www.czasopisma.pan.pl PAN www.journals.pan.pl

ARCHIVES OF ENVIRONMENTAL PROTECTION

vol. 40 no. 1 pp. 3 - 12 2014

VERSITA

PL ISSN 2083-4772
DOI: 10.2478/aep-2014-0003

© Copyright by Polish Academy of Sciences and Institute of Environmental Engineering of the Polish Academy of Sciences, Zabrze, Poland 2014

ECO-FRIENDLY SOAKING PROCESS USING TANNIC ACID AS AN ALTERNATIVE BACTERICIDE

ARIFE CANDAŞ ADIGÜZEL ZENGIN[1]*, SELIME MENTEŞ ÇOLAK[1], GÖKHAN ZENGIN[1], EYLEM KILIÇ[2]

[1]Department of Leather Engineering, Engineering Faculty, Ege University
Bornova 35100 Izmir, Turkey
[2]Department of Leather Engineering, Uşak University, 64200 Uşak, Turkey
*Corresponding authors e-mail: adiguzel.candas@gmail.com

Keywords: Bactericide, leather industry, soaking, tannic acid.

Abstract: Eco-friendly leather processes based on the usage of natural products have become a potentially attractive issue for leather industry during the last few decades. Synthetic protective chemicals like bactericides used in most soaking process are known as hazardous substances and cause tannery effluents with high concentrations of Chemical Oxygen Demand (COD). In the present study, the effect of tannic acid on microorganisms, skin, wool and effluent were investigated in order to demonstrate the applicability of tannic acid in soaking process instead of commonly used bactericides. The bacterial load (cfu/ml), COD and Nitrogen Content (N) of the soaking effluents and Total Kjeldahl Nitrogen (TKN) content of skins and wools were investigated. Application of 0.5 and 1 wt% tannic acid concentrations was more effective than commercial bactericide, while comparable results were achieved by 0.1 and 0.3 wt% tannic acid. The application of tannic acid for soaking process resulted in lower COD and N values of effluents. The results show that tannic acid has the potential to be an alternative, eco-friendly bactericide for leather industry by reducing the pollution of leather soaking process.

INTRODUCTION

New eco-benign systems, either using non-hazardous compounds or developing new production methods have become the raising trend in global industrial activities, due to the increasing health and environmental regulations and restrictions.

Researchers have adopted various approaches to minimize the environmental impact of leather processes taking into consideration the use of chemicals having less toxicity or less environmental impact [5, 27], recovery and/or reuse of water, floats and chemicals in leather processing steps [25, 36, 43] and innovation of processes and products [33, 476].

Soaking is one of manufacturing processes of leather used to restore the original water content of skin. In this process synthetic antimicrobial agents are widely used to prevent microbial growth. However, considering the potential toxicity of these chemicals and requirements imposed by regulating agencies, natural eco-friendly alternative

antimicrobial products have recently become a predominant focus of interest in many fields like textile, leather as well as pharmacy, medicine, cosmetics, food and etc.

In leather industry, the use of several alternative environmentally friendly antimicrobial products has been reported in different processing steps. The antimicrobial effect of *Aloe vera* in fatliquoring process [6], the biocide effect of photo sensitizers [16], bronopol [38] and ozone [39] in soaking process were investigated. The use of vegetable tannins such as quebracho, mimosa, gall-nut, chestnut and valonia as a biocide in leather soaking process was also studied [18] owing to their antimicrobial and antiseptic properties [19]. Tannic acid, which is a commercially important hydrolysable gallotannin, has also got a great deal of attention as an antimicrobial agent due to its antimicrobial, antimutagenic, anticarcinogenic and antioxidant properties [1, 3, 4, 10, 22, 23, 31, 35, 37, 41, 44]. Tannic acid is present in a variety of fruits and vegetables including tea, cocoa, beans, grapes, strawberry, persimmon, especially the bark of the oak species, sumac and myrobalan [1, 11, 40] and also categorized as a 'generally recognized as safe' (GRAS) food additive [1]. In addition, it is not classified as hazardous material according to CLP Regulation (EC) 1272/2008 on classification, labeling and packaging of substances and mixtures [32]. Tannic acid was applied in textile [8], microbiology [45], medical and biomaterial sciences [2, 26] and leather industry [17].

Recent studies on tannic acid in leather industry can be summarized as an application of tannic acid in pickling and tanning processes [17, 20, 21, 46, 52], the reduction of Cr (VI) formation in leather by tannic acid [14] and use of tannic acid in leather dyeing process as a natural dye [29]. According to our knowledge, so far, there has been no reported research on the application of tannic acid as a bactericide in soaking process for leather industry.

In this research, the applicability of tannic acid as an alternative natural antimicrobial agent in respect to common synthetic bactericides used in soaking process were investigated to reduce the negative environmental impact of these protective chemicals.

MATERIALS AND METHODS

Materials

Tannic acid (Merck, Darmstadt, Germany), which is the powder form of tannin with a pH value of 3.5 (100 g/l, H_2O, 20°C), was used as an eco-friendly bactericide in soaking process. The bactericide effect of tannic acid was tested using 100 cm^2 pieces of dry salted raw Metis type sheepskins. To evaluate the antibacterial effect of tannic acid, a commercial bactericide containing benzisothiazolinon (25% conc.) as an active ingredient was used.

Methods

Leather samples were pre-soaked for 2 hours under static conditions and washed for 5 minutes to remove the dirt and remained salt. Then, the samples were put in 2L beakers and soaked with 1L solutions of tannic acid of different concentrations (0.1, 0.3, 0.5 1.0%) as well as with 0.1% solution of commercial bactericide at 27°C. During the soaking process, a shaker provided mechanical mixing for 10 minutes per hour. Also, pH and temperature values of soaking baths were controlled. At 8 and 24 hours of process, samples were taken for the determination of soaking bath properties. In order

to demonstrate the applicability of tannic acid in soaking process, not only the effect of tannic acid as bactericide, but also its effect on soaking effluent and the compositions of skins and wools were determined. A blank bath without any protective chemicals was also analyzed. The results were given as mean values (±SE) obtained with triplicate samples and evaluated statistically.

The enumeration of cultivable bacteria by plate count method (CFU)

The bacterial load of the soaking baths was determined with the samples taken at 8th and 24th hours of process by use of pour plate method. 1ml of each soaking sample was added to 9 ml sterile physiological saline solution for preparing serial dilutions of samples. This procedure was followed by mixing 1 ml aliquots of the appropriate sample dilutions with Plate Count Agar (pH 7; 45°C; Merck) in a sterile petri dish. After plating, the media were incubated at 37°C for 24 hours and the cultivable bacterial cells in 1 ml of a soaking bath were counted. For each experiment, three separate sub-samples from soaking baths were analyzed and the enumeration of bacteria was expressed in log cfuml^{-1}.

The determination of N contents and COD values of soaking effluents

The spent liquor from the soaking operation was analyzed for total N content and COD values (mg·L^{-1}) using standard kits (114763: Nitrogen (total) Cell Test; 114555: COD Cell Test; Merck) with Merck SQ 300 Water and Wastewater Spectrophotometer at 520 and 585 nm respectively. N content and COD load of each soaking effluent was analyzed in triplicate.

The determination of shrinkage temperature and TKN

Shrinkage temperature of leather samples was determined in accordance with TS 4120 EN ISO 3380 [49]. In this method, soaked 5 × 0.2 cm leather specimen was immersed into distilled water which was used as a heating medium for the shrinkage temperatures at or below 98°C and the heater was set to make the temperature of the heating medium increase 2°C per minute. When the first shrinkage was observed, the changes were recorded in every 30 seconds until the last shrinkage.

TKN values of skins and wools were determined in accordance with the Kjeldahl nitrogen method [7, 50] and experiments were performed in triplicate.

Scanning Electron Microscopic (SEM) Examination

The fiber structure of wool of each soaked leathers after soaking process was monitored with TM 1000 Hitachi (Japan) Tabletop Scanning Electron Microscopy with the magnification power 20–10.000 according to methods previously described in the literature [9]. The wool samples were dried at 40°C after soaking process and randomly selected ten separate wool samples from each application were viewed under the SEM without requiring any sample preparation.

Statistical analysis

The data were analyzed with SPSS 15.0 for Windows. Analysis of variance was performed on each attribute and data were analyzed for treatment effects, time effects and treatment by time interactions. When significant interactions were observed, Tukey *post hoc* test was used for multiple comparisons for enumeration of the cultivable bacteria

assessment. Multiple comparisons of chemical oxygen demand, total nitrogen content and total kjeldahl nitrogen results were performed by Duncan test. The evaluation of the linear relationship between tannic acid offer and the CFU, COD, N and TKN results was measured using Pearson's correlation.

RESULTS

The Effect of Tannic Acid on Microbial Load of Soaking Process

The bacterial load of soaking baths with different concentrations of tannic acid and control samples are presented in Table 1.

Table 1. The number of cultivable bacteria expressed as $\log_{10}$cfu per 1 ml after 8th and 24th hours of soaking process

	Treatment					
Time (h)	**Blank**	**0.1% TA**	**0.3% TA**	**0.5% TA**	**1% TA**	**Bactericide**
8	5.93 ± 0.015^{Ba}	4.80 ± 0.012^{Bbc}	4.53 ± 0.004^{Bcd}	4.22 ± 0.003^{Bed}	3.51 ± 0.004^{Be}	5.06 ± 0.017^{Bab}
24	8.03 ± 0.028^{Aa}	7.66 ± 0.008^{Ab}	7.51 ± 0.003^{Ab}	7.28 ± 0.004^{Ac}	6.49 ± 0.005^{Ac}	7.43 ± 0.006^{Ab}

* values in the same columns or rows followed by the same upper or lower case letters, respectively are not significantly different according to Duncan test ($p < 0.01$).
TA, tannic acid; $n = 3$

The bacterial growth at 8 hours of soaking indicated that all the concentrations of tannic acid wt% could prevent the microbial growth under ideal conditions. The microbial growth prevention of 1wt% tannic acid was significantly ($p < 0.01$) higher than the other treatments of tannic acid and control samples. On the other hand, there was no significant difference between control samples and 0.1, 0.3, 0.5 wt% concentrations of tannic acid ($p > 0.01$).

The number of cultivable bacteria in bactericide added soaking process for 24 hours was found significantly lower than the blank sample ($p < 0.01$). Comparable results with commercial bactericide were obtained by using 0.1 and 0.3 wt% of tannic acid ($p > 0.01$). For the same period of time, although 0.1 wt% of tannic acid gave the highest colony forming unit results, it has been a limitation at microbial growth as much as half of the blank sample ($p < 0.01$). It was obvious that the results of 0.5 and 1 wt% of tannic acid were significantly better than the commercial bactericide ($p < 0.01$). An analysis using Pearson's correlation coefficient also indicated that there was a significant relationship between tannic acid offer and number of bacteria (cfu) at 8 and 24 hours of soaking process, $r\ (12) = -1$, $p < 0.000$ and, $r\ (12) = -0.990$, $p < 0.000$ respectively.

Nitrogen and COD Values of Soaking Effluents

The nitrogen value of blank sample for 24 h, presented in Table 2, had the highest value among other effluent samples ($p < 0.05$). This might be because of the heavy infection of the soaking float. Owing to inhibition effect of tannic acid and bactericide on microbial growth, lower nitrogen values were acquired in these effluents. The lowest N content values were obtained from 0.5 and 1 wt% tannic acid application compared to other

soaking groups ($p < 0.05$). However, there were no statistical differences between the groups of 0.1, 0.3 wt % tannic acid and bactericide ($p > 0.05$). In the mean time, the difference between the 0.5 and 1 wt % tannic acid was not statistically significant ($p > 0.05$). Generally, all effluents contain N values in the range between 3 and 10 mg per L [15], that are the limit values specified for directly discharge into the surface water.

Table 2. Total nitrogen (N) content and chemical oxygen demand (COD) values of soaking effluents ($mg \cdot L^{-1}$) for 24 h soaking process

	Treatment					
	Blank	**0.1% TA**	**0.3% TA**	**0.5% TA**	**1% TA**	**Bactericide**
N	4.13 ± 0.38^{a}	$3.23 \pm 0.09^{a,b}$	$3.17 \pm 0.41^{a,b}$	2.27 ± 0.45^{b}	2.43 ± 0.07^{b}	$3.17 \pm 0.20^{a,b}$
COD	1673 ± 40.55^{c}	1683 ± 33.33^{c}	1667 ± 6.67^{c}	1673 ± 21.86^{c}	2043 ± 61.19^{b}	3040 ± 34.64^{a}

* values in the same row followed by the same letters are not significantly different according to Duncan test ($p < 0.05$);
TA, tannic acid; $n = 3$

The COD values of soaking effluents were determined lower than the previously reported values [42, 48]. With the use of 1 wt% of tannic acid instead of bactericide, the COD values of soaking effluents were reduced approximately by 33% (Table 2). The difference between the 1 wt% of tannic acid and bactericide was also found significant ($p < 0.05$). However, no statistically significant difference was found between the soaking applications of blank, 0.1, 0.3 and 0.5 wt% of tannic acid ($p > 0.05$). The highest COD value reduction by 45% was obtained from 0.1, 0.3 and 0.5 wt% tannic acid applications. An analysis using Pearson's correlation coefficient also indicated that there was a significant relationship between tannic acid offer and COD values of effluents, r (12) = 0.853, $p < 0.000$ respectively. In soaking process, the 0.5 wt% tannic acid concentration that has a comparable antimicrobial effect with 1 wt%, may be selected considering both environmental aspects and cost-effectiveness, although the COD value of 1 wt% tannic acid is lower than bactericide.

The Effect of Tannic Acid on Shrinkage Temperature, TKN and SEM

The shrinkage temperature of the skins soaked with increasing concentrations of tannic acid presented only slight differences within the minimum and maximum range of 55 and 57°C. This can be regarded as a supportive indication for not generating any modification in skin structure. Also, the shrinkage temperatures of blank and bactericide samples were determined as 53 and 55°C respectively.

All skin and wool samples provided similar total Kjeldahl nitrogen values that varied between 14.56–15.27% (Table 3). No statistically significant difference was found between TKN values of all wool and skin samples ($p > 0.05$). Scanning electron microscopy analysis of wool for tannic acid applications as well as control samples did not reveal any bacterial attack on fiber structure of wool (Figure 1). The statistically insignificant TKN values of wool were also supported by similar SEM images. Accordingly, bacterial attack in the course of tannic acid application does not cause any adverse structural modifications in skin matrix and wool as supported with TKN values and SEM images.

Table 3. Total nitrogen (TKN) values of skin and wool samples (%) for 24 h soaking process

	Treatment					
	Blank	**0.1% TA**	**0.3% TA**	**0.5% TA**	**1% TA**	**Bactericide**
Skin samples						
TKN	14.57±0.090[a]	14.60±0.063[a]	14.56±0.127[a]	14.87±0.055[a]	14.68±0.072[a]	14.58±0.012[a]
Wool samples						
TKN	15.22±0.165[a]	15.27±0.012[a]	15.21±0.169[a]	15.06±0.063[a]	15.24±0.048[a]	14.97±0.050[a]

* values in the same row followed by the same letters are not significantly different according to Duncan test ($p < 0.05$)
TA, tannic acid; $n = 3$

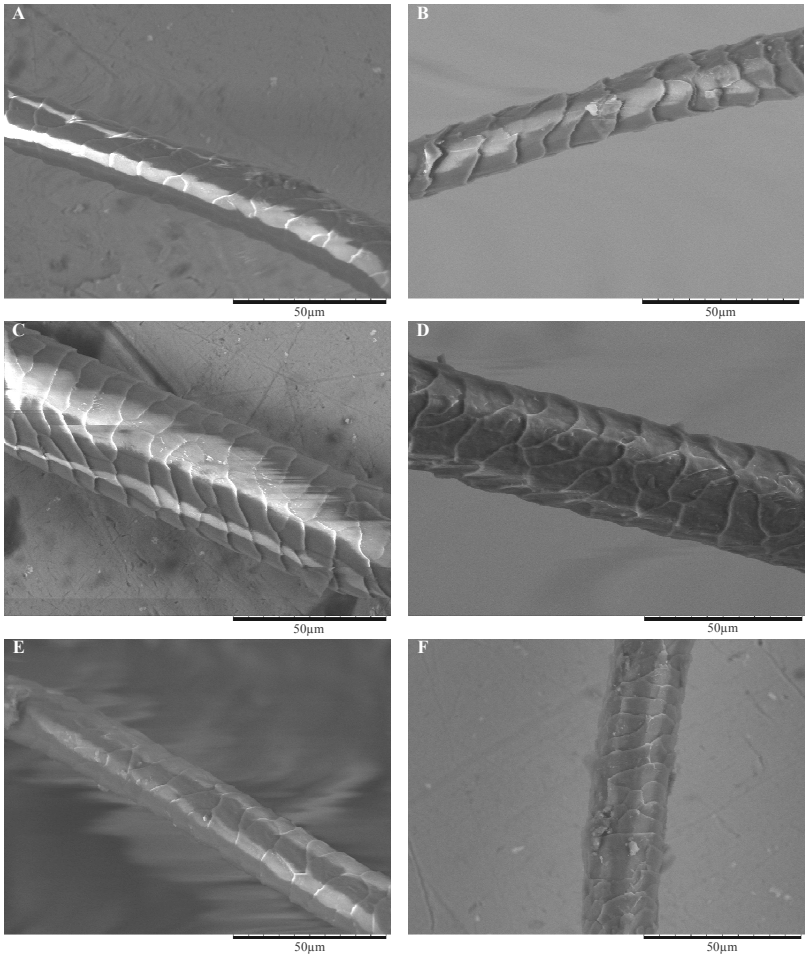

Fig 1. SEM micrographs of wool samples soaked with 0.1% (A), 0.3% (B), 0.5% (C) and 1% (D) of tannic acid, without any bactericide (blank) (E) and with a commercial bactericide 0.1% (F) × 1500

DISCUSSION

The most commonly known synthetic preserving agents used in leather industry have carbamates, benzisothiazolinon, organo-bromine compounds, and oxidative agents as active substances. Their use in leather industry has become the focus because of the chemical safety and consumer health issues considering their toxic effect for wastewater, aquatic organisms and environment. The toxicity of bactericides and fungicides in tannery effluents was confirmed by determination of their extremely high acute and chronic toxic effects on bacteria, algae, zebra fish and daphnids such as *Daphnia magna* and *Danio rerio* [48]. They may impair fertility, cause harm to the unborn child and potential heritable genetic damage [30].

A number of articles have been reported regarding the inhibition effect of tannins on microorganisms. Their antibacterial effect could be largely attributed to their reduction of enzyme activity, dysfunction of cell membrane and deprivation of substrate metal ions and minerals [1, 24, 51]. Amongst tannins, tannic acid was found to be inhibitory to the growth of intestine bacteria such as *Bacteroides fragilis*, *Escherichia coli* and *Clostridium perfringens*. This inhibitory effect was thought to be closely related to the iron-binding capacity of tannic acid [11]. Besides, Chung *et al.* [12] reported that tannic acid inhibited the growth of all selected food-borne bacteria while gallic and ellagic acid could not inhibit any of them. Chung *et al.* [12] and [13] reported that the ester linkage between gallic acid and glucose (to form tannic acid) was important for the antimicrobial potential of these compounds.

Tannic acid significantly inhibited the microbial growth without altering collagen matrix as well as wool structure. All concentrations of tannic acid used in the study, provided an antibacterial effect in comparison to blank samples by decreasing the bacterial load of soaking baths significantly. Tannic acid performed better antimicrobial activity during the first 8 hours of soaking process when compared to commercial bactericide. Commercial bactericide presented similar antibacterial effect during 24 h soaking as 0.1 and 0.3 wt% tannic acid. Tannic acid at the concentrations of 0.5 and 1 wt% fulfilled the requirements of being antimicrobial agent by providing highest inhibition effect on microorganisms. However, it is advisable to apply the tannic acid in lower concentrations with the maximal inhibitory effect on microorganisms due to its polyphenolic structure. Although it is a natural compound, there are limitations in the use of vegetable tanning materials due to their high organic load in the effluent, which results high COD [28, 34]. Nevertheless, lower COD values than 0.1% solution of commercial bactericide were obtained after application of tannic acid at highest tested concentration (1%), which is a good sign of their environmentally friendly structure. In the view of these findings, tannic acid can be appreciated as a natural and eco-friendly bactericide due to its antimicrobial, environmentally friendly and biodegradable properties.

ACKNOWLEDGEMENTS

The authors would like to thank Turkey Prime Ministry State Planning Organization for the supply of equipment (Project no: 2007 DPT 001).

REFERENCES

[1] Akiyama, H., Fujii, K., Yamasaki, O., Oono, T. & Iwatsuki, K. (2001). Antibacterial action of several tannins against staphylococcus aureus, *Journal of Antimicrobial Chemotherapy*, 48, 487–491.

[2] Albu, M.G., Cotrut, C., Titorencu, I., Trandafir, V. & Giurginca, M. (2009). Biomaterials based on collagen and tannic acid, *1st International Leather Engineering Symposium*, Izmir, Turkey, April 29th–May 1st, 377–386.
[3] Andrade Jr, R.G., Dalvi, L.T., Silva Jr, J.M.C., Lopes, G.K.B., Alonso, A. & Hermes-Lima, M. (2005). The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals, *Archives of Biochemistry and Biophysics*, 437, 1–9.
[4] Andrade Jr, R.G., Ginani, J.S., Lopes, G.K.B., Dutra, F., Alonso, A. & Hermes-Lima, M. (2006). Tannic acid inhibits in vitro iron-dependent free radical formation, *Biochimie*, 88, 1287–1296.
[5] Aravindhan, R., Saravanabhavan, S., Thanikaivelan, P., Raghava Rao, J. & Unni Nair, B. (2007). A chemo-enzymatic pathway leads towards zero discharge tanning, *Journal of Cleaner Production*, 15, 1217–1227.
[6] Bitlisli, B.O., Yasa, I., Aslan, A., Cadırcı, B.H. & Basaran, B. (2010). Physical and antimicrobial characteristics of aloe vera treated split suede leather, *Journal – American Leather Chemists Association*, 105, 34–40.
[7] Bradstreet, R.B. (1954). Kjeldahl method for organic nitrogen, *Analitical Chemistry*, 26, 185–187.
[8] Burkinshaw, S.M. & Kumar, N. (2009). The mordant dyeing of wool using tannic acid and $feso_4$, part 1: Initial findings, *Dyes and Pigments*, 80, 53–60.
[9] Cadirci, B.H., Ozgunay, H., Vural, C. & Yilmaz, O. (2010). A New Defect on Leather: Microbial Bio-Film, *Journal – American Leather Chemists Association*, 105, 129–134.
[10] Chen, S.C. & Chung, K.T. (2000). Mutagenicity and antimutagenicity studies of tannic acid and its related compounds, *Food and Chemical Toxicology*, 38, 1–5.
[11] Chung, K.T., Lu, Z. & Chou, M.W. (1998). Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria, *Food and Chemical Toxicology*, 36, 1053–1060.
[12] Chung, K.T., Stevens Jr, S.E., Lin, W.F. & Wei, C.I. (1993). Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds, *Letters in Applied Microbiology*, 17, 29–32.
[13] Chung, K.T., Zhao, G., Stevens Jr, S.E. & Simco, B.A. (1995). Growth inhibition of selected fish pathogens by tannic acid and related compounds, *Journal of Aquatic Animal Health*, 7, 46–49.
[14] Çolak, S.M. & Dandar, U. (2010). The effect of tannic acid usage on the formation of Cr (VI) in leather, *Fresenius Environmental Bulletin*, 19, 1511–1516.
[15] Çolak, S.M. & Kılıç, E. (2008). Deliming with weak acids: Effects on Leather quality and effluent, *Journal – Society of Leather Technologists and Chemists*, 92, 120–123.
[16] Çolak, S.M., Bayramoglu, E.E. & Uluc, D. (2006). Determining the usability of photosensitizers as biocides in the soaking process, *Journal – Society of Leather Technologists and Chemists*, 101, 66–72.
[17] Çolak, S.M., Yapici, B.M. & Yapici, A.N. (2010). Determination of antimicrobial activity of tannic acid in pickling process, *Romanian Biotechnological Letters*, 15, 5325–5330.
[18] Çolak S.M. (2006). Soaking with tannins: The biocidal activity of vegetable tannins used in the soaking float, *Society of Leather Technologists and Chemists*, 90, 193–196.
[19] Cowan, M.M. (1999). Plant Products as Antimicrobial Agents, *Clinical Microbiology Reviews*, 12, 564–582.
[20] Fathima, N.N., Aravindhan, R., Raghava Rao, J. & Unni Nair, B. (2006). Tannic acid-phosphonium combination: A versatile chrome-free organic tanning, *Journal – American Leather Chemists Association*, 101, 161–198.
[21] Fathima, N.N., Saravanabhavan, S., Raghava Rao, J. & Unni Nair. B. (2004). An eco-benign tanning system using aluminum, tannic acid and silica combination, *Journal – American Leather Chemists Association*, 99, 73–81.
[22] Ferguson, L.R. (2001). Role of plant polyphenols in genomic stability, *Mutation Research*, 75, 89–111.
[23] Gali, H.U., Perchellet, E.M., Klish, D.S., Johnson, J.M. & Perchellet, J.P. (1992). Hydrolysable tannins: Potent inhibitors of hydroperoxide production and tumor promotion in mouse skin treated with 12-o-tetradecanoylphobol-13-acetate in vivo, *International Journal of Cancer*, 51, 425–432.
[24] Goel, G., Puniya, A.K., Aguliar, C.N. & Singh, K.(2005). Interaction of gut microflora with tannins in feeds, *Naturwissenschaften*, 92, 497–503.
[25] Gutterres, M., Aquim, P.M., Passos, J.B. & Trierweiler, J.O. (2010). Water reuse in tannery beamhouse process, *Journal of Cleaner Production*, 18, 1545–1552.
[26] Heijmen, F.H., Pant, J.S., Middelkoop, E., Kreis, R.W. & Hoekstra, M.J. (1997). Cross-linking of dermal sheep collagen with tannic acid, *Biomaterials*, 18, 749–754.

[27] Kanagaraj J., John Sundar, V., Muralidharan, C. & Sadulla, S. (2005). Alternatives to sodium chloride in prevention of skin protein degradation – A case study, *Journal of Cleaner Production*, 13, 825–831.
[28] Kanth, S.V., Venba, R., Madhan, B., Chandrababu, N.K. & Sadulla, S. (2009). Cleaner tanning practices for tannery pollution abatement: role of enzymes in eco-friendly vegetable tanning, *Journal of Cleaner Production*, 17, 507–515.
[29] Kim, W.J., Heo, J.S., Shin, S.B. & Ko, J.Y. (2003). Measurement of color difference in the leather dyeing process with natural dyes, *Society of Leather Technologists and Chemists*, 87, 25–29.
[30] Kleban, M. (2008). Preserving agents in the leather production process, *World Leather*, May, 20–24.
[31] Kuo, M.L., Lee, K.C. & Lin, J.K. (1992). Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in the salmonella and cho systems, *Mutation Research*, 270, 87–95.
[32] L353. (2008). Regulation (EC) No 1272/2008 of the European parliament and of the council of 16 December 2008 on classification, labeling and packaging of substances and mixtures, amending and repealing directives 67/548/eec and 1999/45/ec, and amending regulation (ec) no 1907/2006, *Official Journal of the European Union*, 51, 1–1355.
[33] Li K., Chen, H., Wang, Y., Shan, Z., Yang, J. & Brutto, P. (2009). A salt-free pickling regime for hides and skins using oxazolidine, *Journal of Cleaner Production*, 17, 1603–1606.
[34] Liang, W., Xinshen, Z., Jin, H., Yonghong, X. & Kai, X. (2008). On-line determination of tannic acid in leather wastewater by flow injection spectrophometry, *Journal – Society of Leather Technologists and Chemists*, 92, 127–129.
[35] Lopes, G.K.B., Schulman, H.M. & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from fenton reaction by complexing ferrous ions, *Biochimica et Biophysica Acta*, 1472, 142–152.
[36] Morera, J.M., Bartolí, E., Chico, R., Solé, C. & Cabeza, L.F. (2011). Minimization of the environmental impact of chrome tanning: A new process reusing the tanning floats, *Journal of Cleaner Production*, 19, 2128–2132.
[37] Mukhtar, H., Khan, W.A., Wang, Z.Y., Bik, D.P. & Bickers, D.R. (1988). Exceptional activity of tannic acid among naturally occurring plant phenols in protecting against 7,12 dimethylbenz(a)anthracene-, benzo(a)pyrene-, 3-methylcholanthrene-, and n-methyl-n-nitrosourea-induced skin tumorigenesis in mice, *Cancer Research*, 48, 2361–2365.
[38] Muthusubramanian L. & Mitra, R.B.A. (2006). Cleaner production method for the synthesis of bronopol-a bactericide that is useful in leather making, *Journal of Cleaner Production*, 14, 536–538.
[39] Mutlu, M.M., Çadırcı, B.H., Ozgunay, H., Adıgüzel, A.C. & Sari, O. (2009). Ozone as a biocide in soaking, *Journal – Society of Leather Technologists and Chemists*, 93, 18–20.
[40] Nepka, C., Asprodini, E. & Kouretas, D. (1999a). Tannins, xenobiotic metabolism and cancer chemoprevention in experimental animals, *European Journal of Drug Metabolism and Pharmacokinetics*, 24, 183–189.
[41] Nepka, C., Sivridis, E., Antonoglou, O., Kortsaris, A., Georgellis, A., Taitzoglou, I. Hytiroglou, P., Papadimitrou, C. Zintzaras, I. & Kouretas, D. (1999b). Chemopreventive activity of very low dose dietary tannic acid administration in hepatoma bearing c3h male mice, *Cancer Letters*, 141, 57–62.
[42] Preethi, V., Parama Kalyani, K.S., Iyappan, K., Srinivasakannan, C., Balasubramaniam, N. & Vedaraman, N. (2009). Ozonation of tannery effluent for removal of cod and color, *Journal of Hazardous Materials*, 166, 150–154.
[43] Rodrigues, M.A.S., Amado, F.D.R., Xavier, J.L.N., Streit, K.F., Bernardes, A.M. & Ferreira, J.Z. (2008). Application of photoelectrochemical-electrodialysis treatment for the recovery and reuse of water from tannery effluents, *Journal of Cleaner Production*, 16, 605–611.
[44] Sah, N.K., Kumar, S., Subramanian, M. & Devasagayam, T.P. (1995). Variation in the modulation of superoxide-induced single strand breaks in plasmid pbr322 DNA by biological antioxidants, *International Journal of Biochemistry and Molecular Biology*, 35, 291–296.
[45] Saran, S., Isar, J. & Saxena, R.K. (2007). A modified method for the detection of microbial proteases on agar plates using tannic acid, *Journal of Biochemical and Biophysical Methods*, 70, 697–699.
[46] Saravanabhavan, S., Fathima, N.N., Rao, J.R. & Nair, B.U. (2004). Combination of white minerals with natural tannins chrome-free tannage for garment leathers, *Society of Leather Technologists and Chemists*, 88, 76–81.
[47] Sivakumar, V. & Rao, P.G. (2001). Application of power ultrasound in leather processing: an ecofriendly approach, *Journal of Cleaner Production*, 9, 25–33.

[48] Tisler, T., Zagorc-Koncan, J., Cotmana, M. & Drolca, A. (2004). Toxicity potential of disinfection agent in tannery wastewater, *Water Research*, 38, 3503–3510.
[49] TS 4120 EN ISO 3380: (2005). Leather – Physical and mechanical tests – determination of shrinkage temperature up to 100°C, Turkish Standard (TSE), Ankara, Turkey.
[50] TS 4134: (1985). Leather – Determination of nitrogen content and hide substance. Titrimetric method, Turkish Standard (TSE), Ankara, Turkey.
[51] Widsten, P., Heathcote, C., Kandelbauer, A., Guebitz, G., Nyanhongo, G.S., Prasetyo, E.N. & Kudanga, T. (2010). Enzymatic Surface Functionalisation of Lignocellulosic Materials with Tannins for Enhancing Antibacterial Properties, *Process Biochemistry*, 45, 1072–1081.
[52] Zhihua, S. & Guowei, W. (2004). Combination tannage of tannic acid and rare earth, *Journal – Society of Leather Technologists and Chemists*, 88, 72–75.