions at a certain level can be done and valuable amino acids can also be obtained in a simple way by using cheap and available chemicals like Na_2S and H_2O_2. So, the tanners can easily use this method of extraction for treating the limed hair/wool, which is cost effective without posing an economic burden. As this valuable by-product like limed hair and wool are not used in our country and considered as useless waste, so by this extraction method, the demand for amino acids can be full filled for our country. At present, amino acids are used in various branches of industry. It can be a better remedy to reduce environmental pollutions caused during liming operations.

Amino acids are used in various branches of industry as food additives and surface-active agents, in the production of polymeric materials, in electrochemical manufacture etc. [12]. Amino acids are widely used in biotechnology applications and being natural compounds, they can be safely used in pharmaceutical applications [13]. Amino acid-based surfactants have an amino acid residue as a hydrophilic moiety [14].

Amino acids are used for a variety of applications in the industry mainly as additives to animal feed. This is necessary for feeds like soybeans having low levels or lacks some of the essential amino acids like lysine, methionine, threonine and tryptophan [15]. The food industry is a major consumer of amino acids, mainly glutamic acid, used as a flavour enhancer [16] and aspartame as a low-calorie artificial sweetener [17]. Also used for animal nutrition employed in the human nutrition industry to alleviate symptoms of mineral deficiencies like anaemia by improving mineral absorption [18].

The chelating ability of amino acids is used in fertilizers for agriculture to facilitate the delivery of minerals to plants in order to correct mineral deficiencies, such as iron chlorosis. These fertilizers are also used to prevent deficiencies from occurring and improving the overall health of the plants [19]. The remaining production of amino acids is used in the synthesis of drugs and cosmetics [15].

The main purpose of the study is to extract amino acids from the hair dissolving liming waste to reduce pollution load in a tannery.

2. MATERIALS AND METHODS

2.1 Sample collection

Hair dissolving liming wastes was collected from the leather manufacturing workshop, Department of Leather Engineering, KUET, Bangladesh during the unhairing and liming process. The collected samples were washed eventually with distilled water and dried at room atmosphere. Fig.2 shows the hair and wool samples.

Goat hair Sheep wool

Fig.2 Hair and wool samples of goat and sheep

2.2 Reagents and chemicals

Commercial sodium sulphide (Na_2S, Merck, India), hydrogen peroxide (H_2O_2 30%, Merck, India), copper sulphate ($CuSO_4$, Merck, India), sodium hydroxide (NaOH, Loba, India), nitric acid (HNO_3, Merck, Germany), lead acetate ($Pb(CH_3COO)_2$, Merck, India), 1-naphthol (Loba, India), bromine solution (Merck, India), glacial acetic acid (Loba, India), acetic acid (Loba, India), sulphuric acid (H_2SO_4, Merck, Germany), millions reagent (Merck, India), ammonium carbonate (Loba, India) were procured from the local scientific store, Khulna, Bangladesh. The urea was procured from Chittagong urea fertilizer Ltd., Chittagong, Bangladesh.

2.3 Treatment of limed hair and wool

The collected limed hair and wool were treated with the sodium sulphide. After dissolving hair/wool, hydrogen peroxide was for oxidation. Fig.3 shows the treated hair/wool.

Goat Hair Sheep wool

Fig.3 Treated hair/wool with Na_2S and H_2O_2

Table 1 Amounts of wool, Na_2S and H_2O_2

Sample ID	Amount (g)	H_2O (mL)	Na_2S (g)	H_2O_2 (mL)
Wool	5.1477	400	4.5917	5
Limed hair	149.1048	500	4.4824	35

2.4 Decantation

The mixture was decanted for the separation of the immiscible liquid sample from the solid.

2.5 Centrifugation
The entire amount of decanted liquid was centrifuged for 5 minutes to remove the undissolved solid materials.

Fig.4 Centrifugation of decanted liquid

2.6 Filtration
After centrifugation of both of the wool and limed hair solution, these were undergone through filtration to scan out the settled solids from the clear solution.

2.7 Confirmation test for peptide bond

2.7.1 Biuret test
The biuret test (Piotrowski's test) is usually performed for detecting the presence of peptide bonds. The biuret test was done using copper sulphate solution and sodium hydroxide solution [20].
At first 5 mL of sample solution of both of the wool and limed hair were taken into two test tubes. Then, 5 mL of sodium hydroxide solution was mixed with both of the test tubes containing both of the samples. Nearly, 5-6 drops of copper sulphate solution were added to both of the samples containing test tubes, shaken speedily and kept for a while in order to observe the visual colour change.

2.7.2 Xanthoproteic test
This test is usually performed for differentiating between aromatic amino acids such as phenylalanine, tyrosine, tryptophan etc. [21].
This test was performed by adding concentrated 2/3 mL HNO_3 in the sample solution in a test tube. Then, it was cooled under running tap water. About 10 drops of NaOH was then added to the test tube and wait for colour change.

2.7.3 Lead sulphide test
This test is mainly performed for identifying sulphur containing amino acids, such as cysteine and cystine [22].
Lead sulphide test was done with using sodium plumbate solution and sodium hydroxide solution. Sodium plumbate solution was made by 5 mL of NaOH (diluted) was added to 2 mL of lead acetate where a white precipitation of $Pb(OH)_2$ formed and then it was boiled until precipitation dissolves.
At first 2 mL of the sample solution was boiled with a few drops of NaOH for 2 minutes. It was then cooled and a few drops of sodium plumbate solution was added to it and wait for colour change.

$$\text{Cysteine} + 2\text{NaOH} = \text{Na}_2\text{S} \text{ (In presence of heat)}$$
$$\text{Na}_2\text{S} + \text{Pb}(\text{CH}_3\text{COO})_2 = \text{PbS} + 2\text{CH}_3\text{COONa}$$

2.7.4 Sakaguchi test
The Sakaguchi test is usually used for detecting the presence of arginine in proteins [23].
A few drops of 40% NaOH solution was added to 1 mL of sample solution taken in a test tube. Then, few drops of the solution of 1-naphthol were added to it. After that few drops of 5% urea solution was added to it and then bromine solution was added to it and waits for colour change.

2.7.5 Hopkins Cole Test
The Hopkins-Cole test is usually used for detecting the presence of tryptophan in proteins [24].
The indole moiety of tryptophan reacts with glyoxilic acid in the presence of concentrated sulphuric acid to give a purple coloured product. Glyoxilic acid is prepared from the glacial acetic acid by being exposed to sunlight [22].
At first 1mL glyoxylic acetic acid reagent was added to 1mL of sample solution taken in a test tube. Then a few drops of concentrated sulphuric acid were then added to it and wait for the result.

2.7.6 Millons test
The Millons test is performed for detecting the presence of tyrosine in proteins [25]. A few drops of the reagent are added to the test solution, which is then heated gently. A reddish-brown colouration or precipitate indicates the presence of tyrosine residue, which occurs in nearly all proteins [25].
At first, few drops of Millons reagent were added to 1mL of sample solution taken in a test tube and shaking was performed. Then it was gently kept in the water bath for 10 minutes and wait for the colour change.

2.7.7 Histidine test
This test was performed to detect the presence of histidine in proteins [22]. This reaction involves bromination of histidine in acid solution, followed by neutralization of the acid with an excess of ammonia. Heating of alkaline solution develops a blue or violet colouration [22].
At first 5% bromide in 33%, the acetic acid solution was prepared in a volumetric flask. Then a few drops of this solution were added to 1mL of sample solution taken in a test tube. Then the test tube was kept for 10 minutes. After that, a few drops of ammonium carbonate solution was added to it. Then it was kept in the water bath for 10 minutes for a colour change.

2.8 Extraction of amino acids in solid phase
The obtained solutions were placed in the oven at 60°C until total solution dries out and take into the powder form. Approximately after 5 days expected dried out

sticking solid materials were obtained of both of the samples. Then by using a spatula very gently the dried out solid materials were dug off thus producing powdered materials from the solutions.

Limed Hair Sheep Wool

Fig.5 Extracted pulverulence of limed hair and wool sample

2.8 Performance of FT-IR

Fourier-transform infrared spectroscopy (FT-IR) technique was used to obtain an infrared spectrum of absorption or emission of the desired solid powder form by using SHIMADZU FT-IR in the mid regions (4000-500 cm^{-1}).

3. RESULTS AND DISCUSSION

3.1 Characterization of extracted amino acids

3.1.1 Biuret test result

The colour of both of the samples was converted into a light violet colour indicating a positive result. Thus biuret test of both of the samples of wool and limed hair indicates that these samples contain enough amino acids, which were extracted from further powder formation.

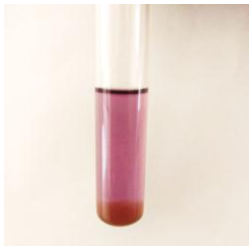

Fig.6 Biuret test

3.1.2 Xanthoproteic test result

After getting the confirmation of amino acids in a sample solution by using the biuret test, the xanthoproteic test was performed.

Fig.7 Xanthoproteic test

A yellow colour was formed that indicates the positive result of this test with the existence of phenylalanine, tyrosine, and tryptophan in the sample solution.

3.1.3 Lead sulphide test result

After getting the confirmation of amino acids in a sample solution by using the biuret test, lead sulphide test was performed. A blackish-brown colour of PbS during testing indicates the positive result of this test with the existence of cysteine in the sample solution.

Fig.8 Lead sulphide test

3.1.4 Sakaguchi test result

After getting the confirmation of amino acids in a sample solution by using the biuret test, Sakaguchi test was performed. Here the appearing red colour during testing indicates the positive result of this test with the presence of arginine in the sample solution.

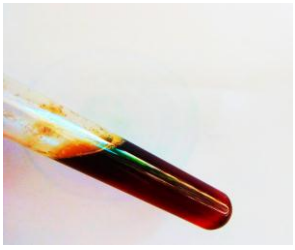

Fig.9 Sakaguchi test

3.1.5 Hopkins Cole test result

After getting the confirmation of amino acids in a sample solution by using the biuret test, Hopkins Cole test was performed. There was not developed any purple violet ring in the test tube that indicates the negative result of this test that means tryptophan was absent.

Fig.10 Hopkins Cole test

3.1.6 Millons test result

After getting the confirmation of amino acids in a sample solution by using the biuret test, Millons test

was performed. There did not occur the creation of reddish brown colour that indicates the negative result of this test that means tyrosine was absent.

Fig.11 Millons test

3.1.7 Histidine test result

After getting the confirmation of amino acids in a sample solution by using the biuret test, Histidine test was performed. No blue colour formation did not appear that indicates the negative result of this test and absence of histidine.

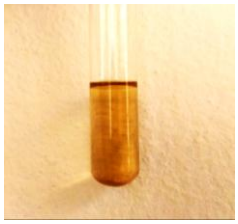

Fig.12 Histidine test

Table 2 Amino acids test result

Test	Result
Biuret test	Presence of protein
Xanthoproteic test	phenylalanine, tyrosine, tryptophan
Lead sulphide test	Cysteine
Sakaguchi test	Arginine
Hopkins Cole test	-
Millons test	-
Histidine test	-

The main amino acids in the hair and wool are histidine, lysine, glycine, arginine, cystine, tryptophan and phenylalanine. So the positive result of performing test indicates that the aim of extraction of amino acids was successful.

3.2 FT-IR result

FT-IR spectrometers are mostly used for measurements in the mid and near IR regions [26]. For the mid-IR region, 2−25 µm (5000–400 cm−1), the most common source is a silicon carbide element heated to about 1200 K. The output is similar to a blackbody [26].

Shorter wavelengths of the near-IR, 1−2.5 µm (10000–4000 cm−1), require a higher temperature source, typically a tungsten-halogen lamp [26].

The long wavelength output of these is limited to about 5 µm (2000 cm−1) by the absorption of the quartz envelope. For the far-IR, especially at wavelengths beyond 50 µm (200 cm−1), a mercury discharge lamp gives a higher output than a thermal source [26].

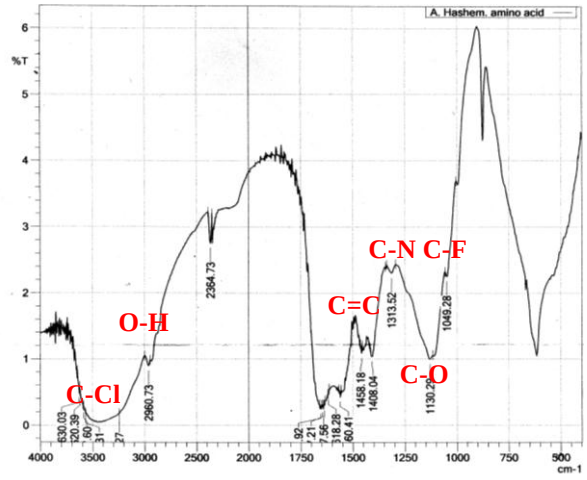

Fig.13 Graphical representation of FT-IR

A graphical representation was obtained from FT-IR where the graph shows different peak points of different wavelength indicating different groups. These groups are present in different amino acids like phenylalanine, tyrosine, tryptophan etc that indicates also a positive result of this process.

After all, amino acids extraction from limed burnt hair minimizes pollution rate. Sample solutions contain phenylalanine, tyrosine, tryptophan, arginine, cysteine which can be used in animal feeds, drugs and cosmetics. The main applications of amino acids are in cosmetic products like keratin shampoo, serum, nail polish products, hair damage repairing products, thermal resistant products in hair straightening, curling, perming and in so many other biomedical uses. The obtained phenylalanine can be used for aspartame production, tryptophan for antioxidants, tyrosine for infusions, arginine for therapy of liver diseases, children growth, cosmetics etc.

4. CONCLUSION

Various amino acids e.g., phenylalanine, tyrosine, tryptophan, cysteine, and arginine are extracted from the hair dissolving liming waste. Amino acid was extracted in a simple reductive-oxidative method with common chemicals. The hair dissolving liming waste could be a great source of amino acids as well as will be reduced pollution in tannery. The extracted amino acids are necessary in our daily life for various purposes. It is the base line experiment and authors need more experiment to development suitable method for amino acids extraction.

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REFERENCES

[1] M. R. Azom, K. Mahmud, S. M. Yahya, A. Sontu, S. B. Himon, Environmental Impact Assessment of Tanneries: A Case Study of Hazaribag in Bangladesh, *International Journal of Environmental Science and Development*, Vol. 3, pp 152-156, (2012).

[2] J. Kanagaraj, M. Rafiuddin Ahmed, N. Samivelu, R. Jayakumar, High Exhaust Chrome Tanning Using Fleshing Hydrolysate, *Journal of American Leather Chemists Association*, Vol. 97, pp 207-214, (2006).

[3] www.bioenergyconsult.com/waste-from-tanneries.

[4] H. Jiang, J. Liu, W. Han, The Status and Developments of Leather Solid Waste Treatment: A mini-review, *SAGE Journals*, Vol. 34, pp 399-408, (2016).

[5] R. Karthikeyan, S. Balaji, P. K. Sehgal, Industrial Applications of Keratin-A Review, *Journal of Scientific and Industrial Research*, Vol. 66, pp 710-715, (2007).

[6] B. Wang, W. Yang, J. McKittrick, M. A. Meyers, Keratin: Structure, Mechanical Properties, Occurrence in Biological Organisms and Efforts in Bioinspiration, *Progress in Materials Science*, Vol. 76, pp 229-318, (2016).

[7] W. Laba, A. Rodziewicz, Biodegradation of Hard Keratins by Two *Bacillus* Strains, *Jundishapur J Microbiol*, Vol. 7, (2014).

[8] P. Staroń, M. Banach, Z. Kowalski, A. Staroń, Hydrolysis of Keratin Materials Derived from Poultry Industry, *Proceedings of ECOpole*, Vol. 8, pp 443-448 (2014).

[9] D. H. Simmonds, The Amino Acid Composition of a Keratin Derivative Extracted from Wool with Alkaline Thioglycollate Solution, (1954).

[10] P. Thanikaivelan, J. R. Rao, B. U. Nair, T. Ramasamai, Recent Trends in Leather Making: Processes, Problems and Pathways, *Critical Reviews in Environmental Science and Technology*, Vol. 35, pp 37-79, (2005).

[11] C. Delolme, F. Jabob, J. Perrier, Impact of Liming Waste on Liming Activity and Leachate Characteristics: A Laboratory and Field Scale Approach, *Waste Management & Research*, Vol. 16, pp 160-174, (1998).

[12] M. S. Sadovnikova, M. V. Belikov, Industrial applications of amino acids, *Russian Chemical Reviews*, Vol. 47, pp 2, (1978).

[13] T. Arakawa, K. Tsumoto, Y. Kita, B. Chang, D. Ejima, Biotechnology applications of Amino Acids in Protein Purification and Formulations, *Amino acids*, Vol. 33, pp 587-605 (2007).

[14] M. Takehara, Properties and Applications of Amino Acid Based Surfactants, *Colloids and Surfaces*, Vol. 38, pp 149-167, (1989).

[15] W. Leuchtenberger, K. Huthmacher, K. Drauz, Biotechnological Production of Amino Acids and Derivatives: Current Status and Prospects, *Applied Microbiology and Biotechnology*, Vol.69, pp 1–8, (2005).

[16] S. Garattini, Glutamic acid, Twenty Years Later, *The Journal of Nutrition*, Vol. 130, pp 901-909, (2000).

[17] L. D. Stegink, The Aspartame Story: A Model for the Clinical Testing of a Food Additive, *The American Journal of Clinical Nutrition*, Vol. 46, pp 204-215, (1987).

[18] Albion Laboratories Inc., *Albion Ferrochel Website*, (2011).

[19] H. D. Ashmead, Foliar Feeding of Plants with Amino Acid Chelates, *Park Ridge: Noyes Publications*, (1986).

[20] C. J. Fenk, N. Kaufman, D. G. Gerbig, *J. Chem. Educ*., Vol. 84, pp 1676-1678, (2007).

[21] Chatterjea, Textbook for Biochemistry for Dental/Nursing/Pharmacy Students, *Jaypee Brothers Publishers*, pp 5, (2004).

[22] Qualitative Analysis of Amino Acid, *vlab.amrita.edu*, (2011).

[23] S. Sakaguchi, Über Eine Neue Farbenreaktion Von Protein und Arginin, *J. Biochem*., Vol. 5, pp 25–31 (1925).

[24] R. A. Joshi, Question Bank of Biochemistry, *New Age International*, pp 64 (2006).

[25] E. O'F. Walsh, An Introduction to Biochemistry, *London: The English Universities Press Ltd*., pp 406–407 (1961).

[26] D. R. Smith, R. L. Morgan, E. V. Loewenstein, Comparison of the Radiance of Far-Infrared Sources, *J. Opt. Soc. Am*, Vol. 58(3), pp 433-434, (1968).