ABSTRACT

RECOVERY AND BIOLOGICAL VALUE OF PROTEIN FROM WASTE
EFFLUENT OF POTATO CHIP PROCESSING METHODS
OF RECOVERY AND BIOLOGICAL VALUE AS AFFECTED BY VARIETY AND TREATMENT

By

Erhard Meister-Clemons

Simple physical-chemical methods for the recovery of protein from waste effluent of potato processing were investigated. The nutritional value of precipitated protein of simulated waste effluent and from whole tubers of three varieties from which it was made was biologically and microbiologically assessed using weanling voles (Microtus pennsylvanicus) and Streptococcus zynogemes.

Heat application (98°) at pH 4 to 4.5 or adjustment of pH with $FeCl_3$ to pH 4 yielded highest amounts of recoverable protein. Acid treatment alone (pH 3 to 3.5) or addition of lime (to pH 11.5) followed by lowering the pH to 9 with either H_3PO_4 or $FeCl_3$ were almost as effective.

Sedimentation of protein was accomplished by settling for one hour or by centrifugation. There appeared little difference in the amount of protein removed by centrifugational forces up to 10,000 x G with only minimal gains from 10 to 40,000 x G.

Protein efficiency indices (PEI), determined in the vole assay, approached those of casein and were equal or better than PEI's of the whole

tubers. The microbiological assay gave similar results, with "biological values" ranging from 66 to 75. In both assays heat and/or acid treatment was least damaging to protein quality.

It was estimated that a third of the crude protein in the waste effluent or approximately 130 kg of dried protein/day could be easily recovered in an average potato chip plant. The quality of the protein was excellent.

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INTRODUCTION

World wide population growth; rising affluence; shortage of resources such as land, water, energy, and fertilizer; and symptoms of ecological overstress caused an international scarcity of major agricultural commodities. Besides social and economic improvements, the alleviation of food shortages and/or protein malnutrition by means of increasing crop yields and quality, developing new food sources, and reducing wastes is part of an integrated system to meet food demands. The traditional approach to increasing production has only limited scope in view of limiting resources. Controlling waste is vitally important. Land is lost through erosion; water through mismanagement; crops by degradation of disease, insects, and rodents; food through poor handling, storage, processing, and retailing.

The importance of potatoes in medieval societies of South America and Europe is history. Today, the potato ranks fourth as a food crop in the world, and second in the U. S. In this country, processed products account for 50% of the potatoes consumed. They help diversify one's diet and reduce preparation time.

Unfortunately, approximately 10-20% of the potato is lost during processing. This contributes to the pollution of public waters. It is in the interest of the public, as well as the potato industry, to recover as much of the valuable nutrients as possible and reduce waste discharge.

To see the problem of waste production, reclamation, and disposal in the right perspective one has to appreciate the following points: (1) It was the introduction of snack and convenience foods by the processing industry that brought the decline in potato consumption to a halt in the 1950's. (2) Potato processing plants are in business to produce an edible product, and are more concerned with creating less waste, than recovering it. (3) Economic exploitation of the waste stream may be possible by large plants, but perhaps only collectively by the smaller chip factories in municipal areas. (4) Types of waste vary within and between plants.

Equipment for the recovery of starch from potato processing water is available, but used only by one plant in Michigan. Potato processors and representatives of the potato starch industry met to discuss methods and their feasibility for collection of this by-product. A survey followed, indicating that some plants could recover up to 1.3 million pounds of starch per year.

This study was undertaken to investigate methods for the recovery of proteins from the waste stream of potato chip processing, and to evaluate the nutritional value of the recovered product.

CHAPTER 1

REVIEW OF LITERATURE

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I. Potatoes: General Considerations

Vegetable foods such as potatoes have been culturally associated with diet patterns of less affluent times and less affluent people. Per capita consumption is inversely related to the rising standard of living because of a shift to more expensive and less available animal food.

As a part of the anti-affluence image, potatoes are primarily thought of as "stomach fillers," being fattening and of low nutritional value. Although, in its fresh state, the potato contains approximately 2-3% crude protein, or 10-17% of the dry weight, it is equivalent or better than the major cereals. Kofranyi and Jekart (1967) reported that in human subjects potato protein is almost equivalent to whole egg protein and better than beef, tuna, whole milk, wheat flour, corn, rice, soybean, and kidney bean protein. Potatoes contain low amounts of fat, and enrich our diet with other essential nutrients such as minerals and vitamins.

A. Historical Background of Potato Processing

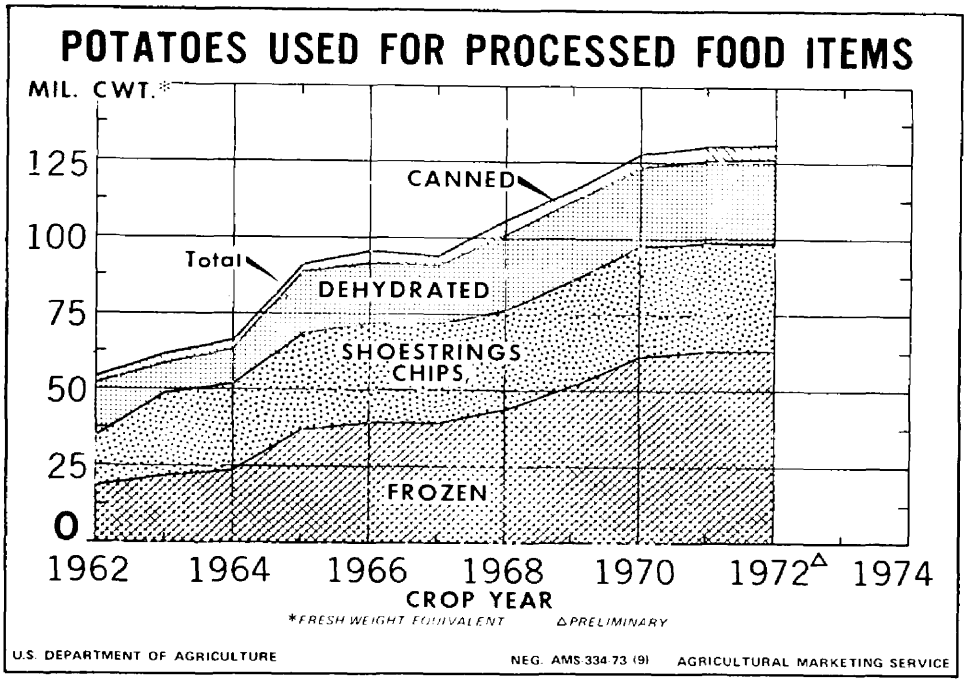
Potatoes in their original form are inconvenient and time consuming to prepare. Increasing involvement of women in activities outside of the household was partly responsible for the decline in potato consumption. The availability of processed snack and convenience foods by the mid fifties halted the decline. Consumption of fresh produce continued to decrease.

Potato processing dates back to at least 200 A.D. when the natives of Peru dehydrated potatoes by allowing them to freeze at night and thaw

during daylight hours, yielding a product called "chuño". In the U.S., potato processing has risen from practically nothing in 1940 to about 30% of all potatoes consumed in 1959 and 52% in 1971.

(Figure 1). Initially potato chips accounted for most of the processing while in 1971 frozen french fries represented the largest share with 40%, followed by chips with 26%, (50% in 1959), dehydration with 20%, starch manufacturing with 6%, and other frozen or canned products accounting for the rest, (American Potato Yearbook, 1974).

Figure 1



II. Pollution Problems of the Potato Industry

Eutrophication caused by discharge of human, agricultural and industrial wastes into rivers is a serious problem, both from an aesthetic and health point of view. Public pressure and concern by the government resulted in the Clean Water Restoration Act of 1972, which calls for elimination

of discharge into navigable waters by 1985 (Federal Register, 1973). The nutritive requirement for growth of algae are, in descending order: CO_2, N, P, K, Mg, Ca, S, Fe, to mention the major ones. Carbon can be biologically oxidized by bacteria. Nitrogen is generally removed with activiated sludge and/or anaerobic denitrification. Breaking the food chain by N-reduction is not particularly favored because of the ability of some algae, especially the objectionable blue-greens, to fix atmospheric nitrogen. A favored method to break the food chain is to remove P. Low cost acid salts of aluminum and iron are capable of reducing P to acceptable levels. Lime can be used to precipitate orthophosphates as calcium hydroxyapatite (Wilcox, 1974).

A. History of Pollution in the Potato Industry

The potato processing industry is one sector of the food industry where serious waste problems are caused by potential foodstuffs.

For many years, the potato industry grew at a very healthy rate and waste waters were dumped in nearby rivers. There was little concern about pollution since most of the larger plants producing frozen french fries or dehydrated products were located in sparsely populated areas. Not until the early sixties did problems become apparent in Maine. Municipal stabilization lagoons have been overloaded in the Red River Valley. Over 25,000 fish were killed in the Snake River of Idaho, and the water supply of the city of Twin Falls was jeopardized (Willard, 1962). These incidents brought the interested parties together for research in this area.

Because of the magnitude, the main effort was directed towards wastes from potato starch factories. In starch manufacturing all solubles, 27% of the dry weight of the potatoes, were dumped into the nearest stream. According to Xander and Hoover (1974) there are many plants processing a

million pounds a day, with a 5-day biochemical oxygen demand (BOD) equivalent to that of a city of 300,000 people. Because of the smaller size of most potato chip factories and the lower pollution potential, the problem seems to be minor, but finding a solution for the large number of plants may be more difficult.

B. Characterization of Processing Wastes from Chip Manufactoring

A composite of waste from a processing plant can be classified into four general categories: screenable, settleable, colloidal and soluble solids. Its composition is largely determined by the process by which it is produced. From a waste treatment point of view potato processing should be broken down in the following areas: (1) washing of unpeeled potatoes, (2) peeling and trimming, (3) slicing accompanied with washing and (4) frying. Therefore, the types of waste vary between; edible and non-edible; cooked and non-cooked; dissolved, fine pulped and particulate matter.

Dirt, silt, stones, and other foreign matter that result from washing of raw potatoes are readily removed by sedimentation in settling ponds (Dostal and Boydston, 1969). The collected solids can be used for land-fill. These wastes shall not be discussed further.

It has been estimated that up to 80% of the organic matter in the factory comes from the peeling process (Graham et al., 1969). Losses are minimized by choosing the best raw material and selecting appropriate peeling processes. Abrasive peeling, the method most widely used by the chip industry, results in effluent consisting of cell debris, granular starch, and soluble cell contents. New, large, round potatoes with smooth skins and shallow eyes produce the least waste (Greig, 1957). Peeling losses as high as 50% have been reported, but Smith (1968) considers losses of 10% as normal. Steam peeling results in lower losses, but the material is

cooked, starch is gelatinized and can no longer be removed by physical means (Dickinson, 1964). Lye peeling results in even lower losses and the waste composition is similar to steam peeling, except for the high concentration of sodium and the high pH (Harrington, 1957). Dry caustic peeling reduced peeling losses by up to 95% and the recovered by-product had a solid content of about 18-20% (Graham et al., 1969).

Washing slices yields a waste stream containing starch granules, nitrogenous constituents (see IV A), minerals, sugars and other solubles. Washing losses are roughly proportional to the new surface exposed by cutting, and are approximately 12% for potato chips compared with 3% for half-inch french fries (Hautela and Weaver, 1971).

III. Disposal and Utilization of Potato Wastes

Laws, bad experiences such as in the Snake River, environmental considerations to preserve these waters for food, water supply, and recreation no longer permit discharge of industrial and municipal wastes into streams, lakes, or tidal waters.

A. Biological Treatment of Wastes

Organic matter can be removed from a solution by micro-organisms through biological oxidation. Local factories may buy the right to dispose of their waste into municipal facilities (Dickinson, 1964), but this is an added financial burden on the potato chip industry. Activated sludge and aerated lagoons could be very effective for BOD removal (up to 95%) Buzell et al., (1964). Difficulties arose from BOD overload, especially during the winter months, which resulted in bad odors and the development of the purple sulphur bacteria, Chromaticium (Olson et al., 1964). In many urban areas, space is not available for large ponds to provide sufficient retention

times. Trickling filters or biotowers seem to offer an alternative (Sproul, 1964). The disadvantage of these conventional methods is that they are destructive, costly and return nothing.

B. Potato Waste for Fuel Production

Haeusler and Malcher (1972) report the successful application of methane fermentation, utilizing gasses given off by methanogenic organisms during anaerobic fermentation as fuel. Wet combustion of waste was proposed as a possible alternative by Hurwitz and Dunday (1960).

C. Agricultural Use of Waste

Dickinson (1964) reported that composting of potato solids with farmyard manure is practical, but the scale is obviously limited. Where sufficient agricultural areas with suitable soils are available, spray disposal is feasible (de Haan et al., 1971). Unswollen starch which does not readily decompose may interfere with consumption and digestion by grazing animals (Dickinson, 1964). Sodium content of caustic which can destroy colloidal structure of soils and pollute surface run-off and ground water are other concerns.

D. Waste Effluent as Culture Media for Micro-organisms

Selected strains of fungii can reduce nitrogen and phosphorous compounds in waste streams to low levels and produce a useful by-product. Waste effluent was used as substrate for: biosynthesis of protein with Candida utilis (Reiser, 1964) and Torula cremonis (Janicki et al., 1964); simultaneous production of protein and antibiotics with yeast and the mold Actinomyces (Wieczer, 1963); synthesis of vitamin B_{12} (Dietrich 1962), acetone, butanol and ethanol (Malcher, 1971); and for alcohol and yeast production (Borud, 1971).

E. Primary Treatment of Effluents for the Recovery of Solid Waste

Screening: Removal of rejected potatoes, trimmed potato fragments, eyes and sprouts is often an essential step not only from a waste treatment point of view, but it also protects pumps and other equipment. The effectiveness of screening is determined by the mesh size. The practical lower limit for mesh size is 6-7 for fixed screens, 120 for drum, 20 for disc and 40 for vibrating screens (Ballance, 1964). Screening will remove approximately 50% of the suspended and 90% of the settleable matter (US-Public Health Service, 1955).

Sedimentation: Settling of 95% of the suspension was achieved during a holding period of seven hours (US-Public Health Service, 1955). It is a very effective method of removing solids but has two major disadvantages: the sludge withdrawn has a dry matter content of only 2-4% and sedimentation tanks are bulky (Ballance, 1964). The sediment could easily be dewatered in a centrifuge to an easily handled product and was an excellent cattle feed (Kueneman, 1964). According to Olson et al. (1964) 5-day BOD is reduced by nearly 75% through primary treatment.

F. Recovery of Starch

Starch granules are insoluble in cold water, but may be suspended and form a slurry. Heating beyond a critical temperature (56-67^{o}C, Schoch and Maywald 1956), causes the granules to swell. This process is referred to as gelatinization, whereby the solubility increases markedly.

Starch granules can be removed by settling through either gravity or centrifigal forces. Commercial hydrocyclones designed for the size of potato chip plants are available, but mechanical separation no longer applies after starch has been gelatinized.

G. Physical-Chemical Treatment for the Recovery of Suspended and Dissolved Solids

Precipitation and recovery of proteins: Proteins, like amino acids, are ampholytes and their solubility is dependant on their functional groups, temperature, pH, ionic strength and the presence of organic solvents and is lowest at the isoelectric point. The solubility of globulin is markedly increased by low concentrations of neutral salts, a phenomenon called salting-in, but proteins are precipitated from aqueous solution by high concentrations. Poly-valent ions are more effective than mono-valent, and salting-out is more effective at the isoelectric point. Isoelectric precipitation of potato protein has been used by Neuberger and Sanger (1942), and Xander and Hoover (1959).

Many ions form insoluble salts with proteins and are used as precipitating agents. Acids such as phototungstic, trichloroacetic, picric, perchloric, etc. form insoluble salts with proteins when the latter are in cation form, and have been used for deproteinizing protein water (Neuberger and Sanger, 1942). The standard method for determining the amount of protein nitrogen is calculated from N precipitated by 10% TCA (AACC, 1967). Heavy metal ions are used for precipitating proteins on the alkaline side of their isoelectric point, but these compounds are of little use for recovery of proteins from a waste stream. Denaturation of proteins which is caused by a loss of its native conformation is generally accompanied with reduced solubility. Most proteins are denatured by heating, freezing-thawing, high concentration of urea, radiation and water miscible solvents. Of these methods heat denaturation for recovery of potato protein has been used by Neuberger and Sanger (1942). Heisler *et al*. (1959) precipitated protein by heating the "fruit juice" to 80°C. Borud (1971) achieved immediate flocculation of proteins by heat treatment of potato juice to 120°C.

Distillation: This process is based on vaporizing the liquid waste, leaving the dissolved solids in an enriched solution, and then condensing the water vapor. This process is more of theoretical interest since high temperature treatment of the waste is undesirable and evaporation under reduced pressure tends to be expensive, e.g. freeze-drying.

Freeze Crystallization: This is another method involving phase change and has been under development in the desalination industry, but has not yet been applied commercially (Probstein, 1972). The use of freezing as a concentration process is based on the fact that when ice is crystallized from an aqueous solution, the ice crystal is pure water, with all of the impurities left in the original solution. Thus, with suitable means to separate the ice from the mother liquid,pure water and high concentration waste could be recovered. Advantages would be: low energy consumption in comparison with distillation and less destruction of proteins and other compounds.

Reverse Osmosis: This process separates salt and other solids from water under the action of a hydrostatic pressure applied across a semipermeable membrane. Energy costs are quite low, but the economic limitation is related to the high cost of membranes and their maintenance. Application of reverse osmosis to wastes from potato starch manufacturing was investigated by Porter et al. (1970). Great difficulties were encountered in a pilot plant study from potato chip effluents by Seyfert (1974; pers. comm.).

Ion Exchange: This is based on absorption and removal of charged molecules by a resin from which they are eluted in a regeneration step. Recovery of free amino compounds from starch waste by ion exchange was investigated by Heisler et al. (1962), amino acids and potassium by

Heisler et al. (1972). Even though the total purification process reduced the chemical oxygen demand by 78% and total solids by 84%, Stabile et al. (1971) found that protein recovery followed by removal of other constituents using ion exchange is economically not feasible.

IV. Composition and Quality of Crude Potato Protein

A. Nitrogen Containing Substances of the Potato

In potato tubers crude protein (N x 6.25) is found in three morphological and physiological forms:

Structural proteins or bound proteins are part of the living cytoplasm and its organelles. They include nucleo- and lipo-proteins, proteins of mitochondria and amylo-plasts, etc. This portion is often referred to as insoluble protein or scleroprotein and it accounts for at least 7-10% of the total nitrogen (Neuberger and Sanger, 1942).

Crystalline proteins were found by some authors in the less starchy subperidermal tissue and in the pith of the tuber. These are deposits of protein crystals, formerly thought to be associated with virus disease. Varietal differences have been described by Hoelzl and Blancher (1959).

Cell sap proteins consist of soluble proteins, classified according to their solubility, and a non-protein fraction composed of short peptides, free amino acids, amides and minor constituents such as vitamins, nucleic acids, etc. This extractable nitrogen fraction, accounting for about 90% of the N, is the portion most extensively investigated. Protein accounts for 25-50% (Neuberger and Sanger, 1942), nucleic acids for 8-12% (Levitt, 1954), most of the remainder can be attributed to amides and amino acids.

Extractable protein. Osborne and Campbell (1896) suggested that the protein present in potatoes was a single substance, a globulin, which they called tuberin. Groot et al. (1947) described, in addition, an albumin now termed tuberinin. Further separation according to solubility by Lindner et al. (1960) yielded six protein fractions, tuberin accounting for more than three-fourths. In more recent work, Luescher (1972) found similar relative amounts.

The albumin particles are corpuscular and the globulin molecules are longitudinal, having a greater dissymetry, a lower solubility, and higher viscosity. The albumin is denatured when heated at 50-60°C. The viscosity thereby increases more than 100-fold, because of the loss of its globular structure (Jirgenson, 1946). He also suggests that tuberin and tuberinin are interconvertible by changing the pH. Acid treatment yielded more tuberin. Lindner et al. (1960) found a very similar composition of the two proteins, but Luescher's data suggests that they differ.

Separation of proteins using paper electrophoresis yielded six complex bands (Zwartz, 1966). Band heights were typical for varieties. Environmental components showed little or no effect, but virus infection drastically changed the pattern. Refined separation by acrylamide electrophoresis resulted in the separation of up to 25 proteins which could be used for variety identification (Loeschke and Stegemann, 1966).

Non-protein nitrogen is mainly composed of: short peptides; free amino acids with glutamic, aspartic, γ-aminobutyric and alanine being most abundant; amides, the principle ones being asparagine and glutamine. These free amino acids and amides have a key role in the nitrogen metabolism of plants as well as animals. Their relative amounts are

determined by the physiological stage of the tuber and are subject to changes by environmental conditions, namely nutrient availability (Mulder and Bakema, 1954).

B. Nutritional Value of the Nitrogenous Substances of the Potato

The nutritional value of crude potato protein compares favorably with other plant proteins. For 258 samples examined, the biological value ranged from 61-89. Low values were caused by either deficiency or surplus of nitrogen fertilization (Schupan, 1959). Nitrogen balance studies with human adults showed that potato protein was superior to most major plant protein and approached the value of whole egg (Kofranyi and Jekart, 1967).

Removal of the skin, that accounted for 7.6% of the dry weight and for about 10% of the total N, led to higher weight gains of growing rats and better N utilization (Chick and Slack, 1949). This difference could partly be explained through the lower digestibility of the insoluble nitrogen present in the skin. The non-protein fraction did not support growth, but gave, in combination with tuberin, a better growth than the latter by itself. This supplementary effect could not be explained in terms of their contents of essential amino acids.

The sulfur containing amino acids are the first to limit growth of animals in potato protein (Schupan, 1958). Supplementing potato protein with methionine increased both digestibility and weight gain of growing voles (Rios *et al*. 1972). Kies and Metzfox (1972) improved nitrogen balance in human subjects who consumed dehydrated potato flakes by adding methionine. Using *Streptococcus zymogenes*, Luescher (1972) found in a seedling population that crude protein was negatively correlated with

both methionine (r = -.45) and the biological value (r = -.55). Most of the variability in the methionine was in the non-protein fraction. Hoff et al. (1971) found that as the total nitrogen content of a variety increased, non-protein N went up but methionine decreased.

C. Protein Evaluation Methods

Determination of protein content in foods is based on measurement of the nitrogen content. There are several methods available, but the Kjeldahl method is most widely used and is standardized (AOAC, 1970). Crude protein is estimated by multiplying the amount of nitrogen by 6.25.

The nutritive value is determined by the amount and the relative availability of the amino acids. Speedy and accurate methods are available for measuring amino acid composition (Speckmann et al., 1956), but they do not indicate the availability. Microorganisms have been used by Luescher (1972), voles by Rios et al., (1972), rats by Peare (1973), and human subjects by Kofranyi et al., (1967) to assess protein quality of potatoes.

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CHAPTER 2

PHYSICAL-CHEMICAL METHODS FOR THE RECOVERY OF PROTEIN FROM WASTE EFFLUENT OF POTATO CHIP PROCESSING

PHYSICAL-CHEMICAL METHODS FOR THE RECOVERY OF PROTEIN FROM WASTE EFFLUENT OF POTATO CHIP PROCESSING[1]

Erhard Meister and Norman R. Thompson[2]

ABSTRACT

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 18.4 metric tons of potatoes per week, could recover approximately 170 kg of dried potato protein (550 kg of food containing 30% of protein). Sedimentation of protein in settling tanks for 60 minutes yielded, after appropriate treatments, the same amounts of recoverable protein as centrifugation at speeds below 10,000 x G. Drum drying of precipitate appears to be the most suitable method to produce a concentrated feed. Improved recovery can be expected from higher protein concentrations, thus use of a hydrocyclone to remove starch granules and recycling of water would be advantageous. Application of heat at pH 4-4.5 was generally more efficient for protein recovery, but lowering the pH to 3-3.5 with no heat gave similar results. Protein yields were improved if the waste was kept in motion during floc formation. Total dry matter reduction was highest if no heat was applied. $FeCl_3$ (at pH 4) was a slightly more effective precipitating agent than either HCl or H_3PO_4.

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From an environmental point of view the latter is not very desirable. Protein recovery was similar when pH was raised to 11.5 and then lowered with either H_3PO_4 or $FeCl_3$ to pH 9, but large amounts of chemicals were required. The composition of the nitrogenous compounds extracted is dependent on the type of water used. This also determines the efficiency of protein recovery. Approximately 30-40% of the crude protein or 80-90% of the coagulable protein, presently wasted, could easily be recovered by any one of the above procedures.

INTRODUCTION

The potato processing industry is one sector of the food industry where serious waste problems are caused by potential food stuffs. Michigans potato chip manufacturers are searching for economical solutions to minimize losses and to meet local and federal standards for the effluent discharged. The Clean Water Restoration Act of 1972 calls for elimination of waste discharged into navigable water by 1985 (Federal Register, 1973).

Most of the potato chip plants are located in municipal 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, primarily from washing of tubers and potato slices, is still inadequate. The waste effluent contains approximately 50% starch and 30% crude protein.

Most of the work on potato waste has been done on effluent from starch manufacturing where the problem is magnified because of the larger size of the factories and only starch is retained and the other solids wasted. In Europe, a process for the recovery of protein has been patented (Vlasblom and Peters, 1958). A starch factory eliminated its waste by a process that called for an investment of 17 million German Marks, of which the Dutch government paid 11 million. The U.S.D.A. investigated the application of ion exchange for the recovery of amino acids, proteins and potassium (Heisler et al. (1972) and organic acids and phosphate (Schwartz et al. (1972). But 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 (Seyfert, 1974, person. comm.). In the small potato chip plants simple methods for by-product recovery are required. Both commercial hydrocyclones for the recovery of starch and a market for the product are available (Pettay, 1975). It was the purpose of this study to examine optimal conditions for recovery of proteins by simple means. Special attention is given to heat treatments, since waste heat generated by the cooker could be recovered in a heat exchanger.

MATERIALS AND METHODS

I. Preparation of Protein Water

Preliminary work was carried out on processing water received from a Detroit potato chip factory. Because of the inconvenience of transporting a dilute solution a long distance and possible compositional changes, it was decided to simulate processing water in the laboratory by preparation of a dilute potato extract. Potatoes were washed thoroughly, peeled and ground in a Waring Blender. The slurry was diluted with tap water (composition, see Table 1) to 10 times its volume. Initially, the resulting juice was centrifuged at 2000 x G for five minutes, the supernatant filtered through paper napkins to remove suspended cell debris and kept at 10° . It was later found that filtering the juice through several layers of cheesecloth followed by settling for 30 minutes produced protein water of a composition similar to the samples obtained from processing plant A (see Table 2).

The potatoes used were Russet Burbanks grown on the Montcalm Experiment Station during the 1974 season. Tubers were kept in cold storage until utilized.

II. Analytical Procedures

The dry matter of the samples was determined by the official AOAC vacuum oven method (AOAC, 1970). The vacuum oven was operated for 12 hours at 70° under partial vacuum.

The nitrogen content of the samples was measured by the official AOAC micro-Kjeldahl method (AOAC, 1970).

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

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

III. Separation of precipitates

Initial attempts to filter the slurries with several size filters were fruitless since starch and protein floc immediately plugged the cloth.

Gravity settling. The influence of concentration on settling times of samples treated with or without heat was investigated. Protein water was prepared as described above with the exception that slurries were diluted either five or ten times their volume to give different concentrations. The pH of the protein water was adjusted with 2N HCl

to pH 4 and one half was heated to boiling (98°) 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 ten minute intervals and analyzed for nitrogen and dry matter. This was repeated twice.

Centrifugation. Separation of protein by 7 different centrifugational forces for 15 minutes was compared with gravity settling for 1 hour. Two different extracts of protein water were heated to 98° at pH 4. Duplicate samples were centrifuged in a laboratory centrifuge (Sorvall Superspeed RC-2) at speeds of 2, 4, 6, 10, 20, 30, and 40,000 x G and residual nitrogen in the supernatant was determined.

IV. Treatments for protein precipitation.

Gravity settling for 1 hour was chosen for the evaluation of each treatment combination. Aliquots of the supernatant were withdrawn with a syringe after 1 hour of sedimentation, placed in screwcap bottles and stored in a refrigerator for analysis.

Heat versus 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 protein water was adjusted with 2N HCl or 2N H_3PO_4 to 8 different pH levels while stirring with a magnetic stirrer. Four 100 ml aliquots of protein water of the different pH levels were placed in Erlenmeyer flasks. They were assigned to water baths of 23°, 60°, 80° and 98°, and heated under a low shaking motion. They were immediatly cooled

in ice water after reaching the desired temperature and allowed to settle.

The same procedure was followed with protein water obtained from a distilled water potato extract, but only temperatures of 23° and 98° were compared.

$FeCl_3$ versus HCl as coagulant. The pH's of 100 ml samples of protein water was adjusted with either 1M $FeCl_3$ or 2N HCl over the same pH range. Samples were agitated for 20 minutes in a shaker at 23° before they were subjected to sedimentation.

Lime, H_3PO_4 and $FeCl_3$ treatment. A newly prepared slurry of CaO and distilled water was used to gradually raise the pH of 3 liters of protein water to pH 12. This was followed by lowering the pH with either 2N H_3PO_4 or 1M $FeCl_3$. 100 ml aliquots were withdrawn at intermediate pH's and subjected to settling.

RESULTS AND DISCUSSION

Two local potato chip plants co-operated in the conduct of this research providing several samples of typical effluents from their plants. Table 1 gives the average dry matter and crude protein content of the samples. Values for the total dry matter content for plant B are of the same magnitude as reported in the literature (Willard et al., 1962). The higher values in plant A are attributable to the use of a hydrocyclone which removes the bulk of the starch and allows partial recycling of effluent. In the light of reduced waste discharge the latter system would be more desirable. Effluent of plant A was

Table 1. Chemical analysis of the water taken from Michigan University campus wells*

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	.03	.46	.73	.01

* Data: D'Itri (1973)

Table 2. Average composition of potato effluent of two local potato chip plants.

processing plant	sample*	dry matter mg/liter	crude protein mg/liter
A	1	11200	4307
	2	8400	2960
	3	9400	3524
	4	10600	3360
B	5	3310	1578
	6	1725	771
	7	1550	547
	8	2100	895

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

simulated for the study of protein recovery.

Smith (c.f. 1968) reports that an average potato plant processes approximately 18.4 metric tons of potatoes per week and uses 460,000 liters of water per day. Assuming that the waste composition of plant B is typical, one can calculate the daily loss of protein per day which amounts to about 385 kg of crude protein, of which approximately a 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 5832 ppm. on the average were acidified, heated to 98° and immediately cooled in ice water. Of 3582 ppm. protein precipitated by 10% TCA, approximately 84% was removed at speeds of 2 to 10,000 x G, 87%, 94%, and 99% settled at 20, 30, and 40,000 x G respectively. The lower centrifugational forces did not yield any appreciable amounts beyond the 82% which was achieved by gravity settling for 60 minutes. The latter yielded a white slurry that could easily be drained off and contained from 6-8.5% dry matter, whereas a pasty cake (15-20% DM) was recovered by centrifugation.

Drying studies with similar wastes from potato starch manufacturing (Strolle et al., 1973) gave good results with double drum drying, where freeze drying would be too expensive and air drying in a conventional tray dryer 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 centrifugational forces would yield a product suitable for drum drying. The slurry from settling would require additional concentration but its volume is about twenty times smaller than the original volume of the waste.

Depending on the individual case, settling or centrifugation might be preferred. In this study separation of protein by settling was chosen because of the large number of samples that had to be treated simultaneously.

Effect of concentration and heat treatment on settling.

Residual crude protein measured over a time span of 80 minutes is given in Table 3. Sedimentation was faster in heat treated samples, but differences were not significant beyond 60 minutes. The significant interaction, time x treatment, indicates that initial sedimentation was faster at low protein concentrations 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 using a hydrocyclone for starch recovery but protein was not recovered.

Table 3. Residual crude protein (ppm) in solution after gravity settling of protein from waste water of low and high protein concentration at pH 4, with and without heat treatment.*

settling time minutes	high concentration 23°C	high concentration 98°C	low concentration 23°C	low concentration 98°C
0	8333 a,A	8337 a,A	4232 a,A	4232 a,A
10	7310 b,A	7107 b,A	3672 b,A	3607 b,A
20	6594 c,A	6054 c,A	3297 c,A	3111 c,A
30	6169 d,A	5631 d,B	3001 bc,A	2829 cd,A
40	5783 e,A	5092 e,B	2801 c,A	2623 de,A
50	5497 f,A	4807 e,B	2742 c,A	2503 de,A
60	5088 fg,A	4838 e,A	2642 c,A	2379 e,A
70	5049 g,A	4827 e,A	2496 d,A	2355 e,A
80	4949 g,A	4875 e,A	2475 d,A	2367 e,A
TCA standard	3898		1874	

* average value of two replications

Studentized range test:

- time comparisons within treatments (within columns):

  Values with the same letter (a,b . . .) are not significantly different.

- temperature means within time and concentration (between columns):

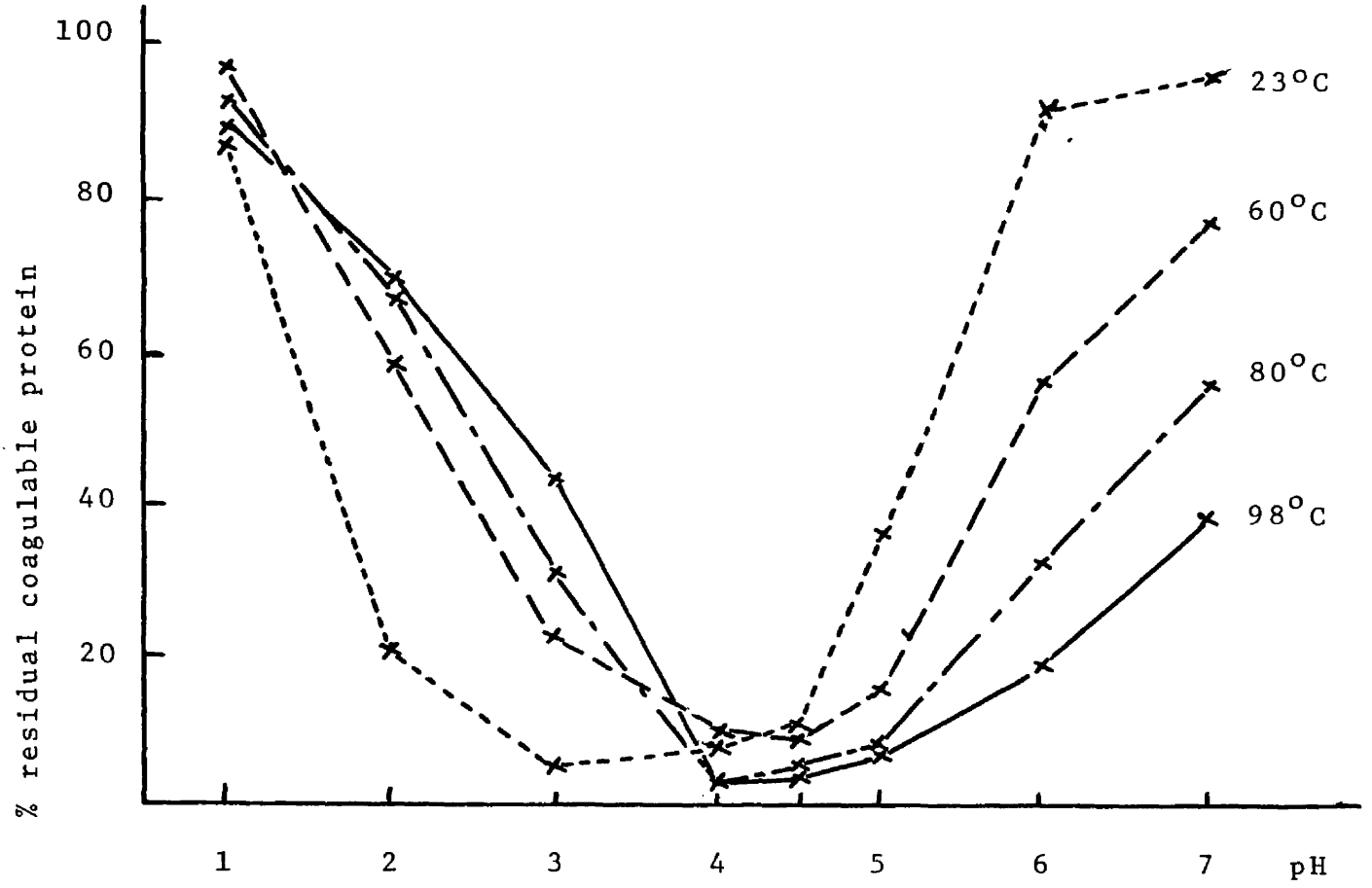
  Values with the same letter (A) are not significantly different.

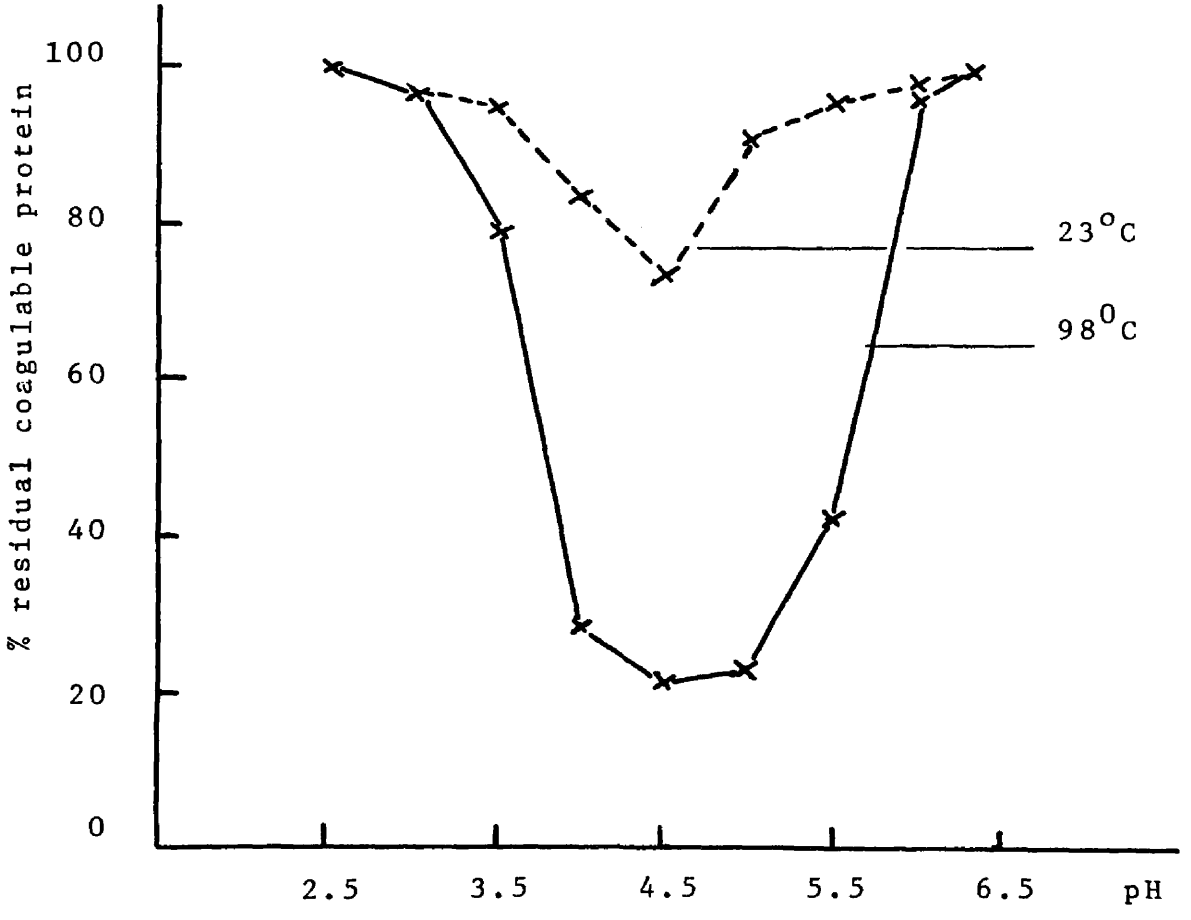
Heat versus pH treatment.

The solubility of proteins is markedly influenced by the pH and is minimum at the iso-electric 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 TCA in precipitating protein. Neuberger and Sanger (1942) compared TCA and heat with NaCl at 80° and found that TCA was slightly more effective. Efficiency was improved at higher NaCl concentration. Preliminary work with neutral salts (NaCl, $(NH_4)_2SO_4$, Na_2SO_4) was not very successful at low salt concentrations and high concentrations are not justifiable in recovery of proteins from a waste stream. The curves in Figure 1 indicate an optimal pH range for protein precipitation from pH 3.5-4.5. The highest temperature was most effective at almost all pH's except the very low ones. The temperature effect was most pronounced at neutral pH and was negligible around pH 4. At higher acidities, room temperature yielded better results. Eighty degrees was included because Heisler et al., (1959) reported that the simplest method of carrying out precipitation was by heating to 80° or acidification to pH 3.0 or slightly below. These data suggest that neither treatment gave 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. His data did not show the optimal pH range observed here.

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

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


100
80
60
40
20
% residual coagulable protein
23°C
60°C
80°C
98°C
1
2
3
4
5
6
7
pH


100
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% residual coagulable protein
23°C
98°C
2.5
3.5
4.5
5.5
6.5
pH

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 ($\sim$ 3.5) good results could be achieved at low temperatures. Aggregation was much improved through the slight shaking motion during floc formation and resulted in faster and better settling.

Using distilled water in the initial simulation of potato waste effluent, a rather surprising observation was made. Sedimentation was rather poor, especially when no heat was applied (Figure 2). These results did not agree at all with the observations made on actual waste water. This suggested the investigation of possible differences in the composition of the proteins extracted (see below) but it also points out the difficulties with comparisons of results of different sources.

If HCl was substituted for H_3PO_4 results were almost identical and are not given. From a cost point of view as well as considerations of adding H_3PO_4 to public water HCl should be preferred.

If one looks only at the protein recovered, heating of the protein water after acidification with HCl appears to be the best procedure. But the decision becomes even more difficult if one also looks at the total dry weight recovered. The original solution contained a total of 14,700 ppm solids of which 4,705 ppm was crude protein. After heating to 98° at pH 4 and separation of the precipitate, 2,744 ppm crude protein and 7,894 ppm solids remained in the

solution. Acid treatment at pH 3 reduced the residual protein to 2,905 ppm but the solids were decreased to 6,853 ppm. It is assumed that this difference in reduction of total solids is primarily due to starch. Potato starch gelatinizes above 50-67° (Schoch and Maywald, 1956), thereby yielding a dispersion of granule fragments, starch aggregates and molecules, which do not settle as readily but contribute to the pollution problem. If recovered, starch could be a valuable feed constituent.

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.

Composition of protein of water extracts and whole tubers.

The major difference between the nitrogenous components in distilled and tap water extracts versus those in the original tubers is the increased non-protein-N in the extracts (Table 4). Of the protein fractions, the water soluble tuberinin and the unknown nitrogen compounds were increased most. 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 but more tuberinin and non-protein-N. It appears that the different response of the two extracts to protein precipitation can be explained partly by the different compositions of the extracts. The distilled water extract contained more of the highly soluble fractions.

Table 4. 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 (12% TCA)	49.8	71.3	58.2

[a]Sample size 60 gm, 116 mg crude protein/gm

[b]Sample size 15 gm, 469 mg crude protein/gm

[c]Sample size 20 gm, 321 mg crude protein/gm

Comparison of ferric chloride and HCl.

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, 1973). Table 5 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. Amine et al. (1972) found in chicken assays that reduced iron ranked second only to ferrous sulfate in efficiency as an iron supplement.

Lime, H_3PO_4 and $FeCl_3$ treatment.

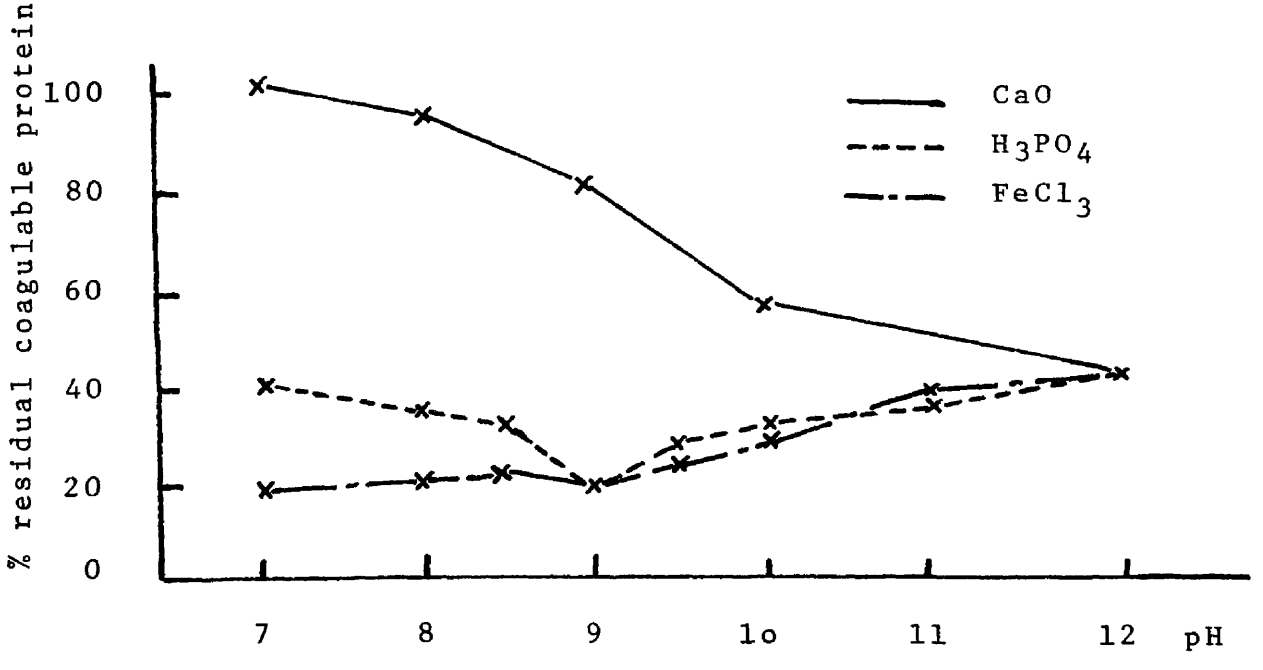
In the sugar beet industry, the Steffen process, using a combination of lime and H_3PO_4 for the recovery of protein has been widely used (Schneider, 1968). Figure 3 summarizes the data of this experiment. Protein yields are continuously increased as the pH is raised to pH 12 and then lowered by H_3PO_4 or $FeCl_3$. The data indicates that lowering the pH to 9.0 with either $FeCl_3$ or H_3PO_4 yielded the highest quantities of recoverable protein. 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, phosphorus can

Table 5. Residual crude protein (ppm) in solution after treatment of protein water with ferric chloride and HCl respectively.

pH	HCl	$FeCl_3$
6.0	3101	2659
5.0	2707	2264
4.0	2501	2167
3.0	2382	2228
2.0	2584	2652
1.0	3024	3207
original concentration	3456	3468
TCA standard	1982	2012

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



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 phosphorous in the effluent stream after the above treatment.

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CHAPTER 3

PROTEIN QUALITY OF PRECIPITATE FROM WASTE EFFLUENT OF POTATO CHIP PROCESSING MEASURED BY BIOLOGICAL METHODS

PROTEIN QUALITY OF PRECIPITATE FROM WASTE EFFLUENT OF POTATO CHIP PROCESSING MEASURED BY BIOLOGICAL METHODS[1]

Erhard Meister and Norman R. Thompson[2]

ABSTRACT

The nutritional value of precipitated protein of simulated waste effluent and of crude protein of whole tubers was biologically and micro-biologically assessed using weanling voles (Microtus pennsylvanicus) and Streptococcus zymogenes. The nutritional value of the protein fraction from Sebago was superior to Russet Burbank and an unidentified variety received from a potato chip plant. The protein efficiency index (PEI) for Sebago was not significantly different from casein. PEI's for precipitated protein were higher than for whole tubers for the latter two varieties but not for Sebago. No differences in PEI's were found between samples heated to 98° and those treated at room temperature. The "biological values" determined by S. zymogenes followed the same pattern for both varietal and treatment differences, giving highest values for Sebago protein. No differences were found between heat and acid (HCl or $FeCl_3$) treatment but values for whole tubers and samples treated with lime and H_3PO_4 or $FeCl_3$ were all lower. This paper demonstrates that a potato chip plant could reduce water consumption and discharged waste by relatively simple means and obtain

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a high quality feed containing approximately 30% protein with a high biological value.

INTRODUCTION

Michigans potato chip industry is searching for methods to reduce processing losses and to economically exploit the waste effluent. After removal of primary wastes by screening, starch accounts for approximately 50% of what remains. A sizable portion can easily be removed with hydrocyclones. The product can be marketed in either dry or wet form (Pettay, 1975). Simple procedures for the recovery of proteins, accounting for 1/3 to 1/2 of the nitrogenous substances in the effluent, were reported by Meister and Thompson (1975). Several treatment combinations reduced the protein in the effluent by 85 to 95%. The recovered material contained approximately 30% protein.

Proteins are subject to alterations by both physical and chemical treatments. These and the source of the protein may affect their nutritional value. It is important to select a procedure for waste reclamation that is least damaging to the protein to obtain a high quality product.

Nitrogen balance studies with human adults showed that potato protein was superior to most major plant proteins and it approached the value of whole egg (Kofranyi and Jekart, 1967). The sulfur containing amino acids are first limiting in potato protein (Schupan, 1958). Weight gains of growing voles were dependent on the potato variety fed and were improved with methionine supplementation (Rios

et al. 1972). "Available methionine" and a "biological" value of potato protein was evaluated with Streptococcus zymogenous (Luescher, 1972). The same organism was used by Ford (1962) to study heat damage to different proteins.

The purpose of this study was to examine the effect of cultivar differences and methods for protein precipitation on the biological value of the recovered potato protein.

MATERIALS AND METHODS

Two cultivars, Russet Burbank and Sebago grown on the Montcalm Experiment Station during the 1974 season and an unidentified variety from a Detroit chip plant were selected for this study. Whole tuber, heat and acid precipitate of each variety were tested and compared to casein as a standard. Abrasive peel from the unknown variety was included as an additional protein source.

Preparation of samples for vole diet.

For whole tuber diets, average size tubers of each variety were selected, washed, autoclaved at 15 p.s.i. for 15 minutes, peeled, sliced and freeze-dried. To simulate waste water, Russet Burbank and Sebago tubers were washed, ground for 10 minutes in a Waring blender, the mash diluted with water to five times its volume, filtered through several layers of cheese cloth and left for 30 minutes to settle the starch. Half of each diluted extract and half of a water sample received from Detroit were acidified with HCl to pH 3.0, samples were stirred for 15 minutes then subjected to 90 minutes of sedimentation.

The other half of each sample was heated to 98° after adjustment of the pH to 4.5, this was followed by immediate cooling in ice water and settling. The peel and the acid precipitates were heated in a waterbath for 15 minutes at 60° to gelatinize the starch. All samples were freeze-dried.

Protein evaluation by animal assay.

In this study meadow voles (Microtus pennsylvanicus) were selected as experimental animals because of their rapid growth and small food requirements.[3] Diets were made up according to methods described by Elliott (1963) and (Shenk and Elliott, 1969). Composition of control and experimental diets is given in Table 1. Freeze dried samples were ground in a Wiley Mill to pass a 40 mesh sieve, and assay diets made up to contain seven percent protein.

In the feeding experiments, weanling voles 12-14 days old and weighing from 12.5-16.0 grams were used. For two days they received a starter diet followed by the experimental diets over a six day period. Food and water were available ad libitum. The animals were closely observed during the feeding trial and weights taken initially and every two days thereafter. The voles were housed individually in plastic-bottomed cages with corncob bedding and non-absorbent cotton for nesting.

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[3]Litters of weanling voles were received from Dr. Elliott, Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan 48824.

Diets were mixed with the amount of water necessary to make a dough-like consistency, then molded into wafers that would fit the vole feeder. They were dried at 105°F for 48 hours, wrapped in aluminum foil and stored at -18° until needed. They were thawed at room temperature for 16-18 hours before weighing and feeding. The moisture content of an aliquot was determined using the vacuum oven method (AOAC, 1970). The food consumption was determined by the loss in weight of the entire feeder.

The experimental design was a randomized block design. The most uniform voles of two litters of the same harem were randomly assigned to the eleven diets forming one block and this was repeated 6 times. At the end of the feeding period, protein efficiency indices (PEI) were computed from gain and food intake.

Protein evaluation by Streptococcus zymogenes.

The "biological" value of protein was assessed microbiologically. Ford's procedure, adapted for potato protein by Luescher (1971) and Peare (1973), was followed. Casein was used as a standard.

Samples containing the equivalent of 50 mg crude protein were placed into 4-ounce screw-cap bottles, 20 ml citrate cyanide buffer was added, and the pH adjusted to 7.2 with 1 N KOH. The samples were heated in a water bath to 56° for 3 hours with intermittent shaking and adjustment of the pH to 7.2, they were diluted to 100 ml with distilled water.

Triplicate portions of 4 ml of the digest were pipetted into 16 x 150 ml test tubes. Two ml of basal medium (see Luescher, 1971) was added and the volume brought to 10 ml with distilled water. After capping each tube, samples were sterilized with flowing steam for 12 minutes, and after cooling one drop of inoculum culture [4], diluted 1:10 with sterilized .85% saline solution, was added and tubes were incubated for 48 hours at 37°.

After incubation, tubes were heated in flowing steam for 10 minutes, stoppered and shaken vigorously, and set aside for 30 seconds. The optical densities of the cultures were then measured with a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer at 580 mμ. The "biological value" of the sample was expressed in percent of growth compared to a tube containing the same amount of casein protein.

The above procedure was carried out on two consecutive days and average values for absorption on each day were used to calculate the "biological value."

Preparation of samples for microbiological assays.

Five tubers of each of the three varieties were chosen at random, four longitudinal slices were cut from the middle of each tuber, quickly frozen, combined and freeze-dried.

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[4]The organism used for these tests was Streptococcus zymogenes NCDO 592, obtained from the National Collection of Dairy Organism, Institute for Research in Dairying, Shinefield, Reading (UK).

Dilute potato extracts were made as described. Aliquot samples were treated as described below:

(a) Acidification with HCl to pH 3.0.

(b) Acidification with HCl to pH 4.5, heated to 98°.

(c) Acidification with $FeCl_3$ to pH 4.0.

(d) Raising pH to 11.5 with lime followed by adjustment to pH 9 with H_3PO_4.

(e) Raising pH to 11.5 with lime followed by adjustment to pH 9 with $FeCl_3$.

All sediments were dialized in cellophane bags[5] for 48 hours to avoid interferences with the assay. The samples were freeze dried, ground in a Wiley mill through a sixty mesh screen and the nitrogen content was determined by the micro-Kjeldahl method (AOAC, 1970).

RESULTS AND DISCUSSION

The protein quality of whole tubers, heat and acid precipitates of three varieties, and a sample of abrasive peel were compared with casein in a feeding trial with weanling voles. A description and the exact composition of the diets is given in Table 1. The analysis of variance for just the three treatments of the three cultivars indicated that treatment effects were less pronounced than varietal differences (Table 2). Food intakes, weight gains and protein efficiency indices (PEI's) are summarized in Table 2. Food consumption was the same for all diets, although differences were found between litters. Examination of the raw data showed that two litters with high initial

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[5] Number 27/100 was obtained from Union Carbide.

Table 1. Diet Composition* (%)

Diets	protein source[a] g	mineral salt[b] g	vitamin mixture[c] g	corn oil g	fiber[d] g	honey g	sugar g	starch[e] g
Casein	8.0	3	2	2.0	20.0	8	8.5	49.5
Burbank tubers	64.8	3	2	1.7	18.5	8	3.5	1.7
Burbank heat prec.	25.7	3	2	2.0	20.0	8	8.5	32.1
Burbank acid prec.	24.1	3	2	2.0	20.0	8	8.5	33.1
Sebago tubers	58.3	3	2	1.7	18.6	8	3.5	7.5
Sebago heat prec.	20.0	3	2	2.0	20.0	8	8.5	37.5
Sebago acid prec.	23.5	3	2	2.0	20.0	8	8.5	33.5
Unknown tubers	46.7	3	2	1.8	18.9	8	3.5	15.2
Unknown heat prec.	21.6	3	2	2.0	20.0	8	8.5	34.9
Unknown acid prec.	24.6	3	2	2.0	20.0	8	8.5	31.9
Unknown peel	58.3	3	2	1.8	13.7	8	8.5	6.8

* adjusted to 7% crude protein.

[a]Protein content of the different sources was: 87%, 10.8%, 27.2%, 12%, 35%, 15.1%, 32.4%, 28.4%, 12%
[b]Salt Mixture W, Nutritional Biochemicals Corp., Cleveland, Ohio.
[c]Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corp., Cleveland, Ohio.
[d]Alpha-cellulose, Nutritional Biochemical Corp., Cleveland, Ohio.
[e]potato starch: cooked, oven-dried and ground.

body weights (14-15-5 g) ate more than the others, but the weight gains were not significantly different. Consequently, PEI's of litters varied considerably. PEI values are in the same range as protein efficiency ratios (PER) found for potato protein in rat feeding trials by Peare (1972). They are much higher than reported for voles by Rios et al. (1972) who fed diets containing 5.28% crude protein, considered to be below the level for minimal growth. PEI values were highest for casein but protein of Sebago compared favorably with it. Values for the other two varieties were lower, especially of whole tubers. With the exception of the heat precipitate of Sebago (SH), PEI's of protein recovered by either method of precipitation were equal or superior to crude protein of whole tubers. Chick and Cutting, (1943) and Slack (1948) found that nonprotein nitrogen alone did not support growth of weanling rats and that tuberin prepared from the sap by heating at 80° at pH 4 was not superior to that of the mixture of protein and non-protein in the whole potato. Only the data from Sebago are in accordance with these results. These findings suggest that the nutritional value of the protein fraction of Sebago is superior to Russet Burbank and the unidentified variety. The presumably better balanced protein of Sebago could compensate for the lower nutritive value of the non-protein fraction. Evidence for such an interpretation is given by the higher PEI's of Sebago protein to protein of the other precipitates, and the fact that PEI's for recovered protein were equal to crude protein of whole tubers for Sebago but not for the other

Table 2. Comparison of litter, variety and treatment effects of vole feeding trial.

Analysis of variance for PEI

source	df	SS	MS	F
litter	5	2.330	.466	3.531*
variety	2	4.066	2.032	15.403**
error (a)	10	1.319	.132	
treatment	2	1.547	.774	7.075*
var. x tmt.	4	1.905	.476	4.355**
error (b)	30	3.277	.109	
total	53	14.445		

*, **, *** significant at P = .05, .01, .001 respectively.

varieties. The relative amount of non-protein N was the same for all varieties (approximately 54-60%).

The abrasive peel from the unidentified variety did not support growth (Table 3). Chick and Slack (1949) have given evidence that removal of the skin and outer cortex, which are the parts richest in "insoluble protein" increased the nutritive value of the remainder in a rat feeding trial. In these trials with field voles, salvage and processing of the skin into a feed is not justifiable because of its low nutritional value.

It was anticipated that the different procedures employed to precipitate the proteins might reduce the nutritional value of the protein. Two major sources of spoilage were expected to occur: (1) non-enzymatic browning (Maillard reaction, 1912) and (2) structural changes such as denaturation and aggregation. The former involves condensation between aldehyde groups of reducing sugars and free amino groups, primarily the ϵ-amino groups of lysine, a reaction that increases rapidly at higher temperatures. The data from the vole feeding trial did not reveal any appreciable differences between heat and no heat treatment. The high moisture content and low pH during heating could have slowed dwn the reaction. Also differences may not have appeared because the second sample was heated (60°) to gelatinize the starch.

Streptoccus zymogenes was considered an ideal organism to test the different varietal and treatment differences. Besides reduced

Table 3. Food intake, weight gain and protein efficiency index (PEI) of eleven diets tested in vole feeding trial. Average values of six voles per diet.

Diets	Food intake g/6 days	Weight gain g/6 days	PEI
Casein	30.0	6.25 a	3.02 a
Burbank tubers	35.8	4.75 cd	1.89 e
Burbank heat prec.	35.5	5.31 bc	2.19 c
Burbank acid prec.	28.4	4.13 de	2.07 c
Sebago tubers	35.3	5.98 ab	2.51 b
Sebago heat prec.	40.3	6.26 a	2.21 c
Sebago acid prec.	28.2	5.82 ab	2.96 ab
Unknown tubers	32.2	3.93 e	1.81 e
Unknown heat prec.	33.8	4.93 c	2.09 c
Unknown acid prec.	35.4	5.41 bc	2.18 c
Unknown peel	34.7	.06 f	.02 d

Studentized range test:

Values with the same letter are not significantly different at the 5% level.

availability of lysine, Ford (1962) demonstrated significant reduction of available arginine, histidine, methionine, valine, leucine, isoleucine, and tryptophan of heated milk and fish protein using the above bacteria. Luescher (1972) successfully used the same organism to discriminate between available methionine of different potato cultivars. The variety Sebago gave a higher biological value for almost all treatments (Table 4). Highest biological values were observed by precipitates of treatment with HCl alone, HCl plus heat and $FeCl_3$. Treatment with H_3PO_4 or $FeCl_3$ subsequent to coagulation with lime gave lowest values, but not different from whole tubers.

Table 4. "Biological value" of crude protein of three potato varieties subjected to six treatments, determined by S. zymogenes.

Treatment	Russet Burbank	Sebago	Unknown
whole tubers	67 D	70 ED	68 CD
[a]heat precipitate	70 CD	75 AB	69 CD
[b]acid precipitate	72 BC	77 A	69 CD
[c]$FeCl_3$ precipitate	72 BC	74 AB	69 CD
[d]CaO and H_3PO_4 prec.	66 D	68 CD	67 D
[e]CaO and $TeCl_3$ prec.	68 C	68 CD	67 D

Studentized range test:

Values with the same letter (A, B, . . .) are not significantly different at the 5% level.

[a]heated to 98° after acidifying with 2N HCl to pH 4.5

[b]sedimentation at 23° after acidifying with 2N HCl to pH 3.0

[c]sedimentation at 23° after acidifying with 2M $FeCl_3$ to pH 4.5

[d]sedimentation at 23° after raising pH to 11.5 with CaO, followed by lowering it to pH 9.0 with H_3PO_4

[e]sedimentation at 23° after raising pH to 11.5 with CaO, followed by lowering to pH 9.0 with H_3PO_4

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A P P E N D I C E S

Appendix 1

Sedimentation of protein from tap water extract of Russet Burbank tubers after heating to 98°C at pH 4.5 by gravity settling and 7 different centrifugational forces. Randomized block design with 2 replications, 8 treatments with duplicate observations per treatment.

rep	gravity	centrifugational forces in 1000 x G						
		2	4	6	10	20	30	40
1	3075	2985	3000	2977	3030	2917	2602	2422
2	2705	2650	2590	2575	2553	2482	2311	2150
mean	2890	2817	2795	2776	2791	2699	2456	2286

Studentized range test:

Underlined means are not significantly different at the 5% level, using the studentized multiple range test.

Analysis of variance

source	SS	df	MS	F
replication	1120504	1	1120504	223.178***
treatment	1210124	7	172874	34.432***
rep x tmt	35144	7	5021	
sample	4168	16		
total	2369941	31		

*** significant at P = .001

APPENDIX 2

Sedimentation of protein from tap water extracts of Russet Burbank tubers at pH 4 as a function of protein concentration, coagulation temperature and settling time. Split plot design with 2 replications, 2 concentrations, 2 temperatures and 8 time intervals (10 min.).

Analysis of variance

source	df	SS	MS	F
replication	1	2551	2551	.04 n.s.
concentration	1	152989866	152989866	2955.23***
error (a)	1	51769	51769	
temperature	1	913923	913923	147.14***
temp x conc	1	307426	307426	49.49*
error (b)	2	12432	6211	
time	8	51636030	6454504	348.66***
time x temp	8	321570	40196	2.17 n.s.
time x conc	8	5763635	720454	38.84***
time x temp x conc	8	419240	52405	2.83*
error (c)	32	592389	18512	
total	71	213010523		

n.s. non-significant at P = .05

*,*** significant at P = .05 and P = .001 respectively

APPENDIX 3

Precipitaion of protein from a distilled water extract of Russet Burbank tubers as influenced by 9 pH and 2 temperature levels. Split plot design with 2 replications, 9 pH levels, room temperature, heating to 98°C and 2 observations per treatment.

Analysis of variance

source	SS	df	MS	F
replication	261623	1	261624	66.41***
pH	8080439	8	1010055	240.94***
error (a)	33536	8	4192	
temperature	2606239	1	2606239	1085.07***
pH * temperature	3374821	8	421852	175.63***
error (b)	21617	9	2402	
sample	15429	36	428	
total	14393704	71		

***significant at P = .001

Residual crude protein (ppm) in solution after pH and heat treatment.*

pH	replication I		replication II		mean	
	23°C	98°C	23°C	98°C	23°C	98°C
6.3	4144	4152	4303	4285	4224 a,A	4218 a,A
6.0	4145	4142	4261	4248	4204 a,A	4195 a,A
5.5	4100	4032	4216	4128	4158 ab,A	3157 a,A
5.0	4026	3099	4143	3216	4084 abc,A	3157 c,B
4.5	3842	2866	3899	2868	3870 bc,A	2867 d,B
4.0	3867	2922	3998	2971	3933 c,A	2947 cd,B
3.5	4107	3665	4227	3904	4164 ab,A	3785 b,B
3.0	4187	4068	4252	4269	4219 a,A	4169 a,A
2.5	4137	4166	4325	4620	4231 a,A	4243 a,A
original sol.	4151		4295		4223	
TCA	2523		2451		2481	

*average values of duplicate sample (ppm)

Studentized range test:

- Temperature means for a given pH:

  Values with the same letter (A,B,..) are not significantly different at the 5% level.

- pH means for a given temperature:

  Values with the same letter (a,b,..) are not significantly different at the 5% level.

APPENDIX 4

Precipitation of protein from a tap water extract of Russet Burbanks tubers by 9 pH and 4 temperature levels. Split plot design with 2 replications, pH levels, 4 temperatures and duplicate observations.

Analysis of variance

source	SS	df	MS	F
replication	3418918	1	3418918	77.374***
pH	5858462	7	5658462	128.051***
error (a)	44189	7	44189	
heat	310339	3	310338	8.891***
pH * heat	500989	21	500989	14.353***
error (b)	83729	24	34904	
sample	238	64		
total	5564227	127		

***significant at P = .001

Residual crude protein (ppm) in solution after pH and heat treatment.*

pH	23°C	60°C	80°C	98°C
6.8	4702 a,A	4344 ab,AB	3917 b,BC	3527 b,C
6.0	4682 a,A	3836 bc,B	3403 bc,BC	3142 bc,C
5.0	3360 b,A	3177 d,AB	2908 c,AB	2786 c,B
4.5	2957 bc,A	2956 d,A	2854 c,A	2745 c,A
4.0	2905 bc,A	2984 d,A	2906 c,A	2744 c,A
3.0	2865 c,B	3537 cd,A	3140 c,AB	3498 b,A
2.0	3339 b,B	3902 bc,A	3992 b,A	4167 b,A
1.0	4646 a,A	4612 a,A	3656 a,A	4706 a,A
original sol.	4705			
TCA	2661			

*average values of duplicate samples and 2 replications.

Studentized range test:

- Temperature means for a given pH:

  Values with the same letter (A,B,..) are not significantly different at the 5% level.

- pH means for a given temperature:

  Values with the same letter (a,b,..) are not significantly different at the 5% level.

APPENDIX 5

Comparison of ferric chloride versus hydrochloric acid as precipitating agent for protein from a dilute potato extract. Split plot design with 2 replications, 2 treatments at 6 different pH levels.

Analysis of variance

source	df	SS	MS	F
replication	1	1097600	1097600	17.77 n.s.
treatment	1	356482	356482	5.77 n.s.
error (a)	1	61778	61778	
pH	6	8703489	1450581	82.58***
tmt x pH	6	932467	155411	8.84***
error (b)	40	702606	17565	
total	55	11854423		

n.s. non-significant at P = .05

*** significant at P = .001

APPENDIX 6

Residual crude protein in solution after raising the pH with CaO to pH 12 followed by lowering it with H_3PO_4 and $FeCl_3$. Randomized block design with 2 replication, 20 treatments and duplicate observations.

pH	CaO	H_3PO_4	$FeCl_3$
7.0	3444	2622	2317
8.0	3432	2577	2320
8.5	--	2541	2717
9.0	3185	2375	2387
9.5	--	2412	2455
10.0	2840	2459	2502
11.0	--	2596	2562
12.0	2657		
original solution	3448		
TCA	2125		

Analysis of variance

source	df	SS	MS	F
replication	1	233820	233820	96.22***
treatment	19	9183968	483366	198.92***
error	59	77593	1315	
total	79	9495381		

*** significant at P = .001

APPENDIX 7

Vole Feeding Trial

Eleven diets were tested in a randomized block design, food intake and weight gain was measured and the protein efficiency index calculated. Replications were made up of voles of two litters originating from the same harem.

Analysis of variance for food consumed

source	df	SS	MS	F
litter	5	1285.54	257.11	3.280*
diets	10	795.32	79.53	1.015 n.s.
error	50	3919.03	78.38	
total	65	5999.89		

Analysis of variance for weight gained

source	df	SS	MS	F
litter	5	9.92	1.98	1.198 n.s.
diets	10	190.41	19.04	11.502***
error	50	82.77	1.65	
total	65	283.11		

Analysis of variance for PEI

source	df	SS	MS	F
litter	5	2.26	.452	4.318**
diets	10	39.56	3.956	37.79***
error	50	5.23	.1046	
total	65	47.07		

n.s. non-significant at P = .05

*,**,*** significant at P = .05, .01, .001 respectively