Protein Quality of Precipitate from Waste Effluent of Potato Chip Processing Measured by Biological Methods

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The nutritional value of precipitated protein of simulated waste effluent and of crude protein of whole tubers was biologically and microbiologically assessed using weanling voles (*Microtus pennsylvanicus*) and *Streptococcus zymogenes*. The nutritional value of the recovered protein was dependent on both the protein quality of the potatoes and the method of recovery. Of three varieties tested, the protein efficiency index (PEI) was highest for Sebago. It was not significantly different from the PEI of casein. No differences were found between the biological value of protein obtained by heat or acid (HCl or $FeCl_3$) treatment of wash effluent, but values for whole tubers and samples treated with CaO and H_3PO_4 or $FeCl_3$ were all lower. This paper demonstrates that a potato chip plant could reduce discharged waste and water use by relatively simple means and obtain a high quality feed containing approximately 30% protein.

The potato chip industry requires methods to reduce processing losses and to economically exploit the waste effluent. Primary wastes and starch can be easily removed by screening and centrifugation. The product can be marketed in either dry or wet form (Pettay, 1975). Simple procedures for the recovery of proteins, accounting for one-third to one-half of the nitrogenous substances in the effluent, were reported by Meister and Thompson (1976). 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.

Nitrogen balance studies with human adults have shown potato protein to be superior to most major plant proteins and approaching 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 potato variety fed and were improved with methionine supplementation (Rios Iriate et al., 1972). "Available methionine" and a "biological" value of potato protein were evaluated with *Streptococcus zymogenes* (Luescher, 1972). The same organism was used by Ford (1962) to study heat damage to different proteins.

The purpose of this study was to determine the biological value of potato protein recovered by different methods from waste effluent of chip processing.

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 chip plant were selected for this study. Whole tubers and heat and acid precipitates of each variety were tested and compared with casein as a standard. Abrasive peel from the unknown variety was included as an additional protein source.

Preparation of Samples for Vole Diet. Average size tubers of each variety were selected, washed, autoclaved at 15 psi for 15 min, peeled, sliced, and freeze-dried. To simulate waste water, Russet Burbank and Sebago tubers were washed and ground for 10 min in a Waring Blendor, and the mash was diluted with water to five times its volume, filtered through several layers of cheesecloth, and left for 30 min to settle the starch. Half of each diluted extract and half of a water sample received from a chip plant were acidified with HCl to pH 3.0, samples were stirred for 15 min and then subjected to 90 min of sedimentation, and the supernatant was drained off. The other half of each sample was heated to 98 °C after adjustment of the pH to 4.5, and this was followed by immediate cooling in ice water and settling. The peel and the acid precipitates were heated in a water bath for 15 min at 60 °C to gelatinize the starch. All samples were freeze-dried.

Protein Evaluation by Animal Assay. Meadow voles (*Microtus pennsylvanicus*) were selected as experimental animals because of their rapid growth and small food requirements. Diets were made up according to methods described by Elliott (1963) and by Shenk and Elliott (1969). Composition of control and experimental diets is given in Table I. Freeze-dried samples were ground in a Wiley Mill to pass a 40 mesh sieve.

To the diets containing 7% protein enough water was added that they could be molded into wafers to fit the vole feeder. The wafers were dried at 40 °C for 48 h, wrapped in aluminum foil, and stored at −18 °C until needed. They were thawed at room temperature for 16–18 h before weighing and feeding, and the moisture content determined using the vacuum oven method (AOAC, 1970). The oven was operated for 12 h at 70 °C under partial vacuum. Food consumption was determined by loss in weight of the entire feeder.

In the feeding experiments, weanling voles 12–14 days old and weighing from 12.5 to 16.0 g were used. For 2 days they received a starter diet followed by the experimental diets over a 6-day period. Food and water were available ad libitum. The animals were closely observed during the feeding trial and weights were taken initially and every 2 days thereafter. The voles were housed individually in plastic-bottomed cages with corncob bedding and nonabsorbent cotton for nesting.

The diets were tested in a randomized block design with six replications. The most uniform voles of two litters of the same harem were randomly assigned to the 11 diets forming one block. 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

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Table I. Diet Composition[f] (%)

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

[a] Protein contents of the different sources were: 87, 10.8, 27.2, 29, 12, 35, 30, 15.1, 32.4, 28.4, and 12%. [b] Salt mixture W, Nutritional Biochemicals Corp., Cleveland, Ohio. [c] Vitamin diet fortification mixture, Nutritional Biochemicals Corp., Cleveland, Ohio. [d] α-cellulose, Nutritional Biochemical Corp., Cleveland, Ohio. [e] Potato starch, cooked, oven-dried, and ground. [f] Adjusted to 7% crude protein.

(1971) and Peare (1973), was followed. Casein was used as a standard.

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

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; (c) acidification with $FeCl_3$ to pH 4.0; (d) raising pH to 11.5 with CaO followed by adjustment to pH 9 with H_3PO_4; (e) raising pH to 11.5 with CaO followed by adjustment to pH 9 with $FeCl_3$.

All precipitates were dialyzed in cellophane bags for 48 h to avoid interferences with the assay. The samples were freeze-dried and ground in a Wiley mill through a 60 mesh screen, and the nitrogen content was determined by the micro-Kjeldahl method (AOAC, 1970).

Samples of casein and precipitates containing the equivalent of 50 mg of crude protein were placed into 4-oz screw-cap bottles, 20 ml of citrate cyanide buffer (Luescher, 1971) was added, and the pH was adjusted to 7.2 with 1 N KOH. The samples were heated in a water bath to 56 °C for 3 h with intermittent shaking and the pH was periodically adjusted; then the samples were diluted to 100 ml with distilled water.

Triplicate portions of 4 ml of the digest were pipetted into 16 × 150 mm test tubes. Two milliliters of basal medium (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 min, and, after cooling, one drop of inoculum culture, diluted 1:10 with sterilized 0.85% saline solution, was added and tubes were incubated for 48 h at 37 °C. (The organism used for these tests was *Streptococcus zymogenes* NCDO 592, obtained from the National Collection of Dairy Organisms, Institute for Research in Dairying, Shinefield, Reading, United Kingdom.)

After incubation, tubes were heated in flowing steam for 10 min, stoppered and shaken vigorously, and set aside for 30 s. 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 with a tube containing the same amount of casein protein.

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 are given in Table I. The analysis of variance for just the three treatments of the three cultivars indicated that treatment effects were less pronounced than varietal differences (Table II). Food intakes, weight gains, and protein efficiency indices (PEI's) are summarized in Table III. Food consumption was the same for all diets, although differences were found between litters. Examination of the data showed that two litters with high initial 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 Iriate et al. (1972), who fed diets containing 5.28% crude protein, considered to be below the level for minimal

Table II. Comparison of Litter, Variety, and Treatment Effects of Vole Feeding Trial; Analysis of Variance for PEI

Source	df	Mean square	F^a
Litter	5	0.466	3.531*
Variety	2	2.032	15.403**
Error (a)	10	0.132	
Treatment	2	0.774	7.075*
Var. × tmt.	4	0.476	4.355**
Error (b)	30	0.109	

[a] *, **, ***, significant at $P = 0.05, 0.01, 0.001$, respectively.

Table III. Food Intake, Weight Gain, and Protein Efficiency Index (PEI) of 11 Diets Tested in Vole Feeding Trial; Average Values of Six Voles per Diet

Diets	Food intake, g/6 days	Wt gain,[a] g/6 days	PEI[a]
Casein	30.0	6.25a	3.02a
Burbank tubers	35.8	4.75cd	1.89e
Burbank heat prec.	35.5	5.31bc	2.19c
Burbank acid prec.	28.4	4.13de	2.07c
Sebago tubers	35.3	5.98ab	2.51b
Sebago heat prec.	40.3	6.26a	2.21bc
Sebago acid prec.	28.2	5.82ab	2.96ab
Unknown tubers	32.2	3.93e	1.81e
Unknown heat prec.	33.8	4.93c	2.09c
Unknown acid prec.	35.4	5.41bc	2.18c
Unknown peel	34.7	0.06f	0.02d

[a] Studentized range test: values with the same letter are not significantly different at the 5% level.

Table IV. "Biological Value" of Crude Protein of Three Potato Varieties Subjected to Six Treatments, Determined by *S. zymogenes*

Treatment	Russet Burbank[e]	Sebago[e]	Unknown[e]
Whole tubers	67d	70ed	68cd
Heat precipitate[a]	70cd	75ab	69cd
Acid precipitate[b]	72bc	77a	69cd
$FeCl_3$ precipitate[c]	72bc	74ab	69cd
CaO and H_3PO_4 prec.[d]	66d	68cd	67d
CaO and $FeCl_3$ prec.[d]	68c	68cd	67d

[a] Heated to 98 °C after acidifying with 2 N HCl to pH 4.5. [b] Sedimentation at 23 °C after acidifying with 2 N HCl to pH 3.0. [c] Sedimentation at 23 °C after acidifying with 2 M $FeCl_3$ to pH 4.5. [d] Sedimentation at 23 °C after raising pH to 11.5 with CaO, followed by lowering it to pH 9.0 with H_3PO_4. [e] Studentized range test: values with the same letter (a, etc.) are not significantly different at the 5% level.

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. Protein recovered by either method of precipitation gave equal or higher PEI's than 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 °C at pH 4 was not superior to that of the mixture of protein and nonprotein N in the whole potato. Only the data from Sebago tend to confirm these results. Crude protein of Russet Burbank and the unidentified variety gave significantly lower efficiency indices than the corresponding protein precipitates. The relative amount of protein N was the same for all varieties (approximately 40–46%) but the PEI's of precipitated Sebago protein were higher. The results suggest that protein nitrogen of Sebago compensated better for the lower nutritional value of the nonprotein N or that the two fractions did supplement each other better than in the other two varieties.

The abrasive peel from the unidentified variety did not support growth (Table III). 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 are 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) nonenzymatic browning and (2) structural changes such as denaturation and aggregation. The data from the vole feeding trial did not reveal any appreciable differences between protein precipitated at 98 °C and at room temperature (25 °C). The high moisture content and low pH during heating might have slowed down the Maillard reaction. Also, differences may not have appeared because the second sample was heated (60 °C) prior to feeding to gelatinize the starch.

Streptococcus zymogenes was considered an ideal organism to test the varietal and treatment differences. The variety Sebago gave a higher "biological value" for almost all treatments (Table IV). Highest "biological values" were observed from 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.

Good agreement was found between PEI's of the vole assay and "biological values" of the *Streptococcus* assay. Varietal differences were found in the nutritional value of crude protein of tubers and the protein precipitates of simulated potato chip effluent. Values were highest for Sebago proteins. No differences were found between protein precipitated from acidified effluent at room temperature (25 °C) or at 98 °C. Values were lower for protein precipitated by a combination of lime and H_3PO_4 or $FeCl_3$.

In addition to good processing characteristics, potato varieties used in chip manufacturing should contain a high percentage of well-balanced protein. A larger protein–N fraction in tubers would result in more easily recoverable protein. Upon recovery this would reduce waste discharged and give higher yields of a salable protein feed. Its quality is expected to be good but will depend on the method of recovery used and the biological value of the potatoes processed.

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