

Development of a Pilot-Plant Process for the Preparation of a Trypsin Inhibitor-Rich Fraction from Potatoes

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As part of a lifetime (2-year) rat feeding program to evaluate the biochemical effects of animal and vegetable trypsin inhibitors (TI) on the pancreas, a process to prepare large quantities of a potato TI concentrate for incorporation into diets was investigated. The trypsin inhibitors first were concentrated by processing 24 000 lb of Kennebec potatoes to remove pulp and starch. The remaining protein water, which contains a variety of protease inhibitors, noninhibitor proteins, and low-molecular-weight impurities, was concentrated to ca. 40% solids by vacuum evaporation. A number of procedures involving (1) heat coagulation, (2) ultrafiltration, and (3) acid precipitation were then investigated to fractionate and concentrate TI. Best results were obtained with method (3) in which acid precipitation was conducted over the range of pH 2-5. At pH 3.5 noninhibitor type proteins were precipitated, and TI was recovered in a subsequent UF separation with only a 10% loss of TI activity.

Introduction

For maximum conversion of raw proteins of soybeans and other food legumes into products with good nutritional quality, moist heat treatment is required to simultaneously inactivate trypsin inhibitors (TI) and transform the raw protein into more readily digestible forms (Rackis et al., 1975; Baker and Mustakas, 1973). Short-term feeding trials indicate that the relative capacities of various sources of TI to inhibit growth and enlarge the pancreas vary widely (Liener and Kakade, 1980). Very little information is available on the effects of various levels of TI activity in the diets of rats over a lifetime (2-year) feeding trial. The quantity of TI required for such tests is substantial. Potatoes are a good source of TI. In commercial processing of potatoes to produce potato starch, a protein water fraction results that contains the water-soluble components of potatoes, including the TIs (Gerry, 1977). The protein water, when concentrated by vacuum evaporation, becomes a rich source of TI. Low-molecular-weight constituents account for about 65% of the solids in potato juice concentrate (PJC) (Porter et al., 1970). Therefore, it would be expected that the TIs could be successfully separated by ultrafiltration (Baker et al., 1979). However, a protein fraction remains that creates problems of foaming during processing, and it first must be removed. The purpose of this study was to develop a pilot-plant procedure to prepare a TI-rich fraction in sufficient quantities for long-term feeding trials.

Materials and Methods

Twelve tons of sprout-inhibited Kennebec potatoes grown in Maine were purchased from the second harvest in August, 1979. The potatoes were processed in the potato-starch pilot plant at USDA's Eastern Regional Research Center at Philadelphia, PA. The pulp and starch fractions were removed, leaving a protein water fraction containing the water-soluble protein and nonprotein components found in potatoes, including the TIs. The large volume of protein water was concentrated under vacuum to 1300 lb at a solids concentration of 40%. The resulting

PJC had a TI activity of 68 000 TI units/mL. The PJC was loaded into 5-gal plastic carboys, frozen, and shipped by air to the Northern Regional Research Center at Peoria, IL. The PJC was stored frozen in a cold room and was removed and thawed as required for experimental purpose.

Analytical Methods

Trypsin inhibitor was determined by the method of Hammerstrand et al. (1981), which evolved primarily from the work of Kakade et al. (1969, 1974). Total solids were determined by evaporation. Nitrogen and ash were determined by the official AOCS methods (1976). Non-protein nitrogen was run by the method of Becker et al. (1940). Glycoalkaloids were determined by the method of Fitzpatrick and Osman (1974).

Equipment and Procedures

Except when the higher concentrations were being evaluated, the PJC was diluted to 2% solids concentration. Eight pounds of PJC was added to 152 lb of distilled water in an agitated 30-gal stainless steel-jacketed kettle. Protein was either coagulated with heat or precipitated with acid as dictated by the experimental conditions. The protein curd was separated in a Sharples Supercentrifuge fitted with a 4-in. bowl. The heat-coagulated or acid-precipitated protein curd was resuspended in distilled water in a Cowles disintegrator. The curd suspension was recentrifuged, and the wash was combined with the original whey. The combined whey fraction was ultrafiltered on a 55-ft² polysulfone module manufactured by Osmonics Inc., Hopkins, MN. The filtered whey solution was then concentrated to a small volume, diluted to near the original volume, and re-ultrafiltered. The module was flushed with 2 × 10 gal of distilled water, and the flushes were combined with the original retentate and freeze-dried. Total solid and TI balances were made on the original diluted PJC and on the resulting protein curd, permeate, and retentate fractions.

Results and Discussion

The composition of PJC is shown in Table I. The true protein is calculated by subtracting the nonprotein nitro-

Table I. Composition of Potato Juice Concentrate

component	% dry basis
apparent protein N \times 6.25	48.1
nonprotein nitrogen (NPN) \times 6.25 = 27.4% ^a	
true protein (N - NPN) \times 6.25 = 20.7%	
ash	26.0
carbohydrates, etc. (by difference)	25.9
	100.0

^a Amino acids, peptides, alkaloids, etc.

Table II. Relative Trypsin Inhibitor (TI) Values of Potato Juice Concentrate (PJC) and Raw, Dehulled, Defatted Soyflour

	mg of TI/ g of sample	mg of TI/ g of protein
soyflour	37	84
PJC	36	174

gen from the total nitrogen and multiplying by 6.25. The nonprotein nitrogenous compounds account for a little more than half the total apparent protein. Trypsin inhibitors amount to 3.6% of the total solids or 7.5% of the total apparent protein. The nonprotein components (ash and carbohydrates) account for approximately half of the total and are of relatively low molecular weight.

The relative TI values of PJC and raw dehulled defatted soyflour are compared in Table II. Based on mg of TI/g of sample, PJC and soy flour are nearly equal. However, when based on mg of TI/g of protein, PJC has nearly twice the TI activity of soyflour. The separation of only the nonprotein impurities from each of these starting materials would yield a fraction much richer in TI when starting with PJC rather than soyflour.

Heat Coagulation of Non-TI Protein. Preliminary laboratory experiments by Professor C. A. Ryan and co-workers at Washington State University, Pullman, WA, indicated that by rapidly heating a 2% dispersion of PJC to 75 °C, holding for 3 min and rapidly cooling, the non-TI protein could be selectively heat coagulated, leaving the TI in solution. This procedure was the basis for our first pilot-plant experiments in the 30-gal, stainless steel-jacketed kettle. However, we could only account for about 8% of the TI in the curd and whey fractions; about 92% was assumed to be heat inactivated during the process. The experiments were repeated using injected live steam as well as jacket steam in an attempt to decrease the heat-up time. Again, the accountability of TI was only about 10%. At this point the batch kettle was abandoned for a plate heat exchanger, in which the PJC was pumped through to get the fastest possible heat-up time. The accountability of TI was only slightly better at 15%, and this method of separation was evidently not going to work in the pilot plant.

Separation of Non-TI Protein by Ultrafiltration. In all, four proteinase inhibitors were found in potatoes by other investigators. Inhibitor I inhibits chymotrypsin, has a molecular weight (M_n) of 39 000 and comprises 24% of the total (Melville and Ryan, 1972). Inhibitor II inhibits chymotrypsin and trypsin, has M_n 21 000 and comprises 37% of the total (Bryant et al., 1976). Inhibitor III inhibits carboxypeptidase, has M_n 4500 and comprises 17% of the total (Ryan et al., 1974). Inhibitor IV inhibits polypeptide chymotrypsin, has M_n 5000 and comprises 22% of the total (Hass et al., 1976). Using a 50 000 M_n module, the TI's, all of which had M_n 39 000 or less, should permeate the membrane while the heavier M_n proteins found in potatoes would be rejected, thus effecting a separation. However, approximately 30% of the TI's were found in the retentate,

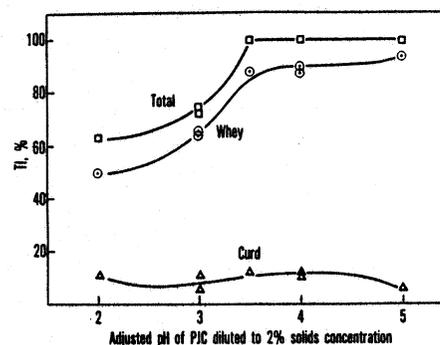


Figure 1. Distribution of trypsin inhibitor (TI) in curd and whey fractions resulting from acid precipitation of protein from potato juice concentrate (PJC).

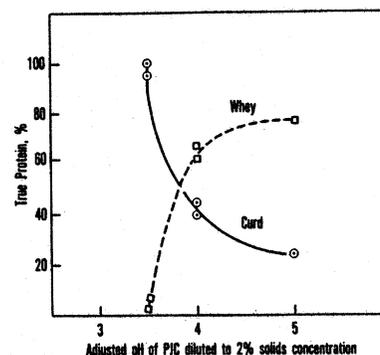


Figure 2. Precipitation of true protein from potato juice concentrate (PJC) with acid at varying pH.

probably due to association, and a satisfactory separation was not obtained. Also, processing difficulties because of severe foaming adversely affected the flux rate.

Acid Precipitation of Non-TI Protein. A series of experiments was run in which protein was precipitated by acid over a pH range of 2 to 5. TI accountability was equally good over the pH range of 3.5 to 5 (Figure 1). Approximately 10% of TI activity was lost to the curd, the balance appearing in the whey. Below pH 3.5, a significant loss of TI activity was observed. Between pH 3.5 and 5, although the accountability of TI was equally good, the amount of non-TI protein precipitated varied (Figure 2). At pH 3.5 almost all of the non-TI protein was precipitated, with less than half at pH 4 and only about 20% at pH 5. It is important to remove as much of this non-TI protein as possible to improve the purity of the isolated TI-rich fraction. Therefore, acid precipitation at pH 3.5 was indicated.

Separation of Low-Molecular-Weight Components. The non-TI components in the whey (obtained by precipitation of curd at pH 3.5) consisted of roughly one-third each of ash, sugars, and nonprotein nitrogenous compounds. The nonprotein nitrogenous compounds consisted mainly of amino acids, peptides, and alkaloids. The whey was ultrafiltered over a 1-K module (1000 M_n cutoff). A high percentage of the low-molecular-weight components was removed in the permeate, but the loss of TI to this stream was less than 5%. The potential toxicity of glycoalkaloids in new potato varieties bred specifically for disease resistance has been recognized. However, no alkaloids were found in the retentate fraction. This separation proved to be quite satisfactory and no further screening of ultrafiltration modules was necessary.

Effects of pH on Ultrafiltration. Acid precipitation of the protein curd was shown to be best at pH 3.5 over the range studied. Portions of the as is whey (pH 3.5) were adjusted to pH 5 and 7 before ultrafiltration to study the

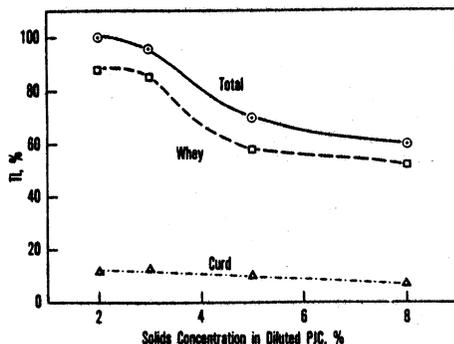


Figure 3. Effect of solids concentration on trypsin inhibitor (TI) accountability in diluted potato juice concentrate (PJC).

effect of pH on the permeate and retentate fractions. The loss of TI to the permeate was approximately 10% greater at pH 7 and over 25% higher at pH 5. Recovery of TI in the retentate fraction was highest at pH 3.5.

Effect of Solids Concentration. All the preceding experiments had been run at 2% solids concentration in the PJC. In the interest of keeping the number of pilot plant runs to a minimum for processing the main bulk of the PJC, it would appear desirable to utilize a higher solids concentration. Experiments were run in which the solids concentration was increased to 5 and 8% (Figure 3). At 5%, the TI accountability decreased to about 70%, and to about 60% at 8%. It appeared that 2% solids concentration was close to ideal. However, we found that we could operate at 3% solids concentrations with only negligible loss of TI activity.

This study has shown that non-TI protein can be separated as an acid curd at pH 3.5, and the low-molecular-weight components in the whey can be removed in the permeate by passing over a 1K ultrafiltration module. The resulting TI-rich retentate, which contained approximately 90% of the TI originally present in the PJC, can then be

freeze-dried for incorporation into diets for the long-term feeding trials.

Acknowledgment

Alkaloid analyses were run by S. Osmon, Eastern Regional Research Center in SEA-AR, USDA, Philadelphia, PA. All other analyses were made by L. T. Black, J. D. Glover, K. A. Rennick, and K. M. MacDonald. Pilot-plant experiments were run by R. L. Brown. Technical advice on potato trypsin inhibitor properties was given by C. A. Ryan, Washington State University, Pullman, WA.

Literature Cited

- American Oil Chemists' Society, "Official and Tentative Methods of Analysis", Vol. 1, 2nd ed.; The Society, Champaign, IL, 1964 (Revised to 1976).
 Baker, E. C.; Mustakas, G. C. *J. Am. Oil Chem. Soc.* **1973**, *50*, 137.
 Baker, E. C.; Mustakas, G. C.; Moosemiller, M. D.; Bagley, E. B. *J. Appl. Polym. Sci.* **1979**, *24*, 135.
 Becker, H. C.; Milner, R. T.; Nagel, R. H. *Cereal Chem.* **1940**, *17*, 447.
 Bryant, J.; Gren, T. R.; Gurusaddaiah, T.; Ryan, C. A. *Biochemistry* **1976**, *15*, 3418.
 Fitzpatrick, T. J.; Osman, S. F. *Am. Potato J.* **1974**, *51*, 318.
 Gerry, R. W. *Poult. Sci.* **1977**, *56*, 1947.
 Hamerstrand, G. E.; Black, L. T.; Glover, J. D. *Cereal Chem.* **1981**, *58*, 42.
 Hass, G. M.; Venkatakrishnan, R.; Ryan, C. A. *Prog. Natl. Acad. Sci.* **1976**, *73*, 1974.
 Kakade, M. L.; Rackis, J. J.; McGhee, J. E.; Puski, G. *Cereal Chem.* **1974**, *51*, 376.
 Kakade, M. L.; Simons, N.; Liener, I. E. *Cereal Chem.* **1969**, *46*, 518.
 Liener, I. E.; Kakade, M. L. "Protease Inhibitors" in "Toxic Constituents of Plant Foodstuffs", Liener, I. E., Ed.; Academic Press; New York, 1980.
 Melville, J. C.; Ryan, C. A. *J. Biol. Chem.* **1972**, *247*, 3445.
 Porter, W. L.; Siciliano, J.; Krulick, S.; Heisler, E. G. *Membrane Sci. Technol.* **1970**, *220*.
 Rackis, J. J.; McGhee, J. E.; Booth, A. N. *Cereal Chem.* **1975**, *52*, 85.
 Ryan, C. A.; Hass, G. M.; Kuhn, R. W. *J. Biol. Chem.* **1974**, *249*, 5495.

Received for review August 21, 1981

Accepted November 6, 1981

Presented at the 181st National Meeting of the American Chemical Society, Atlanta, GA, Mar 29-Apr 3, 1981. The mention of firm names or trade products does not imply that they are endorsed by the U.S. Department of Agriculture over other firms or similar products not mentioned.