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

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

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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