IDENTIFICATION AND DETERMINATION OF THE EASILY HYDROLYZABLE COMPONENTS IN WESTERN RANGE GRASSES

By

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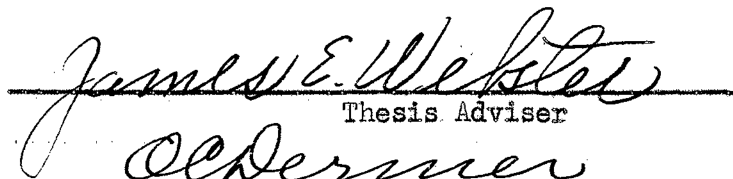
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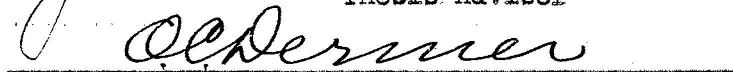

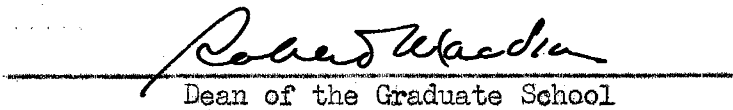
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INTRODUCTION

Many studies conducted in past years have shown that the widely used and well known proximate analysis is not adequate to account for the known differences in feeding value of range grasses for beef cattle. There are indications, however, that the two principal carbohrydrate fractions in the proximate analysis, the crude fiber and the nitrogen-free extract, may possibly account for most of the variation in quality from grass species to grass species. If there were some way to make a detailed analysis of these fractions, this variation in quality might be better explained. Fortunately, such detailed analyses can be made.

One of the routine methods of analysis for carbohydrates employed at the Agricultural Chemistry Department of Oklahoma State University determines the several substances making up these easily hydrolyzed fractions.

REVIEW OF LITERATURE

Farmers and ranchers have known there were differences in grass quality for many hundreds of years (33), but the evaluation of the range plants by chemical methods is a relatively recent undertaking. The first attempt at a chemical analysis of grasses and animal feeds came almost 100 years ago. This first method, with only slight modifications, is still in use today. This method is the well known proximate analysis. Almost all commercial feeds are analyzed in this way, and the results of the analysis are attached by means of tags to the sacks in which the feed is sold. This method reports such general values as crude protein, fat, crude fiber, ash and nitrogen-free extract.

That part of the grass called nitrogen-free extract is found by adding the percentages of all the components of the proximate analysis, and subtracting from 100. In other words, 100 minus (percent protein + percent fat + percent crude fiber + percent ash) = percent nitrogen-free extract. This figure is generally considered to be a measure of the digestible carbohydrate content of the grass. (26)

The carbohydrates are the most abundant of the various grass constituents. When a plant is fed to an animal, the plant carbohydrates furnish more energy for its life processes than any other class of materials.

The carbohydrate content of grasses has been the subject of much experimentation for many years. Is any one fraction of carbohydrates responsible for the superior nutritive quality of one grass species or is a combination of many such fractions responsible? Do the kinds of carbohydrates present determine grass quality, or does the quality of

grass depend only on total carbohydrate quantity? These questions are still being studied. New answers are still being found.

The overall quality of a grass species does not depend entirely on the specific chemical compounds found in it. Most of the food consumed by an animal is broken down, and in some cases further changed by enzyme action. These products can not in general be identified with any particular compound, since simple nitrogen compounds such as amino acids come from a variety of complex proteins, and sugars come from a similarly complicated variety of long-chain carbohydrates. Chemical changes also occur through micro-organismal action, especially in ruminants. The bodies of these microorganisms are then digested in the true stomach of the ruminant animal. (33)

Processes such as these make "grass quality" a most elusive value. With the advance of knowledge in analytical chemistry, and with the better analytical procedures that this brings, it is the hope of all workers in this field that actual chemical composition will become a better measure of quality.

One of the problems being studied at Oklahoma Agricultural and Mechanical College involves the determination of various classes of carbohydrates present in several range grasses grown in northwestern Oklahoma.

Polysaccharides are composed of two classes of sugars, hexoses and pentoses. (32) Some of the hexose polymers which have been reported in grasses are the fructosans (1, 19, 21) and cellulose. (10) Only very small amounts of starches are present. (14, 16, 32) Except for cellulose, the pentosans have a more dominant role in grasses than have the hexosans. Arabans, xylans, mannans, and many others have been shown by several research workers to be present in variable quantities in grasses. (6, 8, 29)

Cellulose, that carbohydrate which make up the framework of grasses, is a glucose polymer, but it is encrusted with many other substances. These substances are of two general types: the hemicelluloses, and lignin. (33) There are several different types of hemicelluloses, but those in grasses are apparently of the polyuronide type, that is, when they are hydrolyzed they yield simple sugars and uronic acids. (33) Hemicelluloses of sheep fescue and sweet vernal grass yield on hydrolysis arabinose, xylose, and a unidentified uronic acid. (4) Those of alfalfa contain for the most part xylose, a little arabinose, and a uronic acid. (31) In alfalfa roots the hemicelluloses consist of glucose, xylose, and a uronic acid which is probably glucuronic acid. (11) Pectins, which are co-polymers of pentoses and uronic acids, have been reported only in relatively small quantities in grasses. (5)

The other chief contaminant of cellulose, and therefore of hemicelluloses, is lignin. Although lignin is generally classified with the carbohydrates, its structure is not definitely known, and it probably is not a carbohydrate at all. (32) Lignin is generally characterized by its resistance to attack by all but the strongest chemical treatments. It is also highly resistant to attack by microorganisms. (32)

Close relationships are thought to exist between pectins, hemicelluloses, and lignin. Norman (27) has summed up the several theories of their formation as follows: pectins undergo transformation to lignin; hemicelluloses or certain groupings thereof may be converted to lignin; polyuronide hemicelluloses are formed from pectins; and all three substances are connected, lignin being formed from pectins through the intermediate stage of polyuronide hemicelluloses.

Since the nitrogen-free extract contains too many components to be studied in the limited time available, this research work was limited to a study of the carbohydrate fraction called fructosans, as determined by mild acid hydrolysis. (32)

The first isolation of a fructose polymer was made by Muntz in 1878. (13) Since that time, many such polymers have been discovered. Most of these were named according to their origin--asparagosin from asparagus, irisin from iris. In recent times, all such polymers have been grouped under the name of "fructosan", referring the different polymers to their common origin, D-fructose.

The fact that all fructosans are not the same may best be illustrated by the following examples.

Inulin (a D-fructose polymer isolated from guayule tissue) on acid hydrolysis gave a quantitative yield of D-fructose. Methylation followed by hydrolysis yielded 3,4,6,-trimethylfructofuranose and a small portion of 1,3,4,6,-tetramethylfructofuranose. The ratio of tri- to tetra- methyl fructose indicates an average chain length of about 28 fructose residues per chain.

The fructosan of barley leaves on methylation and hydrolysis yielded 1,3,4,-trimethyl-and 1,3,4,6,-tetramethylfructofuranose, indicating chains of D-fructofuranose units with 2,6 linkages. These chains, apparently unbranched, are about 10 residues in length. A fructosan isolated from timothy caplocorns shows similar 2,6 linkages, but is 15 to 16 fructose units long.

Irisin (a fructosan from the iris plant), apparently containing a branched-chain structure, yielded on methylation and hydrolysis equal amounts of 3,6-dimethyl- and 1,3,4,6-tetramethylfructofuranose. (10)

As was mentioned earlier, (1, 19, 21) fructosans are known to be present in many range grasses, and as might be expected, considerable variation in quantity has been noted. The amounts vary not only with grass species, but also with locality and seasons of the year. For instance, in ryegrass in England, Norman found as much as 30 percent fructosans, while the same species in Ohio contained only about 5 percent fructosans. (28)

Since research on fructosans has been so extensive, many methods have been proposed for their quantitative determination. These methods range from the earlier, non-specific gravimetric methods (13) to the newer, more specific methods of colorimetry (23) and chromatography. (18)

One method tentatively adopted at Oklahoma Agricultural and Mechanical College consists of boiling an ethanol-extracted sample for 20 minutes in 0.2 N H_2SO_4. This is followed by neutralization, filtration, and finally measurement of the reducing content by a quantitative volumetric technique based on the reducing action of monosaccharides toward an alkaline copper complex. (32) This method determines the easily hydrolyzed components of the grasses. It was originally assumed that fructosans were the only major constituents being removed. This assumption is apparently valid when applied to certain plant tissues, but percentages of "fructosans" seemed much too high when the method was applied to such grasses as were studied in this particular problem. Another more selective method based on the unique ability of fructose to react with resorcinol to form a colored compound (23) showed practically <u>no</u> fructosans to be present, except at certain seasons of the year in one of the ten grasses tested.

Since only small amounts of fructosans are present in the grasses being studied, an attempt was made to determine what sugars were responsible for the excessively high values given by the mild hydrolysis mentioned earlier.

Several sugars were suspected after a literature search. Aspinall and Wilkie discovered arabinose and xylose in oat straw; (3) and Binger and Sullivan, working with orchard grass, found xylose, glucose and galactose, as well as some uronic acids. (8) In a study of forage grasses in Palestine, it was found that the hemicellulose fraction contained mostly pentosans, while the cellulose fractions of the grasses were predominantly hexosans. (9) In all, one or more of 13 substances were possibly present. These were: glucose, fructose, galactose, arabinose, xylose, rhamnose, mannitol, galacturonic acid, gluconic acid, glucuronic acid lactone, arabonic acid, and galactonic acid.

One of the simplest methods for the identification of sugars involves the use of paper chromatography. While certain authorities will not accept this as positive proof, it is sufficiently reliable to identify, at least tentatively, those sugars present. This is the method used in this project.

The principles which govern the separation of sugars on a strip of paper during development of a chromatogram are exceedingly complex, and will not be discussed in this paper. A discussion of this may be found in the article by Comsden _et al_. (12) Suffice it to say that, theoretically, the best separations of sugars are made with filter paper strips approximately 100 times as long as wide, treated with a developing solution such that R_f values are in the neighborhood of 0.2. (16)

To repeat, too little is known about the quality of range grasses, and while this project is chiefly concerned with the quality of the carbohydrates in grasses, it is a part of the whole study of animal nutrition. When the importance of forages, and particularly pasture forage in the economy of animal production, is considered, the need for studies such as this is readily seen.

EXPERIMENTAL METHODS EMPLOYED

Method 1

Easily Hydrolyzable

Carbohydrates

Reagents:

1. 0.2 N H_2SO_4
2. 1.0 N NaOH
3. 0.2374 M $CuSO_4$ (Fehling's " A ")
4. Alkaline tartrate solution, composed of 346 grams Rochelle salt and 100 grams of NaOH per liter of solution. (Fehling's " B ")
5. 1 percent starch solution.
6. 0.200 N $Na_2S_2O_3$ stabilized with 5 grams NaOH per liter.
7. 0.0278 M KIO_3 and 0.261 M KI, stabilized with 1 gram KOH per liter.
8. 5 N H_2SO_4.
9. Saturated solution of potassium oxalate.

Procedure:

Weigh out 1.000 gram of extracted residue and place in a 600 ml. Berzelius beaker. Add 45 ml. 0.2 N H_2SO_4 and boil gently in a reflux condenser for 20 minutes. Then cool, and add 8.6 ml. of 1 N NaOH to the sample. Filter through No. 54 Whatman filter paper and make to volume in a 100 ml. volumetric flask.

Pipette 25 ml. Fehling's " A " and 25 ml. Fehling's " B " solution into a 500 ml. Erlenmeyer flask. Add 50.0 ml. of the sugar solution from

the 100 ml. volumetric flask, and stopper with a Tuttle flask cover. Heat the flask over a burner adjusted so as to bring the solution to a boil in exactly 4 minutes, and continue boiling for 2 to 4 minutes. Add 25 ml. iodate solution, swirl, add 20 ml. of 5 N H_2SO_4 and 20 ml. of saturated oxalate and swirl again. Let stand one minute and titrate with $Na_2S_2O_3$, adding 1 to 2 ml. of starch solution near the end of the titration. In order to calculate results, a blank is required. Proceed as above, but substitute 50 ml. of H_2O for the sugar solution. Calculations are made with the aid of the levulose column in the conversion tables of Munson and Walker. (24)

Method 2

Chromatographic Separation (18)

Reagents:

1. Developing solvent composed of ethyl acetate, acetic acid, and water in a volume ratio of 3:1:3. Mix thoroughly, and separate the organic phase from the water phase. This mixture is subject to spontaneous hydrolysis, and thus should be made fresh for each series to be run.
2. Color reagent prepared by mixing equal portions of 5 N NH_4OH and 0.1 N $AgNO_3$. These should be mixed immediately prior to use. The $AgNO_3$ solution should be kept in a brown bottle.
3. Sugar solutions. Prepare a 1 percent standard solution (w:v) of each sugar suspected to be present in the unknown.

Procedure:

Weigh out 1.0 gram of extracted residue and place in a 600 ml. Berzelius beaker. Add 45 ml. 0.2 N H_2SO_4 and boil gently on a reflux

condenser for 20 minutes. Cool, and neutralize to pH 7.0 with 0.5 N $Ba(OH)_2$. Filter under suction through Whatman No. 5 filter paper, and evaporate over a hot water bath to a volume of 5 to 10 ml. Add a few drops of toluene to each solution, and store in a refrigerator when not in actual use.

Cut a piece of Whatman No. 1 filter paper so that a strip is produced 10 x 50 cm. Place two pencil dots, 3 cm. from each side, 10 cm. from one end of the paper. Place the dots firmly, so that two raised points appear on the opposite side of the paper. These two dots locate the point of application for the sugar solutions. Since the unknown hydrolyzate is pipetted on one of these dots, it is necessary to prepare as many papers as there are suspected sugars in the hydrolyzate. An unknown must be developed with each standard sugar because of individual variations in the filter paper. Serrate the bottom of the paper with pinking shears to facilitate an even flow of solvent through the paper. (30) Using a micropipette, apply about 4 to 7 microliters of solution to one of the raised dots. Do the same to the other dot, using the unknown hydrolyzate. This should be done slowly and carefully, with a hot air blower directed at the spot. The final spot should be about 3 to 5 mm. in diameter. The raised dot allows a more careful application of solution to chromatogram. (7) The paper is now ready for development.

Secure a chromatographic solvent assembly (No. C4250 in the general catalog of the Scientific Glass Apparatus Co. Inc., Bloomfield, N. J. is satisfactory) and a glass chamber about 30 cm. in diameter and 60 cm. high. Wrap a large sheet of filter paper around the inside of the jar, with the bottom edge of the paper resting on the floor of the jar, and the top edge coming nearly to the top of the jar. The paper facilitates saturation of the atmosphere in the chamber with solvent. (22)

Place the prepared papers in the trough, with the spots extending about 3 cm. past the glass hangers of the solvent assembly. Apply a sealer (composed of a paste of starch and glycerol) to the ground surface of the jar, and close the chamber with a glass plate. The glass plate should have a stoppered hole directly over the trough of the solvent assembly.

When the atmosphere of the chamber is saturated, (about 18 hours is sufficient) remove the stopper and fill the trough with the organic layer of the developing solvent. Replace the stopper, and allow the chromatograms to develop for about 40 hours.

Remove the papers from the chamber, and hang them under a hood until they are dry. Mix the color reagents in an evaporating dish, and lay one of the papers on a large clean filter paper. Cut a piece of filter paper slightly larger than the chromatogram, run it evenly through the ammoniacal silver nitrate, let the excess solvent drip off for a moment, and blot the wet paper evenly over the surface of the chromatogram for about 10 seconds. If this is done correctly, the chromatogram will be uniformly damp, but not wet, from the color developer. Use this same procedure for each chromatogram. Hang the papers under a hood again until dry.

Next place a large flat asbestos board on a steam plate. Put the papers on the board, and cover with another sheet of asbestos. After about 10 minutes, the papers can be removed. The sugars spots show up as dark brown spots on a light brown background.

This procedure, with slight modifications, was adapted from the method given by Jermyn and Isherwood. (18) The length of time for development and choice of solvent were the same as that given in the article, but the method of applying sugar solutions to the paper was altered slightly; the solutions

were applied to the raised dots as suggested by Beroza. (7) Color development was also changed slightly, the details being given in the body of the preceding method.

Method 3

Colorimetric Determination of Fructosans in Plant Materials (23)

Reagents:

1. Resorcinol - 1 gram per liter in 95 percent ethanol.
2. HCl solution - 5 volumes of concentrated HCl to 1 volume H_2O.
3. Fructose standard - 100 mg. fructose per 100 ml. H_2O. (prepared daily)
4. Saturated neutral lead acetate.
5. Saturated potassium oxalate.

Procedure:

Weigh out 0.5 gram samples into 150 ml. beakers. Measure into similar beakers 1, 2, 3, and 4 ml. aliquots of the standard solution. Add 20 ml. H_2O to every beaker and heat in a boiling water bath for 30 minutes more, stirring from time to time. Remove from the bath. Cool, add 2 ml. saturated neutral lead acetate to each beaker, and stir thoroughly. Filter through Whatman No. 4 filter papers into 250 ml. volumetric flasks with 2 or 3 good rinsings. Add 2 ml. (or a slight excess) of saturated potassium oxalate to each volumetric flask, make up to volume, shake well and filter through Whatman No. 2 filter papers into 250 ml. beakers.

Take a 5.0 ml. aliquot from each beaker and place in separate colorimeter tubes. Also add 5 ml. H_2O to a tube for a reference point. Then add 5.0 ml. resorcinol and 15.0 ml. HCl to each tube. Heat for 20 minutes in a water

bath at 80 ± 2° C. Cool and read the percent transmission on the Evelyn colorimeter using a 540 millimicron filter. A standard curve is plotted from the readings of the reference tubes, and the concentrations of the unknown are read from the graph.

Method 4

Determination of Pectic Materials
in Dried Samples (20)

Reagents:

1. Versene solution - Dissolve 5 grams of dry ethylenediaminetetraacetic acid tetrasodium salt (from the Bersworth Chemical Co., Framingham, Mass., under the trade name of Versene regular) in 1 liter of water.
2. Pectinase - A commercial Pectinol 100 D (from Rohm and Haas Company, Philadelphia, Pa.).
3. Acetic Acid - Reagent grade, glacial.
4. Sulfuric acid - Reagent grade, concentrated.
5. Ethanol - Purified. Reflux 1 liter of 95 percent ethanol with 4 g. of zinc dust and 4 ml. of 50% sulfuric acid for 24 hours. Distill using all-glass apparatus. Add 4 g. of zinc dust and 4 g. of potassium hydroxide to the distilled alcohol and redistill.
6. Ethanol - 95 percent.
7. Carbazole Reagent - Dissolve 0.15 gram of reagent grade carbazole in 100 ml. of purified ethanol. Solution is slow and stirring is required.
8. Galacturonic acid monohydrate - Reagent grade. Check the purity by titrating 0.5 g. with 0.1 NaOH to pH 8.0. The theoretical equivalent weight of the acid is 212.

Procedure:

Weigh 1.0 gram of 40 mesh or finer extracted residue containing 10 to 40 percent pectin into a 250 ml. beaker and moisten with 95 percent ethanol. Sequester the divalent cations with 200 ml. of 0.5 percent Versene solution. Adjust the pH to 11.5 with 1 N NaOH and de-esterify the pectin and pectinates by holding at 25° C. for 30 minutes. Acidify the mixture to pH 5.0 to 5.5 with acetic acid. Add 0.1 gram of pectinase, stir for about 1 hour, dilute to 250 ml., and filter. Discard the first few ml. of the filtrate, dilute 2 ml. to 100 ml., and take 2 ml. aliquots for color development.

Measure 12.0 ml. of concentrated sulfuric acid into a 25 x 200 mm. culture tube. Cool the tube and contents to about 3° C. in an ice bath and add a 2 ml. aliquot of solution containing 5 to 80 micrograms of de-esterified galacturonide or polymer. Insert a 5 ml. beaker into the mouth of the test tube and mix the contents thoroughly. Replace the tubes in an ice bath and cool to below 5° C. Heat the tube and contents for 10 minutes in a boiling water bath. Cool to 20° C., add 1.0 ml. of 0.15 percent carbazole reagent, mix thoroughly, and allow to stand at room temperature for 25 ± 5 minutes. Determine the intensity of the color using light of wave length 520 millimicrons. Read the samples in sequence, so that the time and temperature from the addition of the carbazole to the color determination are comparable.

Use a standard curve to obtain the concentration of anhydrouronic acid in the samples. To control daily variation in heating time, include a 40 microgram galacturonic acid hydrate standard with each series. Report the results in terms of percent galacturonic acid in the dried sample.

Method 5

Official Method for Determining Pentosans

in Grain and Stock Feeds (25)

Reagents:

1. Hydrochloric acid - Contains 12 percent by weight of HCl. To 1 volume of HCl add 2 volumes of H_2O. Determine the percent acid by titration against standard alkali and adjust to proper concentration by dilution or addition of more HCl, as may be necessary.

2. Phloroglucinol - Dissolve a small quantity of phloroglucinol in a few drops of H_2SO_4. A violet color indicates the presence of diresorcinol. A phloroglucinol that gives more than a faint coloration may be purified by the following method: Heat in a beaker about 300 ml. of the dilute HCl and 11 grams of commercial phloroglucinol, added in small quantities at a time, stir constantly until the phenol is nearly dissolved. Pour the hot solution into a sufficient quantity of the same HCl (cold) to make the volume 1500 ml. Allow to stand at least overnight, preferably several days, to permit the diresorcinol to crystallize. Filter immediately before using. A yellow tint does not interfere with its usefulness. In using, add to the distillate a volume containing the required quantity of phloroglucinol.

Procedure:

Place into a 300 ml. distillation flask such a quantity of sample, 2 to 5 grams, that the weight of phloroglucide obtained will not exceed 0.3 gram. Add 100 ml. of the dilute HCl and several pieces of recently ignited pumice stone. Place the flask on a wire gauze, connect with condenser, and heat, rather gently at first, and then regulate at such a rate as to distill over 30 ml. in about 10 minutes. Pass the distillate through a small filter

paper. To replace the 30 ml. distilled, add a like quantity of the dilute HCl by means of a separatory funnel in such a manner as to wash down particles adhering to the sides of the flask, and continue the process until the distillate amounts to 360 ml. To the total distillate add gradually a quantity of phloroglucinol dissolved in the dilute HCl and thoroughly stir the resulting mixture. The quantity of phloroglucinol used should be about double that of the furfural it is required to treat. The solution turns yellow, then green, and soon there appears an amorphous greenish precipitate that grows darker rapidly until it becomes almost black. Make the solution to 400 ml. with the dilute HCl and allow it to stand overnight. Collect the amorphous black precipitate on an asbestos mat in a weighed Gooch crucible, wash carefully with 150 ml. of H_2O so that the H_2O is not entirely removed from the crucible until the very last, and dry for 4 hours at the temperature of boiling water. Cool, and weigh in a weighing bottle. The increase in weight is considered to be furfural phloroglucide. To calculate pentosans from phloroglucide, use the following formulas:

1. For a weight of phloroglucide, designated by "a" in the following formulas under 0.03 gram:

   Pentosans = (a + 0.0052) x 0.8949.

2. For a weight of phloroglucide "a", between 0.03 and 0.30 gram:

   Pentosans = (a + 0.0052) x 0.8866.

3. For a weight of phloroglucide, "a" over 0.3 gram:

   Pentosans = (a + 0.0052) x 0.8824.

The results of all the preceding analyses on three different grasses are tabulated on the following pages. Graphs of the various percentages of components versus time are also presented.

Table I

RESULTS OF ANALYSIS OF THREE DIFFERENT GRASSES DURING A ONE YEAR PERIOD (1955)

Sample description	Date	True Fructosans ,%	Galacturonic Acid,%	Pentosans,%	Total	Mild Hyd.,%
			Blue Grama			
dormant	2/15/55	0.33	2.05	6.46	8.84	7.57
dormant, trace of green	3/17/55	0.37	1.87	6.60	8.84	7.61
beginning to head	5/25/55	0.16	1.74	5.67	7.57	7,29
seed ripe	7/20/55	0.20	2.06	6.05	8.31	10.19
seed stalks dry, leaves 50% brown	9/14/55	0.08	1.41	6.06	7.55	5.10
Winter dormant with trace of green	11/22/55	0.20	1.33	6.33	7.86	8.40
			Sand Dropseed			
dormant	2/15/55	0.43	1.63	6.44	8.50	7.52
dormant, trace of green	3/17/55	0.44	1.95	6.39	8.78	8.39
green and tender	5/25/55	0.17	1.70	5.15	7.02	7.32
seed ripe	7/20/55	0.21	1.60	5.69	7.50	6.58
seed stalks dry	9/14/55	0.19	1.55	6.05	7.79	5.89
dormant	11/22/55	0.26	1.34	6.40	8.00	8.28
			Western Wheat Grass			
dormant, trace of green	2/15/55	0.62	2.17	6.49	9.28	8.23
dormant to green	3/17/55	0.31	1.50	6.80	8.61	8.81
heading, leaves green but tough	5/25/55	0.39	2.12	7.50	10.01	7.34
some seed ripe	7/20/55	3.81	1.00	5.78	10.59	9.61
green and growing	9/14/55	6.17	1.90	5.95	14.02	11.53
green	11/22/55	3.10	1.31	5.56	9.97	9.18

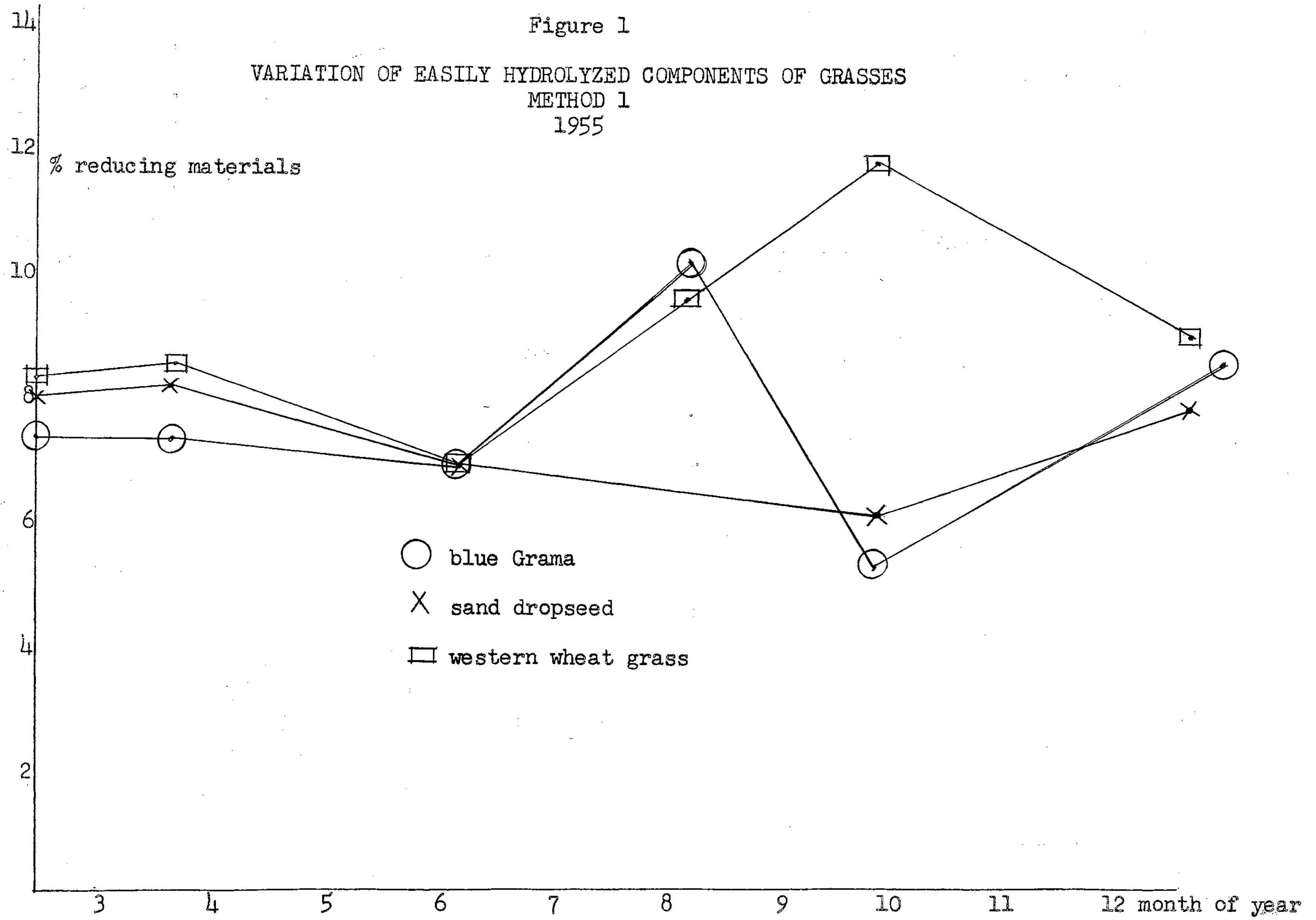
Figure 1
VARIATION OF EASILY HYDROLYZED COMPONENTS OF GRASSES
METHOD 1
1955
% reducing materials
14
12
10
8
6
4
2
3
4
5
6
7
8
9
10
11
12 month of year
blue Grama
sand dropseed
western wheat grass

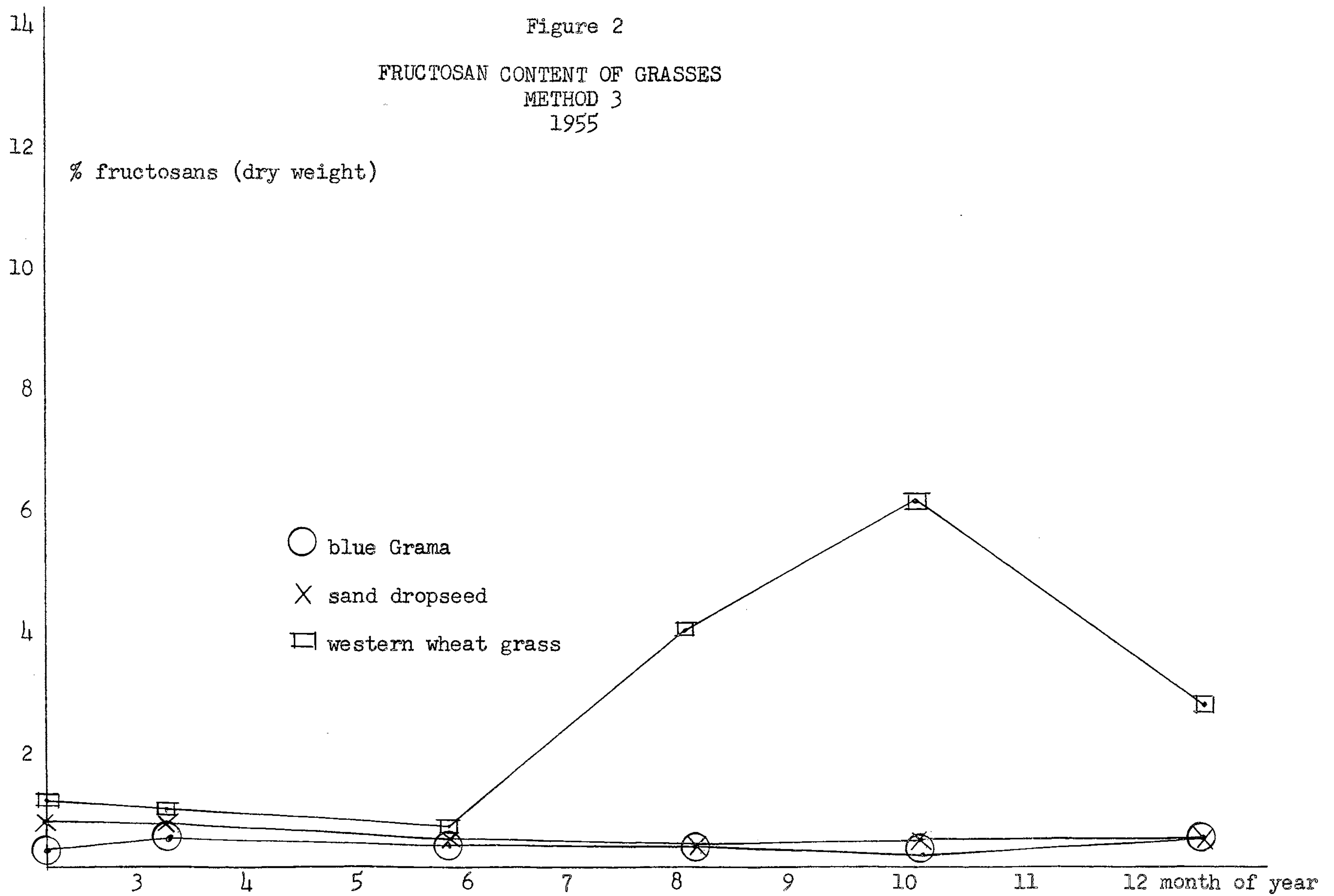
Figure 2
FRUCTOSAN CONTENT OF GRASSES
METHOD 3
1955
% fructosans (dry weight)
14
12
10
8
6
4
2
blue Grama
sand dropseed
western wheat grass
3
4
5
6
7
8
9
10
11
12 month of year

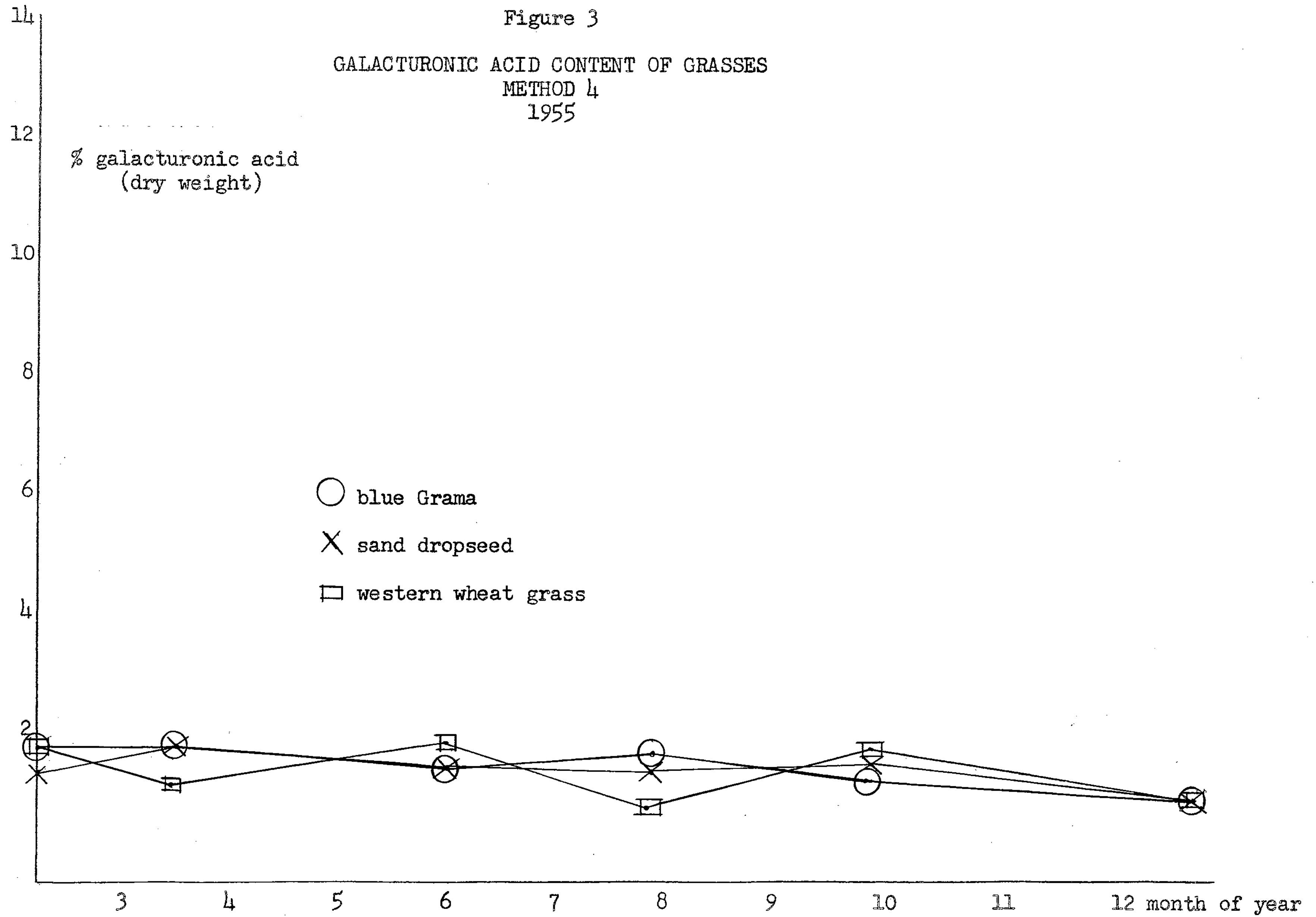

Figure 3

GALACTURONIC ACID CONTENT OF GRASSES
METHOD 4
1955

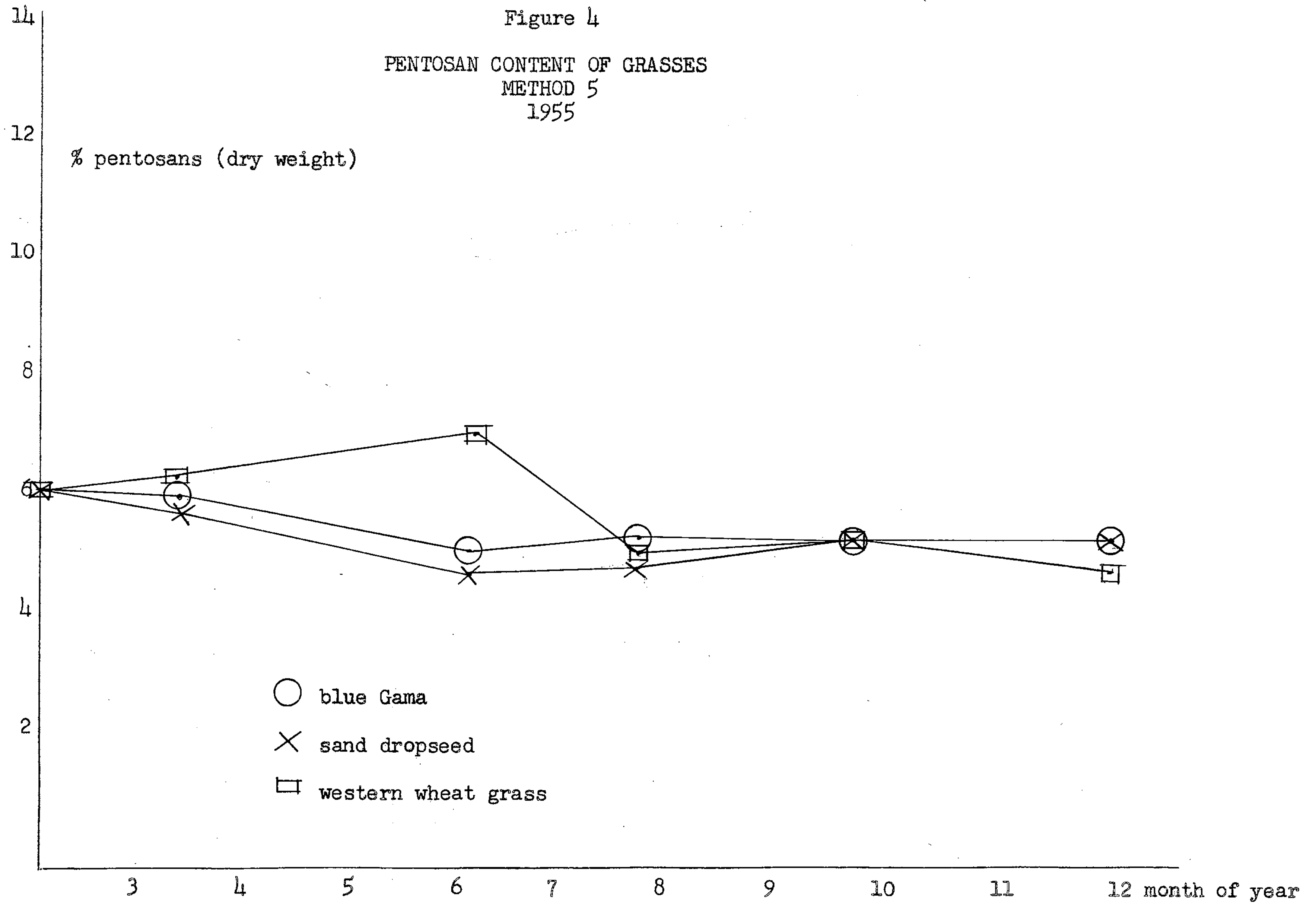

Figure 4
PENTOSAN CONTENT OF GRASSES
METHOD 5
1955
% pentosans (dry weight)
14
12
10
8
6
4
2
blue Gama
sand dropseed
western wheat grass
3
4
5
6
7
8
9
10
11
12 month of year

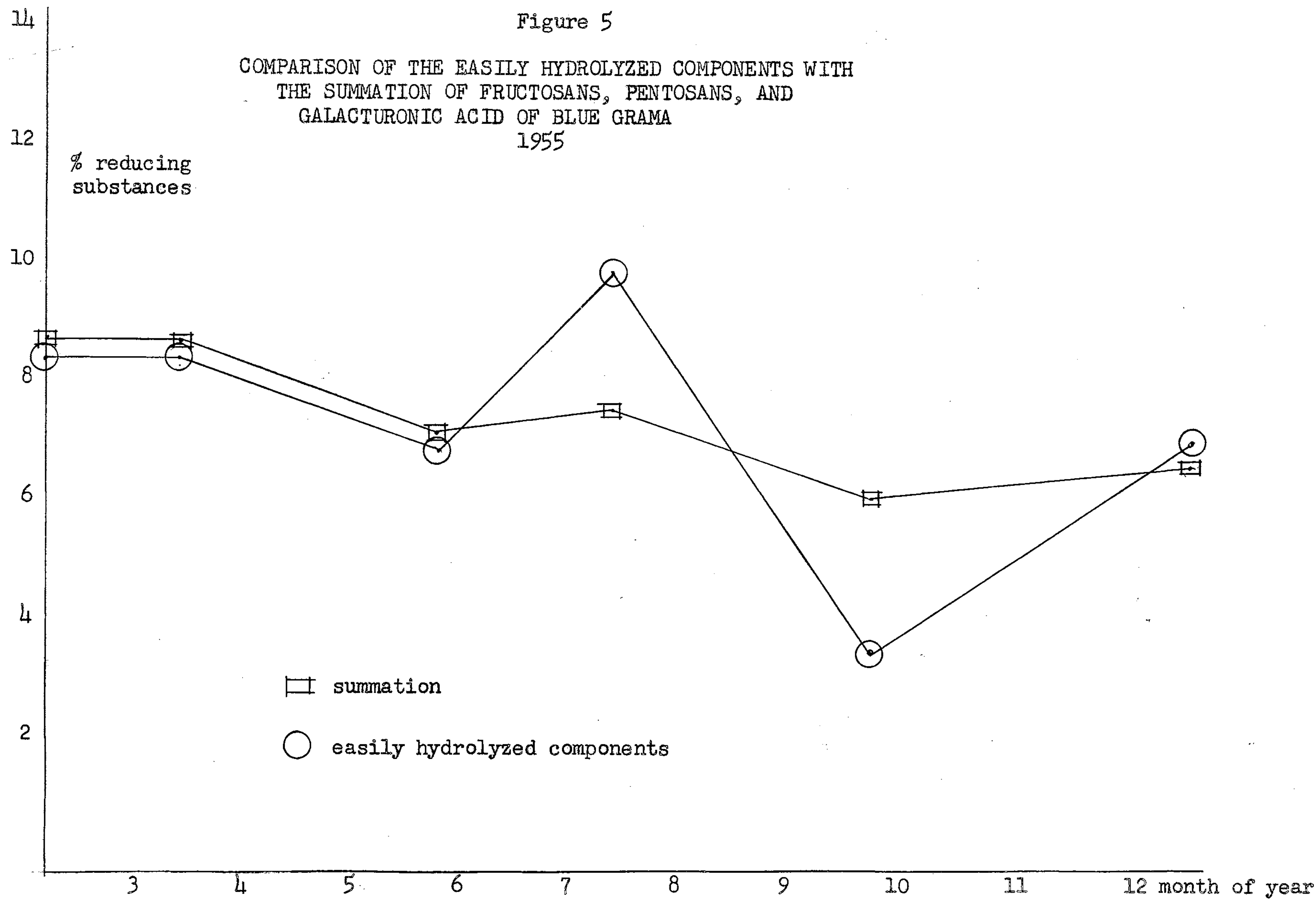

Figure 5

COMPARISON OF THE EASILY HYDROLYZED COMPONENTS WITH THE SUMMATION OF FRUCTOSANS, PENTOSANS, AND GALACTURONIC ACID OF BLUE GRAMA 1955

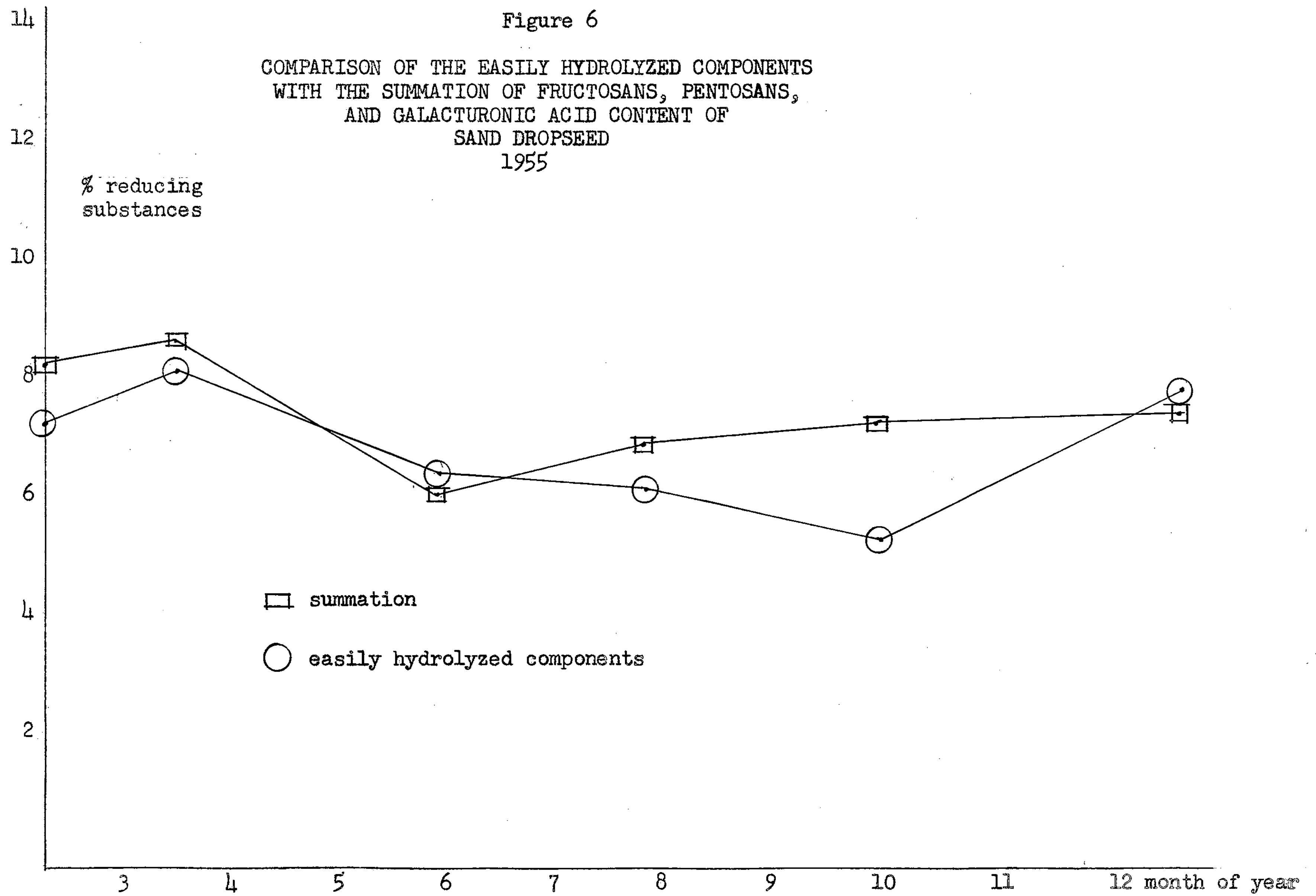

Figure 6

COMPARISON OF THE EASILY HYDROLYZED COMPONENTS WITH THE SUMMATION OF FRUCTOSANS, PENTOSANS, AND GALACTURONIC ACID CONTENT OF SAND DROPSEED 1955

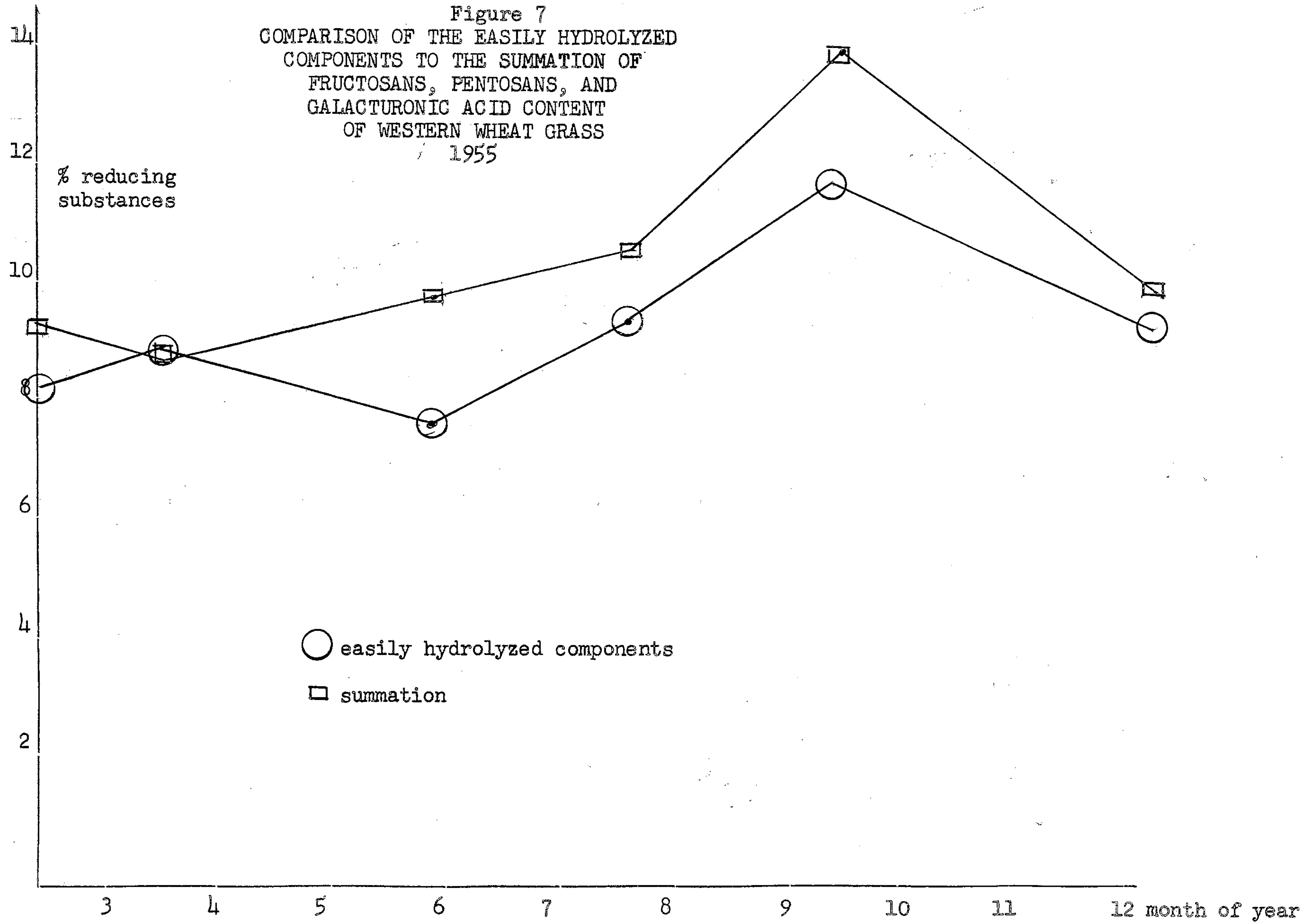
Figure 7
COMPARISON OF THE EASILY HYDROLYZED
COMPONENTS TO THE SUMMATION OF
FRUCTOSANS, PENTOSANS, AND
GALACTURONIC ACID CONTENT
OF WESTERN WHEAT GRASS
1955
% reducing
substances
14
12
10
8
6
4
2
easily hydrolyzed components
summation
3
4
5
6
7
8
9
10
11
12 month of year

DISCUSSION OF METHODS AND RESULTS

The mild hydrolysis method of determining insoluble carbohydrates is the simplest of the methods used in this project. It also gives the highest results. As was mentioned earlier in this paper, there is some question as to what is being hydrolyzed. Only a very small quantity of the total found proved to be fructosans. If one knew what materials were present in the hydrolyzate, and how much of each was present, some conclusions could be made about the analytical value of this method when it was applied to the grasses being studied. As the method stood, it was obvious that some reducing materials were being formed, and since reproducible results were being obtained, certainly some definite fraction, or fractions of polysaccharides were being hydrolyzed.

With this problem in mind, the second method, that of paper chromatography was employed. This procedure is both qualitative and semiquantitative. The primary purpose, however, was to establish just what materials were in the hydrolyzate.

Results of the chromatographic separations showed that arabinose, xylose, fructose, galacturonic acid and two unidentified reducing substances were present. None of the other nine materials mentioned on page 7 were present, at least in large enough quantities to be detected. It is significant that no glucose was discovered in the hydrolyzate. Glucose polymers, such as cellulose, are known to be present in grasses. The only conclusion to be drawn is that the hydrolysis was sufficiently mild that no glucose was released thereby. A corollary to this argument is that there are no glucose polymers in the grasses studied which are susceptible to mild hydrolysis.

A visual examination of the size and intensity of the spots on the developed chromatograms indicated arabinose and xylose to be most abundant, galacturonic acid and fructose next, and finally the two unidentified materials to be least concentrated. Indeed, the amounts of the unidentified reducing substances seemed comparatively insignificant.

The third method was the procedure used to determine the amount of fructosans. The analysis used was that published by McCrary and Slattery (23), and the method is quite suitable for routine analysis. Results of this determination showed that the fructosans content ranged from almost 0 to about 6 percent in the samples tested.

The fourth method is one which had been developed to measure the galacturonic acid content of dried fruits. It is essentially the method of McReady and McComb (20). Reproducibility was satisfactory, with about 10 percent variation observed between duplicate samples. Results secured by this method showed that the galacturonic acid content ranged from 1 to 2 percent in the grasses studied.

Since the arabinose and xylose present in the hydrolyzate are both pentoses, these substances were determined together as pentosans instead of separately. A satisfactory method for pentosan determination was found in the AOAC Methods of Analysis. (25) This official method for pentosan determinatio in grain and stock feeds, was used without modification. As may be seen in Figure 4, the pentosans content ranged from 5 to 7 percent of the dry weight of the grasses.

An inspection of Figures 5, 6, and 7 shows that the easily hydrolyzed components as determined in method 1 approximately equaled, in almost all instances through an entire year's sampling on three different grasses, the sum of the three other methods. There are discrepancies, particularly in

Western Wheat Grass, (Figure 7) but the error usually lies on the side of the summation. That is, when a discrepancy is observed, the higher percentage is usually the sum of the three specific methods.

Since the two lines on each of the last three graphs lie so close together, there is a possibility that one of the three specific methods might have picked up the two unknown reducing materials. Certainly if the two materials had been present in large amounts, or if they were not picked up in one of the other methods, the sum of the results of the three specific methods should have been lower than the easily hydrolyzed components.

SUMMARY

Three different Western range grasses were analyzed for easily hydrolyzed components at intervals during one year. Samples of each of the grasses were analyzed by hydrolysis and paper chromatography. The samples chosen were those which gave the highest percentage of "easily hydrolyzable carbohydrates". Development of the chromatograms showed that arabinose, xylose, fructose, galacturonic acid, and two unidentified materials were present.

Three specific quantitative methods were then used on the ethanol extracted residues of the grasses. A colorimetric determination of fructosans showed that the fructosans content ranged from only traces to about 6 percent of the dry weight of the sample. Galacturonic acid accounted for 1 to 2 percent of the dry sample. From 5 to 7 percent pentosans were found present. The sum of the percentages found by these three methods accounted for practically all of the reducing sugars produced by the original mild hydrolysis when they were calculated as fructosans.

LITERATURE CITED

1. Amos, G. L. and Wood, J. G, Australia J. Exptl. Biol. Med. Sci. 17, 283 (1939).

2. Archbold, H. K. Ann. Botany 2, 403 (1938).

3. Aspinall, H. O., and Wilkie, K. C. B. J. Chem. Soc. 1956, 1072.

4. Bennet, E. J. Biol. Chem. 146, 407 (1942).

5. Bennet, E. J. Plant Physiol. 15, 327 (1940).

6. Bennet, E. J. Plant Physiol. 26, 182 (1951).

7. Beroza, M. Chemist-Analyst. 45, 83 (1956).

8. Binger, H. P.; Sullivan, J. P., and Jensen, C. D. J. Agr. Food Chem. 2, 696 (1954).

9. Bondi, A. H., and Meyer, H. J. Agr. Sci. 33, 123 (1943).

10. Bonner, Plant Biochemistry, Academic Press Inc., N.Y., 1950, pp 71, 66.

11. Burkhart, B. A. Plant Physiol. 11, 421 (1936).

12. Comsden, R., Gordon, A. H., and Martin, A. H. P. Biochem. J. 38, 224 (1944

13. Deer. Intern. Sugar J. 18, 402 (1916).

14. de Grandchamp-Chauden, Andree. Compt rend 231, 1082 (1950) via C. A. 45, 6587g. (1951).

15. Drake, Birger Anal. Chem. Acta. 3, 452 (1949) via C. A. 44, 10571 (1950).

16. Ecklund, S. Kgl. Lantbruks-Hogskol. Ann. 16, 1297 (1943) via C. A. 45, 6755g. (1951).

17. Harper, R. H., and Phillips, T. G. New Hampshire Agr. Expt. Sta. Tech. Bull. 81, 1 (1943).

18. Jermyn, M. A., and Isherwood, F. A. Biochem. J. 44, 402 (1949).

19. Julander, Odell Plant Physiol. 20, 573 (1945).

20. McCready, R. M., and McComb, E. A. Anal. Chem. 24, 1986 (1952).

21. McDonald, E. J. Advances in Carbohydrate Chemistry 2, Academic Press, N. Y. 1946. p. 253.

22. McFarren, E. F., Brand K., and Rutkowski, H. R. Anal. Chem. 23, 1146 (1951).

23. McRary, W. L. and Slattery, M. C. J. Biol. Chem. 157, 161 (1945).

24. _Methods of Analysis_, AOAC, 6th ed. (1945) sec. 41.12.

25. _Methods of Analysis_, AOAC, 7th ed. (1950) sec. 22.39.

26. Moxon, A. L. Feed Age 4, 31 (1954).

27. Norman, A. G. _Biochemistry of Cellulose, Polyuronides, Lignins_, Oxford University Press, 232 pp., 1937.

28. Norman, A. G., Wilsie, G. P., and Gaessler, W. G. Iowa State Coll. J. Sci. 15, 301 (1941).

29. Paech, K. and Tracey, M. V. _Modern Methods of Plant Analysis_, vol. 2, Springer Verlag, Berlin, Gottingen, Heidelberg. p-197, 1955.

30. Partridge, S. M. Nature 164, 443 (1949).

31. Phillips, M., and Davis, B. L. J. Agr. Research 60, 775 (1940).

32. Phillips, T. G., and Smith, T. O. New Hampshire Agr. Expt. Sta. Tech. Bull. 81, 3, 11 (1943).

33. Sullivan, J. T., and Garber, R. J. Penn. State Coll. Agri. Expt. Sta. Bull. 489, 1, 2, 24 (1947).

34. Weihe, H. D. and Phillips, M. J. Agr. Research 74, 77 (1947).

VITA

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