The economic feasibility of the whole process is presently under study. The possibility of using the final extraction residue as a substrate for single-cell protein production is also under active investigation. If this proves to be the case, a final product with a lower fiber and higher protein content could be obtained, with the desirable characteristics in a material intended for the production of concentrates for monogastric animals.

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The Use of Activated Charcoal to Remove or Inactivate Mouse Growth Inhibitors Present in Soybean Whey

Larry J. Tidemann and David J. Schingoethe*

The whey solution (pH 4.4 supernatant) from raw soybean meal was subjected to charcoal filtration procedures as a means of finding a practical method of removing or inactivating the animal growth inhibitors present in this soybean fraction which is currently discarded as a polluting waste product. Filtering the pH 4.4 supernatant through activated charcoal removed or inactivated more than 98% of the trypsin inhibitors, while about half of the carbohydrates and one-quarter of the starting protein remained in the filtrate. When fed in diets to mice, this filtrate slightly reduced their weight gains indicating most of the growth inhibitor activity in the pH 4.4 supernatant was also removed or destroyed. Washing the charcoal successively with 0.3 *N* NaOH, 0.15 *N* NaOH, and 0.2 *N* HCl resulted in the recovery of additional quantities of protein and carbohydrates. The growth inhibitor activity of these fractions was low and diluted enough so as not to cause any marked reductions in growth rates of mice. The protein elution patterns on Sephadex G-25 of the various charcoal filtrated fractions indicated that the charcoal filtrate contained mostly large molecular weight proteins (>than 5000 molecular weight), whereas the 0.30 and 0.15 *N* NaOH filtrates contained primarily peptide and amino-sized compounds, and the 0.2 *N* HCl filtrate contained a small amount of various sized protein and peptides. This treatment scheme of the soybean whey fraction appears to be an effective practical method for recovering a substantial portion of its nutrients for human or animal food use, and at the same time removing or destroying virtually all of the trypsin inhibitors and most of the animal growth inhibitors present in this soybean fraction.

The poor nutritive value of raw (unheated) soybean meal has been attributed to several heat labile components which reduce animal growth (Liener and Kakade, 1969; Mickelsen and Yang, 1966). Most of these compounds are contained in the soybean whey solution, which is the water and acid (pH 4.0–4.5) soluble fraction remaining after acid precipitating most of the soy proteins (Rackis *et al.*, 1963; Schingoethe *et al.*, 1970). The soybean whey contains 20–33% of the total solids and 5–8% of the total protein present in the original soybean meal (Rackis *et al.*, 1963; Schingoethe *et al.*, 1974), that is presently discarded as a waste product of soy protein processing because of these growth inhibitor problems. The objectives of this research were (1) to devise a practical method of separating the proteins and peptides in soybean whey from the carbohydrates, and (2) to devise a practical method of removing or inactivating the growth inhibitors present in the soybean whey solution. The ultimate results could make possible the utilization of at least part of the currently wasted soy by-product for animal or human foods.

Dairy Science Department, South Dakota State University, Brookings, South Dakota 57006.

MATERIALS AND METHODS

Preparation of Soybean Fractions. Soybean whey solution (pH 4.4-S) was prepared from Corsoy variety soybeans by previously described methods (Schingoethe *et al.*, 1970). Fractionation using activated charcoal proceeded as outlined in Figure 1. One liter of the pH 4.4-S was mixed thoroughly with approximately 30 g of activated charcoal (U.S.P., powder, Mallinckrodt Chemical Works, St. Louis, Mo.) before filtering and the resulting filtrate (charcoal filtrate) saved for analysis and growth assays. The charcoal was then washed in order with equal vol-

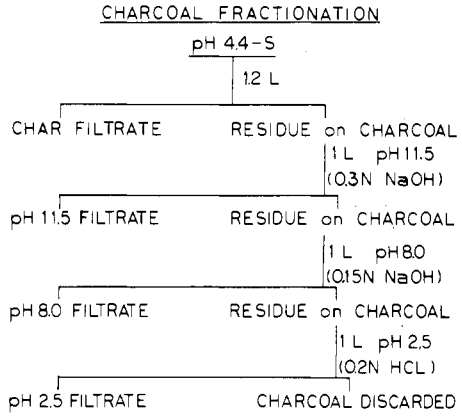

Figure 1. Fractionation of the pH 4.4 supernatant using activated charcoal.

Table I. Composition of Diets Fed to Mice

Ingredient	Amount, g
Salt mix[a]	4.0
Vitamin mix[b]	2.2
Corn oil	5.0
a-Cellulose[c]	1.5
Glucose[d]	37.3
HRSBM[e,f]	$50 - X$
Soybean meal test fraction[f]	$X/100.0$

[a] Wesson modification of Osborne-Mendel Formula, Nutritional Biochemicals Corporation, Cleveland, Ohio. [b] Vitamin diet fortification mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio. [c] Nutritional Biochemicals Corporation, Cleveland, Ohio. [d] Dextrose, J. T. Baker Chemical Corporation, Phillipsburg, N. J. [e] Soybean meal is autoclaved soybean meal (HRSBM) in all cases except for raw soybean meal fraction. [f] Soybean meal fractions replaced part of the HRSBM.

umes of 0.30 *N* NaOH, 0.15 *N* NaOH, and 0.20 *N* HCl and the resulting filtrates (pH 11.5, 8.0, and 2.5 filtrates, respectively) saved. The pH 11.5 filtrate was neutralized to pH 7.0 using 6 *N* HCl immediately after collecting. Samples of each of the filtrates were analyzed for trypsin inhibitor activity (Schingoethe *et al.*, 1970), protein (Warburg and Christian, 1941; AOAC, 1970), and carbohydrates (Badin *et al.*, 1953) prior to lyophilizing to dryness.

Heated raw soybean meal (HRSBM) was prepared by a modification of the procedure of Renner and Hill (1960). Raw soybean meal (RSBM) was autoclaved at 110° (6.8 kg steam pressure) for 15 min. After autoclaving, the meal was dried at room temperature and ground through a 1-mm screen.

Growth Assay Procedure. The test diet composition is shown in Table I. The soybean meal was HRSBM, except for the negative control diet RSBM/2. In that diet, RSBM replaced half of the HRSBM and served as a negative control diet. In previous studies (Schingoethe *et al.*, 1970), RSBM replaced all the HRSBM, which caused marked reductions in feed consumption and weight gains. The soybean meal test fraction replaced part of the 50 g of HRSBM in the diet, based on the quantity of test fraction recovered from 50 g of RSBM as determined by the weight of lyophilized test fraction from a known quantity of starting RSBM. In most cases, two times this amount of charcoal-separated fractions was added to various diets to maximize any growth-depressing responses.

Weanling mice were used as the test animal since previous studies (Schingoethe *et al.*, 1970) showed that mice gave a similar response to that of weanling rats but required less feed. Eight 21-day-old male mice were randomly assigned to each diet and divided into two groups, four mice in each wire meshed cage. Growth trials continued for 5 days. At termination of the experiments, the mice were killed by exposure to diethyl ether, and their pancreases removed and weighed. Feed consumption for each treatment subgroup was determined by weighing the feed fed and subtracting the amount estimated to be remaining in the feeders at termination of the experiment. Because feces were mixed with the feed and there was some feed scattering, there may have been some errors in determining the amount of feed and feces present in the feeders at termination of the experiments. To minimize this error, the mice were fed daily and efforts were made to ensure that no more than 1.5 day's feed supply was available to the mice at any one time. A positive control diet (HRSBM) and two negative control diets (RSBM/2 and pH 4.4-S) were fed during each growth assay to serve as controls for that particular experiment. Growth inhibitor activity was calculated according to the formula of Schingoethe *et al.* (1970), whereby one unit of growth inhibitor activity equals 1% reduction in growth rate below that of the positive control group's (HRSBM) after correcting for any differences in feed intake. Such a calculation made comparisons between experiments more uniform since absolute weight gains and feed intakes sometimes varied quite substantially from one experiment to the next. The correction for difference in feed intake between a treatment group and the positive control increased the accuracy of the growth inhibitor activity calculation, despite the inherent inaccuracies in the feed in-

Table II. Composition of Raw Soybean Meal, the pH 4.4 Supernatant, and Its Various Charcoal-Separated Filtrates

Fraction	Dry matter, g	Protein		Carbohydrate		Trypsin inhibitor act., units/mg[c]
		%[a]	g	%[b]	g	
Raw soybean meal	100.0	46.6	46.60	5.0	5.00	5.56
pH 4.4 supernatant	20.0	15.2	3.01	6.0	1.20	6.67
Charcoal filtrate	8.35	8.7	0.72	7.4	0.62	0.27
pH 11.5 char. filtrate	3.34	14.4	0.48	2.5	0.08	0.00
pH 8.0 char. filtrate	1.25	6.5	0.08	4.6	0.06	0.00
pH 2.5 char. filtrate	2.9	8.2	0.24	4.3	0.12	0.00
Total charcoal fractions as % of pH 4.4 supernatant	79.2		50.5		73.3	1.7

[a] Kjeldahl nitrogen × 6.25. [b] According to the method of Badin *et al.* (1953). [c] According to the method of Schingoethe *et al.* (1970).

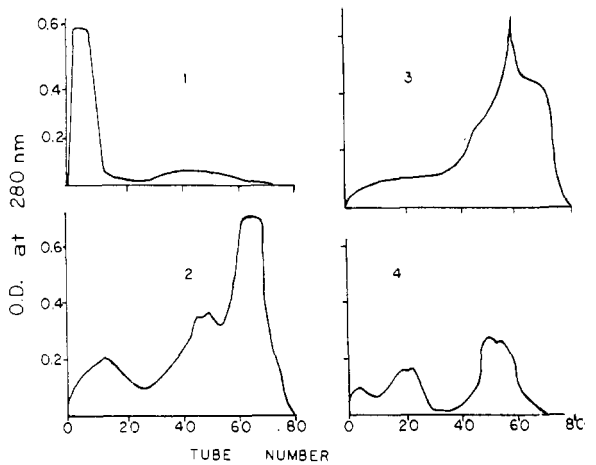

Figure 2. Chromatography on Sephadex G-25 of charcoal filtrate fractions of pH 4.4 supernatant: column dimensions, 5.7 × 107 cm; sample, lyophilized sample dissolved in distilled water; eluting buffer, distilled water; flow rate, 23 ml/min; charcoal filtrate, 1; pH 11.5 filtrate, 2; pH 8.0 filtrate, 3; pH 2.5 filtrate, 4.

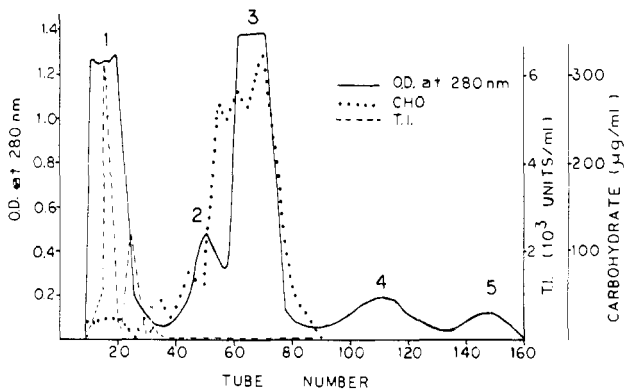

Figure 3. Elution pattern of pH 4.4 supernatant on a Sephadex G-25 column: column dimensions, 5.7 × 107 cm; sample, 2.25 g of lyophilized pH 4.4 supernatant dissolved in 75 ml of distilled water; eluting buffer, distilled water; flow rate, 23 ml/min; OD at 280 nm (—); trypsin inhibitor activity (- - -); carbohydrate concentration (• • •).

take data previously mentioned. An increase in specific activity (*i.e.*, units/g of dry matter) brought about by a fractionation step would also indicate a concentration of growth inhibitor activity by that particular fraction step.

RESULTS AND DISCUSSION

The composition and amounts recovered in the various charcoal-filtered fractions are listed in Table II. The charcoal filtrate contained 42% (8.35/20) of the dry matter present in the pH 4.4-S, and accounted for 24 and 52% of the pH 4.4-S protein and carbohydrate, respectively. Washing the charcoal with solutions at pH 11.5, 8.0, and 2.5 consecutively was performed in attempts to remove as much pH 4.4-S material off the charcoal as possible, and to see if protein or growth inhibitor activity could be concentrated in any one fraction. One may prefer to simply increase the pH stepwise from the starting pH 4.4 to 11.5 as an alternate treatment scheme. The cumulative recovery in these filtrates accounted for another 37% of the dry matter, 27% of the protein, and 22% of the carbohydrate. Thus, nearly 80% of the starting pH 4.4-S dry matter was recovered, which accounted for nearly three-quarters of the carbohydrate and one-half of the protein.

Very low levels of trypsin inhibitor activity were found in the charcoal filtrate and none in any of the other fractions. This indicated that either (1) the trypsin inhibitors were destroyed or inactivated by this treatment regime or (2) the trypsin inhibitors were retained on the charcoal. In either case, it appears that this method may have promise as a means of removing trypsin inhibitor activity from soybean whey fractions.

The amount of charcoal used for this fractionation procedure was more than sufficient to remove as much of the growth inhibitory materials as possible. While quantitative studies were not conducted to determine the capacity of the charcoal, laboratory observations indicated that approximately 10 g of activated charcoal per liter of pH 4.4-S may be necessary for complete removal of the harmful substances.

After lyophilizing the various filtrates that resulted from charcoal fractionation, they were redissolved in distilled water, applied to a Sephadex G-25 column, and eluted with distilled water. This was done as a means of desalting for animal growth assays and also to see if any

Table III. Growth Assay with Mice Fed Various Fractions of pH 4.4 Supernatant (pH 4.4-S) from Raw Soybean Meal (RSBM) Eluted through Charcoal[a]

Test fraction	Amount,[b] g/100 g of diet	Wt gain, g/day	Feed intake, g/day	Growth inhibitor		Pancreas size, % of body wt
				Total units[c]	Units/g of DM[d]	
HRSBM		0.86a[e]	5.4			0.61b
RSBM/2	25.0	0.08b	4.4	94 ± 5.2[f]	72 ± 6[f]	0.91a
pH 4.4-S	10.0	0.11b	3.8	88 ± 8.7	170 ± 32	0.84a
Charcoal filtrate	8.35[g]	0.58a	4.5	14 ± 8.1	68 ± 30	0.64b
pH 11.5 char. filtrate	3.34[g]	0.81a	4.7	5 ± 4.2	70 ± 19	0.58b
pH 8.0 char. filtrate	1.25[g]	0.74a	4.6	13 ± 6.2	207 ± 30	0.58b
pH 2.5 char. filtrate	2.9[g]	0.58a	5.3	9 ± 5.6	71 ± 21	0.61b
SEM[h]		0.12	0.10[i]			0.04

[a] Average of four experiments with eight mice per diet in each experiment. [b] Amount of test fraction. [c] Corrected for amounts of fractions recovered from a known quantity of starting material. [d] Units per gram of dry matter of the test fraction under consideration. [e] Figures in the same column followed by the same letter are not different ($P > 0.05$) using Duncan's new multiple range test (Steel and Torrie, 1960). [f] Means of four experiments ± standard error of the mean (SEM). [g] Twice the amount recovered from 50 g of RSBM. [h] Standard error of the mean. [i] Based on average daily intake per cage.

separation has been achieved by this fractionation scheme.

Figure 2 shows the protein (*i.e.*, $A_{280\,nm}$) absorbancy scan of the G-25 eluent of each of the charcoal filtrates. For a comparison, Figure 3 is the elution pattern of the starting pH 4.4-S using the same G-25 column. The proteins in the charcoal filtrate were primarily those which eluted with the void column, thus comparable in molecular size to the compounds present in peak 1 of the pH 4.4-S (Figure 3). Previous studies (Schingoethe *et al.*, 1974) indicated that peak 1 of the pH 4.4-S contained trypsin inhibitor activity and accounted for 37–45% of the animal growth inhibitor activity present in the pH 4.4-S. The pH 11.5 charcoal filtrate (scan 2, Figure 2) contained a small amount of material which corresponds to peak 1 of Figure 3 and predominantly peaks 2 and 3 of Figure 3. Components of peak 2 of Figure 2 were most abundant in the pH 8.0 filtrate (scan 3, Figure 2). The pH 2.5 charcoal filtrate appeared to contain parts of all portions of pH 4.4-S (scan 4, Figure 2), although predominantly peak 2 (Figure 3) proteins or peptides. Components of peaks 2 and 3 of Figure 3 account for about 60% of the growth inhibitor activity of the pH 4.4-S (Schingoethe *et al.*, 1974).

Data of four mouse growth assays in which the charcoal filtrates were utilized are summarized in Table III. None of the fractions significantly reduced growth rates of mice when added to their diets at twice the levels recovered from a known quantity of RSBM, although growth rates were usually slightly reduced. Thus, only small amounts of growth inhibitor activity were found in any of the charcoal fractions, with no apparent concentration of growth inhibitor activity in any one fraction. Growth inhibition caused by the charcoal filtrate may be attributable to trypsin inhibitors since that is the only charcoal fraction containing substantial amounts of protein of the molecular weight of trypsin inhibitors. The charcoal filtrate also had a trace of trypsin inhibitor activity. Growth inhibitor activity in the other charcoal fractions was more likely caused by small molecular weight growth inhibitors recently isolated (Schingoethe *et al.*, 1970; Schingoethe *et al.*, 1974). None of the charcoal fractions caused pancreas enlargement, indicating that the factor(s) causing pancreas enlargement was either destroyed or retained on the charcoal. If it is valid to assume that the growth inhibitor activities recovered in the various fractions are additive, then approximately 47% of the growth inhibitor activity present in the pH 4.4-S was recovered in the charcoal fractions, 16% of this activity being present in the initial charcoal filtrate. Previous studies (Schingoethe *et al.*, 1970; Schingoethe *et al.*, 1974) indicated that such an assumption is generally true, but sometimes overestimates the total growth inhibitor activity recovered. The recombined fractions were not fed to mice, so such data are not available.

Specific growth inhibitor activity (*i.e.*, units/gram of dry matter) was not significantly higher ($P > 0.05$) in any of the charcoal fractions than in the pH 4.4-S. This indicated that the growth inhibitors present in the pH 4.4-S were not concentrated within any one of the charcoal filtrate fractions. In fact, the specific activity was usually lowered, providing further evidence that this fractionation scheme removed or inactivated most of the growth inhibitory substances present in soybean whey.

This treatment scheme of the soybean whey fraction appears to be an effective practical method for recovering a substantial portion of its nutrients for human or animal food. At the same time most of the animal growth inhibitors present in this soybean fraction can be removed or inactivated. Thus, this treatment regime offers a means of increasing the amount of useful products from soybeans and at the same time reducing the amount of polluting waste products which must be discarded.

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