Nakagawa, Y., Perlmann, G., *Arch. Biochem. Biophys.* **149**, 476 (1972).
Oke, O. L., *Indian J. Nutr. Diet* 8, 121 (1971).
Oppenheimer, H., Barany, K., Hamoir, G., Fenton, J., *Arch. Biochem. Biophys.* **120**, 108 (1967).
Oppenheimer, H., Barany, K., Hamoir, G., Fenton, J., *Arch. Biochem. Biophys.* **115**, 233 (1966).
Paik, W., Kim, S., *Biochemistry* 11, 2589 (1972).
Pearson, A., Spooner, M., Hegarty, G., Bratzler, L., *Food Technol.* **19**, 1841 (1965).
Pirie, N. W., "Proteins as Human Food", Lawrie, K. A., Ed., Avi Publishing Co., Westport, Conn., 1970, pp 46–61.
Polyanovski, O., *Biochem. Biophys. Res. Commun.* **19**, 364 (1965).
Prendergast, K., *Food Trade Rev.* **44**, 16 (1974).
Protein Advisory Group, No. 10, United Nations, New York, N.Y., 1970.
Provensal, M. P., Cug, J. L., Cheftel, J. C., *J. Agric. Food Chem.* **23**, 938 (1975).
Riordan, J., Vallee, B., *Biochemistry* **3**, 1768 (1964).
Stahmann, M., *Econ. Bot.* **22**, 73 (1968).
Subba-Rau, B. H., Ramana, K. V., Singh, N., *J. Sci. Food Agric.* **23**, 233 (1972).
Swift, C., Sulzbacher, W., *Food Technol.* **17**, 224 (1963).
Tanford, C., "The Hydrophobic Effect: Formation of Micelles and Biological Membranes", Wiley, New York, N.Y., 1973.
Tannenbaum, S. R., Ahearn, M., Bates, R. P., *Food Technol.* **24**, 607 (1970).
Wang, J. C. N., Ph.D. Thesis, Cornell University, Ithaca, N.Y., 1975.
Wang, J. C. N., Kinsella, J. E., *J. Food Sci.* **41**, 286 (1976a).
Wang, J. C. N., Kinsella, J. E., *J. Food Sci.*, in press (1976b).
Wofsky, L., Singer, S., *Biochemistry* **2**, 104 (1963).
Woodham, A. A., *Proc. Int. Congr. Nutr., 9th*, **3**, 69 (1972).
Yasumatsu, K., Sawada, L., Moritaka, S., Misaki, M., Toda, J., Wada, T., Ishii, K., *Agric. Biol. Chem.* **36**, 719 (1972).

Received for review February 25, 1976. Accepted May 10, 1976. Supported in part by National Science Foundation Grant No. ENG75-17275.

# Physical-Chemical Methods for the Recovery of Protein from Waste Effluent of Potato Chip Processing

Erhard Meister* and Norman R. Thompson

Simple physical–chemical methods for the recovery of protein from potato chip processing were evaluated. It was estimated that an average potato chip plant, processing 31 metric tons of potatoes per day, could daily recover approximately 170 kg of dried potato protein (550 kg of food containing 30% of protein). Sedimentation or centrifugation after heating, adjusting pH, or both yielded similar amounts of protein. Heat (80–90 °C) at pH 4–4.5 was most effective for protein recovery but total dry matter reduction was highest if no heat was applied. Protein yields were improved if the waste was kept in motion during floc formation. Recovery was similar when pH was raised to 11.5 and then lowered with either $H_3PO_4$ or $FeCl_3$ to pH 9. Approximately 30–40% of the crude protein or 80–90% of the coagulable protein, presently wasted, could easily be recovered.

The potato processing industry is one sector of the food industry where serious waste problems are caused by potentially valuable food materials. Chip manufacturers need simple economical methods to minimize losses and to meet local and federal standards for the effluent discharged (Federal Register, 1973).

Traditionally potato chip plants are located in urban areas where space for conventional treatment or agricultural use of the effluent is not available. Peel, potato fragments, and other particulate solids can be readily removed by screening or settling (Ballance, 1964). Recovery of dissolved and suspended solids in the waste effluent, which results from peeling and slicing, is still inadequate.

Most research on potato waste has been done on effluent from starch manufacturing where only starch is retained. However, Vlasblom and Peters (1958) have patented a process to recover protein. Xander and Hoover (1959) precipitated proteins from potato juice by heat coagulation and recovered amino acids and amides with a strongly basic anion exchanger. Using ion exchange, Heisler et al. (1972) investigated the recovery of amino acids, proteins, and potassium and Schwartz et al. (1972) studied the recovery of organic acids and phosphate. However, Stabile et al. (1971) found that protein recovery followed by removal of other constituents using ion exchange is not economically feasible. Reverse osmosis treatment of waste was investigated by Porter et al. (1970), but great difficulties were encountered in a pilot plant study with potato chip effluents (Seifert, 1974). In the small potato chip plants simple methods for by-product recovery are required. Hydrocyclones for the recovery of starch are available (Pettay, 1975). The purpose of this study is to examine optimal conditions for recovery of proteins by simple means. Special emphasis is given to heat treatments, since waste heat generated by the cooker could be recovered in a heat exchanger.

Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan 48824.

## MATERIALS AND METHODS

**(I) Preparation of Protein Water.** Preliminary work was initiated with processing water from a potato chip factory. Because of the inconvenience of transporting a dilute solution and possible compositional changes, it was decided to simulate processing water in the laboratory. Russet Burbank potatoes were washed thoroughly, peeled, and ground in a Waring Blendor. The slurry was diluted with tap water (composition, Table I) to 10 times its volume. This, after filtering the juice through several layers of cheesecloth followed by settling for 30 min, produced protein water of a composition similar to the

Table I. Chemical Analysis of the Water Taken from Michigan University Campus Wells[a]

| pH | Cl, mg/l. | Alkalinity, mg/l. $CaCO_3$ | Total hardness, mg/l. $CaCO_3$ | Sulfate, mg/l. $SO_4$ | Nitrate, mg/l. N | Tot. Kjeldahl, mg/l. N | Fe, mg/l. | Mn, mg/l. |
|---|---|---|---|---|---|---|---|---|
| 7.5 | 4.9 | 304 | 318 | 21 | 0.03 | 0.46 | 0.73 | 0.01 |

[a] Data from d'Itri (1973).

Table II. Average Composition of Potato Effluent of Two Local Potato Chip Plants

| Processing plant | Sample[a] | Dry matter, mg/l. | Crude protein, mg/l. |
|---|---|---|---|
| A | 1 | 11 200 | 4307 |
| | 2 | 8 400 | 2960 |
| | 3 | 9 400 | 3524 |
| | 4 | 10 600 | 3360 |
| B | 5 | 3 310 | 1578 |
| | 6 | 1 725 | 771 |
| | 7 | 1 550 | 547 |
| | 8 | 2 100 | 895 |

[a] Samples 1 to 4 give average composition of effluent leaving the hydrocyclone on 5 different days in plant A. Samples 5 to 8 were collected on the same day at 4 different locations in factory B.

samples obtained from processing plant A (Table II).

**(II) Analytical Procedures.** The nitrogen content of the samples was determined by micro-Kjeldahl, and dry matter content by the official AOAC vacuum oven method (AOAC, 1970). The vacuum oven was operated for 12 h at 70 °C under partial vacuum.

The terms total, coagulable, and precipitated protein refer to crude protein (N × 6.25), protein coagulable by trichloroacetic acid (10% w/v), and protein precipitated by specified treatments. Precipitated protein is expressed as percent of coagulable protein. All values were adjusted for dilution caused by treatments.

The composition of crude protein in whole Russet Burbank tubers and in both distilled and tap water extracts was determined according to Lindner et al. (1960), outlined by Luescher (1972). Flour of freeze-dried tubers and flour of freeze-dried waste water were each blended with the extracting solutions for 4 min at room temperature. The isolates were dialyzed in cellophane bags against 100 times their volumes of distilled water for 48 h at 4 °C. During dialysis the water was changed 4 times.

**(III) Separation of Precipitates.** Initial attempts to filter the slurries were fruitless since starch and protein floc immediately plugged the filters.

*Gravity Settling.* The influence of concentration on settling times of samples treated with or without heat was investigated. Waste water was prepared as above but diluted either five or ten times its volume to give different concentrations. The pH of the protein water was adjusted with 2 N HCl to pH 4 and one-half was heated to boiling (98 °C) followed by immediate cooling in ice water. The other half was stirred during the time the former was heated and cooled. One liter of each treatment combination was poured into a settling cylinder. Samples were withdrawn from the center at 10-min intervals and analyzed for nitrogen and dry matter. This was repeated twice.

*Centrifugation.* Separation of protein by 7 different centrifugal forces for 15 min was compared with gravity settling for 1 h. Two different extracts of waste water were heated to 98 °C at pH 4. Duplicate samples were centrifuged in a laboratory centrifuge (Sorvall Superspeed RC-2) at speeds of 2000, 4000, 6000, 10 000, 20 000, 30 000, and 40 000*g* and residual nitrogen in the supernatant was determined.

**(IV) Treatments for Protein Precipitation.** Aliquots of the supernatant from each treatment combination were withdrawn with a syringe after 1 h of sedimentation, placed in screwcap bottles, and stored in a refrigerator until analyzed.

**Heat vs. pH Treatment.** The effect of pH on protein precipitation was tested in the range of pH 1 to 7. The pH of 500-ml samples of waste water was adjusted with 2 N HCl or 2 N $H_3PO_4$ to 8 different pH levels while stirring with a magnetic stirrer. Four 100-ml aliquots of protein water from each pH level were measured into Erlenmeyer flasks, which were then placed in water baths at 23, 60, 80, and 98 °C, and subjected to a slow shaking motion while being heated. After reaching the desired temperature they were immediately cooled in ice water and allowed to settle.

The above procedure was repeated using distilled water to make the extract, but temperatures of only 23 and 98 °C were compared.

**$FeCl_3$ vs. HCl as Coagulant.** The pH values of 100-ml samples of waste water were adjusted with either 1 M $FeCl_3$ or 2 N HCl over the same pH range. Samples were agitated for 20 min in a shaker at 23 °C before they were subjected to sedimentation.

**Lime, $H_3PO_4$, and $FeCl_3$ Treatment.** A newly prepared slurry of CaO and distilled water was added to 3 l. of waste water to raise the pH to 12 gradually. This was followed by lowering the pH with either 2 N $H_3PO_4$ or 1 M $FeCl_3$. Aliquots (100 ml) were withdrawn at intermediate pH values and subjected to settling.

## RESULTS AND DISCUSSION

The average dry matter and crude protein content of samples received from two potato chip plants are given in Table II. Values for the total dry matter content for plant B are of the same magnitude as reported by Willard (1962). The higher values in plant A are attributable to the use of a hydrocyclone to remove most of the starch and allow partial recycling of effluent. In the light of reduced waste discharge the latter system is more desirable.

Smith (1966) reports that an average potato plant processes approximately 31 metric tons of potatoes per day and uses 460 000 l. of water. Assuming that the waste composition of plant B is typical, there would be a daily loss of about 385 kg of crude protein, of which approximately one-third to one-half can be easily precipitated. This gives an estimate for easily recoverable protein per day of 170 kg for an average potato chip plant.

**Separation of Protein from Heat Treated Waste Water.** Samples of potato waste water with an initial crude protein concentration of 5830 ppm were acidified, heated to 98 °C, and immediately cooled in ice water. Of 61% coagulable protein, approximately 84% was removed at speeds of 2000 to 10 000*g*; 87, 94, and 99% settled at 20 000, 30 000, and 40 000*g*, respectively. The lower centrifugal forces did not yield any appreciable amounts beyond the 82% which was achieved by gravity settling for 60 min. Gravity settling yielded a white slurry with 6–8.5% dry matter that could easily be drained off. A

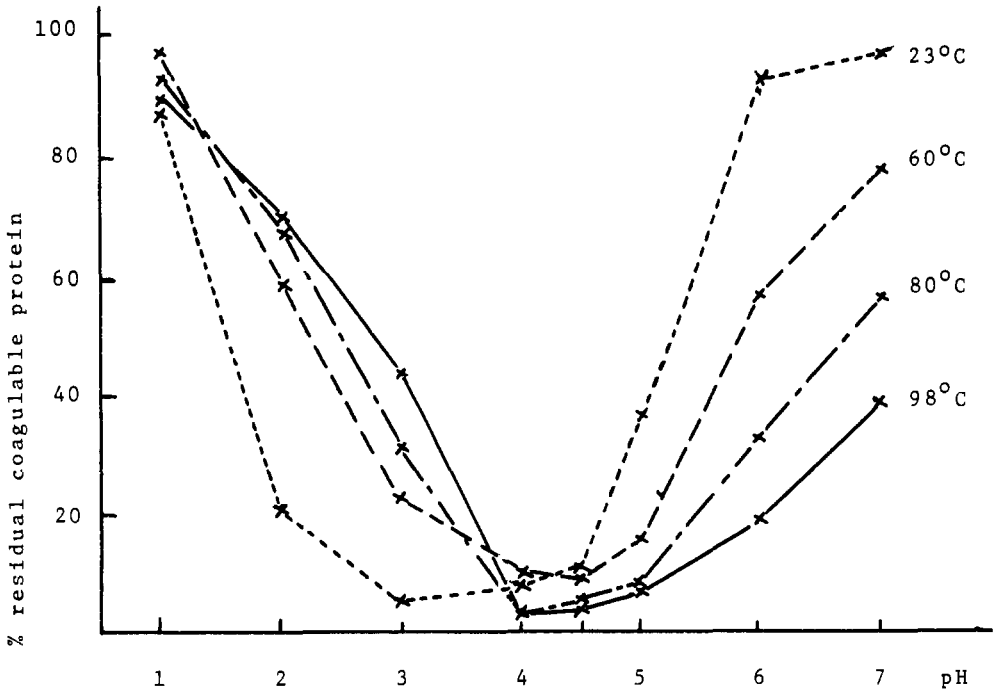


**Figure 1.** The effect of pH and heat treatment on the sedimentation of protein from a tap water extract.

pasty cake of 15–20% dry matter was recovered by centrifugation.

Drying studies with similar wastes from potato starch manufacturing (Strolle et al., 1973) gave good results with double drum drying; freeze drying was too expensive and air drying in a conventional tray drier gave a black, hard, hornlike product. The drum drier required material containing 12–15% solids. The cakes produced by centrifugation at 500 000 rpm contained 25–35% solids and had to be diluted. It appears that low centrifugal forces would yield a product suitable for drum drying. The slurry from gravity settling would require additional concentration.

**Effect of Concentration and Heat Treatment on Settling.** Residual crude protein measured over a time span of 80 min is given in Table III. Sedimentation was faster in heat treated samples, but differences beyond 60 min were not significant. The significant interaction, time × treatment, indicates that initial sedimentation in low protein concentrations was faster but that the percentage of protein settled during the entire period was lower. Strolle et al. (1973) came to the same conclusion using waste from a starch plant. These data suggest that the efficiency of protein recovery could be increased if higher waste strength could be obtained. Water usage should be reduced if possible and the waste effluent recycled. This was achieved in plant A by use of hydrocyclone for starch recovery.

**Heat vs. pH Treatment.** The solubility of proteins is markedly influenced by the pH and is minimum at the isoelectric point. Since a potato extract represents a mixture of different proteins and other constituents we cannot expect a narrow zone of low solubility. The data of this experiment are summarized in Figures 1 and 2. No heat–pH combination was as effective as $Cl_3CCOOH$ (trichloroacetic acid) in precipitating protein. The curves in Figure 1 indicate an optimal pH range of 3.5–4.5 for protein precipitation. The highest temperature was most effective at all but the very low pH values. The temperature effect was most pronounced at neutral pH and was negligible around pH 4. At higher acidities, room temperature gave better recoveries than the heat treatments. Heating to 80 °C or acidification to pH 3.0 or slightly below as suggested by Heisler et al. (1959) did not give optimal responses. Strolle et al. (1973) who used steam injection found that heat alone was not very effective but that lowering the pH improved the efficiency of the treatment.

Table III. Residual Crude Protein (Parts per Million) in Solution after Gravity Settling of Protein from Waste Water of Low and High Protein Concentration at pH 4, with and without Heat Treatment[a]

| Settling time, min | High concn | | Low concn | |
|---|---|---|---|---|
| | 23 °C | 98 °C | 23 °C | 98 °C |
| 0 | 8330a,A | 8340a,A | 4230a,A | 4230a,A |
| 10 | 7310b,A | 7110b,A | 3670b,A | 3610b,A |
| 20 | 6590c,A | 6050c,A | 3300c,A | 3110c,A |
| 30 | 6170d,A | 5630d,B | 3001bc,A | 2830cd,A |
| 40 | 5780,A | 5090e,B | 2800c,A | 2620de,A |
| 50 | 5500f,A | 4800e,B | 2740c,A | 2500de,A |
| 60 | 5090fg,A | 4840e,A | 2640c,A | 2380e,A |
| 70 | 5050g,A | 4830e,A | 2500d,A | 2360e,A |
| 80 | 4950g,A | 4880e,A | 2480d,A | 2370e,A |
| $Cl_3CCOOH$ standard | 3900 | | 1870 | |

[a] Average value of two replications. Studentized range test: time comparisons within treatments (within columns), values with the same letter (a) are not significantly different; temperature means within time and concentration (between columns), values with the same letter (A) are not significantly different.

Although heat coagulation has been proposed by many authors (Vlasblom and Peters, 1958; Borud, 1971; Strolle et al., 1973) our data indicated that at low pH (~3.5) good results could be achieved at low temperatures where protein recovery was essentially the same but reclamation of total solids was much higher. Aggregation was much improved through the slight shaking motion during floc formation and resulted in faster and better settling.

The protein content of the recovered food ranged from 27 to 35%. It was lower for acid precipitate since more starch settled with the proteins.

When HCl was substituted for $H_3PO_4$, results were almost identical and are not given. From a cost point of

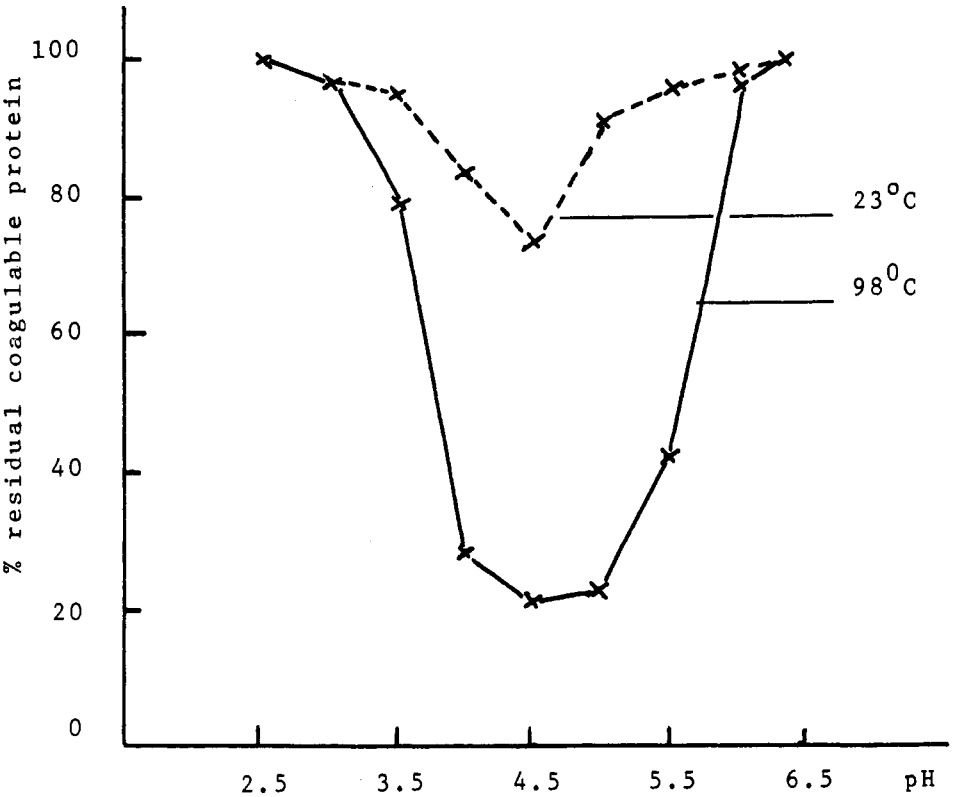


**Figure 2.** The effect of pH and heat treatment on the sedimentation of protein from a distilled water extract.

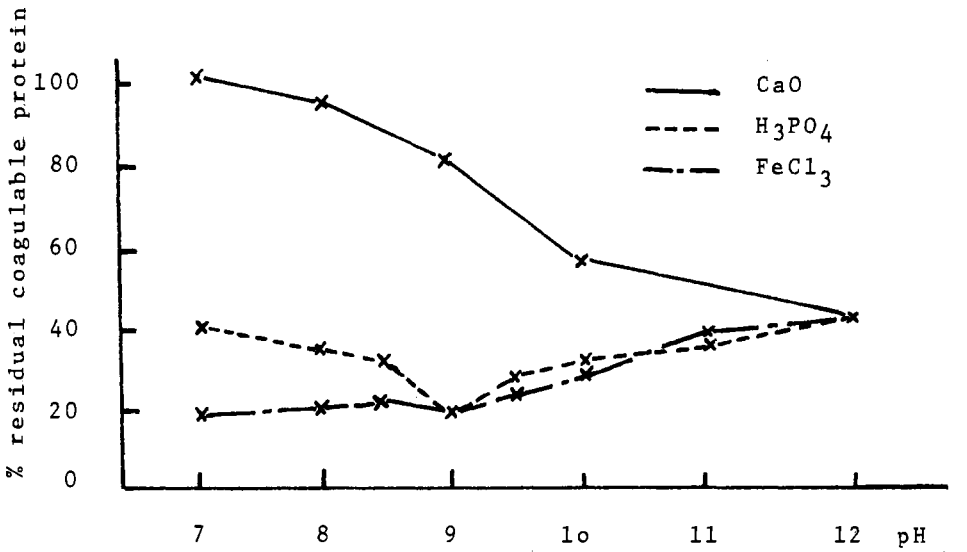


**Figure 3.** Residual protein in solution after raising the pH with CaO, followed by lowering it with either $H_3PO_4$ or $FeCl_3$.

view as well as considerations of adding $H_3PO_4$ to public water HCl should be preferred.

In distilled water extracts sedimentation was poor, especially when no heat was applied (Figure 2). These results did not agree with observations on actual waste water and point out the difficulty of comparing results from different sources.

**Protein Composition of Water Extracts and Whole Tubers.** The major difference between the nitrogenous components in distilled and tap water extracts vs. those in the original tubers is the increased nonprotein N in the extracts (Table IV). Of the protein fractions, the water-soluble tuberinin and the unknown nitrogen compounds comprised larger percentages. Tuberin, the less soluble but major protein fraction in potato tubers and the residue, was markedly lower in both extracts. The distilled water extract contained much less of the tuberin fraction than did the tap water but more tuberinin and nonprotein N. The different response of the two extracts to protein precipitation can be explained partly by the different compositions. The distilled water extract contained more of the highly soluble fractions.

**Comparison of $FeCl_3$ and HCl as Coagulants.** Ferric chloride is one of the principal coagulants used in sewage work. It is cheap, has acid properties, and the trivalent iron ion is a good nucleating site for large floc formation (Daniels, 1974). Table V shows that it compares favorably with HCl; its pH optimum for protein precipitation is higher (pH 4.0) than for HCl (pH 3.0). The advantage of ferric chloride is that the water does not have to be heated. The iron recovered with the protein could add to the nutritional significance of a recovered feed.

Table IV. Composition of Crude Protein of Russet Burbank Potatoes: Whole Tubers, Distilled and Tap Water Extracts

| | Whole tuber, % | Distilled water, % | Tap water, % |
|---|---|---|---|
| Crude protein | 100[a] | 100[b] | 100[c] |
| Protein fractions | | | |
| Tuberin | 39.2 | 13.7 | 27.3 |
| Globulin II | 0.5 | 1.1 | 0.8 |
| Tuberinin | 1.5 | 6.9 | 6.2 |
| Prolamin | 0.8 | 1.2 | 0.6 |
| Glutelin | 0.1 | 0.1 | 0.1 |
| Unknown nitrogen compounds | 0.9 | 4.9 | 4.3 |
| Residue | 7.8 | 4.1 | 4.2 |
| Nonprotein N | 49.2 | 67.8 | 56.4 |
| Nonprotein N (10% $Cl_3CCOOH$) | 49.8 | 71.3 | 58.2 |

[a] Sample size 60 g, 116 mg of crude protein/g. [b] Sample size 15 g, 469 mg of crude protein/g. [c] Sample size 20 g, 321 mg of crude protein/g.

Table V. Residual Crude Protein (Parts per Million) in Solution after Treatment of Protein Water with $FeCl_3$ and HCl, Respectively

| pH | HCl | $FeCl_3$ |
|---|---|---|
| 6.0 | 3100 | 2660 |
| 5.0 | 2700 | 2260 |
| 4.0 | 2500 | 2170 |
| 3.0 | 2380 | 2230 |
| 2.0 | 2580 | 2650 |
| 1.0 | 3020 | 3210 |
| Original concn | 3450 | 3470 |
| $Cl_3CCOOH$ standard | 1980 | 2010 |

**Lime, $H_3PO_4$, and $FeCl_3$ Treatment.** The Steffen process, using a combination of lime and $H_3PO_4$ for the recovery of protein, has been widely used in the sugar beet industry (Schneider, 1968). Applied to waste water protein yields increased as the pH is raised to pH 12 (Figure 3). Maximum precipitation occurred after lowering the pH to 9.0 with either $FeCl_3$ or $H_3PO_4$. Protein recovery was good, but because of the high amounts of Ca in the product its usefulness as protein feed is questionable. It would better qualify as a Ca supplement.

A serious disadvantage of lime and $H_3PO_4$ treatment is that water has to be neutralized before being discharged. Also, phosphate can be readily precipitated with lime above pH 11.8 but its solubility is much increased at pH 9 (Wilcox, 1974), thus leaving high amounts of residual phosphate in the effluent stream after the above treatment.

Recovery of 30–40% of crude protein currently discharged in effluent from potato chip processing can be achieved by any one of the methods tested in the laboratory. The most efficient appears to be heating at pH 4–4.5. When combined with starch recovery the process should be economical.

ACKNOWLEDGMENT

The authors wish to express their appreciation to F. C. Elliott and D. Penner for their assistance in preparing this manuscript and providing lab facilities for the conduct of this work.

LITERATURE CITED

Association of Official Agricultural Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., 1970, pp 122, 858.
Ballance, R. C., International Symposium on Utilization and Disposal of Potato Waste, Proceedings, 1964, pp 200–211.
Borud, O. J., *Staerke* **23**(5), 172 (1971).
Daniels, S. L., *AIChE Symp. Ser.* **136**(70), 266 (1974).
d'Itri, F. M., Water Quality Management, Technical Report No. 31.2, Institute of Water Research, Michigan State University, E. Lansing, Mich., Aug 15, 1973.
*Fed. Regist.* **38**, 13523, 13528 (May 22, 1973); Federal Water Pollution Control Act as amended by Public Law 92-500 of 1972.
Heisler, E. G., Siciliano, J., Krulick, S., *J. Sci. Food Agric.* **23**, 745 (1972).
Heisler, E. G., Siciliano, J., Treadway, R. H., Woodward, C. F., *Am. Potato J.* **36**, 1 (1959).
Lindner, K., Jaschik, S., Korpaczy, I., *Qual. Plant. Mater. Veg.* **7**, 289 (1960).
Luescher, R., Ph.D. Thesis, Michigan State University, East Lansing, Mich., 1972.
Pettay, B., *Chipper*, 50–52 (Jan 1975).
Porter, W. L., Siciliano, J., Krulick, S., Heisler, E. G., "Membrane Science and Technology", Flinn, J. E., Ed., Plenum Press, New York, N.Y., 1970.
Schneider, F., Ed., "Technologie des Zuckers", 2nd ed, Verein der Zuckerindustrie, Hannover, 1968, pp 983–989.
Schwartz, J. H., Krulick, S., Porter, W. L., *J. Sci. Food Agric.* **23**, 977 (1972).
Seifert, J., personal communication, 1974.
Smith, O., Report of PCII director of Research, Proceedings of Production and Technical Division Meetings, Potato Chip Institute International, 1966, p 11.
Stabile, R. L., Turkot, V. A., Aceto, N. C., Proceedings of the National Symposium on Food Processing and Wastes, Denver, Colo., 1971, p 185.
Strolle, E. O., Cording, J., Aceto, N. C., *J. Agric. Food Chem.* **21**(6), 974–977 (1973).
Vlasblom, M., Peters, H., Dutch Patent 87 150 (1957); German Patent 1 021 243 (1958).
Wilcox, R. L., *AIChE Symp. Ser.* **136**(70), 358 (1974).
Willard, M., 12th National Potato Conference, Bakersfield, Calif., 1962, pp 37–38.

Received for review November 13, 1975. Accepted June 14, 1976. Michigan Agricultural Experiment Station Journal Article No. 7351.