PROTEIN REMOVAL FROM GLUTEN-STARCH WASH WATER[1]

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ABSTRACT

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Wheat gluten-starch washing produces large volumes of high Biological Oxygen Demand gluten waste water (GWW). This GWW represents a serious disposal problem as well as a loss of potentially valuable nutrients. By concentrating the GWW in a multiple effect evaporator, a molasses containing appreciable protein can be obtained. However, the protein and suspended starch in the effluent tend to coat the surfaces of the evaporator, causing reduced efficiency and, eventually, clogging of the system. The fouling problem can be eliminated by adding calcium or ferric ions (50 ppm) to the GWW, heating to 85° C by direct steam injection, and removing coagulated protein and suspended solids with a desludging centrifuge prior to evaporation. The clarified supernatant can be readily concentrated to afford a molasses which has potential as an animal feed. The dried precipitate from the centrifuge contained 70 to 73% protein, and had an amino acid profile typical of the soluble proteins of wheat, indicating potential as a food additive.

About 320 million lb of second clears flour are processed each year in the U.S., yielding approximately 40 million lb of gluten, 187 million lb of prime starch, and 25 million lb of low-grade starch. These processes also produce up to 60 million lb of flour solids, which are normally lost as plant effluents. Depending on the degree of water recycling, the effluents contain from 2 to 6% solids. These solids contain approximately 30% protein, corresponding to an annual loss of 18 million lb of protein. In most plants, disposal of all or part of the gluten wash water (GWW) represents a substantial problem. One attempt to alleviate this situation has been an effort to convert such waste to a high-protein, high-starch molasses by removing water from the effluent by evaporation. However, the protein and suspended starch in the effluent generally cause a serious fouling problem in the evaporators due to coating of vessel surfaces. Since the gluten starch wash water consists mainly of heat-labile soluble proteins from wheat, the fouling problem will exist whenever the material is heated over 70° C. One alternative would be an agitated film or wiped surface evaporator. Such equipment, however, is generally designed for concentrating high solids materials, not the dilute materials found in waste streams. Concentration or evaporation of dilute waste streams in most cases would only be a temporary measure until processes are modified to eliminate such waste streams. The purpose of this work was to develop a method to remove the starch and heat-labile proteins from the effluent, thus reducing evaporator fouling, improving the overall efficiency of the process, and affording recovery of a valuable protein. This procedure would allow the alternative use of lower cost evaporators which may be more applicable to the low solids material used in this work. Such a pretreatment could also be used prior to reverse osmosis.

[1]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

It has been previously reported (1,2) that condensed phosphates can be used to remove soluble protein from waste streams without impairing the functional quality of the proteins. The cost and limited supply of phosphate, however, restrict the application of this method to very special cases. The current work reports use of added ferric or calcium ion to increase the heat-sensitivity of proteins, allowing them to be removed from typical GWW by coagulation prior to concentration of the process effluent.

MATERIALS AND METHODS

Preparation of GWW

GWW was prepared by a modification of Knight's method (3) for testing flour for performance in gluten and starch production. Water (500 ml) was slowly added to 1000 g of second clears flour (Centenial Mills, Portland, Oreg.) and blended at low speed with a Hobart A-200 mixer equipped with a dough hook. The dough was worked an additional 5 min after the water addition was completed. The dough ball was then covered with 5000 ml of water and allowed to stand for 1 hr at 21° C. Mixing was then resumed for 7 min and the starch-milk was carefully poured off. The washing-working cycle of the dough was repeated three times and the combined starch-milk extracts were centrifuged in a Westphalia separator (Model LWA-205) equipped with a nozzle head to remove the starch. The supernatant GWW was either frozen, used directly, or freeze-dried.

Analytical Methods

Trichloroacetic acid (TCA) soluble nitrogen, nitrogen, ash, and total solids were determined by AOAC (4) methods. Amino acid analysis was carried out according to Spackman (5) and after 21 hr acid hydrolysis, except tryptophan, which was determined according to Finley *et al.* (6). Cystine and methionine were determined according to Moore (7). Amino acid values requiring correction were corrected according to Kohler and Palter (8). Turbidity was estimated by absorbance at 500 nm.

TABLE I
Effect of pH on Protein Removal from GWW with Various Cations[a]

pH	% Total Nitrogen Removed			
	Ca	Fe	Mg	No Metal Ion
3	39	39	40	23
4	41	57	41	20
5	41	54	41	20
6	40	55	47	21
7	45	40	37	19
8	57	41	34	21
9	53	41	34	15

[a]All metals added at 100 ppm.

Laboratory Scale Treatments

Studies on the effect of pH were conducted by adding 0.1 ml of a 10% solution (100 ppm) of metallic cation (chloride salt) to 100 ml of GWW and adjusting the pH of the solution to the appropriate level with either 1 *N* HCl or 1 *N* NaOH. The solution was weighed, heated to 90° C by injection of atmospheric steam, reweighed, and filtered through S & S 602 filter paper. Samples of the freeze-dried precipitate and filtrate were analyzed and final protein concentrations corrected for dilution due to steam.

Temperature studies were conducted similarly by adding 100 ppm of the metal ions to three 5000-ml aliquots of GWW, adjusting the pH to the appropriate optimum, and slowly heating the samples to 100° C. Aliquots (100 ml) were removed at 5° intervals from 50° to 100° C, filtered, and analyzed as above.

The effective concentration of metal ion was established using an identical procedure; however, the concentrations of added metal ions varied from 0 to 1000 ppm. The pH value used for this study was: iron 5.0, magnesium 6.0, and calcium 8.0. Solutions were heated to 85° C prior to filtration and analysis.

Pilot Scale Test

GWW (80 l.) was prepared as described above and split into two equal aliquots. Ferric chloride (50 ppm) was added to one aliquot and the pH adjusted to 4.0; the other aliquot was not treated and served as the control. The sample containing ferric chloride was heated by steam injection to 85° C, and then centrifuged in a Westphalia (Model C) centrifuge equipped with chamber head. The clarified effluent and the untreated effluent were concentrated in a vacuum pan evaporator at 74° C (liquid temperature) and 29 in. of vacuum to afford molasses samples; the centrifuged sludge was freeze-dried and ground to yield a tan powder.

RESULTS AND DISCUSSION

The GWW used for these studies contained 0.22% protein (N × 5.7) and 0.85% total solids. Precipitation of the protein with TCA removed 84.8% of the soluble

TABLE II
Effect of Metal Ion Concentration on Protein Removal from GWW

Metal Ion Concentration ppm	% Total Nitrogen Removed Metal Ion		
	Ca[a]	Fe[b]	Mg[c]
1000	55	58	51
500	55	59	50
250	56	58	51
100	57	58	51
50	57	59	48
25	40	42	37
0	21	20	21

[a]pH 8.0.
[b]pH 5.0.
[c]pH 6.0.

nitrogen from the freshly prepared GWW. Initially, storage of the GWW was a problem because the TCA soluble nitrogen content of the GWW increased from 15.2 to 33.2% after storage at 4°C for 18 hr. Presumably, this was due to proteolytic enzyme activity which could be eliminated by freezing samples in small plastic containers.

The effects of pH, metal ion concentration, and treatment temperature on the removal of protein from GWW were investigated in an attempt to find optimum conditions. In the presence of 100 ppm of three different metal ions, the effect of pH on the process was established by heating the samples to 90° C. As shown in Table I, the optimum pH for nitrogen removal from GWW varied considerably, from pH 8.0 for calcium ion to between pH 4.0 and 5.0 for ferric ion, and pH 6.0 for magnesium ion. The control with no metal ion added removed a small amount of protein, but the samples remained turbid, showing that both pH and the metal ion are critical to the protein removal. These results suggest that a processor could choose the metal ion for precipitation of protein waste on the basis of the pH of the particular effluent stream. Of the three metal ions studied, it is apparent that iron and calcium were more effective than magnesium for removal of protein from GWW.

The second parameter investigated was the effect of metal ion concentration on the removal of protein from GWW. Solution pH's were adjusted to previously determined optimum levels (pH 8.0 for calcium, 5.0 for iron, and 6.0 for magnesium) and metal ion concentrations were varied from 0 to 1000 ppm. Results of this study are shown in Table II. It is evident that 50 ppm of either ferric ion or calcium ion is sufficient to remove most of the heat-sensitive protein from the solution. Magnesium ion was considerably less effective than either calcium or ferric ion at equal concentrations, and was also less effective below 50

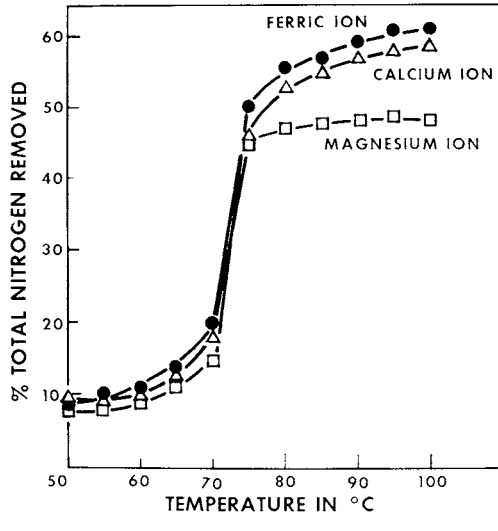

Fig. 1. The effect of treatment temperature on the total nitrogen removed from GWW. All metal ion concentrations were 100 ppm.

TABLE III
Effect of Various Levels of Ferric Ion on Protein Removal from GWW Reconstituted at Various Levels

% Solids in GWW	% N in GWW	Fe Ion Added in ppm	% N Removed
2	0.109	25	27
		50	40
		100	58
4	0.216	50	25
		100	41
		200	58
6	0.327	100	40
		200	47
		300	61
8	0.431	200	38
		300	50
		400	61
		800	62
		1000	61
8	0.431	300 plus 300 ppm Ca	72

TABLE IV
Amino Acid Content of Soluble Proteins Precipitated from GWW by Various Metal Ions[a]

Amino Acid	Amino Acid Content in g/16 g N		
	Calcium	Ferric	Magnesium
Lysine	5.05	4.71	5.20
Histidine	3.65	4.87	3.61
Tryptophan	1.65	2.03	1.85
Arginine	5.90	8.87	6.09
Aspartic acid	5.03	3.98	5.43
Threonine	3.16	3.91	3.31
Serine	4.43	3.95	3.81
Glutamic acid	8.91	11.59	10.81
Proline	5.16	3.87	4.86
Glycine	4.70	3.62	4.05
Alanine	9.32	8.51	8.80
Cystine	1.09	0.94	1.03
Valine	6.73	5.64	5.66
Methionine	1.47	1.25	1.44
Isoleucine	4.29	3.89	3.50
Leucine	8.01	6.40	7.04
Tyrosine	3.96	3.31	3.52
Phenylalanine	4.66	3.76	4.46
% Recovery	87.17	85.10	84.47

[a]Precipitates obtained from heating 50 ppm metal salt solutions at optimum pH to 85°C.

ppm. In combination with heat, ferric or calcium ion can remove approximately 58% of the total nitrogen from GWW. This compares to the 84.8% total nitrogen removed with TCA. After heat-treatment and filtration of solutions treated with 50 ppm or greater metal ions, no detectable turbidity was evident, suggesting complete removal of heat-precipitated protein. It is important to mention that in typical plant operations the composition of GWW can vary greatly, necessitating changes in the levels of metal ion addition to obtain optimum coagulation of the soluble proteins.

The minimum effective temperature for removing protein from GWW in the presence of metal ions was determined in an attempt to minimize energy consumed by the process. Studies were conducted by adding 100 ppm metal ion to GWW, adjusting the pH to the appropriate optimum, and removing aliquots as the solutions were slowly heated to boiling. The results shown in Fig. 1 indicate that: a) all three metal ion systems show similar heat sensitivity, and b) the process is strongly affected by the treatment temperature. For significant removal of protein from GWW, a minimum temperature of 75°C is required. Although temperatures above 75°C do not increase protein removal significantly, they may be required in some applications. For example, treated GWW should be heated to at least the temperature used during evaporation to assure removal of proteins which are heat-sensitive at or near the evaporator operating temperatures. Most commercial GWW would be considerably higher in soluble solids than the GWW used for these model systems. To establish the effectiveness of the heat and metal ion treatment in samples with higher solids contents, freeze-dried GWW was reconstituted to 2, 4, 6, and 8% total solids and treated with various levels of ferric ion and heat. The results shown in Table III suggest that maximum protein precipitation occurs when ferric ion is added at a level equivalent to 0.5% of the total solids in the GWW being treated. This ratio is consistent with the optimum ratios observed in Table II. Addition of higher levels of ferric ion to the 8% solids GWW gave no improvement in N removal. Additionally, it can be seen that the combination of ferric ion and calcium ion precipitates slightly more protein than the ferric ion would have done.

The coagulated proteins isolated from the above studies were analyzed for their amino acid composition to obtain an indication of their nutritive value. The results shown in Table IV indicate that the amino acid composition of the dried protein concentrates varied with the metal ion employed; this can probably be

TABLE V
Results of Pilot Scale Treatment of Treated and Untreated Gluten Wash Water

	Total Solids %	Nitrogen %	Ash %
Gluten wash water (GWW)	0.85	0.046	0.10
Treated GWW	0.85	0.018	0.13
Protein concentrate (PC)	19.2	...	...
Freeze-dried PC	92.0	11.30	...
Concentrated GWW	35.5	1.95	2.65
Concentrated treated GWW	35.2	0.75	3.24

attributed to the sensitivity of various proteins to specific metal ions. The overall amino acid patterns, however, were typical of water-soluble wheat proteins.

The effect of evaporator fouling of protein removal from GWW was established in a pilot-scale comparison. GWW treated at pH 5.0 with 50 ppm ferric chloride and steam injected to 85° C was compared with untreated GWW. Major problems were encountered during concentration of the untreated GWW. The evaporator became fouled, as evidenced by lost efficiency of the system; this was caused by suspended starch and protein in the effluent coating the surfaces of the vessels. Conversely, little or no fouling was observed when the treated GWW was concentrated in the evaporator during the 3 hr of operation. These results indicate that the addition of ferric ion followed by heat coagulation of the protein is a simple method for removing the soluble proteins which cause evaporator problems during concentration of GWW. The total solids, nitrogen, and ash contents of the various fractions obtained from the treatment of untreated GWW and treated GWW are compared in Table V. The total solids and ash contents of the untreated and treated GWW are about the same. However, the nitrogen content of the treated GWW was reduced by 62% as compared to the untreated GWW. The freeze-dried protein concentrate (PC) obtained from the process contained 70 to 73% protein, indicating its potential use as a source of protein. The amino acid composition of this material (Table III) suggests that the proteins may be of high nutritional value because of their high levels of tryptophan, although the sulfur amino acids are low. As expected, the molasses fraction obtained from the treated GWW had a lower nitrogen and higher ash content than the molasses from the untreated GWW. This product also has potential as an animal feed.

SUMMARY

Heat-labile proteins are removed from GWW by heating in the presence of metal ions. The process offers a means of removing heat-labile material from dilute solutions prior to concentration on an evaporator. Removal of these heat-sensitive solids allows the use of a much simpler evaporator. A protein concentrate and a molasses are recovered which have potential as animal feed. It should be noted that evaporation of such waste water is only a temporary measure until processes are developed which produce zero or significantly less waste water. Such a protein recovery system could be incorporated in a process if the waste stream was either more concentrated or of much lower volume than currently produced by gluten washing. Large volumes of dilute waste could not be evaporated over an extended period because of the very high energy consumption of such a process.

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