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Absorption and fixation of vegetable tannins by collagen

Franciela Spier[1], Wagner F. Fuck[1], Manuel Antonio Chagas Jacinto[2], Mariliz Gutterres[1]

[1]Laboratory forLeather and Environmental Studies (LACOURO),Chemical Engineering Post-graduate Program (PPGEQ), Federal University of Rio Grande do Sul (UFRGS), Av. Luiz Englert s/n°, Porto Alegre-RS,Brazil, 90040-040, (51)3308-3954, franciela@ibest.com.br, mariliz@enq.ufrgs.br

[2]Leather laboratory of the Embrapa Livestock Southeast, Rodovia Washington Luiz, km 234, CEP 13.569-706 São Carlos, SP, Brazil, +55 16 3411-5693, manuel.jacinto@embrapa.br.

Abstract

In the process of transforming animal hide into leather, the hide, which is susceptible to putrefaction, is stabilized. The growing interest in clean technologies and environmental requirements led tanners to increase their efforts to develop chrome-free tanning agents. Among the various tanning options, vegetable tanning is a traditional and recognizable method and, associated with some physicochemical properties, it provides the leather with unique characteristics, retaining the natural flaws and markings. As an organic biodegradable material, it is also environmentally friendly. The vegetable tannins are complex mixtures with tanning action provided by their concentration of phenolic substances that react with collagen materials. This study aimed to investigate the ability of hide's collagen substrate to absorb and fix vegetable tannin of *acacia mearnsii* at different pH, through the use of especial analytical and experimental methods. In substrates such as collagen powder, bovine hide powder, and bovine hides, was examined the influence of the pH and tannins offers on the absorption and fixation of acacia vegetable tannin. The absorbed tannin content was determined by UV-visible spectroscopy and by the difference of solids contents in the suspension before and after tanning. The analyses of zeta potential allowed for the elucidation of conditions of the tanning process.

Keywords: tannin absorption, spectrophotometry, zeta potential.

1 – Introduction

After pelting, hides are susceptible to attack by bacteria and microorganisms. The collagen structure of the hides must be stabilized by means of the tanning process to avoid its degradation. Of the existing tanning processes, chrome tannage is the most widely used by the leather industry, because it makes leathers with excellent physical and organoleptic properties.Approximately90% of the tanning process is carried out with chromium(III) salts (Marsal *et al*., 2012). But, chrome tanning has its own disadvantages. In Brazil, leather wastes containing chromium are considered hazardous waste (Class I) and must be placed in special landfills. Therefore increasingly, research has concentrated on alternatives to chrome tanning. Among the innumerable alternative tannages that are currently exploited, the vegetable tannins is one of the most promising options. Vegetable tannins are natural materials which have been consideredas a suitable eco-friendly option to replace chromium (D'aquino*et al*.,2004).

Tannins are defined as naturally occurring water soluble polyphenols of molecular weight between 500 and 20,000Da,which differ from most other natural phenolic compounds in their ability to form insoluble complexes in water with proteins, polysaccharides and alkaloids. This particular reactivity with proteins is named astringency and is the basis for their use in the tanning industry (Khanbabaee and Ree, 2001).They are widespread in the plant kingdom (pteridophytes, gymnosperms and angiosperms), are found in leaves, fruits, bark and wood, and can accumulate in large amounts in particular organs or tissues of the plant (Haslam, 1989).Feature a large value in the interactions between the plant and its ecosystem exercising, eg., the paper *fagoinhibitor* against herbivores or as antimicrobes agents. Tannins are considered plant secondary substances as they are not involved in metabolic pathways. As secondary metabolites, the tannins are phenolic compounds of great economic and ecological interest (Monteiro*et al*., 2005).

The classification of tannins, based on their structures and properties, there are hydrolysable and condensed tannins (Adamczyk *et al*., 2011). Hydrolysable tannins are composed of esters of gallic acid or ellagic acid, gallotannins and ellagitannins respectively, with a sugar core which is usually glucose, and are readily hydrolysed by acids or enzymes into monomeric products (Falcão and Araújo, 2013). Condensed tannins, also known as polymeric proanthocyanidins,are composed of flavonoid units, and are usually more abundant in tree barks and woodsthan their hydrolysable counterparts (Bhat*et al*., 1998).The sources of tannins are very varied. For both hydrolysable and condensed structures, the species rich in tannins are many. Notable for either economic and industrial importance are black wattle or black mimosa bark (*Acaciamearnsii*), quebracho wood (*Schinopsis balansae*), oak bark (*Quercus* spp.), chestnut wood(*Castanea sativa*), mangrove wood, divi-divi (*Caesalpina coraria*), algarobilla chilena, tara, and the bark of several species of pines and firs, not counting even more plants with extractable tannins (Pizzi, 2008).

In this context, this work aimed to investigate the ability of hide's collagen to absorb and fix vegetable tannin of *acacia mearnsii* at different pH, throughout the use of especial analytical and experimental methods.

2 – Materials and Methods

It was evaluated the influence of substrate, pH, kind and offer of tannins on the absorption and fixation of acacia vegetable tannin. For this purpose the following conditions were tested:

Substrate: pickled bovine hide, bovine hide powder, collagen powder;
Vegetable tannin: acacia extract, acacia extract modified;
Concentration: 30%, 45%;
Initial pH: 3.0, 4.5, 6.0.

The tanning processing was carried out in rotating laboratory drums. The process lasted 300 min, and the tannin was added at once and fixed to the end of the process by addition of formic acid. It was added 150 mL of water by pre calculated quantities tannin, the initial pH was adjusted and this solution was added to 5g (wet basis) of the substrate. Taking into account the moisture content of the substrates: 53.3% hide, 16.2 hide powder and 13.7% collagen, the amounts of tannin offered calculated as a percentage of collagen on dry basis, resulted in: 64.2 and 96.3% for hides, 35.8 and 53.7% for hide powder and 34.7 and 52.14% for collagen powder. The materials used were bovine hide powder produced by Fordchungsinstitut für Leder uns Kunsttoffbahnen Freiber containing 0,5% Cr_2O_3, acacia extract and acacia extract modified (clarified), produced by TANAC S.A., collagen hidrolizate produced by Protein Trading and pickled bovine hide.

The moisture content of substrates was determined by analysis of weight loss at 105°C up to constant weight, according ISO 4684.For determination the total solids content of the aqueous medium before and after the tanning, samples containing 20 mL were evaporated and dried in porcelain capsules at 105°C up to constant weight, according NBR 13572. The determination of the tannin concentration in the aqueous medium before and after the tanning was performed with a spectrophotometer T80 + UV/Vis Spectrometer (PG Instruments Ltd.) using a wavelength of 278nm obtained by scanning. The absorbance value was converted to concentration (g.L^{-1}) through a standard curve previously determined for each tannin. The zeta potential of aqueous medium was determined on Zetasizer Nano Series (Malvern Instruments Ltd.) The pH of the substrates and the aqueous medium was also determined.

3 – Results and Discussion

The substrates initially had pH 5,5 (collagen), 4,55 (hide powder) and pickled hide (3,10).The pH of pickled hide was adjusted to pH 5,0 with sodium bicarbonate. The determinations of pH in the aqueous medium during the tanning process for the acacia extract at 30% of initial offer are shown in Figure 1. At a 45% of initial offer for tannin and modified acacia extract the results are very similar (data not shown).

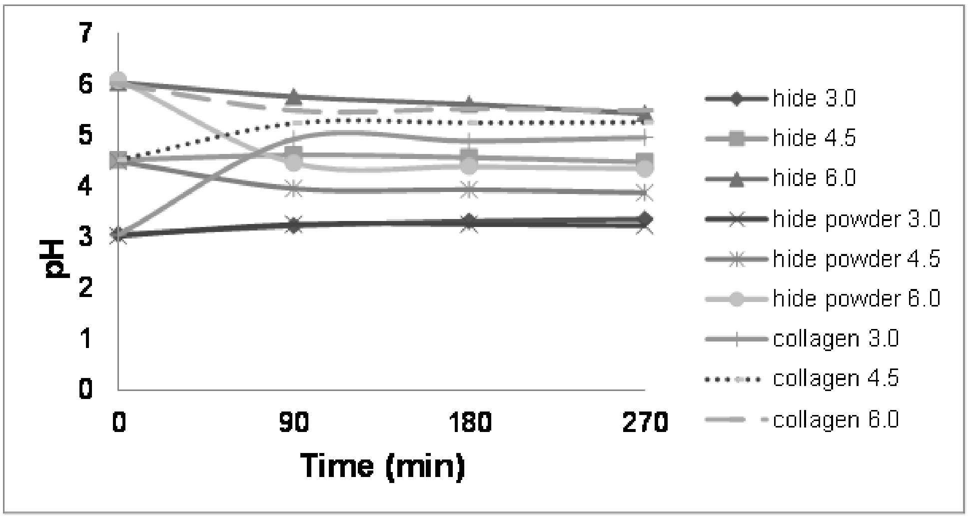

Figure 1. ph monitoring during the tanning process, with acacia extract at 30% of initial offer, for the three substrates and for initial pH 3, 4.5 and 6.

It is observed that the major changes in pH occurred in the assays of tanning aqueous medium with initial pH 6. For the substrates, there was a higher variation of pH in aqueous medium with collagen, followed by those with hide powder. The tendency is that the pH of the substrate and the tanning medium come into equilibrium in the process, since in the case of collagen and hide powder there is less barriers preventing the exchange (hide surface and fiber network), the pH of those mediums approaches the pH of the substrate more quickly.

The total solids content, tannin concentration and zeta potential of tanning baths are presented in Table 1.

Table 1. Total solids content, tannin concentration and zeta potential of tanning aqueous medium.

Assays	Total solids (%)		Tannin concentration (g.L^{-1})	
	initial	final	initial	final
1	1.88	1.51	20.43	15.34
2	1.93	1.49	20.59	15.59
3	1.89	1.51	20.85	16.25
4	2.70	2.20	29.98	23.85
5	2.83	2.24	29.40	23.82
6	2.68	2.27	30.63	24.37
7	1.83	0.46	20.43	1.06
8	1.82	0.51	20.43	1.11
9	1.81	0.60	21.11	1.03
10	1.55	0.41	16.24	0.81
11	1.57	0.46	16.07	0.91
12	1.56	0.46	16.59	0.75
13	0.96	2.62	10.20	1.64
14	1.02	2.66	10.31	1.97
15	0.99	2.68	10.07	1.81
16	1.50	2.53	15.45	1.79
17	1.60	2.79	16.98	1.95
18	1.51	2.64	16.15	1.85
19	1.93	1.60	20.65	16.28
20	1.94	1.66	20.45	16.87
21	1.89	1.67	19.93	16.87
22	2.87	2.48	31.86	25.02
23	2.84	2.44	30.92	24.86
24	2.77	2.47	31.40	24.92
25	1.98	0.79	20.72	4.47
26	1.99	0.86	20.98	4.43
27	1.92	0.85	20.55	4.28
28	1.65	0.57	17.39	2.32
29	1.67	0.58	17.39	2.23
30	1.66	0.59	16.80	2.32
31	1.07	2.92	11.32	4.14
32	1.04	2.91	10.70	4.28
33	1.03	2.87	10.31	4.18
34	1.62	2.85	16.61	5.50
35	1.60	2.81	16.77	5.67
36	1.58	2.86	17.22	5.51

Zeta potential		
Assays	inicial	final
10	-7.71	-5.86
11	-13.2	-10.3
12	-13.3	-12.9

It is assumed that the tanning action exerted by tannins occurs by hydrogen bonds phenolic groups of tanning with certain basic groups linked to the polypeptide chain. Although the strength of the individual hydrogen bond is low, the large number of available hydrogen bonding provides a high stabilization of the collagen structure (Heidemann, 1997).

The tanning process occurs in two stages. First, tannin molecules diffuse into the hide and on a second stage, the molecules are finally fixed during the fixing phase. According to Faber (1990), the factors that influence the diffusion are: particle size, viscosity of the solution, temperature, pH, concentration of tanning, mechanical action and others.

The tannins diffusion increases with increasing pH of the tanning medium. It is associated to the size reduction of tannin particles, and with the modification of the hide swell with pH, and furthermore, with the decrease of affinity and tendency of tannins fixation due to the pH elevation. The increased rate of diffusion occurs clearly only above pH 4 or 5. In the neutral state rate of diffusion is especially high. When diffusion is completed occurs the fixation of the tannin by lowering the pH. For this purpose, organic acids are used (Hoinacki and Gutheil, 1978).

It is verified through the analysis of the results of total solids and tannin concentration at the end of tanning process that the initial pH of aqueous medium did not affect the absorption of tannins for all three substrates studied (Table 1).However, it is possible to observe greater influence of the substrate, the tannin concentration and even the type of tannin used (Figure 2, 3 e 4). Assays 10, 11 and 12 showed the best results, with increased tannin absorption evidenced by lower total solids and tannin concentrations in the residual bath. In these tests with hide powder the tannin was used without modification in its highest concentration (53.7%). The tannins diffusion rate is proportional to the concentration difference of tannin in the aqueous medium and on interfibrillar region of hide. When the difference becomes very small. the diffusion decreases and finally it stops completely. Thus, the diffusion is facilitated by high concentrations of tanning. The tannin used in these tests was not submitted to sulfitation process, where is obtained a clarified tannin with reduction of tanning content and the hide powder used was pre-treated with chromium (0.5%). Furthermore, in the tanning of hide powder, the initial stage of diffusion through the hide layers is eliminated, it is used as a standard substrate, eliminating interference of origin or kind of hide.

When comparing the two methods used to quantify the absorbed tannins, there is a correlation of the results obtained for the hide. However, for the hide powder and collagen substrates, there was no correlation between the remained solids obtained even after filtration quantified the tannin and in the case of collagen, resulting in high total solids content in the tanning wastewater even larger than the aqueous medium at the beginning of tanning. The method using spectrophotometry UV/VIS showed excellent results, wherein the wavelength found in the scan 278 nm is reported in literature (Kardel *et al*., 2013) and tannin concentrations found trough the equation generated on calibration curve are consistent with the real. For example, the assay 31 in which was added 1.7 g/150mL of tannin and was found, through analysis, the value of tannin 1.698g/150mLin the initial aqueous medium. According Kardel *et al*. (2013) vegetable tannins are polyphenols, distributed into condensed and hydrolyzable tannins with an immense structural variability, reaching high degrees of polymerization. Tannins from different sources can react very diverse regarding protein affinity. Differences in tannin structure can occur even between similar plant species. This heterogeneity generate complications regarding a standardized tannin measurement.

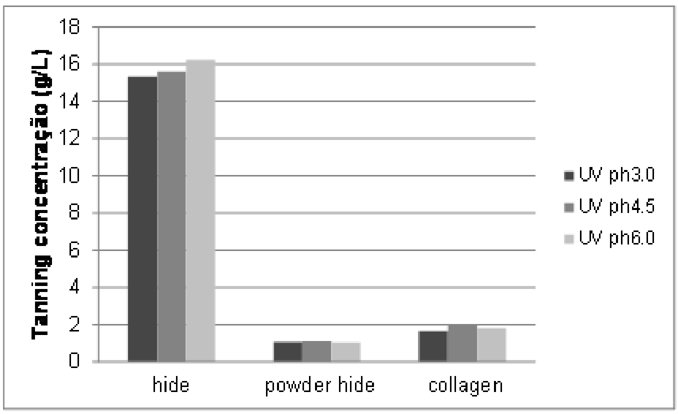

Figure 2. Tannin concentration (g /L) in tanning wastewater, for the three substrates and pH studied, by offer of 30% acacia extract.

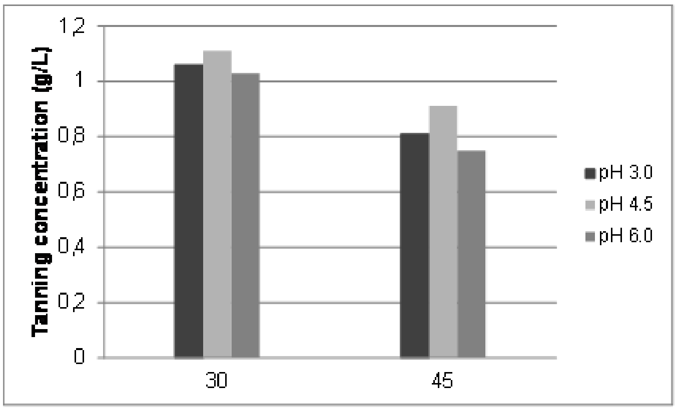

Figure 3. Tannin concentration (g/L) in tanning wastewater, by offer of 30% and 45% of acacia extract on hide powder.

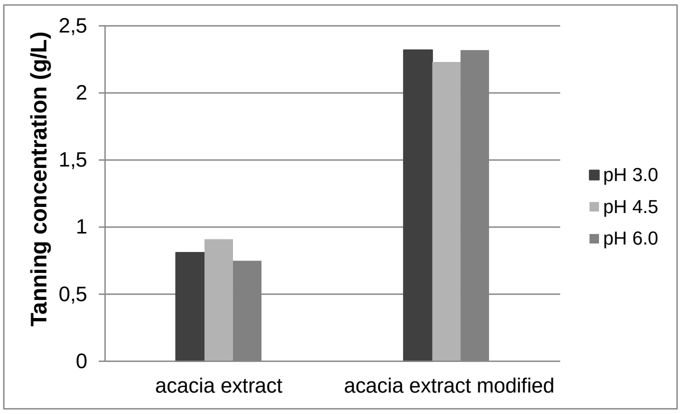

Figure 4. Tannin concentration (g/L) in tanning wastewater, by offer of 45% of acacia extract and acacia extract modified on hide powder.

The reactivity of collagen is assigned to its isoelectric point which depends on the ionization of acid and basic groups of amino acids. The collagen has a multipurpose electrolyte amphoteric character, it works as a dipolar ion.

Zeta Potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticle surface (Mantilla *et al.*, 2007).

In the assays that showed the best results of tannin absorption were performed the determinations of zeta potential (Table 1).It is verified that both tannins, with or without modification have anionic character is in agreement with that reported by Özacar and Sengil (2003) and after the tanning process it approximates the isoelectric point(-13.2 to -10.3).

4 – Conclusion

Under the conditions studied the variation of the pH of tanning aqueous medium did not influence the absorption of tannins during tanning process.

The method using spectrophotometry UV/VIS can be considered a feasible method to perform quantitative analyzes of tannin in tanning aqueous medium, showing excellent results. The procedure is also easy to perform and does not require the use of reagents which ultimately increase the costs.

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6 – References

1. Adamczyk, B., Adamczyk, S., Smolander, A., Kitunen, V. Tannic acid and Norwayspruce condensed tannins can precipitate various organic nitrogen compounds.*Soil Biology and Biochemistry*, **43**, 628–637, 2011.
2. ASSOCIAÇÃO Brasileira de Normas Técnicas. NBR 11057: Couro - Determinação do pH e da cifra diferencial. Rio de Janeiro, 1999b, p.1-3.
3. ASSOCIAÇÃO Brasileira de Normas Técnicas.NBR 13572: Água residuária e banho residual resultantes de curtume — Determinação de sólidos totais e sólidos suspensos totais. Rio de Janeiro, 2012b, p.1-4.
4. Bhat, T.K., Singh, B., Sharma, O.P. Microbial degradation of tannins – A current perspective.*Biodegradation*, **9**, 343–357, 1998.
5. D'Aquino, A., Barbani, N., D'Elia, G., Lupinacci, D., Navigella, B., Seggiani, M., Tomaselli, M., Vitolo, S.Combined Organic Tanning Based on Mimosa and Oxazolidine:Development of Semi-Industrial Scale Process for High-Quality Bovine Upper Leather.*Journal Society Leather Technology Chemistry,* **88**, 47-55, 2004.
6. Falcão, L., Araújo, M.E.M.Tannins characterization in historic leathers by complementary analytical techniques ATR-FTIR. UV-Vis and chemical tests.*Journal of Cultural Heritage*, **14**, 499–508, 2013.
7. Faber, K. Bibliotek des Leders Band 3: Gerbmittel. Frankfurt: Gerbund und Nachgerbund. 2nd Ed. v.29, n.30, p.95, 1990.
8. Haslam, E. Plant polyphenols – vegetable tannins revisited. Cambridge University Press. Cambridge, 1989, 453p.
9. Heidemann, E.Comparison between chrome and vegetable tanning. inferred from collagen bond position.*Das Leder*, **28**, p.99-104, 1997.
10. Hoinacki, E., Gutheil, N.C. Peles e couros: origens. defeitos e industrialização.Porto Alegre: CIENTEC: Novo Hamburgo: CTCCA, 1978, 264p.
11. Kardel**, M.,** Taube**1**, F., Schulz**, H.,** Schütze**, W.,** Gierus**, M.**Different approaches to evaluate tannin content and structure of selected plant extracts – review and new aspects.***Journal of Applied Botany and Food Quality*, 86, 154 – 166, 2013.**
12. Mantilla, C., Pedraza, J., Laverd. D. Utilización de estudios de potencial zeta en elDesarrollo de un proceso alternativo de Flotación de mineral feldespático.*Dyna*, **154**, 65-71, 2008.
13. Marsal, A., Maldonado, F., Cuadros, S., Bautista, M.E., Manich, A.M. Adsorption isotherm. thermodynamic and kinetics studies of polyphenols onto tannery shavings.*Chemical Engineering Journal*, **183**, 21– 29, 2012.
14. Monteiro, J.M., Albuquerque, U.P., Araújo, E.L. Taninos: Uma abordagem da Química à Ecologia.*Quimica Nova*, **28**, 892-896, 2005.
15. Özacar, M., Sengil, I.A. Evaluation of tannin biopolymer as a coagulant aid for coagulation of colloidal particles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **229**,85–96, 2003.
16. Pizzi, A.Tannins: Major Sources. Properties and Applications.*In: Monomers. Polymers and Composites from Renewable Resources*, p.179- 199, 2008.
17. Spencer, C.M., Cai, Y., Martin, R.,Gaffney, S.H., Goulding, P.N., Magnolato. D., Lilley, T.H.,
18. Khanbabaee, K., Ree, T. Tannins: classification and definition.*Natural Product Report*,
19. **18**, 641–649, 2001.