Summary and Conclusions

Hydrogenation of cottonseed oil occurred at slower rates of reaction in a *gamma* radiation field of Cobalt 60 than comparable nonirradiated runs. The *gamma* radiation apparently degraded the triglycerides to form poisons for the nickel catalyst. Free fatty acids and possible carbonyls were probably the poisons produced. The degree of selectivity and *cis-trans* isomerization that occurred during hydrogenation were unaffected by the *gamma* radiation.

Acknowledgment

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Solubility of Cottonseed Proteins in Hydrochloric Acid

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THE MARKED VARIABILITY in the growth response of nonruminants to cottonseed meals, fed as a protein supplement, has created the need for grading cottonseed meals intended for use for this purpose. The problem of developing specifications for cottonseed meals for mixed feeds for poultry and swine has been approached empirically by several investigators. Some progress has been made in the development of such specifications. For example, a correlation has been noted between the solvent power for cottonseed proteins (*e.g.*, the fraction of the total meal nitrogen that will dissolve in a specified period of time) of 0.02 Normal aqueous NaOH and the growth response of chicks fed cottonseed meal as a protein supplement (1, 2). A correlation has been noted also between the comparable solvent power of 0.5 Normal aqueous NaCl and the growth of chicks (1, 3).

A correlation is reported in this paper between the solvent power of a 6 Normal aqueous HCl for cottonseed meal proteins and the growth response of chicks fed the cottonseed meals as a protein supplement. The correlation obtained with the acid solution is as good as those obtained with the two solvents mentioned above.

Experimental

Determinations of the Fraction of the Total Meal Nitrogen-Soluble in HCl Solution. Cottonseed meal samples containing 600 milligrams of nitrogen were weighed into flat-bottom, screw-capped glass bottles. Glass beads and 200 milliliters of constantly boiling hydrochloric acid solution were added to each of the bottles. The capped bottles were shaken vigorously, then clamped on to a rotating shaft in a water bath maintained at 37.5°C. Bottles containing the resulting suspensions were withdrawn for analyses at successive intervals of time. The samples were cooled quickly to room temperature and immediately centrifuged. The supernatant liquid was filtered, and determinations for nitrogen, using the Kjeldahl procedure, were carried out on the clear filtrates.

Determinations of the Fraction of the Total Meal Nitrogen-Soluble in 0.02 Normal NaOH and in 0.5 Normal NaCl Solutions. The methods used for these determinations were the same as those described in other investigations (1, 2, 3).

Determination of Amino Nitrogen. The procedure described by Pope and Stevens (4) was followed in determining the amino nitrogen in the clear filtrates, *vide supra*. It was advantageous, in this study, to double the concentrations recommended by Pope and Stevens for the $CuCl_2$ and Na_3PO_4 solutions. It was necessary, for consistent results, that the suspension of cupric phosphate be prepared daily.

Determinations were carried out on the filtrates before and after the addition of trichloroacetic acid in order to estimate the extent of protein hydrolyses.

Results

Data which are typical of the effect of time on the fraction of the total nitrogen soluble in 6 Normal HCl are recorded in Figure 1. It may be noted that the fraction increases with time; the kinetics of this dispersion of the protein into 6N HCl are not simple however.

Included in Table I are the data for the fraction of the total meal nitrogen found in the 6N HCl after 1 hr. of exposure. Included also are reproductions of the data for the fractions of the total nitrogen of cottonseed meals dispersed in 0.5 Normal NaCl, and in 0.02 Normal NaOH, as recorded by Chang *et al.* (1). The data for the nutritional response of chicks

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TABLE I

Correspondence Between Protein Solubility and Nutritive Value of Meals

Sample number	Sample description and processing history	Nutritive value (%)	Total gossypol content (%)	Total nitrogen (%)	Soluble nitrogen content (%)		
					0.5N NaCl 3 hrs.	0.02N NaOH 1 hr.	6N HCl 1 hr.
535-1	Raw, oily cottonseed flakes	—	0.92	5.64	—	—	91.7
S6-276	Solvent-extracted cottonseed meal prepared at laboratory (standard meal)	100.0	0.14	9.3	—	81.5	82.7
5B [a]	Commercially processed prepressed solvent-extracted cottonseed meal	81.9 [b]	0.71	6.49	47.8	83.4	63.7
10B [a]	Commercially processed prepressed solvent-extracted cottonseed meal	80.9 [b]	0.75	6.71	45.7	80.1	64.1
6C [a]	Commercially processed prepressed solvent-extracted cottonseed meal	71.3 [b]	0.92	7.15	40.7	73.2	58.4
7B [a]	Commercially processed prepressed solvent-extracted cottonseed meal	70.2 [b]	1.07	6.75	36.8	68.9	56.1
1A [a]	Commercially processed prepressed solvent-extracted cottonseed meal	69.2 [b]	0.86	6.35	30.8	68.5	57.6
4A [a]	Commercially processed prepressed solvent-extracted cottonseed meal	66.0 [b]	0.99	6.95	25.5	65.4	50.3
440	Commercial screw-pressed c/s meal	51.1 [c]	0.84	6.31	10.5	41.2	37.5
441	Commercial prepressed solvent extracted cottonseed meal	76.6 [c]	0.81	6.55	42.3	76.2	61.1

[a] These meal numbers correspond to those used in the publications where processing conditions employed and nutritive value obtained on these materials are described (1).
[b] These values are averages of data obtained in independent feeding studies conducted by four different laboratories (1).
[c] These values were obtained by private communication with A. B. Watts.

are those recorded by Chang *et al.* (1) and by Watts.[2]

The determinations of the amino nitrogen in the clarified hydrochloric acid solutions indicated that there was no substantial hydrolysis of the proteins in one hour, *e.g.*, the titers of the solutions, before and after the precipitation of the proteins with trichloroacetic acid, were essentially the same. Therefore the data for the fraction of the total nitrogen dispersed into 6N HCl in one hour are not unduly complicated by a hydrolysis factor, and the data for the quantity of nitrogen in solution after one hour were taken for the computations reported. It may be noted however that the hydrolysis of the proteins was extensive after several hours.

A comparison is made in Table II, where regression and correlation coefficients are tabulated, of the results obtained with the three solvents. The nutritive indices [3] used are from Table I. The odds for the significance of the correlations for each are better than 100:1. Apparently any one of the three solvents is as good as the other two in the grading of cottonseed meals for broiler rations.

Comparable statistical data relating the solvent power of the three solutions for cottonseed meal proteins and the total gossypol of the meals are also included in Table II. The correlations here are not as good as those obtained with the nutritive indices. The odds for significance in the case of the 0.02 N NaOH solution, for example, are less than 10:1.

[2] Nutritional data for meals 440 and 441 were supplied in a private communication by Prof. A. B. Watts, Poultry Husbandry Dept., Louisiana State University.

[3] The nutritive index is defined as the ratio of the growth response of chicks on an experimental cottonseed meal to the growth response of chicks on a cottonseed meal prepared by exhaustive extraction with hexane and then with methyl ethyl ketone.

TABLE II

Comparison of Regression and Correlation Coefficients for a) the Solvent Power for Meal Proteins and Nutritive Value, and b) for the Solvent Power for Meal Proteins and Total Gossypol

	Solvent		
	0.5N NaCl	0.02N NaOH	6N HCl
Coefficient of regression for			
a) solubility on nutritive index	0.96	1.03	1.19
b) nutritive index on solubility	0.92	0.76	0.76
Coefficient of correlation of a) and b)	+ 0.84	+ 0.89	+ 0.95
Coefficient of regression for			
c) solubility on total gossypol	−43.5	−24.5	−36.2
d) total gossypol on solubility	− 0.013	− 0.010	− 0.50
Coefficient of correlation of c) and d)	− 0.76	− 0.50	− 0.76

The correlation coefficient for the nutritive index and total gossypol is −0.72; the odds for significance are less than 50:1. Obviously other compositional factors, in addition to the total gossypol, are of importance in determining the effectiveness of cottonseed meals as a protein supplement for broiler production.

Discussion

It is interesting to note that processing of cottonseed affects solubility of nitrogen in alkali, salt solution, and acid in similar manners, and this decrease in solubility during processing is reflected in a decrease in the nutritive value of the meal when used as a source of protein to nonruminants. There have been many attempts, applied to many protein sources, to relate solubility of nitrogen in one or another solvent to nutritive value, and these attempts have met from time to time with some measure of success. For cottonseed, solubility in dilute alkali has been

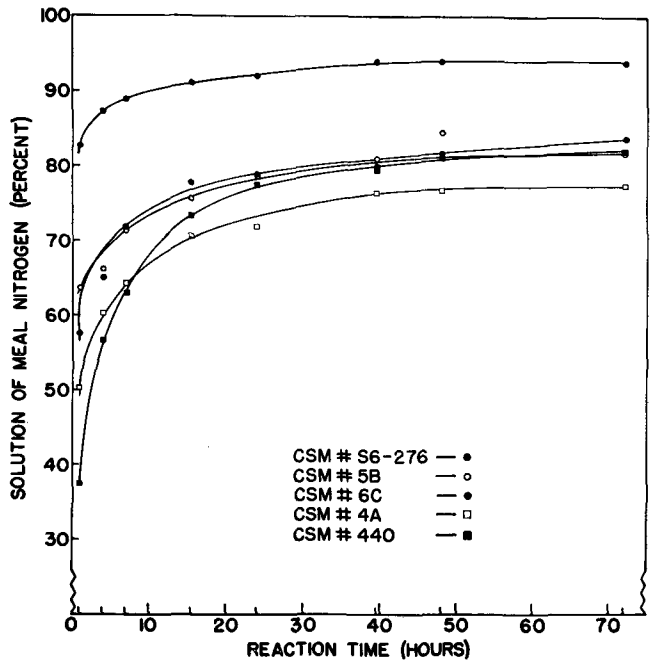

FIG. 1. Rate of solution of cottonseed proteins in 6 normal hydrochloric acid.

successful in certain instances in differentiating between meals of high and low nutritive value but has been less successful in differentiating between meals of intermediate nutritive value or in comparing meals produced by one process with those produced by another process. Similarly for soybean meal, solubility measurements have been successful in limited application.

The fact that the increased resistance to peptization of cottonseed proteins in each of the solvents occurs concomitantly with the impairment of the nutritive value of cottonseed meal suggests that chemical modifications of the proteins are induced because of the application of heat. The solubility studies may well serve as a clue to the type of chemical reactions that take place during the heating of cottonseed meals and might serve as a guide to developing improved processes even though, eventually, solubility may be superseded as means for correlating nutritive value with chemical properties.

The conclusion that the protein solubility data obtained with the three solvents are of equal value in grading of cottonseed meal for broilers is implicit in the results from the statistical computations. The absolute values of the coefficients of correlation are specific to these studies and might be slightly different for studies of other cottonseed meals.

Summary

The correlations between the growth response of chicks to the nine cottonseed meals fed as a protein supplement and the solvent powers of 0.02N NaOH, 6N HCl, and 0.5N NaCl for cottonseed meal proteins are almost identical. The correlations between the solvent power of 6N HCl, 0.02N NaOH, and 0.5N NaCl and the gossypol contents of the meals are not as good as the correlations between the solvent powers of these solutions and the growth response of chicks.

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Certain Uses of the Analysis of Variance with Standard Product Specifications

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EVERYONE will agree that standard product specifications are a modern-day necessity. They ensure constant product quality and a consequent healthy brand growth. The need is even more obvious when the production facilities used are numerous and widely separated and utilize different sources of raw material in different equipment. In some cases the same customer will obtain production from two or even three factories in successive purchases. The usual objective of industry is to minimize the variations in product sufficiently that the changes in source go unnoticed by the consumer. These conditions make it difficult to provide those responsible for the manufacturing process with standard specifications that are capable of being met uniformly. The use of the statistical technique of the Analysis of Variance has provided a helpful answer to this problem, and some discussion of its use is warranted.

Standard specifications may relate to finished product characteristics by which the consumer will be directly influenced in his evaluation of the product, such as color, odor, shape, package outage. However these have little relationship to the real performance of the product. They may apply to characteristics that can be measured objectively as dimension or sudsing efficiency, or subjectively as flavor or odor. Once market research has determined that the characteristic is important to the consumer and once the acceptable level of the characteristic is defined, then production must conform to this level to meet acceptance. The inherent variability of the process must not dictate the quality of the product; instead the necessary level of quality dictates the permissible variation in the process conditions.

If the process imparts greater variability in finished-product quality than the standard specification permits, then the reason for this variability must be isolated and controlled. While it may be that the process itself is not under good enough control, the variability may be caused by raw material nonuniformity or the lack of effective training of operators. It is often found that the variability is more apparent than real, because of sampling difficulties or lack of precision in the analytical techniques used to define the quality level of the production.

In the determination of the relative importance of the numerous possible causes of excessive variability of finished-product quality in order to start the right corrective measures, the Analysis of Variance technique has been used successfully. Obviously if there is no difficulty in meeting the standard specification uniformly, no problem exists and the following is unnecessary.

Where excessive variability does appear to occur however, it is helpful to make a preliminary separation of the total variability into the fraction contributed by the analytical techniques, by the sampling techniques, and by the remainder which will all be considered as process variability. When the relative magnitude of the three classes has been determined, the area is apparent in which to work first to improve quality or its uniformity, and the search for the specific cause or causes of the lack of control can go forward.

There are two specific aspects of the above discussion which will be illustrated by a practical example: *Problem 1.* the determination of the relative magnitudes of the components of variation in a process (this will assist in making decisions as to where in the process suitable adjustments should be made in order to decrease the variability of the finished product.); *Problem 2.* the determination of an optimum