ENZYMATIC MODIFICATION OF THE EXTRACTABILITY OF PROTEIN FROM SOYBEANS (*GLYCINE MAX*)

by

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Thesis Submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in Candidacy for the degree of
DOCTOR OF PHILOSOPHY
in
Biochemistry and Nutrition

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May 4, 1966

Blacksburg, Virginia

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III. INTRODUCTION AND LITERATURE REVIEW

One of the most serious problems facing the world is the insufficiency and the inadequacy of food supply, especially in the developing countries where the food supply per person is diminishing and protein-calorie malnutrition is widespread due to rapid population growth, poor economy, and primitive agricultural practices.

Nutritionists and public health nutritionists are trying very hard to combat protein-calorie malnutrition in the developing countries using various methods. One of the most successful methods they have reached is the development of good quality food mixtures from agricultural products which are indigenous to the country where the mixture is intended to be used. These food mixtures supply most of the nutrients required by humans, and they are cheap enough to be afforded by most of the people. Incaparina developed in Guatemala under the direction of Dr. Nevin S. Scrimshaw, AK-1000 developed by Dr. K. W. King for Haiti, and Pro Nutro produced by Hind Brothers for South Africa are examples of such food mixtures.

It is true that animal protein is far superior to one single plant protein, but it is also far more expensive. This is mainly due to the inefficiency of the animal in converting plant protein into animal protein. Most animals only retain 10-15% of their protein intake. But by development of technological processes for improving the yield and quality of plant protein, the protein resources would be

extended 7-10 times. This can be seen from Table 1, which indicates that plants produce several times more protein per acre of land than animals.

One technological process which might prove to be economically feasible is the use of cellulolytic enzymes for macerating plant fibers thus rendering more protein available for human use.

Soya beans, whose protein content is about 30-40% holds economic, agronomic, and nutritional promise for combatting malnutrition.

A series of processes has been developed involving essentially extraction of seed meals with water, isoelectric precipitation of protein, and drying in order to obtain products with good nutritional qualities as well as long shelf-life under primitive storage conditions. With soybean, however, only 50-80% of the protein is extractable,and Hackler et al. (10) have shown that the unextracted protein is of even higher nutritional quality than that presently recovered. Furthermore, it has been reported that coconut flour protein is of greater nutritional value than that of coconut protein isolate, but the high fiber content of both soybean residue and coconut flour makes them unsuitable for infant feeding. Consequently, any process which will degrade fiber without damaging the protein would make the soy residue and coconut flour suitable for infant feeding. It might also increase the yield of protein isolate and improve its quality.

According to Van Veen (19) the fiber can be reduced by fermentation but only at a considerable risk of bacterial contamination. It is probable

Table I (1)

Comparison of Protein Yield of Plants and Animal Products

Product	Protein Yield (pounds/acre)
Grass	600
Legumes	370
Wheat	269
Milk	90
Beef	54

that bacterial contamination can be avoided by isolating the enzymes which degrade the fiber, thereby making it possible to reduce the fiber in a shorter period of time. Promising indications, that cellulase treatment may be of use in enhancing protein extraction, have been reported by Tazaki et al. (27), Oshima et al. (20), Toyama (30), Ozaki et al. (21), Nisizawa et al. (18), and Watanabe et al. (32). Nisizawa et al. (18) using Irpex lacteus cellulolytic enzymes reported that 78% of soy residue nitrogen has been extracted by this enzyme treatment. It should be mentioned here that Nisizawa et al. used a soy residue which contained 50% of the nitrogen of the soybean seed. Since it has been reported by Hand (11) that water extraction of the dehulled soybean would leave only 16.5% of the whole seed nitrogen in the residue, it can be seen that the actual extraction achieved by Nisizawa et al. enzyme treatment is about 32%.

It is of great importance to conduct animal feeding experiments on products prepared by use of fungal enzymes to find out if these enzyme preparations contain any substance which might be detrimental to health. These experiments would also reveal whether the increase in yield of extractable protein due to enzyme treatment would improve its quality.

Bacterial and fungal enzyme preparations have been used for the purpose of increasing animal protein production. Toyama (29) claims a 35% increase in egg production as a result of adding crude Trichoderma viride cellulase to barley-based rations. Willingham et al. (33) reported

that there was a highly-significant increase in growth and feed utilization when diets containing barley were supplemented with enzymes from bacterial or fungal sources. Later Berg (1959, 1961) (5, 6) reported similar results. Leveil et al. (15) using albino rats reported that cellulase improved the digestibility of Chlorella pyrenoidosa protein.

In this dissertation an attempt has been made to study cultural conditions under which fungal cellulases are capable of rendering the protein of soybeans more readily extractable and to find out the optimum conditions for enzymatic treatment on a scale sufficient to permit evaluation of the nutritive value of the protein isolate in rats.

Six fungi, Pestalotiopsis westerdijkii QM:381, Myrothecium verrucaria QM:460, Aspergillus terrus QM:72f, Chaetomium globosum QM:459, Basidiomycetes sp. QM:806, and Trichoderma viride QM:6a have been screened according to their capacity to elaborate on extracellular enzyme systems capable of enhancing the extractability of soy protein. The most active culture was selected for production of large amounts of the enzyme system. This enzyme system was used for treating the residual fraction of soy beans after water extraction, and both the effectiveness of the enzyme system in improving the recovery of soy protein as soy milk and the nutritive value of soy residue and soy milk before and after treatment were studied.

IV. MATERIALS AND METHODS

Six fungi, Pestalotiopsis westerdjkii QM:381, Myrothecium verrucaria QM:460, Aspergillus terreus QM:72f, Chaetomium globosum QM459, Basidiomycetes sp. QM:806, and Trichoderma viride QM:6a were generously supplied by Dr. E. T. Reese.

Media:

To one gram of soy residue or wheat bran and Solka floc (2:1 wt./wt. ratio) 0.7 ml of a diluted trace mineral mixture* was added. The medium containing soy residue is called "soy residue medium", and that containing wheat bran is called "wheat bran medium."

Preparation of extracellular enzyme system capable of degrading soy fiber:

Trays containing four or five-hundred grams of one of the above media were inoculated with spores of one of the above fungi obtained from potato dextrose agar slants and then incubated for 7-10 days at 28°-32° C. The contents of the trays were extracted for three hours with five volumes of distilled water at room temperature and filtered through cheese cloth. The filtrate was fractionated with ammonium sulfate. The material precipitated between 30-70% ammonium sulfate saturation was collected by centrifugation in a refrigerated centrifuge at 2500 X G for 20 minutes. The precipitate was dissolved in an amount of distilled water equivalent to one-sixth of the original volume of the

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*One ml of Reese et al. (24) trace mineral mixture was diluted 1000 times with distilled water.

filtrate. This enzyme solution was dialyzed on a Sephadex G25 column and the fraction which had all the aryl-β-glucosidase activity was collected.

Preparation of soy fiber:

One gram of soy meal was boiled for one hour in 100 ml of digestion mixture having the following composition:

80 ml acetic acid, 80 ml nitric acid, 32 gm trichloroacetic acid, 720 ml distilled water.

The digested mixture was filtered on cloth. The fibers were collected and washed thoroughly with distilled water to neutrality. The fibers were air dried and very finely ground in an agate mill.

Preparation of soy residue:

Whole soybean seeds were soaked for 12 hours with 3.5 volumes of distilled water, dehulled, extracted with 8.3 volumes of distilled water per kilogram of dry soybeans in a Waring blendor for ten minutes, and filtered through cheese cloth. The filtrate was called "soy milk" and the unextractable fraction was called "soy residue." Both fractions were freeze dried.

Soy-fiber-degrading activity assay:

This assay was essentially the same as that of Li, Flora and King (16) except hydrocellulose was replaced by soy fiber.

Carboxymethylcellulase assay (CMCase Assay):

Hash and King's (12) method was followed.

Aryl-β-glucosidase assay:

King's (14) method was used.

Chemical Analysis:

The Johnson (13) method for microkjeldahl, Somogyi (25) method for reducing sugar and the A.O.A.C. (3) method for proximate analysis were used.

V. RESULTS AND DISCUSSION

1. Selection of Fungus

The fungus which elaborated an extracellular enzyme system having the highest soy fiber-degrading activity and the highest carboxymethylcellulase activity was chosen for further study.

The spores of the previously-mentioned fungi were grown on soy residue medium as mentioned under Materials and Methods for ten days at 28° C. The enzyme system was prepared as mentioned under Materials and Methods, except the gel filtration step was omitted.

The enzyme preparations produced by these fungi were tested for their soy fiber-degrading activity and carboxymethylcellulase activity. The results are shown in Table 2.

Table 2 indicates clearly that Pestalotiopsis westerdijkii elaborates the most active extracellular enzyme system. It was, therefore, chosen for further study.

2. Selection of Cultural Medium

Spores of Pestalotiopsis westerdijkii were grown on soy residue or wheat bran medium, and the enzyme system was prepared as mentioned under Materials and Methods. The activity of the enzyme system produced on each cultural medium was assayed as follows:

One gram soy residue samples were treated with 10 ml distilled water or purified enzyme at pH 4.0 and 37° C for six hours. Toluene was added to prevent bacterial contamination. At the end of the

Table 2

Comparison of soy fiber degrading activity and CMCase activity of extracellular enzyme systems produced by different fungi

Fungus	Soy fiber degrading activity (△Klett)*	CMCase activity units of enzyme per ml**
Pestalotiopsis westerdijkii	- 10	15.9
Chaetomium globosum	- 8	8.8
Trichoderma viride	- 4	1.5
Basidiomycetes sp.	0	6.2
Myrothecium verrucaria	+ 2	13.1
Aspergillus terreus	+ 2	12.0

* △Klett denotes the decrease of turbidity of soy fiber suspension treated with 1 ml enzyme for 1 hour above or below a control soy fiber suspension treated with only buffer.

** One unit of CMCase enzyme is defined as the amount of enzyme per ml which causes the production of ten micrograms of reducing sugar in one hour.

incubation period, the reaction mixtures were filtered by suction on Whatman No. 1 filter paper. The nitrogen content of the filtrates was determined by the microkjeldahl method, and the nitrogen extracted as a result of enzyme treatment was calculated as well as the percent of nitrogen extraction. The results are shown in Table 3 which indicates that Pestalotiopsis westerdijkii when grown on wheat bran medium produces a more active enzyme system for making soy residue protein more available for water extraction than that enzyme system produced on soy residue medium.

3. Optimum Cultural Conditions

a) Optimum temperature

Pestalotiopsis westerdijkii was grown on wheat bran medium for 10 days at 27°, 31.5°, 34°, and 36° C. There was no growth whatsoever at 34° and 36°. At 27° the mold sporulated on the fifth day and on the tenth day at 31.5° C.

The enzyme system was prepared from cultures grown at 27° and 31.5° C. The activity of the enzyme preparations was determined as percent increase in nitrogen extraction and as percent loss in weight of one gram of soy residue when treated for six hours with 10 ml/^{of}enzyme at 37° C and pH 4.8. Table 4 suggests that the optimum temperature for the production of a potent enzyme system is 31.5° C.

b) Age of culture

The mold was grown on wheat bran medium at 31.5° C for 4, 6, 8, and 10 days, and the purified enzyme system was

Table 3

Extraction rates of nitrogen from soy residue by enzyme systems from different media

Source of enzyme	mg nitrogen extracted*	% Extraction**
Wheat bran medium	7	17
Soy residue medium	3	7

*The nitrogen content of the control (9 mg) and the enzyme from wheat bran medium (1 mg/ml) and from soybean residue medium (2.5 mg/ml) are subtracted.

**One gram of soy residue contains 42 mg nitrogen.

Table 4

Nitrogen extraction and loss of weight of soy residue by enzymes produced at different temperatures

Source of Enzyme	% Nitrogen Extraction *	% Loss in Weight
27° C culture	7.8	8.7
31.5° C culture	9.5	19.3

*These values were corrected for nitrogen contributed by the enzyme (1 mg/ml) and by the control (9 mg).

prepared as mentioned. The enzyme activity and the age of culture were studied. The results are shown in Table 5. This table indicates that ten-day-old cultures give the most active enzyme preparation.

4. Optimum Conditions for Enzyme Activity

a) pH optimum

The pH optimum of the purified enzyme system was determined by treating one gram of soy residue with ten ml enzyme preparation and adjusting to various pH values by addition of hydrochloric acid or sodium hydroxide. The mixtures were then incubated at 37° C for six hours and filtered through Whatman No. 1 filter paper by suction. The nitrogen content of the filtrates was determined, and the amount of nitrogen extracted due to enzyme action was calculated. It can be seen from Table 6 that although the absolute amount of nitrogen extracted by the same amount of enzyme at the same pH for two independent experiments differs, the percent of maximum activity agrees very well for the two experiments. The data in Table 6 and Figure 1 indicate clearly that the pH optimum is 4.6.

b) Optimum Temperature

The optimum temperature for soy residue protein extraction was determined as mentioned before under optimum pH except the pH was fixed at 4.6 and the temperature was varied. Figure 2 suggests that the temperature optimum for enzyme activity is 38° C.

Table 5

Effect of culture age on enzyme activity for extraction of soy residue nitrogen

Age of Culture	% Nitrogen Extraction	% Increase over control
4 days	26.3	4.9
6 days	27.9	6.5
8 days	29.2	7.8
10 days	29.6	8.2
Control	21.4	—

Table 6

pH Optimum of enzyme system acting on soy residue protein extraction*

pH	Experiment No. 1		Experiment No. 2	
	mg N Extracted	% of Maximum activity	mg N Extracted	% of Maximum activity
4.4	1.6	28	0.0	0.0
4.6	5.7	100	1.1	100
4.8	2.6	45	0.5	46
5.0	1.7	30	0.3	27

*Control without enzyme treatment resulted in extraction of 9 mg N.

Figure 1.

Effect of pH on the activity of the extracellular enzyme system preparation. Bars indicate highest and lowest of duplicate values.

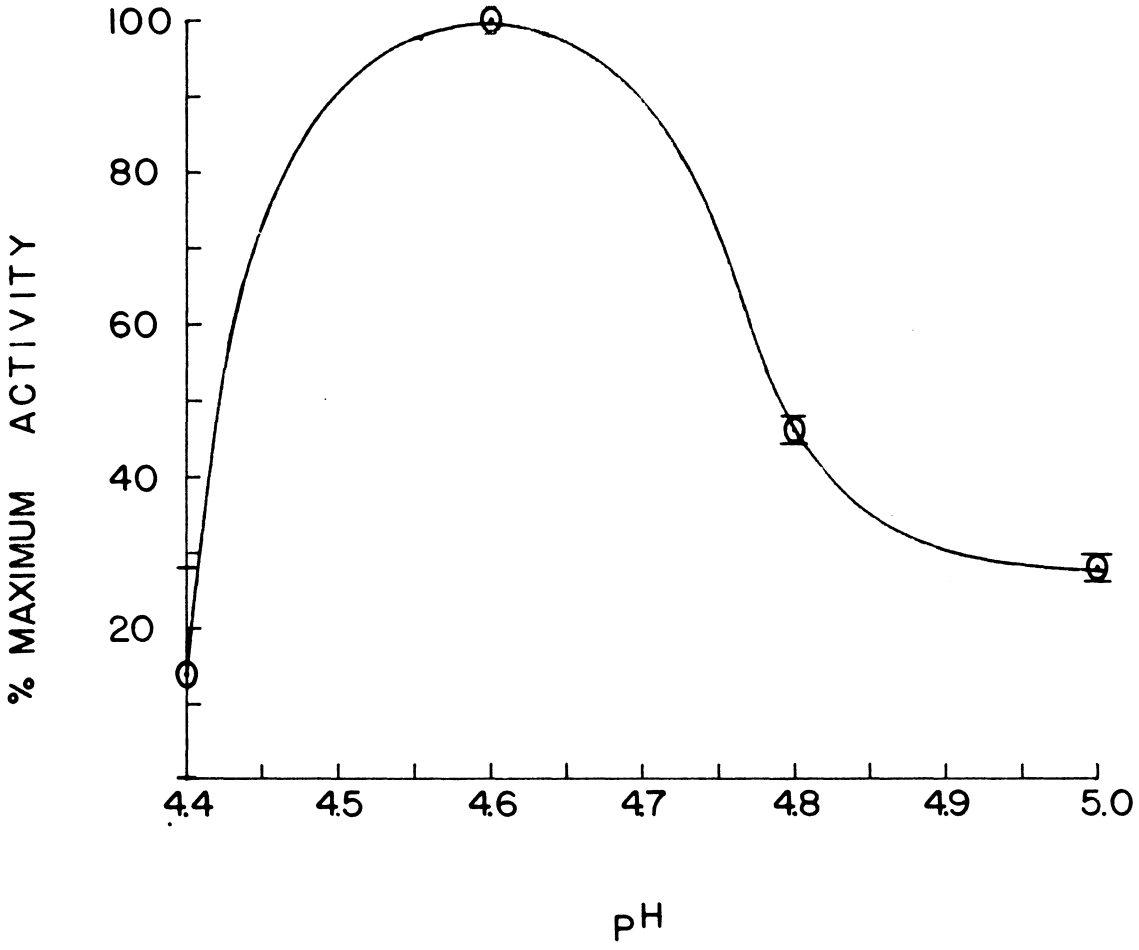
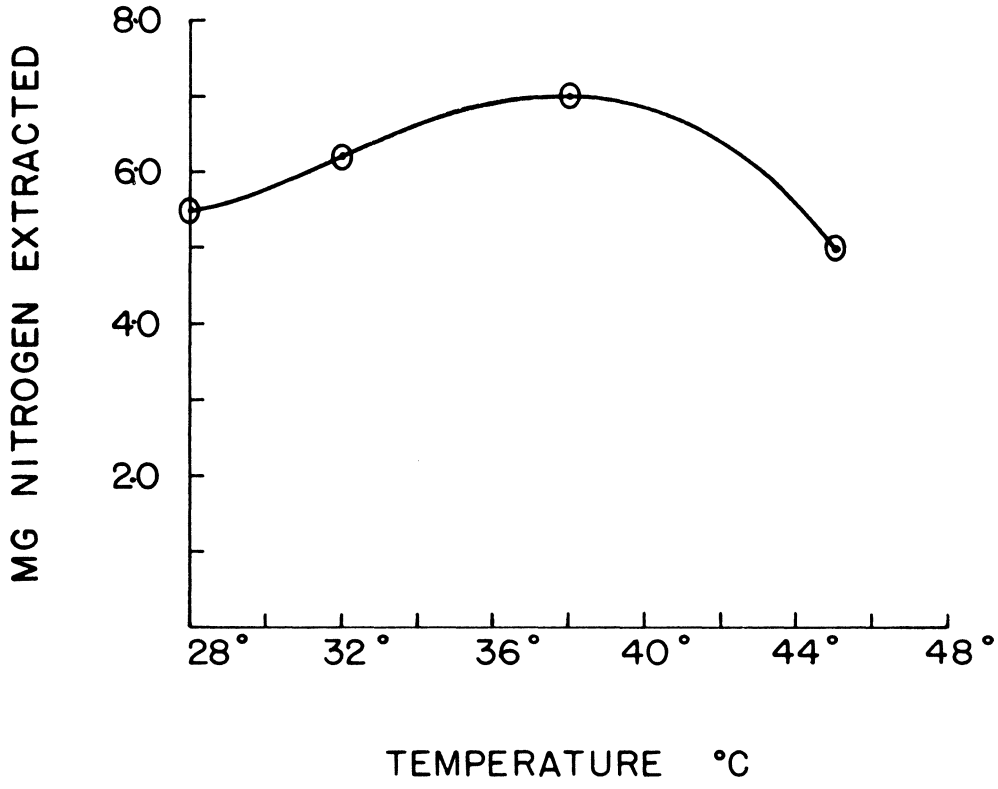

% MAXIMUM ACTIVITY
100
80
60
40
20
4.4
4.5
4.6
4.7
4.8
4.9
5.0
pH

Figure 2

Effect of temperature on the activity of the extracellular enzyme system preparation.


MG NITROGEN EXTRACTED
80
60
40
20
28°
32°
36°
40°
44°
48°
TEMPERATURE °C

c) Optimum amount of enzyme for maximum extraction of protein for soy residue:

Figure 3 indicates the extraction of nitrogen from soy residue increases rapidly with the increase of the amount of enzyme. Then the efficiency of extraction decreases. The same figure indicates that the optimum amount of enzyme to be used for large scale soy residue extraction is 40 ml. Beyond this level added enzyme had little effect.

d) Incubation time vs. enzyme system activity on extraction of nitrogen from soy residue:

Samples of 1 gram of soy residue each were treated with 40 ml enzyme at pH 4.6 and 37.5° C. for different periods of time.

Figure 4 suggests that nitrogen extraction increases rapidly the first 20 hours, and for longer periods of time the increase in nitrogen extraction is slow. It also suggests that the optimum incubation time is 20 hours.

5. Nature of Hydrolytic Products

a) Carbohydrates

Total carbohydrates (28) and reducing sugars (25) liberated by the action of 10 ml enzyme system on one gram soy residue at optimum pH and temperature for six hours were determined. Sugars liberated were identified chromatographically using Trevelyan's et al. (31) method and determined by Somogyi's (25) method after eluting each spot. The results are presented in Table 7 which suggests the presence of hemicellulolytic enzymes. The data also suggest that in addition to the liberation of

Figure 3

Effect of amount of extracellular enzyme system on the rate of nitrogen extraction from soy residue.

One gram soy residue was treated with different amounts of enzymes for 24 hours at pH 4.6 and 37° C. The reaction mixtures were filtered through Whatman No.1 filter paper. Nitrogen contents of filtrates were determined and the percent nitrogen extracted was calculated after correcting for control values.

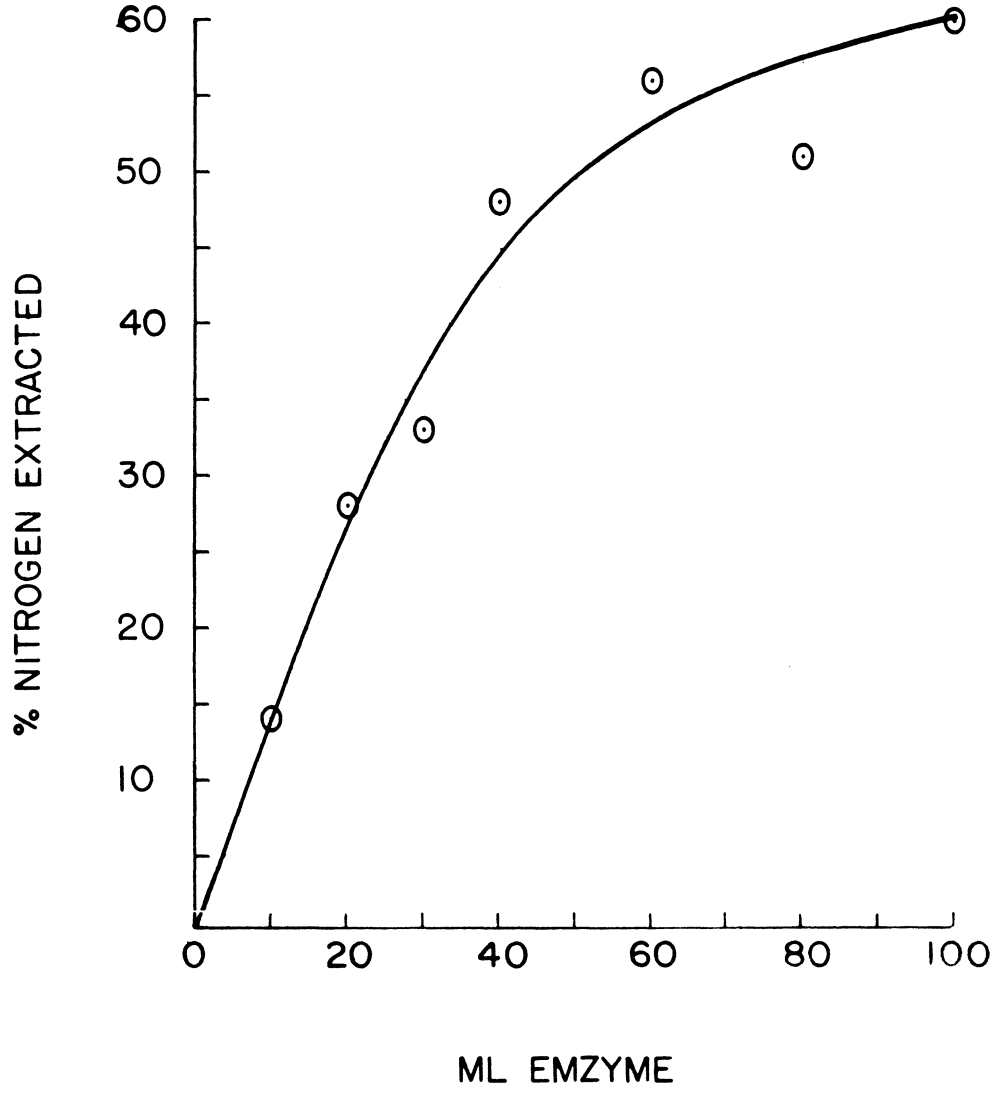

60
50
40
30
20
10
% NITROGEN EXTRACTED
0
20
40
60
80
100
ML EMZYME

Figure 4

Effect of incubation time on the total nitrogen and non-protein nitrogen content of soy residue filtrate after treatment with the extracellular enzyme system.

One gram of soy residue samples was incubated with 40 ml (32 mg nitrogen) extracellular enzyme system preparation at 37° C and pH 4.6 for different periods of time. The reaction mixtures were filtered through Whatman No. 1 filter paper. Nitrogen and non-protein nitrogen were determined in the filtrate. Curve A shows the effect of incubation time on the amount of nitrogen in the filtrate. Curve B shows the effect of incubation time on the non-protein nitrogen content of the filtrate

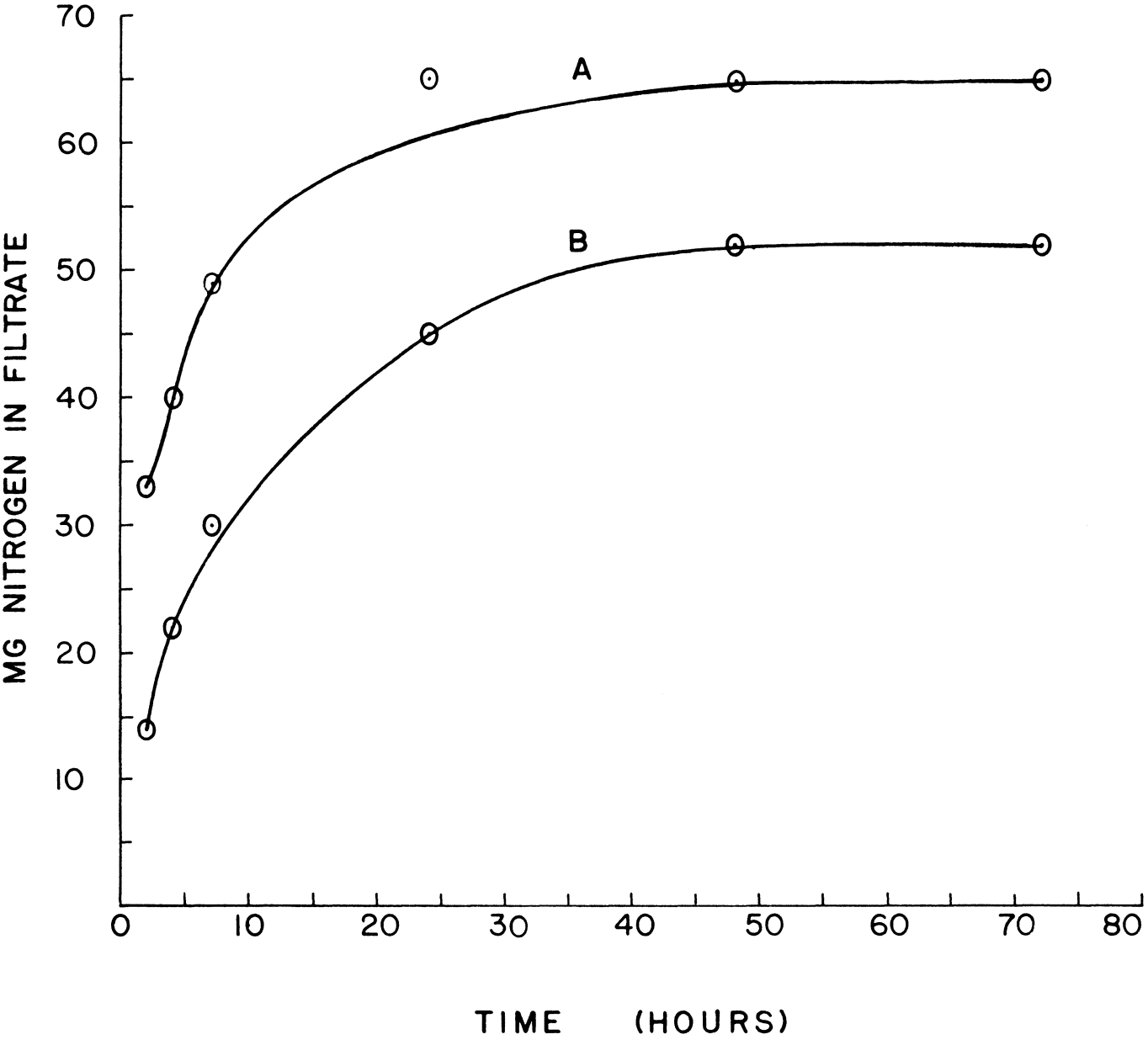

70
60
50
40
30
20
10
MG NITROGEN IN FILTRATE
A
B
0
10
20
30
40
50
60
70
80
TIME (HOURS)

Table 7

Carbohydrate liberated due to the action of enzyme system on soy residue

Carbohydrate	Residue and Boiled Enzyme*	Residue Treated	Increases
	mg	mg	mg
Total carbohydrate	36	128	92
Reducing sugars	38	74	36
Unknown	2.5	2.5	0
Galactose	3.8	18.1	14.3
Glucose	3.8	10.0	6.2
Arabinose	17.5	15.0	0
Xylose	2.5	2.0	0

*Enzyme solution was boiled for 10 minutes.

glucose and galactose other carbohydrates with an average chain length of three were produced.

b) Non-Protein Nitrogen

Non-protein nitrogen produced when one gram of soy residue was treated with 40 ml of enzyme system under optimum pH and temperatures for different periods of time was determined by the method of Becker et al. (4). The results in Figure 4 suggest that there is a rapid increase in the amount of non-protein nitrogen and then a slow increase after 10 hours. When one gram of soy residue was incubated with 40 ml of distilled water for two hours, there was no production of non-protein nitrogen, but when incubated for three days, it was found that 75% of the nitrogen extracted was non-protein nitrogen. In both cases, that is, treating with enzyme or distilled water, toluene was added to prevent bacterial contamination. It can be suggested from these results that the formation of non-protein nitrogen is due to proteolysis by proteases present in both the enzyme system and the soy residue.

6. Feeding Experiments

a) Large scale preparation of untreated and treated soy residue. Two samples of six-hundred grams of soybean seeds each were soaked with 3.5 volumes of distilled water for 12 hours. The soak-water was drained and the seeds were dehulled. The dehulled beans were then homogenized for ten minutes in a Waring blendor with 8.5 volumes of distilled water per kilogram of dried soybean seeds. The homogenates were filtered through two layers of cheese cloth by squeezing. The

filtrates (soy milk) were boiled for one hour at 100° C. then freeze dried. The residues were freeze dried and used for treatment with either distilled water or enzyme* at a rate of 40 ml per gram of dried residue for two days at pH 4.6 and 37.5° C. Toluene was added to prevent bacterial contamination. At the end of the incubation period the mixtures were filtered through Whatman No. 1 filter paper under suction. The filtrates and the residues were boiled for one hour at 100° C. and freeze dried and weighed. The dried filtrates were combined with the two soy milk fractions to produce control milk and treated milk. The material balance of the fractions prepared and their proximate analysis is shown in Tables 8 and 9.

Table 8 indicates that enzyme treatment increases the protein yield in soy milk 20% more than water extraction treatment. The significance of such a treatment can be more appreciated if the following is taken into consideration.

The annual world production of soybean is 40 million tons. If this were processed for production of soy milk and residue, then 3.4 million tons of protein would be left in the soy residue which is not suitable for human use because of its high fiber content. Since enzyme treatment makes 60% of soy residue protein available for extraction, an additional two million tons of protein would be available for human use. If we assume that protein requirement for a human adult weighing

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*Nitrogen concentration in enzyme preparation is 0.8 mg per ml.

Table 8

Dry weights of control and treated soy residue and soy milk and the percentage of original crude soy protein in each fraction

Fraction	Dry Weight	% Protein*
	gm.	
Control Residue	182.5	24.3
Control Milk	304.9	69.8
Treated Residue	79.0	9.4
Treated Milk	392.6	89.5

*one gram of soybean seeds used has 33% crude protein.

Table 9-a

Proximate analysis of soybean fractions

Fraction	% Protein	% Ash	% Ether Extract	% Moisture	% Fiber	% Other	Total
Control Residue	24.7	1.1	16.7	6.4	16.9	34.2	100
Control Milk	43.9	5.5	19.0	3.2	0.3	28.1	100
Treated Residue	22.4	3.1	20.6	4.8	11.9	27.2	100
Treated Milk	41.1	5.7	10.8	8.9	1.6	31.9	100

Table 9-b

Composition of Soybean Fractions

Figures in grams per 100 gm dry matter

Fraction	Protein	Ash	Ether Extract	Fiber	Other
Control Residue	26.3	1.2	17.9	18.1	36.5
Control Milk	45.3	5.7	19.6	0.3	29.1
Treated Residue	23.5	3.3	21.7	12.5	39.0
Treated Milk	45.1	6.2	11.9	1.9	34.9

70 kg is 36 kg protein per year and the protein requirement for a five year old child weighing 20 kg is 12 kg of protein per year, then the amount of protein made available by enzyme treatment would meet the protein needs of 55 million human adults or 165 million children five years old.

b) Amino Acid Composition of Soybean Fractions

The amino acids were determined according to Spackman et al. (26) and tryptophan was determined by the method of Graham et al. (9). The results are summarized in Table 10. The requirement index was calculated for each fraction according to the Rao (22) method. The results are summarized in Table 11 which indicates that all the five fractions have protein of considerably good quality. Table 12 shows the essential amino acid requirements for infants and the percentage of the requirement met when the infant consumes his protein requirement* (23) as treated soy milk. Table 12 also suggests that more than 100% of the essential amino acid requirements for infants are satisfied by treated soy milk protein with the exception of methionine where only 64% of the requirement is satisfied and isoleucine where only 87% of the requirement is satisfied.

c) Determination of Protein Quality of Soybean Fractions.

Diets containing 10% protein from soybean fractions and a control diet containing 10% protein from casein were prepared according to the Derse (7) method. The salt mixture and vitamin mixture used were those recommended by Campbell (8).

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*Protein requirement is 2.6 gm/kg body of protein with biological value of 70.

Table 10

Amino Acid Composition of Soybean Fractions
gm per 100 gm protein

Amino Acid	Control Residue	Control Milk	Treated Residue	Fraction Extracted by Enzyme	Treated Milk
Lysine	8.0	7.3	8.7	9.9	8.0
Histidine	3.7	2.9	3.1	2.5	2.8
Arginine	6.2	6.4	3.5	0.2	4.8
Aspartic	11.5	10.6	10.3	9.5	10.3
Threonine	4.3	3.6	4.0	3.7	3.7
Serine	5.1	4.2	4.0	4.0	4.2
Glutamic	16.7	16.8	11.7	12.8	15.8
Proline	6.3	5.2	4.9	5.1	5.2
Glycine	4.6	3.9	5.0	4.0	3.9
Alanine	4.5	4.0	5.6	4.5	4.2
Cystine	1.4	2.7	4.0	1.6	2.4
Valine	5.4	4.3	5.5	5.3	4.5
Methionine	1.1	1.1	1.0	1.0	1.1
Isoleucine	5.4	4.3	4.6	4.1	4.2
Leucine	8.8	6.7	5.1	6.4	6.7
Tyrosine	2.5	3.4	2.7	2.7	3.2
Phenylalanine	5.3	4.4	4.5	3.7	4.3
Tryptophan	1.3	1.4	1.3	1.3	1.3
Ammonia	2.0	1.8	2.5	3.4	2.2

Table 11

Requirement Index of Soybean Fractions

Protein Source	Requirement Index
Control Residue	89
Control Milk	89
Treated Residue	91
Treated Milk	87
Fraction Extracted by Enzyme	83

Table 12

Essential Amino Acid Requirements for Infants and the Percentage Satisfied by Treated Soy Milk

Amino Acid	Requirements (17) mg/kg	% Satisfied by 2.6 gm Soy Milk Protein
Histidine	34	241
Isoleucine	126	87
Leucine	150	116
Lysine	103	202
Methionine	45	64
Total S-AA	---	---
Phenylalanine	90	124
Total Aromatic-AA	---	---
Threonine	87	111
Tryptophan	22	154
Valine	105	111

Forty-seven male rats of the Sprague-Dawley strain, weighing 45 ± 5 gm each, were fed a casein diet containing 10% protein for five days, then starved for 18 hours to empty their intestinal tracts. The animals were weighed and divided into six groups. Ten animals were allotted for each of several groups including the protein-free diet, casein diet, soy milk control, and treated soy milk diet. Five rats were fed the control soy residue, and two rats were fed the treated residue diet.

The animals were placed in metabolism cages, fed ad libitum for seven days, and then starved for 18 hours. The food consumption was recorded and the animals were weighed and sacrificed with chloroform. The carcasses were dissolved in 50% sulfuric acid solution in a steam bath. The urine and feces were collected. The nitrogen content of carcasses, urine and feces were determined by macrokjeldahl (13).

Table 13 shows weight gains, nitrogen intake, body nitrogen, urinary and fecal nitrogen per rat. It also shows some of the protein quality parameters. Table 13 shows that treated milk is a little superior in protein quality when compared to control soy milk or treated residue. There was a little improvement in the digestibility of treated soy milk. The data in this table also confirm the observation of Hackler et al. (10) that soy residue prepared by water extraction has a high PER compared to soy milk. It can also be seen from Table 13 that there is no sign of toxicity. However, long-term feeding experiments are required for detection of any mycotoxins which might cause any hepatoma

Table 13

Nitrogen Balance and Estimates of Protein Quality of Casein and Soybean Fractions

	Protein free	Casein	Control Milk	Treated Milk	Control Residue	Treated Residue
N-Intake, in gm	0.0	1.02	0.87	0.84	0.66	0.67
Wt. Gain, in gm	- 7.8	24.7	11.0	11.7	10.6	3.5
Urinary N, in gm	0.059	0.094	0.163	0.177	0.154	0.230
Fecal N, in gm	0.039	0.115	0.195	0.159	0.162	0.280
Body N, in gm	1.18	1.82	1.53	1.60	1.54	1.46
Digestibility	--	92	82	85	81	57
Biological value	--	96	85	83	82	68
NPU	--	62	40	49	48	41
NPV	--	6.2	4.0	4.9	4.8	4.1
NPR	--	5.1	3.5	3.7	4.4	2.6
PRE	--	81.6	56.0	59.2	70.4	41.6
PER	--	3.9	2.0	2.2	2.5	0.8

or other indications of toxicity. Because of the close similarity in amino acid composition and requirement index between the soy residue and soy milk prepared by water extraction as seen in Tables 10 and 11, there is no obvious reason to explain why the residue protein is of superior quality.

VI. CONCLUSION

By use of an extracellular enzyme system isolated from Pestalotiopsis westerdijkii about 60% of the nitrogen content of soy residue has been made available for extraction. There was an increase of 20%, in protein yield of soy milk. In addition, there was a small improvement in the protein quality of treated soy milk as well as improvement in its digestibility when compared to control soy milk prepared by water extraction. Additional information is needed to establish the economic feasibility of such treatment. Information is also needed to show if these fungal cellulolytic enzyme preparations offer no hazard to human health. Supplementation of the treated soy milk with sulfur amino acids and isoleucine should make it considerably better as a protein substitute for infants.

VII. SUMMARY

1. Pestalotiopsis westerdijkii is the most suitable producer of cellulolytic enzyme systems among the fungi examined for this study.
2. Wheat bran medium is superior to soy residue medium for the production of the enzyme.
3. The optimum temperature for the growth of Pestalotiopsis westerdijkii and the production of enzyme is 31.5° C.
4. A ten-day growth period is sufficient for the production of enzyme capable of degrading soy residue.
5. The optimum pH and temperature for the enzyme activity are pH 4.6 and 38° C.
6. Forty ml of enzyme and 24 hoursincubation time with one gram soy residue results in the most efficient extraction of protein from soy residue (about 60% of soy residue protein).
7. The amino acid content of the treated soy milk indicates that the first limiting amino acid is methionine or sulfur-containing amino acids. The second most limiting amino acid is isoleucine. The rest of the essential amino acids are present in amounts far more than sufficient to meet the infant's daily requirements.
8. The enzymatic treatment in addition to increasing the protein extraction from soy residue, improved both the protein quality and digestibility of soy milk.

VIII. ACKNOWLEDGMENT

I offer my deep appreciation to all my professors in the Department of Biochemistry and Nutrition at Virginia Polytechnic Institute for all the knowledge they offered me. My sincere thanks is offered to Dr. K. W. King for his guidance and encouragement throughout my stay at V.P.I. I am really lacking in words to show my utmost appreciation to Dr. King who gave me the opportunity to have an experience in the field of nutrition. Many thanks also to Dr. J. P. Fontenot, under whom I had my first course in nutrition, which was well taught and on which I started building my knowledge in nutrition.

The sacrifice and help of my wife is also greatly appreciated.

Last, but not least, many thanks to the staff of the Institute of International Education for giving me the opportunity for higher education and meeting many wonderful people at V.P.I.

IX. BIBLIOGRAPHY

1. Altschul, A. M. 1962. Seed Protein and World Food Problems, Economic Botany, 16, January-March, p. 4.

2. Agricultural Statistics. 1965. United States Department of Agriculture, Washington, D. C.

3. A.O.A.C. 1960. Official Methods of Analysis (9th Ed.) Association of Official Agricultural Chemists, Washington, D. C.

4. Becker, H. C., R. T. Milner, and R. H. Nagel. 1940. Method for the Determination of Non-protein Nitrogen in Soy Meal. Cereal Chem. 17: 447.

5. Berg, L. R. 1959. Enzyme Supplementation of Barley Diets for Laying Hens. Poultry Sci. 38: 1132.

6. Berg, L. R. 1961. Effect of Adding Enzymes to Barley Diets at Different Ages of Pullets on Laying House Performance, Poultry Sci. 40: 34.

7. Derse, P. H. 1958. Evaluation of Protein Quality (Biological Method). J. Assn. Official Agr. Chem. 41: 192.

8. Evaluation of Protein Quality. 1963. National Academy of Sciences-National Research Council. Publication 1100, Washington, D. C.

9. Graham, C. E., E. P. Smith, O. W. Hier, and D. Klein. 1945. An Improved Method for the Determination of Tryptophan with p-Dimethylaminobenzaldehyde. J. Biol. Chem. 168: 711.

10. Hackler, L. R., D. B. Hand, K. H. Steinkraus, and J. P. Van Buren. 1963. A Comparison of the Nutritional Value of Protein from Several Soybean Fractions. J. Nutrition 80: 205.

11. Hand, D. B. 1965. Development of Low-Cost Supplemental Food from Soybeans. New York State Agricultural Experiment Station, Cornell University, Geneva, New York.

12. Hash, J. H. and K. W. King. 1958. On the Nature of the β-Glucosidase of Myrothecium verrucaria. J. Biol. Chem 232: 394.

13. Johnson, M. J. 1941. Isolation and Properties of a Pure Yeast Polypeptidase. J. Biol. Chem. 137: 575.

14. King, K. W. 1956. Microbial Degradation of Cellulose. Tech. Bull. No. 154. Virginia Agricultural Experiment Station.

15. Leveile, G. A., H. E. Sauberlich, and J. A. Edelbrock. 1961. The Influence of Enzyme Supplementation on the Digestibility of Algae. U. S. Army Med. Res. and Nutr. Lab., Fitzsimons General Hospital, Denver, Colorado. Report No. 259, Project 6X60-11-001.

16. Li, L. H., R. M. Flora, and K. W. King. 1963. Purification of β-Glucosidase from Aspergillus niger. Initial Observations on the C_1 of Trichoderma koningii. J. Fermentation Technol. 41: 98, No. 2.

17. Munro, H. N. and J. B. Allison, eds. Mammalian Protein Metabolism. Vol. II. 1964. Academic Press, New York and London.

18. Nisizawa, K. and H. Ozaki. 1964. Effect of Culture Filtrates of Irpex lacteus on the Extraction Rate of Protein from Defatted Soybean. J. Fermentation Technol. 42:415, No. 7.

19. Nutrition Document R9/Add. 2. PGA Meeting of August, 1960.

20. Oshima, J. and Igi, N. Studies on the Extraction of Tea Components Using Cellulase. Proc. 2nd And 3rd Symposia on Cellulase and Related Enzymes, p. 64 (1963). Cellulase Association. Dept. Ferment. Technol. Facult. Engin. Osaka, Japan.

21. Ozaki, H., Sazaki, I. and Tazaki, R. 1962. Third Cellulase Symposium, Osaka, Japan. Original not seen.

22. Rama Rao, P. B., H. W. Norton, and B. C. Johnson. 1964. The Amino Acid Composition and Nutritive Value of Proteins. V. Amino Acid Requirements as a Pattern for Protein Evaluation. J. Nutrition 82: 88.

23. Recommended Dietary Allowances (6th Ed.) 1964. National Academy of Science-National Research Council, Publication 1146, Washington, D.C.

24. Reese, E. T. and Mandels,M. 1957. Induction of Cellulase in Trichoderma viride as Influenced by Carbon Source and Metals. J. Bacteriol. 73: 269.

25. Somogyi, M. J. 1952. Notes on Sugar Determination. J. Biol. Chem. 195: 19.

26. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic Recording Apparatus for Use in the Chromatography of Amino Acids. Anal. Chem. 30: 1190,

27. Tazaki, R. and K. Ouye. 1961. Action of Cellulase on Soybean Oil Meal. Proc. 1st Symposium on Cellulases and Related Enzymes, p. 23.

28. Timell, E. T. 1960. Determination of the Degree of Polymerization of Reducing Pentose and Hexose Oligosaccharides. Svensk. Paperstid 63: 668.

29. Toyama, N. 1962. Personal Communication to Dr. K. W. King.

30. Toyama, N. 1963. Degradation of Foodstuffs by Cellulase and Related Enzymes. Advances in Enzymic Hydrolysis of Cellulase and Related Materials, Pergamon Press, Oxford, London, New York, Paris.

31. Trevelyan, W. E., D. P. Proctor, and J. S. Harrison. 1950. Detection of Sugars on Paper Chromatography. Nature 166: 444.

32. Watanabe and Fukamachi. 1960. Shokuryo Kenkynsno, Kenkyu Kokokn No. 14B, 1-84. Original not seen.

33. Willingham, H. E., L. S. Jensen, and J. McGinnis. 1959. Studies on the Role of Enzyme Supplements and Water Treatment for Improving the Nutritional Value of Barley. Poultry Sci. 38: 539.

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ABSTRACT

Enzymatic Modification of the Extractability of Protein from Soybeans (Glycine max)

Six fungi, Pestalotiopsis westerdijkii QM:381, Myrothecium verrucaria QM:460, Aspergillus terrus QM:72f, Chaetomium globosum QM:459, Basidiomycetes sp. QM:806, Trichoderma viride QM:6a were screened according to their ability to elaborate an extracellular enzyme system which has the highese soy fiber-degrading activity and the highest carboxymethylcellulase activity. Pestalotiopsis westerdijkii QM:481 gave the most potent extracellular enzyme system.

When Pestalotiopsis westerdijkii was grown on a wheat bran medium at a temperature of 31.5° C for ten days, it elaborated the most potent extracellular enzyme system.

It was found that the optimum conditions for this extracellular enzyme system, acting on soy residue for the purpose of making soy residue protein available for extraction, were pH 4.6 and 38° C.

Treating one gram of soy residue, 40 ml (32 mg Nitrogen) enzyme preparation, at the optimum pH and temperature for 20 hours caused the most efficient extraction of protein.

There was an increase in the amount of soluble carbohydrates, reducing sugars, glucose and galactose due to the action of enzyme on soy residue. The amount of non-protein nitrogen in the extractable

fraction of soy residue after treatment with enzyme preparation or water increases with the increase of incubation time even in the presence of toluene which prevents bacterial contamination. This is probably due to the action of proteases from the soybean and enzyme preparation.

In large scale experiments, when 40 ml (32 mg Nitrogen) of the enzyme preparation per gram of soy residue were used, about 60% of the soy residue protein was made available for water extraction and the yield of extractable protein in soy milk was increased by 20%

Feeding experiments using treated or untreated soy residue and soy milk showed that the treated milk contains protein of higher quality and better digestibility when compared to untreated soy milk or treated residue. The most limiting amino acid in these products is methionine. The amino acid composition of these products was very much the same.