SEASONAL VARIATIONS IN THE CARBOHYDRATE COMPOSITION OF TWO SPECIES OF GRAMA GRASSES

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OF TWO SPECIES OF GRAMA GRASSES

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INTRODUCTION

Previous studies have shown that the well known proximate analysis does not give adequate information on the feeding value of range grasses for beef cattle. Indications have been presented showing that the two principle carbohydrate fractions, crude fiber and nitrogen-free extract, may account for most of the variation in the quality of various grass species. This report deals chiefly with the components of the nitrogen-free extract fraction and attempts to identify a component that will give a better measure of the nutritive value of plants than those methods now in use.

CHAPTER II

LITERATURE SURVEY

For many years hays and grasses have been analyzed for their organic constituents by a method which has been for convenience, termed proximate analysis. Proximate analysis includes the determination of protein, crude fiber, ether extract (fat), ash, and nitrogen-free extract. Until recent years this analysis was the major procedure used on hays and grasses to determine their nutritive value.

The portion of proximate analysis called nitrogen-free extract is expressed as 100 percent minus (percent crude fiber plus percent ether extract plus percent ash plus percent protein). This figure is generally considered to be a measure of the digestible carbohydrate content of grasses and includes starches, sugars, hemicelluloses, pentosans, etc. (33).

Savage and Heller (45) made a complete proximate analysis study on various species of western forage grasses. They found that during the spring months, protein, fat, and ash were at a maximum while crude fiber and nitrogen-free extract were at a minimum.

In recent years considerable research has been conducted leading to a fractionation of the various carbohydrate components of the nitrogen-free extract. This type of work has been done in an attempt to learn exactly how valuable this carbohydrate fraction is as a feeding material. Similar characteristics of the component carbohydrates makes

it difficult to obtain a concise definition of this material.

The carbohydrate components or the products of hydrolysis of the nitrogen-free extracts are usually classified under two headings, hexosans and pentosans (40). Cellulose (9) and fructosans (1), (26), (27), are two types of hexose polymers which have been reported. Starch has been reported to be present in only very small amounts (11), (12), (19), (39). With the exception of cellulose, pentosans represent the major portion of the carbohydrate fraction of western grasses. Arabans, xylans, and mannans have been shown to be present in variable quantities (6), (8), (38).

Cellulose, the chief constituent of the carbohydrate fraction, is a glucose polymer. Two carbohydrate fractions which are closely associated with cellulose are lignin and hemicellulose (47). These are encrusted around the glucose polymer.

Lignin is generally characterized by its resistance to all but the strongest chemical reagents. It is of uncertain chemical structure but is usually considered to be a polymer made up chiefly of phenylpropane units. Lignin and the pectins contain the major part of the methoxyl content of a plant (49). Lignin is always closely associated with the cellulose and hemicelluloses of the plant and may be, in part, combined chemically with the carbohydrates of plant cells (21), (41).

Another carbohydrate constituent usually associated with cellulose is a group of pentosans termed hemicelluloses. Isolation and identification of the hemicelluloses from certain western range grasses was the major problem of this study.

The term hemicellulose was first originated by Schulze (46) who isolated carbohydrate fractions from a number of plant materials by

extraction with dilute alkali and subsequent precipitation by acidification. These substances were much more susceptible to dilute acid hydrolysis than cellulose, but were believed to be related to cellulose, possibly as intermediates in its formation. The hemicelluloses of Schulze were, until recently, believed to be true hexosans or pentosans, or more commonly, hexopentosans. It was later shown that these alkaline extracts contained uronic acid groups. Phillips (39) defined two groups of hemicelluloses, cellulosans and polyuronides, cellulosans being a group composed of pentosans and hexosans and polyuronides being products of condensation of certain uronic acids with sugars.

In the conventional proximate analysis, the larger part of the hemicellulose fraction is included in the nitrogen-free extract along with pectins and some lignin. These three substances are closely associated and this association has led to several theories dealing with the formation of hemicelluloses. Bennett (5) proposed that polyuronide hemicelluloses might be derived from pectin, a large portion of which is composed of anhydro-galacturonic acid units. He also made a second proposal: that hemicellulose may be converted to lignin and/or all three substances are interrelated, lignin being formed from pectin through the intermediate stage of polyuronide hemicellulose. His experimental results did not substantiate his theories. No direct evidence could be found linking lignin, pectin, and hemicellulose formation. Norman (34) demonstrated some evidence of a relationship between hemicellulose and lignin in attempting to methylate lignin in the natural state. The methoxyl content of lignin could be increased only after a preliminary hydrolysis. He concluded that the active hydroxyl groups were not free until after hydrolysis and that lignin must, therefore, be attached to some of the

other carbohydrates present, possibly the hemicelluloses. The close association between lignin, pectin, and the hemicelluloses presents many problems in the determination of hemicelluloses.

The first isolation of hemicelluloses was carried out by direct alkaline extraction of the untreated plant material. Schulze (46) used this method when he first isolated the hemicelluloses. O'Dwyer (36) used this process for the isolation of hemicelluloses from wood. These experiments as well as others (2), (5), (35) proved very ineffective in that high concentrations of protein, pectin, and lignin were extracted with the hemicellulose fraction. Various methods (7), (10), (42) have been proposed to remove these substances prior to the alkaline extraction of hemicelluloses. If all of the components except cellulose and hemicellulose are removed from a sample, the preparation is termed holocellulose.

Holocellulose preparations became the major starting material for hemicellulose isolation when Jayme (24) discovered that lignin could be easily removed by treating the sample with sodium chlorite. This discovery led Wise (50) to the development of the acid-chlorite procedure which is widely used today. He found that lignin could be removed almost quantitatively without concomitant loss of hemicellulose.

Information regarding the hemicellulose of forage crops is very meager. Although hemicellulose has sometimes been included in studies of the nutritive value of forages, it was usually determined only as total pentosans (23). Early attempts were made to extract hemicellulose from grass tops without prior delignification (10). Xylose, glucose, arabinose, galactose, and uronic acids were found in these hemicellulose preparations but no quantitative analyses were made. Holocellu-

lose has been prepared from sweet vernal grass by delignification with chlorine gas (4). The hemicellulose extracted from this holocellulose yielded xylose and smaller amounts of arabinose and uronic acids.

Two reports have appeared dealing with change in the hemicellulose content of grasses as they matured, but neither was based on hemicelluloses prepared from chlorite holocellulose. Bennett (3) found that the percentages of hemicellulose in Kentucky bluegrass and also in red clover increased as the season advanced. Richards and Reid (43) found increasing amounts of hemicellulose in the vegetative, boot, and fullbloom stages of timothy hay.

Ely et al. (8) conducted an extensive study on the composition of orchard grass. They found that 60 to 95 percent of the hemicellulose was composed of xylose and glucose while arabinose and galactose comprised from 2 to 18 percent. Later, Routley (44) studied the hemicelluloses of various portions of brome grasses. He reported xylose, arabinose, galactose, glucose, galacturonic acid, glucuronic acid and possibly some mannuronic acid present in variable quantities.

CHAPTER III

EXPERIMENTAL PROCEDURE

Sampling

Collection of Samples. The samples of Bouteloua curtipendula (sideoats grama) and Bouteloua gracilis (blue grama) were taken from the Palatability of the Southern Great Plains Field Station Experimental Range. These samples were collected at various stages of their growth and were oven-dried to preserve them. The samples were then ground through the fine screen of the Wiley cutting mill, oven-dried at 105°C overnight, and stored in sealed containers.

Chemical Analyses

Proximate Analysis. Proximate analysis was run essentially as described in the A. O. A. C. methods (29). Ash was determined by heating the samples at 600°C until a white ash remained. Protein was determined by the Gunning modification of the Kjeldahl procedure. Ether extract was determined in a Goldfisch extractor using anhydrous ether. Crude fiber was run on the extracted residues essentially as directed. Nitrogen-free extract (NFE) was calculated by subtracting from 100 the sum of the percentages secured from the previous determinations.

Carbohydrates. The grass samples were extracted with 80 percent ethanol, and carbodydrate analyses were carried out on both the extract and the

residue. The ethanol was evaporated from aliquots of the extract on a steam bath. The resultant aqueous solution was clarified with lead acetate and the excess lead removed using potassium oxalate. Total sugars were determined on the clarified solutions after inversion with hydrochloric acid. The percent sucrose was calculated as: (percent total sugar minus percent reducing sugar) times 0.95.

The following analyses were made on the extracted residues after they had been ground in a ball mill. Direct acid hydrolysis, which was run according to A. O. A. C. method (30); starch, determined essentially as directed in the A. O. A. C. method substituting Takadiastase for malt extract (31); mild acid hydrolysis, conducted essentially as directed in the method of Phillips and Smith (40); and fructosans, determined colorimetrically using a slightly modified procedure of McRary and Slattery (28).

Lignin was determined using a combination method derived from the procedures of Phillips and Smith (40), Thacker (48), and the A. O. A. C. (32). This method is essentially the procedure of Thacker with minor modifications from these other reports.

Cellulose was determined by the method developed by Patton (37). The sample was digested in an acetic acid (80 percent)-nitric acid mixture (15:1.5 v:v) for 20 minutes. Twenty ml. of 95 percent ethanol was added and the precipitate removed by centrifuging. The precipitate was then washed successively with 95 percent ethanol (twice), hot benzene, hot 95 percent ethanol, and ethyl ether. The difference between the dry weight and the weight after ashing was recorded as the weight of cellulose.

Holocellulose, from which hemicellulose was later extracted, was

prepared using a modification of the method of Wise et al. (50). A 100-gram sample of grass was extracted for approximately 15 hours with ethanol-benzene (1:2.5 v:v) in a large Soxhlet extractor. The sample, after drying, was divided into 4 equal parts to facilitate ease in handling and each part placed in a 500-ml. Erlenmeyer flask. Each portion of the extracted sample was refluxed at 85°C for 4 hours with 250 ml. of 0.5 percent ammonium oxalate. Each portion was then filtered and washed thoroughly with hot water. The above extraction was repeated for approximately 15 hours. The 4 residues were then combined in a 2-liter beaker and 625 ml. of water added. The temperature was raised to 85°C over an open flame. The beaker and contents were immediately removed from the flame and placed in a water bath held at this temperature. Two ml. of acetic acid and 7.5 grams of $NaClO_2$ (sodium chlorite)* were added to the sample at 15-minute intervals for one hour (four additions). The sample was stirred constantly with an electric stirrer in order to facilitate the escape of ClO_2 and to prevent frothing. After one hour the reaction mixture was cooled in an ice bath to 10°C and filtered. The residue was washed with ice water (6 times) and air-dried. This residue is the fraction that is commonly termed holocellulose.

Hemicellulose was extracted from the holocellulose fraction with two concentrations of KOH. A 2.5-gram sample of holocellulose was weighed into a 30 x 200-mm test tube. The tube was filled with KOH solution (4 percent) and stoppered with a rubber stopper. The tube was filled as full as possible to eliminate any air which might oxidize the sample. After sealing the stopper in place with paraffin, the tube was placed

*The acetic acid must be added first or an explosion may occur.

for 2 hours in a constant-temperature bath held at 20°C and the contents stirred every 10 minutes by revolving the tube. The contents of the tube were then filtered through a tared, sintered-glass funnel. The tube and stopper were washed with 50 ml. of 4 percent KOH and 150 ml. of distilled water. The filtrate and washings were combined in a suction flask containing 10 ml. of glacial acetic acid.

The residue was transferred back to the extraction tube and the tube was filled with 24 percent KOH. The extraction procedure was conducted as before. The contents were filtered through the tared funnel a second time and the stopper and extraction tube washed successively with a 25-ml. portion of 24 percent KOH and 250 ml. of water. The filtrate and washings were run into a suction flask containing 45 ml. of glacial acetic acid.

The residue on the funnel was washed with ice water (5 times) and acetone (5 times) and allowed to reach air dryness. It was then weighed and the weight recorded as alpha-cellulose.

The filtrates from the two extractions were placed separately in 2-liter flasks and each suction flask rinsed with 25 ml. of water. The 2-liter flasks were then filled with 95 percent ethanol and allowed to stand overnight. The flasks were shaken intermittently for two hours to facilitate settling of the precipitate. After standing for 24 hours the supernatants were siphoned off as far as possible. The precipitates were transferred to centrifuge tubes and the final precipitates were washed as follows: 95 percent ethanol (4 times), acetone (3 times), and ethyl ether (2 times). The precipitates were then air-dried, weighed, and the weights recorded respectively as hemicellulose soluble in 4 percent KOH and hemicellulose soluble in 24 percent KOH.

Chromatographic Analysis. The hemicelluloses from the 4 and 24 percent KOH extractions were combined for chromatographic analysis since time precluded the examination of each separately.

Hydrolysis of the hemicellulose fraction was carried out on a 400-mg. portion of the sample. This portion of the sample was weighed and placed in a 30 x 200-mm test tube. Ten ml. of 3 percent HNO_3 was added and the sample thoroughly mixed by swirling the test tube. A Tuttle flask cover was placed over the mouth of the tube to condense the vapors and the tube was then placed in a water bath. The temperature of the bath was increased until the boiling point of water was reached and the hydrolysis allowed to proceed for 4 hours. The hydrolyzate was then cooled to room temperature before spotting on a chromatographic sheet.

A 30 x 30 cm. sheet of Whatman No. 1 filter paper was used in the chromatographic run. A base line was drawn 8 cm. from one end and the known sugars and acids (fructose, glucose, rhamnose, galactose, arabinose, xylose, galacturonic acid, and glucuronic acid) plus the hemicellulose hydrolyzate were spotted along this line 3.8 cm. apart. The hemicellulose hydrolyzate spot was neutralized with NH_4OH vapors before developing the chromatogram. The paper was then placed in an air-tight chromatographic cabinet containing a quantity of the non-aqueous phase of the solvent of Jermyn and Isherwood (ethyl acetate:acetic acid:water 3:1:3) (25). After 24 hours equilibration more of the non-aqueous phase of the solvent was introduced into the chromatographic trough which held the paper. The solvent was allowed to drip from the bottom of the paper; a total developing time of 24 hours was allowed.

At the end of the run the paper was removed and dried in a current of air at room temperature. The spots were then developed using the

2-aminobiphenyl hydrogen oxalate developer of Gordon, Thornburg, and Werum (20). This developing reagent causes hexosans to appear greenish-brown, pentosans red, and uronic acids purple.

TABLE I

DESCRIPTION OF SAMPLES

Date	Stage of Growth	Average Height Seed Stalk	Average Height Leaves	Moisture %
	Bouteloua curtipendula (Sideoats Grama)			
5-15-1957	Early spring growth	3-4		59.92
6-20-1957	Heading	6-10		61.37
9-17-1957	Seed ripe and shattering	24	8	42.09
10-28-1957	Seed shattered	24	8	39.69
1-16-1958	Dormant	12	3	12.53
4-1-1958	Dormant	12	3	7.23
	Bouteloua gracilis (Blue Grama)			
5-15-1957	Early spring growth	3		67.82
6-20-1957	Headed and in bloom	10-12		60.15
9-17-1957	Leaves green; stalks dormant	18	7	40.55
10-28-1957	Some green leaves at base of plant; stalks dormant	18	7	33.02
1-16-1958	Mostly dormant; a few green leaves at base	10	3	16.02
4-1-1958	Mostly dormant; a few green leaves	10	3	10.34

TABLE II

PROXIMATE ANALYSIS
AS AFFECTED BY TIME OF HARVEST

(Dry Weight Percentage)

Bouteloua curtipendula (Sideoats Grama)

Date	Ash %	Protein %	Crude fiber %	Ether extract %	NFE %
5-15-1957	11.07	12.44	24.29	2.48	49.72
6-20-1957	10.27	8.63	29.68	2.03	49.29
9-17-1957	10.45	4.44	33.93	2.23	48.95
10-28-1957	10.01	5.69	34.57	2.09	47.64
1-16-1958	9.40	4.44	31.14	2.46	52.56
4-1-1958	8.64	4.31	33.14	2.25	51.66
		Bouteloua gracilis (Blue Grama)			
5-15-1957	10.81	16.06	28.60	3.06	41.47
6-20-1957	10.90	7.44	33.51	2.32	45.93
9-17-1957	9.78	6.19	34.97	2.38	46.68
10-28-1957	11.32	5.31	33.64	2.39	47.34
1-16-1958	10.83	5.19	32.18	1.96	49.84
4-1-1958	10.37	5.06	34.30	2.19	48.08

TABLE III

CARBOHYDRATE ANALYSES IN RELATION TO STAGE OF MATURITY

(Dry Weight Percentage)

Date	Sugars			Mild Acid Hydrolysis	Fructosans	Starch	Total Acid Hydrolysis	Cellulose	Lignin
	Reducing %	Total %	Sucrose %	%	%	%	%	%	%
Sideoats Grama									
5-15-1957	0.48	2.21	1.73	4.02	0.22	5.94	24.06	24.53	5.51
5-20-1957	0.56	2.36	1.80	3.70	0.22	5.02	24.60	23.34	4.87
9-17-1957	0.54	1.97	1.43	4.52	0.19	8.12	26.68	26.64	6.92
10-28-1957	0.61	2.12	1.51	4.27	0.17	7.79	26.78	28.35	7.60
1-16-1958	0.74	1.27	0.53	4.49	0.14	6.97	26.73	27.16	7.72
4- 1-1958	0.23	0.32	0.09	4.37	0.13	7.12	27.77	27.20	8.64
Blue Grama									
5-15-1957	0.47	3.25	2.78	3.43	0.18	9.27	23.57	21.47	3.26
6-20-1957	0.36	1.44	1.08	5.03	0.20	10.89	30.47	23.35	4.30
9-17-1957	0.33	0.97	0.64	4.14	0.12	10.40	28.33	30.40	6.83
10-28-1957	0.41	1.26	0.85	5.24	0.17	9.11	30.26	27.46	6.40
1-16-1958	0.73	1.56	0.83	4.08	0.12	5.16	28.04	27.65	6.73
4- 1-1958	0.17	0.17	0.00	3.70	0.13	6.69	26.78	28.75	5.90

TABLE IV

CRUDE HOLOCELLULOSE AND ITS COMPOSITION

(Dry Weight Percentage)

Date	Crude Holocellulose %	Percentages of Holocellulose					
		Ash %	Lignin %	Protein %	Cellulose %	Hemicellulose ext'd with 4% KOH	Hemicellulose ext'd with 24% KOH
Sideoats Grama							
5-15-1957	59.3	10.20	3.18	8.96	27.28	33.90	8.47
6-20-1957	66.1	9.40	3.04	5.69	31.63	34.66	10.32
9-17-1957	69.9	8.84	2.98	2.56	35.12	35.82	10.72
10-28-1957	71.1	9.73	3.10	3.69	32.41	33.10	9.76
1-16-1958	72.0	10.14	3.16	2.38	31.57	32.46	9.55
4- 1-1958	70.9	9.88	3.02	2.19	33.60	34.35	9.65
Blue Grama							
5-15-1957	61.0	10.04	3.22	9.44	33.51	31.49	9.78
6-20-1957	68.2	9.08	3.44	3.13	32.51	33.17	9.92
9-17-1957	76.1	8.23	3.42	3.25	33.96	35.38	8.93
10-28-1957	77.1	11.03	3.98	3.35	40.88	34.30	9.92
1-16-1958	74.5	10.40	3.00	2.44	35.76	33.10	10.09
4- 1-1958	72.8	9.99	2.82	2.44	42.83	34.34	8.97

TABLE V

A COMPARISON OF TOTAL ADJUSTED* ACID HYDROLYSIS VALUES WITH THE YIELDS OF HEMICELLULOSE

(Dry Weight Percentage)

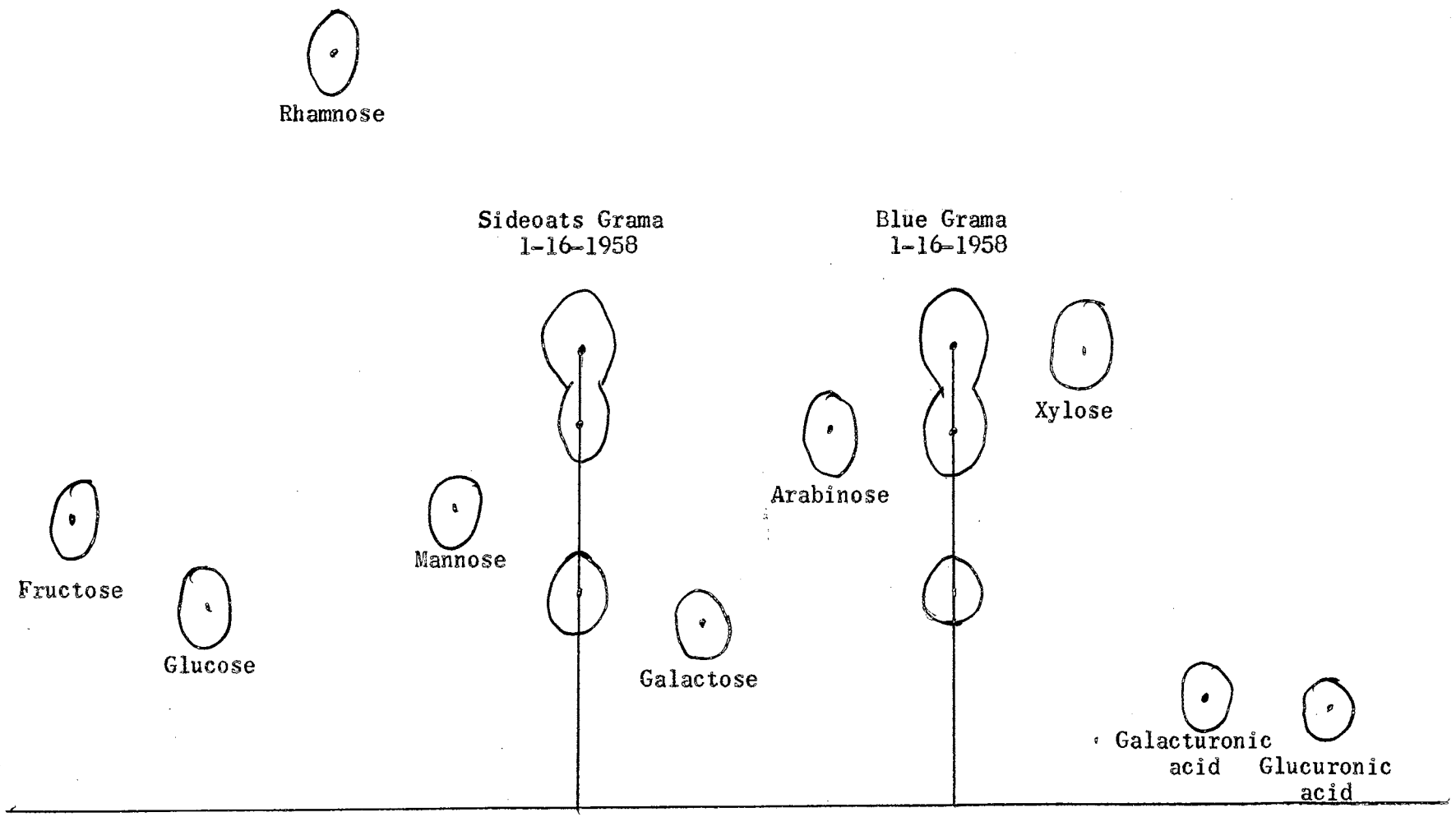
Date	Hemicellulose** %	Percent acid hydrolysis minus approximate percent pectin and percent fructosans
	Sideoats Grama	
5-15-1957	22.47	22.06
6-20-1957	25.35	22.60
9-17-1957	28.77	24.68
10-28-1957	26.85	24.78
1-16-1958	26.91	24.73
4-1 -1958	27.72	25.77
	Blue Grama	
5-15-1957	22.52	21.57
6-20-1957	26.51	28.47
9-17-1957	30.02	26.33
10-28-1957	29.78	28.26
1-16-1958	28.39	26.04
4-1 -1958	28.08	24.78

* Approximate pectin and fructosan values of two percent were secured from previous work.

** The values given for hemicellulose include the heimcellulose fractions from both the 4 and 24 percent KOH extractions. These values have been corrected for ash and they are calculated back to the original sample.

Figure 1

A CHROMATOGRAM OF THE HEMICELLULOSE HYDROLYZATE



CHAPTER IV

RESULTS AND DISCUSSION

The data in Table I give a description of the samples, the time of harvest, and their percent moisture. As shown, the percent moisture was at a maximum in the spring months and decreased steadily to a minimum in the winter months, which is characteristic of a great many range grasses.

Proximate analysis was run in order to get an over-all picture of the chemical composition of the samples and to compare these data with the results of other workers. The data given in Table II agree in general with those reported by Savage and Heller (45). The ash, fat, and protein percentages were at a maximum during the spring months while crude fiber and nitrogen-free extract percentages were at a minimum. Any variation from a steady increase or decrease may be explained by taking into consideration the weather for the specific season being studied. Noteworthy is the fact that with the exception of the spring sampling, the crude fiber and NFE values are relatively constant.

Table III lists a fairly complete carbohydrate study of the two species of grasses. Since only a one-year period was covered it is difficult to establish a definite pattern of changes. However some patterns were observed. For example fructosans, reducing sugars, total sugars, and sucrose tend to decrease as the season advances from spring to winter. Noteworthy also are the very low values for sugars at the end of the dormant season and particularly is this true for sucrose. Cellulose and

lignin tended to increase initially with very little change thereafter. This is in agreement with the results given by Patton (37) in his studies on grama, brome, and oat grasses. Mild acid hydrolysis values remain relatively constant while total acid hydrolysis percentages definitely increase after the flush of spring growth although there is some fluctuation at specific times.

Tables II and III give an over-all picture of the chemical composition of these two varieties of western range grasses.

A study was made of the holocellulose preparations to determine whether this component could be used in describing the nutritive value of a plant. In Table IV it can be seen that the percent holocellulose was at a maximum and fairly constant during the winter season. Data in this table also show that the holocellulose preparations were highly impure. Holocellulose was relatively high in ash content although this value appeared to remain essentially constant. Removal of the lignin was not as complete as expected but this substance, like ash, remained at a relatively constant value. No attempt was made in these tests to remove the protein. The use of pepsin to accomplish this would probably have increased the utility of holocellulose values for describing the composition of the plant. The high degree of impurities in the holocellulose fraction makes it seem doubtful that this fraction can be of much value in interpreting nutritive values. Improved techniques can probably increase the purity of this fraction in future studies.

Extraction of hemicellulose from holocellulose with KOH shows that approximately 75 percent of the hemicellulose is extracted with 4 percent KOH. The acid chlorite treatment apparently renders the major portion of the hemicellulose soluble in dilute alkali. Data in Table IV show that

in all of the samples, 4 percent KOH extracted a high percentage of the hemicellulose that was recovered in these tests.

A comparison was made of the percent of carbohydrates determined in the total acid hydrolysis method minus the approximate percent pectin and percent fructosans to the percentage of hemicelluloses. This comparison is presented in Table V and shows that the percent hemicelluloses extracted with 4 and 24 percent KOH was consistantly higher than that of the acid-hydrolyzable fraction. Since hemicellulose was determined on a semi-micro scale, protein and lignin were not determined on this fraction. This may partially explain why the percent of hemicellulose extracted with KOH was consistently higher than the value obtained through acid hydrolysis since lignin and protein might both be present in this hemicellulose fraction. If these two values had corresponded more closely, direct acid hydrolysis might be used to approximate the percentage of hemicelluloses. Further tests will be necessary before this point can be proven.

Hemicelluloses have been found to vary slightly in composition. Chromatographic analysis carried out on the hemicellulose hydrolyzate of the samples dated 1-16-1958 showed the presence of xylose, arabinose, and glucose. The chromatographic technique employed was semi-quantitative. It demonstrated that xylose and arabinose were the major components of the hemicellulose hydrolyzate, and that glucose was present in a smaller quantity. Another chromatogram developed using the ethyl acetate:pyridine:water (2:1:2) solvent of Jermyn and Isherwood (25) indicated the presence of a trace of uronic acid. This uronic acid was not identified but it was shown not to be glucuronic or galacturonic acids since these acids were used as standards in developing the chromatogram.

CHAPTER V

SUMMARY

The nitrogen-free extracts of two species of western range grasses were studied. A relatively complete carbohydrate analysis was carried out showing seasonal variations which agreed with those which had been previously reported. Holocellulose was prepared but found to be too impure to be of any value in defining the nutritive value of these species. Total acid hydrolysis values minus approximate pectin and fructosan percentages yielded a value that remained consistently lower than the total percentage of hemicellulose that was extracted with 4 and 24 percent KOH. Hemicellulose was corrected only for ash and not for any protein or lignin that might possibly have been present. A closer correspondance between these values might have been obtained if the hemicellulose fractions had been corrected for protein and lignin.

The combined hemicellulose fractions from one sampling period were analyzed chromatographically and found to be composed chiefly of xylose and arabinose with a smaller quantity of glucose present. A trace of one uronic acid was also found but its identity was not determined.

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