Changes Induced by Autoclaving a Solvent-Extracted Cottonseed Meal

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The effect of autoclaving a prime-quality solvent-extracted meal upon its soluble protein fractions was studied to determine chemical differences between cottonseed meals of known and varied nutritive value. Portions of both the original and the autoclaved meal were fractionated, by successive extractions, with water, saline, and dilute alkali. Autoclaving modified the solubility behavior of the cottonseed protein, and reduced the sugar, lysine, and arginine contents of the meal. Results showed that simple measurement of the solubility of nitrogen in the meal, commonly used to indicate the degree of heat damage to a meal, does not reflect the complete change induced in cottonseed protein by heat.

NORMAL COMMERCIAL METHODS of oil extraction from cottonseed include a variety of procedures, such as hydraulic pressing, screw pressing, solvent extraction, and combinations of the latter two. Processing conditions such as temperature, time, and degree of pressing vary between methods and between mills using the same method. Therefore, the processing methods, as well as specific operating conditions, could affect the potential protein quality of cottonseed meals.

Cottonseed meals have been used successfully and extensively as protein supplements in cattle feeds. Until recently, however, relatively insignificant amounts of the meal were included in nonruminant feeds because of the presence of gossypol, an interfering factor in the growth of swine and poultry. Through extensive research on the nature and activity of gossypol and the relationship between processing conditions and gossypol content of the meal (*1*, *2*, *6*), several types of commercial processing methods were shown to allow the production of meals containing 0.04%, or less, of free gossypol. Experimental data demonstrated the suitability of these materials for unrestricted use in poultry diets (*3*, *7*, *23*).

Heat damage incurred during processing affects the protein quality of the meals and the extent of this heat damage varies even within a given method of processing. A rapid, reliable method for estimating the extent of heat damage would be invaluable in controlling commercial processing methods, in developing new processing techniques, and in assessing the quality of any type of cottonseed meal.

Nitrogen solubility in 0.02N sodium hydroxide (*8*) has been used for estimating the nutritive value of cottonseed meal. This method has made possible gross discrimination between good and bad meals; however, under normal operating conditions, it often does not reflect accurately enough the extent of heat damage and the quality of the protein. Additional information was sought by going beyond the solubility itself and extracting the proteins from cottonseed meals to determine the effect of processing upon their amount and quality. This study indicates that, in addition to the actual quantity of soluble nitrogen affected by heat, autoclaving cottonseed meal changed the type of protein material extracted and reduced the lysine, arginine, and sugar content of the meal.

Fractionation of Cottonseed Meals

The meal used in this experiment was prepared on a pilot-plant scale, under controlled conditions, by successive extraction of prime quality cottonseed with petroleum ether and butanone (*9*). Residual solvent was removed by exposing thin layers of the meal to a forced-air draft at temperatures under 100° F. for 16 hours. This extraction reduced free gossypol to 0.02% and total gossypol to 0.2%, and the resulting meal had a high nutritional value for chicks. By autoclaving a portion of this meal for 2 hours, its nutritive value was reduced approximately 70% (*8*). Hereafter,

Table I. Properties of Control and Autoclaved Cottonseed Meal and Their Insoluble Fractions

Sample	Weight, G.	Moisture Content, %	Nitrogen		Nonprotein Nitrogen		Gossypol		Phosphorus		Amino Acid in Protein, %					
			Total,[a] %	% of initial	Total,[a] %	% of meal nitrogen	Total,[a] %	% of initial	Total,[a] %	% of initial	Glutamic	Aspartic	Cystine	Lysine	Arginine	Histidine
Whole meal																
Control	600	6.9	10	..	0.7	7	0.16	..	1.4	..	19.4	9.8	2.1	4.3	11.5	2.4
Autoclaved	600	7.6	10.3	..	1.0	7	0.07	..	1.4	..	19.6	9.6	1.7	2.7	9.8	2.1
Insoluble, pH 6.5																
Control	404	6.6	11.8	83	...	..	0.18	76	1.3	63	...	...	...	...	..	...
Autoclaved	370	5.1	12.3	81	...	..	0.08	71	0.7	32	...	...	...	...	...	...
Insoluble, salt																
Control	283[b]	7.6	9.0	43	...	..	0.27	79	1.5	50	...	...	...	...	...	...
Autoclaved	350[b]	4.0	10.5	65	...	..	0.08	67	0.5	22	...	...	...	...	...	...
Insoluble, pH 10.5																
Control	179[b]	6.3	7.8	25	0.1	1	0.23	43	1.7	36	16.2	10.3	1.7	4.4	8.8	2.2
Autoclaved	340[b]	7.4	12.4	70	0.1	1	0.06	48	0.5	20	19.6	10.9	1.7	3.2	9.7	2.1

[a] Calculated to dry-weight basis. [b] Calculated weight recovered if entire previous residue used.

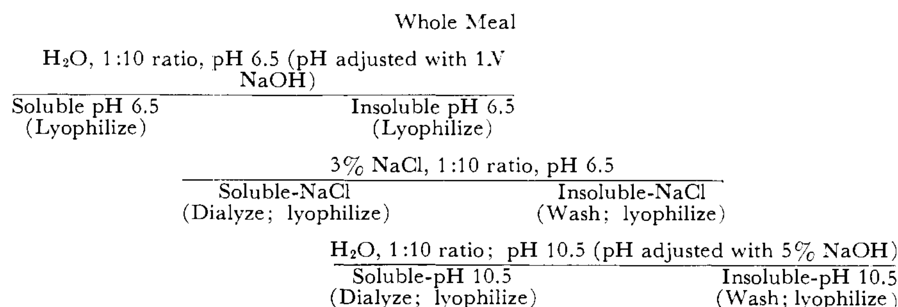
Table II. Properties of Soluble Materials Isolated from Control and Autoclaved Cottonseed Meal

Sample	Weight Recovered, G.	Moisture Content, %	Nitrogen Content: Total,[a] %	Nitrogen Content: % of initial	Relative Amino Acids Content[b]	Phosphorus Content: Total,[a] %	Phosphorus Content: % of initial
Soluble, pH 6.5							
Control	147	6.6	5.5	14	Low in basic acids	1.72	30
Autoclaved	141	5.7	4.2	11	Low in basic acids and cystine	3.19	55
Soluble, salt							
Control	116[c]	2.3	17	35	Typical	0.35	5
Autoclaved[d]	7[c]	...	4[e]	1	Low in basic acids and cystine	6.26[f]	6
Soluble, pH 10.5							
Control	24[c]	8.6	15.4	7	Low in basic acids	0.39	1
Autoclaved	19[c]	6.9	7.8	3	Low in basic acids and cystine	1.43	3

[a] Calculated to dry-weight basis.
[b] Relative to typical amino acid chromatogram of cottonseed meal hydrolyzate.
[c] Calculated weight recovered if entire previous residue used.
[d] Insufficient material to obtain all analyses.
[e] Calculated from data on hydrolyzate prepared for amino acid analysis.
[f] "As is" basis.

these samples are referred to as the control and autoclaved meals, respectively.

The fractionation procedure outlined here:



is similar to that used by earlier investigators on cottonseed meals and other materials (*13*, *15*, *16*). Six hundred grams of each meal were used in two equal portions for the initial extraction, and, for the successive extractions with salt and water at pH 10.5, a portion of each residue was used. By this technique, samples of all soluble and insoluble fractions were provided.

Materials soluble at pH 6.5 and in saline were separated from their respective residues in a basket-head centrifuge. Successful separation of the materials soluble at pH 10.5 was achieved by centrifugation in 250-ml. centrifuge bottles. Each extraction step was completed in an 8-hour period, and, except where dialysis was necessary, the fractions were divided into 250- to 500-ml. portions and frozen immediately after centrifugation. The proteins were precipitated by removing the excess salt and alkali from the saline and alkali-soluble fractions by dialysis for 72 hours against running distilled water at 5° C. on a rocking dialyzer. These materials were frozen within 2 hours after removal from the dialysis sac. To minimize deterioration all samples remained frozen until dried from the frozen state (lyophilized).

Determination of Chemical and Physical Properties

Total nitrogen, free gossypol, and moisture contents were determined by the official methods of the American Oil Chemists' Society (*4*). Total gossypol was determined by the method of Pons, Hoffpauir, and O'Connor (*21*). The phosphorus content of the whole meals and insoluble residues was estimated by the method of Pons, Stansbury, and Hoffpauir (*22*) and of the soluble fractions by the Fiske and Subbarow method (*12*). After extraction and hydrolysis of the sugar, according to official Association of Official Agricultural Chemists methods (*5*), total sugar determinations were made according to the Hanes (*14*) modification of the Hagedorn-Jensen method. Nonprotein nitrogen was estimated by the macro-Kjeldahl determination of nitrogen in trichloroacetic acid extracts of the samples.

The amino acids content of each fraction was determined, qualitatively, by a two-dimensional paper chromatographic method similar to that described by Pernis and Wunderly (*20*). The meal or meal fraction was hydrolyzed by refluxing for 24 hours with twice-distilled 6*N* hydrochloric acid, using 200 ml. of acid for every 100 mg. of nitrogen. Excess acid was removed from the hydrolyzed sample by evaporation to dryness under reduced pressure. The final traces of hydrochloric acid were removed by successive addition and evaporation of three portions of water. The residue was taken up in water and the insoluble material, humins, filtered off through a medium-porosity sintered-glass filter. When excess amounts of water were used during this washing, the filtrate was again concentrated under reduced pressure and the residue taken up in sufficient 10% aqueous isopropyl alcohol to yield a solution containing 6 to 12 mg. of nitrogen per ml.

The paper chromatographic analyses were carried out on washed and buffered Whatman No. 1 paper. The sheets were washed twice with 0.3*N* hydrochloric acid, once with 0.1*N* sodium hydroxide, and three times with water before being buffered by two washings with a boric acid buffer, pH 9. By using a modified Büchner funnel, designed by F. H. Thurber and made at this laboratory from sheets of Lucite, it was possible to wash, buffer, and dry 12 sheets at a time.

The amino acid solution was applied near a corner of the square sheet of buffered paper in 2.5-μl. aliquots until 30 γ of nitrogen had been added. A two-dimensional ascending technique was used to develop the chromatogram. The first developing solvent, 70% aqueous *n*-propyl alcohol, was allowed to irrigate the sheet for 18 hours. After

the sheet was dried for 30 minutes at room temperature, the propanol front was removed. The sheet was then rotated through 90°, and development was completed by irrigation for 22 hours with buffered phenol (100 grams of redistilled crystalline phenol and 30 ml. of the boric acid buffer, pH 9). The chromatogram was dried for 6 to 16 hours at room temperature in a forced draft, and then dipped in a freshly prepared solution of ninhydrin (0.2% in acetone containing 4% glacial acetic acid, v./v.). Color development of the dried chromatogram was completed by heating in a forced-draft oven at 65° C. for 15 to 30 minutes. The amino acids were identified qualitatively by comparison with a map which had been prepared under identical conditions with standard amino acid solutions.

The lysine, arginine, cystine, histidine, and glutamic and aspartic acids content of the meals and final residues was determined by ion exchange chromatographic methods. For the determination of the basic acids, a hydrolyzate was prepared as described above, but the final dilution was accomplished with water to yield a solution containing 2 to 3 mg. of nitrogen per ml. The pH was adjusted to a value of 7. The sample was placed on a column, 0.9 × 15 cm., of Dowex 2 in the acetate form. The column was washed with 100 ml. of water and then with 25 to 50 ml. of an acetate buffer, pH 3.7. These eluents were combined and evaporated to dryness under reduced pressure. The residue was taken up in water to provide a solution containing approximately 0.5 mg. of nitrogen per ml., and 20 ml. of this sample were added to the top of a 3 × 15 cm. column of Dowex 50. The procedure of Moore and Stein (*17*), with corresponding increases in eluting volumes, was used to separate the lysine, arginine, and histidine. The cystine and cysteine present in the materials were converted to cysteic acid by performic acid oxidation (*24*), prior to acid hydrolysis. An ion exchange procedure (*19*) developed at this laboratory was used to separate glutamic, aspartic, and cysteic acids.

In both separations, the eluents were collected in 5- to 10-ml. fractions. The amino acid distribution was estimated by removing aliquots of 5 to 10 μl. from each fraction and applying them to ninhydrin test paper (*25*). Appropriate fractions, those containing the isolated amino acids, were combined and made up to a convenient volume. Aliquots of these solutions were analyzed by the photometric ninhydrin method of Moore and Stein (*18*). Results expressed as per cent amino acid in sample were converted to a protein basis by the relationship:

$$\frac{\%\text{ amino acid in sample}}{\%\text{ nitrogen in sample} \times 6.25} = \%\text{ amino acid in protein}$$

Results and Discussion

The two meals used in this study were prepared under special conditions to provide samples of known and varied nutritive value. The solvent-extracted meal, from which oil and gossypol were removed without heat, was autoclaved for 2 hours to reduce its nutritive value, and also its nitrogen solubility, in 002*N* sodium hydroxide, 65 to 70% (*8*). Other properties of the meal were also affected by this treatment (Table I). The lysine and arginine contents of the protein were reduced 40 and 15%, respectively, the sugar content was reduced approximately 20%, and the total gossypol content was reduced slightly. There was no apparent effect on the total nitrogen, nonprotein nitrogen, or total phosphorus contents of the meal.

Certain general effects of autoclaving on the solubility of the protein components of the meal were noted (Table II). The fractions from both meals which were soluble at pH 6.5 appeared to be similar, but there were striking differences between the salt-soluble fractions with respect to both weight and nitrogen content. Comparison of the fractions soluble at pH 10.5 indicated a slight difference in the amount of material removed and significant differences between their nitrogen contents.

The major portion of the nitrogen of the control sample was extracted during the fractionation procedure, but 70% of the nitrogen of the autoclaved meal remained insoluble. However, paper chromatographic analyses indicated the presence of the complete amino acid spectrum in all fractions. There appeared to be a lower concentration of the basic acids in most of the soluble fractions—i.e., the depth of color of the area representative of lysine, arginine, and histidine on the paper chromatograms was less than that on chromatograms prepared from the whole meals or the insoluble fractions. The cystine content appeared to be low in all soluble fractions from the autoclaved meal.

Quantitative analyses for the abovementioned amino acids and for glutamic and aspartic acids were made on the whole meals and the final insoluble residues. There was a reduction in the lysine and arginine contents of the meal as a result of autoclaving, but the relative concentration of these acids in the residual protein was not affected. Similar reductions in these amino acids have been noticed when soy bean meals were autoclaved for extended periods (*10, 11*).

The nonprotein nitrogen (trichloroacetic acid–soluble nitrogen) of the original meals was similar, accounting for 7% of the total nitrogen. Approximately 5% of the total meal nitrogen could be dialyzed from the fractions of both meals soluble at pH 6.5. This was equivalent to the major portion of the nonprotein nitrogen. In both final residues, approximately 1% of the total nitrogen could be accounted for as nonprotein nitrogen.

In spite of the higher initial sugar content of the control, the major portion of the sugar from both meals was extracted during the first fractionation at pH 6.5.

Comparison of the gossypol contents of the whole meals and their insoluble residues indicated that 70% of the gossypol was insoluble at pH 6.5 and an equivalent amount remained insoluble after the saline extraction. Slightly less than 50% of the gossypol was accounted for in the final insoluble residues from both meals.

The total phosphorus content of the meal was not changed during autoclaving, but the solubility of the phosphorus-containing material was affected. More phosphorus was removed from the autoclaved meals in each step of the fractionation procedure than from the control. Correspondingly, the phosphorus contents of the soluble fractions isolated from the autoclaved meal were high.

Summary and Conclusions

The nitrogenous materials which are soluble at pH 6.5 were not affected by the action of heat. The native globulins and glutelins of the meal, however, were almost completely denatured, where the extent of denaturation was measured by determinations of solubility in saline and in alkali.

It is not known whether the denaturation per se is related to the reduction in the nutritive value induced by the action of heat (*8*).

A large portion of the lysine present in the meal, as well as a portion of the arginine, was destroyed by autoclaving. Confirmation of these results is desired, as the destruction of a portion of these two amino acids by the action of heat could account for a reduction in the nutritive value of the heat-damaged meals.

Significant also is the destruction of sugars, which could be related to the disappearance of both arginine and lysine. The apparent liberation of phosphate from organic combination by the action of heat may be associated with the reduction in nutritive value of the meal.

In order to provide maximum control of the experimental material, this work was done on a specially prepared meal, and the autoclaving was accomplished in laboratory equipment. There are several fundamental differences between the way this meal was heat damaged and the possible ways damage could

occur in commercial processing. The major differences are that, under commercial conditions, oil and gossypol are present when the meal is heated, and both factors could affect the nature of changes that take place in the meal protein. Therefore, extrapolation of these results to commercially processed meal should not be attempted until additional information of the same type has been obtained with commercial meals.

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PROTEIN EFFICIENCY

Relative Nutritive Values of Proteins in Various Foods at Increasingly High Levels of Protein Intake

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The nutritive value of the proteins in various foods at increasingly high levels of protein intake was studied for possible practical application in poultry and livestock feeding. Protein efficiency decreases with increased protein intake because there is greater waste in metabolism with the increased ingestion of protein. In all the foods studied, 15% was the most efficient level of protein intake.

The relative nutritive values of the proteins in corn gluten meal, degossypolyzed cottonseed meal, defatted soybean flour, dried nonfat milk solids, defatted dried whole eggs, wheat germ meal, and defatted germ at 15 to 30% planes of intake were studied. In all these foods, 15% was the most efficient level of protein intake. The protein efficiency decreases with the increased protein intake because there is greater waste in metabolism with the increased ingestion of protein. The supplementation of the proteins in corn gluten meal fed at a 20% level of protein intake, with 0.2% L-lysine and 0.4 DL-tryptophan, resulted in a 17.3% increase in efficiency of utilization.

Table I. Protein Content of Various Foods

	Protein, %
Corn gluten meal	42.1
Cottonseed meal[a]	39.7
Wheat germ meal	28.5
Defatted wheat germ	33.7
Dried nonfat milk solids	35.1
Defatted dried whole eggs	65.6
Defatted soybean flour	51.3

[a] Degossypolyzed by extraction with methyl ethyl ketone.

Experimental Procedure and Materials

Results have been reported on the relative nutritive values of proteins in various foods at levels of protein intake ranging from 5 to 12% (*5*). In this study, information is given on the relative nutritive values of high-protein containing foods fed at planes of intake ranging from 15 to 30%. The foods used furnished all the proteins in the rations and in sufficient amounts to provide the desired levels of proteins. The protein content of the various foods used (Table I) was determined from nitrogen analyses, using the factor 6.25 for the corn gluten meal, cottonseed meal, soybean flour, and eggs; the factor 6.38 for the nonfat milk solids; and the factor 5.83 (*3*) for the wheat germ meal.

The rations also contained 4% of Sure's salt mixture No. 1 (*6*), 7% of