

COMPARISON OF SURFACTANTS PREPARED FROM SKIN FLESHINGS WASTE: A STATISTICAL APPROACH

Beena Zehra*¹ and Syed Shahid Shaukat²

¹Leather Research Centre, PCSIR, D-102, SITE, South Avenue, Karachi-74600, Pakistan

²Institute of Environmental Studies, University of Karachi, Karachi-75270. Pakistan

Correspondence at: (zehrabeena@yahoo.com)

ABSTRACT

In the present study, three surfactant products were prepared from waste protein of skin fleshings waste and then reacted with three different fatty acids. These surfactant products were applied in the same dosage (10%) on a goat skin as a replacement of fatliquor. The results obtained from physical testing of final leathers were compared with a commercial product. All the results such as tear strength (N/mm), tensile strength (N/mm²), bursting load (N), distension at break (mm), water absorption (%) were compared using the ANOVA followed by Duncan's Multiple Range Test. These results were found significant $P < 0.001$. Thus, there is a possibility to convert a waste material into a surfactant product which can improve the leather properties comparable to commercial product.

Keywords: Tanneries Waste, Surfactant, Skin fleshings, Comparison, ANOVA, Duncan's Multiple Range Test.

INTRODUCTION

Leather processing has regularly been associated with high pollution due to the generation of different organic wastes during the traditional manufacturing processes in tanneries (Taylor *et al.*, 1998a). It is generally known that approximately 200 kg leather product is produced from one ton of wet salted hides. Therefore, a massive amount of solid wastes is generated (Langmaier *et al.*, 2008; Veeger 1993). This solid waste creates a major problem for tanneries in terms of both their variety and quantity. A huge amount of skin fleshings waste is reusable in various useful products after necessary modification as reported earlier by Colak *et al.* (2005). Commonly, leather waste processings are associated to the innovative separation and processing procedures for fats, protein and chromium (Colak *et al.*, 2005; Taylor *et al.*, 1998b). The skin fleshings waste is discarded without any further treatment in our country from tanneries. Previously, skin fleshings have been anaerobically digested for the production of methane gas using biomethanation process (Zupancic and Jamec, 2010). These skin fleshings have been used for glue manufacture, enzyme production and animal feed production as reported earlier (Vasudevan and Ravindran, 2007). Limed skin fleshings have been co-digested with biodegradable fraction of municipal solids waste and optimized for biogas production (Shanmugam and Horan, 2009). The skin fleshings have also been used for fishes feed (Sumathi and Sekaran, 2010).

The surfactants from natural non-toxic resources have got unique attention due to their hydrophilic emulsifying group with non toxicity on biological systems and strong antimicrobial activity (Colak *et al.*, 2005). As a result, protein based surfactants have been studied for important applications in various areas, such as foods, cosmetics and pharmaceutical formulations, etc. as reported earlier (Xia *et al.*, 1995). The objective of the present work were 1) isolation of protein from skin fleshings waste 2) conversion into surfactant product and 3) comparison of results after the application of surfactant products in leather as a replacement of fatliquor.

MATERIAL AND METHODS

Tannery chemicals for processing were purchased from local market and used without purification. Fatty acids were purchased from Merck. Centrifugation of surfactant product was done by using variable speed centrifuge model 2010H from Scientific LTD, UK. Samples were conditioned prior to physical testing in a normal atmosphere of temperature $20 \pm 2^\circ \text{C}$ with a relative humidity of $65 \pm 2\%$. Measurement of thickness (SLP 4, IUP4; BS 3144:method 3) by Official methods of Analysis Issue 2001 was carried out. Thickness of prepared leather samples were measured using calibrated thickness gauge apparatus of 50mm diameter. Measurement of tensile strength and percentage elongation (SLP 6, IUP6; BS3144: method 5) by Official methods of Analysis Issue 2001 was carried out. Measurement of tearing Load (SLP 7, IUP8; BS3144: method 6) by Official methods of Analysis Issue 2001 was carried out. Softness of leather was determined with the (IUP36, SLP 37) Official methods of Analysis Issue 2001. It is a non destructive method for determining the softness of leather. This method is applicable to all non-

rigid methods e.g. shoe upper leather, leather goods leather and apparel leather. Leather Softness Tester ST 300 from MSA Engineering Systems, UK was used for this test. This method is based on the principle that a cylindrical rod of definite mass is lower at a specific rate into a firmly clamped area of leather. The distension (mm) of the leather produced was recorded as the softness. The water absorption was measured on Bally Penetrometer model 5220. The dynamic water resistance of processed leathers after application of prepared surfactant products was checked with IUP 10 (SLP-22) Official methods of Analysis Issue 2001. Bally Penetrometer machine was used to measure the water absorption. The motion of machine was adjusted at 50 cycles/min \pm 5 cycles/min. Test samples were prepared and tested according to standard while % of water absorption was calculated after 120 minutes. The percentage of water absorption, W_a , was calculated using the formula:

$$W_a = \frac{(M_1 - M_0) \times 100}{M_0}$$

Where: M_1 is the mass of the sample after 2 h time period, in grams.
 M_0 is the initial condition mass of the sample, grams

Liquid paraffin from Merck was used to check the emulsification of prepared surfactants. Emulsification of prepared surfactants was checked by blending with liquid paraffin with a speed stirrer for continuous 5 minutes in different proportions using liquid paraffin and prepared surfactant products. These emulsions were prepared with the 5-10% of the surfactant while (10%) of the liquid paraffin was used. For the comparison of emulsification results emulsions of the liquid paraffin and protein solution were also prepared in the 100ml graduated plastic test tubes. The stability of prepared emulsions with protein and liquid paraffin was checked by storage of prepared emulsions for 7 days at room temperature (25 °C) in graduated plastic test tubes and covered with plastic caps. A commercial water resisting surfactant was applied in the processing for the comparison of physical testing results. Physical testing of leathers were carried out according to standard test methods and results were quantified by ANOVA and subsequently by Duncan's Multiple Range test (Zar, 1999). The results of physical testing were found significant.

Experimental

Waste skin fleshings were collected then subjected for hydrolysis by 6% alkaline mixture of magnesium oxide and sodium hydroxide (on moisture free weight of skin fleshings) with water in autoclave at 95-110 °C for 2h continuously. Then the protein was reacted with 1:1 ratio with the long chain fatty acids chlorides. Three protein-fatty acids condensation products were prepared by changing the fatty acid source in addition to the Paraffin Wax. The reaction was carried at 160°C minutes with constant stirring by overhead stirrer. When reaction was completed the flasks were cooled at room temperature. Then products were separated by centrifugation at 4500 RPM. The supernatant was water which was confirmed from emulsifying power test, while milky colored surfactant products were in the thick paste form at the bottom of the tubes. The thick form surfactant products were separated with spatula and stored in a cool place till application.

Application of Surfactant Products

Four goat skins were processed up to wet blue by usual chrome tanning process selected for the application of surfactants. Three wet-blue were used for three different prepared surfactant products named as TR1, TR2 and TR3 while the fourth one was processed as a reference with the commercial surfactant product named as TR4. Surfactants were diluted with distilled water (1:5) by stirring for 10 minutes at 60°C. Wet-blues were processed with further tannery processes e.g. Shaving, Washing, Neutralization, Retanning, Washing, Fatliquoring and then Fixation.

RESULTS

After collection of limed skin fleshings, these were processed as given detailed in "material and methods". Alkaline hydrolysis of skin fleshings results in three valueable fractions of which the yields were calculated on moisture free basis. The fats were found 20.31 \pm 0.16, protein hydrolyzate 65.43 \pm 0.20 and sludge or residues were found 14.38 \pm 0.27. These fractions were kept in the fridge in separate flask until further utilization.

Preparation and application of surfactant for water resisting of leather

As a result of the reaction of the fatty acids and hydrolyzed protein as given in 'material and methods' with three different fatty acids white colored waxy surfactant products were obtained. These surfactant products were initially in the liquid form while on cooling at room temperature these were solidified into a thick paste form. These surfactant products were checked for emulsification. Fine emulsions were prepared with the prepared surfactant with liquid paraffin. Emulsions with protein solution and liquid paraffin were showed a gel like behaviour and slightly stable. Prepared crust leathers were evaluated for various physical tests, the results are presented in Table 1. The tear strength (N/mm) varied significantly with the treatments ($F=37.925$, $P<0.001$). The treatment 1 showed highest tear strength, followed by treatments 3 and treatment 4. The lowest tear strength was found in the treatment 2. The elongation (%) varied significantly with the treatments ($F=67.119$, $P<0.001$). The treatment 1 showed highest tear strength, followed by treatments 2 and treatment 3. The lowest tear strength was found in the treatment 4. The tensile strength (N/mm^2) varied significantly with the treatments ($F=43.197$, $P<0.001$). The treatment 2 showed highest tensile strength, followed by treatments 3 and treatment 1. The lowest tensile strength was found in the treatment 4. The bursting load (N) varied significantly with the treatments ($F=14.156$, $P<0.001$). The treatment 2 showed highest bursting load followed by treatments 3 and Treatment 1. The lowest bursting load was found in the treatment 4. The distension at break (mm) varied significantly with the treatments ($F=41.794$, $P<0.001$). The treatment 4 showed highest distension at break, followed by treatments 3 and treatment 2. The lowest distension at break was found in the treatment 1. The water absorption varied significantly with the treatments ($F=492.782$, $P<0.001$). The treatment 4 showed highest water absorption followed by treatment 3 and treatment 1. The lowest water absorption was found in the treatment 2.

Table 1. Physical Characteristics of Resulted Crust Leather from treatments 1-4.

Treatment No.	Tear Strength (N/mm)	%Elongation (mm)	Tensile Strength (N/mm^2)	Bursting load (N)	Distension at break (mm)	Water Absorption after 120minutes (%)
F-values	37.925	67.119	43.197	14.156	41.794	492.782
Treatment 1	23.277±1.508a*	119.399±5.985a*	5.651±0.084c*	312.455±4.933b*	36.598±1.279b*	21.354±0.654c*
Treatment 2	15.443±0.884c*	116.183±3.793a*	7.753±0.498a*	335.525±1.072a*	37.673±0.563b*	19.768±0.200c*
Treatment 3	18.912±0.541b*	113.499±4.86a*	6.92±0.206b*	332.153±2.993a*	38.096±1.527b*	24.580±0.977b*
Treatment 4 (Reference)	17.639±0.304b*	75.85±0.677b*	5.489±0.114c*	267.358±25.791b*	45.378±0.575a*	48.269±1.704a*
LSD 0.05	1.747	8.119	0.534	27.191	2.023	1.959

*standard deviation is given against each result calculated from three observations of each test *Note: Means sharing same letters in a column are non-significant.

The effect of surfactant on tear strength (N/mm) of leather is presented in Table 1. The tear strength was highest in the treatment 1 while little difference was observed in the other three treatments 2, 3 and 4 ($F=37.925$, $P<0.001$).

The effect of surfactant on elongation (%) of leather is presented in Table 1. The elongation (%) was highest in the treatment 1 while little difference was observed in the other two treatments 2, 3 and lowest was found in the treatment 4 ($F=67.119$, $P<0.001$).

The effect of surfactant on tensile strength (N/mm^2) of leather is presented in Table 1. The tensile strength was highest in the treatment 2 while little difference was observed in the other two treatments 1, 4 and lowest was found in the treatment 1 ($F=43.197$, $P<0.001$).

The effect of surfactant on bursting load (N) is presented in Table 1. Some difference was observed in the treatment 2 and 3. The lowest bursting load was found in the treatment 4 ($F=14.156$, $P<0.001$).

The effect of surfactant on distension (mm) is presented in Table 1. The highest distension was found in the treatment 4 while little difference was observed in the other three treatments 1, 2 and 3 ($F=41.794$, $P<0.001$).

The effect of surfactant on water absorption of leather is presented in Table 1. The highest water absorption (%) was found in the treatment 4 while little difference was observed in the treatment 1, 2 and 3 ($F=492.782$, $P<0.001$).

The change in all parameters of physical testing was due to the effect of surfactant. All these results by using standard methods were found significant as analyzed with the ANOVA followed by Duncan's Multiple Range Test. The tested treatments (quality characteristics can be ranked in order of their test qualities (Table 2).

Table 2. Ranking of materials based on their quality characteristics.

Rank	Treatment No.	Mean	n	Non-Significant Ranges
1	4	48.269	3	a
2	3	24.580	3	b
3	1	21.354	3	c
4	2	19.768	3	c

DISCUSSIONS

The animal skin has complex morphological structure. It consists of three layers, outer layer is epidermis, middle layer is dermis, and a lower layer is subcutis. The dermis layer of the skin is used to produce leather and it is composed of connective tissue. It is bound to the skin by looser fibrillar connective tissue which contains bundles of collagen and elastin fibers. During the fleshing process in tanneries, the subcutis layer of the skin is scrapped off through the sharp knives and thrown away from the tanneries as a waste (Simeonova and Dalev, 1996). This skin fleshings waste might be a resource for the production of various valueable products. In this study, these skin fleshings were collected from tanneries and hydrolyzed in alkaline mixture of magnesium oxide and sodium hydroxide. Three different fractions were isolated from hydrolyzed fleshings as previously reported (Zehra *et al.*, 2013). The first fraction was fat content present at the top layer due to light its weight. The second fraction was protein solution and third fraction was insoluble residue at the bottom layer. Second fraction was selected for the preparation of surfactant because of its most purity and richness in amino acids, while the work on the utilization of other two fractions was carried on other types of products such as fertilizer and finishing agent. Extracted protein from leather solid waste has wide applications for different industrial purposes as reported earlier (Nawaz *et al.*, 2009 and 2010; Reis and Beleza 1991; Taylor *et al.*, 1998a ,b; Ohtsuka, 1973; Colak *et al.*, 2005; Gaidau *et al.*, 2007). Protein from renewable sources have surface-active properties which can be further enhanced by some chemical treatments. The protein fatty acid condensates as surfactant products were prepared by condensation reaction of the fatty acids as detailed in “material and methods”. The condensation reaction of the protein with long chain carboxylic acids seems to agree with the findings from those previous reported in literature (Manzo and Fedele, 1985). The condensation of protein with long-chain fatty acids is well reported, whereas the acid may be used in the form of anhydride, chloride or esters (Manzo and Fedele, 1985). Fatliquoring of leather is the last wet chemical process in tannery. These fatliquors may be ionic, cationic or neutral and these can have varing degree of sulphonation and hydrophobic characteristics. This fatliquoring process lubricates the fibres of leather and keeping these fibers from adhering to each other. Presently, traditional fatliquoring agents are being replaced with more recyclable chemicals. This means that the new hydrophobic and oleophobic agents now replace the alkyl phenols and toxic organic halogen compounds. Surfactants can be obtained from molecules that mimic natural amphiphilic structures. The association of a polar amino acid (hydrophilic group) and a non polar long chain compounds (hydrophobic group) builds an amphiphilic structure that allows to obtaining molecules having a high surface activity (Infante *et al.*, 2004). Thus, these lipoamino acids obtained from natural raw materials are the important surface-active molecules for applications in the pharmaceutical, food and cosmetic industries. As a result of their natural structure, they have a low degree of toxicity with resistance to hard water and antimicrobial activity (George *et al.*, 1998) and easily biodegradability (Infante *et al.*, 2004; Infante *et al.*, 1997). The uses of eco-friendly surfactants in various industrial formulations have utilized amino acids as an excellent molecules for modification for amphiphiles (Blunk *et al.*, 2006 ; Infante *et al.*, 2004; Ohta *et al.*, 2003; Roy and Dey, 2007; Tabohashi *et al.*, 2001; Yoshimura *et al.*, 2007). The synthesis of amino acid derived surfactants from naturally occurring lipoamino acids which provides molecules having very lower toxicity for the living things (Brito *et al.*, 2009; Holmberg, 2001; Sánchez *et al.*, 2007) with higher biodegradability in the environment (Infante *et al.*, 1997 and 2004) when compared to usual surfactants. These amino acid based molecules have antimicrobial activity (Infante *et al.*, 1997; Holmberg, 2001). Protein and their hydrolysates are amphotiles with higher surface-active properties which might be further enhanced with some chemical treatment (Bautista *et al.*, 2015). The most important characteristics of the condensates are more surface-activity, improved dispersing, and better stability in hard water in electrolytes solutions as compare to untreated hydrolyzates. Therefore, the products were prepared by protein hydrolyzate and fatty acids and applied to goat skin leather processing (10%) at fatliquoring stage as a replacement of fat liquor as well as a water resisting agent. It has been found that despite hydrophilic action of protein, the use of surface modified substance resulted in

an improvement of the water repellent effect. The significant difference in water absorption was observed in the results. Basically, the absorption of water into leather completes gradually from upper to bottom portion. Therefore, this process must be stopped at any step by the application of proper chemicals. Long chain fatty acids in the series of 16-22 C-atoms are most appropriate for surfactants because they support the formation of oil-in-water emulsions and develop the organoleptic behaviour of leather as well as physical properties when applied in the aqueous solution form (Kaussen *et al.*, 1989). The emulsions of protein and liquid paraffin were found slightly stable. These alkaline emulsions were found to have greater instability while the net charge of collagen protein is lower at the high pH values that reduces the electrostatic forces of the droplet (Santana *et al.*, 2012). Therefore, leather protein gelatin is widely utilized as a gelling agent, although it shows the weak emulsifying properties as compared to other biopolymers such as globular proteins (Karim and Bhat, 2009).

CONCLUSION

Protein-fatty acids condensates as surfactants have been prepared from waste protein extracted from skin fleshings waste. These surfactant products have been successfully applied in leather processing not only as a fatliquor but as water resisting agent. The physical testing results were subjected to ANOVA which were found significant. The results are in agreement with the previous reported work (Bautista, *et al.*, 2015) which might be an interesting area for the preparation of environment friendly surfactants.

REFERENCES

- Bautista, M.E., L. Perez, M.T. Garcia, S. Cuadros and A. Marsal (2015). Valorization of tannery wastes: Lipoamino acid surfactant mixtures from the protein fraction of process wastewater. *Chemical Engineering Journal*, 262: 399–408.
- Blunk, D., P. Bierganns, N. Bongartz, R. Tessorf and C. Stubenrauch (2006). New speciality surfactants with natural structural motifs. *New Journal of Chemistry*, 30: 1705–1717.
- Brito, R.O., E.F. Marques, S.G. Silva, M.L. do Vale, P. Gomes, M.J. Araujo, J.E. Rodriguez-Borges, M.R. Infante, M.T. Garcia, I. Ribosa, M.P. Vinardell and M. Mitjans (2009). Physicochemical and toxicological properties of novel amino acid-based amphiphiles and their spontaneously formed catanionic vesicles, *Colloids Surf. B: Biointerfaces*, 72: 80–87.
- Colak, S., G. Zengin H. Ozgunay Sarikahyah., O. Sari and I. Yuceer (2005). Utilisation of Leather Industry pre-fleshings in Biodiesel Production. *Journal of American Leather Chemists Association*, 100: 134-137.
- Holmberg, K.. (2001). Natural surfactants. *Current opinion in Colloid and Interface Sciences*, 6: 148–159.
- Gaidau, C.M., Ghiga, E. Stepan., D. Taloi and Flipescu (2007). Bioactive protein-based additives and new materials obtained from leather manufacture by-products. *Proceedings of 29th Congress of the International Union of leather Chemists Association*, Washington.
- George, A., J. Modi, N. Jain, P. Bahadur (1998). A comparative study on the surface activity and micellar behavior of some N-acylamino acid based surfactants. *Indian Journal of Chemistry, Section A*, 37A: 985.
- Infante, M.R., L. Perez, A. Pinazo, P. Clapes, M.C. Moran, M. Angelet, M.T. Garcia and M.P. Inardell (2004). Amino acid-based surfactants. *Comptes Rendus Chimie*, 7: 583.
- Infante, M.R., A. Pinazo and J. Seguer (1997). Non-conventional Surfactants from Amino Acids and Glycolipids: Structure, Preparation and Properties. *Colloids and Surfaces, A: Physicochemical and Engineering Aspects*, 49: 123-124.
- Kaussen, M., D. Stockhauson, H.G Hartan and A. Landscheidt (1989). Retanning Process. U.S patent 4813968.
- Karim, A.A and R. Bhat (2009). Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids*, 23: 563-576.
- Langmaier, F., P. Mokrejs, K. Kolomaznik and M. Mladek (2008). Biodegradable packing materials from hydrolysates of collagen waste proteins. *Waste Management*, 28: 549–556.
- Manzo, G. and G. Fedele and A. Colurcio (1985). Utilizzazione della rasatura nella concia al cromo, *Cuoio Pelli Mat. Contianti*, 61 (11): 11-15.
- Nawaz, H.R, B. Zehra, B. A Solangi and U. Nadeem (2009). Recycling of chrome tanned leather solid wastes as a retanning agents: Towards environmental clean leather technology. *Environmentally Sustainable Development*, 3: 298-302.
- Nawaz, H.R., B.A Solangi, U. Nadeem and B. Zehra (2010). Preparation of High Exhaust Chrome from Leather Shavings and Hydrocarbons with its Application in Leather Processing for Green Tanning Technology. *Journal of Chemical Society of Pakistan*, 32: 525-530.

- Ohtsuka, K. (1973). Amino acid seasoning produced from scraps obtained from chrome tanned leather. *Japan Patent*, 7: 329, 145.
- Ohta, A, N. Ozawa, S. Nakashima, T. Asakawa and S. Miyagishi (2003), Krafft temperature and enthalpy of solution of N-acyl amino acid surfactants and their racemic modifications: effect of the amino acid residue. *Colloid Polymer Science*, 281: 363–369.
- Roy S and J. Dey (2007). Effect of hydrogen-bonding interactions on the self-assembly formation of sodium N-(11-acrylamidoundecanoyl)-l-serinate, l-asparaginate, and l-glutamate in aqueous solution, *Journal of Colloid Interface Science*, 307: 229–234.
- Reis, A.D.M and V. Beleza (1991). Utilization of leather waste: animal feedstuff from chrome shavings. *Journal of the Society of Leather Technology and Chemists*, 75: 15–19.
- Santana, R.D.C., A.C.K Sato and R.L.D Cunha (2012). Emulsions stabilized by heat-treated collagen fibers. *Food Hydrocolloids*, 26: 73-81.
- Sánchez, L., V. Martínez, M.R. Infante, M. Mitjans and M.P. Vinardell (2007). Hemolysis and antihemolysis induced by amino acid-based surfactants. *Toxicology Letters*, 169: 177–184.
- Shanmugam, P. and N.J. Horan (2009). Optimising the biogas production from leather fleshing waste by co-digestion with MSW. *Bioresource Technology*, 100: 4117–4120.
- Simeonova, L.S and P.G. Dalev (1996). Utilization of a leather industry waste. *Waste Management*, 16: 765-769.
- Sumathi, C. and G. Sekaran (2010). Nutritional Evaluation of Animal Fleshing as a Fish Meal Replacer in *Labeo rohita*. *Journal of Aquaculture Feed Science and Nutrition*, 2: 6-10.
- Tabohashi, T., K. Tobita, K. Sakamoto, J. Kouchi, S. Yokoyama, H. Sakai and M. Abe (2001). Solution properties of amino acid-type new surfactant, *Colloids and Surfaces B: Biointerfaces*, 20: 79–86.
- Taylor, M.M., L.F. Cabeza, G.L. Dimaio, E.M. Brown, W.N. Marmer, R. Carrio, P.J. Clema and J. Cot (1998). 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.
- Taylor M. M., L. F. Cabeza, G.L. Dimaio, E.M. Brown, W.N. Marmer, R. Carrio, P.J. Clema and J. Cot (1998). 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: 61 – 82.
- Vasudevan N. and A.D, Ravindran (2007). Biotechnological process for the treatment of fleshing from tannery industries for methane generation. *Current Science*, 93: 1492–1494.
- Veeger, L. (1993). Ecological Procedure to Solve the Tannery Waste Problems. *Journal of American Leather Chemists Association*, 88: 326-329.
- Xia, J., Y. Xia and I.A Nnanna, (1995). Structure-function relationship of acyl amino acid surfactants: surface activity and antimicrobial properties. *Journal of Agricultural Food Chemistry*, 43: 867.
- Yoshimura, T., A. Sakato, K. Tsuchiya, T. Ohkubo, H. Sakai, M. Abe and K. Esumi (2007). Adsorption and aggregation properties of amino acid-based N-alkyl cysteine monomeric and N,N'-dialkyl cystine gemini surfactants. *Journal of Colloid Interface Science*, 308: 466–473.
- Zar, J.H. (1999). *Biostatistical Analysis*. 4th edition Prentice. Hall, Englewood Cliffs, New Jersey.
- Zehra, B., O. Hany and S.S. Shoukat (2013). Preparation, application and quantification of retanning products from skin fleshing hydrolyzed protein: A process optimization for waste recycling from tanneries into Product. *International Journal of Advanced Research*, 1: 406-414.
- Zupancic, G.D. and A. Jemec (2010). Anaerobic digestion of tannery waste: semicontinuous and anaerobic sequencing batch reactor processes. *Bioresource Technology*, 101: 26–33.

(Accepted for publication March 2016)