Recovery and Analyses of Hair Proteins from Tannery Unhairing Wastes

Stephen H. Feairheller, Maryann M. Taylor, Wallace Windus, Edward M. Filachione,* and Joseph Naghski

Protein has been recovered from leather tannery unhairing wastes by processes which may be adaptable to commercial use. These wastes are now disposed of in sewage systems and thus constitute a source of pollution that is difficult and expensive to treat. The techniques used in the recovery included filtration or centrifugation, dialysis or ultrafiltration, and precipitation or drying, but not necessarily in that order. Laboratory experiments indicate that about 68% of the protein can be obtained in the form of a slightly colored, odorless solid which is better than 95% protein. Some uses for this recovered protein are suggested by reports in the literature and additional uses are being investigated, including a potential use in human foods.

All of the "wet" operations in the manufacture of leather are important to the quality of the final product and, in addition, are interdependent. One of these operations is primarily concerned with the removal of the hair and epidermis from the hide; however, this treatment also results in a number of more subtle changes in the hide itself and all of these changes have an ultimate effect on the end product. This particular "wet" operation utilizes the effect of alkali and depilatory agents on the keratin of the hair and epidermis to achieve a partial or complete solubilization. Where partial solubilization takes place, the epidermis is destroyed and the hair is loosened so that it can be mechanically removed more or less intact. Where complete solubilization takes place, the fibrous nature of the hair is also destroyed and most of it dissolves. Which of these takes place is determined largely by the amounts of depilatory agents used; but to a certain extent it also depends on the length of time, temperature, and mechanical action, if any, employed. The source of alkali is almost always lime, of which a large excess is used. It is inexpensive and has the additional advantage that its limited solubility allows the presence of a constant supply of alkali without the risk of an excess in solution at any time. The most commonly used depilatory agent is sodium sulfide (or sulfhydrate), the amount of which determines whether the hair is destroyed or not. Others which have been used, either alone or in combination, include amines, cyanides, and mercaptans. The other changes caused by these reagents include, but are probably not limited to, the removal of other noncollagenous materials and the lowering of the isoelectric point of the collagen from near neutrality to about pH 5 by converting primary amide groups to carboxylic acid groups. While the hair may be removed from the hides by other means which do not require the use of these reagents and may be economically competitive, the other changes do not occur; and later processing steps, and thus the final product, are adversely affected. No one to date has developed a combination of "wet" operations using a different unhairing system which resulted in a final product of at least equivalent quality. Therefore, this is the process which the tanning industry will be using in the foreseeable future. An excellent review of this material can be found in three consecutive chapters of the American Chemical Society Monograph No. 134 (Morris, 1956; Merrill, 1956; Lollar, 1956).

Eastern Regional Research Laboratory, Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

About 60–75 million pounds of hair are removed from hides and skins in the U.S. each year by these two processes. At the present time about 50% of this is removed intact (the hair-saving process) and finds use in a variety of products; however, it is not very valuable. The remaining 50% is destroyed and, at present, is washed, along with the unused lime and sodium sulfide, into the sewage system. Thus, approximately 30–38 million pounds of good protein is not only wasted, but becomes a pollutant, the treatment of which may become prohibitively expensive. This total amount of hair processed will increase annually as the number of hides processed also increases; and, in addition, several changes which are presently taking place are going to increase the fraction which is destroyed. Therefore, there are two real incentives for finding a solution to the problem. The first is to eliminate a source of pollution and the second is to recover and utilize a good protein which is presently being wasted.

We have found, as a part of our continuing program on the utilization of animal hides and the elimination of pollution by the tanning industry, that a good yield of a protein can be obtained from these spent unhairing liquors and we have analyzed it chemically. The techniques involved and the results of the analyses are the subject of this report.

EXPERIMENTAL PROCEDURES

The treatments were designed to approximate conditions used in a tannery for dissolving hair as far as concentration of reagents, agitation, temperature, and duration were concerned. To simplify the study, the hair had been clipped from the hide before treatment. Five grams of the hair was placed in 600-ml of water, stirred, and allowed to sit for 20 min. Five grams of a solid material which was reported to be 60% sodium sulfide was added and stirred into solution. After 5 min, 16 g of lime was added and the resulting mixture was allowed to stand at room temperature for 72 hr with no agitation and exposed to the atmosphere. At the end of this time the suspended material was removed by filtration and/or centrifugation and the supernatant was treated in one of three different ways. Sample I was dialyzed exhaustively until the dialyzate had a pH near neutrality and gave a negative test for sulfide. By this time some precipitation had already occurred in the dialysis tube. The suspension was treated with glacial acetic acid to pH 4.8 and the precipitated material was isolated by centrifugation. Sample II was treated with glacial acetic acid directly to pH 4.8 which, of course, caused the evolution of considerable hydrogen sulfide. The resulting suspension was then dialyzed until free of sulfide and the precipitate was collected by centrifugation.

Sample III was also obtained by direct precipitation with glacial acetic acid but was not dialyzed. Instead, air was bubbled through the suspension for several days until the suspension was free of hydrogen sulfide. The product was then isolated by centrifugation. Sample IV was obtained from the residue of the initial filtration by washing with dilute hydrochloric acid solution until free of inorganic salts.

The amino acid analyses were determined on a single column system using a continuous gradient elution buffer and are the results of duplicate determinations. The tryptophan analyses were performed by the same chromatographic technique on alkaline hydrolysates of the proteins (Oelshlegel *et al.*, 1970). The data were calculated and tabulated on an IBM 1130 computer using programs developed at this Laboratory. The nitrogen determinations were Kjeldahl Nitrogens and the sulfur determinations were total organic and inorganic sulfur. The percent yield is on a weight basis and is based on the amount of hair used.

RESULTS AND DISCUSSION

Keratin, in its many forms, has been solubilized by a variety of reagents and has been recovered from these solutions. Probably the first report of this having been done by methods similar to tannery unhairing procedures appeared in 1934 (Goddard and Michaelis, 1934). In this report it was shown that wool could be dissolved by sodium sulfide solutions and then precipitated by acidification. The product was analyzed thoroughly and was shown to be digestible by trypsin and pepsin. In the intervening years many papers and patents have appeared concerning this property of keratins and many uses have been suggested for the product. These include use as a plasma expander (Ewald *et al.*, 1964), as an agent for making stable foams (Anker, 1969), and for spinning fibers (Hervey, 1957; Jones and Mecham, 1948; Happey and Wormell, 1949; Wormell, 1950; Jones and Mecham, 1950; Lundgren, 1946; Lundgren and O'Connell, 1944; Caldwell, 1953; Trotman and Trotman, 1929), as well as for other purposes. It is also reported to be edible and digestible (Koerner *et al.*, 1952).

The analytical data for the material we obtained are given in Table I. The control sample was native hair, which was used as is for treatment but which was washed with water and chloroform before analysis. Sample I was a light cream-colored, fluffy solid. By freeze-drying portions of the supernatant from which Sample I was isolated, there was obtained a very fluffy white solid, but this recovery was not carried to completion. Sample II was denser than Sample I and had a slightly darker color. Sample III resembled Sample II in physical appearance. The aeration process by which Sample III was freed of hydrogen sulfide caused some loss of solid product due to foaming and drying and this is responsible for the lower yield. Sample IV was a dark grey horny material.

While this has no direct bearing on the major proposition of this paper, there are some interesting differences in the relative amounts of the amino acids in the native hair, recovered protein (Samples I, II and III), and the residue (Sample IV). First, the cystine content is considerably reduced in the treated samples and even drastically so in the residue. This is in contradiction of some results published recently (Wronski and Goworek, 1965). The loss is partially accounted for by the formation of lanthionine. There is also a reduction in the amount of serine in all treated samples, which probably indicates that it is being destroyed. There is a reduction in the amount of glycine in the recovered material but an increase in the residue, which probably indicates that the process is separating fractions with different glycine contents. One other minor difference: the amount of the aromatic amino acids is low in the recovered protein and high in the residue.

Table I. Analyses of Recovered Protein Samples (Units are Percent by Weight of Pure Protein)

	Native hair	Sample I	Sample II	Sample III	Sample IV	Hen's egg protein
ASP	6.6	7.2	7.4	6.8	7.0	9.6
THR[a]	6.1	6.0	6.0	5.9	4.7	5.0
SER	8.2	6.6	6.8	6.9	7.2	7.6
GLU	15.1	15.5	15.9	15.4	17.7	12.7
PRO	7.1	7.0	6.1	7.2	6.4	4.2
GLY	4.3	2.6	2.6	2.6	4.9	3.3
ALA	3.6	3.7	3.8	3.7	3.8	5.9
VAL[a]	5.2	5.8	5.3	5.4	5.2	6.9
MET[a]	0.4	0.4	0.4	0.4	0.7	3.4
CYS	12.4	6.4	7.9	8.1	2.9	2.5
Total S	12.8	6.8	8.3	8.5	3.6	5.9
ILE[a]	3.4	3.6	3.8	3.8	3.6	6.3
LEU[a]	7.1	7.6	8.2	7.7	8.1	8.8
TYR	3.7	3.5	3.3	3.3	4.5	4.2
PHE[a]	2.6	2.0	2.4	2.4	3.7	5.7
Total Aromatic	6.3	5.5	5.7	5.7	8.2	9.9
HIS	1.0	0.8	1.0	0.9	1.5	2.4
LYS[a]	3.4	3.0	3.7	3.2	3.8	6.9
TRY[a]	0.5	0.4	—	—	—	1.5
ARG	9.7	10.7	10.5	10.6	9.4	6.1
LAN[c]	0	7.3	3.5	4.0	4.9	—
UNK[b]	0	0.1	1.3	1.6	0	—
N	16.3	16.5	16.8	16.4	11.6	16.0
S	3.7	3.6	5.1[d]	5.9[d]	2.8	—
Yield	—	18	68	50	24	—

[a] Essential amino acids. [b] Unknown, elutes between methionine and isoleucine. Calculated using the leucine color constant. [c] Lanthionine. [d] Based on the amino acid composition, the theoretical values are II, 2.7 and III, 2.9.

Regardless of these differences, the recovered protein has a reasonably good amino acid distribution with the exception of the amount of methionine and tryptophan. For comparison, the amino acid composition of hen's egg protein (converted to a percent by weight of pure protein) is included (FAO, 1970). If the sparing effect of cystine will make up for the deficiency of methionine, then tryptophan would be the limiting amino acid.

It has been reported in the literature (Moran and Summers, 1968) that autoclaved cattle hair alone was not suitable for chicken feed. It had to be supplemented with methionine, lysine, histidine, tryptophan, and glycine or used in combination with a corn–soybean meal as a partial replacement for the latter. The amino acid composition reported for this autoclaved cattle hair is similar to that reported here for the recovered protein and this latter could suffer from the same deficiencies. The autoclaved cattle hair, which was not as free of nonproteinaceous material as was our recovered proteins, was found to be nontoxic to the chickens.

The presence of the lanthionine in the recovered protein should cause no problems. It was almost certainly present in the aforementioned autoclaved cattle hair. The presence of one unknown amino acid (Unk) has been detected in the hydrolysates of these recovered proteins. It elutes between methionine and isoleucine on our system. As indicated in Table I, it accounts for better than 1% by weight of the total amino acids in some samples. Its identity may have to be established before the recovered proteins can be used for human consumption.

The nitrogen and sulfur analyses are interesting. The nitrogen values are high for the recovered protein and low

for the residue, another indication that fractionation may be taking place. The high sulfur contents of the materials which were precipitated from the unhairing liquors directly by the addition of acetic acid are due to the presence of free sulfur. Dialysis of the liquors to the complete removal of the sulfide and alkali before acidification also removed any free sulfur.

The highest yield of product and the best recovery were obtained in Sample II. Better than 90% of the hair was accounted for. The aeration technique, as previously mentioned, caused some mechanical losses and without these may have been as good as the technique used in preparing Sample II. Eventually, a compromise may have to be made between yield and purity. This may in part be determined by the use for which the material is intended.

Some preliminary work is now in progress on spent unhairing liquor obtained from a tannery and, although additional problems have arisen, the results are promising. A product of equal quality can be obtained from these liquors with a little additional effort. We are also in the process of making a large enough quantity of the recovered protein on which to obtain some information regarding its physical properties as well as its effectiveness in animal feeding studies.

There is considerable interest in research along these lines in the tanning industry because of the pressures on it to eliminate pollution. The research reported here and the proposals made may be a step in the right direction to help relieve some of this pressure.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Ruth Zabarsky for the computer calculations and tabulations of the amino acid analysis data.

LITERATURE CITED

Anker, C. A. (to General Mills, Inc.), U.S. Patent 3,464,825 (Sept 2, 1969).
Caldwell, J. R. (to Eastman Kodak Co.), U.S. Patent 2,625,490 (Jan 13, 1953).
Ewald, R. A., Anderson, P., Williams, H. L., Crosby, W. H., *Proc. Soc. Exptl. Biol. Med.* **115,** 130 (1964).
FAO, Amino Acid Content of Foods and Biological Data on Proteins, 122, Rome, 1970.
Goddard, D. R., Michaelis, L., *J. Biol. Chem.* **106,** 605 (1934).
Happey, F., Wormell, R. L., *J. Textile Inst.* **40,** T855 (1949).
Hervey, L. R. B. (to Rubberset Co.), U.S. Patent 2,814,851 (Dec 3, 1957).
Jones, C. B., Mecham, D. K. (to United States of America), U.S. Patent 2,447,860 (Aug 24, 1948).
Jones, C. B., Mecham, D. K. (to United States of America), U.S. Patent 2,517,572 (Aug 8, 1950).
Koerner, E. C., Ehrhardt, H., Haigh, F. R., Kirchoff, J. (to Botany Mills, Inc.), U.S. Patent 2,591,945 (April 8, 1952).
Lollar, R. M., in "The Chemistry and Technology of Leather, Vol. I," F. O'Flaherty, W. T. Roddy, R. M. Lollar, Eds., Reinhold, New York, N.Y., 1956, p 297.
Lundgren, H. P., *Silk J.* **23,** (no. 269), **48** (no. 270), 32 (1946).
Lundgren, H. P., O'Connell, R. A., *Ind. Eng. Chem.* **36,** 370 (1944).
Merrill, H. B., in "The Chemistry and Technology of Leather, Vol. I," F. O'Flaherty, W. T. Roddy, R. M. Lollar, Eds., Reinhold, New York, 1956, p 257.
Moran, E. T. Jr., Summers, J. D., *Poultry Sci.* **47,** 570 (1968).
Morris, W., in "The Chemistry and Technology of Leather, Vol. I," F. O'Flaherty, W. T. Roddy, R. M. Lollar, Eds., Reinhold, New York, 1956, p 229.
Oelshlegel, F. J., Jr., Schroeder, J. R., Stahmann, M. A., *Anal. Biochem.* **34,** 331 (1970).
Trotman, S. R., Trotman, C. R., Wolsey Ltd., Brit. Patent 329,766 (April 4, 1929).
Wormell, R. L., *Brit. Rayon Silk J.* **26** (no. 309), 55 (1950).
Wronski, M., Goworek, W., *Zesz. Nauk. Uniw. Lodz., Ser. 2* **19,** 79 (1965); *Chem. Abstr.* **64,** 14460e (1966).

Received for review September 20, 1971. Accepted November 12, 1971.

Preparation and Isolation of Acid-Catalyzed Hydrolysates from Wheat Gluten

Catherine Aranyi* and E. J. Hawrylewicz[1]

The rate of acid-catalyzed hydrolysis of gluten to polypeptides was investigated using hydrolytic agents that form single-phase systems with gluten. Controlled digestion on commercial gluten preparations and on laboratory-extracted samples could be achieved with similar end effects. The rate of partial hydrolysis of gluten was considerably improved by disrupting its disulfide linkages before digestion. Hydrolysis was followed by measuring the number of cleaved peptide bonds, the number of degraded amide groups, and the changes in relative viscosity. The hydrolysates were purified by fractionation on Sephadex gel. Preparations from unoxidized gluten could be obtained in acetic acid-soluble or water-soluble forms and showed similar characteristics when examined by analytical test, viscosity and sedimentation measurements, and by gel filtration. Hydrolysates originating from the oxidatively cleaved gluten samples were soluble in water or sodium hydroxide and showed increased fragmentation of the protein by all experimental methods applied.

Since wheat gluten protein can be readily produced commercially and has unique amino acid distribution, efforts were directed to prepare from it novel products with potential for industrial application. Peptides from gluten with appropriate chemical modification could be suitable for uses such as films, adhesives, coatings, and surfactants. The specific objective of the work reported here was to develop a controlled acid-catalyzed hydrolysis method and to isolate and purify the polypeptide mixture obtained.

Life Sciences Research Division, IIT Research Institute, Chicago, Illinois 60616.
[1] Present address: Mercy Hospital Medical Center, Chicago, Illinois 60616.

MATERIALS AND METHODS

Hydrolysis experiments were conducted on gluten extracted from wheat flour in our laboratories, on Vital Gluten, a product of the Hercules Powder Company, and on a laboratory-extracted gluten sample that had been exposed to performic oxidation to cleave its disulfide bonds.