INSECTS AS POTENTIAL PESTS AND CONTAMINANTS OF TEXTURIZED SOY PROTEIN

by

WILLIAM E. PURSLEY

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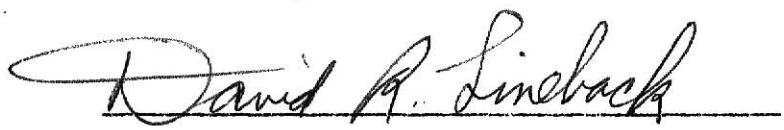

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INTRODUCTION

World food shortages becoming more prevalent, and affecting more of the population, have lead to more interest in increasing the utilization and efficiency of existing foodstuffs. Increased interest in by-passing livestock feeding, increasing efficiency of vegetable protein in the human food chain, and utilizing feedstuffs in human foods have resulted in much research on vegetable proteins in human foods. With the development and advancement of extrusion processing, vegetable proteins are being processed into several utilizable forms. Textured vegetable proteins, for example, are being used in several food products as mixes, meat replacements, or meat product extenders.

The purpose of this research was to determine the acceptance of a texturized soy product and soy flour by ten common stored products insects. Procedures for recovering and counting insect fragments from texturized soy protein were tested to find a reliable, convenient procedure. The effect of processing on insect fragments was also examined.

REVIEW OF LITERATURE

Until 1942 very little was known concerning insect pests of edible soy products. At this time the Soy Flour Association became concerned over possible insect problems and requested the Division of Entomology and Economic Zoology, University of Minnesota, to make a detailed investigation of insects likely to injure edible soy products in storage.

Using the confused flour beetle, Tribolium confusum (J. duV.), Mickel and Standish (1946) reported that the rate of larval development decreased

so greatly that little chance of major infestation existed on either soy flour or soy grits.

Lin and Richards (1952) continued this work at the University of Minnesota to better understand the unsuitability of soy flour for the confused flour beetle. They reported that longer developmental times were required for confused flour beetles on soy flour versus cereal flours. It was discovered that, with the addition of dried yeast, the insects performed nearly equal to those on cereal flours. They also found that the addition of pure B-vitamins and cholesterol improved the food value of soy flour, however, neither sterol nor B-vitamins wholly accounted for the deficiency of soy flour as food for this beetle.

From the mid-1950's through the 1960's, research on soy and insects was directed toward resistance found in soy. Lipke et al. (1954) used the confused flour beetle to study effects of various known inhibitors found in soy on the beetle. They found only minor response and suggested the presence of an undescribed toxin.

In the 1960's, research teams from Hebrew University, Rehovoth, Israel, [Birk and Applebaum (1960), Birk et al. (1962), Ishaaya and Birk (1965)] reported various effects of different soy inhibitors on Tribolium castaneum (Hbst.), Tenebrio molitor (L.), rats and chicks. They found trypsin inhibitors, protease inhibitors and saponins as the major inhibitors in soy which appeared effective against insects.

Su et al. (1972) tested soybean saponins and their calcium salt against Sitophilus oryzae (L.) and found them effective in controlling this weevil when dusted on wheat. Soybean meal and defatted soybeans in high dosages also gave some protection when dusted on wheat.

Partida (1973), using fourteen different stored products insects on sound soybeans, soybean which were one-third damaged, and soybean oil meal, reported that only Lasioderma serricone (F.), Rhyzopertha dominica (F.), Tribolium confusum (J. duV.), and T. castaneum (Hbst.) appeared capable of surviving on soybean oil meal. None were successful on sound or one-third damaged soybeans.

Highland (1974) tested eleven cereals and blends of wheat, soy, corn, and oats with ten species of stored products insects and found that rapid infestations could occur in any of the tested blends.

MATERIALS AND METHODS

Test Media

Source. Soy flour and texturized soy protein (TSP) used in the insect acceptability tests were supplied by Garvey Mills, Inc., Wichita, Kansas.

Sterilization and Storage. Soy flour, TSP, and control media were placed in the Department of Grain Science and Industry cold room held at -23°C for at least two weeks to destroy any live insects that might have been in the products and then stored at 5°C in the Stored Products Laboratory cold room to prevent any subsequent infestation.

Particle Size. Standard particle size tests were run on TSP using U.S. Standard sieves and shaking for 10 min. using a Ro-tap shaker. Overs of each sieve were weighed and recorded. Calculations were made using standard formulas for the Modulus of Uniformity, Modulus of Fineness, mean particle size, and number of particles per gram.

Standard U.S. sieve sizes included in screen tests were; 3, 4, 6, 8, 10, 14, 20, 28, 35, 48, 65, 100, 150, 200, and 270. Screen sizes used in calculating Modulus of Uniformity and Fineness were; 3, 4, 8, 14, 28, 48, and 100.

Moisture Equilibrium Tests. Saturated solutions of NaCl, NaBr, KBr, KCl, and K_2CO_3 were prepared in plastic containers (29.5 cm. x 19.5 cm. x 10 cm) with tightly fitting snap-on lids. Saturated solutions and their corresponding relative humidity (R.H.) are as follows (Hubbard, 1957):

Solution	Relative Humidity
KCl	84.7%
KBr	80.7%
NaCl	75.8%
NaBr	57.0%
K_2CO_3	43.8%

Chambers were allowed to equilibrate in a room with a constant temperature of 27.7°C at the specific R.H.'s. After 48 hrs., approximately 2 gm. of TSP and soy flour were individually placed in 9 cm. petri dishes without covers. Three replications of each were placed in the different R.H. chambers. Samples were allowed to equilibrate and then removed to determine corresponding moisture contents.

At 27.7°C and 70 ($\pm$ 2%) R.H., moisture contents were also determined on samples of soy flour and TSP used in the acceptability tests under rearing room conditions. Moisture tests of samples under identical conditions as that used in insect acceptability tests were run after two months.

Moisture Determination. Moisture contents of both TSP and soy flour were determined by using a standard air-oven method (AACC Method 44-15, "Cereal Laboratory Methods," 1962). Moisture contents of the samples were determined using the following equation:

$$\frac{\text{(wt. of dish + wet sample)-(wt. of dish + dry sample)}}{\text{(wt. of dish + wet sample)-(wt. of dish)}} \times 100 = \% \text{ moisture}$$

Sample Sizes. Three replications for each time period and insect were used for TSP, soy flour and control media. Fifty-gram samples, weighed on an Ohaus Triple Beam Balance, were placed in one-pint, narrow-mouthed glass canning jars. The jar rings were fitted with 80 mesh brass strainer cloths and 7 cm. kelthane-treated filter papers were placed outside the brass cloth to prevent possible mite infestation. Samples were allowed to equilibrate in the rearing room for one week before insects were introduced.

Control Media. Control media used for insect acceptability tests were stock rearing room culture media supplied by Stored Products Laboratory, Department of Entomology, Kansas State University or locally purchased materials. Media used for control samples were:

Whole Wheat Flour - Red flour beetle, Tribolium castaneum (Hbst.)
Confused flour beetle, Tribolium confusum (J. duV.)
Cigarette beetle, Lasioderma serricorne (F.)

Rolled Oats - Saw-toothed grain beetle, Oryzaephilus surinamensis (L.)
Flat grain beetle, Cryptolestes pusillus (Schonh.)
Merchant grain beetle, Oryzaephilus mercator (Fauv.)

Wheat - Rice weevil, Sitophilus oryzae (L.)
Lesser grain borer, Rhyzopertha dominica (F.)

Cornmeal - Indian meal moth, Plodia interpunctella (Hbn.)

Chicken Mash - Warehouse beetle, Trogoderma varabile (Beal)

Insects

Source. All but one insect species were obtained from Stored Products Insect Laboratory cultures, Kansas State University.

The red flour beetle, confused flour beetle, saw-toothed grain beetle, flat grain beetle, lesser grain borer, and Indian meal moth were originally found in Kansas, dates unknown. The cigarette beetle was obtained from a package of Kellogg's All-Bran in Kansas, August, 1966. The merchant grain beetle was obtained from Savannah, Georgia in 1964. The rice weevil was obtained in Kansas prior to 1955. These cultures were maintained in the rearing room.

The warehouse beetle, T. varabile (Beal), was obtained from the Stored Product Insects Laboratory, USDA Grain Marketing Research Laboratory, Manhattan, Kansas. This culture had been maintained in a stock media (ingredients unknown).

Collection and Introduction of Insects. One-to seven-day-old adult insects were removed from stock cultures using standard wire mesh screens. This was done for all but the Indian meal moth and warehouse beetle. Adults were spread out in a 35-cm. diameter pan, then collected and counted as they were aspirated into 25-mm. x 100-mm. plastic tubes. Fifty adult insects were placed on media in individual narrow-mouth glass jars. The jars were then placed in the rearing room.

Indian meal moths and warehouse beetles were collected by removing portions of culture media from stock cultures and spreading it out in 35-cm. diameter pans. Using feather weight forceps, 30 late instar larvae were collected randomly, placed in individual glass jars containing media and placed into the rearing room.

Assessment of Populations. Three jars of each media and insect species were removed from the rearing room at intervals of 2, 4, and 6 months. Insects were removed by sieving; live adults were counted and the number recorded.

Indian meal moth adults were collected and counted in a moth collecting cage (a glass top and front, wooden sides with arm holes, and cloth sleeves).

X-rays were also used to check lesser grain borer and rice weevil populations since both species have the potential to develop inside larger TSP particles. Radiographs were made using a General Electric Grain Inspection Unit with Eastman Kodak, Type M x-ray film. A 1.5-min. exposure was used at 20 kv. and 5 ma.

Insect Fragments

Source. Procedures for recovering and counting insect fragments from TSP were tested using soy flour with a known concentration of fragments. The soy flour was then texturized and various methods of recovery and counting were examined.

Insect fragments were produced by grinding dried red flour beetles, confused flour beetles, and cigarette beetles mixed in 200 gm. of soy flour through a Wiley Mill using a 20-mesh grinding screen. This mixture was then mixed with approximately 5 lbs. of soy flour and later blended into 100 lbs. of soy flour using a S. Howes Horizontal Double Ribbon Blender for ten minutes. Defatted soy flour used in the fragment count tests was supplied by Far Mar-Co., Inc., St. Joseph Division, St. Joseph, Mo. Insect fragment numbers were determined using an acid-digestion technique and recorded for soy flour which was later processed into TSP.

Texturizing Soy Protein. Soy flour containing a known insect fragment count was processed through a Wenger [1] X-25 extruder to produce TSP with insect fragments. To produce TSP, soy flour was added to the feeder and moisture was added (as steam at a rate of approximately 200 ml. of water/min.) during conditioning. Steam was also supplied to the steam jacket surrounding the barrel of the pressure cone. Temperature of the conditioned flour was continuously increased as it was subjected to high shear and compression action by the screw conveyor. Temperature of the mixture at the outlet of the chamber was in the range of 107°-121°C. Water was added at the beginning of the extrusion barrel at an approximate rate of 75 lbs./hr. A single-hole die (0.375 inch diameter) was used with a rotating 8-blade cutter to produce the TSP. TSP was then dried in a hot air drier to approximately 13% moisture content, collected, and bagged. Bags were properly marked and placed into cold storage until the product could be tested for insect fragments by various micro-analytical techniques. Particle size of TSP was reduced so that all particles could go through a No. 4 sieve prior to micro-analytical testing.

Insect Fragment Recovery. Standard analytical methods, together with several modified procedures, were tested and evaluated for isolation and detection of insect contaminants in TSP. Numbers of fragments per sample for each test procedure were compared to a standard procedure used on soy flour containing a known number of insect fragments (AACC Method 28-41, "Cereal Laboratory Methods," 1962). Preliminary tests eliminated some techniques because of procedural difficulties.

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[1] Wenger Mixer Manufacturing, Sebetha, Kansas

Insect Fragment Examination. Insect fragments were examined and identified using a wide-field Bausch & Lomb binocular microscope. Fragments were examined on ruled filter paper in petri dishes using an insect probe. Texture, color, shape, and recognizable characteristics were used in identifying fragments (Kurtz and Harris, 1962). Insect fragments were counted and the numbers recorded.

Further examination, to help define some questionable identifications of fragments, was done using the scanning electron microscope.

Scanning Electron Microscopy. Using a scanning electron microscope, fragment material was observed to determine whether fragments were vegetable or animal matter. Fragments to be determined were mounted on circular (9-mm. diameter) specimen studs using 3M double-sided adhesive tape. The mounted samples were coated with a 100-200# Å layer of gold with a high-vacuum, electron-evaporation apparatus (Kinney Corp., Boston, Massachusetts). Each sample was examined by an Auto Scan (ETEC Corp., Hayword, California) scanning electron microscope at 20 k.v. at the magnifications indicated.

RESULTS AND DISCUSSION

Moisture Equilibrium Determination. Moisture equilibrium of both TSP and soy flour media was determined at different relative humidities using five different salt solutions. Results are listed in Table 1.

After 13 days equilibration, moisture contents of samples were determined in all but 84.7% R.H. (KCl). Samples at 84.7% R.H. had started to mold, so it was necessary to repeat this test. Mold infestation was also observed in both TSP and soy flour at 80.7% R.H. (KBr solution) after two weeks at 27.7°C.

The equilibrium chamber was cleaned and new solutions were made for 84.7% R.H.. Samples were again introduced and had equilibrated after eight days. Moisture contents were determined.

Equilibrium relative humidity:moisture content relationships for TSP and soy flour at 27.7°C are shown in Figure 1. The curves indicate that TSP had a higher moisture content than soy flour under these conditions. Critical moisture contents of TSP and soy flour were not tested against test insects to determine whether different moisture contents at the same relative humidity were significant.

Moisture contents of TSP and soy flour used in insect acceptance tests were determined under test conditions (approximately 27.7°C and 70% $\pm$ 2% R.H.). Average moisture contents found (TSP, 14.4%; soy flour, 13.3%) were slightly greater than data shown in Figure 1.

Particle Size Distribution of Texturized Soy Protein. Particle-size distribution data were calculated using standard wire sieves and standard formulas for modulus of uniformity and modulus of fineness. Mean particle

Table 1: Average moisture content in texturized soy protein and soy flour at five different relative humidities*

Relative Humidity %	TSP	Soy Flour
42.7	7.7	7.4
57.0	9.2	8.8
75.0	14.5	14.4
80.7	19.1	17.5
84.7	22.0	22.0

*Appendix A

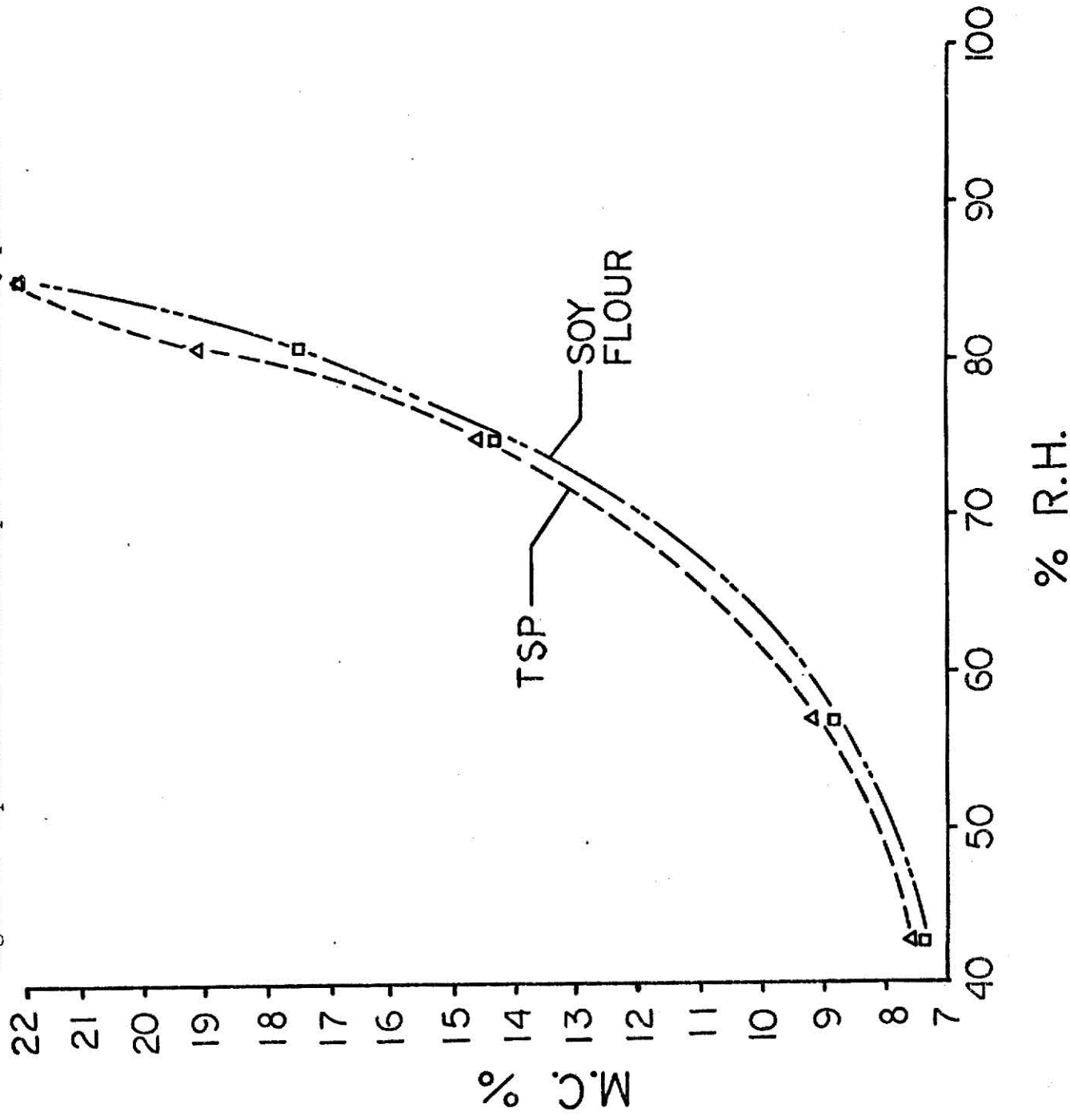

Figure 1: Equilibrium relationships of texturized soy protein (TSP) and soy flour

Table 2: Particle size analysis of texturized soy protein

Samples	Modulus of uniformity*	Modulus of fineness*	Surface area (sq. cm./gm.)	Mean particle size (microns)	Particles per gm.
1	6:4:0	4.51	22.005	2291.3	141.7
2	5:5:0	4.46	22.525	2228.5	148.0

*Appendix B

size, and number of particles per gram were computed using a particle-size analysis program. Data are shown in Table 2.

Although insect acceptance tests, using particle size as a variable, were not conducted for this research, observations indicate that both flour beetles [T. castaneum (Hbst.) and T. confusum (J. duV.)] appear better adapted to finer particle sizes. Particle-size data are given here as a reference to the TSP used. Several different forms are available, and only one has been tested in this study.

Determination of Species Capable of Infesting Soy Flour. Ten common stored product insects were tested to determine if they could infest soy flour under standard rearing room conditions. Results indicate that four of the insects used showed some success and that only one of these appears capable of serious infestations (Table 3).

Both flour beetles maintained a population on soy flour, but did not exhibit the capability of increasing their population. After six months, few red flour beetle larvae could be found and it appeared that possible cannibalism of dead adults and larvae by adult beetles, due to dietary stresses, were the cause. This could have been true for the confused flour beetle also; although it maintained a higher population than the red flour beetle, its populations were relatively small. After six months, several confused flour beetle larvae of all sizes were observed in the flour media.

After six months, adults of both red and confused flour beetles were removed from test jars leaving the larvae alone under rearing room conditions for two months. The number of adults that developed from these larvae are given in Table 4. Results correlate with those found earlier and those found by Michel and Standish (1946), using the confused flour beetle. As

Table 3: Susceptibility of soy flour to ten common stored products insects

Months after introduction:	2		4		6	
Insect	Average no. live adults	Average no. dead adults	Average no. live adults	Average no. dead adults	Average no. live adults	Average no. dead adults
Red flour beetle	45.0	7	51.0	13	55.0	23.3
Confused flour beetle	51.3	0.6	50.3	1.3	84.0	4
Cigarette beetle	104.6	66.3	-[a]	-[a]	-[a]	-[a]
Saw-toothed grain beetle	0	46.5	0	0	0	0
Merchant grain beetle	1	49.0	0	0	0	0
Flat grain beetle	8.3	35.3	0	0	0	0
Rice weevil	0	50.3	0	0	0	0
Warehouse beetle	0	0	0	0	1	1
Indian meal moth	0	20	0	17	0	18
Lesser grain borer	0	50.6	0	0	0	0

[a]Symbol: - = no data collected.

Table 4: Average number of larvae developing into adults during a two-month period after adults were removed at six months from soy flour and texturized soy protein

Insect	Soy flour	TSP
Red flour beetle	6.67	.67
Confused flour beetle	25	1.3
Warehouse beetle	0	0
Lesser grain borer	0	0

stated by these authors, "This insect could not develop normally on samples of soy flour and soy grits. The rate of larval development was so greatly decreased that serious infestation seemed unlikely." It appears that neither flour beetle is capable of extensive infestation in soy flour, but their ability to survive in a soy flour media may indicate that, if mixes or blends of other materials were introduced, present limitations could be removed. Prolonged storage under conducive conditions could result in a considerable loss due to insect damage from either of these species.

Infestation and insect damage due to the warehouse beetle was also exhibited, but problems occurred in using this insect. The warehouse beetle appeared to enter diapause or to have been in diapause at the beginning of the test. The culture in which the beetles were taken was an old culture and not well maintained. It appeared that there was stress on the individuals at the beginning of the test and, as a result, normal development was never reached. In comparing the individuals on soy flour to those on whole wheat, the whole wheat cultures appeared to be developing at a more normal rate after four or five months. After removing adults at six months, adults were not found after checking at eight months while larvae of all stages were found in both media. Continued evidence of diapause was possibly due to a poorer balance of nutritional requirements found in soy flour versus whole wheat flour; however, it occurred in all samples tested.

Indirectly related to these tests, warehouse beetle larvae were placed in individual plastic containers without food or water and left on a desk top at temperatures varying from 18°C to 30°C. After ten months, 17 out of 25 larvae originally placed in containers were still alive. three of the original 25 had chewed their way out of the containers. The warehouse beetle should be considered a very adaptable animal and one which could be a possible pest in soy flour.

Test results show the cigarette beetle quite capable of supporting and producing infesting populations in soy flour. After introducing fifty individuals into culture jars, evidence of larval development could be observed after two weeks. At five weeks, new adults had emerged and at eight weeks live adult numbers had increased two-fold (Table 3). Number of dead adults after eight weeks averaged 66.3. Cultures were checked at the end of thirteen weeks and the population had died because the media was exhausted.

Table 3 indicates that the cigarette beetle is a possible pest of soy flour. The cigarette beetle is a strong flier with a rapid developmental period, produces a high number of progeny, and is able to penetrate packaging well. It seems to possess the needed attributes for becoming a serious pest problem in this product.

As shown in Table 3, the saw-toothed grain beetle, merchant grain beetle, flat grain beetle, rice weevil, Indian meal moth and lesser grain borer found soy flour unacceptable. While all expressed a high level of fitness on their individual control media, none of these species appear to be a threat to soy flour.

Determination of Species Capable of Infesting Texturized Soy Protein. Ten common stored products insects were tested to determine if they could infest TSP under standard rearing room conditions. Results (Table 5) indicated that only two species showed any success in increasing its population. Indications of survival and reporduction were also found in four other species; however, their populations were limited.

Results (Table 5) indicated that neither flour beetle found TSP an acceptable media; neither expressed fitness on TSP diet. After two months larvae were observed in both cultures. After four months, numbers of live adult red

Table 5: Susceptibility of texturized soy protein (TSP) to ten common stored products insects

Months after introduction:	2		4		6	
Insect	Average no. live adults	Average no. dead adults	Average no. live adults	Average no. dead adults	Average no. live adults	Average no. dead adults
Red flour beetle	48.7	3.3	13.3	48.3	7	53.3
Confused flour beetle	53.0	1.7	56.3	1.0	51.3	4.3
Cigarette beetle	448.0	128.0	-[a]	-[a]	-[a]	-[a]
Saw-toothed grain beetle	1.0	46.3	0	0	0	0
Merchant grain beetle	1.7	43.7	0	0	0	0
Flat grain beetle	0	14.0	0	0	0	0
Rice weevil	0	48.3	0	0	0	0
Warehouse beetle	0	0	1.0	3.0	3.7	8.3
Indian meal moth	0	24.7	2.0	34.7	0	28.7
Lesser grain borer	51.3	50.3	6.0	102.6	1.0	208

[a]Symbol: - = No data collected.

flour beetles decreased while confused flour beetle live adult numbers remained constant. Observed viable larval numbers in both cultures had also decreased and after six months few larvae could be observed in either set of jars.

Adults of both red and confused flour beetles were removed from test jars after six months. Larvae were then left alone and placed under rearing room conditions for an additional two months. Table 4 shows the number of adults that developed. As found using soy flour, little success of survival was exhibited by either flour beetle and the threat of serious infestations seems unlikely.

The warehouse beetle may be capable of infesting TSP; however, as explained previously in discussing tests using soy flour, these insects appeared to have either entered a state of diapause or were in diapause at the beginning of the test. In comparing the individuals on TSP to those on whole wheat, little difference between them existed with neither appearing to have normal development. After six months the adults were removed leaving the larvae in the media. These were rechecked after a period of two months. While several larvae were found of all sizes, no newly developed adults were found (Table 4).

Although the warehouse beetle did not develop on TSP, it remains a serious pest because of its ability to survive on the media. From a contamination viewpoint, this could be a serious problem.

The lesser grain borer, while appearing successful in the earlier months of the test, eventually died. At the end of two months the population had doubled. At the end of four months the number of live adults decreased, but the total number, including dead adults, remained constant. After six months only one live adult per jar remained, while the total number of live and dead

adults had increased by a factor of four. A few viable larvae were observed in the media so adults were removed and jars were placed back in the rearing room for further observation. After an additional two months, new adults were not found and evidence of viable larvae was not observed.

A few possible reasons as to why the lesser grain borer could not maintain a population should be emphasized: 1) while normal development occurred in whole wheat (control), stress due to a less acceptable media (TSP) could be to great for diseased populations; 2) at no time during the test did live adult numbers increase beyond the original population; and 3) failure of larvae developing into adults may have occurred because of disease or lack of certain essential nutritional requirements.

It is possible that the lesser grain borer could be a pest of TSP but further investigations are needed. As shown in Figure 2, lesser grain borer larvae were able to bore into TSP particles and develop much like they do in cereal grains (U.S.D.A., 1965). They should be considered a possible threat because they penetrate packages well, are strong fliers, and produce a high number of progeny in a short period of time. They possess many of the characteristics which could make them a serious pest.

The cigarette beetle is capable of producing infesting populations in TSP. After introducing fifty individuals into culture jars, evidence of larval development was observed after two weeks. After five weeks, new adults had emerged and at eight weeks live adult numbers had increased by a factor of nine. Cultures were checked at the end of thirteen weeks and the population had died because they had exhausted the media. Unfortunately it was too late to add food and the test was terminated.

Table 5 shows the high degree of acceptability of TSP to the cigarette beetle. As explained previously in relation to soy flour, the cigarette beetle appears capable of becoming a serious pest in soy products.

Figure 2: Radiograph prints of lesser grain borer, Rhyzopertha dominica (F.), development within texturized soy protein particles. (A) early instar larvae; (B) late instar larvae; (C) pupae; (D) emergence

B

D

A

C

As shown in Table 5, the Indian meal moth had limited success in developing a population on TSP. After four months, several larvae of all stages were observed (after successful development of most of the original larvae), and a few had apparently developed into adults. When these cultures were rechecked at the end of six months, viable larvae were not observed and the population had died.

While the Indian meal moth does not appear capable of developing populations on TSP, it remains possible that it could be successful on blended products. Under prolonged storage conditions, where other more acceptable material are stored along or in the general vicinity, serious damage to the product can result from a relatively few individuals due to webbing associated with the Indian meal moth.

As shown in Table 5, the saw-toothed grain beetle, merchants grain beetle, flat grain beetle and the rice weevil found TSP an unacceptable media. These species do not appear to be a threat to TSP in this form.

Mites and psocids were found in both soy flour and TSP, generally in unsuccessful cultures. Except for their general classification, further identification of the individuals was not attempted. They were successful in both media where they had apparently infected cultures. These infestations probably had little, if any, effect in the results obtained. They should be considered a possible pest in soy flour and TSP products though because of their apparent success, especially in products going out-of-condition.

Analysis of Various Isolation and Detection Methods in Determining Insect Contamination in Texturized Soy Protein. Insect fragment numbers were determined for soy flour used to make TSP using a standard procedure (AACC

Table 6: Number of insect fragments found per sample (25 gm.) in texturized soy protein using six various procedures

	Procedure						
	1[a]	2[b]	3[c]	4[d]	5[e]	6[f]	Known[g]
	42	15	103	63	65	28	81
	32	14	71	59	79	48	84
	54	3	88	79	55	43	52
	52	2	74	71	44	39	67
	26	7	56	80	55	25	63
	—	—	—	61	—	45	—
Σx	206	41	392	413	298	228	347
n_1	5	5	5	6	5	6	5
Σx^2	9,084	483	32,006	28,853	18,452	9,108	24,779
$\bar{x}$	41.2	8.2	78.4	68.8	59.6	38	69.4
S D	12.2	6.1	17.8	9.2	13.1	9.4	13.2

[a]Ground using standard AACC Floatation Method

[b]Unground using standard AACC Flotation Method

[c]Ground boiling 20-30 min. in 5% hydrochloric acid solution

[d]Ground autoclaving for 20 min. at 121°C and 20 psi in 5% hydrochloric acid solution

[e]Unground boiling 20-30 min. in 5% hydrochloric acid solution

[f]Unground autoclaving for 20 min. at 121°C and 20 psi in 5% hydrochloric acid solution

[g]Standard AACC Method 28-41

Method 28-41). Various isolation and detection procedures (Appendix C) were then tested using TSP to evaluate these methods against a known standard. Table 6 lists the numbers of insect fragments found in each procedure, including the number found using the standard procedure on soy flour.

To test results from various procedures, a one-way analysis of variance (Appendix D) on the result was calculated using a LSD Test ($P < .05$) to determine the significance of variations found with respect to the known standard value.

In procedures 1 and 2, modifications of the standard flotation method, results were significantly lower than the standard value. It appears that protein layers formed during extrusion processing bind insect fragments between the layers. Without some method of separation, most insect fragments remained bound.

A significant variation was also found in procedure 6. From the results in Table 6, it appears that boiling TSP in 5% hydrochloric acid for 20-30 minutes is more effective in releasing fragments from TSP than autoclaving at 121°C at 20 psi for 20 minutes. A better separation of insect fragments from TSP is achieved through boiling for 20-30 minutes as in procedures 3, 5, and standard AACC Method, than autoclaving at 121°C at 20 psi for 20 minutes as in procedures 4, 6, and modified AACC Method using the autoclave.

Variations in insect fragment numbers were also found when comparing ground and unground samples. A possible increase in insect fragments due to grinding seems likely when comparing procedures 3 (ground) and 5 (unground), and 4 (ground) and 6 (unground). Procedure 6, when using both unground and autoclaving techniques was found unreliable in giving results.

Under the conditions given in these tests and those found in procedures 3, 4, and 5, there were no significant differences found in results when

compared to the standard. However, these tests were run on only one TSP product and several variations exist when other products are considered. Particle size and distribution, configuration, corrections due to changes in moisture content, and increase in fragment numbers due to size reduction in grinding are all variables which must be considered when determining the most desirable procedure for fragment determination in various products.

If a procedure for fragment analysis is required on TSP, procedures 3, 4, and 5 are all acceptable. If the product is difficult to digest because of size or configuration, it might be necessary to reduce the particle size. However, when using this procedure, the fragments are likely to also be reduced in size increasing their total numbers.

In conclusion, these tests (3, 4, and 5) indicate that contaminants found in soy flour prior to extrusion processing are not significantly changed during processing. Therefore, it appears more advantageous to conduct insect fragment analysis prior to processing, if the original flour is available. Any contaminant analysis required to insure the integrity of the product after processing could follow procedures 3, 4, and 5 with consideration given to variations due to different products.

Analysis of Vegetable and Animal Fragments Using a Scanning Electron Microscope. Similar characteristics of both animal and vegetable fragments were examined using a scanning electron microscope to further define variation of each in classification. Though various diagnostic characteristics found could be used, common classification techniques were used and found adequate for testing purposes.

SUMMARY

The primary objectives of this study were to determine whether ten different common stored products insects were capable of infesting and developing on texturized soy protein (TSP) or soy flour products, and to find a reliable and convenient procedure for recovering and counting insect fragments in TSP.

Under environmental conditions of 70% relative humidity and 27.7°C, the ten species were tested on both soy flour and TSP. Populations of the cigarette beetle developed on both and this insect appears quite capable of becoming a serious pest under favorable conditions. Red and confused flour beetles apparently are unable to produce serious numbers on either product, but both were capable of maintaining small populations and should be considered possible pests. The lesser grain borer appears capable of developing and producing progeny on TSP, but for unknown reasons it could not maintain a viable population. Further investigation is required before it can be considered a serious pest. While only a few progeny were produced by the Indian meal moth on TSP, serious damage could be caused by larval webbing of the product.

Since Trogoderma larvae tested were in diapause during the test period, results were inconclusive. Indications are that the Trogoderma larvae could be a pest of both soy flour and TSP because of their ability to survive on unfavorable food, becoming contaminants.

A revised acid digestion technique, after reducing texturized particles so they would pass through a No. 4 sieve, was the most acceptable recovery technique tested. While other procedures were found acceptable, they were either less convenient or possible alterations in the procedures were judged to make the results unpredictable. Problems can occur in confusing vegetable

fragments and insect fragments during micro-analytical examinations due to similar characteristics of both. Efforts were made to further define various characteristics of each, but common classification techniques were finally used and found adequate for testing purposes.

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APPENDICES

APPENDIX A

Moisture Content (%) of TSP and Soy Flour Samples

Relative Humidity %	TSP	Soy Flour
84.7	21.944	21.937
	22.165	22.089
	21.897	22.064
80.7	19.005	17.550
	19.294	17.583
	19.041	17.340
75.8	14.451	14.287
	14.578	14.217
	14.491	14.602
57.0	9.082	8.790
	9.324	8.776
		8.844
43.8	7.542	7.651
	7.795	7.331
		7.248

APPENDIX B

PARTICLE SIZE TEST ON TSP

Sample 1

Modulus of Uniformity

Screen Size	Percent Over	
3	0	
4	0	56.75 ÷ 10 = 5.675 = 6
8	56.75	
14	39.87	
28	2.44	42.31 ÷ 10 = 4.231 = 4
48	0.47	
100	0	.94 ÷ 10 = 0.094 = 0
Pan	0.47	

Modulus of Fineness

Screen Size	Percent Over	
3	0	X 7 = 0
4	0	X 6 = 0
8	56.75	X 5 = 283.75
14	39.75	X 4 = 159.0
28	2.44	X 3 = 7.32
48	0.47	X 2 = 0.94
100	0	X 1 = 0
Pan	0.47	X 0 = 0

451.01 ÷ 100 = 4.51

APPENDIX B

PARTICLE SIZE TEST ON TSP

Sample 2

Modulus of Uniformity

Screen Size	Percent Over	
3	0	
4	0	51.52 ÷ 10 = 5.15
8	51.52	
14	45.38	
28	2.49	47.87 ÷ 10 = 4.78
48	0.092	
100	0	0.55 ÷ 10 = 0.05
Pan	0.461	

Modulus of Fineness

Screen Size	Percent Over	
3	0	X 7 = 0
4	0	X 6 = 0
8	51.52	X 5 = 257.60
14	45.38	X 4 = 181.51
28	2.49	X 3 = 7.465
48	0.09	X 2 = 0.184
100	0	X 1 = 0
Pan	0.46	X 0 = 0

446.759 ÷ 100 = 4.46

APPENDIX C

PROCEDURE 1

Isolation and Detection of Insect Contamination in Texturized Soy Protein

I Materials, Reagents and Solutions

A. Materials
1. Erlenmeyer trap flask - 1000 ml.
2. Buchner funnel
3. Filtering flask
4. Ruled filter paper
5. Petri dishes
6. Balance

B. Reagents and Solutions
1. Alcohol, 60% isopropyl
2. Tween 80-alcohol solution (40 ml. polyoxyethylene sorbitan mono-Oleate added to 210 ml. 60% isopropyl alcohol, mix and filter)
3. EDTA-60% alcohol solution (Versene solution - dissolve 5 g. tetrasodium salt of ethylene diamine-tetraacetic acid in 100 ml. water, add 150 ml. isopropyl alcohol, mix and filter)
4. Naptha gas
5. Glycerine solution - 1 part glycerine to 1 part water

II Method

A. Procedure
1. Grind TSP using a Wiley Mill with a 20-mesh screen and weigh 25 gm. into a 1-liter trap flask
2. Add 300 ml. 60% alcohol and mix
3. Add 250 ml. Tween 80-60% alcohol solution and mix
4. Add 250 ml. EDTA-60% alcohol solution
5. Add 70 ml. naptha gas and mix
6. Fill flask so that gasoline layer is in neck of flask
7. Stir gently at 5 min. intervals for 20 min.
8. Let stand for 1 hr.
9. Trap off gasoline layer onto ruled filter paper using Buchner funnel
10. Transfer the filter paper to a petri dish to which has been added several drops of glycerine solution
11. Add 40 ml. gasoline and allow 1.5 hr. before trapping off second extraction on separate ruled filter paper.
12. Transfer the filter paper to a petri dish to which has been added several drops of glycerine solution

PROCEDURE 2

Isolation and Detection of Insect Contamination in Texturized Soy Protein

I Materials, Reagents and Solutions

A. Materials
1. Erlenmeyer trap flask - 1000 ml.
2. Buchner funnel
3. Filtering flask
4. Ruled filter paper
5. Petri dishes
6. Balance

B. Reagents and Solutions
1. Alcohol-60% isopropyl
2. Tween 80-alcohol solution (40 ml. polyoxyethylene sorbitan monooleate added to 210 ml. 60% isopropyl alcohol, mix and filter)
3. EDTA-60% alcohol solution (Versene solution - dissolve 5 g. tetrasodium salt of ethylene diamine-tetraacetic acid in 100 ml. water, add 150 ml. isopropyl alcohol, mix and filter)
4. Naptha gas
5. Glycerine solution - 1 part glycerine to 1 part water

II Method

A. Procedure
1. Weigh 25 gm. of TSP into a 1-liter trap flask
2. Add 300 ml. 60% alcohol and mix
3. Add 250 ml. Tween 80-60% alcohol solution and mix
4. Add 250 ml. EDTA-60% alcohol solution
5. Add 70 m. naptha gas and mix
6. Fill flask so that gasoline layer is in neck of flask
7. Stir gently at 5 min. intervals for 20 min.
8. Let stand for 1 hr.
9. Trap off gasoline layer onto ruled filter paper using Buchner funnel
10. Transfer the filter paper to a petri dish to which several drops of glycerine solution has been added.
11. Add 40 ml. gasoline and allow 1.5 hr. before trapping off second extraction on separate ruled filter paper
12. Transfer the filter peper to a petri dish to which several drops of glycerine solution has been added

PROCEDURE 3

Isolation and Detection of Insect Contamination in Texturized Soy Protein

I Materials, Reagents and Solutions

A. Materials
1. Balance
2. Beakers - 800 ml.
3. Stirring rods with rubber policemen
4. Hot plate
5. Separatory funnels - 1000 ml. equipped with rubber drain tubes fitted with pinch clamps
6. Buchner funnel and filtering flask
7. Petri dishes
8. Ruled analytical filter paper

B. Reagents and Solutions
1. Hydrochloric acid solution - 5%
2. Mineral oil
3. Tween 80-alcohol solution (1 part polyoxyethylene sorbitan monooleate to 5 parts 50% alcohol)
4. Naptha gas
5. Glycerine solution - 1 part glycerine to 1 part water

II Method

A. Procedure
1. Grind TSP using Wiley Mill with a 20-mesh screen and weigh 25 gm. into an 900-ml. beaker
2. Add with stirring, 30 ml. of the Tween 80-alcohol solution
3. Add 20 ml. of mineral oil
4. Heat to boiling, with intermittent stirring to prevent TSP from scorching. Boil for 20-30 min.
5. Remove beaker from burner and allow to cool. Cold water (about 200 ml.) may be added to help cooling
6. Pour 30 ml. of kerosene or lead-free gasoline into the 1000-ml. separatory funnel
7. Quantitatively transfer the digested sample from the beaker to the separatory funnel. Rinse the beaker and stirring rod with hot water
8. Allow the sample to settle for 30-45 min. Drain off the sediment so the oil-gas layer is about 1.5 to 2 inches from the bottom. Refill the separatory funnel and repeat
9. Fill the separatory funnel with water and let settle for 30-45 min. Drain off the sediment retaining the oil-gas layer in the separatory funnel
10. Filter the material remaining in the funnel through ruled analytical filter paper using the Buchner funnel.
11. Transfer the filter paper to a petri dish to which has been added several drops of glycerine solution

PROCEDURE 4

Isolation and Detection of Insect Contamination in Texturized Soy Protein

I Materials, Reagents and Solutions

A. Materials
1. Balance
2. Beakers - 800 ml.
3. Stirring rods with rubber policemen
4. Autoclave
5. Separatory funnels - 1000 ml. equipped with rubber drain tubes fitted with pinch clamps
6. Buchner funnel and filtering flask
7. Petri dishes
8. Ruled analytical filter paper

B. Reagents and Solutions
1. Hydrochloric acid solution - 5%
2. Mineral Oil
3. Tween 80-alcohol solution (1 part polyoxyethylene sorbitan monooleate to 5 parts 60% alcohol)
4. Naptha gas
5. Glycerine solution - 1 part glycerine to 1 part water

II Method

A. Procedure
1. Grind TSP using Wiley Mill with a 20-mesh screen and weigh 25 gm. into an 800-ml. beaker
2. Add with stirring, 30 ml. of the Tween 80-alcohol solution
3. Add 20 ml. of mineral oil
4. Place in and autoclave, heat at 121°C and 20 psi for 20 min.
5. Remove beaker from autoclave and allow to cool. Cold water (about 200 ml.) may be added to help cooling
6. Pour 30 ml. of kerosene or lead-free gasoline into 1000 ml. separatory funnel
7. Quantitatively transfer the digested sample from the beaker to separatory funnel. Rinse the beaker and stirring rod with hot water
8. Allow the sample to settle for 30-45 min. Drain off the sediment so the oil-gas layer is about 1.5 to 2 inches from the bottom. Refill the separatory funnel and repeat
9. Fill the separatory funnel with water and let settle for 30-45 min. Drain off the sediment retaining the oil-gas layer in the separatory funnel
10. Filter the material remaining in the funnel through ruled analytical filter paper using the Buchner funnel
11. Transfer the filter paper to a petri dish to which has been added several drops of glycerine solution

PROCEDURE 5

Isolation and Detection of Insect Contamination in Texturized Soy Protein

I Materials, Reagents and Solutions

A. Materials
1. Balance
2. Beakers - 800 ml.
3. Stirring rods with rubber policemen
4. Hot plate
5. Separatory funnels - 1000 ml. equipped with rubber drain tubes fitted with pinch clamps
6. Buchner funnel and filtering flask
7. Petri dishes
8. Ruled analytical filter paper

B. Reagents and Solutions
1. Hydrochloric acid solution - 5%
2. Mineral oil
3. Tween 80-alcohol solution (1 part polyoxyethylene sorbitan monooleate to 5 parts 60% alcohol)
4. Naptha gas
5. Glycerine solution - 1 part glycerine to 1 part water

II Method

A. Procedure
1. Weigh 25 gm. of TSP into 800-ml. beaker
2. Add with stirring, 30 ml. of the Tween 80-alcohol solution
3. Add 20 ml. of mineral oil
4. Heat to boiling, with intermittent stirring to prevent TSP from scorching. Boil for 20-30 min.
5. Remove beaker from burner and allow to cool. Cold water (about 200 ml.) may be added to help cooling
6. Pour 30 ml. of kerosene or lead-free gasoline into the 1000-ml. separatory funnel
7. Quantitatively transfer the digested sample from the beaker to the separatory funnel. Rinse the beaker and stirring rod with hot water
8. Allow the sample to settle for 30-45 min. Drain off the sediment so the oil-gas layer is about 1.5 to 2 inches from the bottom. Refill the separatory funnel and repeat
9. Fill the separatory funnel with water and let settle for 30-45 min. Drain off sediment retaining the oil-gas layer in the separatory funnel
10. Filter the material remaining in the funnel through ruled analytical filter paper using the Buchner funnel
11. Transfer the filter paper to a petri dish to which has been added several drops of glycerine solution

PROCEDURE 6

Isolation and Detection of Insect Contamination in Texturized Soy Protein

I Materials, Reagents and Solutions

A. Materials
1. Balance
2. Beakers - 800 ml.
3. Stirring rods with rubber policemen
4. Autoclave
5. Separatory funnels - 1000 ml. equipped with rubber drain tubes fitted with pinch clamps
6. Buchner funnel and filtering flask
7. Petri dishes
8. Ruled analytical filter paper

B. Regents and Solutions
1. Hydrochloric acid solution - 5%
2. Mineral oil
3. Tween 80-alcohol solution (1 part polyoxyethylene sorbitan monooleate to 5 parts 60% alcohol)
4. Naptha gas
5. Glycerine solution - 1 part glycerine to 1 part water

II Method

A. Procedure
1. Weigh 25 gm. TSP into 800-ml. beaker
2. Add with stirring, 30 ml. of the Tween 80-alcohol solution
3. Add 20 ml. mineral oil
4. Place in an autoclave, heat at 121^{o}C and 20 psi for 20 min.
5. Remove beaker from autoclave and allow to cool. Cold water (about 200 ml.) may be added to help cooling
6. Pour 30 ml. of kerosene or lead-free gasoline into the 1000-ml. separatory funnel
7. Quantitatively transfer the digested sample from the beaker to the separatory funnel. Rinse the beaker and stirring rod with hot water
8. Allow the sample to settle for 30-45 min. Drain off the sediment so the oil-gas layer is about 1.5 to 2 inches from the bottom. Refill the separatory funnel and repeat
9. Fill the separatory funnel with water and let settle for 30-45 min. Drain off the sediment retaining the oil-gas layer in the separatory funnel
10. Filter the material remaining in the funnel through ruled analytical filter paper using the Buchner funnel
11. Transfer the filter paper to a petri dish to which has been added several drops of glycerine solution

APPENDIX D

ANALYSIS OF VARIANCE TABLE

One-Way, Unequal

Source	d.f.	S.S.	M.S.	F
Between	7	19,058.58	2,722.65	19.06
Within	35	5,000.86	142.88	
Total:	42	24,059.44		

INSECTS AS POTENTIAL PESTS AND CONTAMINANTS OF TEXTURIZED SOY PROTEIN

by

WILLIAM E. PURSLEY

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Preliminary tests indicated that few insects found soy flour and TSP as acceptable diets. The cigarette beetle, Lasioderma serricorne (F.), was the only insect found which indicated fitness on both media tested. Flour beetles, Tribolium castaneum (Hbst.) and Tribolium confusum (J. duV.), and warehouse beetle, Trogoderma varabile (Beal), were found as possible pests on soy flour. Both flour beetles, warehouse beetle, Indian meal moth, Plodia interpunctella (Hbn.), and lesser grain borer, Rhyzopertha dominica (F.), indicated limited success on TSP.

Upon examining various isolation and detection methods to determine insect contamination in TSP, a modified standard AACC acid-digestion procedure proved satisfactory. Tests indicate that no significant changes due to extrusion processing occur. Therefore, it appears more advantageous to determine contaminant analysis prior to processing.