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Md. Nur-E-Alam
Leather Research Institute, Bangladesh Council of Scientific and Industrial Research (BCSIR), Savar, Dhaka-1350, Bangladesh

Nasifa Akter
Leather Research Institute, Bangladesh Council of Scientific and Industrial Research (BCSIR), Savar, Dhaka-1350, Bangladesh

Kanish Fatema
Leather Research Institute, Bangladesh Council of Scientific and Industrial Research (BCSIR), Savar, Dhaka-1350, Bangladesh

Md. Abul Kashem Azad
Leather Research Institute, Bangladesh Council of Scientific and Industrial Research (BCSIR), Savar, Dhaka-1350, Bangladesh

Shimul Chakma
a) Leather Research Institute, Bangladesh Council of Scientific and Industrial Research (BCSIR), Savar, Dhaka-1350, Bangladesh
b) Delhi Technological University, Delhi, 110042, India

Md. Anwar Arfien Khan
Institute of Mining, Mineralogy and Metallurgy, BCSIR, Joypurhat, Bangladesh

Correspondence:
Md. Nur-E-Alam
Leather Research Institute, Bangladesh Council of Scientific and Industrial Research (BCSIR), Savar, Dhaka-1350, Bangladesh
Email: nalam1980@yahoo.com

Acid hydrolysis of untanned proteinous wastes from tannery industry in Bangladesh

Md. Nur-E-Alam, Nasifa Akter, Kanish Fatema, Md. Abul Kashem Azad, Shimul Chakma, Md. Anwar Arfien Khan*

Abstract

Leather tanning industry, considered one of the polluting industries, poses various environmental threats to its every sphere. It produces various types of solid wastes as well as liquid wastes. Among the solid wastes, untanned raw trimmings contribute for about 5-7% of the total quantity of raw materials processed. If suitably utilized, these by-products of tanning industry could be a useful resource for various applications. This research work deals with utilization of raw trimmings of tannery solid waste to protein hydrolysate by acetic acid with varying acid concentration, acid solution, temperature and time. The maximum about 76% protein hydrolysate was obtained at 1.5M acid concentration and 80ºC.

Keywords: Raw trimmings, Untanned proteinous solid wastes, Acid hydrolysis.

INTRODUCTION

Various leather tanning processes are found in the history around 8,000 BCE in the early Stone Age. After that, the Egyptian people are said to have invented the plant-based tanning process using various bark and gum of trees [1]. However, modern tanning process involves numerous steps to convert raw hides and skins into imputrescible substance and generates huge quantities of solid and liquid wastes. On an average, converting of one metric ton of rawhide this process produces 200 kg of tanned leather, 250 kg of non-tanned waste, 200 kg of tanned waste leather, and 50,000 kg of liquid waste (Table 1). That means, about 50% leather mass losses during the tanning process [2].

Tannery sector of Bangladesh considered as the most polluting industries which is well established and ranked fourth in terms of earning foreign exchange. The first tannery of Bangladesh was established at Narayanganj by R.P. Shaha in 1940. After that the tannery industry was shifted to Hazaribagh, Dhaka [4]. Now all tanneries of Hazaribagh are relocated to Savar Tannery Estate to protect the environment.

Raw hides & skins are the by-products of meat industry. At the beginning of the tanning process, a large number of un-tanned solid wastes are generated which are mainly composed of proteinous substance and account for about 5-7% of the total quantity of raw materials processed. Proper utilization of these waste proteinous substances can crab the hazardous environmental pollution [5]. Glue, composite leather board, gelatin, and collagen can be produced by these wastes. Various researchers have been reported about the treatment of these wastes into valuable products. Enzyme like papain, neutrases [6], acids like phosphoric acid [7], sulphuric acid [8] propionic acid [9] hydrochloric acid [10] and alkali such as sodium hydroxide [11], magnesium oxide, calcium oxide [8] etc. are used as hydrolyzing agents for converting tannery solid waste to valuable products. Environmentally benign ultra sound technology can also be used to accelerated the hydrolyzing process [12]. In this study work, acetic acid (CH_3COOH) is used as hydrolyzing agent to extract the protein hydrolysate from untanned raw trimmings from tannery industry in Bangladesh.

MATERIALS AND METHODS

Material preparation

Un-tanned trimmings were collected from Savar tannery estate, Savar, Dhaka and washed with excess amount of tape water to remove salt, dirt, dung, blood and other impurities. Hairs were removed by liming process using calcium oxide (CaO) and finally unhaired trimmings were delimed by washing several times

with water. After that dried in open air.

Then the dried trimmings were cut into small pieces for hydrolysis. Acetic acid (CH_3COOH) was procured from Merck Ltd. Glassware's (Pipette, Beaker, Conical flask, measuring cylinder, Test tube, etc.) used were of Borosil/Ranken. Magnetic hotplate, stirrer, Kjeldahl apparatus, etc., were used. As acetic acid is used as a sour agent added in vinegar, pickled vegetables, and sauce with dilute concentration (4 to 8 % by mass), this was chosen as a hydrolysis agent in this work.

Table 1: Environmental Input of leather processing, adapted from [3]

Raw hide (1 ton)				
Leather	**Solid wastes / Byproducts**		**50^3 liquid effluent**	
200 kg	Untanned wastes:		COD	235-250 kg
	Raw trimming	120 kg	BOD	100 kg
	Fleshing	70-230 kg	Suspended solids	150 kg
			Chromium	5-6 kg
	Tanned wastes:		Sulfide	10 kg
	Tanned splits	115 kg		
	Trimming + Shavings	100 kg		
	Dyed / Finished:			
	Buffing	2 kg		
	Trimmings	32 kg		

a) Raw trimmings of hides and skins **b)** Solid wastes duped in nearby tannery's yard

Fig. 1: Solid wastes from tanneries discarded without any treatment

Experimental procedure

The study was performed in a batch process in a series of beakers equipped with stirrers by stirring the dried raw trimmings using acetic acid with varying concentrations and temperatures. The detailed experimental methodology is given in fig 2.

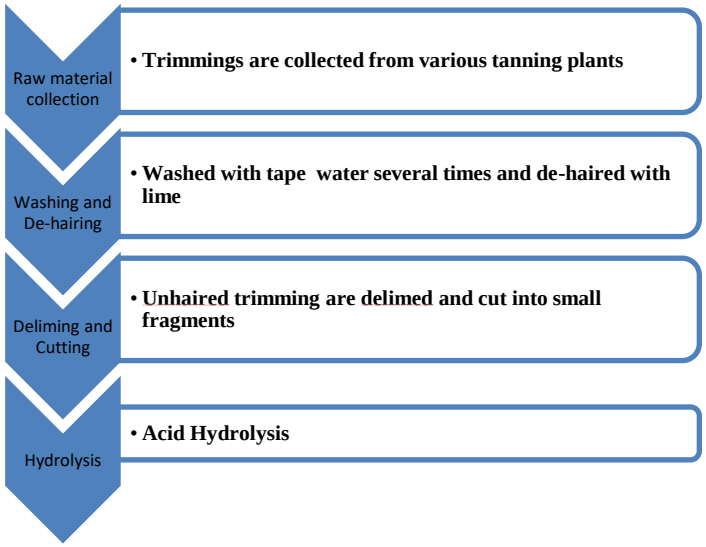


Fig. 2: Experimental Procedures of Hydrolysis

The effect of acid concentration on the protein hydrolysis can be carried out with the different amount of acid concentration to fixed acid solution and temperature. 50 gm of cleaned and dried raw trimmings was dissolved in 5 ml of 0.25M, 0.5M, 1.0M and 1.5M acetic acid solution. Added extra 400 ml of distilled water to each and temperature was selected at 40ºC. Cleaned and dried raw trimmings of 50gm was taken in beaker with best acid concentration and fixed temperature of 40ºC. The effect of temperature on the protein hydrolysis was carried out with best acid concentration and its solution. The effect of hydrolysis time (hour) was carried out with best acid concentration, its solution and temperature.

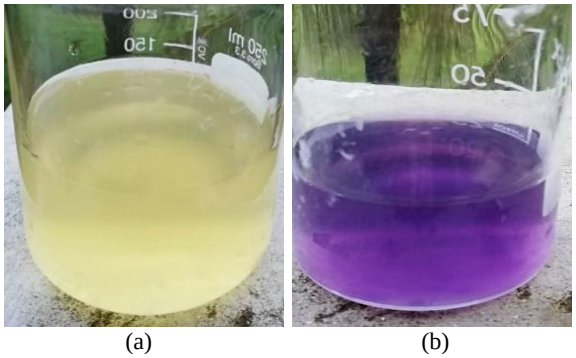
Analysis

Protein of the extracted hydrolysate was identified by the biuret test and protein concentration was determined by the Kjeldahl method in a digester Gerhardt (Germany). The Biuret test is based on the ability of Cu (II) ions to form a violet-colored chelate complex with peptide bonds (-CONH- groups) in alkaline conditions. This test confirms the presence of proteins in the sample. In this test, 2 ml of extracted hydrolysate solution was taken in a dry test tube. Added 3 drops of 10% NaOH and 3-6 drops of 0.5% $CuSO_4$ to the sample test tube [13]. In Kjeldahl method 0.5g of hydrolysate sample was taken in a Kjeldahl flask and digested with 15 ml concentrated sulfuric in the presence of a mixture of Na_2SO_4 and $CuSO_4$ in ratio of and distilled into a 4% boric acid solution. The borate anions formed was titrated with 0.5M H_2SO_4, which was converted to nitrogen in the sample [13].

RESULTS AND DISCUSSION

Biuret and Kjeldahl methods

The change of color of the extracted hydrolysate from grey (a) to purple color (b) after the addition of biuret reagent was shown in figure 3 which represents the presence of proteins in the extracted hydrolysate from raw trimmings.



(a) (b)

Fig. 3: Biuret test **a)** before the addition of biuret reagent, **b)** after addition of biuret reagent.

Protein hydrolysis of tannery raw trimmings by acetic acid was performed with the parameters of acid concentration, acid concentration solution, temperature, and hydrolysis time. Protein concentrations (crude protein) of the extracted hydrolysates were determined by the Kjeldahl method.

Effect of acetic acid concentration on protein hydrolysate

By hydrolyzing of 50gm of raw trimmings by various acid concentration (M) with fixed acid solution (5ml) and temperature (40ºC) the effect of acetic acid concentration (M) on protein hydrolysis of tannery raw trimmings was carried out. It is shown from the fig.4 that percentage of protein was increasing with increasing the concentration of acetic acid from 0.25 to 1.5 molar (M). Since acetic acid is a weak acid and used as a sour agent added in vinegar, pickled vegetables and others with dilute concentration, it is taken as a hydrolyzing agent in this works. As analytical grade acetic was used in this research, the concentration of acid was not used more than 1.5M. Maximum about 32% protein hydrolysate was extracted from 1.5M acid concentration.

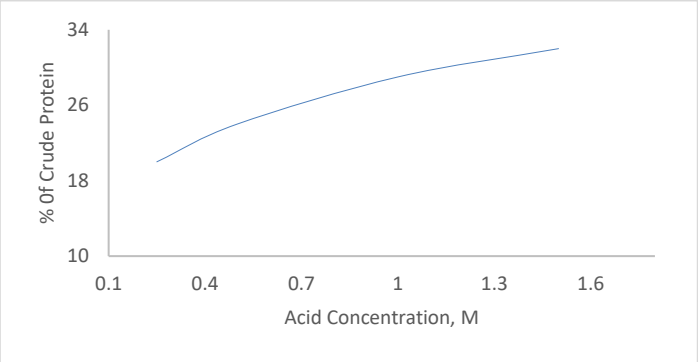


Fig. 4: Effect of acetic acid concentration on protein hydrolysis

Effect of concentration solution on protein hydrolysate

In figure 5, the effect of acetic acid concentration solution (ml) on protein hydrolysis of tannery raw trimmings is shown. Solution of 5ml, 10ml, 15ml and 20 ml of 1.5M acetic acid were taken for each 50gm raw trimmings. From the figure, it is shown that percentage of yield was increasing with the increasing acid solution and maximum yield was found at 20ml solution of 1.5M acetic acid.

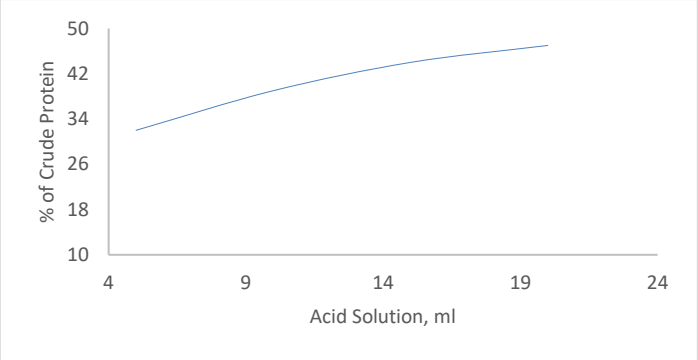


Fig. 5: Effect of acetic acid solution on protein hydrolysis

Effect of temperature on protein hydrolysate

The effect of temperature on hydrolysis of tannery raw trimmings using best acid concentration (1.5M) and concentration solution (20ml) with temperature variation from 40 to 110ºC is shown in figure 6. On heating, trimmings, mainly composed of collagen, are disintegrated and dissolved very quickly. The maximum percentage of protein hydrolysate was obtained about 58 at temperature 80ºC. Temperature above this, the denaturation of protein was occurred and hence yield declined. As raw trimmings are biological materials, the temperature should not increase to high due to avoiding denature of protein.

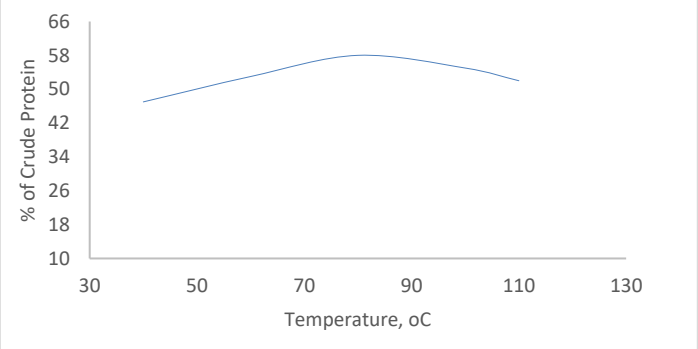


Fig. 6: Effect of temperature on protein hydrolysis

Effect of hydrolysis time on protein hydrolysate

The effect of hydrolysis time (hour) at 1.5M acetic concentration, 20ml acid solution, and 80ºC temperature is shown in figure 7 which depicts about 76% protein hydrolysate after 18 hours of hydrolysis.

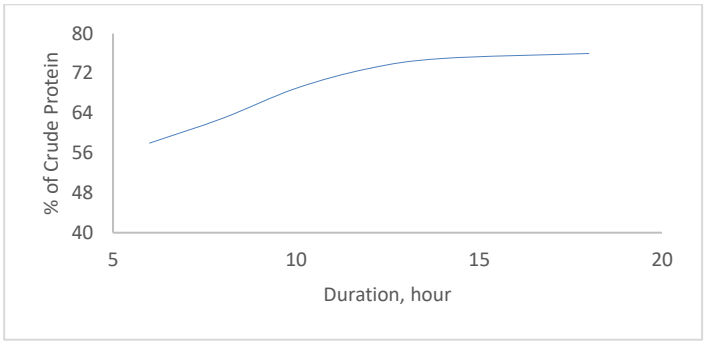


Fig. 7: Effect of hydrolysis time on protein hydrolysate

Raw trimmings hydrolysis and final product are shown in fig. 8.

(a) (b)

Fig. 8: a) Raw trimmings hydrolysis, **b)** Final product

According to recent studies, collagen or gelatin extracted from untanned raw trimmings of hides and skins has huge application in the field of packaging, biomedicine and cosmetics. A kind of biodegradable packing material can be prepared from gelatin extracted by acetic acid from raw trimmings blended with poly vinyl alcohol (PVA). Burn-healing membrane was prepared by enzymatic hydrolysis of pig skin collagen. Again, hybrid films can be prepared using collagen extracted from trimmed skin by acetic acid and then blended with starch/soy protein [15]. In food industry, gelatin plays a major role to stabilize ice-cream and other frozen foods. Collagen can bind large fat quantities as an emulsifier in meat products. However, it has an important application in pharmaceuticals and medical sectors. As for example, outer covering of capsules is made from gelatin [16]. Tissue adhesive, vascular grafts, aortic heart valves, drug delivery matrices, wound dressing, and tissue engineering scaffold can also be made from collagenous materials derived from leather trimmings [3].

CONCLUSION

Tanning industry due to its huge amount of solid and liquid wastes generation, create a grate problem to the environment. The solid waste which is mainly composed of proteinous substance can be converted into various valuable products by proper treatment. In this study works, only acetic was used as hydrolyzing agent and got about 76% protein hydrolysate at 1.5 M. Meanwhile it is possible to increase the efficiency of the process by treatment with some other chemicals especially enzymes. Finally, we recommend evaluating the effect of various pretreatment processes for this type of treatment.

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Conflict of interest

Authors declare no conflict of interest.

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