ISOCYANATE-FUNCTIONALIZED COLLAGEN HYDROLYSATES AS PRETANNING AGENTS FOR ORGANIC WET-WHITE LEATHER

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The present paper aims to synthesize and test a pretanning agent based on isocyanate-functionalized collagen hydrolysates of low molecular mass (0.9 ÷ 3.6 kDa; polypeptides that include 8 to 32 amino acids), obtained starting from hides wet wastes, and used without further purification. Raw colloidal suspension resulted after hydrolysis was centrifuged to separate insoluble particles, and then was repeatedly filtered to retain coarse particulate and fatty maters. The clarified solution was concentrated by vaporization up to 35 % w/v dry matter, mixed with 10 % v/v dimethyl sulfoxide (DMSO), and maturated overnight under efficient mixing, in a hermetically closed reaction vessel, at ambient temperature. Using a solution of diisocyanate in DMSO, the molecular mass of the product was further increased by cross-linking, in parallel with the functionalization in the virtue of a small amount of free isocyanate groups that remain unreacted. The functionalized collagen hydrolysate was characterized by chemical (total nitrogen content, the amount of free carboxyl, amino, and isocyanate groups) and instrumental methods (infrared spectroscopy). The fraction of increased molecular mass after cross-linking was determined by comparative dialysis through membranes with 3.5 and 12 kDa. Pretanning ability of the functionalized hydrolysates was estimated by gelatin precipitation, and tested on sheep pelts. An increase of 6 ºC was measured for the shrinkage temperature. Pretanned pelts were gradually dehydrated (preventing the local drying) and drum-tumbled, and then was treated once again with the same functionalized product, in a short concentrated float. A supplemental increase of shrinkage temperature with 8 ºC was measured.

Key words: collagen hydrolysate, functionalization, diisocyanate, pretanning agent.

INTRODUCTION

Coordinative chromium salts still remain the preferred industrial tanning agents, and the classical vegetable tannins were only partially replaced in retanning recipes. The objective of replacing these "old fashioned" products currently faces to two challenges: (i) the fact that the definitions of the most important leather characteristics and properties were developed in relation with them, and were imposed by their use, and (ii) their technological efficiency and their tanning efficacy / price ratio are unrivalled in comparison with other tanning / retanning products. The race to develop replacing products is imposed nowadays by the ecological constraints, and by the increasing demands of the modern consumer, the "natural" or "organic" terms being enforced by marketers.

During the time, different products were tested as organic tanning / retanning agents. Due to their crosslinking potential, small-molecular, oligo- and poly-meric chemical compounds are sporadically used at industrial level, but, excepting some (di)aldehydes, none of them were largely accepted.

Functionalized compounds of natural origin represent a current investigated alternative. Protein hydrolysates could support a range of functionalizing reactions to

become the "core" of poly-functional crosslinking (and possibly tanning) capable products.

The present paper discuss the way to produce, and then tries to validate, a "reconstructed" artificial polypeptide bearing isocyan chemical groups, in small but necessary amount to endow the "core" with tanning / retanning ability.

The high reactivity of isocyanate group and the rapid kinetics of its reaction with dehydrated dermis gave tanners hope for finding a practical solution to the issue of producing organic tanned leather. Seventy years ago, the crosslinking ability of small-molecular diisocyanates was first exploited to replace the classical tanning agents (Putnam, 1947). The method remained inapplicable at industrial scale because it imposes the work in organic solvents.

The use of oligo- and/or poly-isocyanate blocked derivatives as (pre)tanning agents is also well known and was proposed long time ago (Milligan *et al*., 1983; Träubel *et al*., 1998; Ebbinghaus *et al*., 2006), but their practical application is severely limited by three drawback factors: (i) the costs of producing them, (ii) their limited diffusivity into the pelt, when working in usual tanning conditions, and (iii) the need to work at relatively high temperatures to complete the de-blocking and effective crosslinking.

Collagen hydrolysates were also tested, individually or in association with other small molecular compounds, as polyfunctional physical crosslinkers, which could assist tanning / retanning processes (Cantera *et al*., 2000; Cantera *et al*., 2002; Aslan *et al*., 2006). The main drawbacks in using protein hydrolysates consist in: (i) their intrinsic putrescibility, (ii) their small molecular volume, and (iii) their low reactive chemical groups. A combined chemical transformation which concomitantly increases the molecular mass and the overall reactivity of protein hydrolysates could be beneficial in producing tanning / retanning compounds. The controlled reaction between some diisocyanates and collagen hydrolysates was used in our work in order to diminish the above mentioned deficiencies.

EXPERIMENTAL DATA AND DISCUSSIONS

The collagen hydrolysate we used was obtained according a classical method, starting from pelt / limed split wastes (Constantinescu *et al*., 2015). The physical-chemical characteristics of the final liquid form are resumed in Table 1.

Table 1. The characteristics of the collagen hydrolysate, in liquid form

The determined characteristics	Measuring unit	Determined value
Dry substance	% w/v	9.64
Minerals content in dry substance	% w/w	0.39
Total fatty matters in dry substance	% w/w	8.74
Total nitrogen in dry substance, TN	%	14.47
"Leather substance" in dry matter (TN x 6.28)	% w/w	90.85
Amount of carboxyl groups in dialyzed product	mEq / g	2.92
Amount of α-amino groups in dialyzed product	mEq / g	0.71
Amount of ε-amino groups in dialyzed product	mEq / g	1.74
Mass fraction of the molecules below 3.5 kDa	% w/v	57.41
Mass fraction of the molecules between 3.5 and 12 kDa	% w/v	39.12
Mass fraction of the molecules above 12 kDa	% w/v	3.47
Hydrolysis yield (= total solubilized / initial mass)	% w/v	84.21

The raw collagen hydrolysate in liquid form was further chilled at 17 °C, filtered through a coarse mesh, centrifuged at 3200 g, and ultrafiltered through a membrane having a MWCO value of 3.5 kDa (in tangential flow regime, discarding the retentate). A final dry matter of 5.84 % and a "leather substance" (equivalent with the total polypeptide content) of 5.45 % resulted, representing a purifying yield of about 56.53 %. The fatty maters were completely removed by the purifying method. Figure 1 depicts the acid/base bounding capacity and the buffering capacity of the purified product, based on which were calculated the amounts of reactive groups of polypeptides.

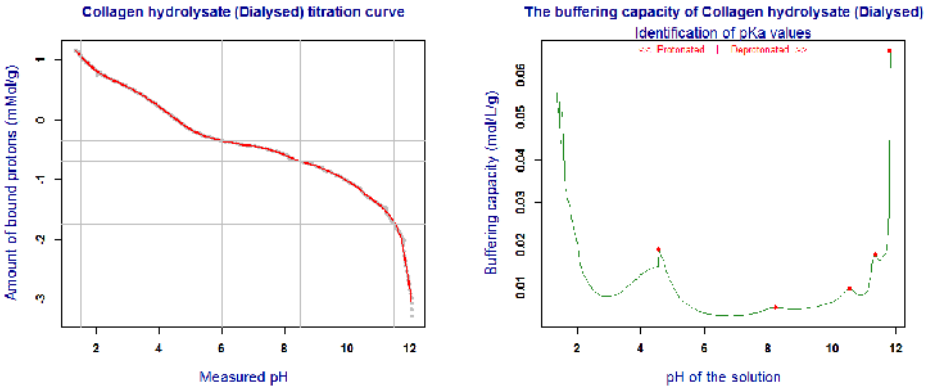

Figure 1. Proton bounding curve and buffering capacity of the purified collagen hydrolysate

The purified hydrolysate was first concentrated by evaporation up to a total polypeptide content of about 35 % w/v, and then 10 % v/v dimethyl-sulfoxide (DMSO) was slowly added, under efficient mixing. The resulted solution was maturated overnight under efficient mixing, in a hermetically closed reaction vessel, at ambient temperature (of about 18 °C). The traces of the resulted colloidal solid (about 0.16 % w/v) were removed by a second centrifugation.

In order to "reconstruct" an extended macromolecular edifice, the polypeptide mixture (initially having a molecular mass in the range of 0.9 ÷ 3.6 kDa, which represents the equivalent of 8 to 32 amino acids length polypeptides) was subjected to a controlled cross-linking with 0.3 % w/w hexamethylene diisocyanate (HMDI) dissolved in 5 % v/v anhydrous DMSO (relative to the volume of the hydrolysate solution). To prevent local precipitation, the HMDI solution was added dropwise, under efficient stirring. Concomitantly, the temperature of the reaction mixture was increased up to 60 °C. After a warm maturation of four hours (at 60 °C, under stirring), the resulted colloidal suspension was further maturated, overnight, at the ambient temperature. Second maturation concluded with the suspension coarsening and settling. The resulted flocks were then separated by centrifugation (3200 g, 30 min.). The slurry was re-suspended in water and centrifuged for three more times. The final sediment was suspended in 10 % v/w DMSO, and, after a warm maturation (4 hours, at 60 °C, under stirring in a hermetically closed autoclave; the dry matter content was finally determined), 3 % w/w isophorone diisocyanate (IPDI) (relative to the volume of the dry matter of the suspension), dissolved in 10 % v/v anhydrous DMSO, was dropwise added. At the end of four hours of efficient stirring, the reaction mixture was decanted

and the resulted flocks were separated by centrifugation. The sediment was further slowly dried, at an increasing temperature from 60 to 95 °C, under permanent hot air flow. After each hour of drying, the final product was crushed and the grinded, to obtain a powder. Finally, the obtained solid powder was ball-milled in the presence of solid starch, in a ratio of 1 : 3 w/w, and of $NaHCO_3$, in a ratio of 1 : 0.5 w/w.

Figure 2 presents the ATR FTIR spectra of the initial purified collagen hydrolysate, the HMDI crosslinked product, and the final IPDI functionalized product.

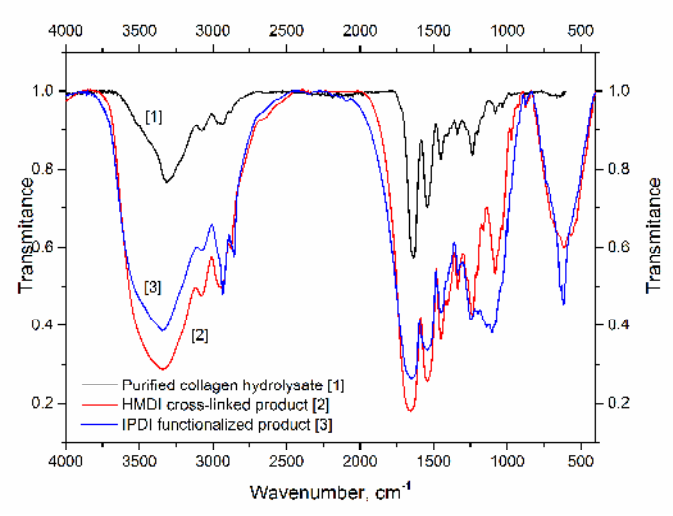

Figure 2. The FTIR spectra of the raw and functionalized products

The increase of molecular mass after the HMDI mediated crosslinking was determined by the amount of un-dialyzed soluble product, using membranes with 3.5 and 12 kDa. The obtained values were of 0.64 % w/v, and 81.30 % w/v, respectively.

The amount of free isocyanate groups was measured by gas chromatography, according the method of Xie and Chai (Xie and Chai, 2016). The values were 0.03 mEq/g in the case of the HMDI crosslinked product, and of 0.84 mEq/g in the case of the IPDI functionalized product.

The (pre)tanning ability of the final functionalized product was estimated by gelatin precipitation, and tested on sheep pelts. An increase of 6 ºC was measured for the shrinkage temperature after a treatment with 6 % w/w in a float of 150 % warm water (30 °C). Pretanned pelts were further gradually dehydrated (preventing the local drying) and drum-tumbled, and then was treated once again with the same amount of functionalized product, in a short float (80 % water, 45 °C). A supplemental increase of shrinkage temperature with 8 ºC was measured. The treated sheep leather were partially dried under vacuum, tumbled for 15 min., two time staken on a hydraulic machine, dried, re-humidified, staken again, wheeled in two steps, and finally tumbled. Soft, light brown sheep leather with a shrinkage temperature of 61 °C resulted.

CONCLUSIONS

We developed a procedure to „reconstruct" an extended polypeptide edifice starting from low molecular collagen hydrolysates, which, after the functionalization with a nonsymmetrical diisocyanate, gained (pre)tanning properties.

1. The molecular mass increasing was induced by the controlled crosslinking of collagen hydrolysates with hexamethylene diisocyanate.
2. The functionalization was performed using isophorone diisocyanate.
3. An increase of 14 °C was measured for the shrinkage temperature of the sheep leather in humid state; after drying and mechanical finishing the measured shrinkage temperature was of about 61 °C.

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