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

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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

The proximate analysis of forages does not adequately classify the constituents of the crude fiber and nitrogen-free extract fractions. Cellulose and hemicelluloses are the major constituents of these two fractions and are arbitrarily distributed between them in varying amounts depending upon the material studied. Holocellulose (cellulose and hemicelluloses) prepared by acid chlorite delignification of forages gave excellent recoveries of the water insoluble carbohydrates in forages. A system of forage analysis including holocellulose determinations would account for all of the extractive-free forage dry matter without large percentages of undetermined fractions (nitrogen-free extract). Cellulose and hemicellulose have similar digestibility coefficients for ruminants which justify combining these fractions for analytical purposes. Holocellulose preparations that retain all of the water insoluble carbohydrates of forages make an excellent material for studying the contents of alpha cellulose, hemicellulose, hemicellulose fractions, and the components of each of these fractions from various forages.

with 200 ml. of hot water and then transferred to a fritted glass funnel with a stream of acetone. A final wash with Skellysolve F was made to facilitate drying in vacuo at 50° C. This material is described as the "extractive-free" forage. For convenience in weighing and handling, the extractive-free forage sample was allowed to come in equilibrium with atmospheric moisture.

Holocellulose was prepared according to the procedure outlined by Wise, Murphy, and D'Addieco (*20*) with minor modifications. Total carbohydrates were calculated for comparative purposes as the lignin-free crude fiber and nitrogen-free extract fractions, according to the following formula:

Total carbohydrates =
(crude fiber + n.f.e.) − lignin.

The theoretical holocellulose contents of the various feeds were calculated as the lignin and protein-free organic matter of the extractive-free feed dry matter:

Theoretical holocellulose =
100 − (ash + protein + lignin)

(Ash, protein, and lignin as per cent of extractive-free feed dry matter)

Results and Discussion

The proximate composition, the lignin, and calculated total carbohydrate contents of the 10 feeds studied are presented in Table I. The variation in the composition of the feeds studied is shown by the range in content of the various constituents as follows: protein from 3.2 to 22.0%, crude fiber from 22.7 to 42.9%, nitrogen-free extract from 32.3 to 63.2%, and lignin from 5.5 to 13.5%.

The composition (protein, ash, and lignin) of the dry matter of extractive-free feeds is given in Table II. These analyses were used for the calculations of the theoretical holocellulose contents of the various feeds which are also given in Table II.

Orienting experiments with four feeds (corn silage, wheat straw, young orchard grass, and alfalfa silage) were conducted to (*a*) ascertain if appproximately all of the theoretical holocellulose was retained following various acid chlorite treatments, (*b*) determine the number of acid chlorite treatments required for the holocellulose yield to approximately equal the theoretical yield, (*c*) know if the holocellulose yields from the four feeds studied would approximate the theoretical holocellulose contents when a uniform number of treatments were used, (*d*) determine the lignin and nitrogen contents of the holocellulose preparations from the four feeds with various acid chlorite treatments, and (*e*) study variations in the composition of holocellulose prepared from feeds varying in lignin, protein, and crude fiber contents.

Table III shows the yields of holocellulose obtained from each of the four feeds with each of six acid chlorite treatments. These results indicate that two acid chlorite treatments retain essentially all of the holocellulose in these four feeds. Three treatments resulted in small losses of holocellulose with two of the four feeds, four treatments resulted in losses with three of the four feeds, and five and six treatments caused additional decreases in the amount of holocellulose recovered from each of the four feeds. Recoveries of from 97 to 103% of the theoretical holocellulose are considered satisfactory. The authors are aware of inherent errors in the calculation of the theoretical holocellulose contents. Analytical errors are also possibly involved in determinations of the ash-, protein-, and lignin-free holocellulose contents. These results indicate that two delignification treatments yield approximately 100% of the theoretical holocellulose contents of these four forages.

The yields of crude holocellulose, prepared from these feeds and the ash-, protein-, and lignin-free holocellulose contents are presented in Figure 1. The lignin and nitrogen × 6.25 contents of the ash-free crude holocellulose prepared from the four feeds are also presented in Figure 1. Delignification apparently occurred most rapidly during the first two or three treatments and at a slower rate with subsequent treatments. The amount of apparent lignin remaining in crude holocellulose prepared from forage is evidently not related to the lignin content of the original forage because the young orchard grass and wheat straw had 5.5 and 13.0% of lignin, respectively, in the original feed dry matter, and 2.5 and 0.5%, respectively, of apparent lignin in the ash-free holocellulose prepared by using six delignification treatments. Several reports (*12*, *13*, *20*) have shown that it is undesirable to attempt complete removal of lignin from wood holocellulose because of the concomitant loss of carbohy-

Table I. Per Cent Composition of Dry Matter of Feeds

	Protein	Ether Extract	Crude Fiber	N.F.E.	Ash	Lignin	Total Carbohydrates
Soybean hay	11.0	0.9	42.9	36.7	8.5	13.5	66.1
Wheat straw	3.2	1.4	42.4	48.1	4.9	13.0	77.5
Clover hay	10.7	1.7	37.8	43.6	6.2	12.7	68.7
Mature orchard grass	11.1	2.3	35.1	46.3	5.2	9.3	72.0
Timothy hay	6.7	1.9	34.2	52.9	4.3	10.7	76.4
Alfalfa hay	14.5	1.5	31.7	45.0	7.3	10.0	66.7
Lespedeza hay	13.8	3.0	30.5	45.5	7.2	12.6	63.4
Young orchard grass	22.0	3.0	24.1	44.4	5.8	5.5	63.0
Alfalfa silage	17.8	3.0	37.9	32.3	9.0	12.5	57.7
Corn silage	7.4	2.3	22.7	63.2	4.4	6.9	79.0

Table II. Per Cent Composition of Dry Matter of Extractive-Free Feeds

	Protein	Ash	Lignin	Theoretical Holocellulose
Soybean hay	9.2	4.5	16.6	69.7
Wheat straw	2.1	3.1	13.4	81.4
Clover hay	10.5	2.8	15.5	71.2
Mature orchard grass	9.4	1.9	11.3	77.4
Timothy hay	5.9	2.0	13.1	78.0
Alfalfa hay	14.4	2.4	15.4	67.8
Lespedeza hay	14.3	3.8	15.8	66.1
Young orchard grass	22.5	1.7	7.4	68.4
Alfalfa silage	10.8	2.7	17.1	69.4
Corn silage	5.4	1.3	12.2	81.1

drates with the more drastic treatment required. The authors recognize that conventional lignin determinations of prepared holocellulose are unsatisfactory and should not be accepted as absolute values. Coldwell and DeLong (*7*) found that lignin isolated from holocellulose had a much lower methoxyl content than the lignin from the corresponding extractive-free tissue. This suggests that the acid chlorite treatment may remove interfering substances that contain methoxyl groups, or the treatment may demethoxylate the lignin. Their results also showed that the nitrogen content of the holocellulose lignin was lower than the nitrogen content of the lignin from the original material, and the holocellulose lignin was tough, elastic, and nearly colorless compared to the dark chocolate brown friable material isolated from the original material (*7*). The holocellulose lignin (material insoluble in 72% sulfuric acid) is quite different from lignin isolated from the original material by the same method.

The nitrogen content of crude holocellulose was highest when forage of high nitrogen content was delignified by acid chlorite treatment. Retention of nitrogen in the holocelluloses prepared with three acid chlorite treatments from corn silage, alfalfa silage, wheat straw, and orchard grass were 10.7, 14.1, 18.6, and 29.8%, respectively, of the original forage nitrogen. Pretreatment of the forage samples with pepsin digestion before the acid chlorite treatment results in considerable reduction of the nitrogen retained in the prepared holocellulose but concurrent decreases in the recoveries of holocellulose occur (*9*). Incomplete removal of protein from forage materials during treatment with acid chlorite has been reported by Bennett (*4*) and Adams and Castagne (*1*). Bennett (*4*) found 53.0 and 55.7% of the original nitrogen retained in holocellulose prepared from Timothy hay and Kentucky bluegrass, respectively. He also found 43.9, 32.5, and 32.4% of the nitrogen retained in holocellulose prepared from oat straw, cornstalks, and corncobs, respectively.

Figure 1. Yields of holocellulose from four forages and lignin and nitrogen contents of crude holocelluloses prepared with various acid chlorite treatments

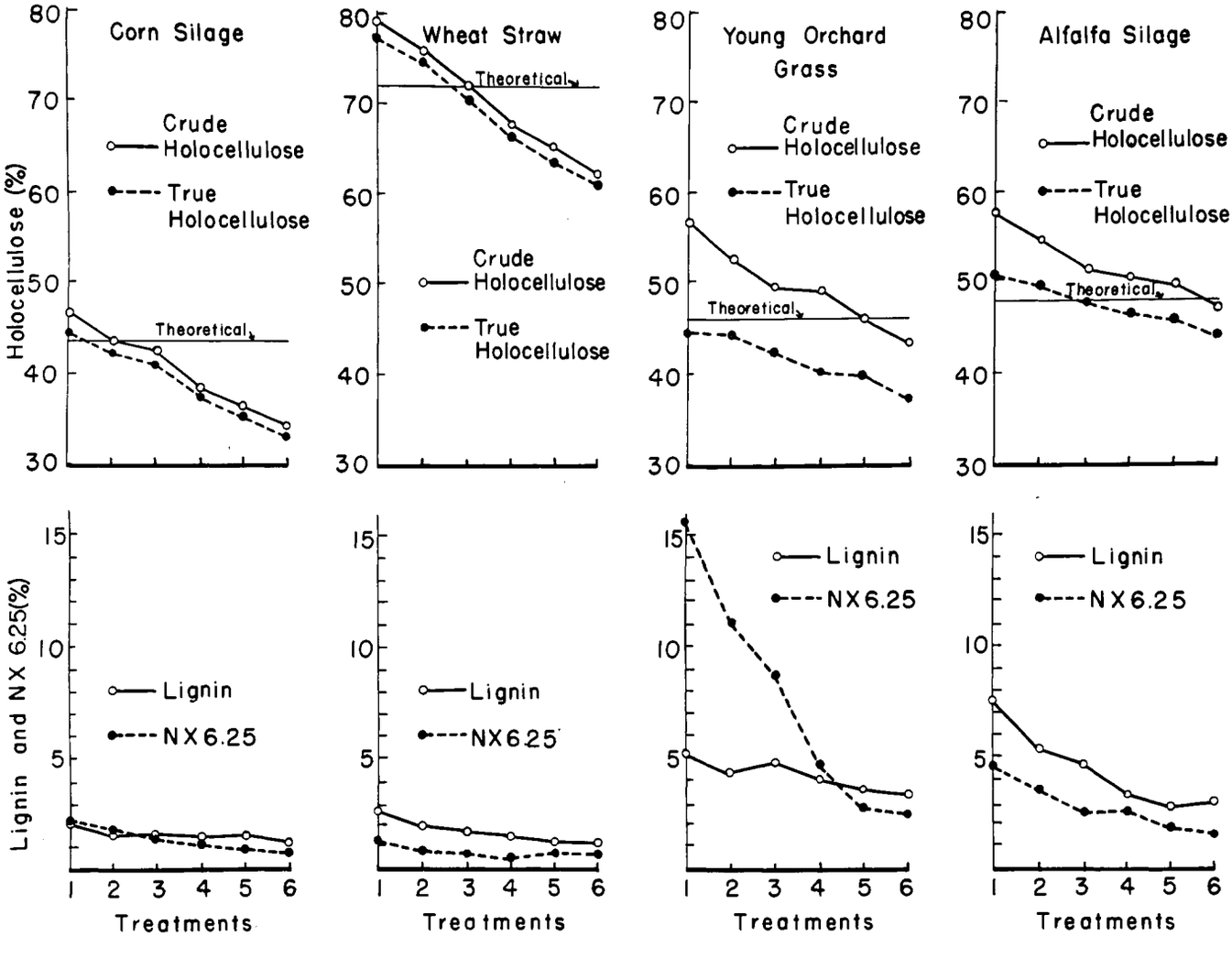

Table III. Yields of Holocellulose[a] Obtained from Four Feeds Using One to Six Delignification Treatments

Treatments	Corn Silage	Wheat Straw	Young Orchard Grass	Alfalfa Silage	Average
1	102	107	97	106	103
2	97	103	97	103	100
3	95	102	93	100	98
4	86	92	89	97	91
5	82	88	85	96	88
6	77	85	81	93	84

[a] Figures given are yields of ash-, protein-, and lignin-free holocellulose as per cent of theoretical.

Adams and Castagne (*1*) studied five straws and found from 7.5 to 13.7% of the nitrogen of the original straw was retained in the holocellulose preparations that yielded approximately 100% recovery of the theoretical holocellulose.

The data in Table III and Figure 1 indicate that two acid chlorite delignification treatments may be suitable for satisfactory recoveries of holocellulose from a variety of feeds with large differences in composition.

Holocellulose yields were determined with ten feeds by preparation with one, two, and three acid chlorite delignification treatments with each forage. The pure holocellulose contents (ash-, protein-, and lignin-free), calculated as the percent of the theoretical holocellulose from each of these feeds and with each treatment, are presented in Table IV. Average recoveries of the theoretical holocellulose were 101. 99, and 96% from the 10 forages when using one, two, and three acid chlorite treatments, respectively. Two acid chlorite treatments with the conditions of this experiment are proposed as satisfactory for recoveries of approximately all of the cellulose and hemicellulose in one fraction (holocellulose) from a variety of feeds. The holocellulose recoveries ranged from 97 to 103% when two acid chlorite treatments were used. There is evidence that losses of carbohydrate material occur in several of the forages when three treatments were used. Eight of the ten feeds studied had less than 97% recoveries of the theoretical holocellulose when three treatments were used. These results indicate that a uniform acid chlorite delignification treatment may be used with feeds varying widely in composition. Further studies should provide a basis for improved procedures for the preparation of holocellulose from forage plants. The method used in this investigation has not been modified to provide what may be optimum conditions for the preparation of satisfactory holocellulose from the materials studied.

Whistler *et al.* (*19*) have shown that the lowest lignin contents of holocellulose preparations from corncobs are obtained when sodium chlorite is added in four portions totaling the weight of the cob and when added at 15-minute intervals. They also showed that the efficiency of delignification decreased with changes in pH above or below 4.5. Harwood (*11*) compared the composition of holocellulose samples prepared from wheat straw by the methods of Wise *et al.* (*20*) and Whistler *et al.* (*19*). The milder reaction conditions employed by Whistler *et al.* (*19*) reduced the losses of pentosans and uronic acids that occurred when using the Adams and Castagne (*1*) modification of the Wise *et al.* (*20*) procedure.

Table IV. Yields of Holocellulose Obtained with 1, 2, and 3 Acid Chlorite Delignification Treatments

Treatments	Hollocellulose[a] Yield as % of Theoretical 1	2	3
Soybean hay	98	99	97
Wheat straw	107	103	102
Clover hay	99	99	95
Mature orchard grass	102	98	94
Timothy hay	100	97	94
Alfalfa hay	100	98	93
Lespedeza hay	98	97	92
Young orchard grass	97	97	93
Alfalfa silage	106	103	100
Corn silage	102	97	95
Av.	101	99	96

[a] Ash-, lignin-, and protein-free holocellulose.

The holocellulose fraction represents from 66 to 81% of the dry matter of the extractive-free feeds studied. This fraction would ordinarily be distributed between the crude fiber and nitrogen-free extract fractions by the conventional proximate system of analyses (*14–17*). Holocellulose preparations that retain all of the carbohydrate material in extractive-free feeds should make an excellent starting material for studies of the content of alpha cellulose, hemicellulose, hemicellulose fractions, and the components of each of these fractions from various forages.

Summary

Acid chlorite delignification of ten forages varying widely in composition gave an average of 99% (97-103%) recovery of the theoretical holocellulose when two treatments were used.

Holocellulose contents of the feeds studied ranged from 66.1 to 81.4% of the extractive-free feed dry matter.

Two acid chlorite treatments gave recoveries of essentially all of the cellulose and hemicelluloses but did not give minimum lignin and nitrogen contents of the prepared holocellulose.

Excessive acid chlorite treatment resulted in apparent loss of carbohydrates from the holocellulose fraction.

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