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RESEARCH ARTICLE

Preparation , application and quantification of retanning products from skin fleshing hydrolyzed protein: A Process Optimization for waste recycling from tanneries into products

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Abstract

Skin fleshing wastes from tanneries are generated during leather processing when flesh in the limed hide or skin is removed with the sharp knives. These skin fleshing wastes from tanneries contain highest protein content and currently being wasted into the dumping sites or in open areas, consequently creating the solid waste disposal problem for tanners. In this study skin fleshing wastes were first hydrolyzed with alkaline mixture of sodium hydroxide and magnesium oxide for the recovery of protein fraction. This isolated protein was further treated with soluble starch solution in distilled water and acrylic acid solution in distilled water with the small addition of initiator such as sodium metabisulphite and potassium persulphate. The reaction was completed in 2h and finally whitish colored polymeric retanning product was prepared. This retanning product was subjected for application in leather processing at (5%, 10% and 15%). Prepared leathers were tested with standard physical tests and compared with reference leather processed with commercial retanning product. The most significant results in leather were obtained by applying 10-15% of prepared retanning product. Data from physical testing was quantified with statistical analysis and it was found significant.

Introduction

Skin fleshing wastes from tanneries are generated in the pre-tanning process when flesh in the limed hide or skin is get rid of by means of sharp knifes. These skin fleshing wastes from tanneries contain the highest collagen protein content and currently being wasted in the open areas, thus creating the solid waste disposal problem in tanneries (Ravindran *et al*., 2008; Kumar *et al*., 2008). These skin fleshing from tanneries have been used for glue manufacture, enzyme production and animal feed production (Vasudevan and Ravindran, 2007). Limed skin fleshing wastes have been co-digested with biodegradable fraction of municipal solids waste and optimized for biogas production (Shanmugam and Horan, 2009). The enzymatic hydrolysates of waste collagen proteins obtained from leather, edible meat product casings,etc. of mean molecular mass 20–30 kDa have been reacted with dialdehyde starch (DAS) to produce biodegradable hydrogels used for packaging materials such as food, cosmetic and pharmaceutical products (Langmaier *et al*., 2008) and thermoreversible hydrogels (Langmaier *et al*., 2001). These skin fleshings have been used for the production of protein based feed for fish cultures. The growth profile was study on he Labeo rohita for duration of eight weeks by supplements with prepared feed. This feed was prepared by raw animal fleshing chemically treated with hydrogen peroxide (Approximately 3%), sunflower oil (approximately 10%) and groundnut oil was used in the feed as a lipid source while wheat flour and rice bran (approximately 14%) were used as a carbohydrate source for fermentation. This feed was compared with reference standard feed formulation.

The feed effects were characterized by taking mean final weight, feed conversion rate and survival rate. The results of study show that there was no significant change ($p<0.05$) compared with the reference diets. Therefore, an economical feed for aquaculture can be prepared (Sumathi and sekaran, 2010).

Although, research has been reported in the previous studies to reutilize the collagen protein hydrolyzate from fleshing in the tanning phase (Castiello *et al*., 2005). But very few studies are related to the reutilization of these skin fleshings protein in the leather products. Therefore, in this study investigations were carried out for the utilization of recovered fleshing waste protein into a useful retanning agent by modification with other chemicals. This process optimization through the standard test of prepared leather will be helpful for waste minization into dumping sites and economically beneficial. The usefulness of applied retanning product is quantified with the statistical analysis using ANOVA and Duncans multiple range test.

Material and Methods

Raw skin fleshings wastes were collected from tanneries of SITE area karachi. Protein was recovered from skin fleshings using alkaline mixture of sodium hydroxide and magnesium oxide. Moisture content in skin fleshings was carried out according to SLC 3 (IUC 5), pH was checked by (ASTM, D,1293-99), ash by (ASTM, D, 2617-96: SLC 6, IUC-7), fat by ISO 4048: 1977). While for physical testing sample cutting was carried out by (BS-3144 IUP-1/EN ISO 2419: 2006), conditioning of leathers was using (SLP3, IUP 3; BS 3144: method 2, 2001), thickness by SLP4 , IUP4; BS 3144: method 3), tensile strength and elongation at break by (BS-3144, IUP-6/EN ISO 3376: 2002), distension and strength of grain by ball burst (SLP 9, IUP /9;BS3144: method 8). Universal Testing Machine from Tinius Olsen was used for physical testing of prepared leathers. Data from physical testing parameters of all four treated leathers was subjected to analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was also used to compare the means of all four treatments (Zar, 1999).

In a typical preparation, soluble starch (62 grams on dry basis) was dissolved in 300ml of water at 80^0C with stirring in the three neck round bottom reaction flask attached with magnetic heating system. Then protein solution containing 27.0 gram of protein (on dry basis) was added and stirred for 10 minutes with heating to homogenized completely. Thereafter, 2.5 gram of sodium metabisulphite was added and stirred for 5 minutes while heating was also maintained. Acrylic Acid (150 grams) was dissolved in sufficient amount of water (pH of the solution was 8.0). Potassium persulphate (5 grams in 150ml of water) was also prepared by only stirring. Prepared acrylic acid solution and potassium persulphate solutions were added drop by drop simultaneously in the reaction flask through two different necks of flask. The reaction was proceed for further 2 h while heating was maintained at 80^0C with constant stirring. Then, retanning product was cooled at room temperature. Finally, the pH of the prepared retanning product (5% in water) was adjusted at 6.5 with solution of sodium bi carbonate in water which was suitable for retanning. Retanning product from fleshing protein was whitish in color and stored in a cool place till application. Some part of the product was tested for general biuret test with the help of copper sulphate and potassium hydroxide reagent used for the presence of peptides which was shown negative (no violet coloration was appeared in the test tube) and confirm the reaction that protein was grafted with the applied acrylic monomers in the presence of initiators used such as potassium persulphate after 2h contineous reaction and unreacted protein was not present in the retanning product. The biuret test was also performed in the protein solution which was showed violet coloration due to the peptide linkage.

Application of Retanning Product

Goat skin wet blue in good condition with full grain (without any types of defects) was selected to treat with the prepared retanning agent. It was cut into four equal parallel halves. One was treated as reference processed with commercial retanning product while other three wet blue pieces were processed as experimental (application of prepared retanning product). Prepared retanning product was applied in experiments (10%, 15%, 20%) named as S1, S2 and S3 respectively wile reference was named as R1.

Application of Retanning Products

Goat skin wet blue shaved at 1.0mm $\pm$ 0.5 mm processed at Leather Research Centre, PCSIR (SITE, Karachi) by conventional chrome tanning process were selected for the application of retanning product. This wet blue was cut into four equal size pieces , three pieces were treated with retanning product (5%, 10% ,15%) whereas one piece was treated with the commercial retanning product for comparision of results. These mechanical opertions were carried according to conventional methods as used in the tanneries for chrome tanned leather into final leather. These were washing, neutralization,

retanning, fatliquoring and drying. At the end, leather was washed with excess of water and horsed up overnight approximately 12h then set out at room temperature. All chemicals used in the mechanical operations were given based on shaved weight of each wet blue piece.

Results and Discussion

Skin fleshings wastes were found pH 12.0, ash was found 29.39% while fat was found 17.3 %. These yields were calculated on moisture free basis of raw skin fleshing wastes. The objective of this study was recycling of protein fraction from skin fleshing wastes into valueable retanning agent product after some modification with chemicals. Since, it has proved through experiments that protein hydrolysates from leather wastes when modified with vinyl monomers and applied as a retanning agent good results were obtained especially good stretch and filling properties (Jianzhong *et al*., 2004). On the other side, modified starch products for retanning of leather have been also studied earlier on goat chrome tanned leather and found excellent softness, tightness and fullness while other mechanical properties such as tensile strength, tear strength and elongation at break were also improved (Zhen and Jianzhong, 2002). Therefore, these both materials (hydrolyzed protein and starch) were selected for the preparation of retanning product. In this reaction, graft copolymerization of starch with vinyl monomers has been studied in the presence of oxidizer such as potassium persulphate (Shenghua *et al*., 2005) . Whereas, the reaction of amino acids of protein has been investigated an esterification with starch as earlier reported (Kapusnaik *et al*., 1999). However, in this reaction complex formation is not completely clear by mechanism. As a result, further investigations to find out the mechanism of reaction is in progress. Prepared retanning product in three different percentages was applied these were 10%, 15% and 20% as given detailed in material and methods section based on shaved weight of each wet blue piece. The results of each treatment (10%, 15%, 20%) are given as S1, S2 and S3 respectively. The effluent obtained after the application of each retanning product from retanning process was clear (not turbid) which showed that the exhaustion of retanning agent was excellent. Reference leather was processed in the same condition by applying commercial retanning product with 10% dosage (results are given as R1). To evaluate the potential of modification in hydrolyzed protein as a retanning agent, physical tests such as tear strength (N/mm), tensile strength (N/mm^2), percentage elongation at break (mm), bursting force at break (N), thickness of leather were performed using standard test methods. Parallel test in same condition were also performed in reference leather R1. The results of physical tests are presented in table1 to table 3. Statistical analysis of physical testing data was performed using ANOVA and Duncans multiple range test. These quantified test results are also presented in various tables (4-19) in each separate table. Some difference was observed in the physical tests of leathers however, results were found under the limit of standard specification which confirmed the suitability of prepared retanning agent in leather. Moreover, color of leather was dark in the treatment 3 in which retanning product (20%) was applied which indicates that some negative effect of increasing the weight ratio of starch. While 10 % and 15% of retanning products in leather were found suitable due to the suitability for color and smoothness of grain was found in leathers.

Table 1: Physical Testing of Resulted Leathers after Application of Retanning Products

Sample	Thickness(mm)	Width(mm)	Area of Cross Section (mm^2)	Tensile Load (Newton)	Elongation (%)	Tensile Strength (N/mm^2)
R1	1.82±0.094	10±0.00	18.33±1.02	353.886±30.818	194.776±29.262	20.158±1.06
S1	1.48±0.05	10±0.00	14.86±0.05	263.166±21.93	155.486±8.785	17.682±1.247
S2	1.34±0.183	10±0.00	12.56±0.83	104.944±1.710	163.395±13.073	7.943±0.037
S3	1.25±0.083	10±0.00	13.4±1.83	147.710±36.269	135.476±19.193	10.863±2.018

Table 2: Physical Testing of Resulted Leathers after Application of Retanning Products

Sample	Thickness(mm)	Tearing Load (N)	Tear Strength(N/mm)
R1	1.96±0.11	130±10.938	66.596±9.156
S1	1.55±0.02	91.00±15.099	58.626±8.977
S2	1.05±0.09	24.496±2.516	23.210±1.746
S3	0.95±0.08	34.216±3.027	36.036±5.650

Table 3: Pysical Testing Of Resulted Leathers after Application Of Retanning Products

Sample	Distension at break (mm)	Bursting Load (N)
R1	58.74	602.50
R1	58.75	514.83
R1	54.03	504.33
S1	69.04	564.16
S1	81.05	559.83
S1	63.48	570.83
S2	31.01	257.66
S2	31.24	240.50
S2	37.30	226.00
S3	27.59	350.33
S3	38.20	347.16
S3	36.20	345.26

Table 4: One Way ANOVA Completely Randomized for Tearing Load (N) of Leather Samples (N)

Source	SS	df	MS	F	P
Main Effects St	22274.179	3	7424.726	81.785	0.000
Error	726.264	8	90.783		
Total	**23000.443**	**11**			

Table 5: Duncan's Multiple Range Test for Tearing Load of Leather Samples (N)

Error Mean Square = 90.783
Degree of Freedom = 8
Significanc Level = 0.05
LSD 0.05 = 17.937

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	130.277	3	a
2	2	91.000	3	b
3	3	34.221	3	c
4	4	24.499	3	c

Table 6: One Way ANOVA Completely Randomized for Tear Strentgth (N/mm) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	3614.176	3	1204.726	24.329	0.0002
Error	396.133	8	46.516		
Total	**4010.313**	**11**			

Table 7: Duncan's Multiple Range Test for Tear Strength (N/mm) of Leather Samples

Error Mean Square = 49.516
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 13.249

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	66.597	3	a
2	2	58.627	3	a
3	3	35.916	3	b
4	4	23.210	3	b

Table 8: One Way ANOVA Completely Randomized for Thickness (mm) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	1.970	3	0.656	87.867	0.000
Error	0.050	8	0.007		
Total	**2.030**	**11**			

Table 9: Duncan's Multiple Range Test for Thickness (mm) of Leather Samples

Error Mean Square = 0.007
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 0.162

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	1.966	3	a
2	2	1.550	3	b
3	3	1.056	3	c
4	4	0.95	3	c

Table 10: One Way ANOVA Completely Randomized for Distension at break (mm) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	3102.928	3	1034.309	31.214	0.0001
Error	265.080	8	33.135		
Total	**3368.009**	**11**			

Table 11: Duncan's Multiple Range Test for Distension at break (mm) of Leather Samples

Error Mean Square = 33.135
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 10.838

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	71.190	3	a
2	2	57.173	3	b
3	3	33.996	3	c
4	4	33.186	3	c

Table 12: One Way ANOVA Completely Randomized for Brusting Load (N) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	217907.085	3	72635.695	90.966	0.000
Error	6387.910	8	798.488		
Total	**224294.995**	**11**			

Table 13: Duncan's Multiple Range Test for Brusting Load (N) of Leather Samples

Error Mean Square = 33.135
Degree of Freedom = 8
Significanc Level = 0.05
LSD 0.05 = 10.838

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	71.190	3	a
2	2	57.173	3	b
3	3	33.996	3	c
4	4	33.186	3	c

Table 14: One Way ANOVA Completely Randomized for Area of Cross Section (mm^2) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	58.309	3	19.436	14.424	0.0014
Error	10.780	8	1.347		
Total	**69.089**	**11**			

Table 15: Duncan's Multiple Range Test for Area of Cross Section (mm^2) of Leather Samples

Error Mean Square = 1.347
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 2.185

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	18.333	3	a
2	2	14.866	3	b
3	4	13.400	3	bc
4	3	12.566	3	c

Table 16: One Way ANOVA Completely Randomized for Tensile Load (N) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	114679.771	3	38226.590	55.613	0.000
Error	5498.937	8	687.367		
Total	**120178.708**	**11**			

Table 17: Duncan's Multiple Range Test for Tensile Load (N) of Leather Samples

Error Mean Square = 687.361
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 49.363

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	353.888	3	a
2	2	263.166	3	b
3	4	147.710	3	c
4	3	104.944	3	c

Table 18: One Way ANOVA Completely Randomized for Elongation (%) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	5465.533	3	1821.844	4.948	0.0314
Error	2945.529	8	368.191		
Total	**8411.069**	**11**			

Table 19: Duncan's Multiple Range Test for Elongation (%) of Leather Samples

Error Mean Square = 368.191
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 36.128

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	194.776	3	a
2	3	163.395	3	ab
3	2	155.486	3	b
4	4	135.476	3	b

Table 20: One Way ANOVA Completely Randomized for Tensile strength (N/mm^2) of Leather Samples

Source	SS	df	MS	F	P
Main Effects St	293.704	3	97.901	57.827	0.000
Error	13.543	8	1.692		
Total	**307.248**	**11**			

Table 21: Duncan's Multiple Range Test for Elongation (%) of Leather Samples

Error Mean Square = 1.692
Degree of Freedom = 8
Significance Level = 0.05
LSD 0.05 = 2.449

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	1	20.158	3	a
2	2	17.682	3	b
3	4	10.863	3	c
4	3	7.943	3	d

Conclusions

- This study reperesents that the waste protein from skin fleshing wastes can be utilized as a beneficial product after some modification with the chemicals.
- Prepared leather retanning agent from skin fleshing protein by reacting with starch and acrylic acid in the presence of sodium metabisulphite and potassium persulphate showed better characteristics in the processed final leather.
- Retanning Product (10-15%) was found suitable for good color for crust.
- Waste skin fleshings may be a good alternative source for preparation of retanning agent and decreasing the problem of disposition of such wastes. This study may be applicable for other modifications with different acrylic monomers and apply in different types of leather for future studies.

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