Environmental Engineering and Management Journal

November 2019, Vol. 18, No. 11, 2355-2362
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

CHARACTERIZATION OF COLLAGEN DERIVED PRODUCTS PREPARED BY USE OF ALKALI AND DAIRY BY-PRODUCT

Gökhan Zengin[1]*, Arife Candaş Adıgüzel Zengin[1], Eylem Kılıç[2], Urana Dandar[1], Altan Afşar[1], Dmitry Valeryevich Shalbuev[3]

[1]Ege University, Faculty of Engineering, Leather Engineering Department, 35100, Izmir, Turkey
[2]Uşak University, Faculty of Engineering, Material Science and Nanotechnology Engineering Department, 64100 Uşak, Turkey
[3]East Siberia State University of Technology and Management Leather and Fur Technology, Water Resources and Commodity Research, 670013, Ulan-Ude, Siberia, Russia Federation

Abstract

Leather processing is one of the highly polluting industries, due to generation of significant quantities of tanned and untanned wastes. Untanned wastes are less contaminated with chemicals and a better source of high value products such as collagen rather than tanned or finished leather wastes. In the present study, collagen dissolution substances (CDS) were prepared from bovine limed split wastes as value added products, by treating leather wastes with sodium hydroxide and fermented dairy by-product. After dissolution processes, the samples were lyophilized and their morphologies were examined with table top scanning electron microscopy (TSEM). The structures of CDS were characterized by X-Ray Diffraction (XRD), differential scanning calorimeter (DSC), and Fourier Transform Infrared Spectrometer (FTIR). In addition to the organoleptic evaluation of CDS, total Kjeldahl nitrogen and fat content, distribution of fatty acid methyl esters and spectrophotometric color determination of CDS were also investigated. The characteristics of CDS revealed that the alkali treatment caused a high degree of dissolution and led to deformation of the collagen structure. In comparison to alkali dissolution, CDS obtained with fermented dairy by product were found to have acceptable chemical and colloidal properties due to the conservation of collagen polypeptide bond structure. Following the results obtained, it may be concluded that fermented dairy by-product may find use for the hydrolyzation of collagen.

Key words: bovine waste, chemical analysis, collagen, dairy by-product

Received: February, 2019; Revised final: March, 2019; Accepted: March, 2019; Published in final edited form: November, 2019

1. Introduction

Every stage of manufacturing process generates different types of waste and by-products. These process outputs can be used as a raw material for manufacturing other products (Souissi et al., 2018). Like many other manufacturing industries leather industry is regarded as a polluting industry and it is responsible for generation of large amount of solid wastes due to beamhouse operations, in which raw skins or hides are converted into imputrescible final products through chemical and mechanical processes. The solid wastes generated throughout leather manufacturing processes can be classified as untanned hides/skins (trimmings, fleshing wastes), tanned leathers (shaving wastes, buffing dust) and wastes from finished leathers (trimmings from finished leather) (Ozgunay et al., 2007).

The amount of waste released due to transformation of 1000 kg of rawhide into leather resulted almost 200 kg of leather final product, along with 250 kg of non-tanned and 200 kg of tanned waste (Buljan et al., 2000; Cabeza et al., 1998; Shakilanishi et al., 2017; Sundar et al., 2011). Disposal of untanned skin pieces, which are primarily composed of interwoven fiber known as collagen, cause serious

* Author to whom all correspondence should be addressed: e-mail: gokhan.zengin@ege.edu.tr; Phone: +902323114407; Fax: +902323425376

environmental problems in terms of soil and underground water resources pollution and odor produced in the course of their putrefaction (Ozgunay et al., 2007).

Therefore, considering their negative effects on environment and the generation in larger amounts, recovery of these wastes are essential (Langmaier et al., 2008). Various attempts have been made by researchers to find application areas for these types of leather wastes (Alptekin et al., 2012; Zengin et al., 2012; Zengin et al, 2013a; Zengin et al., 2013b).

The collagen containing waste from leather industry can be utilized for producing collagen product by using various methods. And these recovered collagenous products can be used in a wide range of application areas such as gelatin (Cot et al., 1999; Mokrejs et al., 2009), biomaterials (Sahiner et al., 2014a), bio-composites (Ficai et al., 2013; Ramnath et al., 2012; Sahiner et al., 2014b), bio-fiber for medicine (Amsaveni et al., 2013), cosmetics (Langmaier et al., 2001), dental composites (Meena et al., 1999), packaging material (Langmaier et al., 2008), additives (Zhang et al., 2006) and energy source (Kanagaraj et al., 2006; Tanattı et al., 2018). Especially they attract a great deal of attention in bioengineering applications due to their natural proteinous structure, biodegradability (Sundar et al., 2011), low toxicity, low immune response, which enables reconstitution of tissue (Yihui et al., 2013).

Although a variety of methods were reported for production of collagen derived substances from leather wastes such as acidic (Ahmad et al., 2010; Sun et al., 2017), alkali (Haiming et al., 2009; Mu et al., 2003; Yihui et al., 2013) and enzymatic (Selvakumar et al., 2012; Sun et al., 2017; Yuanglong et al., 2012), no study has been found in literatures up to date about collagen dissolution substance production by use of dairy by-product, which is a fermentative production of lactic acid using whey protein.

Fermentative product of whey is a by-product of cheese production, and it is of relative importance in the dairy industry due to the large volumes of production. Lactic acid containing fermentative product may be used as an alternative source to extract collagen from leather wastes. To the best of our knowledge, use of dairy by-product for this purpose has never been reported. Therefore, the aim of this work was to extract and characterize collagen dissolution products from leather industry wastes using dairy by-product and compare it with the products obtained by conventional alkali method. In the first step, the extraction of collagen from untanned leather wastes was elaborated using dairy by-product, which enables dissolution of collagen containing waste by means of lactic acid, fermentative product of whey. In the second step conventional alkali extraction method was applied and characteristics of both products were compared to investigate the potential use of dairy by-product.

2. Material and methods

2.1. Material

Wastes from untanned hide were obtained from limed fleshings process was used as collagen source. Caseic whey (Zharnikova et al., 2016) by-product (liquid waste) of the dairy industry that is formed in curd production was obtained from a dairy factory. Analytical grade chemicals were used for the dissolution process of collagen.

2.2. Methods

Collagen dissolution substances (CDS) were obtained from limed fleshing wastes by treating with dairy by-product (Coll-D) and sodium hydroxide (Coll-S) in accordance with the relevant patents (Leonova et al., 2016; Shalbuev et al., 2008). The dairy-milk composition used in dissolution of collagen was prepared on the basis of secondary products of the dairy industry (Shalbuev et al., 2013; Shalbuev et al., 2014), namely pasteurized curd whey with fat content up to 0.1%. Collagen dissolution products were lyophilized in a freeze dryer.

2.2.1. Organoleptic evaluation of CDS and spectrophotometric color measurements

The evaluation of Coll-S and Coll-D was performed organoleptically in addition to spectrophotometric color measurements. Collagen dissolution substances were examined and evaluated by five different leather specialists by scoring 1 to 6 point where 6 represents the extremely strong, annoying and disgusting smell according to the related standard (Anonymus, VDA 270). In addition to odor test, the color of the collagen dissolution substances was also examined organoleptically.

The spectrophotometric color measurements of CDS were determined by Minolta CM-3600A spectrophotometer (Konica, Japan). The measurements were performed according to the Commission Internationale de l'Eclairage (CIE) Lab color system (McLaren, 1983).

2.2.2. Morphological characterization of CDS by TSEM

The morphologies of freeze-dried collagen dissolution substances were observed by TM1000 table top scanning microscopy at x100 and x1000 magnification (Dandar et al., 2014).

2.2.3. X-Ray diffraction (XRD) analysis of CDS

Structural characterization of collagenous materials was carried out by means of a Rigaku Ultima IV X-ray diffraction system. The patterns were collected at 30 kV and 40 mA for 45 min, in the angular range from 2 to 90 θ and step interval was 0.05°.

The identification of the patterns was performed with software containing data maintained by the International Centre for Diffraction Data Files.

2.2.4. Fourier transform infrared spectrometer (FT-IR) analysis of CDS

Fourier transform infrared spectroscopy technique was used for determination of the functional groups of the freeze-dried collagen dissolution substances. Spectrums were collected by transmitted light FTIR (Perkin Elmer Spectrum 100 with ATR) at a resolution of 4 cm^{-1} at the interval of 4.000 – 600 cm^{-1} at room temperature (Zengin et al., 2011).

2.2.5. Differential scanning calorimetry (DSC) analysis of CDS

The thermal behavior of the collagenous materials were analyzed with differential scanning calorimetry using Shimadzu Plus 60 DSC in the temperature ranged from 5 to 150°C with a constant heating rate of 5°C/min under a nitrogen gas flow. The hydrated ~3 mg CDS samples were sealed in an aluminum pan due to the physiological behavior of collagen as described in literatures (Tang et al., 2003; Wenger et al., 2008).

2.2.6. Total Kjeldahl nitrogen (TKN) and fat content of CDS

The total kjeldahl nitrogen values of CDS samples were determined in accordance with the related method (TS 4134, 2009). A closed solvent extraction system (SES) was used to determine the fat content of freeze-dried CDS samples. The samples were loaded into the SES system and dipped into dichloromethane and kept at 120°C for 100 minutes (Zengin and Afsar, 2011).

2.2.7. Fatty acid contents of CDS

Contents of fatty acids of CDS were analyzed by Gas chromatography (GC; Agilent 6890) with FID detector (Zengin and Afşar, 2011). The extracted fats of CDS samples were trans-esterified prior to analysis in accordance with TS EN ISO 12966-2 (2012) and the analysis was performed with TS EN ISO 12966-4 (2016).

3. Results and discussion

3.1. Organoleptic evaluation of CDS and spectrophotometric color measurements

The display of collagen dissolution substances and results of organoleptic evaluations and color measurements are shown in Fig.1 and Table 1 respectively. According to the organoleptic results, collagen dissolution substances are yellow in color, but Coll-S obtained by alkali treatment has more intense color compared to Coll-D which was obtained using dairy by-product. Besides, Coll-D has a specific pungent smell and resulted in more porous and light colored form after lyophilization process (Fig. 1).

The color measurement results showed that Coll-S has higher b values, which indicate the yellow color, compared to Coll-D and different ΔE values reveals the color difference between the collagen dissolution substances. These results were found compatible with the organoleptic results (Table 1).

3.2. Morphological characteristics of CDS

The surface morphologies of the CDS samples observed by Tabletop Scanning Electron Microscopy at different magnifications are given in Fig. 2. Different surface morphologies were observed from Coll-D and Coll-S. The pore size of Coll-S was found smaller compared to Coll-D but homogenous surface porosity was obtained from the collagen dissolution substances.

3.3. XRD results of CDS

The X-ray patterns of the collagen dissolution products are presented in Fig. 3.

(a) (b)

Fig. 1. Collagen dissolution substances obtained by treatment of sodium hydroxide (a) and by-product of dairy industry (b) and their lyophilized forms

Table 1. The color measurement values of lyophilized Coll-S and Coll-D

	L	*a*	*b*	*ΔE*
Coll-S	91.86±1.8	-0.62±0.43	10.81±2.64	13.21
Coll-D	89.90±1.42	-0.23±0.15	8.11±1.4	12.36

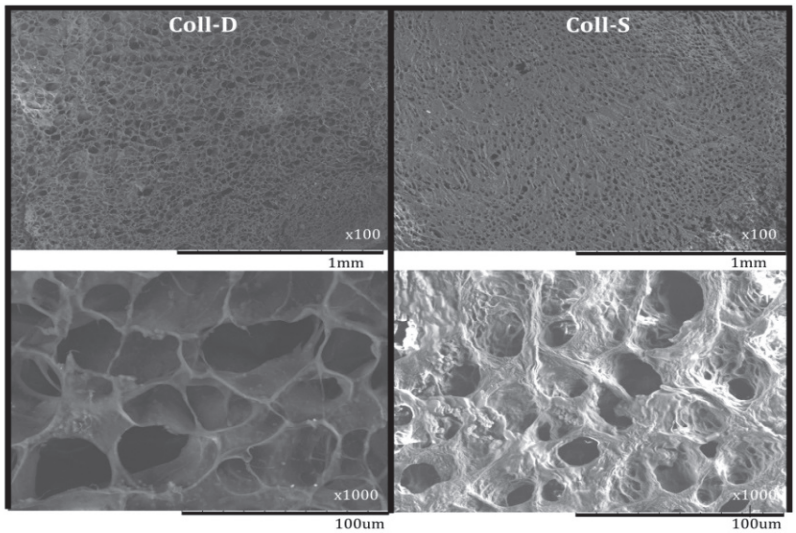

Fig. 2. Surface morphologies of the CDS observed by TSEM at 100x and 1000x magnifications

It is thought that the peaks obtained from CDS by fermented dairy by-product showed up due to the interaction between amino and carboxyl group after the hydrolysis. XRD data of the CDS obtained from alkali condition confirm the FTIR spectra. The results of the study are found compatible with previously published study (Ficai et al., 2013; Ramnath et al., 2012).

The peaks obtained at 7.00-7.500 and 19.50-20.00º in 2θ diffraction angle are the characteristic peaks for the collagen molecule and the first peak is associated with the triple helix structure of the collagen (Sun et al., 2017; Zhang et al., 2011).

3.4. FT-IR results of CDS

The characteristics and the change occurred in macromolecular composition of collagen products are visualized with FT-IR spectroscopy, and their spectrum is shown in Fig. 4.

The FT-IR spectrum of CDS verified that the vibrational mode of amide peaks switched to lower frequencies about 3300 cm^{-1} due to the binding to a peptide chain, although the amide group showed peaks in the bands between 3400-3440 cm^{-1} ranges and for this study, the amide bands were observed at 3315 cm^{-1} and 3293 cm^{-1} for Coll-S and Coll-D respectively. The major bands between 2923-2926 cm^{-1} can be assigned to amid B band. The reason for lower transmittance values of alkali treated sample and sharp peaks observed around 1000-1405 cm^{-1} could be attributed to the significant conformational change due to the alkali effect (Lee et al., 2001; Meena et al., 1999). Due to the C=O absorption the amid bands which constitutes the main structure of collagen were found 1600-1700 cm^{-1} for amid I, 1500-1550 cm^{-1} for amid II, and 1200-1300 cm^{-1} for amid III. It was determined that the peaks of CDS were shifted to wave number 1629-1633 cm^{-1} and 1540-1548 cm^{-1} (Andronescu et al., 2010). No shift was occurred from the spectrum of Coll-S and it was thought that the better dissolution was obtained due to the decrease of intensity of transmittance for the amid I band at 1632 cm^{-1} and wave number shift for amid II. Besides, the lower intensity of transmittance for Coll-S was considered as a serious deterioration of collagen protein. The infrared spectrum of Coll-D showed that amide I, amide II, and amide III bands provided sharp peaks compared to Coll-S.

3.5. DSC results of CDS

The melting point of CDS treated by alkali (Coll-S) and dairy by-product (Coll-D) are observed by the use of DSC technique and results were given in Fig. 5. On set of transition and main denaturation transition were evaluated due to the shrinkage temperature of collagen in DSC thermogram and typical denaturation temperature for natural polymer consist of type I collagen and triple helix structure such as rawhide and skin were observed between 65 to 77°C (Miles et al., 1995; Tang et al., 2003; TS 4134 2009).

Covington specified that denaturation temperature of the untanned bovine hide collagen was found 60°C by using DSC (Covington, 2009). Collagen denaturation temperature were also found as 67°C, and 70°C, in studies regarding investigation of thermal behaviors of historical leather and parchment leather as raw collagen based material respectively (Budrugeac and Miu, 2008; Budrugeac et al., 2011; Cucos and Miu, 2010).

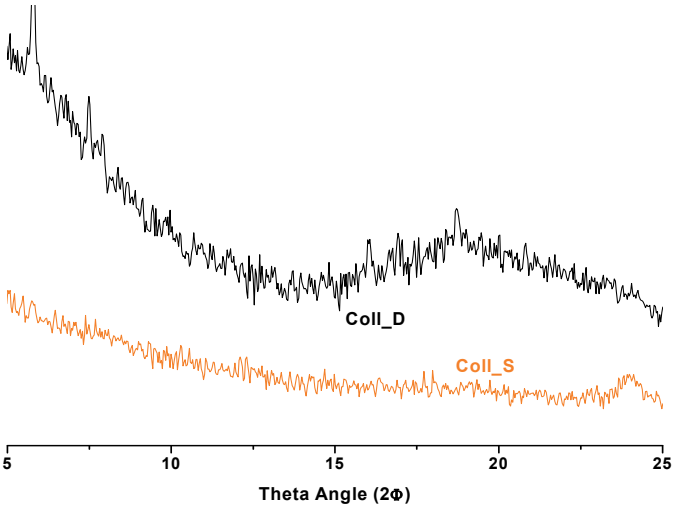

Fig. 3. XRD diagram of Coll-D and Coll-S

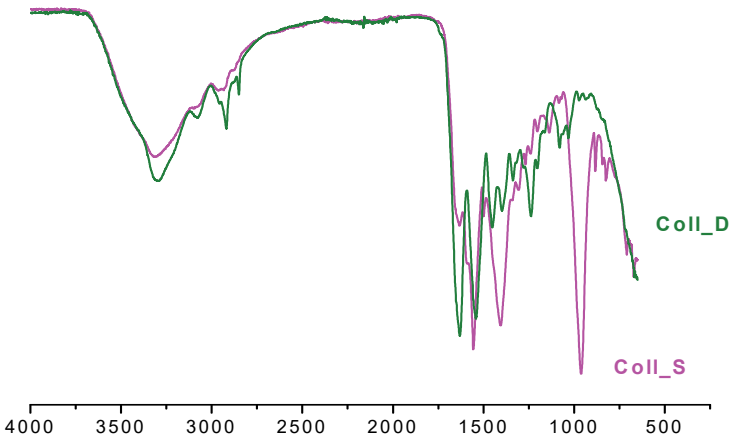

Fig. 4. The FT-IR spectrum of Coll-D and Coll-S

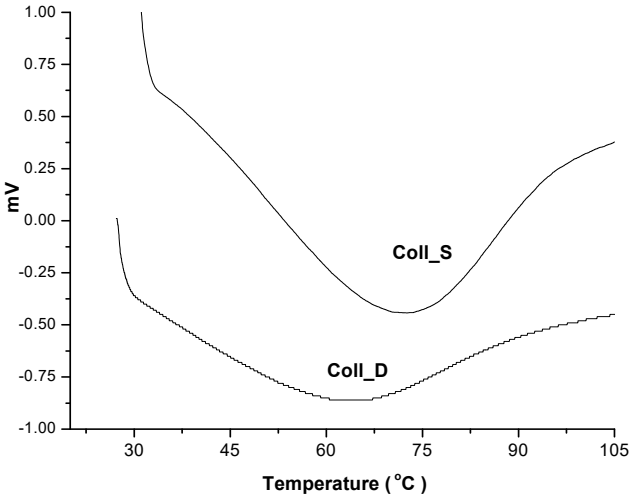

Fig. 5. DSC curves of Coll-S and Coll-D

In the present study, endothermic peaks are observed at T_d 72°C and 64°C for Coll_D and Coll_S, respectively.

3.6. Results of total Kjeldahl nitrogen (TKN) and fat contents analysis of CDS

The total Kjeldahl nitrogen and fat content of Coll-S and Coll-D are given at Table 2. Nitrogen content of Coll-S was found lower than Coll-D. Buljan et al. (2000) specified that 1.5% total kjeldahl nitrogen and 1.2% fat were generated as waste during the leather manufacturing process. The fat content of Coll-D was found higher than Coll-S and this result was attributed to the use of fermented dairy by product in the production method of collagen dissolution substances.

3.7. FAME results of CDS

The FAME distribution of Coll-S and Coll-D are shown in Table 3. The FAME results show the differences in fatty acid distribution of Coll-S and Coll-D, and the fats obtained from different collagenic sources by researchers. The fats obtained from the skin pieces removed by the mechanical processes during biodiesel production (Alptekin et . al, 2012) and the fatty acid distribution of the fat obtained by beef tallow (Moraes et al., 2008) are found similar in terms of fatty acid methyl ester ratio except from the oleic acid (C18:1n9c).

Table 2. Total Kjeldahl nitrogen and fat contents of CDS

	Coll-S	***Coll-D***
TKN (%)	3.80±0.14	7.17±0.07
Fat Content (%)	0.21±0.01	1.42±0.03

C16:0 (Palmitic Acid), which is the main fatty acid of fatty acid methyl ester, had the highest proportion for the Coll-D and had the second highest proportion for the Coll-S. But other distributions of FAME belonging to Coll-S and Coll-D were differentiated.

4. Conclusions

In the present study different types of collagen dissolution substances obtained by use of sodium hydroxide and fermented dairy by-product, were investigated in terms of morphological, structural and chemical characteristics and following conclusions have been drawn.

a. Although the collagen dissolution substances were derived from bovine limed split wastes, they showed different characteristics in their morphologies and macromolecular compositions. The difference characteristic of Coll-S was attributed to a decrease occurred in inter- and intramolecular cross-linking compared to Coll-D.

b. Coll-S had a smaller pore size in comparison to Coll-D but Coll-D had more porous structure, lighter in color in addition to its specific smell.

c. The structural characteristics of CDS revealed that Coll-S had a serious deterioration in terms of collagen protein compared to Coll-D and their molecular weight was found lower than the collagen but higher than the collagen hydrolysate.

d. The chemical compositions and FAME distributions of CDS were found different depending on the production method. The total Kjeldahl nitrogen and fat content of Coll-D was found higher. The amount of palmitic acid in fatty acid methyl esters was determined in highest proportions for CDS but the other FAME distributions and their amounts were differentiated.

Consequently, the results obtained from the present study revealed that use of fermented dairy by-products for the production of collagen dissolution substances provided mostly comparable to and in some aspects better properties than conventional method. Furthermore, it enables finding use for an industrial by-product, instead of using a chemical product.

Table 3. Distribution of fatty acid methyl esters of CDS, collagen, and animal fats (%)

FAME	***Coll-S***	***Coll-D***	***Alptekin et al. (2012)***	***Moraes et al. (2008)***
C8:0	0.75	0.26	-	-
C10:0	1.65	1.28	-	-
C12:0	0.80	1.46	-	-
C14:0	3.36	8.17	0.3	2.68
C14:1	-	0.73	-	-
C16:0	20.85	32.6	27.25	26.17
C16:1	2.50	1.25	5.1	1.90
C17:1	-	-	-	1.74
C17:0	5.45	1.84	0.94	-
C18:0	7.38	17.44	13.23	33.69
C18:1n9t	8.20	2.16	-	-
C18:1n9c	22.89	5.71	42.06	30.09
C18:2n6c	5.00	-	2.04	0.76
C18:3			0.16	-
C20:0			0.21	0.30

Collagen dissolution product obtained by fermented diary by-product might have a great potential in biomaterial utilization due to its special physicochemical properties and preserved collagen conformational structure.

Therefore, characterization of collagen-based products obtained with alternative methods is essential for investigating the applicability of these wastes in different industrial fields and economic benefits can be provided by conversion of an industrial solid waste into a valuable product

Acknowledgements

Authors thank to Scientific and Technological Research Council of Turkey (TUBITAK) under the project number 211T129 for the financial support as well as Turkey Prime Ministry State Planning Organization (Project no: 2007 DPT 001) for the equipment supply.

References

Ahmad M., Benjakul S., Nalinanon S., (2010), Compositional and physicochemical characteristics of acid solubilized collagen extracted from the skin of unicorn leatherjacket (*Aluterus monoceros*), *Food Hydrocolloids*, **24**, 588-594.

Alptekin E., Canakci M, Sanli H., (2012), Evaluation of leather industry wastes as a feedstock for biodiesel production, *Fuel*, **95**, 214-220.

Amsaveni M., Anumary A., Ashokkumar M., Chandrasekaran B., Thanikaivelan P., (2013), Green synthesis and characterization of hybrid collagen–cellulose–albumin biofibers from skin waste, *Applied Biochemistry and Biotechnology,* **171**, 1500-1512.

Andronescu E., Ficai M., Voicu G., Ficai D., Maganu M., Ficai A., (2010), Synthesis and characterization of collagen/hydroxyapatite: Magnetite composite material for bone cancer treatment, *Journal of Materials Science: Materials in Medicine,* **21**, 2237–2242.

Anonymus, VDA 270, (1992), *Determination of the Odor Behavior of Automotive Interior* Materials (Bestimmung des Geruchsverhaltens von Werkstoffen der Karftfahrzeug-Innenausstsattung), Verband der Automobilindustrie e.V. (English Version), On line at: https://www.vda.de/en/services/Publications/vda-270-determination-of-the-odour-characteristics.html

Budrugeac P., Cucos A., Miu L., (2011), The use of thermal analysis methods for authentication and conservation state determination of historical and/or cultural objects manufactured from leather, *Journal of Thermal Analysis and Calorimetry*, **104**, 439-450.

Budrugeac P, Miu L., (2008), The suitability of DSC method for damage assessment and certification of historical leathers and parchments, *Journal of Cultural Heritage*, **9**, 146-53.

Buljan J., Reich G., Ludvik J., (2000), Efficiency of leather manufacturing. Mass balance in leather processing, US/RAS/92/120 Regional Programme for Pollution Control in the Tanning Industry in South-East Asia, United Nations Industrial Development Organization, 16-21.

Cabeza L.F., Taylor M.M., Di Maio G.L., Brown E.M., Marmer W.N., Carrio R., Celma P.J., Cot J., (1998), Processing of leather waste: pilot scale studies on chrome shavings. Isolation of potentially valuable protein products and chromium, *Waste Management*, **18**, 211-218.

Cot J., Manich A.M., Marsal A., Fort M., Celma O.J., Carri R., Choque R., (1999), Processing of collagenic residues. Isolation of gelatin by the action of peroxochromates, *Journal of the American Leather Chemists Association*, **94**, 115-128.

Covington T., (2009), *Collagen and Skin Structure (Chapter 1),* In: *Tanning Chemistry the Science of Leather*, The Royal Society of Chemistry, Cambridge, United Kingdom, 25-26.

Cucos A., Miu L, (2010), The suitability of the DMA methods the characterization of recent and historical parchment and leathers, *International Journal of Conservation Science*, **1**, 13-18.

Dandar U., Çolak S.M., Deveci R., Adıgüzel Zengin A.C., Zengin, G., (2014), Skin characteristics of *Cervus elaphus* L. from Republic of Tuva in Russia, *Journal of the American Leather Chemists Association*, **109**, 141-150.

Ficai A., Albu M.A., Birsan M., Sonmez M., Ficai D., Trandafir V., Andronescu E., (2013), Collagen hydrolysate based collagen/hydroxyapatite composite materials, *Journal of Molecular Structure*, **1037**, 154-159.

Haiming C., Min C., Longli L., Zhiqiang L., (2009), Chemical and physical behaviour of collagen fibre in alkaline solutions, *Journal of The Society of Leather Technologists and Chemists*, **93**, 140-144.

Kanagaraj J., Velappan K.C., Babu C., Sadulla S., (2006), Solid wastes generation in the leather industry and its utilization for cleaner environment - a review, *Journal of Scientific and Industrial Research*, **65**, 541-548.

Langmaier F., Mladek M., Kolomaznik K., (2001), Collagenous hydrolysates from untraditional sources of proteins, *International Journal of Cosmetic Science*, **23**, 193-199.

Langmaier F., Mokrejs P., Kolomaznik K., Mladek M., (2008), Biodegradable packing materials from hydrolysates of collagen waste proteins, *Waste Management*, **28**, 549-556.

Lee C.H., Singla A., Lee Y., (2001), Biomedical applications of collagen, *International Journal of Pharmaceutics*, **221**, 1-22.

Leonova E.G., Shalbuev D.V., Radnaeva V.D., Sovetkin N.V., (2016), Studying the possibility of using caseic whey for close-cycle technology of pickling fur raw materials, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **7**, 670-678.

McLaren K., (1983), The colour science of dyes and pigments, Adam Hilger Ltd, Bristol, England, 186.

Meena C., Mengi S.A., Deshpande S.G., (1999), Biomedical and industrial applications of collagen, *Indian Academy of Sciences (Chemical Sciences)*, **111**, 319-329.

Miles C.A., Burjanadze T.V., Bailey A.J., (1995), The kinetics of the thermal denaturation of collagen in unrestrained rat tail tendon determined by differential scanning calorimetry, *Journal of Molecular Biology*, **245**, 437-446.

Mokrejs P., Langmaier F., Mladek M., Janacova D., Kolomaznik K., Vasek V., (2009), Extraction of collagen and gelatine from meat industry by-products for food and nonfood uses, *Waste Management & Research*, **27**, 31-37.

Moraes M.S.A., Krause L.C., Cunha M.E., Faccini C.S., Menezes E.W., Veses R.C., Rodrigues M.R.A., Caramão E.B., (2008), Tallow biodiesel: properties

evaluation and consumption tests in a diesel engine. *Energy Fuels,* **22**, 1949-1954.

Mu C., Lin W., Zhang M., Zhu Q., (2003), Towards zero discharge of chromium-containing leather waste through improved alkali hydrolysis, *Waste Management*, **23**, 835-843.

Ozgunay H., Colak S., Mutlu M.M., Akyuz F., (2007), Characterization of leather industry wastes, *Polish Journal of Environmental Studies*, **16**, 867-873.

Ramnath V., Sekar S., Sankar S., Sankaranarayanan C., Sastry T.P., (2012), Preparation and evaluation of biocomposites as wound dressing material*, Journal of Material Science Materials in Medicine*, **23**, 3083-3095.

Sahiner M., Alpaslan D., Bitlisli B.O., (2014a), Collagen-based hydrogel films as drug-delivery devices with antimicrobial properties, *Polymer Bulletin*, **71**, 3017–3033.

Sahiner M., Butun S., Alpaslan D., Bitlisli B.O., (2014b), Preparation of collagen based composite materials with synthetic polymers for potential wound dressing applications, *Hacettepe Journal of Biology and Chemistry*, **42**, 63-69.

Selvakumar P., Chuan Ling T., Covington A.D., Lyddiatt A., (2012), Enzymatic hydrolysis of bovine hide and recovery of collagen hydrolysate in aqueous two-phase systems, *Separation and Purification Technology*, **89**, 282-287.

Shakilanishi S., Narasimhan K.C.B., Chittibabu S., (2017), Exploration of chrome shaving hydrolysate as substrate for production of dehairing protease by *Bacillus cereus* VITSN04 for use in cleaner leather production, *Journal of Cleaner Production*, **149**, 797-804.

Shalbuev D., Zengin G., Leonova E., Zengin A.C.A., Sovetkin N., Zharnikova E., Tceherova S., Titova I., (2014), *Investigation of Physicochemical and Biological Changes in the Collagens Under the Influence of the Prokaryotic Organisms-Acids System*, Proc. 5th Int. Conference on Advanced Materials and Systems, Bucharest, Romania, 273-278.

Shalbuev D.V., Zharnikova E.V., (2013), Method of obtaining collagen dissolution products. Russian Federation, RU Patent, No. *2486258C1*.

Shalbuev D.V., Beketova T.S., (2008), Method of producing protein hydrolysate, Russian Federation, RU Patent, No. *2375385C1*.

Souissi N., Jridi M., Slama R.B., Rebah F.B., Nasri M., (2018), Sardinelle by-product hydrolysates as nitrogen source for microbial growth and protease production, *Environmental Engineering and Management Journal,* **17**, 2677-2688.

Sun L., Hou H., Li B., Zhang Y., (2017), Characterization of acid- and pepsin-soluble collagen extracted from the skin of Nile tilapia (*Oreochromis niloticus*), *International Journal of Biological Macromolecules*, **99**, 8-14.

Sundar V.J., Gnanamani A., Muralidharan A., Chandrababu N.K., Mandal A.B., (2011), Recovery and utilization of proteinous wastes of leather making: a review, *Reviews in Environmental Science and Bio/Technology,* **10**, 151-163.

Tanattı P., Şengil İ.A., Özdemir A., (2018), Treatment of biodiesel wastewater by solvent extraction: evaluation of kinetic and thermodynamic data, *Environmental Engineering and Management Journal,* **17**, 2657-2665.

Tang H.R., Covington A.D., Hancoock R.A., (2003), Use of DSC to detect the heterogeneity of hydrothermal stability in the polyphenol-treated collagen matrix, *Journal of Agricultural and Food Chemistry*, **51**, 6652-6656.

TS 4134, (2009), Leather-determination of nitrogen content and hide substance (Titrimetric method), *Turkish Standard (TSE)*, Ankara, Turkey.

TS EN ISO 12966-2, (2012), Animal and vegetable fats and oils- Preparation of methyl esters of fatty acids- Part 2: Preparation of methyl esters of fatty acids *Turkish Standard (TSE)*, Ankara, Turkey.

TS EN ISO 12966-4, (2016), Animal and vegetable fats and oils-Analysis by gas chromatography of methyl esters of fatty acids-Part 4 Determination by capillary gas chromatography, *Turkish Standard (TSE)*, Ankara, Turkey.

Yihui C., Guoyin L., Yun J., (2013), Rheological behavior of alkali solubilized collagen from limed bovine split wastes, *Journal of the Society of Leather Technologies and Chemists,* **97**, 195-199.

Wenger M.P.E., Horton M.A., Mesquida P., (2008), Nanoscale scraping and dissection of collagen fibrils, *Nanotechnology*, **19**, 1-11.

Yuanglong C., Min C., Xiajou C., Wenghua Z., Xuepin L., Bi S., (2012), Enzymatic hydrolysis of skin shavings for preparation of collagen hydrolysates with specified molecular weight distribution, *Journal of the Society of Leather Technologies and Chemists*, **96**, 16-20.

Zengin G., Afşar A., (2011), Use of natural fat emulsions in fatliquoring process and investigation of fatty spue formation, *Journal of the American Leather Chemists Association*, **106**, 83-91.

Zengin G., Ozgunay H., Mavioğlu Ayan E., Mutlu M.M., (2012), Determination of dyestuffs remaining in dyeing processes of vegetable-tanned leathers and their removal using shavings, *Polish Journal of Environmental Studies*, **21**, 479-506.

Zengin G., Kılıç E., Adıgüzel Zengin A.C., Dandar U., Afşar A., Shalbuev D., (2013a), *Characterization of Collagen Hydrolysates Prepared using Different Organic Acids and Dairy By-Product*, Proc. IX Int. Scientific-Practical Conference Leather and Fur in XXI Century: Technology, Quality, Environmental Management, Education Congress, Ulan-Ude, Republic of Buryatia, Russia, 293.

Zengin G., Kılıç E., Adıgüzel Zengin A.C., Dandar U., Afşar A., Shalbuev D., (2013b), *Characterization of Collagen Hydroysates Prepared using Different Organic Acids and Dairy By-Product*, Proc. XXXII. Congress of International Union of Leather Technologists and Chemists Societies, Turkey, 155.

Zhang Z., Li G., Shi B., (2006), Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes, *Journal of the Society of Leather Technologies and Chemists*, **90**, 23-28.

Zhang F., Wang A., Li Z., He S., Shao L., (2011), Preparation and characterization of collagen from freshwater fish scales, *Food and Nutrition Sciences,* **2**, 818-823.

Zharnikova E.V., Shalbuev D.V., Radnaeva V.D., (2016), Effects of various techniques of untanning of leather shavings on the properties of the protein hydrolysate, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **7**, 670-678.