1948

Lignin, Cellulose and Crude Fiber Changes in Maturing Western Wheatgrass: (Agropyron smithii Rybd.)

Chester R. Anderson

LIGNIN, CELLULOSE AND CRUDE FIBER CHANGES IN MATURING WESTERN WHEATGRASS

(*Agropyron smithii* Rybd.)

By

CHESTER R. ANDERSON

A Thesis Submitted to the Graduate Faculty of

South Dakota State College of Agriculture and Mechanic Arts

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

August, 1948

This is to certify that, in accordance with the requirements of South Dakota State College for the Master of Science Degree, Mr. Chester Anderson has presented to this committee three bound copies of an acceptable thesis, done in the major field; and has satisfactorily passed a two-hour oral examination on the thesis, the major field, CHEMISTRY, and the minor field, MATHEMATICS.

Head of Major Department

August 26, 1948
Date

Head of Minor Department

Rep. of Graduate Committee

ACKNOWLEDGEMENT

The author expresses appreciation to the staff of the Experiment Station Chemistry Department for helpful Suggestions and Criticisms.

INTRODUCTION

It can be said from observations that plants become more woody as they mature. A young plant is relatively tender and supple, whereas an older plant is tougher and has greater strength of structure. The nature of this woodiness and its effect upon the digestibility of plants and plant products by animals is of some concern.

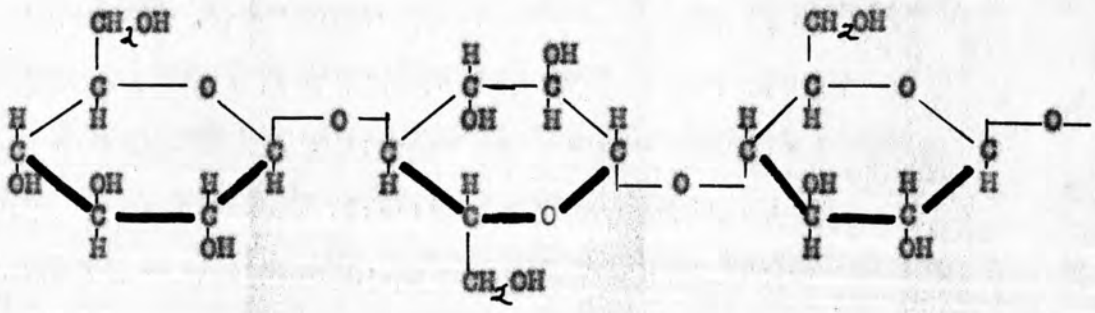
In the analysis of feeds crude fiber is determined by an empirical chemical method which was worked out over seventy-five years ago by Henneberg and Stohmann at the Weende experiment station (1, 2), as quoted by Maynard (3). This method removes the proteins, sugars and starch, and the remaining residue contains most of the cellulose and other complex polysaccharides. It has been known that some of the lignin, which is indigestible by animals, is removed with the proteins, sugars and starch and, thus, is not measured by the crude fiber method.

The recognition of lignin as a separate entity is important because of its dominant influence on the degree of digestibility of feeds (3). Lignin is not only indigestible itself, but it also lowers the digestibility of other feed constituents by encrusting them and making them physically unavailable to the digestive process (4).

Cellulose, which makes up a large portion of the crude fiber

fraction of feeds, is broken down to simpler digestible carbohydrates by the action of enzymes secreted by symbiotic microorganisms of the digestive tract. The degree of utilization of cellulose, thus, depends upon the microorganisms present in the digestive system. These microorganisms are most abundant in the complex digestive tracts of horses and ruminants and are more or less lacking in the simpler digestive systems of other animals (5).

True cellulose is a polymer of glucose units united in a beta linkage as follows:



According to Haworth and his colleagues, as quoted by Fearon, cellulose has a molecular weight of the order of 30,000 and contains 100 to 200 glucose units (5, 6).

Structural cellulose in the plant cell wall includes a considerable amount of pentosans, mostly xylan, which should be considered as an integral part of the natural cellulosic fabric of the tissue (7). Much of the xylan is hydrolized by dilute acid except when it is firmly incorporated into the cellulose bundle as in structural cellulose. Xylose, which is formed on acid hydrolysis, can be absorbed into the blood stream and converted into glycogen by the

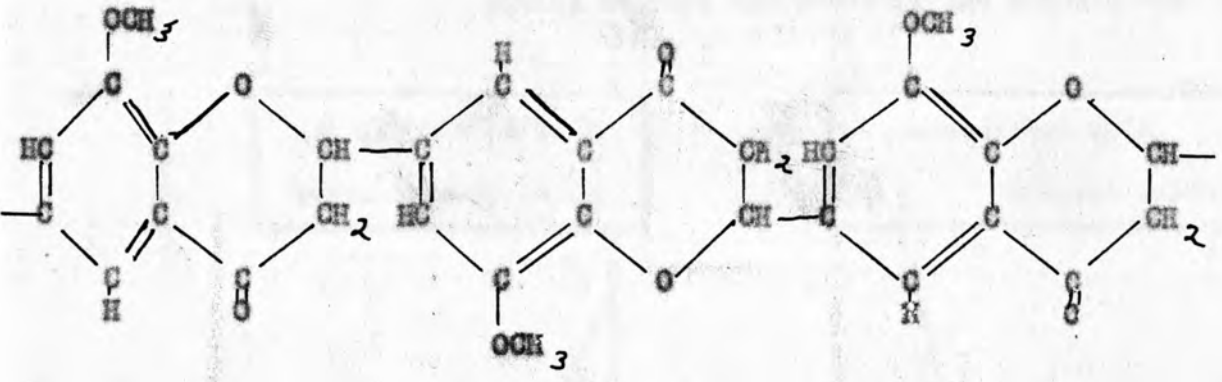
liver. However, xylose is not always fully utilized by the animal, probably because glucose can be used without being first converted into glycogen (8).

Xylan and other pentosans also belong to that group of polysaccharides called hemicelluloses, which are less complex than cellulose and easily hydrolysable into simple sugars. Some uronic acids are present in the hemicellulose group. They are formed by oxidation of the terminal carbinol group of the sugar molecule. Polyuronide formation is not unusual, and there is some indication of the polyuronides being associated with lignin formation. A chart on the differentiation of the hemicelluloses from "The Biochemistry of Cellulose, The Polyuronides, Lignin, etc." by A. G. Norman is shown in Plate 1 (7).

Lignin is in chemical combination with the hemicelluloses and, as the plant tissues age, the lignocellulose physically encrusts the structural cellulose and other cell wall constituents (5). The relation between cellulose and the hemicelluloses is more of a physical nature with the possibility of forces of the nature of Van der Waals being involved (2).

The processes involved in the separation of lignin, cellulose and crude fiber by the methods used here are mainly of a physical nature involving solubilities. Because of the strong reagents ured there are some reaction products formed in the lignin determination. In, fact, lignin has never been known to have been separated in its natural form. It very probably is an heterogeneous substance, but it nevertheless consistently yields propylphenol derivatives upon

high pressure hydrogenation, and there is confirmatory evidence for the essentially aromatic nature of lignin (10, 11, 12, 13). Russell has synthesized a polymeric material with solubility characteristics and general behavior identical with gymnosperm lignin which is a poly-8-methoxydihydrobenzopyrone (14), as follows:

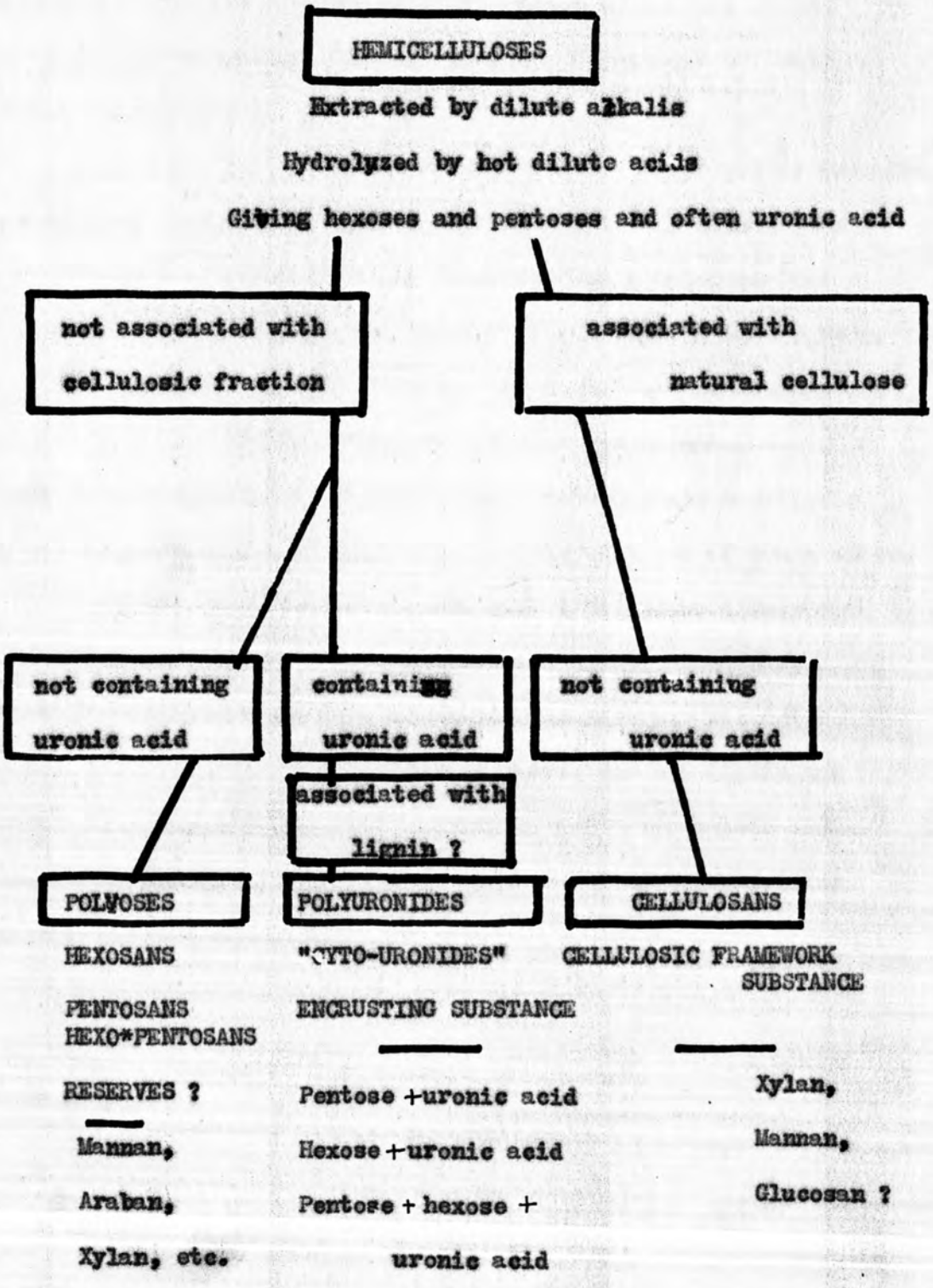


Lignin is easily chlorinated. When wood is chlorinated, and the excess chlorine and hydrochloric acid is washed out with alcohol, and the wood is treated with alcoholic ethanolamine, lignin, which is equal quantitatively to that determined by the sulfuric acid method is removed. The skeletal substance which remains is holocellulose (9).

EXPERIMENTAL

The samples of Western Wheatgrass (Agropyron smithii Rydb.) used in this experiment were collected during the summer of 1945 from a small plot on the south side of the Chicago and Northwestern railroad right-of-way four miles west, one mile north and one-half mile east from Volga, South Dakota. The collections were all made between 10 and 12 o'clock in the morning and all on sunny days,

Plate 1.


HEMICELLULOSES
Extracted by dilute alkalis
Hydrolyzed by hot dilute acids
Giving hexoses and pentoses and often uronic acid
not associated with cellulosic fraction
associated with natural cellulose
not containing uronic acid
containing uronic acid
not containing uronic acid
associated with lignin ?
POLYOSES
POLYURONIDES
CELLULOSANS
HEXOSANS
PENTOSANS
HEXO*PENTOSANS
RESERVES ?
Mannan,
Araban,
Xylan, etc.
"CYTO-URONIDES"
ENCRUSTING SUBSTANCE
Pentose +uronic acid
Hexose +uronic acid
Pentose + hexose + uronic acid
CELLULOSIC FRAMEWORK SUBSTANCE
Xylan,
Mannan,
Glucosan ?

except the day sample number 7 was collected, which was overcast. Moisture, sugar and protein were determined by members of the staff of the Experiment Station Chemistry Department of South Dakota State College.

Lignin and cellulose were determined by the method of Crampton and Maynard as modified by A. R. Patton (15). All data in the analyses in this experiment are calculated on a moisture free basis with a final drying temperature of 103 degrees Centrigrade.

Crude fiber was determined by the method of the Association of Official Agricultural Chemists (16). Large amounts of crude fiber were prepared for fractionation into cellulose and lignin by the same procedure as used for the determination of crude fiber. Also the crude fiber method was run only through the dilute acid extraction part of the determination, and larger amounts were prepared for fractionation into cellulose and lignin. All determinations are presented on a percentage basis, and the lignin and cellulose contents of the acid extraction and the complete crude fiber extraction are converted to their respective proportions of the original material.

Fats (ether extract) and ash were also determined so that the value of the nitrogen free extract (N.F.E.) could be calculated.

Methods

Lignin: Duplicate one gram samples of grass previously dried at 103 degrees C. and ground to 0.4 mm. were weighed out and extracted with ether for 4 hours to remove the fats. The dried residue was

transferred into 50 ml. glass-stoppered Erlenmeyer flasks and 40 ml. of a 2.0 o/o solution of pepsin in 0.1 N HCl were added to digest the protein. The mixture was allowed to stand overnight at 40 degrees C. in a water bath. The undigested residue was recovered by filtration through 200 mesh bolting silk in a Gooch crucible. Successive washings with hot water and hot alcohol to remove the pepsin solution, hot benzene to remove residual fats, and hot alcohol and ether for drying were carried out while the material was still in the bolting silk and Gooch crucible. The washed residue was transferred to a 100 ml. beaker, and the ether was allowed to evaporate. The residue was moistened with 4 ml. of 37 o/o formaldehyde as a hardening agent. The beaker was partly immersed in a cold water bath to prevent the temperature from rising above 70 degrees C. Then 4 ml. of 72 o/o sulfuric acid was added and allowed to penetrate the sample (2 minutes) to start a dissolving process without excessive charring. Then 6 ml. of concentrated sulfuric acid ~~was~~ were added and stirred vigorously with a glass rod to aid in solution, which was complete in from 10 to 15 minutes. When the sample was dissolved, 85 ml. of a granulating reagent consisting of a 1:6 mixture (by volume) of chloroform and glacial acetic acid was stirred in, and the whole was poured into 500 ml. of distilled water in a liter beaker. The chloroform was driven off by boiling gently under the hood for 15 minutes. The solution then cleared and the lignin settled in granular form. A Gooch crucible with medium length asbestos and suction was used for filtering. The lignin was washed in not less than 200 ml. of 5 o/o HCl and dried at 105 degrees C. and the lignin

determined by loss on ignition after heating to 800° C.

Cellulose: Duplicate one gram samples of grass previously dried at 105 degrees C. and ground to 0.5 mm. were weighed out and placed in 6-inch Pyrex centrifuge tubes. Fifteen ml. of 80 o/o acetic acid and 1.5 ml. of concentrated nitric acid were added. Water-filled crucibles with a top diameter of 30 mm. were placed into the necks of the centrifuge tubes to serve as a simplified reflux condenser, and the mixture was boiled for 20 minutes on a sand bath. Three or four perforated glass beads were added to prevent excessive bumping. The centrifuge tubes were cooled with cold water and the crucibles were removed. About 20 ml. of alcohol was added and mixed well with a stirring rod. The tubes were centrifuged until the precipitate settled, and the supernatant liquor was decanted by suction through a glass tubing drawn out to a fine opening. The material was washed in the centrifuge tube successively with alcohol (2 washings), hot benzene to remove fats, hot alcohol and ether for drying. The residue, still in the centrifuge tube, was dryed at room temperature until no noticeable ether odor remained, and placed in the oven at 105 degrees C. for one hour. It was cooled in a desiccator, and weighed. A few drops of alcohol were added, the tube was placed in a muffle furnace and heated to 650° C. It was cooled and weighed again. Loss in weight on ignition was recorded as percent cellulose.

Crude fiber: Duplicate one gram samples of grass previously dried at 105 degrees C. and ground to 0.5 mm., were extracted with ether for 4 hours. The residue was extracted by boiling with 1.25 o/o sulfuric acid for one-half hour. A few drops of amyl alcohol were added to prevent frothing. The acid extracted material was washed from the cloth with hot 1.25 o/o NaOH and boiled under the reflux for one-half hour. The acid and alkali extracted material was then filtered through a Gooch crucible with suction, washed with water, and dried at 105 degrees C. After cooling and weighing the crucibles were put into the furnace and the material ashed. The loss in weight is percent crude fiber.

DATA AND DISCUSSION

The data for the determinations of lignin and cellulose in these nine samples are given in Table I. The percent of lignin varies from slightly over 12 to almost 17 in the four month period during which the samples were taken. The increase is quite steady and is in general agreement with similar experiments on other grasses (15, 17). The lignin appears to approach a value that is approximately half of the amount of cellulose as the season advances, indicating that the rate of increase in lignin is greater than the rate of increase of cellulose.

The percent of cellulose ranges from almost 29 to well over 31, which is not much of an increase compared to the increase in lignin. However, it must be considered that the "structural cellulose" may have made quite an increase and very probably did because it is considered to be from the pentosans in hemicelluloses that lignin is formed (5). The data of Table I is shown on a bar

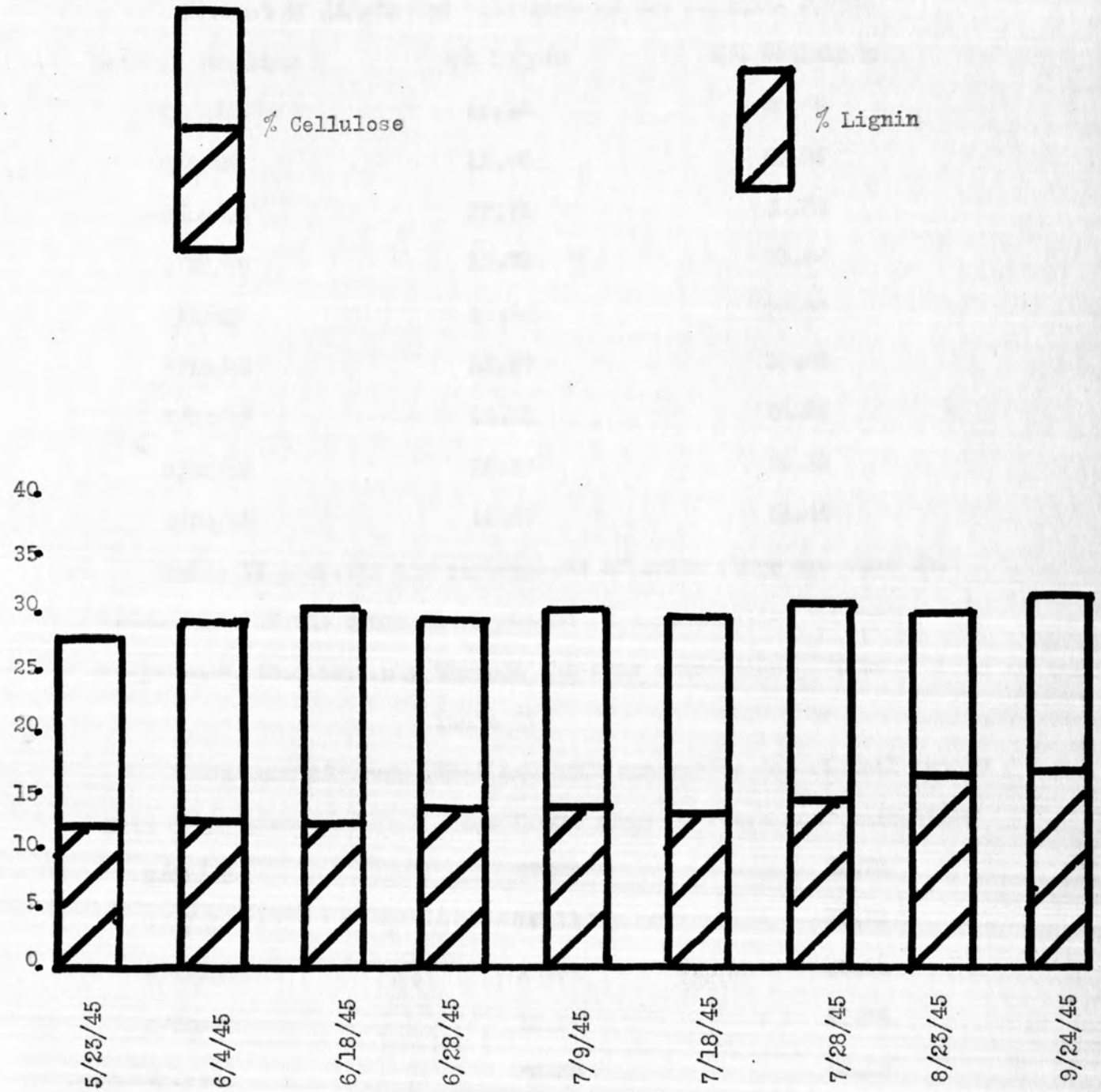


Figure 1.

Percent of Lignin and Cellulose in the Original Sample

chart in Figure 1.

Table I

Percent of Lignin and Cellulose in the original sample

Date of sampling	o/o Lignin	o/o Cellulose
5/23/45	12.14	28.78
6/4/45	12.60	29.62
6/18/45	12.71	30.79
6/28/45	13.76	29.67
7/9/45	13.59	30.60
7/18/45	13.27	29.96
7/28/45	14.21	31.08
8/23/45	16.13	30.16
9/24/45	16.67	31.43

Table II presents the percentages of crude fiber and also the percentages of the crude fiber method of determination run only "half way", that is, only through the acid extraction. It is

Table II

Percent of Crude fiber and acid extraction in original sample

Date of sampling	o/o Crude fiber	o/o acid extraction
5/23/45	27.84	52.74
6/4/45	28.11	51.78
6/18/45	29.51	53.84
6/28/45	32.15	56.04
7/9/45	30.33	55.00
7/18/45	31.25	52.88
7/28/45	30.48	55.18
8/23/45	29.67	54.35
9/24/45	29.49	54.68

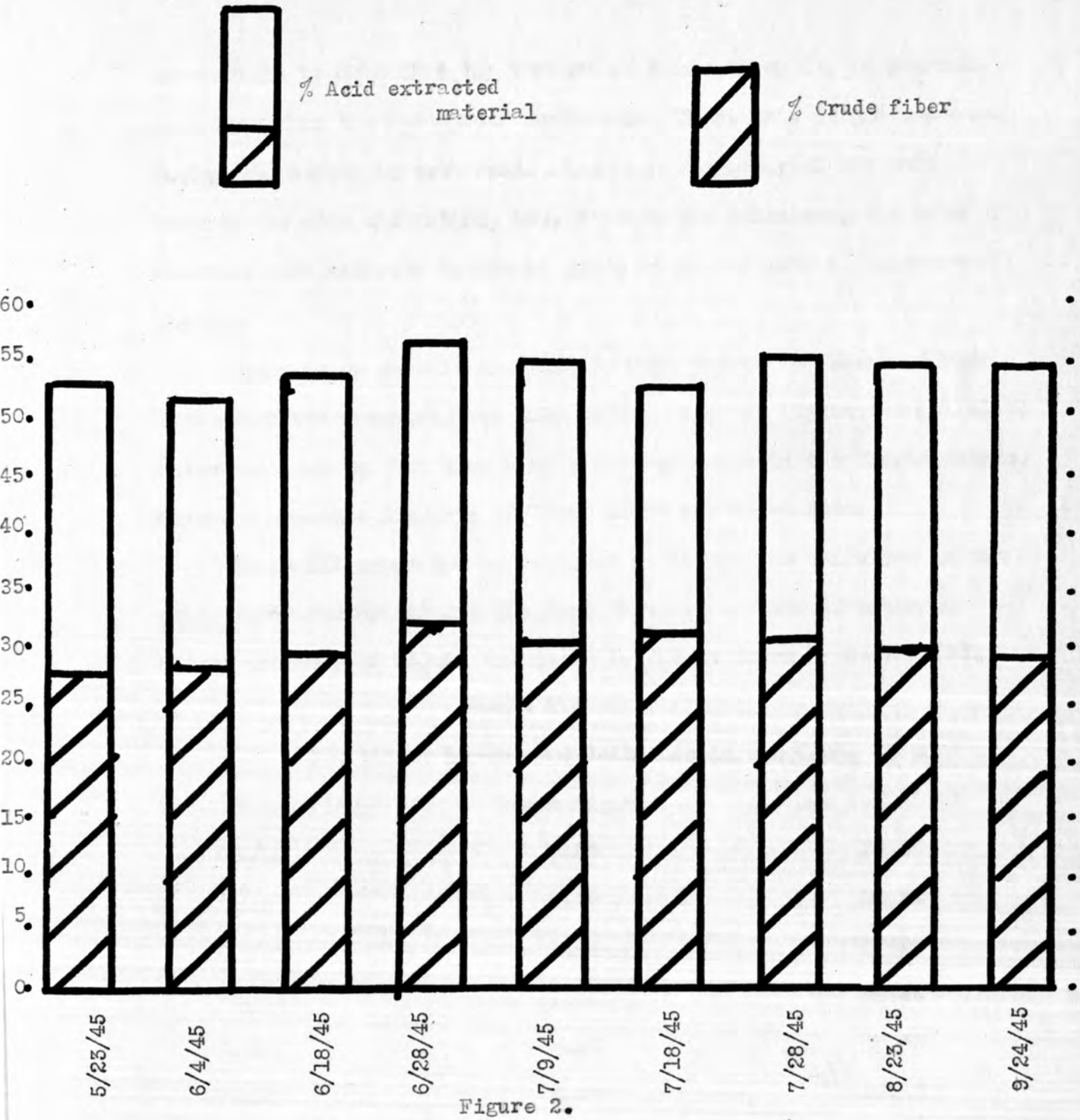


Figure 2.

Percent of Crude Fiber and Acid Extracted Material

interesting to note that the percent of crude fiber is, in general, just less than the percent of cellulose. There is a slight increase during the season in both crude fiber and the material run only through the acid extraction, but, as with the cellulose, the rate of increase with maturity is not as great as is the rate of increase of lignin.

There is an overall increase in both the crude fiber and the acid extracted material, but they differ from the lignin and cellulose determinations in that they have a maximum value in the fourth sample. Figure 2 presents the data of Table II in bar chart form.

Table III shows the percentages of lignin and cellulose in the crude fiber and brings out the fact that crude fiber is composed almost entirely of lignin and cellulose as is shown by Norman (9).

Table III

Percent of Lignin and Cellulose in the Crude Fiber

Date of sampling	o/o Lignin	o/o Cellulose
5/23/45	8.10	86.53
6/4/45	8.24	89.28
6/18/45	7.42	87.45
6/28/45	7.05	84.32
7/9/45	6.38	84.52
7/18/45	6.44	83.13
7/28/45	6.87	85.09
8/23/45	7.75	86.67
9/24/45	7.87	85.60

There is much more cellulose than lignin in the crude fiber (Figure 3).

As the plant matures there is a general decrease in the percentages of cellulose and lignin in the crude fiber even though there is an increase in lignin, cellulose and crude fiber in the original plant material. Such a condition is not unusual because the pentosans may become physically encrusted and not affected to as great an extent by the dilute acid and alkali of the crude fiber method as they are by the strong reagents used in the lignin and cellulose determinations. There is considerable variation in the decrease, the lignin reaching a minimum in sample number five and the cellulose a minimum in the sixth sample.

Table IV

Percent of Lignin and Cellulose in Crude fiber converted to original

Date of sampling	o/o Lignin	o/o Cellulose
5/23/45	2.26	24.03
6/4/45	2.32	25.10
6/18/45	2.19	25.81
6/28/45	2.37	27.09
7/9/45	1.94	25.63
7/18/45	2.01	25.98
7/28/45	2.09	25.94
8/23/45	2.31	25.89
9/24/45	2.32	25.07

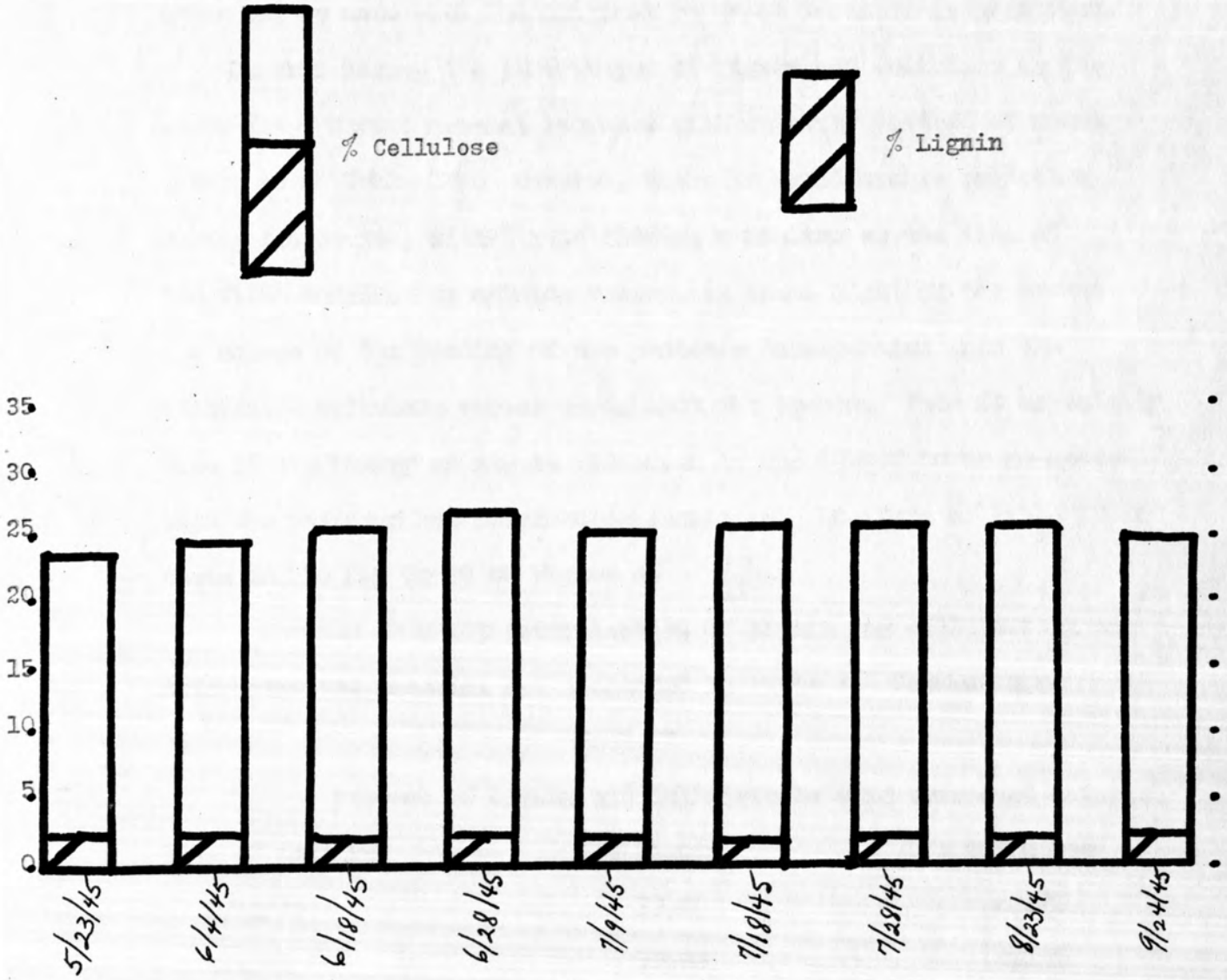


Figure 4.

Percent of Lignin and Cellulose in Crude Fiber Converted to Original

In order to bring the percentages of lignin and cellulose in the crude fiber to the basis of the original material so that comparisons can be made with the original material Table IV is presented.

On this basis, the percentages of lignin and cellulose in the crude fiber show a general increase with maturity instead of a decrease as in Table III. However, there is considerable variation during the season, with lignin showing a minimum at the time of the fifth sample. An opinion concerning these might be the amount and nature of the bonding of the pentoses incorporated into the structural cellulose varies throughout the season. This is especially true if the theory of lignin formation is considered to be connected with the pentosan and polyuronide formation. The Data of Table IV is shown in the Bar Chart of Figure 4.

Results from the determination of lignin and cellulose in the acid extracted material are presented in Table V. Whereas the

Table V

Percent of Lignin and Cellulose in acid extracted material

Date of sampling	o/o Lignin	o/o Cellulose
5/25/45	19.68	46.73
6/4/45	19.95	46.91
6/18/45	19.12	48.71
6/28/45	19.96	51.59
7/9/45	20.62	52.56
7/18/45	19.49	50.85
7/28/45	20.56	51.59
8/23/45	21.81	49.46
9/24/45	20.27	52.04

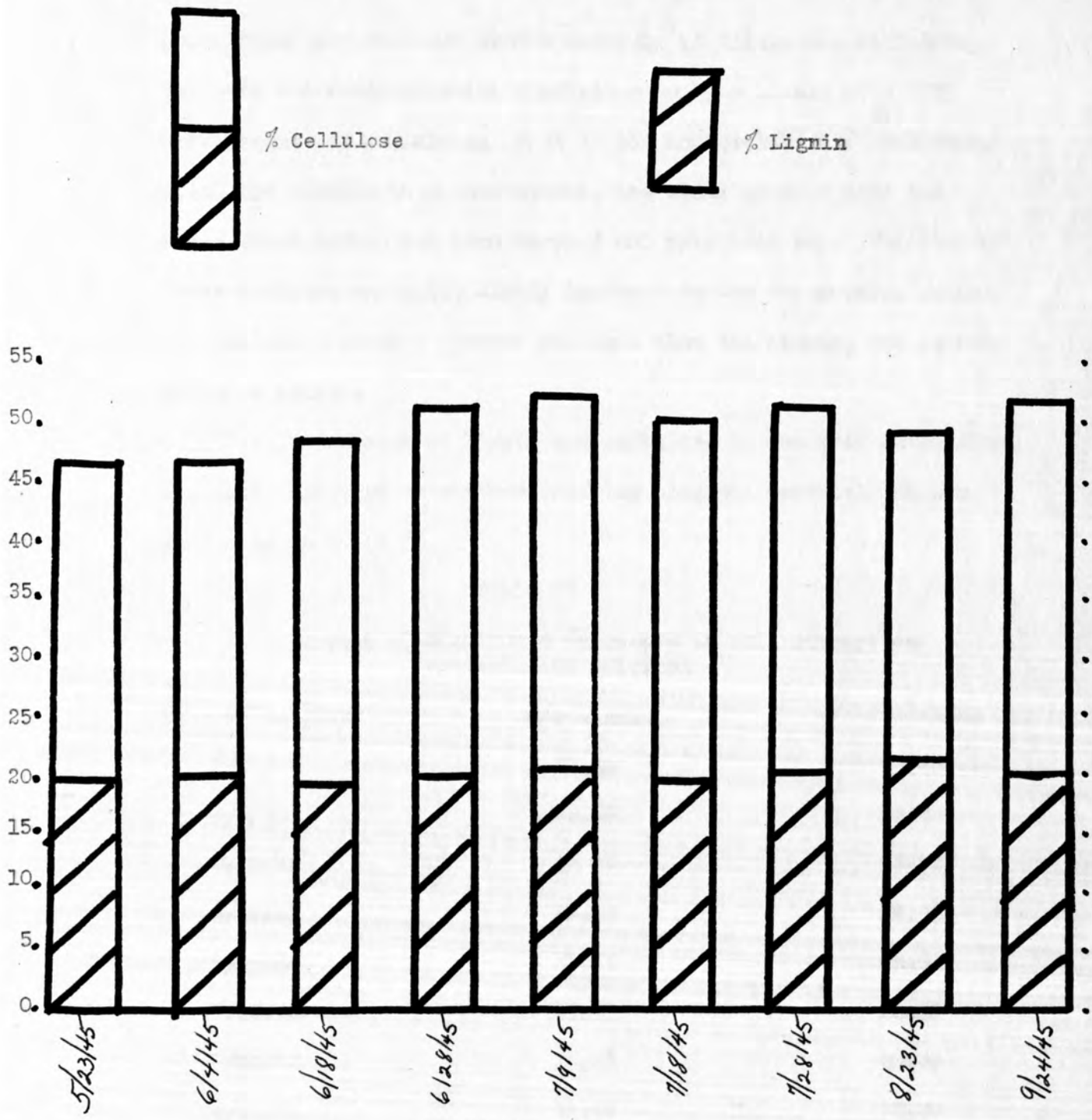


Figure 5.

Per cent of Lignin and Cellulose in Acid Extracted Material

crude fiber was composed almost entirely of lignin and cellulose, the acid extracted material consists of only a little more than half lignin and cellulose. Most of the hemicelluloses and pentosans, which are soluble in dilute alkali, are still present when the crude fiber method has been carried out only half way. The lignin shows a slight but quite steady increase during the growing season. The cellulose shows a greater increase than the lignin, but is not quite as steady.

The percentages of lignin and cellulose in the acid extraction are also converted to the basis of the original material and are presented in Table VI.

Table VI

Percent of Lignin and Cellulose in acid extraction converted to original

Date of sampling	o/o Lignin	o/o Cellulose
5/25/45	10.38	24.65
6/4/45	10.33	24.29
6/18/45	10.29	26.23
6/28/45	11.19	28.91
7/9/45	11.34	26.91
7/18/45	10.31	26.63
7/28/45	11.35	28.47
8/23/45	11.85	26.94
9/24/45	11.06	28.46

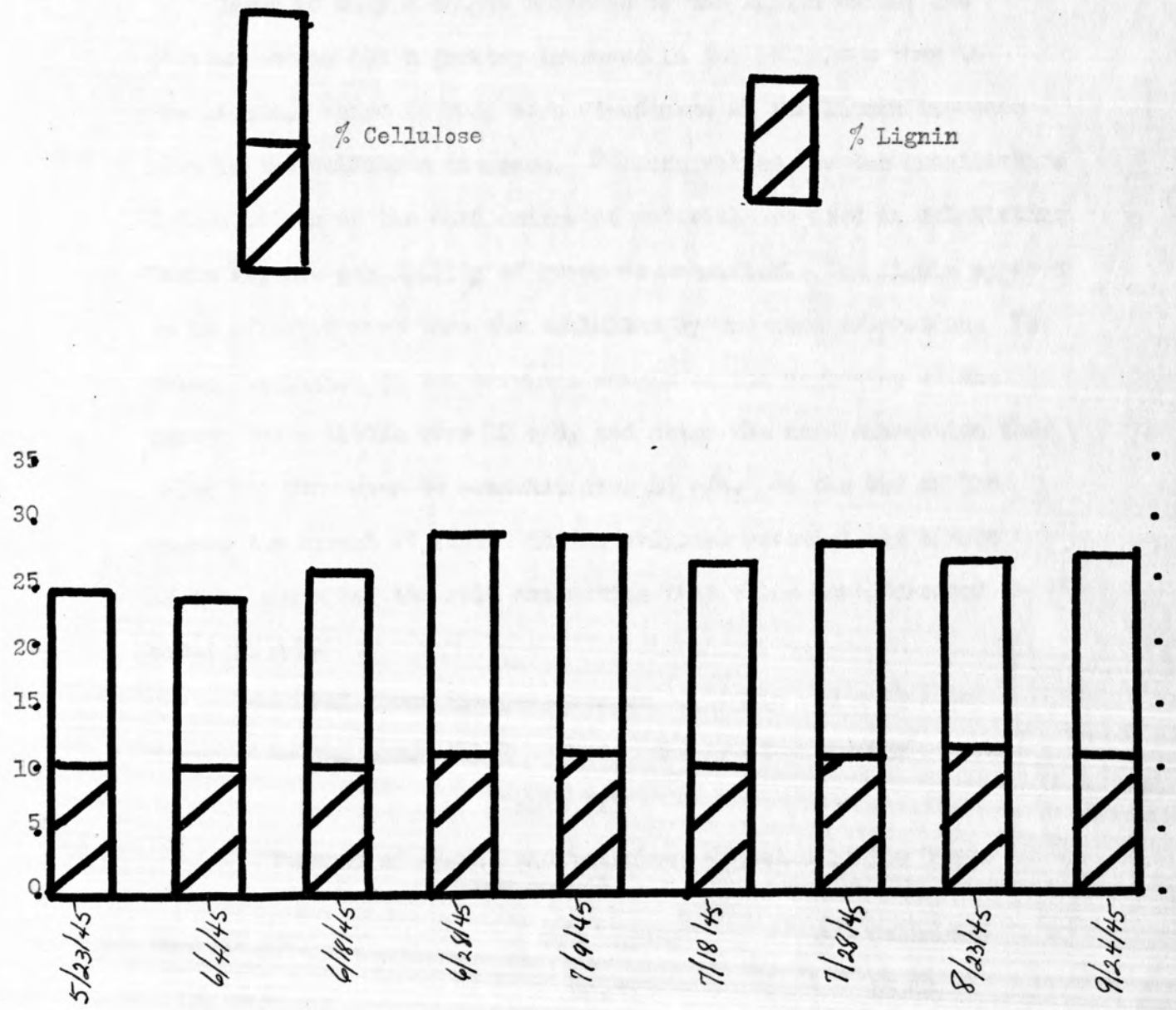


Figure 6.

Percent of Lignin and Cellulose in Acid Extracted Material Converted to Original

There is only a slight increase in the lignin during the growing season and a greater increase in the cellulose than in the lignin. There is much more steadiness in the lignin increase than in the cellulose increase. Because values for the quantitative determination of the acid extracted material are used in calculating Table VI, the possibility of error is magnified. The lignin appears to be affected more than the cellulose by the acid extraction. The value for lignin in the original sample at the beginning of the season was a little over 12 o/o, and after the acid extraction that value has gone down to somewhat over 10 o/o. At the end of the season the amount of lignin in the original material was almost 17 o/o, and after the acid extraction that value has decreased to about 11 o/o.

Table VII shows the percentages of lignin and cellulose digested by the crude fiber method. The greater portion of the

Table VII

Percent of Lignin and Cellulose digested by the Crude fiber method

Date of sampling	o/o Lignin	o/o Cellulose
5/23/45	81.38	16.50
6/4/45	81.59	15.26
6/18/45	82.77	16.17
6/28/45	83.50	8.70
7/9/45	85.72	16.24
7/18/45	84.85	13.28
7/28/45	85.29	16.54
8/23/45	85.68	14.16
9/24/45	86.25	20.24

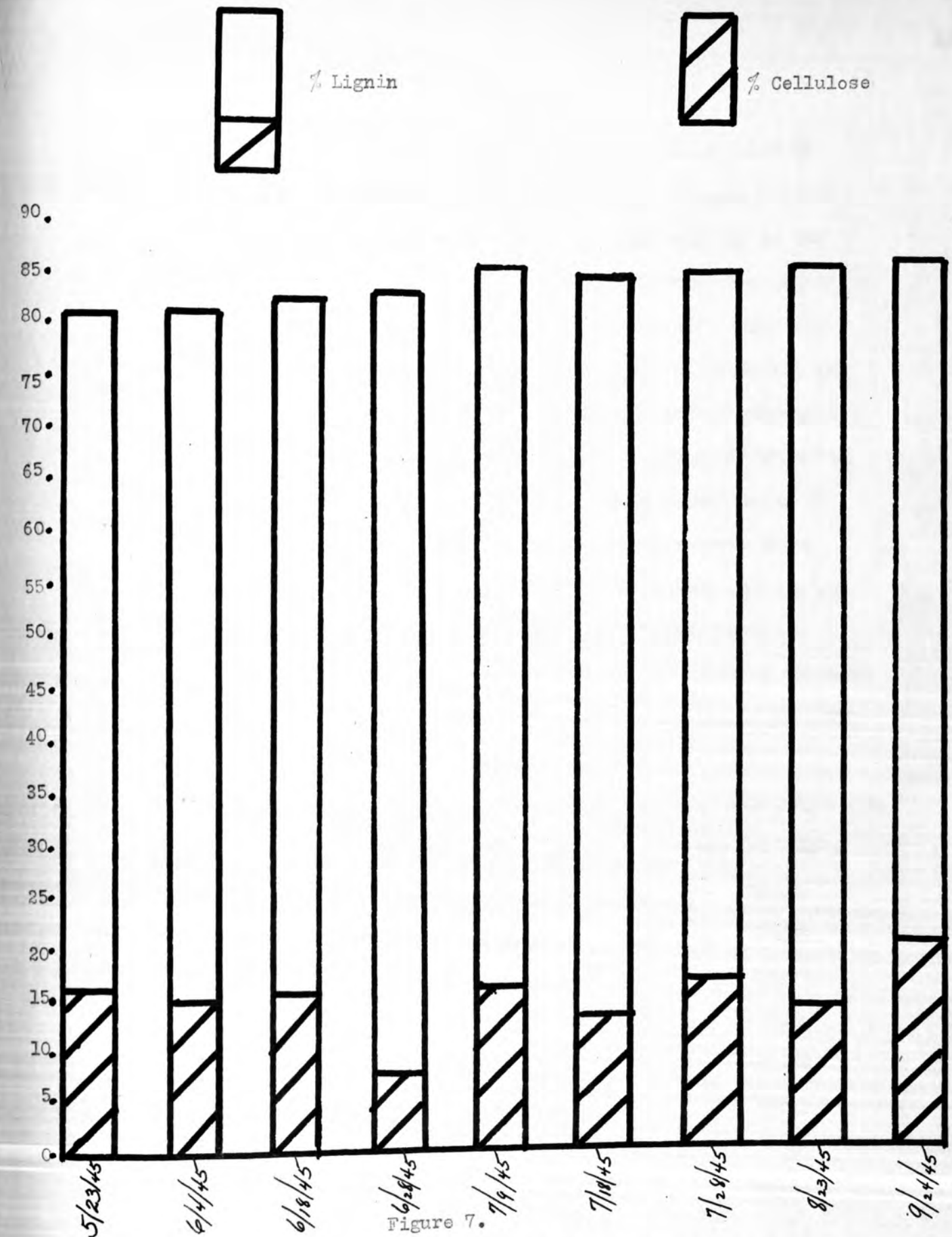


Figure 7.

Percent of Lignin and Cellulose Digested by the Crude Fiber Method

lignin is removed by this method because of the solubility of lignin in alkali. The cellulose is not removed to such a great extent by the crude fiber method, but the dependability on the amount that might be removed is not as great as with the lignin.

There is a definite increase in the amount of lignin removed as the plant matures, that is, as the season advances, the lignin becomes more soluble in dilute alkali. Such a condition would indicate that the crude fiber method is not applicable for the determination of lignin as an indigestible constituent, at least not directly. Of course, solubility by the crude fiber method would not indicate digestibility by an animal anyhow, because digestibility in the animal must include absorption.

The percentages of lignin and cellulose digested by the acid extraction are given in Table VIII. There is not nearly as much

Table VIII

Percent of Lignin and Cellulose digested by the acid extraction

Date of sampling	o/o Lignin	o/o Cellulose
5/23/45	14.50	14.35
6/4/45	18.02	17.99
6/18/45	19.04	14.81
6/28/45	18.68	2.56
7/9/45	15.82	5.52
7/18/45	22.31	11.11
7/28/45	20.13	8.40
8/23/45	26.53	10.68
9/24/45	34.32	9.45

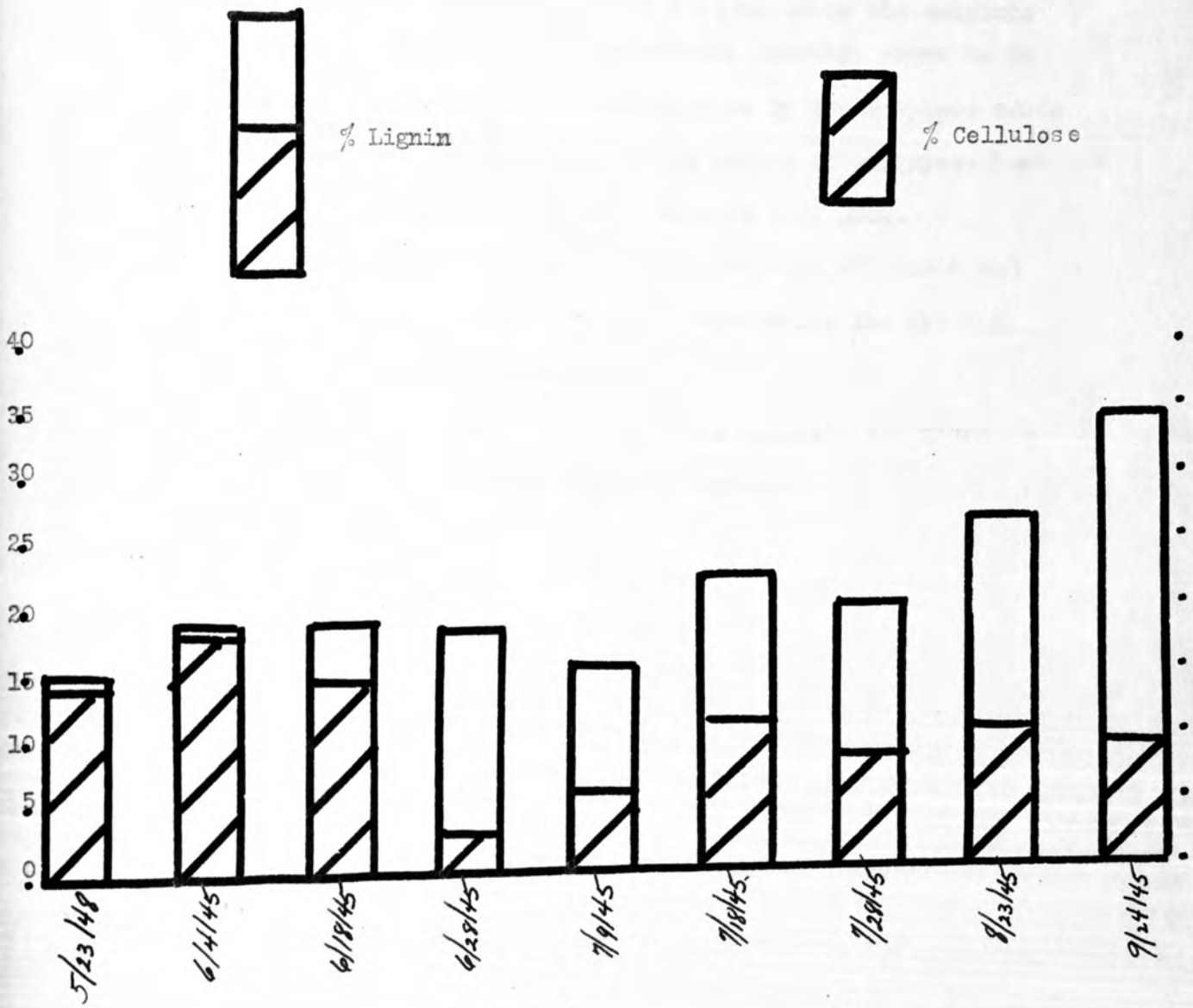


Figure 8.

Perc ent of Lignin and Cellulose Digested by the Acid Extraction

lignin removed by the acid extraction alone as by the complete crude fiber determination. The cellulose, however, seems to be affected as much by the acid extraction as by the complete crude fiber method. The dependability of the amount of cellulose that might be removed by the acid extraction is very poor.

Figure 8 shows the changes in the percents of lignin and cellulose digested by the acid extraction during the growing season of the Western Wheat grass.

Data on sugar, fat, ash and protein analyses are given in Table IX along with the nitrogen free extract.

CONCLUSION

From the increase in lignin with maturity it seems that a determination of the lignin content of plant tissue is a good indication as to the woodiness of the material. Also, if the woodiness or lignification of the tissue is to be a measure of digestibility, alkaline extraction should be avoided in a direct determination of the fiber content, unless lignin is determined.

SUMMARY

Nine samples of Western Wheat grass were analyzed for lignin, cellulose, crude fiber, and the acid extraction of the crude fiber method. Lignin and cellulose were then determined on the crude fiber and the acid extracted material, and the values converted to the original so that the percentages of lignin and cellulose removed by the crude fiber and the acid extraction methods could be compared. The acid extraction removed some lignin and cellulose, and the crude fiber method removed most of the lignin but very little more cellulose than the acid extraction.

Lignin, cellulose and crude fiber increased with maturity. The amount of cellulose removed by the crude fiber method or the acid extraction alone showed variation throughout the season and only showed a general trend toward a decrease in the amount removed by the acid extraction as the season advanced.

Table IX

Date of Sampling	5/23/45	6/4/45	6/18/45	6/28/45	7/9/45	7/18/45	7/28/45	8/23/45	9/24/45
Percent Protein	14.51	13.27	13.98	11.56	8.59	9.87	8.65	8.82	6.55
Percent Fat	2.31	2.75	3.07	5.53	2.57	3.49	3.95	3.89	4.64
Percent ash	7.58	6.59	7.14	7.14	7.16	7.54	7.40	7.49	7.71
Percent Crude fiber	27.84	28.11	29.51	32.13	30.33	31.25	30.48	29.87	29.49
Total	52.84	50.52	53.70	54.36	49.65	52.15	50.48	50.07	48.39
N. F. E. %	47.16	49.48	46.30	45.64	50.35	47.85	49.52	49.93	51.61
Sugar MG/GR.	35.33	35.50	25.74	34.94	35.82	46.35	42.50	42.05	40.65

BIBLIOGRAPHY

1. Hanneberg, Wilhelm and Friedrich Stohmann. Beitrage zur Begrundung einer rationellen Futterung der Wiederkauer. Vol. I., Schwetschke u. Sohn, Brunswick, 1860.

2. Hanneberg, Wilhelm and Friedrich Stohmann. Beitrage zur Begrundung einer rationellen Futterung der Wiederkauer. Vol. II., Schwetschke u. Sohn, Brunswick, 1865.

3. Maynard, L. A., Animal Nutrition, 2nd Edition McGraw-Hill Book Co. Inc., New York, 1947.

4. Furstenberg, A., R. Murdfield and J. Konig, Chemical Abstracts 1, 458 (1907).

5. Fearon, W. R., An Introduction to Biochemistry, 3rd Edition Grune and Stratton, Inc., New York, 1947.

6. Haworth, W. N., Structure, Function and Synthesis of Polysaccharides, Bakerian Lectures, Roy. Soc., London (1944).

7. Norman, A. G., The Biochemistry of Cellulose, The Polyuronides, Lignin, etc., Oxford University Press, London, 1937.

8. Peters, J. P., and D. D. Van Slyke, Quantitative Clinical Chemistry, Interpretations Vol. I, 2nd Edition The Williams and Wilkins Co., Baltimore, 1946.

9. Wise, L. E., Wood Chemistry, A. C. S. Monograph No. 97, Reinhold Publishing Corp., New York, 1944.

10. Brewer, Charles P., Lloyd M. Cooke and Harold Hibbert, Studies on Lignin and Related Compounds. LXXXIV. The High Pressure Hydrogenation of Maple Wood Hydrol Lignin., J. Am. Chem. Soc. 70, 57-59 (1948).

11. Baker, Samuel B., Taylor H. Evans and Harold Hibbert, Studies on Lignin and Related Compounds. LXXXV. Synthesis and Properties of Dimers Related to Lignin., J. Am. Chem. Soc. 70, 60-63 (1948).

12. Baker, Samuel and Harold Hibbert, Studies on Lignin and Related Compounds. LXXXVI. Hydrogenation of Dimers Related to Lignin., J. Am. Chem. Soc. 70, 63-67 (1948).

13. Pepper, James M. and Harold Hibbert, Studies on Lignin and Related Compounds. LXXXVII. High Pressure Hydrogenation of Maple Wood., J. Am. Chem. Soc. 70, 67-71 (1948).

14. Russell, Alfred, Interpretation of Lignin. I. The Synthesis of Gymnosperm Lignin., J. Am. Chem. Soc., 70, 1060-1067 (1948).

15. Patton, A. R., Seasonal Changes in the Lignin and Cellulose Content of Some Montana Grasses, J. Animal Science, 2, 59-69 (1943).

16. Association of Official Agricultural Chemists, Methods of Analysis, 5th Edition Association of Official Agricultural Chemists, Washington, D. C., 1940.

17. Kuppers, J. H., L. L. Husoff and D. M. Seath, Seasonal Variations of Carotene and Other Nutritionally Important Constituents in the Two Pasture Grasses Dallis and Carpet, J. Agricultural Research 77, 55 (1948).