APPLICATION OF THIOL AMINO ACIDS IN A REDUCTIVE LIMING PROCESS

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Thiol containing amino acids cysteine and homocysteine were used as dehairing agents in an alkaline reductive liming process. It is shown that complete dehairing is achieved with amounts of 8 % w/w of the thiol amino acids, rendering them as effective as sodium sulfide on a molar basis. Cysteine and homocysteine can be used to establish a completely sulfide free liming process without fundamental changes of the remaining process parameters. Furthermore a method is presented for the preparation of homocysteine from lower-cost methionine. This method comprises a two step process including the demethylation of methionine in sulfuric acid, resulting in the formation of dimeric homocystine, and the subsequent reduction of homocystine to homocysteine with tin in acidic solution. Identity and purity of the reaction products were proved by HPLC analysis. The effectivity of freshly prepared homocysteine as a dehairing agent is comparable with commercial homocysteine thiolactone.

Keywords: Liming process, thiol, dehairing

INTRODUCTION

Traditional reductive liming processes employ sodium sulfide in alkaline medium. Sodium sulfide is an effective and low-cost dehairing agent. On the other hand, sodium sulfide is a highly toxic compound, requiring safety precautions and extensive waste water treatment. The formation of lethal hydrogen sulfide from sodium sulfide in tanneries is still not completely prevented. Thus the substitution of sodium sulfide by a non-hazardous alternative is a long held concern. Many variants have been investigated and reported in the literature in the last decades, including reductive liming with dimethylamine (Somerville *et al.*, 1963), oxidative liming with hydrogen peroxide or other peroxo-compounds (Bronco *et al.*, 2005; Gehring *et al.*, 2003; Marmer *et al.*, 2003; Marmer and Dudley, 2005), oxidative liming with sodium chlorite (Covington, 2009), and enzymatic liming (Dettmer *et al.*, 2013; Paul *et al.*, 2001; Kanagaraj, 2009), Sivasubramanian *et al.*, 2008). None of these technologies became established except for niche applications. This is due to a multitude of disadvantages, including toxicity, high costs, corrosive reaction conditions, incomplete dehairing or insufficient grain quality.

The approach of the present work is the use of thiol containing amino acids as dehairing agent in a reductive liming process. Cysteine and homocysteine, which are the most prominent representatives of this chemical family, show no toxic properties. As thiol compounds they are able to act as reducing agents, resulting in the formation of dimers (cystine, homocystine). The redox potential of the cysteine/cystine pair ($E^0 = 0.40 \text{ V}$) is in a similar range with sodium sulfide ($E^0 = 0.48 \text{ V}$) (Covington, 2009). Homocysteine with a side chain, extended by one methylene group in regard to cysteine, is stable only as the thiolactone form (see Figure 1). The commercial price of the thiolactone is approximately 25 times the price of sodium sulfide, making it an unpromising competitor. The amino acid methionine is the methylated form of homocysteine and costs only twice as much as sodium sulfide. Thus the second approach in this work is the development of a method for the transformation of

methionine into homocysteine. A variety of chemical conversion reactions is reported in the system methionine-homocysteine-homocystine (Butz and du Vigneaud, 1932; Lavine and Floyd, 1954; Baernstein, 1932; Wagner, 1957; Nekrassova *et al.*, 2003). Many of them require expensive reagents or hazardous reaction conditions or are developed for analytical purposes on a small scale. With regard to a possible application on industrial scale we focused on a two step process, in which methionine is transformed into homocystine, which subsequently is reduced to homocysteine thiolactone. The reaction principles were taken from the literature (Butz and du Vigneaud, 1932; Lavine and Floyd, 1954; Schöberl and Wagner, 1958; Skakun-Todorović and Albahari, 1979) and adapted with regard to a potential future upscaling.

The present work demonstrates the suitability of cysteine and homocysteine as reductive dehairing agents in a liming process and presents a laboratory method for the production of homocysteine thiolactone from methionine.

Figure 1. Structural formulae of (1) cysteine, (2) homocysteine, (3) homocysteine thiolactone, (4) methionine

MATERIALS AND METHODS

Materials

For liming experiments, L-cysteine (98 %) was purchased from abcr, DL-homocysteine thiolactone hydrochloride (99 %) was from Alfa Aesar. For the two step conversion process, DL-methionine (99 %) was purchased from abcr, tin powder (100 mesh, 99.5 %) was from Alfa Aesar. For analytical purposes in HPLC, DL-homocysteine (95 %, Sigma), DL-methionine (99 %, abcr), DL-homocystine (≥ 98 %, Sigma), DL-homocysteine thiolactone hydrochloride (99 %, Alfa Aesar) and DL-methionine methylsulfonium chloride (99 %, Sigma) were used as standards.

Liming and Tanning

Liming experiments with cysteine and homocysteine thiolactone were performed on a laboratory scale each with ca. 200 g bovine hide. Commercial cysteine and homocysteine thiolactone as well as homocysteine thiolactone solution, prepared from methionine, were used as reductive agents. The process steps were: washing (200 % water); liming (100 % water, cysteine or homocysteine, lime) at pH 12.5 over night with perpetual motion and occasional pH control; reliming (100 % water, 1.5 % lime) over night; washing (200 % water). Experiments were repeated with sodium hydroxide instead of lime at pH 13.5 under otherwise identical conditions. The amount of amino acid in the liming step varied and was 6, 8, and 10 %, regarding the hide weight.

The resulting pelts were delimed and pickled and subsequently tanned in a standard chrome tanning process using 8 % chromium sulfate at pH 2.7 for 16 h. The resulting wet blues were neutralized, washed and air dried.

Conversion of Methionine to Homocystine

Methionine (120 g) was boiled under reflux at 135 °C in 800 ml of sulfuric acid (64 %) for 3 h. After cooling down the solution was poured on 2400 g ice. Ammonia (13.5 %, ca. 2000 ml) was added until pH of 7.5 was reached. The precipitate was filtered, washed with water and dried. The reaction yielded 44 g homocystine.

Conversion of Homocystine to Homocysteine Thiolactone

Homocystine (44 g) was suspended in 100 ml water and dissolved by addition of 100 ml concentrated hydrochloric acid. 26 g tin powder were added, and the mixture was heated on a water quench (60 °C, 5 h). After cooling down the mixture was diluted with water and then ammonia was added until pH of 7.5 was reached. The precipitate (tin hydroxide) was filtered. 900 ml of the filtrate were concentrated in a rotary evaporator to 100 ml. This solution, containing 8 g homocysteine, was used in a liming experiment on 100 g bovine hide under conditions described above.

Identity and purity of homocystine and homocysteine thiolactone were verified by HPLC using an amino acid analyzer "Biochrom 30 plus" (Onken, Gründau, Germany), where the amino acids were separated chromatographically, derivatized with ninhydrin, and adsorption measured at wave length 570 nm. Homocysteine, methionine, homocystine, homocysteine thiolactone, and methyl methionine were used as standards.

RESULTS AND DISCUSSION

Liming with Cysteine and Homocysteine

Liming of bovine hides with cysteine or homocysteine under alkaline conditions yielded good results with all tested amino acid concentrations. While with 6 % amino acid some residual hair was visible in particular samples, dehairing was complete for the higher amounts (8 and 10 % amino acid). Figure 2 shows exemplarily pelts and wet blues, obtained by liming at pH 12.5 with 8 % cysteine and homocysteine, respectively (upper row). Pelts, which were treated in a sodium hydroxide liming process at pH 13.5, were likewise dehaired, but severely swollen (Figure 2, lower row).

The amount of amino acid needed for complete dehairing was higher than the usual amount of sodium sulfide, if calculated on a weight basis in regard to the hide weight (2.5 % sodium sulfate vs. 8 % amino acid). This was expected, since molar mass and reaction mechanism of these two different reagents differ. The dehairing effect is mainly based on the reductive opening of stabilizing disulfide bonds in the hair keratin. This is achieved by the action of one mole sulfide per mole disulfide bond, while in the case of amino acids two moles are needed. Taking into account the different molar masses of sodium sulfide (78.05 g/mol) and cysteine (121.16 g/mol) or homocysteine (135.18 g/mol), it can be deduced, that 8 % w/w of amino acid correspond to 2.3 - 2.6 % w/w of sodium sulfide on a molar basis. Thus the effectivity of cysteine and homocysteine as dehairing agents is considered comparable to that of sodium sulfide.

In contrast to sodium sulfide, liming with cysteine or homocysteine is a hair saving process.

Figure 2. Pelts and wet blues from bovine hides after liming at pH 12.5 (upper row) and pH 13.5 (lower row) with 8 % cysteine (left) or 8 % homocysteine thiolactone (right)

Preparation of Homocysteine from Methionine

Based on literature data a two step process was chosen to convert methionine into homocysteine. This method uses common chemicals, like sulfuric acid, ammonia, hydrochloric acid and tin, which will be advantageous for future upscaling in terms of costs and availability. Figure 3 shows the chemical reactions proceeding in the two process steps.

Figure 3. Reaction scheme for (A) the conversion of methionine (4) into homocysteine (5) with methyl methionine (6) and sulfur dioxide as byproducts, and (B) the reduction of homocysteine (5) to homocysteine thiolactone (3)

In the first step (A) methionine was demethylated in sulfuric acid, resulting in the formation of the dimer homocystine. Sulfur dioxide and the sulfonium salt of methyl methionine were detected as byproducts. After neutralization homocystine precipitated and was obtained in pure form by filtration and drying. In the second step (B) homocystine was reduced to homocysteine thiolactone by tin powder in acidic solution. In the original literature excess tin is precipitated with hydrogen sulfide (Schöberl and Wagner, 1958; Skakun-Todorović 1979). Since the aim in this study was the application of homocysteine in a sulfide free liming process, the use of hydrogen sulfide was

avoided. Ammonia was used instead in order to neutralize the solution and precipitate tin hydroxide, which was then filtrated. The remaining solution contained pure homocysteine. It was concentrated by means of a rotary evaporator until a volume was reached which complied with the conditions of the liming experiment. The neutralized and concentrated homocysteine solution was directly added in the liming step. The results were consistent with the results, presented above with commercial homocysteine thiolactone.

Purity and identity of the reaction products after the two process steps were confirmed by HPLC analysis, using commercial compounds as internal standards. Figure 4 shows the HPLC profiles of the precipitate after step (A), and the neutralized solution after step (B).

Figure 4. HPLC chromatograms of reaction products after step (A) and step (B); product after step (B) was measured freshly prepared (“fresh”) and after 3 months storage (“matured”); the standard contains homocysteine (2), homocysteine thiolactone (3), methionine (4), homocysteine (5), and methyl methionine (6)

The products of the two reaction steps proved to be pure homocysteine (product (A)) and homocysteine (product (B)), respectively. The freshly prepared homocysteine solution contained homocysteine besides a considerable amount of homocysteine thiolactone. When the HPLC analysis was repeated after 3 months of storage, the thiolactone nearly completely disappeared and converted into the open-chain form.

CONCLUSION

Cysteine and homocysteine were successfully used as dehairing agents in a reductive liming process. The process parameters are identical to the parameters of conventional sulfide based liming processes. Thus it is possible to establish a completely sulfide free liming technology by replacing sodium sulfide with cysteine or

homocysteine while retaining all remaining process parameters. This is an important step towards sustainable sulfide free liming and a promising alternative to the recent developments in enzymatic unhairing.

Since commercially available homocysteine is a relatively expensive reagent compared with cysteine, a method was established for the preparation of homocysteine from the lower-cost methionine. The proposed two step process uses common and nonhazardous chemicals, which favors future upscaling and implementation in established process chains. On a laboratory scale the method converted methionine into homocysteine with a 36 % yield.

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REFERENCES

- Baernstein, H.D. (1932), “The determination of methionine in proteins”, *J. Biol. Chem.*, 97, 663-668.
- Bronco, S. *et al.* (2005), “Oxidative unhairing with hydrogen peroxide: development of an industrial scale process for high-quality upper leather”, *J. Am. Leather Chem. Assoc.*, 100(2), 45-53.
- Butz, L.W. and du Vigneaud, V. (1932), “The formation of a homologue of cystine by the decomposition of methionine with sulfuric acid”, *J. Biol. Chem.*, 99, 135-142.
- Covington, A.D. (2009), *Tanning Chemistry: The Science of Leather*, RSC Publishing, Cambridge.
- Dettmer, A. *et al.* (2013), “Environmentally friendly hide unhairing: enzymatic hide processing for the replacement of sodium sulfide and deliming”, *J. Cleaner Prod.*, 47, 11-18, <https://doi.org/10.1016/j.jclepro.2012.04.024>.
- Gehring, A.G. *et al.* (2003), “Rapid oxidative unhairing with alkaline calcium peroxide”, *J. Am. Leather Chem. Assoc.*, 98(6), 216-223.
- Kanagaraj, J. (2009), “Cleaner leather processing by using enzymes: a review”, *Adv. Biotech.*, 4, 13-18.
- Lavine, T.F. and Floyd, N.F. (1954), “The decomposition of methionine in sulfuric acid”, *J. Biol. Chem.*, 207, 97-106.
- Marmer, W.N. *et al.* (2003), “Rapid oxidative unhairing with alkaline hydrogen peroxide”, *J. Am. Leather Chem. Assoc.*, 98(9), 351-358.
- Marmer, W.N. and Dudley, R.L. (2005), “Oxidative dehairing by sodium percarbonate”, *J. Am. Leather Chem. Assoc.*, 100(11), 427-431.
- Nekrassova, O. *et al.* (2003), “Analytical determination of homocysteine: a review”, *Talanta*, 60(6), 1085-1095, [https://doi.org/10.1016/S0039-9140\(03\)00173-5](https://doi.org/10.1016/S0039-9140(03)00173-5).
- Paul, R.G. *et al.* (2001), “The use of neutral protease in enzymatic unhairing”, *J. Am. Leather Chem. Assoc.*, 96, 180-185.
- Schöberl, A. and Wagner, A. (1958), “Schwefelhaltige Aminosäuren”, in Müller, E. *et al.* (ed.), “Houben-Weyl Methods of Organic Chemistry Vol. XI/2, 4th Edition: Amines II”, Georg Thieme Verlag, Stuttgart.
- Sivasubramanian, S. *et al.* (2008), “Ecofriendly lime and sulfide free enzymatic dehairing of skins and hides using a bacterial alkaline protease”, *Chemosphere*, 70, 1015-1024, <https://doi.org/10.1016/j.chemosphere.2007.09.036>.
- Skakun-Todorović, M.B. and Albahari, S.R. (1979), “Preparation of L-Cysteine-³⁵S hydrochloride by reduction of L-Cystine-³⁵S”, *J. Labelled Comp. Radiopharm.*, 16(6), 711-716, <https://doi.org/10.1002/jlcr.2580160508>.
- Somerville, I.C. *et al.* (1963), “Rapid unhairing with dimethylamine”, *J. Am. Leather Chem. Assoc.*, 58, 254-268.
- Wagner, H. (1957), “Process for the production of homocysteine compounds”, US Patent 2,935,529.