Coprecipitation of Acid Cheese Whey and Tannery Waste and Recovery of Protein

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Summary

Combining acid cottage cheese whey and the lime–sulfide effluent from tannery unhairing processes spontaneously coprecipitates the whey proteins with the large peptides and proteins of the tannery waste. The flocculation of the denatured protein material also carries down the hide pigments, excess lime, and the casein fines from the whey. The clear supernatant contains lactose, sulfur in various states of oxidation, free amino acids, peptides, and ammonium salts, but no detectable macromolecular proteins. The recovered solid products, which contain more than 20% of the original nitrogen, appear to have a good balance of essential amino acids although actual composition varies with the composition of the raw wastes. Feed supplements may possibly be obtained by this method from two presently wasted industrial effluents.

INTRODUCTION

One of the major technical problems which has received wide attention in industrialized nations in the last decade is pollution of available water sources by industrial wastes. Recovery of feed or food value from certain of these wastes may defray at least part of the costs associated with disposal.

Whey from cheese manufacture contains proteins of excellent quality at a level of ½ to 1%. Sweet whey may be directly dried and sold (although the cost of the fuel to evaporate the water may soon make the process uneconomical). Acid whey is less amenable to drying because of its low pH, which causes some of the lactose to dry in the hygroscopic amorphous form,[1] and most acid whey is currently wasted.

Precipitation of the proteins from whey by heat denaturation is a classic and relatively straightforward way to recover most of them.

The whey may be treated by itself[2] or casein or other materials may be present, in which case the term coprecipitation is used.[3]

Coprecipitation of proteins can be expected to occur maximally when each protein forms a complex with the other of a kind which covers up water-seeking hydrophilic groups. To irreversibly coprecipitate soluble globular proteins, one must destroy their native structure which, in general, consists of hydrophilic residues surrounding a central hydrophobic core.[4] When these oil-like interior residues are exposed to water, they preferentially form intermolecular bonds of hydrophobic or other types and precipitate from solution. Such "irreversible denaturation" is the usual mechanism by which heating renders proteins insoluble.

The major whey proteins, α-lactalbumin and β-lactoglobulin, are, however, rather resistant to denaturation by heat especially at the pH and concentration at which they occur in acid whey.[5,6] Both of these proteins are stabilized in their native conformations by internal disulfide bonds,[7,8] and, at least in the case of β-lactoglobulin, irreversible denaturation with the simultaneous appearance of new sulfhydryl groups has been thoroughly established.[9–12] As a corollary, it has also been shown that addition of reagents capable of reductively breaking disulfide bonds increases the rate and extent of thermal denaturation under certain conditions.[12,13]

Another industrial waste that has been of concern for many years is the lime–sulfide effluent from the unhairing or hair-pulping of hides, a necessary step in leather manufacture. This tannery unhairing effluent (TUF) is an opaque, noxious liquid of very high pH (12–13) which contains up to 400 ml/liter insoluble solids[14] (such as fat, lime, semidissolved hair, and pigments), 5–6 g/liter sulfide as Na_2S, and up to 50 g/liter dissolved solids. TUF has a five-day biological oxygen demand (BOD) of over 19,000 ppm,[14] and contributes over 50% of the total effluent BOD of the tannery.[15] Many studies are being carried out worldwide on methods of disposing of this material,[16,17] which also contains quantities of proteinaceous substances.[14] This "protein" consists of dissolved hair and its breakdown products[18] removed from the hide during the unhairing process. Chemically, it is mostly keratin with its numerous disulfide crosslinks reduced[19] or broken by other mechanisms.[20] These soluble peptide chains and smaller oligopeptides contain —SH groups from the original disulfide crosslinks of the keratin, kept in reduced form by the excess $S^=$ and SH^- ions in the TUF. Along with these materials, there are other sulfur-containing substances such as cysteine, sulfides, polysulfides, and thiosulfate

and sulfite ions,[21] all originating from the same sources. Such materials should comprise an ideal reagent for starting and maintaining a chain reaction of disulfide reduction, unfolding, and intermolecular sulfhydryl interchange,[22] which can lead to aggregation and precipitation of other proteins.[13]

The author therefore decided to investigate the possibility that these two waste liquids, acid whey and spent unhairing liquor, might act as coprecipitants for each other, as well as partly meet each other's requirements for neutralization.

MATERIALS AND METHODS

TUF samples, obtained from Ocean Leather Co.,* Newark, N.J., were the exhausted unhairing liquors not diluted with rinse water. Batches were stored immediately in filled stainless steel drums and refrigerated upon arrival at our laboratory, approximately 40 hr after filling. Experiments reported here were carried out within six weeks after receipt of the samples. The pH of one batch of the material dropped from 12.8 when received, to 12.3 after one month; nitrogen analysis remained constant at 4.11 mg N/ml on material skimmed free of floating solids.

Acid whey was obtained refrigerated from LeHi Dairies, Allentown, Pa., and was used within 48 hr of manufacture. The pH of batches as-received varied between 4.39 and 4.61, and nitrogen analyses varied between 1.0 and 1.3 mg N/ml on samples centrifuged to remove casein fines, and between 1.2 and 1.4 on representative uncentrifuged samples.

Kjeldahl digestions were done with a Hg-free K_2SO_4 catalyst. pH values were measured directly with a standard glass electrode; no corrections were made for high alkalinity or sodium content. Centrifugation was done in 200 or 500 ml bottles at 2000 rpm in a swinging bucket centrifuge. Ashing was done to constant weight at 600°C. Amino acid analysis was carried out, following a 24 hr hydrolysis at 110°C, in evacuated tubes with constant boiling (5.8*M*) HCl. Performic acid oxidation to determine cysteine as cysteic acid was carried out by the procedure of Moore,[23] and the evaporated oxidized product was hydrolyzed as above.

* Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Spontaneous Precipitation

Various proportions of TUF and acid whey were mixed at room temperature to a total of 100 ml. It was found that within specific ratios, black, heavy precipitates were formed within a few minutes. These settled readily and tended to clear the liquid of solid as well as some semisolid floating material. In the range from 20 to 40% TUF the precipitates were easily centrifuged to a semisolid self-adhering pellet which was separated easily from the supernatant by screening because the partially dissolved hair was coagulated and trapped in the pellet. Calculations based on nitrogen analyses of the supernatants (Fig. 1), show that a maximum of 17% of the total nitrogen can be spontaneously precipitated.

Repeatability

The experiment was repeated a week later with fresh whey but the same batch of TUF (Fig. 1, lower solid line). A slightly different degree of precipitation is not surprising, since the TUF, with a pH above 12, is known to contain slightly less recoverable protein with increased storage time,[14] and different batches of whey have varying protein and nonprotein nitrogen contents. The

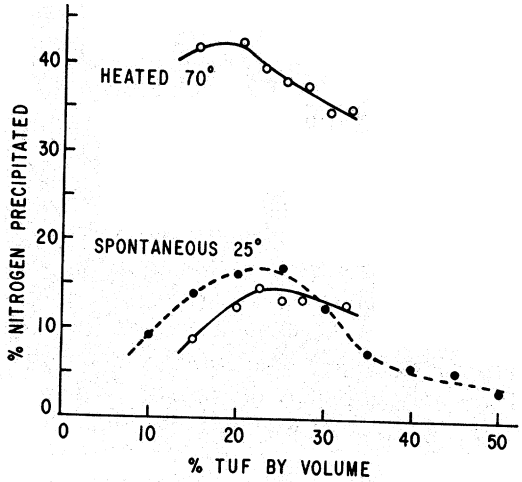

Fig. 1. Percent of soluble nitrogen precipitated from acid whey–TUF mixtures. Dashed line, experiment 1; solid curves, experiment 2.

experiments illustrated were carried out with TUF stored at 5°C for one month; better precipitation could be expected with fresh material.

Further Precipitation by Heat

Supernatants from the second experiment were placed in a water bath and heated slowly to 70°C (over a 15 min period). Heavy white proteinaceous precipitates were visible after 5 min and were separated by centrifugation after the samples cooled. An additional 20–25% of the original nitrogen was precipitated by this heat treatment giving a total of close to 40% (Fig. 1, upper solid line).

In contrast, untreated TUF, subjected to the same temperature–time cycle, lost between 1 and 2% of its nitrogen on centrifugation. When TUF was adjusted to pH 9.55 (which in two experiments required between 8 and 10 g H_2SO_4/liter TUF) and treated identically, about 3% of the nitrogen was removed in the precipitate.

Acid whey (pH $\approx$ 4.6) treated the same way became turbid, but the turbidity would not centrifuge out. When the whey was first adjusted to pH 9.55 (1.05 g NaOH/liter whey required), a visible flocculent precipitate formed, but 98% of the nitrogen remained in the cloudy supernatant. The whey proteins are known to irreversibly denature at this high pH,[5,7,9] but they remain essentially in solution, and only reacidification can precipitate them quantitatively.

Completeness of Precipitation

In the range between 15 and 30% TUF, all soluble "proteins" were precipitated by heating the mixture. This was verified by examining tenfold concentrations of the 15, 25, and 30% supernatants by ultracentrifugation and acrylamide gel electrophoresis in pH 8.6 tris-borate buffers. No sedimenting protein peaks could be seen and no bands could be visualized by amido black stain on the gel. The supernatant still contained essentially all the lactose of the whey, the nonprotein nitrogen, and the excess sulfide.

In the mixture range between 20 and 40% TUF, the spontaneous precipitate was recoverable in a physically tough pellet; above 40% TUF the pH of the mixture remained too high to coagulate the semidissolved hair; below 20% the mixing produced a fluffy precipitate which did not pack well when centrifuged. Nevertheless, a certain amount of leeway is built into the proportions; this

may be valuable with wastes such as these, which come from batch processes.

Since the supernatant was free of macromolecular proteins, the precipitate must include all of the protein of the whey (60–65% of the nitrogen[2]) plus all the material precipitable from the TUF by lowering the pH to 7–9.5 and by reaction with the whey proteins. In a mixture with a 3:1 whey-to-TUF ratio, this would indicate that roughly 90% of the nitrogen of the recoverable material had its origin in the whey, if the assumption is made that the same amount of TUF nitrogen reacts at this pH as would be precipitated by acid.[14] Again, this value would be dependent on the composition and history of the liquids used.

Spontaneous and Heated Precipitates

Sixteen hundred ml acid whey were mixed at 25°C with 400 ml TUF; pH 9.5. The material spontaneously precipitating was centrifugally separated first, and the clear supernatant was then heated

TABLE I
Preparation and Analysis of Coprecipitates

Experiment	2	3	4
TUF[a] (mg N/ml)	6.07	4.56	9.54
Whey (mg N/ml)	1.00	1.19	1.23
% TUF by volume	20.00	25.00	25.00
pH mixture	9.50	7.00	10.8[b]
	Spontaneous precipitate	Mixed precipitate	
% N	7.24	5.80	4.70
% Ash	32.60	34.20	27.60
% P	2.82	5.70	3.98
% Fat	ND[c]	2.20	1.40
	Heated precipitate		
% N	11.32		
% Ash	9.05		
% Recovery of N in solid product	ND	20.8	23.2

[a] TUF is tannery unhairing effluent.
[b] pH too high; reduced to 9.55 with acid. See text.
[c] ND means not determined.

to 80°C by injected steam to precipitate more protein. The products, "spontaneous" and "heated precipitates," were dried and analyzed (Table I, experiment 2). Amino acid analyses of these separated products are presented in Table II, columns 2 and 3. No analysis for tryptophan was attempted.

Preparation and Analysis of Mixed Precipitate

Four and 12 liter experiments were carried out at a ratio of 25% TUF (Table I, experiments 3 and 4). The two experiments used different batches of TUF from the same tannery; however, batch 4 was more highly concentrated and alkaline. In addition, the pig-

TABLE II
Amino Acid Analyses of Coprecipitates

	g/100 g product			
Residue	spontaneous ppt.[a]	heat ppt.[a]	mixed product[b]	whole lactalbumin[c]
Lys	3.04	5.20	1.34	9.7
His	0.81	1.35	0.35	1.8
Arg	2.40	3.73	1.41	3.1
Asp	3.73	5.90	1.77	11.1
Thr	2.35	3.87	1.32	5.2
Ser	2.18	3.98	1.43	4.8
Glu	6.90	11.11	3.78	17.7
Pro	3.05	4.98	1.70	4.6
Gly	1.14	1.84	0.80	2.5
Ala	1.84	2.96	0.97	7.0
Cys	3.50	5.54	1.72	2.7
Val	2.73	3.65	1.12	5.3
Met	0.83	1.34	0.85	1.8
Ileu	2.04	3.26	1.12	6.7
Leu	4.38	6.70	2.05	12.0
Tyr	1.66	1.22	0.92	3.2
Phe	1.53	2.40	1.45	4.0
Trp	ND[e]	ND	ND	1.7
Sum of amino acids	44.1	69.0	24.1	103.4
Crude protein[d]	45.2	70.8	29.4	

[a] 20% TUF, expt. 2.
[b] 25% TUF, expt. 4.
[c] Mean of data of Bloch and Weiss.[27]
[d] 6.25 × % nitrogen.
[e] ND means not determined.

ment load was exceptionally high. When mixed with three times its volume of acid whey, the resultant pH of 10.8 was too high to allow coagulation. Instead of using additional acid whey, the pH was lowered to 9.55 with concentrated H_2SO_4 (1.98 g acid/liter) when coagulation began; this demonstrates that the process may be adapted to various volumes and concentrations of TUF and whey. Steam was injected into the mixture until 70°C was reached, and the settled precipitate was centrifuged after cooling. The slurry (experiment 4, approximately 15% solids) was lyophilized and analyzed for amino acids. A flow sheet for this experiment (4) is presented as Figure 2; 97.5% of the Kjeldahl nitrogen is accounted for. However, in this product, a larger percentage of nonprotein nitrogen was found. This may be due to the heavy pigment load (especially large amounts of humin were seen on acid hydrolysis). Tryptophan was not determined. Differences in final composition which may be expected can be seen by comparison of data in Table II. The last column gives an analysis from the literature for "lactalbumin," i.e., the mixture of all whey proteins in their

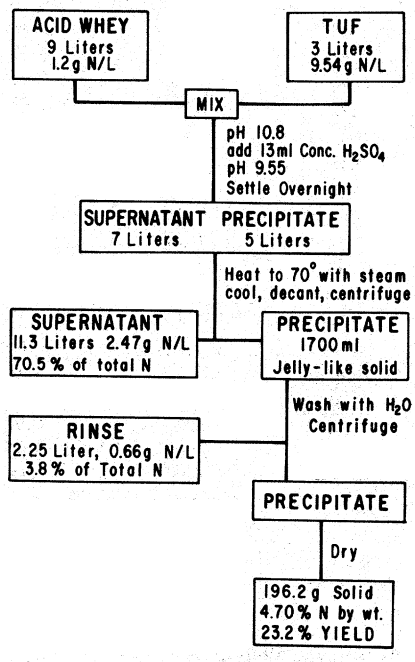

Fig. 2. Flow sheet for preparation of mixed product from experiment 4. Spontaneous precipitate allowed to settle overnight, then heated to 70°C by injected steam.

natural ratios. This column is on a 16% nitrogen basis. Hence, the increase in proportion of cysteine found in the coprecipitates provides evidence of disulfide interchange reaction between the two components.

Possible Utility of Coprecipitates

Examination of the amino acid data and comparison with similar data for whole egg[24] shows that the precipitated products have high contents of essential amino acids, running from 200–300% of whole egg in the case of cysteine, 100–140% for lysine, threonine, and leucine, and from 55–90% of the whole egg values for valine, methionine, leucine, isoleucine, and the combined aromatic amino acids phenylalanine and tyrosine. Tryptophan may be assumed to be present since the whey proteins are reasonably good sources of this amino acid.

If the material were to be considered as a chicken feed supplement, the high ash contents might be an added nutritional factor, as the need for lime in layers' diets is well known.[25]

Nature of the Supernatant

The problem of disposing of the liquid portion still remains. However, an experiment was conducted to show that the oxygen demand of the combined effluent is reduced from the total oxygen demand of the uncombined whey and TUF. In this experiment (not tabulated) chemical oxygen demand (COD) was measured. The acid whey used contributed 480 g COD (6.8 liter $\times$ 70.6 $\times$ 10^3 mg/liter). The TUF contained 153 g COD (3.7 liter $\times$ 41.33 $\times$ 10^3 mg/liter) and the clear supernatant carried 530.2 g (11.1 liter $\times$ 47.8 $\times$ 10^3 mg/liter), which shows a 16% reduction in COD. This protein-free supernatant contained, in addition to the lactose of the whey, quantities of free lysine and arginine (identifiable on high voltage paper ionograms, run in pH pyridine–acetic acid buffers), other free neutral amino acids, and some di-, tri-, and possibly higher peptides. Eight to 10% of the nitrogen present was in the form of ammonium ions, as judged by a 5 min steam distillation from 5% NaOH. The total sulfur content was 26 mg/ml and total solids were 8.5% by weight. Such a material may be an excellent source of nutrients for growing single cell organisms, whether it be for the production of nutritional materials or for rapid disposal in an activated sludge-type sewage treatment.[26] Its clarity is also an advantage.

CONCLUSION

The possibility of combining acid whey and tannery unhairing effluent, two waste liquids individually difficult to dispose or utilize, and recovering from them a product which appears to be rather well balanced nutritionally, and leaving a clear, protein-free liquid waste seems to be a process worthy of evaluation. Its economic practicality depends, of course, partially on the distance between sources of supply of the two waste liquids, which will vary greatly in different localities, and on the monetary value that can be assigned to the solid product. In addition, this point should be made: when a mixture of tannery unhairing effluent and acid whey is made in these general proportions, the pH may fall as low as 7. At pH below 9, H_2S is evolved rapidly when the mixture is heated. On a large scale, it would be necessary to absorb this toxic gas in alkali, in which case calcium or sodium sulfide could be at least partially regenerated and used again in the unhairing process. Capital expenditure must be considered here *vìs a vìs* costs of other disposal methods, environmental as well as fiscal.

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