NUTRITIONAL VARIATION IN COTTONSEED

Effect of Autoclaving in Presence and Absence of Gossypol on Solvent Extracted Cottonseed Meal

MARJORIE Z. CONDON, Southern Regional Research Laboratory, Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, La.

EDITH A. JENSEN, National Cottonseed Products Association, New Orleans, La.

A. B. WATTS and C. W. POPE, Agricultural Experiment Station, Louisiana State University, Baton Rouge, La.

Before the processing conditions in cottonseed oil mills can be modified to produce meals of consistently high nutritional quality, the individual effects of heat and bound gossypol must be understood. This investigation was undertaken to determine the effect of autoclaving in the presence and absence of gossypol on certain chemical and nutritive properties of a cottonseed meal of initially high quality. Chick feeding experiments indicated a progressive decrease in the protein quality index of the meals as time of autoclaving increased. This reduction was paralleled by similar decreases in solubility of nitrogen of the meals in 0.02*N* sodium hydroxide. There appeared to be no direct relationship between any other property of the meals and their nutritive value to chicks. Samples to which 1% gossypol was bound during autoclaving appeared to be equivalent, chemically and nutritionally, to control samples autoclaved for the same time.

COTTONSEED MEAL, because of its high protein content, has long been considered an excellent supplement for animal feeds and has been used to particular advantage in the feeding of beef and dairy cattle. Its use in poultry and swine feeds has been limited, however, due to the presence of growth inhibiting substances and to reductions in protein value which, as in other oilseed and cereal proteins, may occur during processing. In attempting to broaden the utilization of cottonseed meal, research has been conducted to determine the nature of the interfering substances and to develop methods of processing which would remove these substances but maintain protein availability at the maximum level (*2*). In early investigations (*7, 22*) and through the combined efforts of scientists connected with the U. S. Department of Agriculture, the National Cottonseed Products Association, state experiment stations, and industrial laboratories (*2*), it has been demonstrated that there is generally little interference to growth of swine and poultry when the free gossypol content of a meal is reduced to the level of 0.04% or less. (Free gossypol is defined as that portion of the total gossypol extractable by aqueous acetone during a one-hour extraction period.) Using meals with a free gossypol content of 0.02%, Milligan and Bird (*15*) showed that cottonseed meal could be fed at a level of 70% of the diet without any evidence of toxicity. However, even these meals varied widely in nutritive value. These variations, which occur during processing, may be associated with the heat damage to the protein, and/or with a reaction between gossypol or other meal constituents and protein's reducing its availability. The latter assumption is based on the increase in the bound gossypol content which usually accompanies processing and the reduction in the free gossypol content. (Bound gossypol is defined as the difference between the total gossypol and free gossypol contents.)

Examination of a number of commercial meals indicated a relationship between some of their chemical and physical properties and their nutritive value for rats (*9*). For example, changes in the electrophoretic patterns of the soluble protein material appeared to parallel those in nutritive value (*10*). However, these observations were made on commercial meals and it was not possible to separate the variables influencing the protein value of the meal—i.e., heat and bound gossypol. Olcott and Fontaine (*17*) had demonstrated that when oil-free, gossypol-free cottonseed meal was autoclaved, the reduction in nutritive value of the meal for weanling rats was proportional to the time of autoclaving. Using microbiological methods for amino acid assay, Kuiken (*12*) showed no changes in the amino acid contents of oil-free, gossypol-free meals autoclaved alone for 10 minutes but a 20% reduction in lysine availability if similar meals were autoclaved in the presence of gossypol and oil. These experiments suggested that the nutritive value of cottonseed meal can be reduced by heat but that binding of gossypol during autoclaving has a complementary effect. Lyman *et al.* (*13*) after considering this information proposed a chemical index of nutritive value which depends on the nitrogen solubility in 0.02*N* sodium hydroxide and the bound gossypol content of the meal. An investigation of the individual effects of heat and bound gossypol appeared to be a basic requirement for production of consistently high quality meals and for an understanding of the relationship between processing conditions and nutritional value. Therefore, this investigation was undertaken to determine the effects of autoclaving in the presence and absence of gossypol on a cottonseed meal of initially high quality. The conditions used were similar to those reported by Olcott and Fontaine (*17*) and Kuiken (*12*).

Sample Preparation

The meal used in this series of experiments was produced from prime quality cottonseed by successive extractions with hexane and butanone, reducing the free gossypol content to 0.02% and the total gossypol content to 0.2%. The solvents were evaporated by a minimum amount of heat to avoid damage to the protein material (*5*). This type of meal has been well characterized because it has been used as a standard in nutri-

Table I. Chemical and Physical Properties and Protein Quality Indices of Cottonseed Meal Autoclaved in Presence and Absence of Gossypol

Autoclaving time, min.	*0*	*5*			*15*	*30*		*60*	*120*
Gossypol added	0	0	1%[a] in oil	1%[b] in acetone	0	0	1%[a] in oil	0	0
Moisture content, %	7.6	9.0	8.3	8.0	8.6	8.0	8.8	8.8	9.6
Soluble carbohydrate[c] content, %	12.1	13.0	10.9	10.9	11.7	12.2	11.0	11.0	8.9
Gossypol content[c]									
Total, %	0.209	0.187	0.885	1.015	0.157	0.209	1.01	0.130	0.076
Free, %	0.024	0.009	0.025	0.026	0.008	0.004	0.013	0.005	0.004
Nitrogen content[c]									
Total, %	10.4	10.5	10.3	10.2	10.6	10.2	10.2	10.8	10.8
Soluble in NaCl, %	56.7	32.5	28.4	27.4	16.7	12.5	14.3	10.2	13.0
Soluble in NaOH, %	81.9	75.5	69.5	70.7	73.0	56.3	55.5	38.2	29.0
Phosphorus content[c]									
Total, %	1.47	1.46	1.44	1.43	1.55	1.42	1.43	1.59	1.60
Inorganic, %	0.149	0.168	0.161	0.166	0.170	0.204	0.219	0.247	0.329
Acid soluble, %	1.30	1.37	1.36	1.36	1.37	1.37	1.35	1.41	1.43
Protein quality index	100	108	92	96	91	66	70	54	31
Chemical index[d]	96	89	79	70	86	66	55	45	34

[a] 1% by weight of meal added as 2% solution of gossypol in refined and bleached cottonseed oil.
[b] 1% by weight of meal added as 0.6% solution of gossypol in acetone.
[c] Analyses reported on dry weight basis.
[d] Chemical index = $\frac{\%\text{ nitrogen solubility in } 0.02N \text{ NaOH}}{\text{total gossypol content, \%}}$; if total gossypol content is less than 0.85%, 0.85 used.

tional investigations of cottonseed meals produced under various conditions. The excellent nutritional quality of this meal as compared to other cottonseed meals has been demonstrated in feeding trials with rats and chicks, and in microbiological estimations of nutritive value (*9*, *12*, *13*).

The meal sample (1500 grams) was spread in a layer 1.5- to 2.0-cm. thick in a borosilicate glass dish, and autoclaved at 120° C. Gossypol, prepared according to the procedure of King and Thurber (*11*), was added to the meals by methods recommended by Kuiken (*12*). When the gossypol was added to the meal in a solution of acetone, the acetone was evaporated at room temperature, and the meal sample ground through a 2-mm. mesh screen before autoclaving. In other experiments, gossypol dissolved in prime quality, refined, and bleached cottonseed oil was mixed with the meal. Following autoclaving all samples were ground to pass a 2-mm. mesh screen. Meals to which oil had been added were extracted with commercial petroleum ether at room temperature prior to grinding and re-extracted following grinding until the residual oil content was reduced to the level in the original meal (approximately 0.3%). Residual free gossypol was removed from the autoclaved meals by extraction with 2% aqueous butanone at room temperature until the free gossypol content was 0.03% or less (*5*). All extracted meals were reground before chemical and nutritive investigations.

Determination of Chemical And Physical Properties

The total nitrogen, free gossypol, and moisture contents were determined according to official methods of the Am. Oil Chemists' Soc. (*3*). Total gossypol was determined by the method of Pons *et al.* (*18*). The water-soluble carbohydrate fraction was estimated by use of the anthrone reagent according to a modified method similar to that of McCready *et al.* (*14*) and Viles and Silverman (*20*). It involved diluting a water extract of cottonseed meal to contain approximately 0.002 gram of carbohydrate material, reacting this material with a 0.1% solution of anthrone in 95% sulfuric acid, developing the blue-green color by heating at 100° C. for 7.5 minutes, determining the absorbance of the solution at a wave length of 625 mμ, and estimating the per cent of carbohydrate from a previously prepared standard curve. Browning was determined by extracting the meal with a mixture of water, acetone, 4*N* hydrochloric acid, and 30% metaphosphoric acid, developing the color at room temperature, determining the absorbance of the clear filtrate at 380 mμ, and comparing the value obtained with that for the original meal (*4*). Nitrogen solubility in 0.5*N* sodium chloride was determined by the method of Olcott and Fontaine (*16*), and the solubility in 0.02*N* sodium hydroxide by the method of Lyman *et al.* (*13*). The distribution of phosphorus was estimated by the method of Pons, Stansbury, and Hoffpauir (*19*). Electrophoretic patterns were obtained on ethylamine barbital buffer extracts of the meal as outlined by Karon *et al.* (*10*).

Determination of Protein Quality Index

The protein quality indices of the meals were determined by feeding trials on groups of 10-day old chicks. Except for several minor modifications, the method used for evaluation of protein quality was that of Heiman *et al.* (*8*). Immediately after hatching, the chicks were placed on a standardization ration composed of yellow corn meal fortified with vitamins and minerals. This ration was fed for a 10-day period to allow the chicks to utilize all remaining embryonic protein material. At the end of this time, the chicks were weighed individually, and divided into groups by weight. The chicks in each group did not differ in weight by more than 5 grams. The same number of chicks from each weight group was placed in each ration group. This technique reduced, considerably, the individual variation in each experimental lot.

Duplicate lots of the 10-day old chicks were placed on the various rations. These were formulated to supply 12% protein (nitrogen × 6.25); 6% was supplied by yellow corn meal similar to that used in the standardization ration. The other 6% protein was supplied by the cottonseed meal under investigation. Vitamins and minerals were added and equal parts of starch and sugar were used to make the ration up to 100 parts. The composition of a typical ration, in parts by weight, was: yellow corn meal, 65.0; cottonseed meal, 12.8; starch, 9.0; sugar, 9.1; steamed bone meal,

2.0; oyster shell flour, 1.0; salt, 0.5; and 0.6 parts supplied by the following mixture of vitamins and minerals: manganese sulfate, 8.0 grams; BY-21, 25.0 grams; B_{12} supplement (Merck), 25.0 grams; choline supplement (25%), 20.0 grams; cod liver oil (2250A–750D), 227.0 grams. The chicks were kept on this diet for a period of 2 weeks. At the end of this time they were weighed individually, and group feed consumption was determined. The total 2-week gain was divided by the protein consumed during the period, and the result expressed as the gain per gram of protein consumed. The protein quality index for each meal was determined by dividing the average gain per gram of protein for each sample by that obtained for the standard cottonseed meal and multiplying by 100. The standard deviation did not exceed 10% of the mean.

The test ration used in this determination of protein quality index was similar to that used in practical chick diets, because half of the protein was supplied by cereal and half by a protein supplement. It was designed to test the effectiveness of cottonseed meal or any protein supplement to support the growth of chicks under standard conditions. The protein intake was maintained at a suboptimum level (12%), so that small differences between protein supplements could be evaluated. Since all nutrients other than protein were present at recommended levels and the rations differed only in the source of supplementary protein, variations in growth of the chicks were due to differences in the ability of the supplementary protein to support growth (*21*). The term, protein quality, as used in these experiments, is defined as the ability of the protein supplement, cottonseed meal, to promote growth under established conditions. The protein quality index represents the numerical comparison between each sample and a standard reference cottonseed meal.

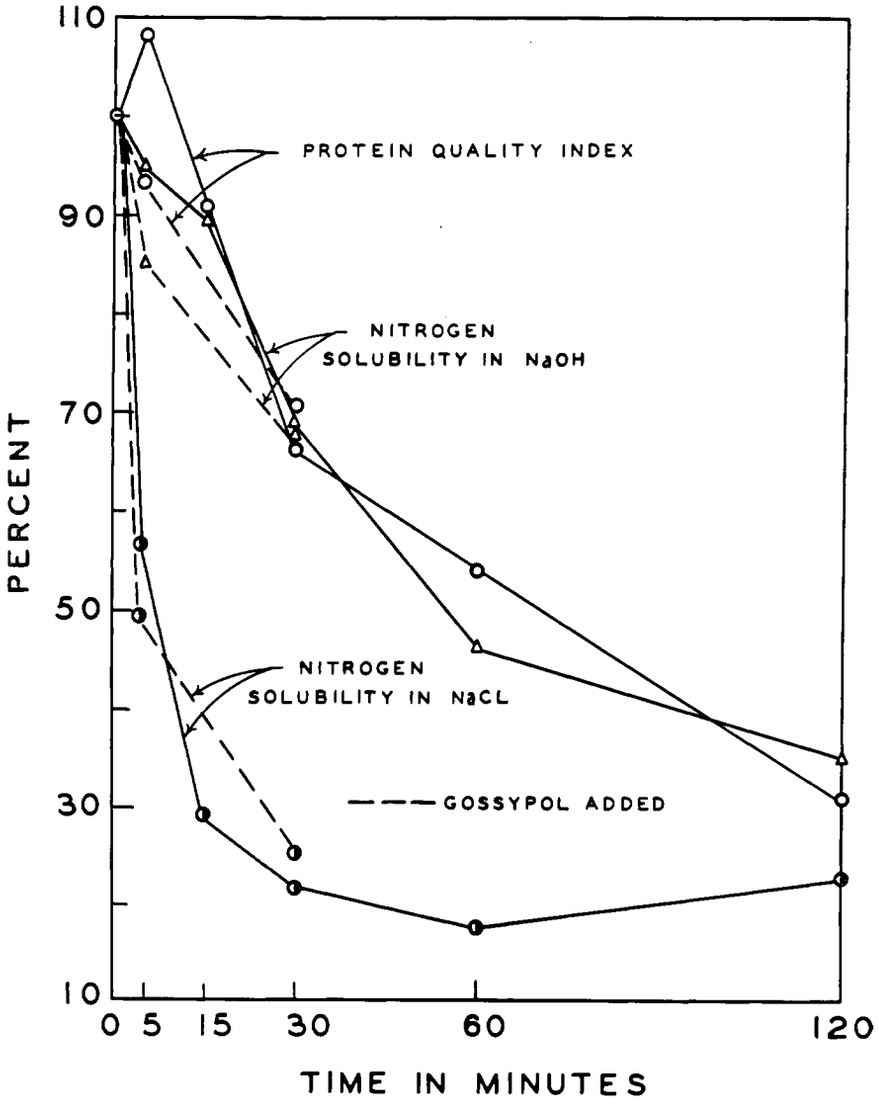


Figure 1. Effect of autoclaving in presence and absence of gossypol on protein quality index and nitrogen solubility of solvent extracted cottonseed meal—expressed as per cent of original

Effects of Autoclaving

The 1500-gram portions of the butanone extracted cottonseed meal were subjected to autoclaving in intervals ranging from 5 to 120 minutes. The samples were analyzed to determine comparatively protein quality index, nitrogen solubility, phosphorus distribution, soluble carbohydrate content, and electrophoretic pattern with the results as shown in Table I and Figures 1 and 2.

There was a slight increase in the protein quality index of the meal after 5 minutes of autoclaving, but, thereafter, a progressive decrease as length of time of autoclaving increased. The nitrogen solubility of the meals in dilute sodium hydroxide also decreased with increasing periods of autoclaving. Comparison of the nitrogen solubility in alkali with the protein quality index, by plotting the values on the same scale in terms of percentage decrease, illustrated the parallelism between these properties. No such parallelism was evident when the nitrogen solubility in sodium chloride and the protein quality index were compared.

There was no significant change in the soluble carbohydrate content after autoclaving for periods up to 1 hour. After autoclaving for 2 hours, there was an indication of the production of Browning pigments. There did not seem to be any significant changes in the phosphorus distribution as a result of autoclaving.

The electrophoretic patterns paralleled those reported in a previous publication (*10*) and appeared to fall into two groups. Patterns of those meals which had undergone no reduction in protein quality index—i.e., those autoclaved up to 15 minutes—were similar to the original and contained only two major components, *A* and *B*. After longer periods of autoclaving, the two major components merged, and the presence of a minor component, *X*, was noted. The results are shown by two representative patterns in Figure 2. Just as indicated in the previous publication (*10*), a closer correlation of electrophoretic patterns with other properties is limited by the lack of solubility of the protein material from samples having very low protein quality indices.

Effects of Autoclaving in Presence of Gossypol

After autoclaving for 5 minutes in the presence of 1% gossypol, which had been mixed with the sample either in oil or acetone, the total gossypol contents of the meals were 0.9 and 1.0%, respectively. Because the free gossypol content of all samples was less than 0.03%, the gossypol was bound almost quantitatively. The chemical and physical properties and protein quality indices of these samples, Table I, appeared to be equivalent to those of a control sample auto-

claved for a similar period of time. Two samples, one to which acetone and one to which oil had been added prior to autoclaving for 5 minutes, also had properties similar to those of the control. (Data for these samples were identical to those for the control autoclaved alone; therefore, they were not included in the table.) The electrophoretic patterns of these samples were identical with those of the control sample and would fall into Group I, indicating no change in protein quality index. Addition of 1.5% gossypol in acetone to the meal prior to autoclaving for 5 minutes resulted in a sample containing 1% total gossypol and having properties similar to those reported for others autoclaved for 5 minutes. Apparently, only 1% gossypol can be bound to the meal under these conditions.

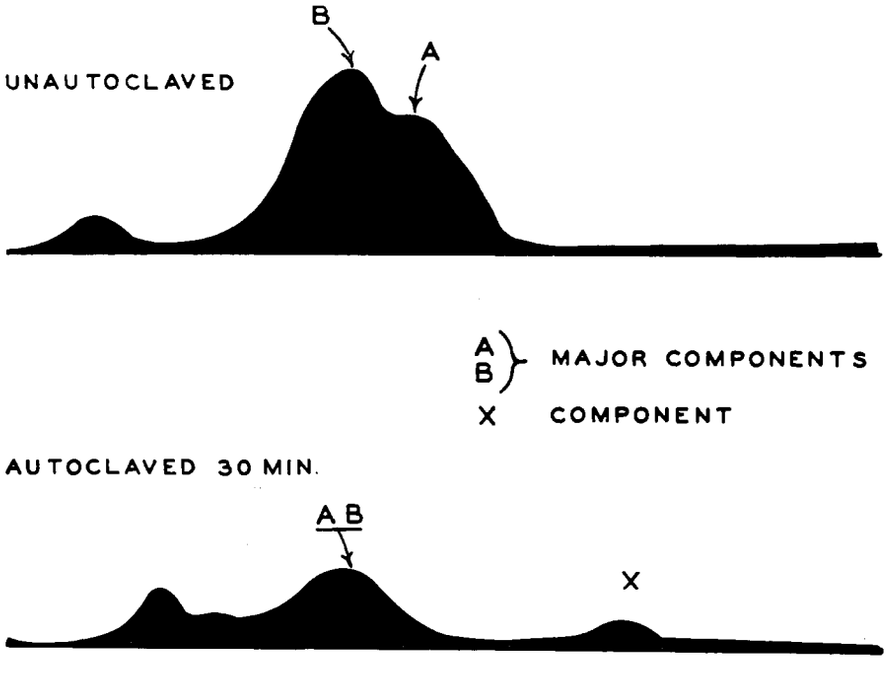
Comparison of those chemical and physical properties which were measured, and the protein quality index of a meal autoclaved in the presence of 1% gossypol in oil for 30 minutes, with one autoclaved alone and with one autoclaved in the presence of oil for a similar period of time, also, indicated no effect of binding of this amount of gossypol on these properties. The electrophoretic patterns of these samples were identical and indicated a meal having a low protein quality index.

Discussion

By using a cottonseed meal which had an initially high protein quality index, a low gossypol content, and a high nitrogen solubility in dilute alkali, it was possible to determine the relative contributions of autoclaving and of binding of gossypol during autoclaving to changes in the chemical and physical properties, and in the protein quality indices of the meal. Comparison of these data illustrated that progressive reduction in protein quality index during autoclaving was paralleled by the decreases in nitrogen solubility in 0.02N sodium hydroxide. These reductions in protein quality index and nitrogen solubility did not seem to be associated with the changes in any other meal constituents which were measured. These other changes occurred either after short periods of autoclaving, as those in free gossypol content, or only after extended periods of autoclaving, as in the soluble carbohydrate and total gossypol contents. The phytin content of the meal, which has been known to suppress the solubility of the seed meal proteins (*6*), did not appear to be responsible for the changes in nitrogen solubility, as there was no change in the acid soluble phosphorus fraction which is composed principally of phytin (*19*).

During a 5-minute autoclaving period, which had been shown to have no deleterious effects on the protein quality index of the meal, binding of 1% added gossypol was accomplished. This bound gossypol did not reduce the protein quality index of the meal. Similarly, binding of 1% added gossypol during a 30-minute autoclaving period caused no greater reduction in protein quality index than that obtained when the meal was autoclaved alone for 30 minutes. From the results, binding of 1% gossypol under these conditions did not lower the protein quality index of a high quality meal and did not have an additive effect in the transformation of the meal of high quality to one of low quality.

Figure 2. Representative electrophoretic patterns of cottonseed meals of high and low protein quality index



Kuiken (*12*) reported a reduction in lysine availability in samples of cottonseed meal autoclaved in the presence of gossypol and oil but no reduction if the samples were autoclaved alone or in the presence of oil. Although the data reported here indicated no differences between meals autoclaved alone and in the presence of gossypol and oil, the amino acid contents have not been determined.

In addition to the relationship between nitrogen solubility in dilute alkali and nutritive value, Lyman *et al.* (*13*), noted an association between the gossypol content and the growth rate index of a series of commercial meals. On the basis of these data, they proposed the following chemical index of nutritive value:

$$\text{Chemical index} = \frac{\%\ \text{nitrogen solubility in } 0.02N\ \text{NaOH}}{\text{total gossypol content, } \%}$$

where the value 0.85 is used if the total gossypol content is 0.85% or less.

By substituting the chemical data for the samples reported here in this formula, reductions in chemical indices of meals autoclaved in the absence of gossypol were similar to the changes in protein quality index. However, when gossypol had been added to the meals, the chemical indices indicated reductions in nutritional quality which were not evident in the chick feeding tests. Where the toxic effects of gossypol have been eliminated by reducing the free gossypol content to 0.04% or less (*1*), any effect of gossypol on the nutritive value of a meal must be due to the amount of bound gossypol present. Since the gossypol bound during autoclaving failed to influence the nutritive value of this high quality meal whereas other investigators noted a relationship between the nutritive values of certain meals and their gossypol contents (*13*), there appears to be at least two types of bound gossypol. The role of bound gossypol in the nutritive evaluation of cottonseed meal remains a major problem.

Comparison of the protein quality indices and nitrogen solubilities in 0.02N sodium hydroxide of a group of commercially prepared meals indicated fair but not strict correlation between these properties. The indications are, therefore, that one (perhaps the major) cause of variations in nutritional quality of cottonseed meals, provided the free gossypol content is 0.04% or less, is the amount of heat to which they are subjected. This damage is reflected by changes in the nitrogen solubilities of meals in dilute alkali, and, where other factors have been eliminated, this solubility measurement may be used to estimate the protein quality index of the meal. In regular commercial processing, however, other factors will probably exert additional influence on the nutritional quality of the

meals, and the effects of these factors may not be reflected solely by the measurement of nitrogen solubility in dilute alkali. Therefore, it is conceivable that any chemical estimation of nutritive value, applicable to all types of cottonseed meal, will include not only determinations of nitrogen solubility in 0.02*N* sodium hydroxide or its equivalent but also determinations of other changes in meal properties or constituents.

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FORAGE CONSTITUENTS

Yields of Holocellulose Prepared from Various Forages by Acid Chlorite Treatment

RAY E. ELY and L. A. MOORE

Dairy Husbandry Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

Studies of holocellulose prepared from common forages are quite limited. Reports of holocellulose prepared from materials other than wood are principally concerned with preparations derived from corn cobs (*4, 19*), straw (*1, 2, 4, 11*), cornstalks (*4–6*), Kentucky bluegrass (*4*), timothy hay (*4*), oat hay (*5*), and mixed hay (*5*).

Since the cellulose and hemicellulose fractions represent the major part of most forage materials and contribute substantially to the energy value of forages for ruminants, it was desirable to study the possibility of separating these polysaccharides from forage in a single fraction (holocellulose). Comparable digestibilities of cellulose and hemicellulose by ruminants (*10, 18*) justify combining these fractions from a physiological viewpoint.

The objectives of this study were to find a satisfactory delignification treatment for the preparation of holocellulose that would retain all of the cellulose and hemicellulose fractions from a variety of forages, ascertain if a uniform delignification treatment would be suitable for the preparation of holocellulose from forages varying widely in composition, and calculate the recoveries of theoretical holocellulose in holocellulose preparations from various forages.

Experimental Procedure

Ten forages were chosen that had a wide range in the amounts of the various constituents as determined by a proximate analyses. The forages used consisted of seven hays, two silages, and one straw.

Analyses of the forage samples for protein, ether extract, crude fiber, nitrogen-free extract, and ash contents were made according to procedures described by the Association of Official Agricultural Chemists (*3*). Lignin was determined by the method of Ellis, Matrone, and Maynard (*8*). Preparations of extractive-free feed samples were made by the following procedure: A 20-gram sample of air dry ground forage was wet with 50 ml. of hot water (80–90° C.) by stirring with a spatula in a beaker. The wet sample was transferred to the Waring Blendor jar by washing from the beaker with an alcohol-benzene mixture (33% ethyl alcohol–67% benzene by volume) using a total of 300 ml. One hundred milliliters of absolute ethyl alcohol were added and the sample was extracted for 7 minutes. The residue was filtered on a large fritted glass funnel of coarse porosity, washed with additional alcohol-benzene and finally with Skellysolve F. Ten grams of the alcohol-benzene extracted residue were then extracted with 400 ml. of hot water (95° C.) for 7 minutes in a Waring Blendor and the residue was filtered on a folded glass cloth (No. G.C.110 glass cloth from Filpaco Industries Inc., Chicago 16, Ill.) in a 4.5-inch diameter glass funnel. The residue was washed