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Research Article

Preparation of Double Action Surfactant using Protein Hydrolyzate from Fleshing Waste and Its Utilization as a Lubricant with Retanning Property in Leather Making

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Abstract: Leather fleshing are generated during beam house leather processes when lower layer of skin is scrapped off through sharp knife. Generally these are thrown out in land area as waste material without any proper treatment. Collagen protein hydrolyzate was recovered by alkaline hydrolysis and further reacted with fatty acid ester product from glycerol. The reaction was promoted by direct addition of alkaline catalyst. The yield of fatty acyl amido carboxylic acid based surfactant was found excellent calculated based on raw extracted collagen protein hydrolyzate. In this study, prepared product was applied in wet blue split from cow hide as a fatliquoring agent with additional retanning property. Investigations were also focused on the characteristics of prepared leather to evaluate the effect of product on resulted leather. The results of standard physical characteristics such as tensile strength, tear strength, % age Elongation at break, softness, etc. showed significant improvement with lubricating effect on leather through smoothness of surface. In this way, a waste is converted into a useful surfactant product.

Keywords: Leather fleshings, protein hydrolyzate¸ protein based surfactants, cow split leather

1. INTRODUCTION

Solid waste management in most of the developing countries is a major environmental challenge as reported by Daskalopolous [1], because leather processing commonly has been associated with high pollution due to the generation of different organic wastes during traditional manufacturing processes in tanneries as reported by Taylor et al [2]. It is generally known that approximately 200 kg leather product is produced from one ton of wet salted hides. Therefore, a huge amount of solid wastes is generated as previously described [3-5].

This solid waste creates a major problem for tanneries in terms of both their variety and quantity. However a huge amount of proteineous waste is reusable in various useful products after necessary modification as earlier reported [6-7]. But the ways of the leather waste processing are associated to the innovative separation and processing procedures for fats, protein and chromium [2, 6]. Leather fleshing mainly consists of protein, fat and water with dirt as earlier reported [7-8].

Skin fleshings are one of the major solid wastes generated during pretanning operations of leather processing. These skin fleshings contain high protein content (50–60%) and collagen (3–5%) content which are currently being wasted, thus creating the solid waste disposal problem in tanneries as reported in previous research [9-10]. In the previous studies, skin fleshings have been anaerobically digested for the production of methane gas using biomethanation process as reported earlier [11]. These skin fleshings have been used for glue manufacture, enzyme production and animal feed production as reported earlier [12]. Base-catalyzed transesterification

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reaction of raw fleshing oil for the production of fuel has been investigated by preparation of ester product as an alternative fuel as well as a feed stock in lubricant production or cosmetic industry [13]. Limed skin fleshings have been co-digested with biodegradable fraction of municipal solids waste and optimized for biogas production as reported earlier [14]. Transesterification reactions of raw fleshings oil using methanol as the alcohol and alkali catalyst was reported to be preferred in the case of low acid value, sodium hydroxide was used as a catalyst in the reaction have been reported by several authors[15-19]. 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 [3] and thermoreversible hydrogels as reported in previous study [20]. Leather fleshings have been used for the production of protein based feed for fish cultures. Growth profile was study on the Labeo rohita for duration of eight weeks by supplements with prepared feed. This feed was prepared by raw animal fleshings chemically treated with hydrogen peroxide (approximately 3%), sunflower oil (nearly 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 as reported earlier [21].

Protein-based surfactants have got special attention due to their hydrophilic emulsifying group with no toxicity on biological systems and strong antimicrobial activity as reported earlier [6]. Consequently protein-based surfactants have been studied for important applications in various areas, such as foods, cosmetics and pharmaceutical formulations, etc as reported earlier [22]. However, high tech purification is needed for such sophisticated product that may be cost effective. In this work, we have investigated on simple and safe side application of this extracted collagen hydrolyzate protein after modification with fatty acid ester into surfactant product for cow split leather.

2. MATERIALS AND METHODS

Tannery Chemicals for processing were purchased from local market and used without further purification. Centrifugation of product was done by using Variable Speed Centrifuge Model 2010H from Scientific LTD, UK. Samples were conditioned prior to physical testing in a standard atmosphere of temperature 20±2 ºC and Relative humidity 65±2 %. The samples were tested according to standard methods [23-29] using a Universal Testing Machine (H5KS) from Tinius Olsen Ltd, UK, having a uniform speed of separation of jaws of 100± 20 /mm for tensile strength and % elongation, tear strength and tearing load. Leather softness tester (ST300) from SATRA was used for softness. Calibrated Equipments were used for testing of leather.

2.1 Leather Fleshing Treatment for Recovery of Protein Hydrolyzate

Leather fleshing wastes (grayish brown colored obtained after fleshing of skins through knife) were received from the tanneries of SITE Area, Karachi stored in refrigerator till further use. After deliming fleshing (1 kg) were hydrolyzed in autoclave at 110 ^{o}C for two hours with the addition of water, 4% magnesium oxide and 2% sodium hydroxide. After two hours fleshings were completely liquefied and resulted a dark colored viscous mass having some insoluble particles. These particles were re-filtered and the solution was freezed. Three fractions were isolated after hydrolysis process using separating funnel as briefly described in Scheme 1. Second fraction was used in this experimental work while other two fractions consisting fats and sludge respectively would be used in other research experiments as per their possible applications. Total solids of all three fractions were determined using the official method as well as delimed leather fleshings as in Table 2.

2.2 Preparation of Amino Acid based Surfactant

In the first step, 250 g protein was dissolved in 200 mL water by heating and stirring to prepare

homogenized solution .Then pH of the protein solution was checked (9.0) and it was used for further reaction. Long chain fatty acid (Lauric acid) 0.1 mole was reacted with 110 g glycerol by adding 1 mL of concentrated high strength Sulfuric acid as a catalyst. This prepared fatty acid ester was added into above protein solution flask. Then, the mixture was heated and stirred for one hour at 60-65^{0}C. During the reaction pH of mixture was maintained at 11-13 by adding drop by drop freshly prepared sodium hydroxide solution. Product was cooled at room temperature and washed twice with water. Transfer the contents in Centrifuge tubes and white Waxy lump product was obtained in the thick paste settled at the bottom of tubes. Prepared surfactant was collected stored in a cool place till further used. The confirmation of product was done through FTIR spectras. The pH of the product was 11.0 and adjusted at 6.5-7.0 with diluted acetic acid solution to apply in leather processing at fatliquoring step.

2.3 Application of the Product

A cow hide was processed up to wet blue stage by conventional chrome tanning process. After preparation wet blue was kept for ageing for 3-4 days at room temperature. A split was achieved using the splitting machine. Then it was shaved at 1.2 mm thickness by shaving process and cut into two equal halves parallel to backbone. Condition of wet blue pieces was normal without any defects. First, both wet blues were washed with 200% water and added 0.2%Acetic Acid, second wash was carried out with 15% water for 15 min each. Wet blues were then basified with 2% Sodium Formate and 0.2% Sodium Bicarbonate for 90 min. Drain the float and Wash. Then 3% dye and 3% (Edaplin MK) was given with 100% water for 15 min drumming 4% (Tanigan O.S) and 12% Quebracho for 30 min drumming. Left Overnight in the drum for thorough dyeing of leather. Next day drum was run for 5 min. Then three times washing was carried with 250% water each for 15 min. Then solution of prepared surfactant 10% in 100% warm water (60^0 C) was applied by continuous drumming for 60 min. After that, 0.3% Formic Acid was given by drumming for 15 min and then 2.5% Chromium Sulfate by drumming for 40 min. Drain the float and Leather was washed two times with water. Finally float was drained. Leathers were horse-up in tannery area then dried at room temperature. The other wet blue was processed with the same procedure and chemical only surfactant dose was applied with 10% commercial synthetic (Derminol ASN) fatliquor. All chemicals were added on shaved weight of wet blue.

3. RESULTS AND DISCUSSION

We have worked on very simple but most important surfactant from the protein hydrolyzates recovered by the hydrolysis of leather fleshing wastes under alkaline conditions as shown in Scheme 1. These solid wastes can be converted into useful by products in many ways such as filler, lubricant, glue, surfactants, etc. as earlier [31]. Before synthesis of product all three fractions were characterized for their contents, physical appearance and % age yield on dry basis (Table 1). Total solids were also determined to calculate the active matters in the material (Table 2). Second fraction showed amino acid composition similar to collagen as previously described [31]. There are two types of methods for the production of surfactants from

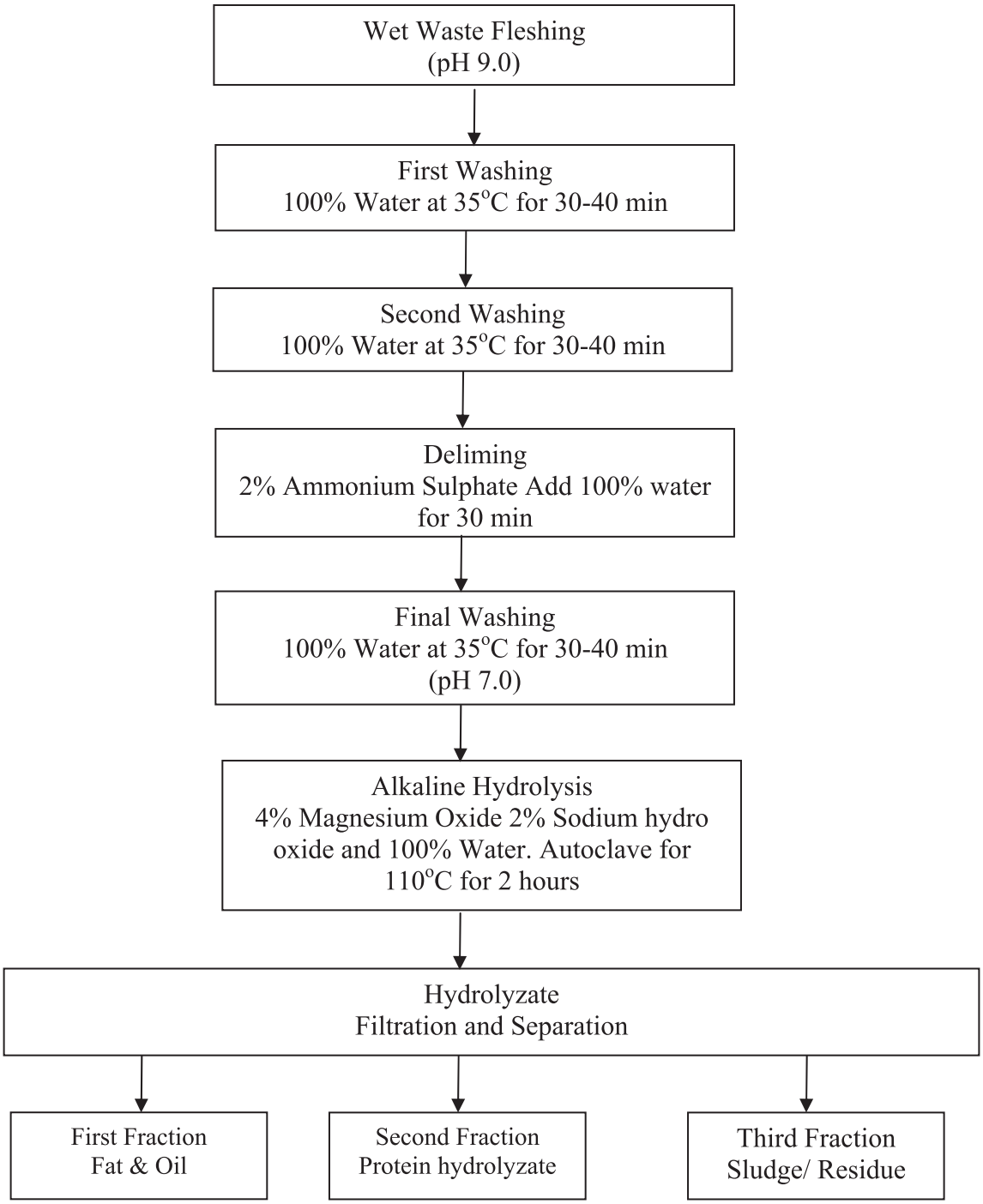
Table 1. Characteristics of recovered fractions**.**

Recovered fractions*	% Yield	Appearance	pH
Fat	20.63	Light Creamy	4.0
Protein Hydrolyzate	65.14	Light Brown	9.0
Sludge /Residue	14.23	Blackish Grey	9.0

*Yield was calculated on moisture-free basis

Table 2. Determination of total solids.

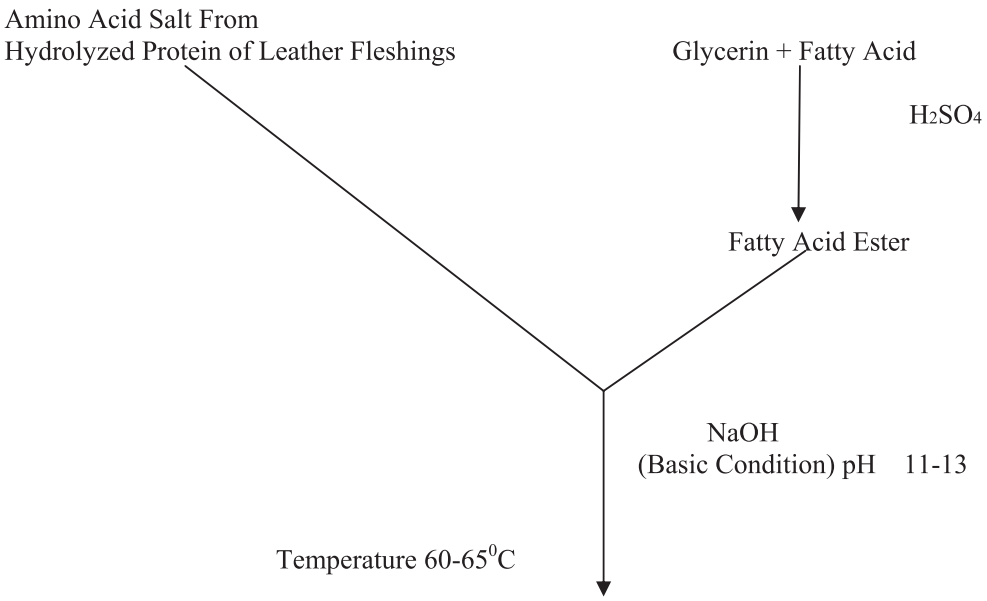
Sample Material	Total Solids (%)
De-limed Fleshings	33.40
Recover Fat	48.74
Protein Hydrolyzate	19.99
Sludge/Residue	23.62



Scheme 1. Separation of fractions from wet raw fleshing waste material.

hydrolyzed protein: 1) biotechnological and 2) chemical methods [32-35]. The well known is the condensation with long-chain carboxylic acids where the acid may be used in the form of chloride, anhydride or various esters as reported earlier [36]. The extracted protein from leather fleshings contain 20 different amino acids in different proportions, these all amino acids reacts with the fatty acid ester of the glycerol in the basic condition as described in the material and methods, thus finally formed a fatty acyl amido carboxylic acid based surfactant as shown in Scheme 2.

This product has the special characteristics due to the two structural features: 1) they contain a long



Scheme 2. Fatty acyl amido carboxylic acid based surfactant.

chain alkyl group for hydrophobic moiety and they contain amido and carboxylic groups acids group for hydrophilic moiety [37]. This product has been applied in the solution form with warmed water in selected wet blue. The prepared surfactant has free base form sites that will react with the acidic sites of leather. In this way the product gives the retanning effect in addition to lubrication. Good result was observed when leather was soaked in the solution and constant drumming for specific period usually about 30 min to 90 min [38]. To study the comparison of standard results of leather, another wet blue was cut in the same size and processed in the similar conditions. The product showed uniform distribution in the leather due to the good interaction of charges. The prepared leathers were evaluated for physical tests. Three samples from each leather were tested and the results are given in (Table 3). All results were improved as compare to reference leather. The difference in shrinkage temperature of surfactant applied leather and the reference was 2 ˚C which is common due to the structural difference in natural skin. The product has increased the strength and the suppleness of leather with lubrication of fibers as shown in the (Table 3). The improvement in all physical properties is due the retanning effect of amino acid. Further research on double action of surfactant will clear the work mechanism.

Table 3. Physical characteristics of the resulted leathers.

Physical Test	Surfactant Applied leather	Reference leather applied Commercial Product
Thickness (mm)	3.46±0.14*	3.23±0.25
Tensile Force at break (N)	297.82±12.73	257.55±6.87
Tensile Strength (N/mm^2)	8.39±0.69	7.811±0.67
Tearing Load (N)	191.00±9.75	152.71±3.69
Tear Strength (N/mm)	78.18+3.98	49.194±2.02
Softness (mm)	3.96±0.37	4.1±0.26

*Standard deviation, calculated from 3 observations of each test.

4. CONCLUSIONS

Protein hydrolyzate from leather fleshing has been separated from other fractions and further converted in to a double action surfactant using fatty acid ester. Application of product has resulted normal soft leather with improved physical characteristics and smooth surface. Therefore, it is concluded that this surfactant from waste protein can be utilized for lubrication with additional retanning benefits.

5. REFERENCES

1. Daskalopolous, E. An integrated approach to municipal solid waste management. *Resources Conservation and Recycling* 24(1): 33-50 (1998).
2. Taylor M.M., L.F. Cabeza, G.L. Dimaio, E.M. Brown, W.N. Marmer, R. Carrio, P.J. Clema & J. Cot. Processing of Leather Waste: Pilot Scale Studies on Chrome Shavings. Part I. Isolation and Characterization of Protein Products and Separation of Chrome Cake. *Journal of American Chemists Leather Association* 93(3): 61–82 (1998).
3. Langmaier, F., P. Mokrejs, K. Kolomaznik, & M. Mladek. Biodegradable packing materials from hydrolysates of collagen waste proteins. *Waste Management* 28: 549–556 (2008).
4. Taylor, M.M., L.F. Cabeza, G.L. Dimaio, E.M. Brown, W.N. Marmer, R. Carrio, P.J. Clema & J. Cot. Processing of leather waste: Pilot scale studies on chrome shavings: Part II Purification of chrome cake and tanning trials. *Journal of American Leather Chemists Association* 93: 83-98 (1998).
5. Veeger, L. Ecological procedure to solve the tannery waste problems. *Journal of American Leather Chemists Association* 88: 326-329 (1993).
6. Colak, S., G. Zengin, H. Ozgunay, Sarikahyah, O. Sari & I. Yuceer . Utilisation of leather industry prefleshings in biodiesel production. *Journal of American Leather Chemists Association* 100: 134-137**(**2005).
7. Eastoe, J.E., The amino acid composition of mammalian collagen and gelatin. *Journal of Biochemistry* 61**:** 598-600 (1955).
8. Gildberg, A., I. Batista & E. Strom. Preparation and characterization of peptone obtained by a two-step enzymatic hydrolysis of whole fish. *Biotechnology and Applied Biochemistry* 1: 413-423 (1989).
9. Ravindran, B., S.L. Dinesh, J.L. Kennedy & G. Sekaran, Vermicomposting of solid waste generated from leather industries using epigeic earthworm Eisenia foetida. *Applied Biochemistry and Biotechnology* 151: 480–488(2008).
10. Kumar G.A , N. Nagesh, T .G. Prabhakar & G. Sekaran. Purification of extracellular acid protease and analysis of fermentation metabolites by Synergistes sp. utilizing proteinaceous solid waste from tanneries. *Bioresource Technology* 99: 2364–2372 (2008).
11. Zupancic, G.D. & A. Jemec. Anaerobic digestion of tannery waste: semicontinuousand anaerobic sequencing batch reactor processes. *Bioresource Technology* 101: 26–33 (2010).
12. Vasudevan N. & A.D. Ravindran. Biotechnological process for the treatment of fleshing from tannery industries for methane generation. *Current Science* 93: 1492–1494 (2007).
13. Colak, S., G. Zengin, H. Ozgunay, H. Sarıkahya & L. Yuceer. Utilization of leather industry prefleshings in biodiesel production. *Journal of American Leather Chemists Association* 100: 137–141 (2005).
14. Shanmugam, P. & N.J. Horan. Optimising the biogas production from leather feshing waste by co-digestion with MSW. *Bioresource Technology* 100: 4117–4120 (2009).
15. Phan, A.N. & T.M. Phan. Biodiesel production from waste cooking oils. *Fuel* 87: 3490–3496 (2008).
16. Ozgunay, H., S. Colak, G. Zengin, O. Sarı, H. Sarıkahya & L. Yuceer. Performance and emission study of biodiesel from leather industry pre fleshings. *Waste Management* 27: 1897–1901 (2007).
17. Chung, K., J. Kim & K. Younglee. Biodiesel production by transesterification of duck tallow with methanol on alkali catalysts. *Biomass Bioenergy* 33: 155–158 (2009).
18. Bhatti, H..N., M.A. Hanif, M. Quasim, & A. Rehman. Biodiesel production from waste tallow. *Fuel* 87: 2961–2966 (2008).
19. Demirbas, A. Biodiesel from waste cooking oil via base-catalytic and supercritical methanol transesterification. *Energy Converervation and Management* 50: 923–927 (2009).
20. Langmaier, F., M. Stibora, M. Mladek, & K. Kolomaznik. Gel–sol transitions of chrome-tanned leather waste hydrolysate. *Journal of Society of Leather Technologists and Chemists* 85: 100–105 (2001).
21. Sumathi, C., & G. Sekaran. Nutritional Evaluation of Animal Fleshing as a Fish Meal Replacer in *Labeo rohita*. *Journal of Aquaculture Feed Science and Nutrition* 2: 6-10 (2010).
22. Xia, J., Y. Xia & I.A. Nnanna. Structure-function relationship of acyl amino acid surfactants: surface activity and antimicrobial properties. *Journal of Agricultural Food Chemistry* 43: 867 (1995).
23. Method for Conditioning of leather (SLP3, IUP 3; BS 3144: method 2) Official Methods of analysis Issue (2010).
24. Measurement of Distension and Strength of Grain by Ball Burst (SLP 9, IUP /9; BS3144: method 8), Official Methods of Analysis Issue (1996).
25. Measurement of Leather Softness (IUP36, SLP 37) Official Methods of Analysis Issue (2001).
26. Measurement of Thickness (SLP 4 , IUP4; BS 3144:method 3) Official Methods of Analysis Issue (2001).
27. Measurement of Tearing Load (SLP 7, IUP8; BS3144: method 6) Official Methods of Analysis Issue (2001).
28. Measurement of Tensile Strength and Percentage Elongation (SLP 6, IUP6; BS3144: method 5) Official Methods of Analysis Issue (2001).
29. Measurement of Shrinkage Temperature (SLP

18,IUP16;BS3144: Method 17).

30. Ramamoorthy, G., P.K. Sehagal & Mahendrakumar. Improved uptake of basic chromium salts in tanning operations using keratin hydrolysate. *Journal of Society of Leather Technologists and Chemists* 73:168-170 (1989).
31. Simeonova, L.S. & P.G. Dalev. Utilization of leather industry waste. *Waste management* 16(8): 765-769 (1996).
32. George, A., J. Modi, N. Jain, P. Bahadur. A comparative study on the surface activity and micellar behavior of some N-acylamino acid based surfactants. *Indian Journal of Chemistry* 37: 985 (1998).
33. Tabohashi, T., K. Tobita, K. Sakamoto, J. Kouchi, S. Yokoyama, H. Sakai & M. Abe. *Solution properties of amino acid-type new surfactant: Colloids and Surfaces, B*: *Biointerfaces* 20: p.79–86 (2001).
34. Sánchez, L., M. Mitjans, M.R. Infante, M.T. García, M.A. Manresa & M.P. Vinardell. The biological properties of lysine-derived surfactants. *Amino Acids* 32: 133–136 (2007).
35. Holmberg, K. Natural surfactants. In: *Current Opinion in Colloid and Interface Sciences, 6 ed.*, p.148–159 (2001).
36. Manzo, G., G. Fedele & A. Colurcio. Utilizzazione della rasatura nella concia al cromo, Cuoio Pelli Mat. Contianti 61 p. 11 (1985).
37. Chen, Q., S. F. Chen & Z.Y. Li. Preparation and properties of amino acid based surfactants. *Chinese Chemical Letters* 10: 789-792 (1999).
38. Allen, A.S. & D.S. Finlay. Leather Softening. *United States Patent* 5603733 (1997).