Condensed Phosphates for Precipitation of Protein from Gluten-Washing Effluent

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ABSTRACT

Washing wheat flour doughs for production of vital gluten and starch produces a waste effluent containing from 1 to 6% solids. Such effluents were treated with various condensed phosphates at pH levels 2.0 to 8.0 to complex and precipitate proteins. Metaphosphate, pyrophospate, tripolyphospate, trimetaphosphate, tetrametaphosphate, and hexametaphosphate were studied. Lower pH was generally more effective, especially with hexametaphosphate. Nearly 90% of the protein can be precipitated and recovered. Gel electrophoresis and gel filtration suggest that various phosphates were specific for various proteins in the effluent. The excess phosphates can be removed from residual effluent after protein removal by treatment with calcium ions at neutral or slightly alkaline pH. Treatment of the protein precipitates with alkali resolubilizes the protein, and the phosphate can then be removed by ion exchange.

In 1938, Perlmann and Hermann (1), using egg albumin, demonstrated that protein forms reversible complexes with phosphates. After formation of the protein-phosphate complex, the complex was redissolved with salt solutions, and the protein was eventually reprecipitated by the continued addition of salt. Several years later, Nitschmann et al. (2) used condensed phosphates to fractionate human plasma proteins, and they reported the fractionation to be a function of hydrogen ion concentration and total ionic strength. At low concentration dramatic differences were observed between the various oligophosphates. Spinelli and Koury (3) used various condensed phosphates for precipitation of sarcoplasmic fish proteins. They investigated the effects of pH and phosphate concentration on the degree of protein removal from solution. It was demonstrated that low levels of phosphate facilitated removal of more than 90% of the soluble protein from a 1% solution.

In the present work we have studied the effect of the various phosphates on material similar to effluent streams from gluten-washing plants. The purpose was to reduce the total solids of the effluent of gluten-washing operations and recover a protein concentrate which may be useful for food products or animal feed.

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. Up to 60 million lb. of flour solids disappear in the form of effluent. The effluent solids contain about 30% protein or a total of 18 million lb. of protein. In one plant, the effluent is mashed with other grain components and fermented for alcohol recovery. In the case of most other plants, it is discarded.

MATERIALS AND METHODS

Preparation of Effluent

Effluent was prepared by a modification of Knight's (4) method for testing flour for performance in gluten and starch production. Using a Hobart[1] A-200 mixer equipped with a dough hook and operated at low speed, 700 ml. of water was slowly added to 1,000 g. of first clears flour and the dough worked an additional 5 min. The dough ball was then covered with 5 liters water and let stand for 1 hr. at 21°C. At the end of 1 hr., mixing was resumed for 7 min. The starch milk was carefully poured off and 5 liters water was placed on the dough, which was again worked for 7 min. The washing was repeated twice more until the starch milk totaled 20 liters. The starch was removed by centrifuging 5 min. at 2,000 r.p.m. in 250 ml. bottles in an International size 2 centrifuge, yielding a supernatant effluent of 0.84% solids. The supernatant was either mixed directly with the phosphate solutions or was freeze-dried for reconstitution at a later time.

Phosphates and Their Use

Metaphosphoric acid [60% $NaPO_3$ (MW = 102) 40% HPO_3 (MW = 80)], reagent grade was obtained from Mallinckrodt. Sodium acid pyrophosphate (MW = 222), sodium tripolyphosphate (MW = 368), and sodium hexametaphosphate (MW = 1,490) were obtained from Monsanto Chemical Co. and the MWs are those given by Monsanto. Sodium tetrametaphosphate (MW = 484) was prepared according to the method of Bell et al. (5). Trimetaphosphate (MW = 305.92) was obtained from Stauffer Chemical Co.

The various phosphates were dissolved in distilled water to form 0.25M stock solutions. Effluent was treated with these stock solutions to yield final phosphate concentrations from 0.005 to 0.05M. The pH was adjusted with 1N hydrochloric acid or 1N sodium hydroxide. Water was added to bring the samples to a final volume such that the original effluent always represented two-thirds of the total volume. Within 1 min. after treatment, solutions were centrifuged at 2,000 r.p.m. for 5 min.

Trichloroacetic acid-soluble nitrogen was determined according to the AACC method for proteolytic acitivity in flour malt from the TCA addition to the end (6).

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[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.

Protein remaining in solution after phosphate and/or calcium precipitation treatments was determined on a Technicon AutoAnalyzer by a modification of the Technicon recommended procedure (7).

The reagents were as follows:

1) Folin phenol reagent 0.74N (370 ml./liter), obtained from Fisher.
2) Buffer: 5% sodium carbonate in 0.2N sodium hydroxide.
3) Copper tartrate (made fresh daily): 1 g. copper ($CuSO_4 \cdot 5H_2O$), 2 g. sodium-potassium tartrate in 1 liter of water.
4) Standards: bovine serum albumin 0.10 to 1.60 mg./ml.

The AutoAnalyzer was connected as shown in Fig. 1.

Phosphorus was determined according to the method of Allen (8). Hexose was determined according to the method of Dubois et al. (9). Moistures or total solids were determined according to the AACC methods (6).

Chromatography on Sephadex G-100 in aluminum lactate buffer pH 3.1 (μ = 0.1) containing 4M urea was carried out in a K 15/30 column (Sephadex). Elution patterns were determined by UV absorption at 280 nm. with a Buchler Uviscan II column monitor.

Disc electrophoresis was carried out as described by Sastry and Virupaksha (10) in aluminum lactate buffer pH 3.1 containing 4M urea. After electrophoresis, staining, and destaining, microdensitometer tracing of the gel pattern was made with a Canalco Model F microdenisitometer.

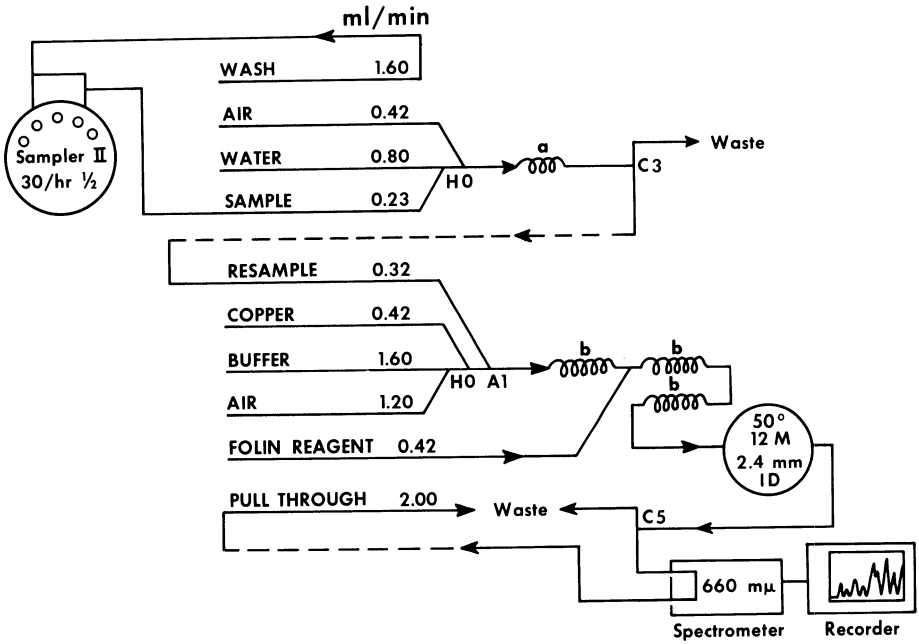

Fig. 1. AutoAnalyzer setup for Folin Lowry protein determination. a, mixing coil, 14 turn, 2.4 mm. i.d. b, mixing coil, 28 turn, 2.4 mm. i.d., Technicon Connector numbers used for all connectors.

RESULTS AND DISCUSSION

The effect of pH on protein removal from effluent containing 0.05M concentration of the various phosphates is shown in Fig. 2. The pH of the waste water before treatment ranged from 5.7 to 6.0. Typically 12 ml. of N HCl was required to adjust 1 liter of waste water with 0.84% solids and 0.05M hexametaphosphate to pH 2.0. The effluent was centrifuged at 2,000 r.p.m. immediately after phosphate addition and pH adjustment.

It can be seen as the pH went down the phosphates became more effective as precipitants. At the lower pH levels hexametaphosphate and tetrametaphosphate were the most effective precipitants at the 0.05M phosphate concentrations.

In an attempt to evaluate the effectiveness of the phosphates on effluent of higher solids content the freeze-dried effluent was reconstituted at various levels from 1 to 10% solids. Figure 3 shows that there was a sharp loss in the effectiveness of hexametaphosphate as the effluent concentration increased from 1 to 2% solids. From 2 to 7% solids there was little difference in the effect of the

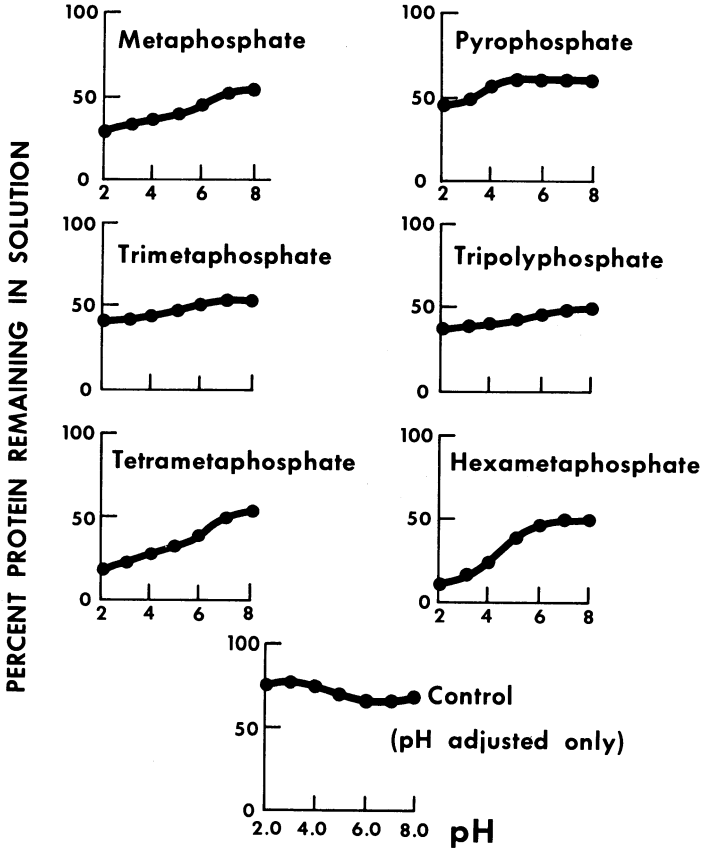

Fig. 2. The effect of pH on the potency of various phosphates as precipitants for protein from gluten-starch wash water.

hexametaphosphate; but above 7% solids, effectiveness decreased further. Results comparable to those shown for hexametaphosphate were obtained with the other condensed phosphates.

The effect of phosphate concentration (0.005 to 0.05M) on protein removal at pH 2.0 in the original effluent is shown in Fig. 4. While protein removal generally increased with increasing phosphate concentration, the individual phosphate behaved very differently. Tetrametaphosphate was very effective at low concentration, while at slightly higher concentration hexametaphosphate became more effective. There was a tendency for effectiveness to increase with molecular weight of the phosphate, except for metaphosphate.

Although not shown in Fig. 4, levels of some of the condensed phosphates can be lowered considerably and still obtain protein precipitation at pH 2.0. While tetrametaphosphate appears most effective at low concentration, we chose to use the commerically available hexametaphosphate for studies at low levels (1.25×10^{-5} to 5.0×10^{-3}M). Approximately 60% of the protein was removed, adding hexametaphosphate from 2.5×10^{-4}M to 5.0×10^{-3}M. At lower levels turbidity was observed, but there was little or no apparent precipitation. These data suggest that hexametaphosphate could simply be added to effluent, along with pH adjustment, and considerable protein removed. Such low level phosphate addition would eliminate the need for phosphate recovery.

Protein-hexametaphosphate precipitates obtained by using different phosphate concentrations and pH levels were freeze-dried and their phosphorus:nitrogen

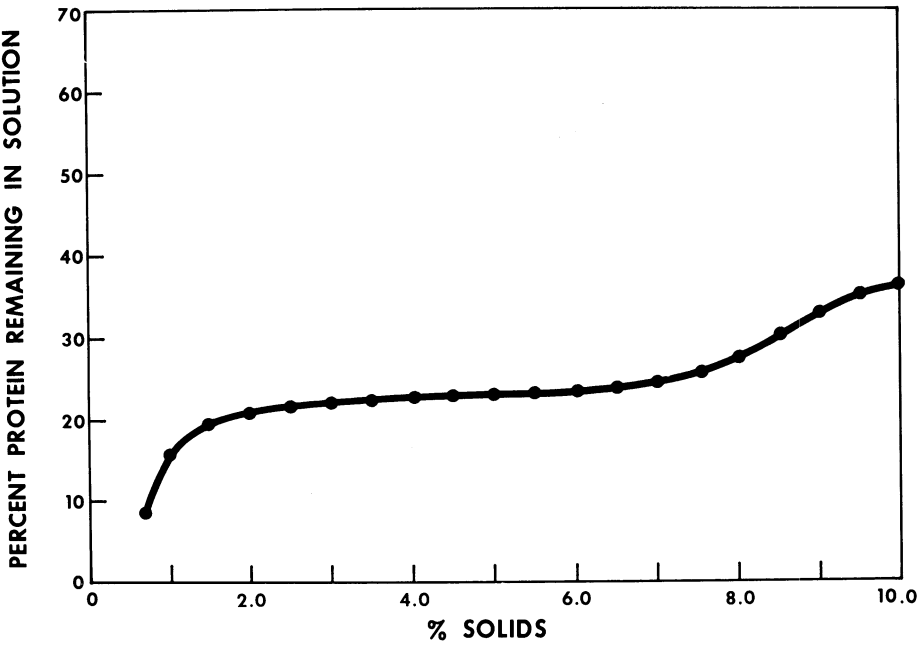

Fig. 3. Effectiveness of 0.05M hexametaphosphate at pH 2.0 for protein removal from effluents of varying concentration.

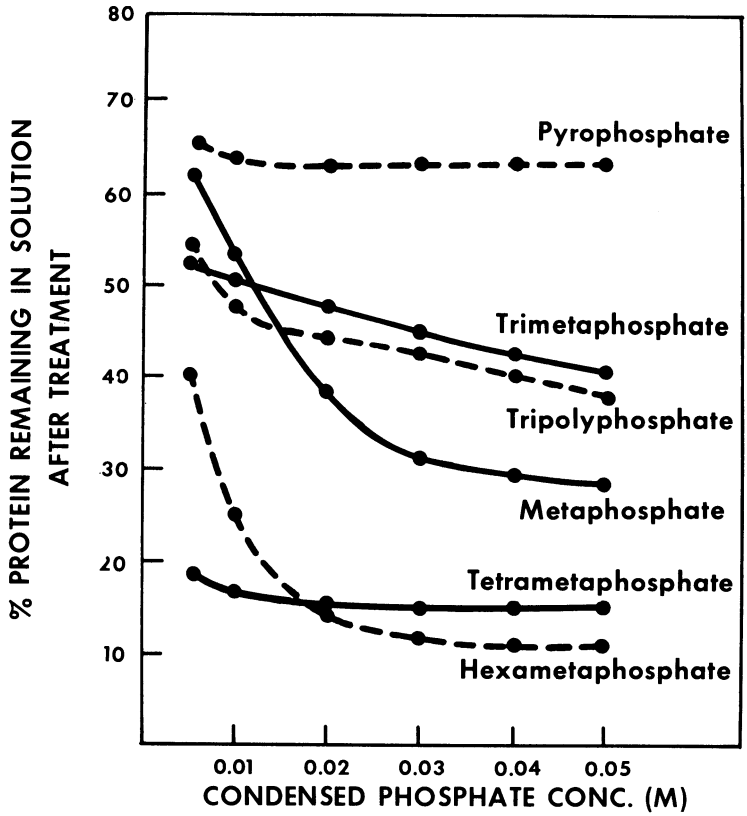

Fig. 4. The effect of phosphate concentration on protein remaining in solution.

TABLE I. PHOSPHOROUS:NITROGEN (wt.:wt.) RATIO FOR HEXAMETAPHOSPHATE AND TRIPOLYPHOSPHATE-PROTEIN COMPLEXES AT VARIOUS pH LEVELS AND PHOSPHATE CONCENTRATIONS

	Molarity of Hexametaphosphate			
pH	0.005	0.01	0.02	0.05
2.0	0.545	0.550	0.597	0.607
2.5	0.550	0.561	0.601	0.599
3.0	0.530	0.542	0.554	0.550
3.5	0.405	0.435	0.429	0.495
4.0	0.309	0.318	0.321	0.382
4.5	0.251	0.271	0.288	0.301
5.0	0.221	0.223	0.219	0.231
	Molarity of Tripolyphosphate			
2.0	0.487	0.493	0.534	0.535
2.5	0.485	0.489	0.540	0.530
3.0	0.470	0.485	0.500	0.511
3.5	0.362	0.370	0.402	0.422
4.0	0.274	0.281	0.318	0.347
4.5	0.241	0.255	0.269	0.298
5.0	0.208	0.210	0.215	0.221

(P:N) ratio determined. The P:N ratios are reported in Table I. The concentration of hexametaphosphate did not affect the P:N ratio, but pH did. At lower pH levels P:N ratio increased 2.5 times, indicating a pronounced change in the nature of the complex.

It was shown in Fig. 4 that the various phosphates precipitated differing amounts of protein from the effluent. An attempt was made to determine if these differences were caused by complexing of different specific proteins, quantitative differences with the same proteins, or both. After precipitation with various phosphates at pH 2.0, the solution was centrifuged and 2 ml. of the soluble

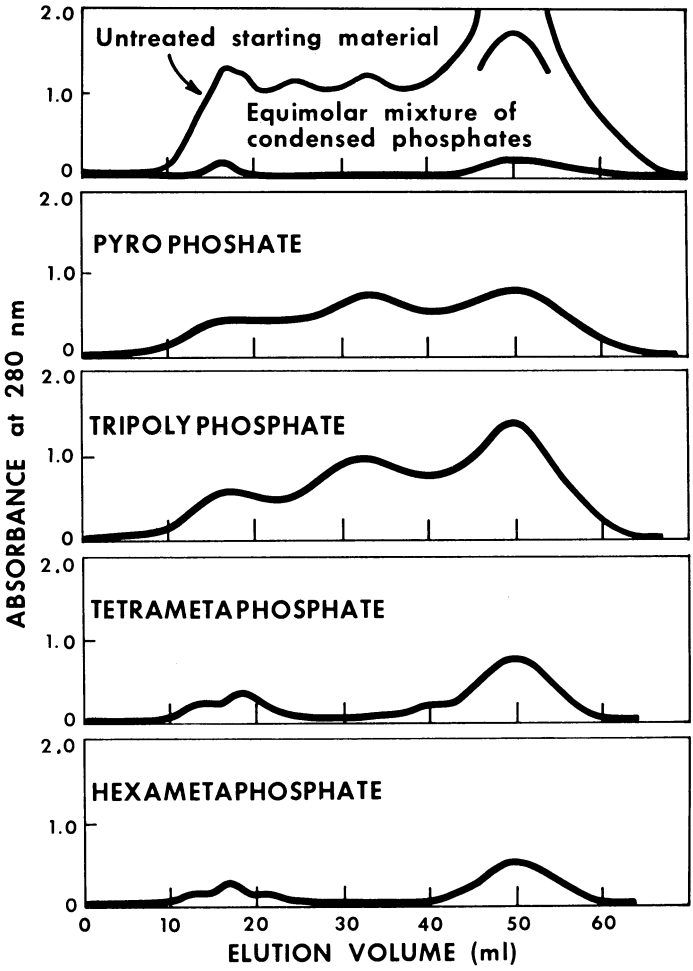

Fig. 5. Elution patterns from Sephadex G-100 (aluminum lactate buffer pH 3.1, 0.1M) of the proteins remaining in solution after treatment of effluent with various phosphates at pH 2.0. Untreated starting material acidified and diluted to the same pH and total dilution as phosphate-treated samples; thus, comparison of the curves provides an indication of quantitative differences.

supernatant was placed on a Sephadex G-100 column equilibrated with aluminum lactate buffer (pH 3.1, $\mu = 0.1$) containing 4M urea. The column was then developed with the same buffer. Figure 5, which shows the Sephadex elution patterns, indicates both quantitative and qualitative differences in the proteins precipitated by the various phosphates. The quantitative effect can best be seen in the peak eluted with 51 ml. of buffer. The qualitative effect can be seen in the various ratios in the first four peaks.

In an attempt to confirm the column chromatographic observations, disc gel electrophoresis was carried out on preparations of the various protein-phosphate complexes. The complexes were prepared by reacting fresh effluent with 0.05M phosphate at pH 2.0 and recovered by centrifuging. The precipitate was resuspended in distilled water, the pH was adjusted to 8.0 with CaO, the precipitate was removed by centrifugation, and the supernatant was exhaustively dialyzed against distilled water and freeze-dried. The untreated control had a P:N ratio of

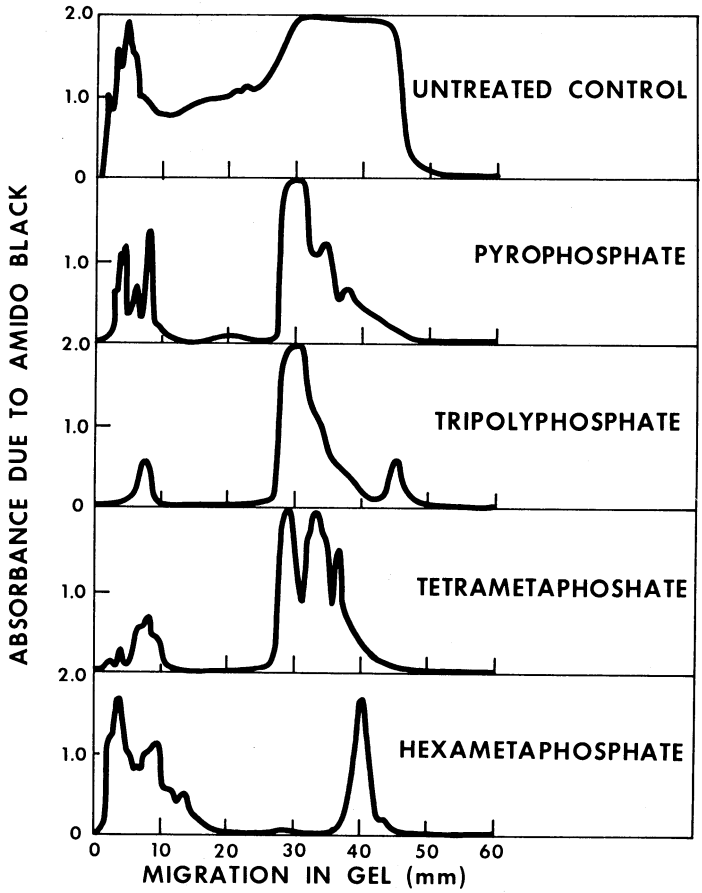

Fig. 6. Microdensitometer tracings from electrophoretograms of the protein precipitated by treatment of effluent with various condensed phosphates.

0.153. All treated samples were in the same range, ±0.01. The freeze-dried material was dissolved to form a 1% solution in aluminum lactate buffer (pH 3.1, $\mu = 0.1$) containing 4M urea and electrophoresed as described previously (10). Figure 6 shows the microdensitometer tracings and verifies the qualitative differences in the proteins precipitated by the various phosphates. Note the differences between tripolyphosphate and hexametaphosphate. These data suggest that the condensed phosphates may have potential for separation of particular portions of the soluble protein from wheat. Differences can also be observed in the supernatants after electrophoresis by the same method.

To remove excess non-complexed phosphates from the effluent after phosphate treatment and removal of the precipitated protein-phosphate complex, the pH was adjusted to 7.0 with a 1M suspension of calcium oxide and the calcium phosphate was centrifuged out. At pH 7.0 and 0.05M phosphate, calcium oxide and centrifugation effected removal of 91.2% of metaphosphate, 90.1% of pyrophosphate or tripolyphosphate, 94.5% of trimetaphosphate, 95.2% of tetrametaphosphate, and 97.3% of hexametaphosphate.

TABLE II. ANALYSIS OF WASTE STREAMS[a] FROM COMMERCIAL GLUTEN PLANTS BEFORE AND AFTER TREATMENT WITH A MIXTURE OF PHOSPHATES FOLLOWED BY CALCIUM OXIDE

	Total Solids %	Protein %	Protein Removed %	TCA-Soluble Protein N X 5.7	Carbohydrate %	Carbohydrate Removed %
Before treatment						
1	1.110	0.268		0.06		
2[b]	2.296	0.570		0.22	1.03	
3[b]	1.791	0.353		0.15	0.42	
After treatment[c]						
1	0.831	0.06	74.9[d]	0.06		
2	0.92	0.03	78.6[d]	0.19	0.40	62.6
3	0.55	0.19	77.6[d]	0.15	0.21	50.0

[a]These were selected streams and do not necessarily represent the character of the total effluent.

[b]Samples were 3 days in delivery from plant: proteolytic activity is suggested by the high TCA-soluble nitrogen.

[c]Samples were adjusted to 0.01M each of metaphosphate, pyrophosphate, tripolyphosphate, tetrametaphosphate, and hexametaphosphate at pH 2.0, centrifuged, adjusted to pH 7.0 with CaO, and centrifuged. Values are corrected for dilution so as to have comparable values before and after treatment.

[d]Corrected for TCA-soluble protein to reflect only the effect on TCA-insoluble proteins.

Effluent samples without the fiber fraction were obtained from commercial gluten-washing plants and were treated with the various phosphates (final concentration 0.05M phosphate, results not shown) and an equimolar mixture (0.01M each of the five phosphates). Precipitated protein was centrifuged out. The supernatant effluent was treated with calcium oxide to adjust the pH to 7.0 and centrifuged to remove excess phosphate as the calcium salt. Protein was determined

on the residual effluent by the Folin Lowry (7) method. Table II shows the effectiveness of the treatments in removing protein and carbohydrates from the effluents. Effluents 2 and 3 were nearly 3 days old before they arrived (held under toluene) and had TCA-soluble nitrogen values nearly four times as high as effluent 1, which was treated 10 min. after sampling. The high TCA values indicate substantial proteolytic activity during transit (given in Table II).

Table II shows that considerable carbohydrate was removed from commercial effluents by the phosphate followed by calcium oxide treatment. A small amount (2 to 5%) of the carbohydrate was precipitated in the first stage or during protein-phosphate complexing, but the majority was precipitated with the addition of the calcium oxide. It is assumed that the carbohydrate material is adsorbed on the calcium phosphate precipitate.

In summary, it appears that condensed phosphates can be effectively used to remove protein and some carbohydrate from gluten starch-washing plant effluents. Biochemical oxygen demand of the waste water can thus be significantly reduced. The soluble proteins can be recovered, and may offer potential for food or other uses.

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