Investigations on the determination of the crude fiber content of some feed materials
by James L Milne

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Chemistry
Montana State University


Abstract:
Certain modifications of the present official crude fiber determinations have been investigated. Particular emphasis has been given to the effects of varying temperature and pressure on the hydrolytic processes involved.

The effectiveness of various hydrolytic agents used singly or in combinations was studied. The comparative results of the modifications are discussed.

# INVESTIGATIONS ON THE DETERMINATION OF THE CRUDE FIBER CONTENT OF SOME FEED MATERIALS

by

JAMES L. MILNE

A THESIS

Submitted to the Graduate Faculty

in

partial fulfillment of the requirements

for the degree of

Master of Science in Chemistry

at

Montana State College

Approved:

B. L. Johnson
Head, Major Department

Leon Johnson
Chairman, Examining Committee

J. A. Nelson
Dean, Graduate Division

Bozeman, Montana
July, 1951

TABLE OF CONTENTS

## I. ABSTRACT

Certain modifications of the present official crude fiber determinations have been investigated. Particular emphasis has been given to the effects of varying temperature and pressure on the hydrolytic processes involved.

The effectiveness of various hydrolytic agents used singly or in combinations was studied. The comparative results of the modifications are discussed.

## II. INTRODUCTION

The term crude fiber as used in this thesis is defined as the organic material which remains insoluble after a feed material has been extracted with di-ethyl ether and subjected to the action of hydrolytic agents under stated conditions of time, temperature, and concentrations.

The results of recent collaborative study on feed analyses carried out by several state Agricultural Experiment Stations indicated that a wide variation in the crude fiber content of a feed sample could be expected. It was noted from this collective study that those Experiment Stations situated in the higher elevations of the country usually reported higher crude fiber values than those Experiment Stations which were located in the areas of lower elevation. The purpose of this investigation was two-fold. The first problem of interest concerned the possible effects of elevated temperatures and pressure on the hydrolitic reactions involved in the crude fiber determinations. It was hoped that these variables in the present analyses could be effectively standardized so that the variations in the results brought about by the difference in altitudes could be eliminated. The second purpose of investigation was to establish the efficiency of various hydrolytic agents which could be used singly or in combinations.

## III. EXPERIMENTAL PROCEDURE

A. Materials.-

Four different livestock feeds were selected as the basic materials for this investigation. These feeds were 1, oat hay, 2, a standard mixture dairy feed containing soy bean meal, 3, alfalfa hay, and 4, barley. Feeds No. 1 and No. 3 were considered representative of feed materials having a high crude fiber content while feeds No. 2 and No. 4 were selected because of their relatively low crude fiber percentage. The feeds were air-dried and ground in a Wiley Mill to pass through a 40-mesh screen. After grinding, the samples were air dried and stored in tightly closed glass jars.

B. Crude Fiber Determinations Using Official Method.-

Several crude fiber determinations were made on each feed material according to the Official and Tentative Methods of Analysis of A.O.A.C. (14). The results of these analyses are tabulated in Table I.

TABLE I

| Standard Crude Fiber Percentages Obtained by the Official Methods of Analysis | | | | |
|---|---|---|---|---|
| Feed | No. of Tests | Average Percentage | Highest Percentage | Lowest Percentage |
| 1 | 6 | 26.56 | 27.85 | 25.40 |
| 2 | 6 | 7.43 | 7.55 | 6.30 |
| 3 | 6 | 25.51 | 26.12 | 25.00 |
| 4 | 6 | 3.56 | 3.85 | 3.05 |

It will be noted Table I contains the information concerning the highest, the lowest, and the average of the determinations for each feed.

C. Crude Fiber Determinations by Elevating Temperature and Pressure.-

In order to control the temperature and pressure, the hydrolytic reactions were conducted in a standard laboratory autoclave.

1. Autoclave.- The autoclave was equipped with the usual pressure gauge and a thermometer. Furthermore, this particular autoclave was so constructued that the pressure could be varied at will by means of an adjustable diaphragm.

2. Containers.- The determinations were carried out in 500 ml Erlenmeyer flasks. In each case, except where otherwise noted, 50 ml of the hydrolytic agent were employed.

3. Pressure.- During the first experiments a pressure of 10 lbs. per square inch was held, but it was later noted that when the pressure was increased to 15 lbs. per square inch the crude fiber percentage was reduced considerably. Therefore, most of the experiments were conducted at a pressure of 15 lbs. per square inch. The recorded temperature at this pressure was 116°C.

4. Time.- Various time intervals ranging from 5 to 20 minutes were used in this investigation. However, it was found that a time interval of ten minutes was the most satisfactory. Therefore, all the experiments recorded in this paper except those employing barium hydroxide were run using a standard time interval of 10 minutes.

5. Hydrolytic Agents Employed.- Various acid and alkaline hydrolytic agents were employed in this study. The alkaline reagents employed were sodium hydroxide and barium hydroxide. Sulfuric acid, hydrochloric acid, and a mixture of hydrochloric acid and acetic acid, were the acid

hydrolytic agents used. In all cases the hydrolytic reactions were preceded by the usual ether extraction as designated in the Official Methods. The temperatures employed during the drying and ignition of the crude fiber residues were the same as those indicated in the Official Methods.

6. General Procedure.- Two grams of the ether extracted feed material were placed in a 500 ml Erlenmeyer flask and 50 ml of the hydrolytic agent were added. In addition, it was found necessary to add a small amount of asbestos and a few boiling chips. The flasks were placed in the autoclave which had been set for 15 lbs. per square inch. The steam was turned on and after the pressure reached 15 lbs. the time was noted. The time interval indicated in all experiments was that interval from the time the autoclave reached 15 lbs. per square inch until it was turned off. After cooling, the flask and contents were removed, and the contents filtered through filter cloth. The residue was then washed with successive applications of water and, finally, with acetone. After the residue had been removed from the filter cloth, it was dried and ignited according to the usual Official Methods. In order to overcome the filtration difficulties encountered in feeds No. 2 and No. 4 by filtration, separation by centrifugation was used. Two grams of ether extracted feed material were placed in a 100 ml centrifuge tube and 20 ml of hydrolytic agent were added together with some boiling chips and a small amount of asbestos. The tube was stoppered and placed in the autoclave at the pressure and time mentioned above. The tube was removed, cooled, and centrifuged five minutes. The supernatant liquid was poured off and the residue was thoroughly agitated with water and after centrifuging again the resultant

liquid decanted. This process was repeated three times. The final washing was with acetone. The residue was removed from the tube, dried, and ignited according to the Official Methods.

D. Enzymatic Procedure Followed in the Crude Fiber Determination.-

In order to find how close the chemical crude fiber resembled the digestive crude fiber, the enzymatic reaction was conducted using papain and taka diastase in a properly buffered solution.

Procedure.- Two grams of dry ether extracted sample were placed in a 250 ml Erlenmeyer flask and 0.5 grams of taka diastase added and 100 ml of phosphate buffer pH 4.8. The mixture was stirred and placed in the incubator at 37.5°C. The feed was allowed to remain in the incubator for 48 hours with occasional stirring. After 48 hours the feed was removed, filtered through coarse filter paper, and washed with distilled water. The feed was returned to the flask and 0.5 grams of papain with a small amount of L cystiene and 100 ml of phosphate buffer pH 7.2 were added. The feed was kept in the incubator 48 hours with occasional stirring. After 48 hours the feed was removed, filtered, and washed. After removal from the filter paper the residue was dried and ignited according to the usual Official Methods.

## IV. EXPERIMENTAL RESULTS

The results of the first experiment on feed No. 1 are show in Table II. The general procedure was followed until the removal of the flask from the autoclave when 5 ml of 6 N hydrochloric acid were added to the flask in order to neutralize the sodium hydroxide remaining in the mixture. From here on the procedure was the same as described previously. The normality of the sodium hydroxide is the same as the normality used by the Official Methods. The increase is approximately .05 on each change of normality.

TABLE II

Crude Fiber Percentages for Feed No. 1 Using NaOH as the Hydrolytic Agent

| Normality of NaOH | No. of Tests | Time Minutes | Pressure lbs/sq.in. | Crude Fiber Per Cent | O.M.* Per Cent |
|---|---|---|---|---|---|
| 0.316 | 2 | 10 | 10 | 34.10 | 26.56 |
| 0.316 | 2 | 15 | 10 | 44.80 | 26.56 |
| 0.316 | 2 | 20 | 10 | 35.00 | 26.56 |
| 0.347 | 2 | 5 | 15 | 34.70 | 26.56 |

* Official Method

It will be noted that the percentage crude fiber as obtained here was far greater than that obtained by the Official Methods.

The next analysis was conducted on feed No. 3 which resembled feed No. 1 in chemical composition. The general procedure was followed for the times indicated, the hydrolytic agent and the pressure were the same as used on feed No. 1. The results of this analysis are shown in Table III.

TABLE III

| Crude Fiber Percentage For Feed No. 3 Using NaOH as the Hydrolytic Agent | | | | | |
|---|---|---|---|---|---|
| Normality of NaOH | No. of Tests | Time Minutes | Pressure lbs/sq. in. | Crude Fiber Per Cent | O.M. Per Cent |
| 0.347 | 2 | 5 | 15 | 25.00 | 25.51 |
| 0.347 | 2 | 10 | 15 | 25.20 | 25.51 |
| 0.347 | 2 | 5 | 15 | 28.00 | 25.51 |

The results of this analysis show a very close approach the range of the crude fiber percentages obtained by the Official Methods. The results indicated that the time of hydrolysis had little effect on the results of the analysis.

A complete test of feed No. 3 was conducted using sodium hydroxide of three concentrations, and varying time of hydrolysis. The results of these analyses using concentrations of 0.316 N, 0.347 N and 0.407 N sodium hydroxide and obtained by times from 5 to 20 minutes show good agreement with the results of the Official Method.

TABLE IV

| Crude Fiber Percentages For Feed No. 3 Obtained Using 15 lbs. per Square Inch Pressue and NaOH as the Hydrolytic Agent | | | | | |
|---|---|---|---|---|---|
| Time Minutes | No. of Tests | Varying Normalities of NaOH | | | O. M. Range Per Cent |
| | | 0.316 | 0.347 | 0.407 | |
| | | Crude Fiber Per Cent | | | |
| 5 | 2 | 28.10 | 26.70 | 24.90 | 26.12 - 25.00 |
| 10 | 2 | 27.05 | 26.15 | 25.00 | 26.15 - 25.00 |
| 15 | 2 | 28.55 | 26.80 | 26.70 | 26.15 - 25.00 |
| 20 | 2 | 29.60 | 27.40 | 27.60 | 26.15 - 25.00 |
| Average | | 28.30 | 26.80 | 26.25 | 25.51 |

The results shown in Table IV indicate that the Official results could be duplicated with feed No. 3 using an ether extracted sample.

Feed No. 2 was analyzed using the general procedure and varying concentrations of sodium hydroxide. The results shown in Table V indicate that the lumps formed during hydrolysis material. The normality of sodium hydroxide was larger than previously indicated since lower concentrations of sodium hydroxide had obtained no usable results.

TABLE V

| Crude Fiber Percentages for Feeds No. 2 Using NaOH as the Hydrolytic Agent, 15 lbs. per Square Inch Pressure and a Time of 10 Minutes | | | |
|---|---|---|---|
| Normality of NaOH | No. of Tests | Crude Fiber Per Cent | O. M. Per Cent |
| 0.407 | 2 | 32.5 | 7.43 |
| 0.830 | 2 | 29.7 | 7.43 |
| 0.98C | 2 | 34.1 | 7.43 |

The general procedure for centrifugation was followed, but 10 ml of 6 N hydrochloric acid was added before the centrifuging the first time. However, the results as shown in Table VI indicated that this method was not feasible for this feed.

TABLE VI

| Crude Fiber Percentages for Feed No. 2 Using Centrifugation for Separation, NaOH as Hydrolytic Agent, 15 lbs. per Square Inch Pressure and a Time of 10 Minutes | | | |
|---|---|---|---|
| Normality of NaOH | No. of Tests | Crude Fiber Per Cent | O. M. Per Cent |
| 0.407 | 3 | 39.95 | 7.43 |
| 0.615 | 3 | 41.65 | 7.43 |
| 0.830 | 3 | 39.50 | 7.43 |

The results of the sodium hydroxide analysis of feeds No. 1 and No. 3 indicated that the alkali hydrolysis worked well for feeds with high crude fiber percentages, but the results of feeds No. 2 and No. 4 indicated that sodium hydroxide was not the proper hydrolytic agent.

A series of experiments were performed using barium hydroxide as the hydrolytic agent. Barium hydroxide solutions were prepared having normalities of 0.160 and 0.320. Feeds No. 1 and No. 2 being typical examples of each group were hydrolyzed with barium hydroxide at 15 lbs. per square inch pressure for twenty minutes. The results as shown in Table VII indicated a decrease in organic residue when the higher concentration of barium hydroxide was used.

TABLE VII

| Crude Fiber Percentages Obtained Using $Ba(OH)_2$ as the Hydrolyzing Agent, 15 lbs. per Square Inch Pressure and a Time of 20 Minutes | | | | |
|---|---|---|---|---|
| Feed | No. of Tests | Normality $Ba(OH)_2$ | Crude Fiber Per Cent | O. M. Per Cent |
| 1 | 2 | 0.160 | 43.00 | 26.56 |
| 1 | 2 | 0.320 | 39.45 | 26.56 |
| 2 | 2 | 0.160 | 32.15 | 7.43 |
| 2 | 2 | 0.320 | 28.70 | 7.43 |

The experiments using barium hydroxide 0.352 N was conducted on feeds No. 1 and No. 2 and the results showed further reduction in the crude fiber percentage. The results are shown in Table VIII.

TABLE VIII

| Crude Fiber Percentages Using 0.355 N $Ba(OH)_2$ as Hydrolytic Agent, 19 lbs. per Square Inch Pressure and a Time of 30 Minutes | | | |
|---|---|---|---|
| Feed | No. of Tests | Crude Fiber Per Cent | O. M. Per Cent |
| 1 | 2 | 34.35 | 26.56 |
| 2 | 2 | 24.20 | 7.43 |

No further experiments were conducted using barium hydroxide as the normality of the last solution was 0.355 N and no higher normalities could be obtained using $Ba(OH)_2 \cdot 8H_2O$ unless a warm solution was used. HANDBOOK OF CHEMISTRY AND PHYSICS (10) indicates that 5.4 g of $Ba(OH)_2 \cdot 8H_2O$ dissolves in 100 ml of water at 15°C. As room temperature is generally about 24° the saturation point had about been reached.

The next group of hydrolytic agents used was the acids. The first acid employed was the 0.255 N sulfuric acid of the Official Methods. The increase is approximately .05 on each change of normality. The feeds tested were No. 2 and No. 4 as feeds No. 1 and No. 3 had been tested successfully with sodium hydroxide. The general procedure was followed using sulfuric acid, 0.255 N, for varying times at 15 lbs. per square inch pressure.

The experiments were continued using higher concentrations of sulfuric acid. The results of the sulfuric acid experiments are shown in Table IX and give percentages of almost double those obtained by the Official Methods.

TABLE IX

| Crude Fiber Percentages Obtained Using Sulfuric Acid as Hydrolytic Agent and 15 lbs. per Square Inch Pressure | | | | | |
|---|---|---|---|---|---|
| Feed | No. of Tests | Normality $H_2SO_4$ | Time Minutes | Crude Fiber Per Cent | O. M. Per Cent |
| 2 | 2 | 0.255 | 5 | 13.95 | 7.43 |
| 2 | 2 | 0.255 | 10 | 12.45 | 7.43 |
| 2 | 2 | 0.255 | 15 | 11.30 | 7.43 |
| 2 | 2 | 0.303 | 5 | 14.00 | 7.43 |
| 4 | 2 | 0.303 | 5 | 7.70 | 3.56 |
| 2 | 2 | 0.352 | 5 | 14.40 | 7.43 |
| 4 | 2 | 0.352 | 5 | 7.35 | 3.56 |

Feed No. 4 was analyzed using sulfuric acid of three concentrations and varying times at 15 lbs. per square inch pressure. The results are shown in Table X and indicate no reduction in the crude fiber percentage was accomplished by either increasing the concentration of the hydrolytic agent or the time of the reaction.

TABLE X

| Crude Fiber Percentage for Feed No. 4 Using Sulfuric Acid as the Hydrolytic Agent and 15 lbs. per Square Inch Pressure | | | | | |
|---|---|---|---|---|---|
| Time Minutes | No. of Tests | Varying Normalities of $H_2SO_4$ | | | O. M. Range Per Cent |
| | | 0.255 | 0.303 | 0.352 | |
| | | Crude Fiber Per Cent | | | |
| 5 | 2 | 7.10 | 6.50 | 6.40 | 3.85 - 3.05 |
| 10 | 2 | 6.90 | 6.45 | 7.10 | 3.85 - 3.05 |
| 15 | 2 | 6.85 | 7.35 | 6.65 | 3.85 - 3.05 |
| 20 | 2 | 7.35 | 6.90 | 6.50 | 3.85 - 3.05 |
| Average | | 7.05 | 6.90 | 6.65 | 3.56 |

As there was no great reduction of the average percentage to the Official percentage the experiments using sulfuric acid were not carried further.

Hydrochloric acid alone and in combination with acetic acid was used as the hydrolytic agent. The general procedure was followed using the following acid reagents: (A) a mixture of 50 ml 0.266 N hydrochloric acid and 50 ml 0.290 N acetic acid; (B) 0.497 N hydrochloric acid and (C) 0.976 N hydrochloric acid. The time for the hydrolysis was 10 minutes and the pressure 15 lbs. per square inch. The results are shown in Table XI.

TABLE XI

| Crude Fiber Percentages Using Acid Reagents | | | | | |
|---|---|---|---|---|---|
| | | REAGENTS | | | |
| Feed | No. of Tests | A | B | C | O. M. |
| | | Crude Fiber Per Cent | | | Per Cent |
| 1 | 2 | 33.30 | 32.75 | 32.80 | 26.56 |
| 2 | 2 | 13.35 | 13.25 | 12.70 | 7.43 |
| 3 | 2 | 37.70 | 33.15 | 32.40 | 25.51 |
| 4 | 2 | 7.90 | 7.25 | 7.50 | 3.56 |

The results of these experiments gave crude fiber percentages considerably above the Official Method percentages and these experiments were not continued.

After the completion of the acid analysis of the feeds, a combination of acid and alkali reagens was used. The first analysis of this type was conducted using the reagents of the Official Method, but instead of boiling the feed material for thirty minutes the feeds were hydrolyzed at 15 lbs. per square inch pressure for ten minutes. The feed materials

were washed and filtered between acid and alkali hydrolysis. The crude fiber percentages obtained were slightly below the Official Method percentages in the case of feeds No. 1, No. 2, and No. 3, while feed No. 4 was within the Official range as this percentage approached the highest Official Method percentage. Another analysis was conducted using stronger reagents and the crude fiber per cent for feed No. 4 went down. Table XII shows the crude fiber percentage obtained from these analyses and the variations from the Official Method percentage.

TABLE XII

| Crude Fiber Percentages Using 15 lbs. per Square Inch Pressure for 10 Minutes During Both Acid and Alkali Hydrolyzing Periods | | | | |
|---|---|---|---|---|
| Feed | No. of Tests | Reagents | Crude Fiber Per Cent | Variations From O. M. Average |
| 1 | 2 | Reagents of O. M. | 20.65 | -5.91 |
| 2 | 2 | Reagents of O. M. | 5.70 | -1.73 |
| 3 | 2 | Reagents of O. M. | 19.75 | -5.76 |
| 4 | 2 | Reagents of O. M. | 5.10 | +1.54 |
| 1 | 2 | 0.25 N $H_2SO_4$<br>0.40 N NaOH | 21.55 | -5.01 |
| 2 | 2 | 0.50 N HCl<br>0.40 N NaOH | 5.60 | -1.83 |
| 3 | 2 | 0.50 N HCl<br>0.32 N NaOH | 18.75 | -6.76 |
| 4 | 2 | 0.50 N HCl<br>0.40 N NaOH | 3.55 | -0.01 |

It is evident from the results of this last series of experiments that this method of analysis is the best of the methods used for all of the feed materials in obtaining percentages near the range of the Official percentages.

With the conclusion of the preceding experiments it was thought advisable to check the crude fiber percentages by using enzymes. The experiments were conducted on duplicate samples. This analysis followed the procedure given for enzymatic analysis in experimental procedure. The results of this analysis in Table XIII gave the crude fiber percentages far greater than those obtained by the Official Method.

TABLE XIII

| Crude Fiber Percentages Using Enzymatic Agents | | | |
|---|---|---|---|
| Feed | Crude Fiber Per Cent | O. M. Per Cent | Variations From O.M. Per Cent |
| 1 | 53.40 | 26.56 | +26.84 |
| 2 | 31.30 | 7.43 | +23.87 |
| 3 | 51.70 | 25.51 | +26.19 |
| 4 | 20.60 | 3.56 | +17.04 |

It is noted from the table that feeds No. 1 and No. 3 have a crude fiber percentage of double the Official percentage, and feeds No. 2 and No. 4 have a percentage of about four times the Official percentages.

## V. DISCUSSION

In this discussion the method of the Official crude fiber determination will be compared with the method employed in this investigation. The mechanisms of the reactions will also be discussed and the use of various reagents employed in the crude fiber determination.

The time of the hydrolysis as given in the official methods is thirty minutes for both acid and alkali reactions. Though the official method of analysis is used for the crude fiber determination in Montana, no way of compensating for differences in atmospheric pressure is taken into consideration. As the temperature of the boiling point of a solution goes down approximately $6^{\circ}C$ per kilometer rise in altitude, it is evident that boiling temperature of the hydrolytic agents will be less in Montana than in the coastal regions. Bidwell (1) pointed out marked variations in the different laboratories throughout the country and Frapes (7) reported variations in the crude fiber results by laboratory locations. Though these reports were made more than 25 years ago nothing was done in order to standardize the temperature used in the crude fiber determination.

From a logrithmetic representation of the Arrhenius equation

$$\log b = \log A - \frac{E}{2.303\ RT}$$

the effect of temperature changes on the reaction rate can readily be seen for A, E, and R are constants and only T varies. Therefore, any change in the temperature of the reaction changes the reaction rate in direct relation, i.e., increase in temperature increases the rate and decrease in temperature decreases the rate. For this reason the autoclave

was used in order to increase the temperature of the reaction during the hydrolytic process. The temperature of the reaction as recorded by the autoclave thermometer was 116°C.

This increase in pressure not only increases the boiling point of the mixture, but decreases the time of hydrolysis at the maximum temperature. The volume of the hydrolytic agent used by the Official Method is 200 ml for both acid and alkali. The volume, using an autoclave pressure of 10 to 19 lbs. per square inch, is 50 ml, a decrease of 150 ml of reagent. The use of a smaller volume of acid and alkali in the investigation causes no large deviation from the large volume of the Official Methods not using pressure. A factor considered here in the using of 50 ml of reagent was the boiling over of the mixture while the pressure was decreasing.

The time of hydrolysis of the Official Method is 30 minutes with the mixture boiling for that time or at a maximum temperature for 30 minutes. By using an autoclave in which to conduct the hydrolysis the time of hydrolysis is reduced to 10 minutes. From the experimental results the changes in time were shown to have no effect on the amount of crude fiber obtained from each feed. However, a time of 10 minutes at maximum temperature was selected as most satisfactory for all feeds.

Winton (19) begins his crude fiber analysis by saying "Extract a 2 gram sample with ether" and this continues with the usual procedure. An agricultural chemistry text of the same date begins the discussion of crude fiber analysis in the same way though hydrochloric acid is used instead of sulfuric acid in the procedure. Bidwell and Bopst (3).

use ether extraction to precede the crude fiber determination. In the methods using inorganic acids and alkali reagents for the most part the ether extraction was used. The chief reason for the removal of the fat material by ether extraction is that if the fats are not removed the formation of soaps with the alkali reagent make separation of the residue much more difficult. This is especially true if a heavier alkali is used as the hydrolytic agent. Also, there are few feeds which will be analyzed for crude fiber only.

Changes in the concentration of reagents have been suggested by J. H. and E. W. Voelcher (18), W. Lepper (13) and others. The concentrations have values from 2.0 per cent to 3.125 per cent for both acid and alkali reagents. The results of these experiments using a higher concentration indicates that the time of the reaction may be reduced as much as ten minutes. Also the volume of the reagents may be reduced using a higher concentration of acid and alkali. From the experimental results it has been shown that both acid and alkali hydrolysis is necessary in order to duplicate the official results on all types of feed samples. It has been shown that acid removes some of the non fibrous material but not all of it. At the same time the alkali removes most of the non fibrous material in the hay type feeds, but removes little of the same material in the case of grain and mixed samples.

The acid hydrolysis of carbohydrates reduces the starches and sugars to simple sugars in most cases glucose. Acids in general do not effect the cellulose molecule unless the hydrolysis is conducted over a long time interval in which cases some fragmentation occurs. Alkali agents

reacting with the starch molecule cause the formation of a gel. The gel formed is not filterable. The use of alkali in hydrolyzing feeds No. 2 and No. 4 caused the formation of lumps in the mixture which remained at the end of the hydrolyzing period. When the lumps were broken open with a stirring rod there was no evidence of a reaction having taken place. Alkali reagents have little effect on the cellulose molecules except over long time intervals, in which case fragmentation occurs. However, the concentration of the alkali must be greater than the ones used in this work.

Proteins are readily hydrolyzed into simple amino acids and soluble peptides by both sulfuric and hydrochloric acid. For quantitative hydrolysis, times of several hours are used to boil the acids containing the protein. The reaction of the acid with the protein molecule probably caused incompleted hydrolysis to amino acids dipeptides, peptones, polypeptides, etc., soluble in the wash water.

Proteins are readily hydrolyzed by alkali reagents. The alkali hydrolysis is very rapid and will work readily in cases as crude fiber analysis where the qualitative and quantitative analysis of proteins is not desired.

The enzymatic analysis of the sample was conducted in order to see how close to a biological crude fiber the acid and alkali hydrolytic end products were approaching. The main objection to the enzymatic analysis is its slowness as compared with inorganic methods. Woodson and Mackenzie (20) used the enzyme pangestin to determine crude fiber percentages. They found that the values obtained for the crude fiber

percentages were about four times the amount obtained with the official methods. The results of my paper were well in keeping with the results obtained in this paper.

The ether extracted feed was first treated with Taka-diastase to remove the carbohydrate material. After the feed had been washed papain was added to the sample with a small amount of cysteine to activate the enzyme. The papain removes the protein material. After these reactions have been completed the crude fiber is obtained in the usual way.

After comparing the official methods with the enzymatic method, it is seen that the organic residue, called crude fiber, is not the same in both methods. The enzymatic method removes carbohydrates and proteins while the acid and alkali reagents remove these products and possibly other materials.

## VI. SUMMARY

From the experimental results of this series of experiments the following conclusions can be stated:

1. Crude fiber is considered a variable quantity dependent on the following factors: (a) the temperature of the reaction mixture, (b) the concentration of the reagent or reagents used, and (c) the type of reagent used.

2. Results of the Official Method on feeds with a high crude fiber content may be approximated by the use of a single hydrolytic reagent at elevated temperatures.

3. In feeds containing larger proportions of starch but low crude fiber percentages according to the Official Methods, unreliable results are obtained using a single reagent at elevated temperatures, and

4. Enzymatic hydrolysis of feed materials employing the combination of proteolytic and carbohydrate splitting enzymes were found to yield a considerably higher quantity of organic residues than obtained by the chemical means of the Official Methods.

## VII. BIBLIOGRAPHY


1. Bidwell, G. L., J. Assoc. Off. Agr. Chem., 5 55-7 (1921).
2. Bidwell, G. L., J. Assoc. Off. Agr. Chem., 5 421-22 (1922).
3. Bidwell, G. L. and Bopst, L. E., J. Assoc. Off. Agr. Chem., 5 58-9 (1921).
4. Bidwell, G. L. and Bopst, L. E., J. Assoc. Off. Agr. Chem., 5 422-24 (1922).
5. Chamberlain, J. S., ORGANIC AGRICULTURAL CHEMISTRY, MacMillan, 126, 265, 267 (1924).
6. Fairley, T. and Burrell, B. A., J. Soc. Chem. Ind., 37 155-64 (1918).
7. Fraps, G. S., Assoc. Off. Agr. Chem., 6 333-44 (1923).
8. Glasstone, S., ELEMENTS OF PHYSICAL CHEMISTRY, D. Van Nostrand (1944).
9. Gortner, R. A., OUTLINES OF BIOCHEMISTRY, John Wiley and Sons (1938).
10. HANDBOOK OF CHEMISTRY AND PHYSICS, Chemical Rubber Publishing Co., 30 Edition (1947).
11. Hill, G. A. and Kelly, L., ORGANIC CHEMISTRY, Blakiston (1943).
12. Hinshelwood, C. N., KINETICS OF CHEMICAL CHANGE, Oxford (1942).
13. Lepper, W., Z. Tierernahr Futtermittelk, 4 217-19 (1940).
14. Official and Tentative Methods of Analysis, Am. Off. Agr. Chem. 408-9 (1945).
15. Norman, A. G., J. Agr. Sci., 25 529-40 (1935).
16. Sherman, H. C., Thomas, A. W., and Baldwin, M. E., J. Am. Chem. Soc. 41 231 (1919).
17. Sumner, J. B. and Somers, F. G., CHEMISTRY AND METHODS OF ENZYMES, Academic Press, Inc., (1943).

18. Voelcher, J. A. and Voelcher, E. W., Analyst 43 31 (1918).

19. Winton, Al., MICROSCOPY OF VEGETABLE FOODS, John Wiley and Sons, 2nd Edition 17 59 (1916).

20. Woodson, H. T. and Mackenzie, H., Cereal Chem., 22 158-60 (1945).