(REFEREED RESEARCH)

ÇEVRE DOSTU RETENAJ UYGULAMALARI: TABAKHANE KİRLİLİK YÜKÜNÜ AZALTMAK İÇİN TABAKLAMA SONRASI SÜREÇLERDE TRANSGLUTAMİNAZ ENZİMİNİN (TGase) ROLÜ

ECO-FRIENDLY RETANNING APPLICATIONS: ROLE OF TRANSGLUTAMINASE ENZYME (TGase) IN POST TANNING PROCESSES FOR TANNERY POLLUTION LOAD REDUCTION

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ABSTRACT

Tanning industry is under increasing pressure owing to the environmental polution arising from leather manufacturing processes. Clean manufacturing processes are of vital importance in order to minimize the effects of the manufacturing process and product on the environment. In clean manufacturing processes, the use of enzymes or enzyme aided methods instead of the traditional technologies look promising among the new alternative technologies targeting the minimization of environmental polution. Transglutaminase enzyme(TGase) is a transferase class enzyme which is commonly used in food manufacturing to change the functional properties of the proteins. The most crucial characteristic feature of TGase enzyme is that the increase in the generation of cross links.In this study, the possible use of TGase enzyme in retannage process was investigated, and the effects of TGase enzyme on the properties of the leather and the pollution load of the process wastewaters was presented. Three different groups was formed and in the first group, the optimum TGase proportion was determined by means of using a variety of 1, 2, and 3% TGase enyzme respectively. In the second group, during the retannage process no filling agents were used in fatliquoring and dyeing processes. Where as in the third group a traditional retannage method was performed. In all three groups the fatliquoring and dyeing processes were carried out by the same procedure. The pollution load of the wastewaters, the hydrothermal stability of the leathers, color properties, tensile strength, elongation at break and tear load and organoleptic properties were investigated for each group. The result of the study has shown that the enzyme aided retannage method was improved the physical properties of the leathers in comparison to the other two methods employed. As for the hydrothermal stability of the leathers; the stability was found to be higher in the samples aided by TGase enzyme. The most remarkable result among the methods was obtained by the pollution load of the wastewaters.. It has been revealed that the use of TGase enzyme in retannage process was reduced the demand of chemical oxygen(COD) by a rate of 47% and the total nitrogen by a rate of 50.4% in contrast with the traditional retannage method.

Keywords: Ecofriendly, Transglutaminase enzyme, Leather, COD, Retanning

ÖZET

Deri sektörü, deri üretim işlemlerinden kaynaklanan çevre kirliliği nedeniyle artan bir baskı altındadır. Üretimin ve ürünün çevreye olan etkisini en aza indirmek açısından temiz üretim yöntemleri büyük önem taşımaktadır. Temiz üretim süreçlerinde, geleneksel teknolojilerin yerine enzimlerin kullanımı veya enzim destekli yöntemler, çevre kirliliğini azaltmak açısından yeni alternatif teknolojiler arasında umut vaad etmektedir. Transglutaminaz (TGase) enzimi, gıda sistemlerinde, proteinlerinin fonksiyonel özelliklerini değiştirmek için yaygın olarak kullanılan transferaz sınıfı bir enzimdir. TGase enziminin en önemli özelliği çapraz bağ oluşumunu arttırmasıdır. Bu çalışmada, deri üretimi sırasında, retenaj işleminde TGase enziminden yararlanma olanakları araştırılarak, TGase kullanımının, deri özellikleri ve proses atıksularının kirlilik yükü üzerine etkisi ortaya konulmuştur. Araştırmada üç farklı grup oluşturulmuştur. Birinci grupta; %1, %2 ve %3 olmak üzere farklı oranlarda TGase enzimi kullanılarak optimum TGase enzimi oranı tespit edilmiştir. İkinci grupta; retenaj işleminde yağlama ve boyama işlemi dışında hiçbir dolgu maddesi kullanılmamıştır. Üçüncü grupta ise; geleneksel bir retenaj yöntemi uygulanmıştır. Tüm gruplarda yağlama ve boyama işlemleri aynı şekilde yapılmıştır. Her bir yöntemde ortaya çıkan atıksuların kirlilik yükü ile derilerin hidrotermal stabilitesi, renk özellikleri, çekme dayanımı ve uzama, yırtılma yükü dayanımı ve organoleptik özellikleri araştırılmıştır. Çalışma sonuçları; enzim destekli retenaj yönteminin derilerin fiziksel özelliklerini diğer iki

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yönteme göre iyileştirdiğini göstermiştir. Derilerin hidrotermal stabilitesi ise, TGase kullanılan örneklerde diğer deri örneklerine göre daha yüksek bulunmuştur. Yöntemler arasında en çarpıcı sonuç ise, proses sonucu oluşan atıksu kirlilik yükünde elde edilmiştir; retenaj işleminde TGase kullanımın kimyasal oksijen ihtiyacını geleneksel retenaj yöntemine göre yaklaşık %47, toplam azot miktarının ise; %50,4 oranında azalttığı ortaya konmuştur.

Anahtar Kelimeler: Çevre dostu, Transglutaminaz enzimi, Deri, KOİ, Retenaj

1. INTRODUCTION

Until recent years, the concern rooted in the pollution caused by the production of leather, legal restrictions and the social pressure have tended towards the absence of toxic materials in the end products. But today, the restrictions have expanded and changed; banning the chemicals used in the production process from having toxic materials and the use of eco-friendly and natural materials have gained importance.

The leather industry deals with proteinous skin material for the conversion of leather and this generates huge amount of solid and liquid wastes giving rise to pollution that needs to be overcome by introducing sustainable cleaner technologies[1]. Leather manufacturing involves several processes in which various hazardous chemicals are used. Production and disposal of these chemicals after use is responsible for a large proportion of the environmental load of leather products[2,3]. Conventionally, prosessing of skin is performed by use of aggressive and/or toxic chemicals, regarding toxicological and environmental aspect as well as the low specifity of most chemicals, there is high need for alternative technologies. New products and production processes designs in which bestavailable techniques, clean technologies are used and environmental impacts are minimised during along an innovative conversion[4].

Retanning is an important operation to impart organoleptic properties to wet-blue leathers by reducing the short falls of the tanning process. Different chemicals such as acrylates, syntans, resins, vegetable tanning extracts and aldehydes can be used in the retanning prosses. There are various retanning agents available in literature. Vegetable, synthetic tanning agents (syntans), formaldehyde and acrylic type of materials play a major role in the retanning process[5,6]. Retanning agents which are used synthetically prepared from phenol/formaldehyde sources that are useful in providing properties. These agents contain many non-tannins/tannins that are not fully taken-up by the leather during processing and generates as pollutants in the effluent[7]. When selecting the synthetic tanning agents (syntans), in addition to characteristics it gives to leather, it is important to consider its environmental performance: chemicals that not consumed by leather can contribute to effluent with high values of COD. To make it worse, very often the COD load they produce is not biodegradable and requires specific and costly tertiary treatment[8,9].

Syntans are sulphonated condensation products of hydroxyl-substituted aromatic compounds (phenol, cresol or naphthalene) with formaldehyde and often with amides. They can cause a high COD and the degradation of sulphonated polyphenols is aerobically and anaerobically insufficient. The degradation products of the sulphonated polyphenols (and the phenols themselves) are strong pollutants. These substances are hardly reduced by adsorption on particulate matter and are highly mobile. Even though they have low acute aquatic toxicity, their persistence and mobility are classified as negative to the environment with respect to resins are derived from aliphatic compounds such as polyurethanes, dicyandiamide and melamine. Resins contain (low) concentrations of free formaldehyde and inorganic fillers.

Acrylic acid condensates exist in a vast number of derivatives. Since polyelectrolytes on the basis of acrylic acid condensates are used in the treatment of drinking water, it is assumed that the acrylic acid condensates behave similarly and are precipitated because they are absorbed on organic particulate matter. Acrylic acids and their condensates are anaerobically and aerobically biodegradable and drinking water quality. When syntans react with proteins, ecological problems are occur. Adverse effects on fish and bacteria in biological treatment plants (blocking activity of the bacteria) have been recorded[8]. Therefore clean production methods become more of a significant issue for the leather industry.

Over the recent years, transglutaminase enzyme in the modification of food borne proteins such as meat, milk and grain have been started to use. In different literatures, it has been stated that transglutaminase enzyme catalyses the isotopic bonds between amino acids or peptides, causing molecular and intermolecular cross links and improving the functional properties of the proteins[10,15]. TGase, an extracellular enzyme of the class of transferases, is produced commercially via traditional fermentation by the microorganism *Streptoverticillium moboarense[16,18].* The enzyme acts in wide ranges of pH and temperature (pH 5.0–8.0, optimum temperature of 50 ^{o} C, but with activity between 40 and 70 ^{o}C), its Ca^{2+} independent, and its activation requires no special cofactors[17,19]. These characteristics, allied to the fact that the enzyme has been recognised by the scientific community as a safe substance for human ingestion, and as a GRAS (Generally Recognized as Safe) substance by the FDA (Food and Drugs Administration) since 1998, make TGase very attractive for the food industry[20].

Transglutaminases can modify proteins by incorporating amines, and affecting intra- and intermolecular cross-links or deamidation, causing profound changes in their molecular structure[21,24]. The resulting bridges are called Ɛ-(γ-glutamyl) lysine bond deamidation[18]. In foods containing protein Ɛ-(γ-Glu) lysine bond occurs in rapid succesion preceding other reactions. This reaction continues until there is no glutamin or lysine left in the setting[25].

In previous research were determined ability of transglutaminase to crosslink native collagen by Collighan[26]. In this study, it was aimed to study of the possible use of transglutaminase enzyme in the retannage

process of leather as a proteinic structure and investigate the visible effects of such method on the properties of leather and the pollution load of process effluent.

2. MATERIAL AND METHODS

Transglutaminase enzyme (TEGEN 220 DM) was supplied from Benosen Chemicals and Food Co. Ltd. The enzyme activity of MTGase was 120 units/g. Conventionally chrome tanned total 15 sheep skins (9 skins for enzyme treatment, 3 skins for CR-1 and 3 skins for CR-2) were used as the raw material of this study. Skins were obtain from a tannery in İzmir. The chemicals used for leather processing were of commercial grade.

2.1. Methods

2.1.1. Experimental TGase retanning procedure

Nine chrome tanned sheep skins were used for the study. The pH of the skins were adjusted to 6.5 using neutral syntan and $NaHCO_3$. Enzyme treatment was carried out in the new bath. The skin was treated with 1% dye auxiliary agent and 3% acid dye along with 30% water (percentages based on shaved weight). Experiments were performed with varying concentrations of enzymes, such as 1, 2, and 3% in the same bath. The skins were processed for 2 hours with enzyme, than 12% combined fatliquor was added in the same bath (Table 1). The process liquor from the trials was analyzed for the pollution load. Based on the shrinkage temperature values of the retanned leathers, physical properties and organoleptic assessment of leathers, 2% concentration of enzyme was optimized (Table 1)

2.1.2. Control retanning process

Along with the retannage process performed by using TGase enzyme in the study, two different control groups were formed to reveal the differences in waste water and leather features that result from filling process during retannage. In both groups, leather's pH level was arranged to 6.5 as mentioned above and retannage was carried out in new bath. First control group was named as CR-1 and neither filling material nor enzyme was used in this group during retannage. Only dyeing and fatliquoring were performed during the process. For this reason, 3 chrome tanned sheepskin were used (Table 2) . Second group was named as CR-2 and a retannage formula used for garment leather production was randomly chosen and applied. According to this recipe, filling, fatliquoring and dyeing processes were done. Enzyme was not used in this group, either (Table-3).

2.2. Analysis of spent liquors from retanning

2.2.1. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) in the exhausted retanning process liquor was tested with Merck Cell Test kit by Merck Spectroquant Move 100 spectrophotometer.

2.2.2.Total nitrogen

Total nitrogen was measured using Merck cell test kit by Merck Spectroquant Move 100 spectrophotometer.

2.3. Physical properties of leather

The leather samples were taken from sampling location and prepared in accordance with “sample location” and conditioned according to “sample preparation and conditioning TS EN ISO 2418 and TS EN ISO 2419 standarts respectively prior to analysis[27,28].

The double edge tear load and tensile strength tests were carried out in accordance with the ISO 3377-2:2002 and ISO 3376:2011 standards respectively [29,30]. The tensile strength and tear load of the dyed leathers were measured using a Shimadzu AG-IS Test Apparatus.

Table-1: Experimental Retanning Process

Chemicals	%	^{0}C	Time
Water	30	40	.
Dye auxiliary agent	1		10’
Dye	3		1h
Enzyme	**X** X:1,2,3%	55	2h
Water	+70	60	
Combined fatliquor	12		1h
Formic acid	1		30’ pH 3.8
Washing			

Table-2: CR-1 Retanning Process

Chemicals	%	^{0}C	Time
Water	30	40	
Dye auxiliary agent	1		10’
Dye	3		1h
Water	+70	60	
Combined fatliquor	12		1h
Formic acid	1		30’ pH 3.8
Washing			

Table-3: CR-2 Retanning Process

Chemicals	%	^{0}C	Time
Water	30	40	.
Dye auxiliary agent	1		10’
Dye	3		1h
Melamine resin	2		20’
Dicyandiamide resin	2		20’
Fenolic syntan	2		20’
Acrylic syntan	1		20’
Water	+70	60	
Combined fatliquor	12		1h
Formic acid	1		30’ pH 3.8
Washing			

2.4. Hydrothermal stability measurements

The shrinkage temperature of the leathers was measured according to the ISO 3380:2002 standard method using a shrinkage temperatures test apparatus[31].

2.5. Reflectance and colour difference measurements of leathers

In order to identify the color differences between experimental research and control leathers, Konica Minolta CM- 3600A spectrophotometer was used. Measurements were performed according to CIELAB color coordination, under the conditons of CIE 100 standart observer angle and CIE standart D65 light source[32,33].

2.6. Scanning electron microscopic (SEM) analysis of leathers

Scanning electron micrographs of the cross section of the leathers were taken by HITACHI TM-1000 tabletop scanning electron microscope with x150 magnification.

2.7. Organoleptic evaluation of leather

Each treated leather sample was evaluated by three evaluator with respect to handle, fullness, grain (break), and color. A value from 1 to 5 was assigned for each parameter, with 1 being the worst and 5 being the best. From these ratings, an overall evaluation was determined and this value (from 1 to 5) was reported.

2.8. Statistical analysis

The effect of skin type, region and interaction of these two factors were analyzed. The statistical analysis of data was performed using Minitab for Windows (ver.14.0, Minitab Inc., State College, PA). Analysis of variance was performed was on each attribute (p< 0,01). Duncan's post hoc test was used for multiple comparisons when significant interactions were observed.

3. RESULT AND DISCUSSION

3.1. Characterization of wastewaters

Retannage process was performed to improve incomplete features of tanned leathers. Filling materials that are used in retannage have negative effects on process waste water pollution load, along with the improvements such as softness, fullness and fastness. When waste water's pollution load was examined in the study, it was seen that there were significant differences (p<0,01) among three TGase groupes (1,2,3% TGase, control (CR-2) and conventional retannage groupe (CR-2) significant interaction effect(tab-4). CR-1 group leather samples had the lowest COD values when compared to other trials. The COD values of CR-1 group were detected as 4 010 mg/l. As it is known, retannage process consists of fatliquoring, dyeing and filling processes. In order to identify the pollution load resulting from filling process, no filling material was used in CR-1 group. Only fatliquoring and dyeing processes were done. The COD values of this group were lower due to the lack of the filling process. The COD value of CR-2 group was obtained as 22 670 mg/l. When CR-1 and CR-2 groups were compared, it was seen that filling process had substantially increasing effect on waste water pollution load (Tab-3, Fig 1).

When the COD values of CR-2 group were compared with the results of the trials treated with TGase enzyme; it has been detected that there was an increase of 47% in leather samples that were treated with 1% TGase, 39% in samples treated with 2% TGase, 31% in samples treated with 3% TGase.

The results of the study have shown that enzyme–aided retannage process reduced the COD values of retannage waste water significantly. When the total N values of the processes were examined, it was determined as 2.37 mg/l in CR-1 group. This was the lowest value among the methods used in this study. It was an expected result that CR-1 group's leather had a low N value because of the missing filling materials used in retannage process. The total N value of the leathers that were treated according to CR-2 method was found as 31.50 mg/l. CR-2 total N results were considerably high when compared to CR-1 group and showed a total N load magnitude that arised from the filling process (Fig 1).

When the total N rates of the waste waters were examined, it has been detected that samples that are treated with 1% and 2% TGase had lower N rates compared to CR-2 group's samples. Also, leather samples that were treated with 1% TGase gave the lowest total N rates after CR-1 group. It was seen in Figure 3 that as TGase enzyme use increases, total N rate in waste water increases at the same rate. It was thought that this increase may result from protein part existing in enzyme's structure.

Table 4. Study results for 1, 2,3 % TGase, CR-1 and CR-2.

	1 %TG	2% TG	3% TG	CR-1	CR-2
Total N (mg/l)	15.62±0.71^{d}	26.75±0.75^{c}	72.00±0.82^{a}	2.37±0.26^{e}	31.50±1.05^{b}
COD (mg/l)	12020.00±209.28^{d}	13800.00±792.59^{c}	15700.00±505.89^{b}	4010.00±75.27^{e}	22670.00±771.88^{c}
Tensile Strength N/mm^2	30.80±1.18^{a}	31.01±1.56^{a}	30.05±1.18^{a}	22.14±1.04^{b}	24.58±1.12^{b}
Elongation (%)	87.99±2.41^{b}	88.22±1.00^{b}	91.74±0.72^{a}	84.59±0.83^{c}	82.41±0.83^{c}
Tear Strenght(N)	40.97±1.84^{b}	55.00±0.79^{a}	37.79±1.04^{c}	35.38±0.81^{d}	26.49±0.64^{e}
Shrinkage Temperature (^{o}C)	110.0±0.82^{a}	114.0±0.82^{a}	114.0±0.00^{a}	110.0±0.82^{b}	113.0±0.82^{a}

$^{a, e}$ Values in the same columns or rows followed by the same lower case letters, respectively are not significantly different according to Duncan test (p<0.01).

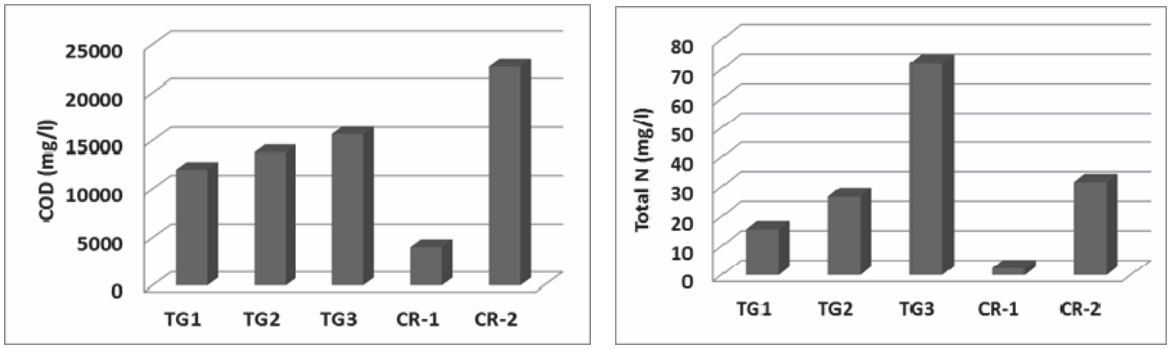

Figure 1. Graphical representation of COD and total N results of control groups and experimental process

3.2. Characterization of leather properties

Samples for physical analysis were taken from four different retanning application that presented table-3. Significant interactions ($p<0.01$) between TGase groupes, CR-1 and CR-2 groupes were observed all physical analysis (tab-4).

The tensile strength is one of the important parameter that gives information on the quality of leathers. There were significant ($p<0.01$)differences in tensile strength TGase groupes and CR-1 and CR-2 (table-4).When TGase group leathers tensile strength was compared to CR-1 group leathers, it was revealed that depending on the enzyme rate, it increased the shrinking endurance between the rates of 26.32% and 28.11%. When CR-2 group leathers were compared with CR-1 group leathers, it has been detected that CR-2 process increased the shrinking rate by 10.30%. It was seen that this rate was lower compared to the increase in tensile strength of leathers treated with enzyme (Fig 2). When the leather samples treated with different rate of TGase enzyme were examined among each other, it has been seen that results were very similar. When all leather samples were examined in respect to % elongation, it has been detected that the extension of leathers that are treated with TGase enzyme had the highest elongation and depending on the usage of enzyme increase, the elongation increased too (Fig 3).

When the tear strength of leathers was examined, the highest tear strength was observed in leather samples that were treated with enzyme. Among the retannage samples that were enzyme-assisted, it has been detected that leathers treated with 2% enzyme showed better tear strength properties. Besides CR-1 and CR-2 groups had lower tear strength values (Fig 4). When the physical properties of leathers were examined, best results in respect to shrinking temperature were received from leather samples that were treated with TGase enzyme. Shrinkage temperature gives information about hydrothermal stability of leathers. When shrinkage temperature results were examined, it has been seen that leather samples treated with enzyme and CR-2 group leathers showed similar results along with little differences (Fig-5).

The color changes in leathers treated with TGase enzymes in different concentrations were measured with spectrophotometer and results were shown in table 5. LAB color coordination system's (CIELAB) three color coordination; "L" shows color's fairness (L= 0, black; L= 100, white), "a" shows the place on red and green axis (when + a is red, –a is green), "b" shows the place on yellow and blue axis (when +b is yellow ,–b is blue). Color differences that emerge among leathers were shown by ΔE rates. When ΔE values were examined after TGase enzyme implementations, the color difference was observed between the leathers that were treated with enzyme–aided retannage and control group samples. When CR-2 (fatliquoring, dyeing and filling process applied) group leather samples and CR-1 (filling process not applied) group samples were compared, the color difference was determined due to the filling process impact on color intensity. Colors closest to black were acquired from CR-2 group leathers. It has been observed that black color of the leather samples in enzyme-aided retannage was lighter (brighter) than CR-1 group. When TGase enzyme applications were compared among each other, the closest color to black was acquired from 2% enzyme implementation and 1 and 3% enzyme applications show very similar black color rates. Besides, when a* values were examined, it could be seen that color of CR-1 leather samples and leathers that were treated with enzyme came from green, while the redness had detected in CR-2 group. It is thought that this redness enables leathers to have darker color (black). Also, there were differences in greenness value of leathers that were acquired through control and enzyme applications (greener) same effect was remarkable in enzyme applications too. When "b*"value, which shows yellowness and blueness values, was examined, the same situation in "a*" value was seen too. It was observed that CR-1 control sample and leathers that were processed with enzyme implementation had blueness in their color, but CR-2 control group had yellowness in their color. Also, in the blueness values of leathers the control group and enzyme implementations had differences (more blue in color). Also the same effect was presented in enzyme applications.

The SEM images of the leather samples treated with enzyme aided retannage had shown better orientation of leather fibre gap. The best result had been received from the samples that were treated with TGase among the enzyme group. (Fig 6).

Organoleptic evaluation of leathers

Subjective evaluation results of leather samples were shown in fig-7. When leather samples were subjectively considered according to color, leathers in CR-2 group gave the darkest color. The leather samples treated with TGase had lighter color than CR-1 and CR-2 groups.

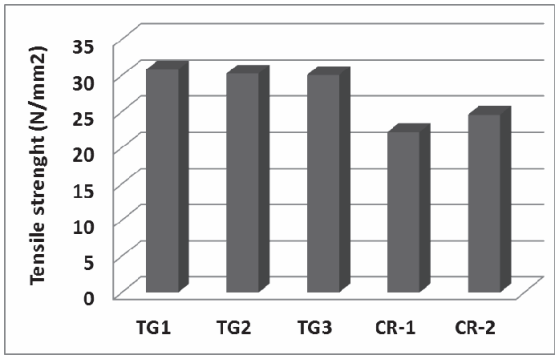

Figure 2. Tensile strength of leather samples

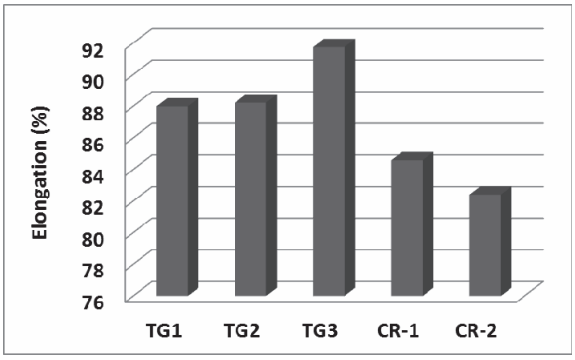

Figure 3. Elongation of leather samples

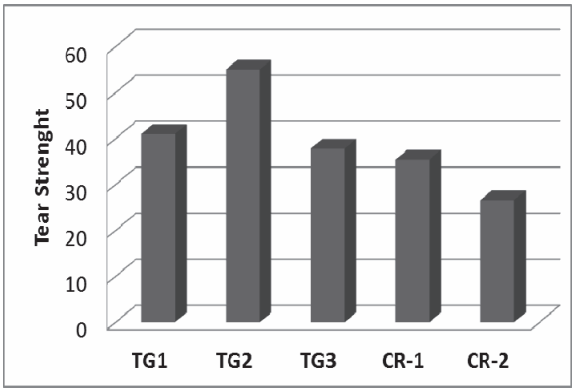

Figure 4. Tear strength of leather samples

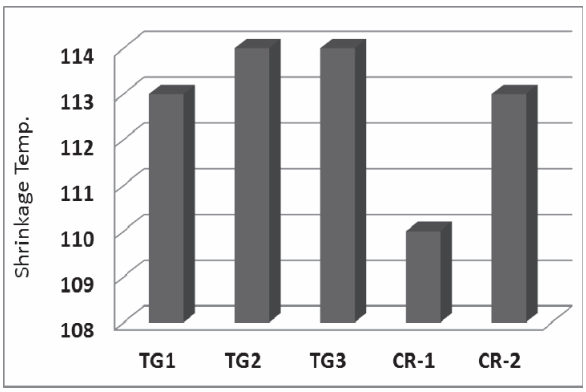

Figure-5. Shrinkage values of leather samples

Table 5. Colour differences of leather samples

	L	a	b	ΔL	Δa	Δb	ΔE
CR-1	20.338	-1.082	-0.23	-78.58	-0.966	0.345	78.584
CR-2	19.19	0.11	0.406	-79.604	0.422	0.174	79.61
1% TGase	25,316	-1.648	-0.6	-73.6	-1.534	-0.476	73.618
2% TGase	22.44	-1.456	-0.516	-76.478	-1.346	-0.225	76.49
3% TGase	24.778	-1.198	-0.815	-74.15	-1.084	0.22	74.146

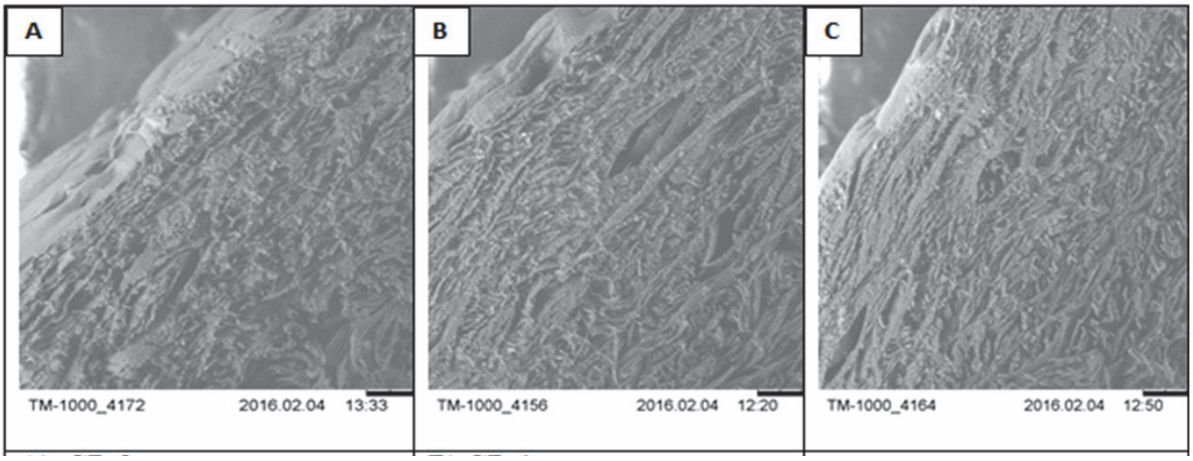

Figure 6. Scanning electron micrographs (150× magnification) showing the cross-section view of (A) CR-1: (B) CR-2: (C) 2% TGase retanned leathers

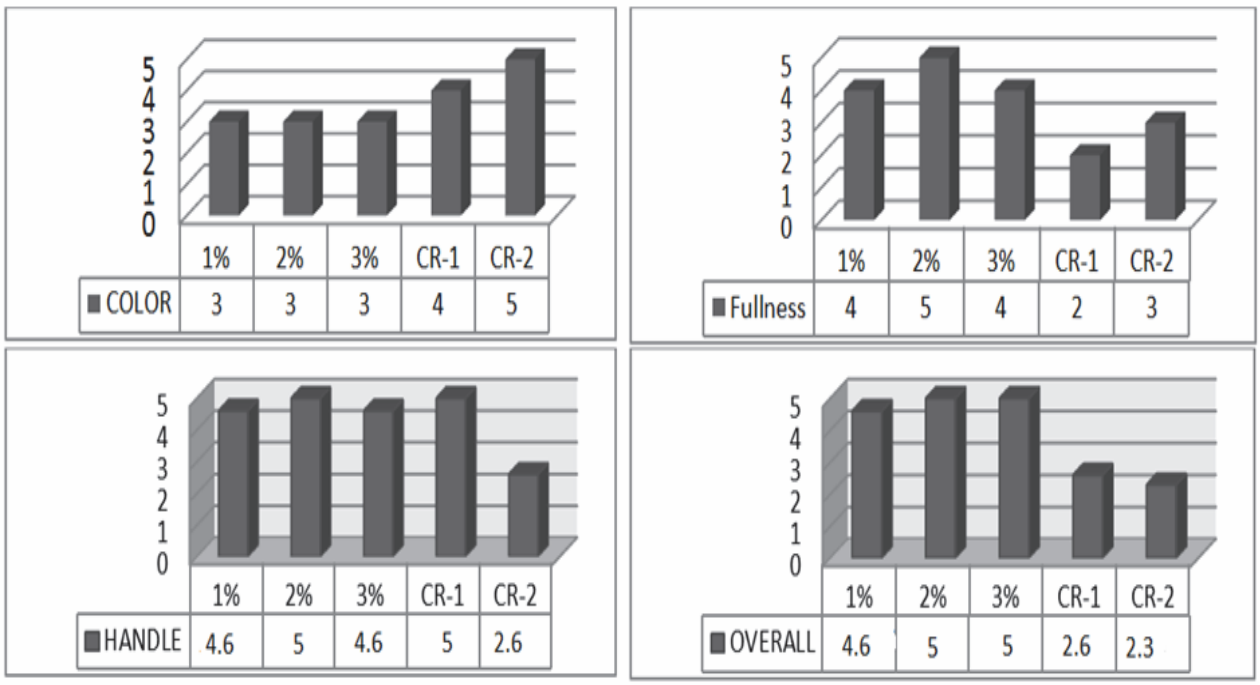

Figure 7. Subjective evaluation of control, conventional retanning , 1% TGase, 2% TGase and 3% TGase samples (Color, handle, fullness, and overall) using rating scala of 1=worst to 5= best).

When leather samples that were treated with TGase are compared among each other, it was concluded that their colors were very similar. When leather samples were examined from the point of fullness, it was seen that best results were obtained by leather samples treated with enzyme. Organoleptic evaluations revealed that CR-1 group leathers had the least fullness. Among the leather samples that were treated with TGase enzyme, the samples processed with 2% enzyme gave the highest level of fullness. Handle examination showed that best results were received from the leather samples treated with 2% TGase enzyme. In overall evaluation, all the leather samples treated with enzyme were better than the ones in CR-1 and CR-2 groups. Besides, the leathers treated with 2% enzyme had better results.

CONCLUSIONS

The results of the study showed that enzyme-aided method reduce the pollution rate of the waste water generating from retannage process. Especially in the retannage process performed by 2% of TGase enzyme, 47% COD (chemical oxygen demand) reduction was determined compared to traditional method. This result showed that enzyme-aided method was more environmentally friendly than the traditional retannage process. Besides, the use of enzyme in retannage process was improved the mechanical properties such as thermal behaviour and tear strenght as well as the increase in softness. It was concluded that experimental samples were more suitable for soft leather production than the control group sample.

TGase enzyme used in the study is acknowledged as a safe material by FDA and also it is used as food additives. Because of the fact that any other filling material except TGase was used in research leathers during retannage, it doesn't include harmful materials that are present in some filling materials which are used for traditional retannage processes and it is more natural. As is known, formaldehyde is used during the production of many filling materials that are used for traditional retannage processes. Leathers processed with such kind of filling materials carry the risk of formaldehyde formation. Formaldehyde is carcinogen. However, leather samples processed with TGase did not carry the same risk. This situation was important both for human health and ecosystem. Apart from this, the leathers produced with enyzme- aided systems were less likely to include harmful materials in solid wastes after mechanic processes such as buffing and cutting sides. Also enzyme–aided system will contribute to reduce the cost of treatment of waste water.

The results of the research show that enzyme–aided retannage process is more environmentally friendly when compared to the traditional methods. Also enzymatic processes are more usable methods as alternatives for overcoming the pollution problems that result from leather industry.

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REFERENCES

1. Kanagaraj J. T., Senthilvelan R.C., Panda, S. 2015 “Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: a comprehensive review”, *Journal of Cleaner Production* 89, 1-17.
2. 2.Marsal, A., Cot, J., Boza, E.G., 1999 “Oxidizing unhairing process with hair recovery. Part I. Experiments on the prior hair immunization” *J. Soc. Leather Technol. Chem.* 83, 310-315.
3. Palanisamy T., Jonnalagadda R.R., Balachandran U.N., 2004 “Progress and recent trends in biotechnological methods for leather processing”. *Trends Biotechnol*. 22, 181-188.
4. Karaman D., Gülümser G., 2016, “Possibilities of Usage of Alkali Aluminosilicates as Tanning Material in Chromium-free Leather Production” *Tekstil ve Konfeksiyon* 26(1), 117-124.
5. 5.Jaisankar S.N., Gupta S., Lakshminarayana Y., 2010 “Waterbased anionic sulfonated melamine formaldehyde condensate oligomer as retanning agent for leather processing” *J. Am. Leather Chem. Assoc.*105, 289-296.
6. Kanagaraj J., Senthilvelan T., Panda R.C., 2015 “Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: a comprehensive review”, *Journal of Cleaner Production* 89, 1-17.
7. Zhang B., Xu L., Ding K., 2011”Dyeing/cross linking property of natural iredoid to protein fibers” *J. Am. Leather Chem. Assoc.* 106 (4), 121-126.
8. Buljan J., Kral I., 2015 “The framework for sustainable leather manufacture”, UNIDO.
9. 9.Michael B., Michele C., Stefan R.,et all 2015 “Best Available Techniques (BAT) Reference Document for the Tanning of Hides and Skins, Industrial Emissions Directive” 2010/75/EU *Integrated Pollution Prevention and Control*.
10. 10.Larre S, Henle T and Klostermeyer H . 1998 “Hydrated Gluten Modified by A Transglutaminase” *Nuhrung* 42(3/4):155-157.
11. 11.Mizuno A, Mitsuiki M and Motoki M., 1999 “Glass Transition Temperature of Casein as Affected by Transglutaminase” *Journal of Food Science*, 64(5):796-798.
12. 12.Gomez-Guillen M.C, Sarabia A.I, Solas M.T., 2001 “Effect of Microbial Transglutaminase on the Functional Properties of Megrim (L. Boscii) skin gelatin” *J Sci Food Agric*. 81: 665-673.
13. 13.Sharma R, Lorenza P.C., Qvist K.B,. 2001 ”Influence of Transglutaminase treatment of Skim Milk on the Formation of Ɛ-(γ-glutamyl) lysine and tha Susceptiblility of Individual Proteins Towards Crosslinking” *Inter Dairy Journal*, 11:785-793.
14. 14. Piersma S.R, Pijpekamp A, Wijgaards G., 2002 “Quantitation and Localisation of (in vitro) Transglutaminase-catalysed Glutamine Hydroxylation Using Mass Spectrometry” *Enyme and Microbial Technology,* 30:266-272.
15. Kurt Ş., Zorba Ö., 2004 “Transglutaminaz ve Proteinlerin Modifikasyonununda Kullanımı (Transglutaminase and its use for protein Modification” *Gıda* 29 (5), 357-364.
16. 16.Ando H., Adachi M., Umeda K., et al. 1989 “ Purification and characteristics of a novel transglutaminase derived from, microorganisms” *Agricultural and Biological Chemistry*, 53(10), 2613–2617.
17. 17.Motoki M., Seguro K., 1998 “Transglutaminase and its use for food processing” *Trends in Food Science & Technology*, 9(5), 204–210.
18. 18.Yokoyama K., Nio N., Kikuchi Y., 2004 “Properties and applications of microbial transglutaminase” *Applied Microbiology and Biotechnology*, 64(4), 447–454.
19. 19.Zhu, Y., Rinzema, A., Tramper, J., et al., 1995 “Microbial transglutaminase: A review of its production and application in food processing” *Applied Microbiology and Biotechnology* 44(3–4), 277–282.
20. 20.Aalami, M., & Leelavathi, K. (2008). Effect of microbial transglutaminase on spaghetti quality. Journal of Food Science, 73(5), C306–C312.
21. 21.Celis, F.T., 2009 “Efeito da reticulação induzida pela transglutaminase e o glutaraldeido sobre as propriedades das microparticulas obtidas por coacervação complexa. Campinas: Universidade Estadual de Campinas.
22. 22.Dube, M., Schäfer, C., Neidhart, S., et al., 2007 “Texturisation and modification of vegetable proteins for food applications using microbial transglutaminase” *European Food Research and Technology*, 225(2), 287–299.
23. 23.Lorenzen, P.C., 2007 “Effects of varying time/temperature-conditions of preheating and enzymatic cross-linking on techno-functional properties of reconstituted dairy ingredients” *Food Research International*, 40(6), 700–708.
24. GasparA.L.G, Pedroso de Góes-Favoni S., 2015 “Action of microbial transglutaminase (MTGase) in the modification of food proteins: A review”, *Food Chemistry* 171, 315–322.
25. 25.Kuraishi C., Yamazaki K., and Susa Y., 2001 “Transglutaminase: Its Utilization in the Food Industry” *Food Reviews International*, 17(2):221-246.
26. Collinghan, R, Clara, S., Li, X., Parry, J. and Griffin, M. 2004 “Transglutaminases as tanning agents for the leather industry, JALCA, 99(7), pp.293-302.
27. 27.TS EN ISO 2418, 2006 “Chemical, physical and mechanical and fastness tests-Sampling location” Turkish Standard Institute (TSE), Ankara, Turkey.
28. TS EN ISO 2419, 2006 “Physical and mechanical tests-Sample preparation and conditioning” Turkish Standard Institute (TSE), Ankara, Turkey.
29. ISO 3377-2: 2002 “Part 2: Double edge tear. International Organization for Standardization”, Geneva.
30. ISO 3376: 2011 “Determination of tensile strength and percentage extension. International Organization for Standardization”, Geneva.
31. ISO 3380: 2002 “Determination of shrinkage temperature. International Organization for Standardization”, Geneva.
32. Zengin, A.C.A., Crudu, M., Maier, S.S., et al., 2012 “Eco-leather: Chromium-free Leather Production using Titanium, Oligomeric Melamine Formaldehyde Resin, and Resorcinol Tanning Agents and the Properties of the Resulting Leathers” *Ekoloji*, 21(82), 17-25.
33. Zengin,, G., Tekin, Y.E., Zengin, A.C.A., 2016 “Saraciyelik Derilerde Yağlayıcı Akrilik Polimerin Yaşlanma Üzerine Etkisi” *Tekstil ve Mühendis* , Available online: 31 Mart 2016