CHEMISTRY & CHEMICAL TECHNOLOGY

Vol. 10, No. 3, 2016 Chemical Technology

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STRUCTURAL TRANSFORMATIONS OF COLLAGEN CONTAINING RAW MATERIALS UNDER ALKALINE TREATMENT

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Received: February 02, 2015 / Revised: March 11, 2015 / Accepted: September 15, 2015

Abstract. In the research of the rawhide structural transformations in chemical-and-colloidal processes under the action of alkaline dispersion, collagen of the dermis gets free of preservative agents and globular proteins. The conditions of rawhide alkaline treatment, under which the structure of a semi-finished product preserving amino-acid composition of collagen of the dermis is formed, have been outlined. A comparative analysis of the developed technologies of a leather semi-finished product formation from cattle rawhide has been carried out.

Keywords: rawhide, collagen of the dermis, structure, semi-finished product.

1. Introduction

Due to a great variety of collagen containing raw materials, a multi-level stage of technological processes fulfillment in the process of its treatment using the reactive chemicals of varying nature and wide range, and also strict requirements for a set of finished leather materials properties, there is an objective problem of the development of their production innovation technologies basing on systematic chemical-and-colloidal and structural research. It is caused by the fact that at each stage of a technological cycle, a structure of a leather semi-finished product is formed, and it significantly affects the efficiency of all succeeding stages and final material properties.

Well-known works in the field of rawhide soaking and liming (SL) generally refer to technological aspects of this problem. Moreover, much attention is paid to the process of hide unhairing. In particular, in [1, 2] the soaking of raw and preserved cattlehide in the presence of sodium carbonate, non-ionic surfactants and proteolytic enzymes was considered. As a result of the studies, it was found that the duration of the process of preserved rawhide soaking ought to last at least 4–6 [1] and 8 h [2], and even twice less at 296 K. Furthermore, the completeness of the technological process was monitored according to the cattlehide increase in weight and technological solution properties.

The authors of the work [3] used lipolytic enzymes along with non-ionic surfactants during rawhide soaking and unhairing. By doing so, the use of non-ionic surfactants was reduced and wastewater ecological properties were increased. The application of calcium peroxide, amines, enzymes and hydrogen peroxide [4] during hide unhairing gave an opportunity to discard sodium sulphide from the technological process. In addition, the use of oxidizing mixture with sodium hydroxide and percarbonate for hide unhairing [5] in the presence of alkaline protease at the second stage of liming contributed to hide formation upon the completion of the entire technological cycle with high end-use properties. The work is dedicated to the issue of the content of the interfibrillar matrix extracted from rawhide [6]. The authors have shown that the amount of proteoglycans extracted from the dermis depends upon the duration of soaking and liming processes.

Considering a multi-level stage and duration of soaking and liming processes there is an objective necessity to conduct a set of chemical-and-colloidal and structural studies during the process of soaking and liming, which determine a hide structure formation at all the following stages of the technological cycle of rawhide transformation into polyfunctional leather materials.

The work presents the research findings of the process of rawhide alkaline treatment, diffusion of alkaline dispersion into the hide dermis structure and reverse diffusion of nonfibrous components into a solution and evolutionary changes of dermis structure properties for a scientific explanation of a semi-finished product structure primary formation technology and an efficient conducting of the following technological processes of polyfunctional material production.

2. Experimental

The wet salted cattle rawhide was used for the investigation, which was collected into batches by taking into account the weight of the hide. Sodium carbonate, sodium hydrosulphide CAS 6721-80-5, sodium sulphide and calcium hydroxide produced according to the standards were used for liming of collagen containing raw materials. The concentration of alkaline agents was varied in the range of 4–35 g/dm^3.

Alkaline solution persorption by the dermis was determined by a weight-change method [7], the structuring degree of hide semi-product – by hydrothermal resistance according to the temperature of beginning the sample shrinkage at the speed of heating 2–3 K/min, enzyme-thermal resistance – by the time of a sample section complete destruction under the action of pancreatine. Deffusion depth of $Ca(OH)_2$ into the dermis structure was determined using a MBR-3 microscope after the section staining with phenolphthalein. Gelatins liquation from a semi-finished product was measured using PHEC-56M photoelectric calorimeter according to the calibration schedule "optical density–dry residue percentage" with a wavelength of 520 nm. D water was a reference standard.

The amino acid composition of collagen of the dermis on a spirit-acetone drying was determined by means of ion exchange chromatography [8] using an automatic T-339 acid analyzer made by "Mikrotechna" company (the Czech Republic) and Ostin LGANB sulfopolystyrene ion exchange resins in a Li-citrated buffer single-column mode. Semi-finished product sponginess was calculated using the results of titrimetric testing of actual and supposed samples density of alcohol and ester drying using the impregnating liquid – kerosene. The structural changes of a semi-finished product after soaking and liming were determined using "REMMA-102" electronic microscope (Ukraine) [9]. The samples for the structural studies were prepared in the form of cross-sections about 1 mm thickness from a semi-finished product by alcohol and ethereous evaporation, accompanied by sputtering their surface with a silver layer of 3–5 nm thickness. The further technological processes of polyfunctional hide formation after liming were carried out according to the industrial technology of JSC "Chinbar".

3. Results and Discussion

3.1. Physico-chemical Rawhide Properties at Structural Transformations

Deep rawhide structural transformations are caused by a set of colloidal and chemical processes which begin at the stage of soaking and liming treatment, under which amide intermolecular bonds are breaking up, which facilitates the active interaction of chemical reagents with collagen of the dermis. At the same time, a crucial influence on the rawhide structural transformations is made by both the solution content and the conditions of their interaction with collagen of the dermis. The results of the studies how the temperature and Na_2CO_3 concentration affect the process of NCl, globular proteins diffusion and desorption are given in Table 1.

One can see from Table 1, with the increase in temperature, Na_2CO_3 concentration and duration of the process at this stage of rawhide liming the degree of its rehydration increases. The temperature increase by 8 K allows to reduce reactive chemical concentration of Na_2CO_3 threefold and the action time by 37–39 % for 5–5.5 h. Therefore, conduction of the rawhide soaking process at 301 K provides a maximum similarity of a semi-finished product by its moisture content to the native

Table 1

Kinetics of rawhide middle layer and its components moisture changes under reverse diffusion

Duration of soaking, h	Moisture content, %, at the temperature, K		Solution concentration, g/dm^3		
			NaCl	Globular proteins, at K	
	301*	293**		301	293
0	47.5	47.5	0	0	0
2.5	57.0/59.0	56.0/58.0	78.0	2.73	2.16
5.0	62.5/64.5	60.5/63.0	107.0	3.64	2.62
6.0	63.5/65.5	61.0/64.3	126.0	4.26	2.87
8.0	65.3/66.5	62.3/65.0	138.0	4.94	3.93
9.0	65.7/67.0	62.5/65.0	141.0	5.21	4.31

Notes: The nominator and denominator respectively show the value without the use of sodium carbonate and with its concentration of, g/dm^3: * – 4, ** – 12 state, which accounts for about 70 % [10], while a maximum mobility of collagen of the hide dermis elements is achieved, and it will facilitate an effective chemical reagents diffusion into the dermis structure. At the same time, a reverse diffusion of globular proteins and 69.0 % of NaCl, as a preserving agent, happens at the temperatures under investigation. In the subsequent alkaline treatment of a semi-product, which includes rinsing, there is practically a full discharge of its structure from these components.

A further interaction between the hide dermis and more active chemical alkaline reagent $Ca(OH)_2$ is the destruction of chemisorbed and chemical bonds between albumins, globulins, mucopolysaccharides, lipids and the elements of collagen fibrous structure [11]. Further removal from the rawhide into the technological dispersion takes place during mechanical deformation of a semi-finished product in the moving device. It is worth noting that elastin and reticulin remain in the hide dermis structure under alkaline treatment due to their high chemical resistance.

The studies of $Ca(OH)_2$ diffusion process into the dermis (Fig. 1) are characterized by a high initial speed with a subsequent slowdown. At the same time, the process intensity is increasing with the increase of reagent concentration, and the duration of the reagent penetration at the depth of 70 % of the dermis thickness is decreasing twofold with its 35 g/dm^3 concentration in comparison with a minimum concentration in the technological dispersion. It can be caused by an efficient interaction of $Ca(OH)_2$ dispersion with collagen of the dermis, as a result of which its structure is becoming more homogenious. At the same time, amide bonds of an ionic type between collateral residuals of collagen polypeptide chains break up and free carboxy groups form complex compounds with $Ca(OH)_2$ [12]. Along with this, a part of a diffused alkaline reagent is located in macropores of a semi-finished product in a free state.

As a result of chemical interaction of alkaline dispersion with collagen of the dermis, its further evolutional changes happen within the next 12 h. In the course of a long interaction of a semi-finished product with $Ca(OH)_2$ dispersion, moisture absorption of hydrophilic samples is increasing significantly (Table 2).

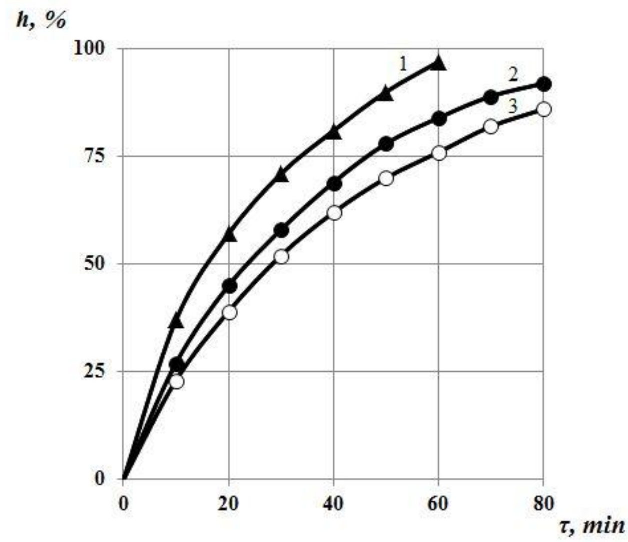

Fig. 1. Kinetics of $Ca(OH)_2$ diffusion into the skin dermis at 301 K and concentration, g/dm^3: 35 (1); 15 (2) and 5 (3)

The results of the research show that after alkaline treatment of the semi-finished product by means of $Ca(OH)_2$ and Na_2S dispersion its moisture content depends on the ratio of the technological dispersion/raw material reaching the maximum decrease at the highest size (variant 3). The reason for this is that increased dispersion of alkaline reagent particles at a lower concentration and the corresponding value of diffusion in the semi-finished product promote water absorption. It should be noted that the minimum water absorption is observed for the samples without previous watering before alkaline treatment (variant 4) containing preservative agents, or at minimum water expense (variant 1).

Table 2

Properties of a semi-finished product after alkaline treatment

Parameter	Parameter values according to the variant				
	1	2	3	4*	5
Technological dispersion/raw material ratio, dm^3/kg	0.5	1.0	1.5	1.5	3.0
Calcium hydroxide concentration, g/dm^3	30.0	15.0	10.0	10.0	5.0
Sodium sulfide concentration, g/dm^3	36.0	18.0	12.0	12.0	6.0
Moisture content, %	78.0	81.0	81.5	77.7	84.5
Hydrothermal stability of the rawhide, K	331	327	327	333	330
Semi-finished product area, %	89.0	92.0	93.0	88.0	89.0

Note: *without rawhide soaking

The results of the research of the semi-finished product after alkaline treatment of minimum and maximum water absorption values correspond to higher values of hydrothermal stability of the samples. This may indicate a simultaneous impact on the structure of the semi-finished product as alkaline reagents interaction with dermis collagen and the formation of internal strains that react on hydrothermal stability decrease due to excessive dermis collagen absorption as a result of high hydrophilicity of the sorbed alkaline reagents. Minimum values of hydrothermal stability point to the largest degree of fibrillar structure division and its potential availability to further effective technological treatments.

Summing up the above-said, the most remarkable changes in corium collagen take place at the concentration of $Ca(OH)_2$ 10–15 g/dm^3 and the proportion of technological dispersion/raw material 1.0–1.5/1.0. Certain increase of hydrothermal stability of the rawhide is stipulated by its excessive absorption, when elastic dermis properties increase. Under these conditions of alkaline semi-finished product treatment, the maximum yield of the leather semi-finished product area is achieved that shows deeper structural changes of dermis collagen.

At the same time the amount of the destroyed collagen – gelatine after alkaline treatment of the semi-finished product at different temperatures can show the state of the semi-finished product structure (Fig. 2). The considerable increase of the amount of the melted gelatine starting from the temperature 301 K confirms the beginning of destructive processes in the inter- and inner molecular bonds of the collagen macromolecules. Thus, as the results of the conducted investigations show it is reasonable to carry out the soaking-liming processes of the rawhide material at the temperatures below 301 K.

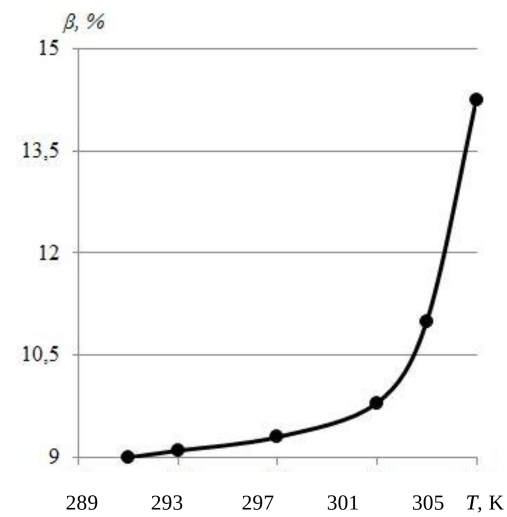

Fig. 2. Dependence of the amount of the melted gelatine *b* from the rawhide on the liming temperature

The study of the amino acid composition of the semi-finished product limed at 301 K confirms this (Table 3). As the results of the investigation of the corium hydrolyzates have shown, the content of amino acids in the samples limed at 293 and 301 K practically does not differ. Certain difference between the glycine amount and the theoretically calculated value 33.3 % [13] can be explained by the presence of non-collagen proteins in the collagen of the dermis.

Table 3

The amino acid compositions of the corium collagen hydrolyzate of the ox hide before and after liming

Amino acid	The amount of amino acids of the corium, mol %		
	Before liming	Liming temperature, K	
		293	301
Alanine	11.92	11.76	11.88
Arginine	5.24	5.18	5.13
Asparaginic asid	4.77	4.82	4.92
Valine	2.67	2.70	2.59
Glycine	28.43	28.64	28.81
Glutaminic acid	9.16	9.12	9.21
d-Hydroxylysine	0.69	0.72	0.63
Histidine	0.63	0.61	0.57
Lysine	2.98	2.96	2.93
Hydroxyproline	6.96	7.17	7.29
Proline	13.79	13.82	13.68
Methionine	0.51	0.53	0.58
Isoleucine	1.37	1.34	1.38
Leucine	2.94	2.97	2.86
Serine	3.92	3.59	3.62
Tyrosine	0.54	0.56	0.57
Threonine	1.96	2.03	1.91
Phenylalanine	1.52	1.48	1.44

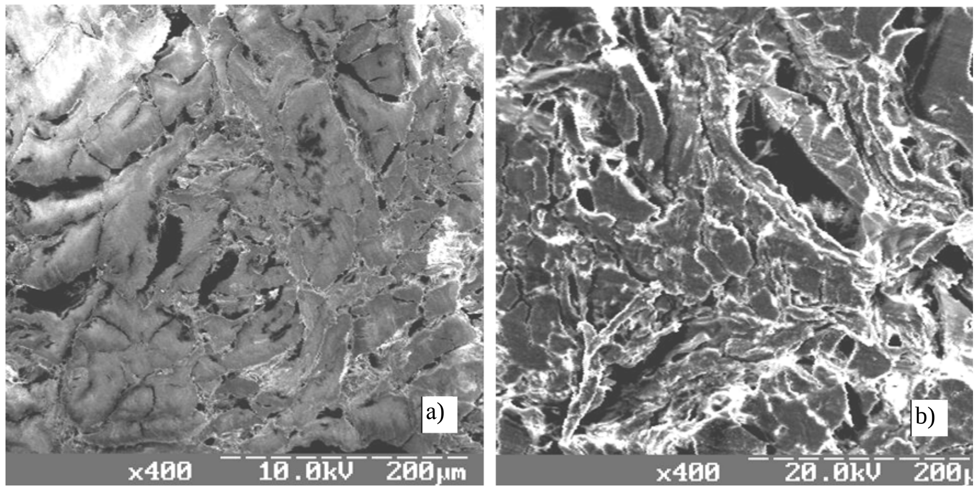

Fig. 3. Submicroscopic images of the skin cross –section after soaking of a bull-calf's skin (a) and its liming (b)

The results of the electronic-microscopic research are the direct evidence of the essential structural transformations of the skins corium during leather material formation using soaking and liming processes (Fig. 3). The raw skin after soaking has consolidated fibrous structure (Fig. 3a), certain fibrils and elementary fibres of which are combined into bundles, between which there are empty spaces, which are filled after soaking with the dispersion of alkaline reagents. Under the action of these reagents during the next liming, the structure of corium becomes more homogeneous (Fig. 3b), as a result of the further absorbing of the alkaline dispersion. At the same time, corium collagen is temporarily structured by Ca^{2+} ions.

The conducted research of the macrostructure change during raw hide formation gives the possibility to detect the peculiarities of releasing the corium collagen structure from soluble proteins – globulins and albumins, mucopolysaccharides, partially lipids and their substitution by cluster water solutions of chemical reagents, and owing to this the raw hide is being formed preserving substantially the initial corium structure of the raw skin. This is due to the existence of the technological dispersion in the created fibrous porous structure of the raw hide, and it fills interfibrillar and interfibrous spaces, which have been created during the process of liming, and it impedes the essential approaching of the structural elements and the whole structure consolidation. Later, at a certain technological stage of leather material formation, the cluster water is displaced by the relevant chemical reagents. As a result of this mechanism of raw skin formation and finished material, a considerable rate of the reagents' diffusion in the soaking and liming processes is being achieved.

Thus, the research of the changes of the colloid–chemical characteristics and structure of the corium collagen under the action of alkaline agents gives the ground to consider that the increasing of temperature up to 301 K will facilitate the effective formation of the leather half – finished material during further stages of its processing.

3.2. Technologies of Rawhide Soaking and Liming

As a result of integrated studies in soaking and liming processes of hides, the technologies were developed and used later to create the technologies of polyfunctional leather materials. The scope of limed semi-finished product largely depends on the type of rawhide technology features and its further processing. If the scope of applicability is limited for small rawhide leather material, in the case of large raw materials, due to the expansion of applied technology processing, their range and polyfunctionality significantly increase. The effectiveness of the use of raw materials in the second case can be significantly raised by increasing the area of semi-finished products in multiple doubling and its convenience of cutting out received material.

The developed technologies for soaking and liming of cattlehide differ significantly from previously existing cost values of chemicals and water, treatment duration of raw materials, saving electricity and environmental efficiency (Table 4). All developed technologies are implemented at an elevated temperature of 281–283 K. The Technology 1 [10] provides processing of hides in one stage and decreases the cost of reagents and water by 2.2–2.3 times, electricity – by 1.7, duration of treatment – by 2.3 and the content of sulfides and calcium hydroxide – by 1.9 and 4.4 times, respectively.

Table 4

Characteristics of soaking and liming technologies of wet salted rawhide processing

Characteristics	Technology SL			
	1	2	3	Previously existing
Materials consumption, kg/t	58.5	62.5	78.0	129.3
including Na_2S +NaSH	20.0	20.0	12.0+9.0	29.5
$Ca(OH)_2$	24.0	30.0	33.0*	80.8
Water consumption, m^3/t	5.0	4.2	4.8	11.5
General duration, h	19.0	20.0	19.0	44.5
– soaking	6.0	5.0	5.0	8.5
– liming	12.0	14.0	13.0	34.0
– washing	1.0	1.0	1.0	2.0
The content in the exhaust solution, g/dm^3				
sodium sulphide	4.3	4.8	4.6	8.0
calcium hydroxide	3.6	4.0	4.2	16.0
keratin	19.0	0.8	0.5	19.0
Power consumption, kW/t	22.7	18.6	20.6	38.3

Note: * sodium hydrosulfide

Table 5

Physical and chemical characteristics of limed pelt

Characteristics	Technology SL			
	1	2	3	Previously existing
Hydrothermal stability, K	328	329	327	327
Enzymatic and thermal stability, min.	58	57	53	51
Gelatin rendering at 330 K, %	9.8	10.3	10.7	12.5
Pelt deformation under load, %				
1	11.0	13.0	12.0	6.0
5	33.0	30.0	32.0	25.0
elastic	32.0	34.0	29.0	41.0
residual	34.0	37.0	39.0	27.0

In contrast to Technology 1, Technologies 2 and 3 [12, 13] are carried out at two stages and are fundamentally different in providing recycling hair. These technologies provide cost reduction in chemicals by 1.7–2.0 times. Therewith the keratin content in waste solutions is reduced by 24–38 times compared with the technology of one-stage liming.

Analysis of the properties of limed pelt (Table 5) shows that after liming the semi-finished product formed by the developed technology is characterized by parameters, the values of which do not differ from previously existing technologies. However, slightly less rendering of gelatin from limed pelt may indicate a higher thermal stability of dermis collagen obtained by the developed technology.

At the same time by their elastic and plastic properties the limed semi-finished product obtained by the developed technology is characterized by increased distorting of less dense peripheral areas of hides and lower values of elastic deformation. This will facilitate the hide structure formation with high yield area.

Thus, according to the obtained research results of the soaking and liming process, efficiency analysis of developed technologies, including properties of limed semi-finished products indicates the possibility of their efficient use, depending on specific conditions of industrial production.

4. Conclusions

In the research of the structural transformations of rawhide in chemical-and-colloidal processes under the action of alkaline dispersion, collagen of the dermis gets free of preservative agents and globular proteins thus facilitating the accessibility of hydrophilic functional groups to interreact with reactive chemicals. The conditions of wet salted rawhide alkaline treatment, under which the chemical-and-colloidal state of the rawhide approaches its primary steam state, have been outlined. It occurs with increasing temperature of the process to 301 K and threefold sodium carbonate decreasing concentration in the process solution. At further rawhide calcium hydroxide liming, there is an evolutional division of collagen of the dermis fibrillous structure with its active participation forming complex collagen compounds and increasing the dermis water absorbing power.

The conditions of rawhide alkaline treatment, under which the amino-acid composition of the dermis collagen is preserved, the optimum watering degree of which can provide the efficient conducting of the further processes and operations of leather materials formation, have been determined. The technologies of cattle rawhide soaking and liming, which were developed and implemented into the industrial production, refer to material-, energy-saving and ecologically efficient ones. The Technology 3 of two-stage soaking and liming, which is the basis for the follow-up studies of the polyfunctional leather materials formation, can be considered to be the most advanced.

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СТРУКТУРНІ ПЕРЕТВОРЕННЯ КОЛАГЕНВМІСНОЇ СИРОВИНИ ПРИ ЇЇ ЛУЖНОМУ ОБРОБЛЕННІ

Анотація. *В результаті досліджень структурних перетворень шкіряної сировини у колоїдно-хімічних процесах під дією лужних дисперсій колаген дерми звільняється від консервуючих агентів і глобулярних білків. Встановлені умови лужного оброблення шкіряної сировини, за яких формується структура напівфабрикату зі збереженням амінокислотного складу колагену дерми. Проведений порівняльний аналіз розроблених технологій формування шкіряного напівфабрикату з сировини великої рогатої худоби.*

Ключові слова: *шкіряна сировина, колаген дерми, структура, напівфабрикат.*