A COMPARATIVE STUDY OF METHODS FOR THE QUANTITATIVE ESTIMATION OF CELLULOSE, HEMICELLULOSE AND LIGNIN

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A COMPARATIVE STUDY OF METHODS FOR THE QUANTITATIVE ESTIMATION OF CELLULOSE, HEMICELLULOSE AND LIGNIN

by

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INTRODUCTION

At present, the attention of research both in the purely theoretical and also in the practical field seems to be turning more to the importance of proper nutrition. In this connection we find research in the use of different products or by-products in the development of different feeds either from an economic standpoint or because some substance essential to a properly balanced ration may be gotten from certain of these products or by-products. Much research is being done on sources of protein, essential elements, vitamins, fats and the carbohydrate complex. A large amount of interest lies in the availability of these various feed constituents to the animal.

Since the protein, fat, and less complex carbohydrates are quite readily utilized by all animals, the availability here is not a question, unless they are tied up in some manner with the less easily digested portion of the feed. This less digestible complex usually consists of a large portion of the so-called roughage. In herbivorous animals, adaptations exist for utilization of feeds very high in the less digestible complex. We have such animals as the cow, deer, sheep, goat, camel and others with the rumen as the first organ of the digestive system, while in the horse we find the caecum and colon greatly enlarged. In many animals including humans, there is no adaptation provided for utilization of these difficultly digestible constituents.

Much work has been done towards finding a suitable means of determining the digestibility of feeds, but due to the unknown chemical structure of these complex substances making up the less digestible portion, the field of analysis seems to be in a constant state of change. Feeds have been analyzed in a good many ways, as will be shown later in this paper.

Feeding stuffs have been analyzed on the basis of protein, ash, crude fat and crude fiber. A combination of the nitrogen-free extract and crude fiber has also been used as a scheme of analysis. More recently, the trend has been to divide the less digestible complex into substances which could be more or less defined by chemical structure. This appears to give a much better basis for analysis than did former methods of dividing the complex into indefinite and varying chemical fractions. The complex has been divided more recently into cellulose, hemicellulose, and lignin. The analysis of these compounds in the feeds has formed the more recent work on methods of feed analysis. There have been many methods brought forth for the analysis of cellulose, hemicellulose and lignin, but the purpose of this thesis was to compare results by the three more prominent methods; namely, those of Olmstead and Williams (1), Crampton and Maynard (2), and Davis and Miller (3). In order to produce comparative results, the same samples were analyzed by all three methods. This thesis was the result of a great deal of interest concerning the feasibility of using one or a combination of these methods in determining digestibility coefficients in some nutritional investigations.

HISTORICAL

Analysis of feeding stuffs

The history of feed stuff analysis is rather old and quite stagnant. Generally, the methods of feed analysis may be divided into two groups; namely, chemical and enzymatic or combinations of both.

The first significant method was the old hay-equivalents brought out by Thaer in 1809. He estimated that ninety pounds of dry hay or clover, or vetch, or alfalfa, or sainfoin, or 200 pounds of potatoes or 266 pounds of carrots, or 350 pounds of rutabagas with tops, or 460 pounds of beets with tops, or 525 pounds of radishes, or 600 pounds of white cabbage were equal to 100 pounds of meadow hay in feeding value. The failure here to consider the protein fraction led to inconsistent results. In 1831, Davy suggested evaluating feeding stuffs by the amount of extract that was removed by digestion with hot water. In 1844, Bouissingault proposed the evaluation of feed stuffs upon the basis of their nitrogen content as compared with ordinary hay of 1.34 per cent nitrogen.

The analysis of feeds proceeded more or less along the old hay-equivalent methods until Hennenberg broke away from it entirely. Hennenberg divided his analyses into five parts: fats, proteins, carbohydrates, ash, and moisture. The sum of these five determinations subtracted from one hundred gave Hennenberg his nitrogen-free extract. Even at this early date, Hennenberg saw possible discrepencies in his crude fiber determination.

In 1864 Hennenberg and Stohmann proposed a weak acid and base digestion which we know today as the Weende crude fiber method. This method has remained essentially the same and has been adopted by the Association of Official Agricultural Chemists with only slight modifications. Bedwell (4) and Lewellin (5) have developed methods with only slight modifications of

Hennenberg's original one.

Sherman (6) in 1896 made probably the earliest attempt in the United States toward modifying Hennenberg's old scheme of analysis. He divided his method into five parts. The soluble carbohydrates were removed by heating with two and one-half per cent hydrochloric acid for two hours. He next treated the residue with malt extract and treated it in the same way as the first filtrate, to yield starch. The residue was then treated with one to two and one-half per cent sulfuric acid and alcohol, made up to two per cent sulfuric and boiled for six hours, after which reducing power was determined to get the free pentosans. Ash and proteid was then determined on the residue which had been previously weighed. Another sample of residue was treated with one per cent sodium sulfite and heated. This residue was weighed and corrected for proteid and ash and the loss was equivalent to the lignin and allied substances. The residue from the above minus proteid, if present, was considered to be cellulose.

In 1899, Brown and Beistle (7) modified Sherman's scheme during their work on dried brewers' grains. Their scheme consisted of crude fat, moisture, sugar, dextrin, starch, lignic acids, lignin, cellulose, pentosans, protein, and ash. These gave a total of 96.01 per cent out of a possible one hundred, and it is one of the best results ever obtained with this type of analysis.

In 1904, Simon and Lobrish (8) developed a new method for feed analysis, namely, the use of strong potassium hydroxide and hydrogen peroxide. In the same year Konig (9) proposed the use of glycerol sulfuric acid.

In about 1910 Tollens and his pupils introduced a method for the estimation of pentosans, as calculated from the yield of furfural.

Steigler in 1913 developed a method in feed stuffs analysis involving the use of ten per cent hydrochloric acid and a stream of air. Some years

later, (1930) Fellenberg used nitric and acetic acids in feed stuffs analysis, which today has become (with modifications) the generally used method for cellulose. In 1931 Scharr and Kurschner modified this somewhat by using acetic acid, nitric acid, and trichloracetic. In this same year Remy (10) introduced probably the first enzymatic method, in which he used enzymes to isolate cellulose, lignin, and hemicellulose from the starch, protein and fat. He compared his values with the crude fiber values, and found a great loss of the indigestible residue. Williams and Olmstead (1) in 1935 proposed the use of pancreatin digestion preliminary to their determination of hemicellulose, cellulose, and lignin. In 1936 Horwitt, Cogwill, and Mendel (11) first introduced the use of pepsin, diastase, and trypsin digestion. In the same year Norman (12) extracted and hydrolyzed the sample before analyzing the lignin. The method of Crampton and Maynard (2) in 1938 involved the use of pepsin before the determination of lignin. Davis and Miller (3) in 1939 proposed a method which made use of pepsin, clarase, and trypsin digestions before the lignin was determined.

Cellulose

In 1857 Schulze proposed the first method for the determination of cellulose. His method depended on the removal of lignin and the other non-cellulose carbohydrates by means of a mixture of nitric acid and potassium chlorate. This method was modified by Hennenberg in 1868 and by Hoffmeister in 1888. Many other modified methods were presented between the years of 1890 and 1910 by such chemists as Lange (1895), Klason (1903), Muller (1911), Koning (1913).

In 1897 Buhler secured a phenolic lignification patent which was later used by Kalb and Schoeller as a method for the determination of cellulose. In about 1906 Cross and Bevan developed a method which is commonly used today. The method made use of chlorine. This method was modified by Dean

and Tower (13) in 1907, Schorger (14) in 1917, Ritter (15) in 1924, and by Jenkins (16) in 1930. The modifications have consisted mainly in the manner in which chlorine is added to the determination. Opferman (17) in 1921 proposed a method for the determination of alpha, beta, and gamma cellulose. The insoluble portion in seventeen and one-half per cent sodium hydroxide is filtered and washed and weighed as alpha cellulose. The alkaline filtrate is acidified with acetic acid, precipitating out the beta cellulose, which is dried and weighed. The gamma cellulose is obtained by the difference between the total and the sum of the alpha and beta forms. In 1923 Bray and Andrews (18) used the same method except that they dissolved the alpha cellulose in seventy-two per cent sulfuric acid and titrated with potassium dichromate. The alkaline filtrate was divided into two portions, one of which was titrated with potassium dichromate, giving the beta and gamma cellulose. The second portion was acidified with ten per cent sulfuric to precipitate the beta cellulose, and the filtrate was then titrated with dichromate giving the gamma cellulose.

Kalb and Schoeller (19) in the same year brought out their method based on Buhlers phenolic delignification, in which the sample was treated with dry phenol and hydrochloric acid for several hours. In 1923 Sieber (20) modified the Cross and Bevans method by more carefully detecting the end point, thus preventing the cellulose from being attacked. In 1926 Kohmota and Sakaguchi (21) modified the Cross and Bevans method by extracting with two and one-half per cent potassium hydroxide previous to the determination. Waksman and Tenny (22) in 1927 presented a scheme of analyses for feed stuffs in which the cellulose was precipitated from the acid filtrate with alcohol. In the same year Kiesel and Semeganovski (23) proposed a method in which eighty per cent sulfuric acid was used to dissolve the cellulose. This mixture was diluted with water and heated for five hours. The

glucose present was then determined by one of the conventional methods.

Kurschner and Hoffer (24) in 1931 proposed a new method for cellulose which consisted of heating the sample with nitric acid and ninety-six per cent ethanol. Schmidt, Tuan-Chi Tang and Jandelbauer (25) in the same year introduced a method which used chlorine dioxide and pyridine. This treatment was supposed to remove lignin and everything else except the cellulose. Phillips (26) in 1932 introduced a new method using chlorine gas and sodium sulfite to remove the lignin. The delignified product was bleached with one-tenth per cent potassium dichromate and rendered colorless with sulphurous acid. It was then washed with ammonia and water and weighed as cellulose.

A novel method based on an idea that all cellulose, regardless of source, is equivalent colorimetrically was introduced by Paloheimo and Volavarra (27) in 1933. The sample is treated with alkali to remove all non-cellulose substances which are soluble in seventy per cent sulfuric. The residue is now taken up in seventy per cent sulfuric which is diluted to fifty per cent sulfuric and then a solution of potassium iodide-iodine, which gives an intense red color, is added. Olmstead and Williams in 1935 (1) introduced a method for cellulose in which the sample was first digested in a buffer-bile and pancreatin solution. The enzyme residue was treated with twenty-one and four-tenths normal sulfuric acid, which is diluted. The mixture is hydrolyzed and the reducing sugars (both total and non-fermentable) are determined from which the cellulose is calculated. Acharya (28) in 1936 proposed a method for cellulose involving the use of hypochlorous acid and sodium sulfite in removing lignin, etc. The carbon and furfuraldehyde content is then determined in the residue from which the cellulose and xylan content are calculated. In the same year Zizka and Kohler (29) introduced the method which is used as such or in a modified form in many cellulose methods today. The method involved the prolonged heating of the sample with a mixture of one

hundred parts of ethyl alcohol to twenty of nitric acid (d 1.29).

In 1937 Launer (30) introduced a simple volumetric procedure for the determination of alpha, beta and gamma cellulose by oxidizing with potassium dichromate. A micro method was introduced by Strepkov (31) at about the same time. The sample was treated with seventy-two per cent sulfuric. The solution was then neutralized and the glucose determined by the potassium ferrocyanide method. In 1938 Ubaldini (32) introduced a method using ten parts of eighty per cent acetic to one part of nitric acid. This oxidizes the xyloid and huminic lignites. The cellulose could be determined by treating the residue with eighty per cent sulfuric at room temperature, and then analysing for glucose. In 1938 Crampton and Maynard (2) introduced a method involving a slight modification of Kurschner and Hanak's (33) method, using an acetic acid-nitric acid mixture. One year later, 1939, Wise, Peterson and Harlow (34) introduced the use of a mixture of ethanolamine, chlorine water and sodium sulfite for isolating the cellulose.

Hemicellulose

Although the literature contains many references to attempts at estimating hemicellulose, there are practically no methods which are entirely satisfactory. Much more attention has been given by research workers to the separation into various fractions of the hemicellulose complex rather than the quantitative estimation of the whole complex. Tollens suggested probably the first method for the quantitative estimation of hemicellulose. Although the method was essentially an empirical one which requires adherence to a strict set of conditions, it has been used up to the present day. His method depends on the conversion of hemicellulose into furfural by distilling with ten to twelve per cent hydrochloric acid and precipitating it. The chances for error are obvious, because other plant constituents

such as pectin and uronic acids also yield furfural. Krober in 1900 modified Tollens method slightly using phloroglucinol as the precipitating agent. The method as outlined by Korber is now used and called Tollens furfural method. Much work has been done on the use of various precipitating agents for furfural. Dox and Plaisance (35) made an extensive study on the use of barbituric acids. 2,4 - dinitrophenylhydrazine has also been used as a precipitant. The serious objections to Tollens method or any of its modifications seem to be that any hemicellulose under investigation may consist of hexosans, pentosans, glucuronic acid, and galacturonic acid. The latter three constituents of hemicellulose give different yields of furfural. Hexosans, on the other hand, yield practically no furfural when boiled with ten to twelve per cent hydrochloric acid. Thus, the hemicellulose must be rather simple in composition in order to be determined by the furfural method. Dore (36) in 1920 determined the loss of weight for woods when treated with five per cent sodium hydroxide. Norman in 1929 described a method for the determination of hemicelluloses in cereal straws, based on the furfuraldehyde method. Preece (37) in 1931 introduced a method in which the hemicelluloses are actually isolated and weighed. The method is rather tedious and from all reports, apparently not too accurate. This was the first attempt to isolate hemicelluloses directly. Many methods have been developed based on the principle that plant material freed from sugar and starch and then hydrolyzed with dilute acid gives reducing sugars. The reducing sugars in the hydrolysate are then determined by the usual methods. The result is usually expressed in terms of glucose, although this sugar makes up only a small part of the reducing compound. A more serious objection is the fact that the hydrolysate may contain hexoses, pentoses, uronic acids, and possibly other substances which may reduce Fehlings solution. The reducing value then is quite conflicting, a nd it is practically impossible to obtain

a value which could be considered as representing hemicellulose. Olmstead and Williams (1) have modified this method. They have determined the total reducing sugars on the filtrate from their lignin determination and then fermented out their hexoses and determined their non-fermentable reducing sugars. The non-fermentable reducing sugars were interpreted as pentosans and the difference which was composed of the fermentable sugars was used for the calculation of cellulose.

Lignin

Numerous methods have been described in the literature for the quantitative estimation of lignin. Much has been done in the field of wood and paper chemistry in separating lignin from its combination with other plant constituents. Direct and indirect methods are employed, and the latter depend upon the estimation of some characteristic group, such as methoxyl, from which the per cent of lignin may be calculated. The indirect methods have proven very unsatisfactory and have been generally discarded. In the direct methods lignin is separated from the cellulose and other carbohydrates associated with it and weighed.

The direct methods may be subdivided into the following classes: (1) Those depending on dissolving all carbohydrate constituents and leaving lignin as the residue, and (2) those that dissolve the lignin and thus separate it from the cellulose and other carbohydrate material present. Treament with seventy-two per cent sulfuric acid and fuming hydrochloric may be considered as examples of the first class, whereas the method of Mehta (38) may be considered as an example of the second class, in which the lignin is dissolved away from the rest of the sample.

In 1883 Flechsig (39) showed that seventy-two per cent sulfuric acid will hydrolyze cellulose in the cold. In 1910 this fact led Ost and

Wilkening (40) to a method for the quantitative estimation of lignin. Their method has been modified by a great many people as to time, temperature and strength of acid. In 1923 Klason (41) modified the method by suggesting the use of sixty-four or sixty-six per cent sulfuric acid instead of seventy-two per cent. Waksman and Stevens (42) have used eighty per cent sulfuric for the isolation of lignin.

Fuming hydrochloric acid (d. 1.212 - 1.223 at 15°C.) was first used by Willstätter and Zechmeister in about 1913 (43) for the isolation of lignin. This method has been applied to the determination of lignin by many workers in its original or somewhat modified form. In 1914 Konig and Rump (44) isolated lignin by heating the material with one per cent hydrochloric acid under five atmospheres of pressure. Wenzl (45) in about 1923 used a mixture of phosphorous pentoxide and concentrated hydrochloric acid to remove polysaccharides, in determining lignin. The lignin isolated by the sulfuric or fuming hydrochloric acid methods contains some nitrogen and inorganic matter. Correction should be made for these. Waksman (42) determined the nitrogen in the crude lignin, and the weight thus obtained, when multiplied by 6.25 is deducted from the weight of the crude lignin. The assumption is that the nitrogen is in the form of protein. In 1922 Mahood and Cable (46) presented what is known as the original seventy-two per cent sulfuric acid method, which is, perhaps, most generally used in the estimation of lignin today. In the same year Phillips (47) introduced what is known as the original fuming hydrochloric acid method. This was modified by Goss and Phillips in 1936 (48). They compared both the original and modified seventy-two per cent sulfuric acid and fuming hydrochloric acid methods and found that the modified fuming acid method probably gave lignin results nearer the true lignin value. Olmstead and Williams (1) in 1935, Davis and Miller in 1939 (3) and Crampton and Maynard in 1938 (2)

used slight modifications of the seventy-two per cent sulfuric acid method.

Crude Fiber

The old Einhoff procedure originating in about 1860 and later used by Hennenberg and Stohmann in 1864 as part of their scheme of analysis of food stuffs has never been changed as far as the method itself is concerned. The method as used today is almost identical with the one first used by Hennenberg. In the literature only one reference to a change in the crude fiber method was found, and that was by Ring (49) in which a round-bottom flask was used under reflux instead of an open flask. He avoided foaming by blowing a stream of air through a constricted glass tube whose tip was about one and a half or two centimeters above the level of the solution.

Nitrogen-free Extract

The so-called nitrogen-free extract of foods and feeding stuffs has been a subject of discussion among Agricultural Chemists since it was first introduced by Hennenberg and Stohmann nearly eighty years ago. The term represents something very complex and indefinite. Hennenberg and Stohman secured its general adoption through their efforts to bring about improvement of current methods of feed stuff evaluation at that time. Davy attempted something similar at the beginning of the nineteenth century. He extracted feed with hot water and supposed this extract to correspond to what Hennenberg and Stohmann extracted with acid and alkali. Davy was of the opinion that the greater the amount extracted with hot water, the more valuable were the feeding stuffs. The nitrogen-free extract of Hennenberg's was equal to the total dry organic materials minus the sum of the crude fiber and the protein. There were many serious objections to this procedure, so that little use is made of it today in the field of nutrition.

DESCRIPTION

Cellulose

Cellulose is perhaps the most abundant organic compound occurring in nature. Celluloses are the principal constituents of the cell walls of all higher plants. Although cellulose is most familiar to the average person in the form of pure cotton or filter paper, this is about the only case in which cellulose is found relatively pure. Cellulose is scarcely ever found in a relatively pure condition, except in the cases previously mentioned. Bragg has said that cellulose is preëminently the molecule of growth in the vegetable world. The cellulose from the cotton boll on extraction with alcohol and ether to remove fat, treatment with boiling dilute alkali and washing gives a product which is ninety-nine and eight-tenths per cent cellulose and five-hundreths per cent ash and is resistant to extraction with seventeen and one-half per cent sodium hydroxide in the cold. This product has been the starting point for all researches on the constitution and properties of cellulose. However, in most cases in plants where cellulose plays a structural role, the cellulose does not occur in a pure or easily purified state, but it is found in intimate association with other cell wall constituents. Earlier it was believed that cellulose existed in combination with cell wall substances such as lignin, pectin, cutin and others to form lignocellulose, pectocellulose, and cutocellulose. This theory was discarded, although some botany textbooks still list such compounds. The chief point of support for the theory existed in the fact thay lignin could not be readily extracted from jute fiber and thus it was concluded that lignin must be combined chemically with cellulose. Bailey and Norris (50) found the existence also of an amorphous cellulose in combination with other polysaccharides in certain mucilages, but this appears to be due to the physical properties of the polysaccharide. The modern

and generally accepted theory of steric structure of cellulose rules out the possibilities of cellulose acting as a structural constituent in these cell wall combinations. Although the structural cellulose of the cell wall cannot be combined in a true chemical sense with any other cell wall constituent, its separation from them is exceedingly difficult. No single method will accomplish this without attacking the cellulose also. This makes the determination of cellulose a difficult task.

Structural celluloses are largely composed of the same polysaccharide as the cotton hair (alpha cellulose), although not necessarily in the same state of molecular aggregation. The remainder is another polysaccharide or mixture of polysaccharides firmly retained. The associated polysaccharides found in most structural cellulose is a xylan, although in the Gymnosperms both mannan and xylan are found. A means of describing this associated material was suggested by Hawley and Norman (51) who proposed calling this associated material "cellulosan".

Constitutionally, cellulose has generally been accepted as being composed of a long chain (giving fiber strength) of 1,4-beta glucose anhydride formula. Some proofs of this structure are:

(1) Almost quantitative yield of glucose from cellulose on hydrolysis.

(2) Hydrolytic production of crystalline fragments of the cellulose chain consisting of two, three and four glucose units.

(3) Formation of a triacetate.

(4) Formation of a well-defined crystallizable octacetate of cellobiose by acetylation.

(5) Identification of beta glucosidic residues and a simple chain structure by the study of rotations and kinetics of cleavage products of cellulose hydrolyzed by strong acids at moderate temperatures.

Thus the cellulose molecule is considered to be a chain of considerable length, the individual unit of which is anhydro beta glucose, the linkage between the units being between the one and four carbon atoms, with the terminal carbon lying alternately on one side of the chain and then the other. There seems to be a great deal of discrepancy on chain length and molecular weight. One group working on physical and chemical properties point to a chain length of one-hundred fifty to two-hundred fifty units. Another group using viscosity measurements gives a chain length as seven to eight-hundred units. Studies seem to indicate a maximum chain length of over twelve-hundred units with a molecular weight (maximum) of over two-hundred thousand. The micro structure of cellulose is in units called micelles, consisting of about sixty cellulose chains or one-hundred to one-hundred twenty glucose units.

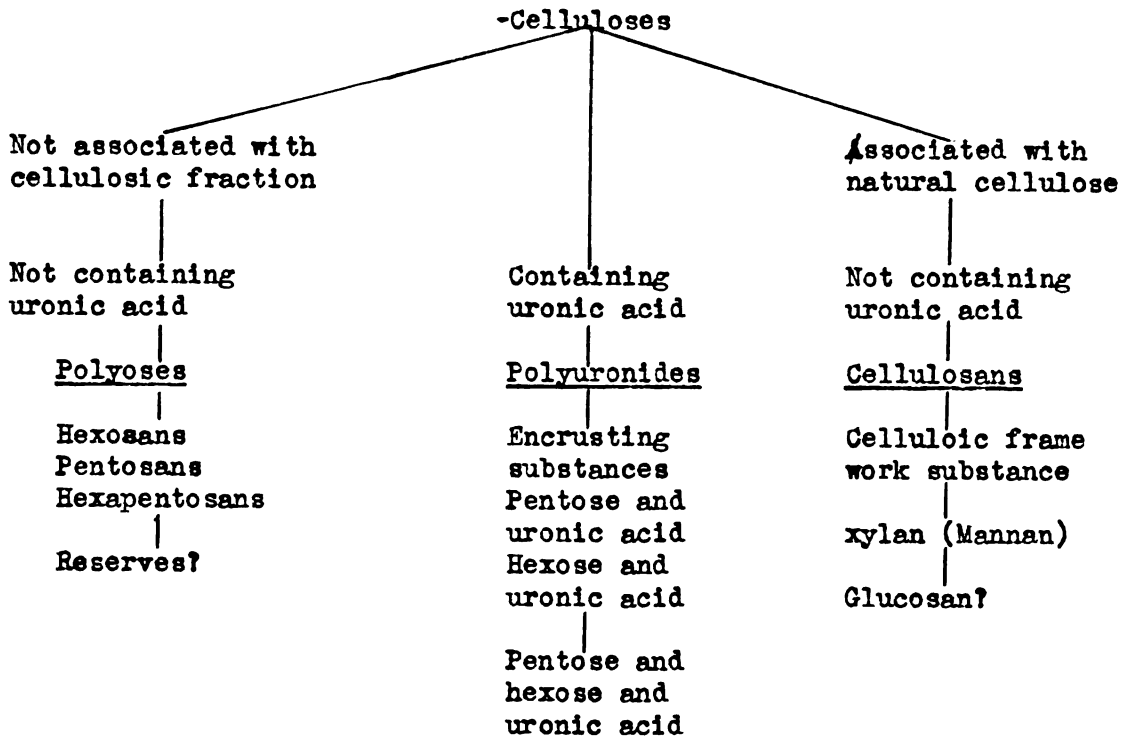
Cellulose is rather inert and has an exceptionally high tensile strength. It is insoluble in water, and on treatment with cold seventeen and one-half per cent sodium hydroxide, alpha cellulose is left as the residue. Cellulose is soluble in seventy to eighty per cent sulfuric acid, and in forty-two per cent hydrochloric acid. Certain inorganic salts such as zinc chloride and hydrochloric acid will also dissolve cellulose. Cellulose swells in the uptake of water, but it is not directional. Cellulose is decomposed by many organisms, such as aerobic and anaerobic bacteria, mesophilic and thermophilic fungi, actinomyces and even protozoa. The attack on cellulose is thought to begin with an exo-enzyme, cellulase, which liberates the glucose units from the cellulose chain with subsequent fermentation, the products formed depending on the organism.

Hemicellulose

If one considers the methods available for the determination of hemicellulose, one is compelled to agree with Norman that "the chemistry of the

hemicelluloses remains to be written." Their complex structure and physical properties make examination very difficult. The generic term "hemicellulose", a very unfortunate one, originated in 1892 from the work of Schultze. These substances are much more susceptible to acid hydrolysis than cellulose, but were believed to be related in some way, probably as intermediate in its formation, hence the name.

Various sugars (arabinose, xylose, and galactose) have been isolated from the hydrolysate of hemicelluloses. The preparations usually contained more than one sugar. Until the last decade, the hemicelluloses of Schultze were believed to be true hexosans or pentosans or hexo-pentosans containing both units. The presence of sugar acids of the uronic acid type has also been demonstrated in hemicelluloses, and the true hexosan or pentosan, except cellulosan, is believed to be very rare. Glucuronic and galacturonic acids are commonly found in plant materials. Mannuronic acid has been isolated in certain type of morine algae. The occurrence of mannuronic acid in plant hemicelluloses is quite improbable, due to the hexose to pentose formation through the formation of uronic acid being incomplete in the mannose series. The pentose member of the mannose series would be d-lyxose which has never been known to occur naturally. There are configurational groups of hemicelluloses: (1) the glucose series (d-glucose, d-glucuronic acid, and d-zylose) and (2) the galactose series (d-galactose, d-galacturonic acid and l-arabinose). Xylose usually predominates in the first group; galactose, in the second. A definition of hemicelluloses might be: those plant constituents soluble in dilute alkali (cold four to ten per cent NaOH), much more easily hydrolyzed than cellulose, and appearing to be used by plants in growth as a reserve food. Norman (52) has given a concise definition and differentiation:



Much work has been done on the fractionation of hemicelluloses, but due to imperfections of the extraction methods, the chemistry of the hemicelluloses has been complicated to a great extent.

Hemicelluloses as previously mentioned may be extracted from plant tissues by alkali and are readily hydrolyzed by hot dilute mineral acids to give reducing sugars and uronic acids. They are non-reducing and in spite of the presence of uronic groups, they do not exhibit acid properties. Many celluloses may be precipitated from alkaline solution by Fehlings solution, copper salts, lead acetate and other metallic salts. They may be methylated and acetylated by the usual methods, although it is difficult to carry these reactions to completion. With alkali and carbon disulfide, a zanthate may be obtained. The majority of hemicelluloses give either no color or a slightly greenish color with iodine. The hemicelluloses are optically active and usually are more or less strongly levo-rotatory.

In young tissue cells only small amounts of hemicellulose are found, combined with about fifty per cent true cellulose and some pectic substances, but no lignin. In old tissue cells we find a great deal more

hemicellulose, up to twenty per cent combined with about fifty per cent true cellulose; up to twenty per cent lignin and practically no pectic substances.

Hemicelluloses have an important influence on the biological decomposition of the plant material in which it is found. They are readily fermented by mixed cultures of micro organisms and especially the fungi. Evidence has been found by Fenny and Waksman (53) that in aerobic rotting of plants, the hemicelluloses are the first constituents removed.

Lignin

Although lignin has been studied by chemists for about a century, much of the chemistry of this substance is still imperfectly understood. Much progress has been made in recent years in the studies concerning lignin structure, and much still remains to be done. From time to time, investigators have proposed probably structural formulae for lignin, but most of them lack any definite chemical evidence to support them. This is primarily due to the fact that a lignin isolation method has never been developed which would isolat e lignin in the pure state. Thus no means of determining its purity is available. Lignin is an amorphous substance ranging in color from light tan to a black, depending on the method used for its isolation. Evidently, lignin is so tied up with the cell wall encrustations that it is very difficult to separate in a very pure state. The isolation methods employed have either not produced pure lignin or they have altered the reactive groups present. The greater part of the work on the structural formula, consequently, is of doubtful value. The general assumption seems to be that lignin is homogeneous in nature, but there is a large amount of evidence to show that this is not the case. Lignin found in different species is not identical. Even the nature of

lignin in one plant depends on the age and nature of the tissue.

Lignin may either be a complex mixture of compounds with similar properties but of unrelated chemical structure or may have the same basal form varying in minor ways only, such as side-chains or substituted groups present or in chain length or degree of polymerization. Due to the work of Hilpert, a third idea of structure may be added. This considers that lignin has no real existence, but is merely an artifact or a reversion product formed by the action of acid on certain of the methylated carbohydrates. The idea that lignin is a mixture of related compounds is most generally accepted and has a certain amount of evidence in support of it. Lignin is found chiefly in the middle lamella and mainly in layers in the secondary wall. The proportions of carbon, hydrogen, and oxygen found on analysis of lignin are not those of a carbohydrate. The carbon content is too high and the oxygen, too low. The methoxyl group is the only group of the lignin molecule that is universally accepted as being present. Many phenolic compounds have been isolated from lignin, supporting the existence of the aromatic ring in the structure of the lignin molecule. Klason has attempted to show that lignin is a derivative of coniferyl alcohol by polymerization and oxidation. Fuchs (54) has suggested a very complex aromatic ring structure with methoxyl groups substituted around the rings. Freudenberg has suggested a quite simple lignin structure, somewhat like the pattern of cellulose.

Lignin exhibits a notable resistance towards strong acids, while alkalies dissolve it more or less readily. Organic solvents have little effect on it. Lignin is very easily oxidized by such agents as hypochlorites, hydrogen peroxide, potassium permanganate and ozone, giving lower fatty acids and di-basic acids. Lignin may be very rapidly nitrated with nitric acid or nitrogen peroxide in the cold, but sulphonation requires

more drastic conditions. In the presence of strong acids, lignin condenses with aldehydes and ketones to give more or less insoluble products of the phenol-aldehyde type. Lignin is unsaturated, as shown by its combination with bromine. Lignin may be methylated fairly easily. It gives the Molisch test, the phloroglucinol test, and the indol test. Considerable work has been done in recent years on the microbial decomposition and digestibilities of lignin in the animal body, but much of this work is very contradictory. Many investigators claim that lignin is digested in part by certain animals, while others deny this fact. Generally there is considerable evidence to show that lignin in plant material is relatively resistant to microbial attack, at least under anaerobic conditions. There is data indicating that when alkali lignin is fed to cows and dogs, there is an increase in benzoic acid (as hippuric acid) eliminated in the urine, the benzoic acid apparently coming from the aromatic portion of the lignin. Csonka, Phillips and Jones (55) have shown that the lignin was in part dimethoxylated in these feeding experiments. Some of the higher fungi have been found to be capable of using a part of the lignin from such sources as straw and timber. Under anaerobic conditions, isolated acid lignin has been shown by Boruff and Buswell (56) as probably having a bacteriostatic action, for on addition to an actively fermenting glucose substrate, gas production instantly ceases and cannot be revived by further additions of glucose. Alkali lignin similarly has a restrictive action. The only lignin derivative examined, phenol-lignin, was found to be decomposed by a number of bacteria and fungi.

Nitrogen-free Extract

As determined by the customary procedure for the analysis of feeding stuffs, the nitrogen-free extract is the largest component of the rations

of animals, representing forty to seventy per cent of the total dry matter. The nitrogen-free extract serves as a source of energy for the body processes and for the deposition of fat. It is not the total amount or the caloric content of the extract that makes it so important nutritionally, but only that fraction of the gross energy which is available to the body. This availability is controlled by digestibility and other metabolic factors, which are related to the chemical nature of the nitrogen-free extract. Thus the nigrogen-free extract in order to be of real value has to be broken down into units which can give some answer as to the availability of the feed. The term obviously does not represent a single constituent, but a residiuum of numerous undetermined substances of variable nutritive value, whose calculation by difference is multiplied by the errors in the methods for determining fat, protein, ash, and especially crude fiber.

The nitrogen-free extract contains starch, hemicellulose, lignin, pectin, and various related substances. Starch as such is almost entirely digested by the animal under the action of enzymes. On the other hand, there are no enzymes secreted by animals which will digest cellulose, or lignin, and this appears to hold true for most of the groups of compounds making up the hemicellulose. Several investigators have failed to find a pectin or an inulin digesting enzyme in animal tissues.

In assuming that the nitrogen-free extract comprises the more readily digestible group of plant constituents, it was quite commonly believed that during treatment with hot dilute acids and bases, only sugars, starches and hemicelluloses were dissolved. However, it has been shown that various polysaccharides and polyuronides and varying amounts of lignin were also extracted. Recent criticisms of the nitrogen-free extract have been based on the fact that no consideration has been given to the lignin fraction. No definite relationship between the hemicellulose and lignin

fractions in the nitrogen-free extract can be established, since their distribution in plant materials varies with the plant and its degree of maturity. The hemicellulose fraction of the extract consists not only of the polymers of hexoses and pentoses, but also of mixtures of these.

Crude Fiber

That portion of feeding stuffs which is insoluble in hot one and twenty-five hundreths per cent sulfuric acid and hot one and twenty-five hundredths per cent sodium hydroxide after correction for ash is called "crude fiber". The original investigators knew that the crude fiber product did not have a constant chemical composition as was shown by the varying percentages of carbon, hydrogen, and oxygen. The indigestible residue, (the portion of the feed not attacked on passage through the digestive system and consisting chiefly of cellulose, hemicellulose, and lignin) contains according to many workers fifty percent more indigestible material than did the crude fiber product. In the crude fiber product, practically all of the hemicellulose is lost and some of the lignin. Coleman (57) and nine colloborators analyzing bran found the crude fiber results unsatisfactory. Remy (10) found in comparing his enzymatic method with the crude fiber method that the latter caused a fifty per cent loss in the indigestible residue.

This seems to indicate that the crude fiber product is defined by the method used to isolate it. Hennenberg himself recognized, as previously mentioned, that his product was quite variable, but he hoped it would serve a purpose in feed analysis methods for the time being, for he thought that soon methods would be developed for determining the various constituents of feeding stuffs. He used the term "crude fiber" because he knew it was of a variable nature. How disappointed he would be today if he saw

that his hopes were yet far from realized.

It seems, therefore, at least from the standpoint of research, that more specific methods for the determination of the complex carbohydrates are needed. This is particularly true for the rations of herbivorous animals. The major role which the higher carbohydrates play as energy foods, especially for herbivora, and the fact that feeds measured by the present methods which appear to have similar composition are found to be quite different in feeding values emphasize the need for the development of more exact chemical and biological methods of analysis.

METHODS AVAILABLE

That the digestibility of the dietary carbohydrate does not follow its partition into crude fiber and nitrogen-free extract with any marked certainty, especially in the case of roughages, is evident from data collected over a period of years. Published literature contains ample evidence that the crude fiber of forages may be as well digested as the protein, as is shown by the work of Jones and Newlander, Mitchell and Hamilton, and Morrison. It would seem, therefore, that if partition of the carbohydrate fraction could be made into parts which were either biological or chemical units, the usefulness of the feeding stuffs analysis in predicting probable feeding value would be enhanced. Most workers on evolving their schemes of analysis of the indigestible residue thought that a partition of the carbohydrate fraction into useful biological or chemical units, would become largely a consideration of the chief constituents of the cell wall carbohydrates, lignin, hemicellulose, and cellulose.

Cellulose

Two general groups of methods exist for the determination of cellulose: (1) those depending on the removal of everything but cellulose in the plant material, and (2) those depending on the hydrolysis of cellulose to glucose and its determination. Methods coming under the first class would be those of Cross and Bevan (chlorinating to remove all but the cellulose), and Crampton and Maynard (removal of all encrustating substances by means of nitric-acetic acid mixture). There are many methods which are on the same idea as these examples mentioned, but these are the most general. The method of Crampton and Maynard was used in the present work. Under the second class any of the copper reduction methods may be used to determine the glucose. The Shaffer-Somagyi method used by Olmstead and Williams was

also employed in these studies.

Hemicellulose

There is no absolutely satisfactory method available at present for the quantitative estimation of the hemicelluloses. The available methods may be conveniently divided into three types. In the first type, the plant material is freed from sugar and starch, hydrolyzed by dilute acid and the reducing sugars determined by any of the available methods. The second type of method depends on the measurement of furfural secured on boiling the material with mineral acids. The third type depends upon the isolation of the hemicellulose and the weighing of it. All of the methods have weaknesses. Olmstead and Williams modified the first method by determining the total sugars before and after fermentation with yeast. In this manner, they secured both the cellulose and hemicellulose. This procedure was used in the present studies.

Most investigators seem to stay away from determining hemicelluloses because of their complex and variable make-up. They prefer to determine the indigestible residue by an enzymatic method and the lignin and cellulose by separate methods. By subtracting the sum of the cellulose and lignin from the indigestible residue, they secure the hemicellulose values. They have termed this difference, not hemicelluloses, but "other carbohydrate material". Davis and Miller (3) have used this scheme, and it was also used in comparing it with other methods in these studies.

Lignin

Numerous methods have been described previously for the determination of lignin. Generally they may be classified as direct and indirect. The indirect methods have been discarded. The direct methods have consisted of isolating lignin by removing other carbohydrates associated with it. The

methods available are divided into two general methods: (1) seventy-two per cent sulfuric acid, and (2) fuming hydrochloric acid method. These both have been modified, so that we actually have four types available. Olmstead and Williams (1) used the sulfuric acid method but modified it by using sixty per cent sulfuric acid instead of seventy-two per cent. Crampton and Maynard (1) used the seventy-two per cent method, but modified it by treating with formaldehyde and using a granulating mixture of chloroform and acetic acid. Davis and Miller (3) used the seventy-two per cent sulfuric acid but modified it by finally diluting the acid mixture out to three per cent sulfuric and refluxing for two hours. A comparison of all three of these methods on the same materials was carried out in the present investigation.

EXPERIMENTAL

Scope of Problem

A great deal of interest has been created in this department in some nutritional work on the digestibility of certain foods. Much work has been carried on and suitable methods for analysis were found to be quite essential. It was thought an interesting problem could be made in seeing how some of these methods compared with each other on the same material. For a scheme of analysis of the nutritional work, it was decided to divide it into the newer units of cellulose, hemicellulose and lignin. The methods chosen for comparison on these three constituents were those of Olmstead and Williams (1) Crampton and Maynard (2) and Davis and Miller (3).

In working on the lignin determination, highly irregular results were found, and it was thought that there might be a relationship between the method of treating the sample before the lignin determination and the lignin value. This idea was carried out in some work which will be described later in this paper.

In some nutritional work being carried on here, there arose the question as to the effect of particle size and heat treatment on the analytical results. Bran was chosen as the substance and crude fiber as the method, and investigations were carried out, the description and summary of which will follow later.

Samples

1 - Composition

The samples used were fecal and food samples collected in the course of a study of the coefficients of digestion of various foods, such as apples, lettuce, cabbage, bran and others. The experiment consisted of feeding a basal diet for one week, followed by the basal diet plus a

supplement of some food, the digestibility of which was to be determined. The subjects were fed weighed amounts of all constituents of the diet and the samples used in this work were the fecal and food samples collected during the experiment.

The basal diet was composed of the following foods: potatoes, meat, cheese, milk, sugar, tea, bread, puddings (cornstarch), jello, jam, crackers, cookies, ice cream. The experiment lasted eight weeks, the plan being given below.

First week -- basal

Second week -- basal and all bran

Third week -- basal

Fourth week -- basal and prunes

J.L., C.T., L.O. (by three people)

basal and celery

H.P., A.S., S.C. (by three people)

Fifth week -- basal and all bran

Sixth week -- basal and lettuce

L.O., H.P., S.C. (by three people)

basal and cabbage

J.L., A.S., C.T. (by three people)

Seventh week - basal

Eighth week -- basal and oranges

J.L., H.P., S.C. (by three people)

basal and apples

C.T., L.O., A.S. (by three people)

2 - Treatment

The material was dried on steam baths in eight inch evaporating dishes for about five to six days.

The samples were ground in a Wiley mill and portions extracted with ethyl ether for the various analyses.

Methods of Olmstead and Williams

Work leading up to the methods

Methods of analysis of cellulose, hemicellulose, and lignin depend first upon removal of protein, fats, resins, gums, and starch. Olmstead and Williams first tried Remy's enzymatic method which consisted of: pepsin-HCl, neutral malt diastase, pancreatin-sodium carbonate. By this method, they found the starch completely removed, also the proteins, fats, and resins were adequately removed, but with a substantial loss (twelve per cent) in the hemicellulose. Successive analysis of the steps indicated that the loss was essentially in the malt diastase treatment. Leurs found in his study of the malt enzyme, zytase, that it split hemicellulose. They then tried another plant diastase, taka diastase, but found a much greater loss of hemicellulose (thirty-one per cent). Finally, they found that animal diastase, pancreatin in neutral solution, removed starch without concurrent loss of hemicellulose. The proteolytic enzymes of the pancreatin are quite efficient and the amyloytic power is not greatly reduced at a pH of eight. To obtain simultaneous digestion of protein and starch, the pH of the digest was adjusted to eight and the hemicellulose recovery indicated that none of it was lost in the feces and wheat bran. However, when the treatment was applied to air-dried vegetables, as much as a forty per cent loss occurred. In fatty materials, bile salts may be important in the pre-treatment. It has already been indicated that cellulose is dissolved by sixty to seventy-two per cent sulfuric acid and lignin is dissociated from it. Thus, it would appear that the first treatment for the quantitative analysis of lignin, cellulose, and hemicellulose is the treatment with strong sulfuric

acid after the enzymatic digestion. Olmstead and Williams found seventy-two per cent sulfuric acid at six to ten degrees had a tendency to char; the pentosan recovery was low, and the lignin value was high. Jenkins found that a lignin-like material was formed from pentose by treatment with seventy-two per cent sulfuric acid. Seventy-two per cent sulfuric acid caused a loss of hemicellulose, but sixty per cent sulfuric acid did not. The optimum for pentosans was fifty per cent sulfuric acid. With fifty per cent sulfuric acid, the cellulose is precipitated on being diluted to four per cent. Sixty per cent sulfuric acid does not appreciably decrease the yield of pentose and converts cellulose between ninety-six to ninety-eight per cent; therefore, sixty per cent sulfuric acid was adopted by Olmstead and Williams.

In using strong sulfuric acid for dissolving cellulose and then diluting to hydrolyze the cellulose into glucose, Williams and Olmstead found that the maximum reduction was obtained at the end of two and one-half to six hours. Consequently, three hours was selected as the optimum time. The higher the temperature, the less time was needed. Sixty per cent sulfuric acid at six to ten degrees reached an optimum between sixteen and thirty-four hours.

To summarize, the principle of the analysis is this: Twenty-one and four-tenths normal sulfuric acid under controlled conditions dissolves the cellulose and hemicellulose completely and dissociates them from the lignin. The twenty-one and four-tenths normal acid is diluted to four per cent and boiled for three hours. The lignin is precipitated quantitatively, whereas the cellulose and hemicellulose are converted into their constituent simple sugars, which are soluble. The lignin is then filtered and weighed. The cellulose and hemicellulose, now in the form of simple sugars, are determined by copper reduction. The non-fermentable reduction

representing the hemicellulose, and the fermentable the cellulose and mannans. Reduplication of results depends primarily on one's familiarity with copper reduction technique. Williams and Olmstead also stress the importance of adjustment of the sugar solutions to neutrality before analysis.

Description of the methods

The reagents necessary are:

(1) Buffer-bile salt solution which consists of fifty cc. of .2M potassium acid phosphate, twenty-three and four-tenths cc. of .4N sodium hydroxide, six and six-tenths cc. of water, and two grams of sodium taurocholate.

(2) A pancreatin-sodium chloride solution prepared daily and consisting of one-hundred cc. of eight and one-half per cent sodium chloride, and ten grams of pancreatin, the mixture being shaken for one-half hour and filtered.

(3) A sixty per cent sulfuric acid solution is made by diluting six hundred cc. of C.P. ninety-five per cent sulfuric acid to one liter.

If the sample is dry, it may be weighed directly. In the case of feces, if the fresh stools are to be used for analysis, the moist weight is obtained and multiplied by four, and this amount of water added. The diluted sample is then ground in a ball mill for twenty minutes until it passes through a twenty-mesh seive. Twenty-five cc. of this mixture is then used in the determination. The sample is then transferred to a fifty cc. glass stoppered bottle, steam sterilized at fifteen pounds pressure for thirty minutes and then cooled below fifty degrees. This kills the spores and gelatinizes the starch. Twenty cc. of the buffer bile solution, five cc. of the pancreatin-sodium chloride solution and a few drops of

toluene are added, and the sample incubated for three days at forty-five degrees C., with occasional shaking. The mixture is then filtered through one-hundred twenty-five mesh silk cloth or centrifuged. The filtrate containing the sugars, and non-carbohydrate material is discarded. The residue containing the non-sugars hemicellulose, cellulose, and lignin are washed with two hundred cc. of water, fifty cc. of hot alcohol, twenty-five cc. of hot benzene, and finally twenty-five cc. of ethyl ether. The residue is transferred to a fifty cc. glass-stoppered container before the ether is completely evaporated. The residue is then dried in an oven at seventy degrees C. for two hours or until residue is dry. Twenty cc. of chilled (six to ten degrees C.) twenty-one and four-tenths normal sulfuric acid is added, and the flask is briskly shaken at hourly intervals (particularly during the first five hours) and is kept in the refrigerator for twenty-four hours. The mixture is then rapidly diluted to three hundred cc. with distilled water, refluxed for three hours, cooled to room temperature, filtered through a loose layer of asbestos in a Gooch crucible and washed successively with water, hot alcohol, benzene, and ether. The residue is dried at one hundred and ten degrees, weighed, ignited, and reweighed, and the loss of weight calculated as lignin. The filtrate containing the cellulose, hemicellulose, and uronic acids as reducing sugars, is neutralized with fifty per cent sodium hydroxide to phenol red. The filtrate is then diluted to five hundred cc. Forty cc. aliquots are fermented by the Somogy washed yeast procedure, and the reducing sugars are determined by the Shaffer-Somogy method. The total reducing sugars are determined with aliquots of the unfermented filtrate by the Shaffer-Somogyi method also. The non-fermentable reduction is interpreted on the xylose-arabinose curve and multiplied by the factor .88 to convert pentose to pentosan (hemicellulose). The fermentable reduction is interpreted on the glucose curve

and multiplied by the factor .9 to convert it to cellulose.

Data

The samples as described previously in this paper were analyzed for cellulose, hemicellulose, and lignin by these methods outlined by Olmstead and Williams. The results of these analyses will be found in tables I to V, inclusive

Methods of Crampton and Maynard

Work leading up to the methods

As a result of the work of such men as Normen and Jenkins, Goss, Phillips, and others, Crampton and Maynard decided upon using some form of the seventy-two per cent sulfuric acid method for lignin. The special problem in the case of forage and animal feces lies in the removal of protein without a simultaneous removal of part of the lignin. According to the present information, lignin is soluble in varying degrees in dilute alkali (hot or cold), boiling water, and dilute mineral acid (1.25 per cent sulfuric acid at 100° C.). Pre-treatment by enzyme digestion, however, would seem to be a suitable possibility. In searching for a suitable method, they studied the work of Olmstead and Williams, who in studies on human diets and feces had used a buffered pancreatin solution at a pH of eight. In their proposal, cellulose and hemicellulose were determined on the filtrate remaining on the removal of lignin. Inasmuch as a slight amount of hemicellulose is removed by the alkaline pancreatin solution, a correction is made by analysing an additional sample of the diet, omitting pancreatin. Lignin values are taken from the sample digested with pancreating. If lignin were isolated from both the enzyme and non-enzyme residues, any effect on the pre-treatment on the lignin would be seen. When this treatment or procedure was tried with sheep and steer diets of grain

and hay, the results in every case showed a smaller lignin value in the pancreatin treated sample. It seems probably that the long exposure (seventy-two hours at forty-five degrees C.) in a buffered solution at a pH of eight, using sodium hudroxide as the alkali, might have dissolved some of the lignin and thus resulted in lower lignin values. Another difficulty was encountered in the use of the Williams and Olmstead procedure. It was found impossible in the case of animal diets to get complete solution of the enzyme residue in the concentrated sulfuric acid. Subsequently, when a lignin balance was attempted in a steer digestion trial, twenty-five per cent more lignin was recovered from the feces than was consumed.

Pepsin, on the other hand, is active in an acid medium (pH 1-2) and it has not been shown that lignin is soluble in dilute mineral acid at the temperature employed. The effectiveness of this enzyme in removing proteins was uncertain in view of studies by Horwitt in which not more than eighty-nine per cent of the nitrogen was removable from spinach leaf by pepsin digestion. There was the possibility, however, that the protein of the materials would be reduced by pepsin digestion to a level no longer seriously interferring with the lignin determination. If the hypothesis is accepted, that lignin is not utilized by the animal, the usefulness of the pepsin pre-treatment and subsequent analytical steps would be indicated by a lignin balance trial.

The problem of rapidly dissolving the undigested residue in the strong acid was solved by Poss and Hill, who found that lignified tissues dissolved promptly (10-15 minutes) in seventy-two per cent sulfuric acid if first moistened with formalin. With acid in contact with the sample just a short time, the chances of the formation of substances from the carbohydrates (pentoses and hexoses) which might add to the lignin values, was presumably largely avoided (Ritter). The use of a granulating reagent

(chloroform and acetic acid) in the precipitation of the lignin to hasten the time necessary for filtration was proposed by Ross and Patter.

It seemed, therefore, that pepsin digestion of the ether extracted sample, followed by solution of the residue in seventy-two per cent sulfuric acid and subsequent precipitation of lignin according to Ross and Hill, and Ross and Patter procedures, could be successfully used for the lignin determination in animal feed and feces.

Description of the methods

A one gram sample of feces or feed is extracted with ether and dried in an oven. It is then placed in a fifty cc. glass-stoppered flask and forty cc. of the two per cent solution of pepsin in .1 N hydrochloric acid is added. This is digested at forty degrees for twelve hours with frequent shaking, especially during the first four or five hours. The mixture is then filtered through bolting silk or centrifuged. The filtrate containing sugars, protein and other substances is discarded.

The non-digestible residue containing cellulose, hemicellulose, and lignin is then washed with hot alcohol, benzene, and ether and transferred to a one-hundred cc. beaker and the last traces of ether are removed with mild heat. The residue is moistened with four cc. of forty per cent formaldehyde. Four cc. of seventy-two per cent sulfuric acid is added and the mixture allowed to penetrate the sample for two minutes. Six cc. of concentrated sulfuric acid is then added and the mixture is stirred vigorously to dissolve the sampke, which would be complete in ten to fifteen minutes. The beaker during this time is partially immersed in a water bath to keep the temperature from rising above seventy degrees C. or so. When the sample has dissolved, thirty-five cc. of the granulating reagent (1:6 mixture by volume of chloroform and acetic acid) is stirred in and the

the mixture poured into an eight hundred cc. beaker containing five hundred cc. of distilled water. The mixture is boiled gently until all the chloroform has been driven off (15 minutes). The solution should be clear and the lignin should settle in granular form. The mixture is then filtered through a Gooch with suction. The filtrate containing the hemicellulose and cellulose is discarded. The residue is washed with two-hundred cc. of five per cent hydrochloric acid and dried, then weighed. It is then ignited and reweighed, and the loss in weight is calculated as lignin.

Kurschner and Hoffer have a procedure for the determination of cellulose in which the sample is freed of non-cellulose, organic constituents by digestion with alcoh ol and nitric acid. Kurschner and Hanak had another method in which they substituted acetic acid for the alcohol in the digestion reagent, and changed the time for boiling from two or three successive one hour periods to twenty minutes of boiling. These gave practically the same results for feces, but lower values from certain feeds were secured from the acetic-nitric acid reagent. A possible explanation might lie in a difference in the resistance of the cellulose fractions of mature hay and grain as compared to those of immature grasses. Certainly the acetic-nitric acid mixture is the more powerful reagent. These results together with its greater simplicity led Crampton and Maynard to use the acetic-nitric acid reagent for their determination of cellulose. It was found, however, that alcohol was preferable to water for the first washings to free the cellulose from the digestion reagent. Centrifuging after each washing facilitated the washing operation.

A one gram air-dried, ether-extracted sample is placed in a one hundred and fifty cc. round bottom flask. This flask must be wide necked and fitted with a reflux condenser. Fifteen cc. of eighty per cent acetic and one and one-half cc. of concentrated nitric acid are added and the mixture is boiled

gently for twenty minutes. The mixture is then transferred to a fifty cc. centrifuge tube and after twenty cc. of alcohol (ethyl) are added and the tube shaken, it is centrifuged for ten minutes and the supernatent is discarded. This washing is repeated and finally the residue is washed with a stream of hot alcohol from a wash bottle into an alundum crucible. (A carborundum product called "Alfrax" and "firefrax" was used.) The residue in the crucible is then washed successively with hot benzene, hot alcohol, and finally with ether, using suction during the filtering. The residue is dried, weighed, ignited, and reweighed, and from the loss in weight the cellulose is calculated.

Data

The samples as were used in the Olmstead and Williams determinations were again used here. They were analyzed according to the scheme of Crampton and Maynard which has just been described. Tables VI-X give the results obtained.

Methods of Davis and Miller

Work leading up to the methods

In certain digestibility studies on forage crops, a method was required which would yield information that could not be obtained by the usual methods. In addition to giving the desired information, the method should be sufficiently simple so as to be readily adapted to routine laboratory procedure. Davis and Miller tried a number of methods and suggested several modifications which appear to have a number of advantages over former procedures.

They proposed a method combining both enzymatic and chemical procedures. Previous dry ether extraction before enzymatic treatment was found

to give a more uniform enzymatic action. They also found it necessary to autoclave the samples before adding the enzymes in order to prevent mold growth. The methods proposed by Williams and Olmstead, Horwitt, and by Cogwill and Mendel, were used on a sample of Red Top grass cut early in June. Davis and Miller found that the Williams and Olmstead method gave higher results for the undigested residue than the Horwitt, Cogwill and Mendel method. Both methods gave approximately the same results when used on samples of feces.

The effectiveness of various treatments in removing nitrogen from a sample of Red Top was tested and the results of this study showed that enzymatic digestion was best. In subsequent work, it was adopted for preliminary treatment of the sample. The Horwitt, Cogwill and Mendel methods were modified by using a smaller volume and reducing the amount of pepsin and trypsin. It was found the amount of pepsin and trypsin did not have a pronounced effect on the amount of nitrogen removed from the sample if more than fifty milligrams per gram of sample was used. The trypsin extract was filtered to prevent any undissolved material from increasing the weight of the residue.

Davis and Miller also conducted tests on the fineness of the sample and showed that best results were obtained when the entire sample passed through a five-tenths millimeter screen. Apparently, the enzymatic action is more uniform if the particles are small. The amount of material digested is greater in fine than in coarse samples.

After the enzymic digestion, the lignin is determined. Davis and Miller observed that the concentration of the acid is the important factor. The results showed that the concentration must be above sixty-five per cent for grasses. Below this, they found the results too high.

The time and temperature factors of the seventy-two per cent sulfuric

acid method were studied by Ritter and Seborg and Mitchell. The results they obtained using wood were confirmed in the present experiments on grass. Formaldehyde was used by Crampton and Maynard to bring about quicker solution of the sample and to improve the rate of filtration. Davis and Miller, however, found that the use of formaldehyde increased the yield of lignin, and consequently its use.

Description of the methods

The details of the Davis and Miller method are as follows: A one gram sample is extracted with anhydrous ether for sixteen hours. The sample is transferred to a one-hundred cc. wide mouth, glass stoppered flask after having been dried. The sample is moistened with water and autoclaved at eighteen pounds pressure for one hour. Fifty cc. of .1 Nlhydrochloric acid and one-tenth gram of pepsin (1:3000) are added and the mixture is filtered and washed with water, after which it is returned to the flask by means of sixty cc. of an aqueous extract of trypsin containing one-tenth gram of trypsin powder. The solution is made slightly alkaline with sodium hydroxide and incubated for ninety six hours. The mixture is again filtered, washed with water, alcohol, and ether. It is dried at one-hundred and ten degrees C. for one to two hours and weighed; this is the undigested residue. All filtrations are made through two-hundred mesh bolting silk, although centrifugation was used throughout the present studies. The residue is transferred to aluminum dishes for final drying and weighing.

The undigested residue is hydrolyzed with five per cent sulfuric acid for one hour. The mixture is filtered, washed, with hot water, and finally with alcohol. The residue is transferred to a one-hundred cc. beaker and placed in a freezing bath and twenty cc. of seventy-two per cent sulfuric acid is added with constant stirring. After fifteen minutes it is removed

and the reaction is allowed to continue at room temperature for forty-five to sixty minutes. The mixture is stirred constantly during the reaction period. The sample is then transferred to a one liter flask and diluted to three per cent by weight of sulfuric acid and refluxed for two hours. The mixture is filtered and the residue is washed with hot water. The residue is dried, weighed, ignited, and reweighed, and the loss in weight is reported as lignin. The final filtration should be carried out within thirty minutes after the completion of the refluxing. If the solution is allowed to become cold, it is difficult to filter and wash, and if the analysis is completed, the results are invariably too high.

Cellulose was determined the Davis and Miller scheme by the acetic-nitric acid method of Kurschner and Hanak, which also is the one used by Crampton and Maynard.

Davis and Miller made no attempt to separate the remaining components of the carbohydrate complex. These "other carbohydrates" can be obtained by difference.

Data

The samples previously used were subjected to the Davis and Miller scheme of analysis. The sum of the lignin and cellulose values were subtracted from the weighed undigested residue. This value was considered as representing "other carbohydrates" probably mostly hemicellulose. The results of these samples as analyzed by the Davis and Miller methods are shown in tables XI-XV.

Work on the nitrogen-lignin value

Description of the work

In analysing various samples by the Olmstead and William's method and also by the Crampton and Maynard methods, lignin values were obtained which

were quite inconsistent. The Olmstead and Williams values were much lower than the Crampton and Maynard. These inconsistencies were experienced by others in a comprehensive fiber study in 1939. It was therefore decided to investigate the residue. This was done by determining the nitrogen content of the original samples before digestion. The same samples were digested by the three enzymatic methods. The residues were collected, washed, and dried, and nitrogen determinations made by the micro Kjeldahl method. The methods giving high lignin values were suspected of not having the nitrogen removed sufficiently by the particular enzymatic digestion process employed.

Data

The samples used previously were again selected for this phase of the problem. The summary of results of these digestion studies in which the relation of nitrogen present to lignin values was being determined is presented in tables XVI-XX.

Particle Size and Heat Treatment

Description of the work

In some recent nutritional work here, the question arose to whether or not the methods of drying the samples as well as grinding might not be influencing the analytical results. The samples had been dried (both food and fecal) by heating five to six days on a steam bath. The grinding was done in a Wiley mill, which gave a fairly fine particle size. It was decided to take some All-Bran and moisten it to form a paste, and this was placed on a steam bath and heated for a period of six days. As the water evaporated off, more water was added and stirred into the bran. At the end of the heating period the sample was divided into two parts. One part was ground in a Hobart mill adjusted to give a grind of slightly larger

particle size than the Wiley mill. The other part was finely ground in a ball mill. Another portion of the bran which had not been treated with heat was also divided into two parts, each part being ground in the same manner as were the two parts of the heat-treated bran. These four samples, two different grinds on two samples, one treated with heat and the other not, were analyzed for crude fiber.

Data

The results of these studies on the relation of particle size and heat treatment to the crude fiber value are presented in Table XXI.

DISCUSSION

Cellulose Determinations:

In studying column one of tables I-V, VI-X, containing the results of the cellulose determinations by the methods of Olmstead and Williams, Crampton and Maynard, and Davis and Miller respectively, one can see a more or less definite variation between the methods. Inasmuch as Davis and Miller used the Crampton and Maynard method, the results were obviously the same. The Olmstead and Williams method gave results on the fecal material much lower than the method of Crampton and Maynard. The average percent variation is about 25-50%. The Olmstead and Williams method offers numerous places in which errors might enter in to give low results, if the Crampton and Maynard method is to be taken as the correct value for the per cent cellulose. First of all, there is an enzymatic digestion and transfer in the Olmstead and Williams method. Secondly, there is treatment with acid and hydrolysis. Thirdly, there is an indirect final step, in using a copper reduction method for the total sugars on one aliquot, while fermenting the hexoses out and running another copper reduction procedure for the non-fermentable sugars. These values are both read from curves and the difference between the total and non-fermentable sugar reduction is taken as the cellulose value. Thus, one can easily see that with so many transfers of the sample and stages in the determination, and the determining of the sugars by copper reduction and differences between two reductions, that numerous errors might enter into the analyses to give the low results as shown in tables I-XVIII when the Olmstead and Williams method is compared with that of Crampton and Maynard. On the other hand, in the Crampton and Maynard method, the ether extracted sample is weighed out directly into the reaction flask, the reagents are added and the contents heated for 20 minutes and filtered. The reagent takes out everything but the cellulose

and a small amount of ash which does not interfere, because after ignition the ash is still present and the cellulose has been determined in a direct manner, with no transfers except in filtering the final product. One can see that when the analyses depend on finding the amount of a given substance by determining one of its groups or parts or end products, a great number of factors present themselves to be solved or regulated by definition, any variation of which usually results in analytical error. The only place that one may have to be cautious in the Crampton and Maynard method is to be sure in rinsing the final product into the weighing crucible or centrifuge tube that all particles adhering to the sides are removed by rinsing the tube with hot alcohol. The food samples, although a few are missing and two or three other samples are somewhat out of agreement, have the same trend; that is, the Crampton and Maynard method gives higher results. Although the samples analysed by the Olmstead and Williams method were done in duplicates on the same filtrate (originating from a one gram sample) the checks were not any closer and in most cases not as close as they were in the Crampton and Maynard method, in which the duplicates were analysed at different times on separate one gram samples.

Hemicellulose determinations

Although Crampton and Maynard did not have any definite method for the determination of hemicellulose in their scheme of analysis, they presented an alternate plan, that is, estimation by difference of the total hemicelluloses plus any other carbohydrates not cellulose. The results for hemicellulose by the method of Davis and Miller were calculated as follows: The indigestible residue from the enzymatic treatment of the sample was washed, dried and weighed. This residue included mostly cellulose, hemicellulose, and lignin, with perhaps a very small percentage of

protein plus carbohydrates which might not have been previously removed. Davis and Miller suggested subtracting the sum of the cellulose and lignin from the total indigestible residue and calling this difference "other carbohydrates", rather than hemicellulose. In studying column two in tables I-V, XI-XV, and table XVII, one can notice how closely the so-called "other carbohydrates" portion of the Davis and Miller scheme agrees with the hemicellulose values as obtained by the method of Olmstead and Williams. Both are indirect methods. The fact that the results by the method of Davis and Miller check fairly well with the Olmstead and Williams procedure seems to indicate that material involved in the former may represent largely the hemicellulose fraction. The Davis and Miller method seems to give almost consistently higher values than the Olmstead and Williams with both the food and fecal samples. The average variation between Davis and Miller values and Olmstead and Williams values is about .27. The method of Olmstead and Williams seems to give better checks on duplicates because they are all aliquots from the same filtrate, whereas the duplicates on the Davis and Miller method are upon two different one gram samples and were also obtained by difference, which would tend to cause a little more variation between duplicates.

Lignin Determinations

The results of the lignin determinations, which are given in column three of tables I-XV, show a definite variation. A summary average is shown in table XVIII. The method of Olmstead and Williams gives the lowest values, whereas that of Crampton and Maynard gives the highest values. The values by the Davis and Miller method are intermediate. The results are fairly consistent for both the fecal and the food samples. The Crampton and Maynard method gives an average lignin value range of from

twenty-four to twenty-eight per cent on the food samples. The average range for the lignin value by the Olmstead and Williams procedure is from about four and one-half to eight per cent on the fecal samples and from two-tenths to three-tenths per cent on the food samples.

The Davis and Miller procedure average range gives values of twelve to eighteen per cent on the fecal material and from seven-tenths to three and six-tenths per cent on the food samples. All of these methods use the seventy-two per cent sulfuric acid method with modifications either of time, temperature or concentration of the sulfuric acid.

The Crampton and Maynard value seemed much too high as compared with either of the other two values. This is probably due in part to the enzymatic treatment, because the nitrogen value of the residue was relatively very high.

The literature contains many references to the effects of the presence of nitrogen, aldehydes and sugars in the enzymatic residue, as well as the effect of time, temperature and acid concentration on the lignin value. It is known that some carbohydrates, such as sugars, xylose, arabinose, fructose and sucrose, will in the presence of strong acids (72% sulfuric acid or stronger), such as used in the lignin determinations by the sulfuric acid method, give substances similar in physical and chemical properties to lignin. It is also known that the presence of nitrogen in protein form gives fission products which will condense with the lignin and thus give high lignin values. Aldehydes also condense with lignin and this may account for high values. Some of the higher saccharides containing a pentose yield furfuraldehyde to some extent in the lignin determination, and this may condense with the lignin. Fatty substances are also capable of increasing the apparent lignin value, but due to their extraction with ether these were reduced to a negligible amount in the above determinations. The method

of Crampton and Maynard was found to give an enzymatic residue extremely high in nitrogen, which may have been partly responsible for the high lignin values. In the same method, formaldehyde was used, which has been found by many workers to be a cause of high results. This would seem to fit in with the idea that aldehydes may condense with the lignin to give humin-like substances and subsequently high values. The use of seventy-two per cent sulfuric acid has also been found by some workers to produce some charring of the sample and high lignin values may also result from this. The Crampton and Maynard method makes use of this seventy-two per cent sulfuric acid. The Olmstead and Williams method, as well as the Davis and Miller method, has a more thorough enzymatic digestion procedure, the Olmstead and Williams procedure making use of pancreatin, while the Davis and Miller procedure makes use of pepsin, clarase and trypsin. These procedures both take out more nitrogen than does the Crampton and Maynard method. The Olmstead and Williams method makes use of sixty per cent sulfuric acid and subsequent dilution and hydrolysis. The Davis and Miller method uses seventy-two per cent sulfuric acid for forty-five to fifty minutes, followed by subsequent hydrolysis after dilution to five per cent sulfuric. The use of this strength acid and for a period as long as fifty minutes may have given results slightly high as compared with the Olmstead and Williams values. The methods and the reasons for the values may be summed up as follows:

Method	Type of Values	Steps in the method which might give low lignin values	Steps in the method which might give high lignin values
1. Olmstead and Williams	1. Lowest values	1. 60% sulfuric instead of 72% 2. dilution and hydrolysis 3. good enzymatic digestion 4. chilled temperature (6-10°C) during contact of conc. acid with sample	1. 24 hours that sample and conc. acid are in contact
2. Davis and Miller	2. Intermediate	1. good enzymatic digestion 2. hydrolyzed after dilution of acid to 5%	1. 72% sulfuric acid used. conc. acid and sample 2. allowed to stand together for 45-50 minutes
3. Crampton and Maynard	3. Highest values	1. acid and sample allowed to be in contact only 10-15 minutes	1. use of formaldehyde 2. use of 72% sulfuric acid 3. enzymatic digestion not good 4. too much nitrogen present 5. reaction allowed to reach room temperature or higher 6. no dilution of acid followed by hydrolysis

In a rough average, the Davis and Miller method seems to average three times the Olmstead and Williams value, while the Crampton and Maynard values average about five to six times the value by the Olmsteadnand Williams procedure. Some idea as to the reproducibility of results on the various methods may be gained from the fact that duplicates by the Crampton and Maynard method had a grand average of .64 per cent difference between them, while the methods of Davis and Miller and Olmstead and Williams had exactly the same reproducibility, both having a grand average difference between duplicates of .06%.

Nitrogen-lignin values

The purpose of carrying out this study has been discussed previously in this paper. The results are shown in tables XIX-XXIII. With a few exceptions, there seems to be a relationship between the amount of nitrogen left in the residue after the enzymatic digestion and the lignin value derived from this residue. The resulting percentages of nitrogen are given both on the basis of the digested residue and the original undigested sample. The fecal samples all seem to hold true to the pattern, that those residues containing the largest percentages of nitrogen also give the highest lignin values. The food samples, with the exception of the basal diet, do not rigidly follow this pattern. This may be due to presence of substances other than nitrogenous compounds in the residue after enzymatic digestion. Substances such as carbohydrates containing pentoses might yield furfuraldehyde in the lignin procedure and this subsequently combined with the lignin to form humus-like condensation products, thus giving rise to high lignin values without the nitrogen content of the residue being correspondingly high. This may be the reason for irregularities of the lignin values of the food samples in columns two and three of

table XXIII. In studies made last summer by other workers, it was found that the residue obtained by the Crampton and Maynard method frequently contained as much nitrogen as the original material.

Particle-size and heat-treatment results

The results of the study of the effect of particle size and heat treatment on the crude fiber values are shown in table XXIV. They indicate that in general particle size and heat treatment have little or no effect on the crude fiber values. The ball mill grind on both heat-treated and non-heat treated bran differed by only .19 per cent on four sets of four samples each. The coffee mill grind on both the heat-treated and non-heat treated bran differed by only from .4 to .5 per cent on four sets of four samples each. The difference between the heat-treated coffee mill and ball mill grind was only about .9 to 1.0 per cent. The difference between the non-heat treated coffee mill and ball mill grinds was about .19 per cent. These variations are within the limits of accuracy of the crude fiber determination.

CONCLUSIONS

1. The Olmstead and Williams cellulose determination gives lower values than does the Crampton and Maynard procedure.
2. The Olmstead and Williams method for lignin gives lower values (one-fifth to one-sixth) than those of the Crampton and Maynard method and one-third that of the Davis and Miller procedure.
3. The Davis and Miller "other carbohydrates" fraction has about the same value as the hemicellulose fraction determined by the Olmstead and Williams method.
4. The Crampton and Maynard enzymatic residue averages contain more nitrogen than do the Olmstead and Williams or Davis and Miller.
5. The Olmstead and Williams enzymatic residue contains the least amount of nitrogen.
6. The Olmstead and Williams enzymatic procedure seems superior because it removes more nitrogen and is faster than that of Davis and Miller.
7. There appears to be a relationship between the percentage of nitrogen remaining the enzymatic residue and the lignin value. In general, the higher the percentage of nitrogen in the residue, the higher the lignin value.
8. The Crampton and Maynard cellulose procedure seems to give better checks on different samples than does the Olmstead and Williams method.
9. The Olmstead and Williams method for hemicellulose seems to give better checks than the Davis and Miller determination of "other carbohydrates".
10. The procedure of Davis and Miller and Olmstead and Williams gives duplicate checks of about the same differences for lignin. Both give better checks than do the Crampton and Maynard procedure.

11. The Crampton and Maynard method for cellulose seems to be a better one than the method of Olmstead and Williams in that it is simpler and more direct.

12. Particle size does not seem to have any effect on the value of crude fiber on the same sample of bran.

13. Heat treatment does not seem to have any effect on the crude fiber value of the same sample of bran.

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A P P E N D I X

TABLE I

Olmstead and Williams

First Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	1.04	1.09	1.06	.45	.35	.40	2.58	2.49	2.53
O	2.47	2.45	2.46	3.05	3.00	3.02	4.16	4.26	4.21
P	1.05	1.01	1.03	2.56	2.41	2.48	5.57	5.84	5.70
T	2.61	2.81	2.71	.91	.93	.92	4.95	4.98	4.96
SC	1.62	----	1.62	1.78	----	1.78	4.78	4.08	4.43
L	5.30	5.20	5.25	3.49	3.61	3.55	4.18	4.47	4.32
Average	2.34	2.51		2.04	2.06		4.37	4.35	
First Week Food Sample Basal	1.16	----	1.16	0.31	----	0.31	.218%	----	.218

TABLE II

Olmstead and Williams

Second Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	9.20	9.10	9.15	11.79	12.06	11.94	5.50	5.41	5.46
O	9.91	9.80	9.85	13.20	13.45	13.32	6.57	6.60	6.58
P	9.72	9.66	9169	12.45	12.97	12.71	6.48	6.33	6.40
T	9.10	8.98	9.04	11.18	11.07	11.12	5.73	5.50	5.61
SC	9.27	9.20	9.23	10.29	9.78	10.03	6.04	6.00	6.02
L	8.51	8.39	8.45	9.67	10.21	9.94	5.03	5.13	5.08
Average	9.28	9.18		11.43	11.59		5.89	5.82	
Second Week Food Sample All-Bran	3.25	3.29	3.27	.45	.45	.45	.60	.60	.60

TABLE III

Olmstead and Williams

Fourth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sampel 1	Sample 2	Average 1 and 2
S	3.11	2.99	3.05	.48	.46	.47	3.55	3.41	3.48
O	2.20	2.08	2.14	2.64	2.90	2.77	10.63	11.41	11.52
P	1.65	1.75	1.70	.62	.67	.64	4.35	4.53	4.44
T	4.04	4.16	4.10	2.02	1.57	1.79	8.82	8.77	8.79
SC	1.92	1,87	1.89	.19	.19	.19	3.93	3.89	3.91
L	4.84	5.01	4.92	2.74	2.82	2.78	8.66	8.65	8.65
Average	2.96	2.97		1.44	1.43		6.65	6.77	
Fourth Week Food Sample Prunes	-	-	-	-	-	-	-	-	-
Celery	-	-	-	-	-	-	-	-	-

TABLE IV

Olmstead and Williams

Sixth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Ave. 1 and 2
S	1.51	1.10	1.30	.12	.12	.12	6.21	6.37	6.29
O	.94	.99	.96	.17	.17	.17	3.55	3.57	3.56
P	1.19	1.19	1.19	.64	.69	.66	5.33	5.31	5.32
T	1.88	1.64	1.76	.25	.37	.31	4.02	4.02	4.02
SC	1.93	1.95	1.94	1.02	1.10	1.06	5.30	5.34	5.32
L	4.32	4.49	4.40	1.29	1.37	1.33	3.91	3.83	3.87
Average	1.96	1.89	.58	.63		4.72	4.74		
6th Week Food Sample Lettuce	1.37	1.30	1.33	.052	.041	.216	.228		.222
Cabbage	1.63	1.66	1.64	.201	.167	.184	.290	.256	.273

TABLE V

Olmstead and Williams

Eighth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Ave. of 1 & 2
S	2.80	3.20	3.00	.50	.62	.56	6.27	6.29	6.28
O	2.1	1.83	1.96	.40	.40	.40	5.29	5.71	5.50
P	3.68	3.79	3.73	.125	.75	.437	4.37	4.58	5.55
T	-	-	-	-	-	-	-	-	-
SC	3.68	3.61	3.64	1.33	1.22	1.27	3.55	3.43	3.49
L	5.83	5.67	5.75	.37	.37	.37	2.81	2.93	2.87
Average	3.61	3.62		.54	.67		4.48	4.58	
8th Week Food Sample									
Apples	1.11	1.11	1.11	.326	.381	.353	.300	.317	.308
Oranges	1.18	1.21	1.19	.054	.0864	.070	.228	.206	.217

TABLE VI

Crampton and Maynard

First Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 & 2	Sample 1	Sample 2	Average of 1 & 2	Sample 1	Sample 2	Ave. of 1 & 2
S	-	-	-	-	-	-	-	-	-
O	2.71	3.46	3.08	-	-	-	25.10	23.91	24.50
P	2.65	2.64	2.64	-	-	-	32.00	32.90	32.45
T	3.02	3.06	3.04	-	-	-	25.70	25.98	25.84
SC	3.33	3.30	3.31	-	-	-	34.30	37.60	35.95
L	6.41	6.49	6.45	-	-	-	20.30	21.80	21.05
					Y				
Average	3.62	3.79		-	-	-	27.48	28.43	
Food Sample Basal	1.13	1.22	1.17	-	-	-	6.00	6.02	6.01

TABLE VII

Crampton and Maynard

Second Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 & 2	Sample 1	Sample 2	Average of 1 & 2	Sample 1	Sample 2	Ave. of 1 & 2
S	13.61	13.50	13.55	-	-	-	31.76	30.75	31.25
O	12.96	13.16	13.06	-	-	-	24.18	23.90	24.04
P	-	11.80	-	-	-	-	-	-	-
T	11.74	11.80	11.77	-	-	-	26.04	25.07	25.55
SC	13.14	13.90	13.52	-	-	-	21.68	22.00	21.84
L	11.76	12.50	12.13	-	-	-	27.59	27.45	27.52
Average	12.64	12.97		-	-	-	26.25	25.83	
Food Sample All-Bran	1.93	1.84	1.88	-	-	-	12.02	11.93	11.97

TABLE VIII

Crampton and Maynard

Fourth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	3.98	3.85	3.91	-	-	-	24.60	24.89	24.74
O	4.81	5.03	4.92	-	-	-	31.74	31.92	31.83
P	5.37	5.32	5.34	-	-	-	25.30	25.68	25.49
T	4.04	4.00	4.02	-	-	-	22.64	22.92	22.78
SC	4.77	4.72	4.74	-	-	-	27134	27.25	27.24
L	7.14	7.12	7.13	-	-	-	31.82	32.51	32.16
Average	5.01	5.00		-	-	-	27.24	27.52	
Food Sample Prunes	1.20	1.29	1.24	-	-	-	2.95	3.08	3.02
Celery	1.30	1.35	1.32	-	-	-	4.21	4.25	4.23

TABLE IX

Crampton and Maynard

Sixth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Ave. of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	6.69	6.56	6.62	-	-	-	30.65	29.99	30.32
O	4.43	4.80	4.61	-	-	-	21.56	21.63	21.59
P	4.95	4.91	4.93	-	-	-	24.57	25.77	25.17
T	6.60	6.30	6.45	-	-	-	23.93	24.34	24.13
SC	5.96	5.90	5.93	-	-	-	26.21	26.20	26.20
L	6.62	6.80	6.71	-	-	-	24.16	24.74	24.45
Average	5.87	5.87		-	-		25.18	23.77	
Food Sample Cabbage	1.90	2.00	1.95	-	-	-	4.14	4.19	4.16
Lettuce	1.22	1.27	1.24	-	-	-	2.70	2.79	2.74

TABLE X

Crampton and Maynard

Eighth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	5.52	5.57	5.54	-	-	-	28.48	28.80	28.64
O	7.00	7.36	7.18	-	-	-	21.65	20.98	21.31
P	8.70	8.27	8.48	-	-	-	24.26	24.26	24.26
T	-	-	-	-	-	-	-	-	-
SC	8.35	8.50	8.42	-	-	-	26.14	25.70	25.92
L	8.79	8.70	8.74	-	-	-	20.50	20.50	20.50
Average	7.67	7.68		-	-		24.20	24.04	
Food Sample Oranges	1.36	1.37	1.36	-	-	-	7.89	7.83	7.86
Apples	1.46	1.33	1.39	-	-	-	6.22	5.82	6.02

TABLE XI

Davis and Miller

First Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Ave. of 1 & 2
S	*	-	-	-	-	-	-	-	-
O	2.71	3.46	3.08	3.67	-	3.67	17.28	17.36	17.32
P	2.65	2.64	2.69	1.84	-	1.84	11.89	11.85	11.87
T	3.02	3.06	3.04	3.31	-	3.31	11.86	12.11	11.98
SC	3.33	3.30	3.31	1.73	-	1.73	9.94	9.88	9.91
L	6.41	6.49	6.45	3.03	-	3.03	15.29	15.29	15.27
Average	3.60	3.79		2.71	-	-	13.25	13.29	
Food Sample Basal	1.13	1.22	1.17	-	-	-	.49	.50	.49

TABLE XII

Davis and Miller

Second Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	13.61	13.50	13.55	11.12	-	11.12	10.79	10.77	10.78
O	12.96	13.16	13.06	-	-	-	10.32	10.90	10.61
P	-	-	-	-	-	-	-	-	-
T	11.74	11.80	11.77	-	-	-	11.93	11.61	11.77
SC	13.14	13.90	13.52	9.33	-	9.33	11.94	11.36	11.65
L	11.75	12.50	12.13	10.90	-	10.90	10.90	11.45	11.17
Average	12.64	12.97		10.45	-		11.17	11.21	
Food Sample All-Bran	1.93	1.84	1.88	.65	-	.65	3.39	3.87	3.63

TABLE XIII

Davis and Miller

Fourth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	3.98	3.85	3.91	.54	.31	.42	18.26	17.67	17.96
O	4.81	5.03	4.92	5.51	5.72	5.61	21.29	21.41	21.35
P	5.37	5.32	5.34	1.99	2.90	2.44	14.84	15.05	14.94
T	4.04	4.00	4.02	2.35	-	-	18.21	18.27	18.24
SC	4.77	4.72	4.74	.22	-	-	12.95	12.76	12.85
L	7.14	7.12	7.13	-	-	-	22.41	23.08	22.74
Average	5.01	5.00		2.12	2.97		17.99	18.04	
Food Sample									
Prunes	1.20	1.29	1.24	.3	.09	.19	.98	.93	.95
Celery	1.30	1.35	1.32	.13	.22	.17	.73	.75	.74

TABLE XIV

Davis and Miller

Sixth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	6.69	6.56	6.62	.24	-	.24	11.80	12.40	12.10
O	4.43	4.80	4.61	-	-	-	13.75	14.01	13.88
P	4.95	4.91	4.93	.96	-	.96	14.68	14.46	14.57
T	6.60	6.30	6.45	1.63	-	1.63	9.42	9.60	9.51
SC	5.96	5.90	5.93	3.00	-	3.00	13.66	13.28	13.47
L	6.62	6.80	6.70	1.27	-	1.27	10.63	10.68	10.65
Average	5.87	5.87		1.42	-		12.32	12.40	
Food Sample									
Cabbage	1.90	2.00	1.95	.52	-	.52	1.03	1.08	1.05
Lettuce	1.22	1.27	1.24	.037	-	.037	.95	1.05	1.00

TABLE XV

Davis and Miller

Eighth Week

Name	% Cellulose			% Hemicellulose			% Lignin		
	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2	Sample 1	Sample 2	Average of 1 and 2
S	5.52	5.57	5.54	.65	-	.65	18.76	18.67	18.71
O	7.00	7.36	7.18	.60	-	.60	15.10	15.50	15.30
P	8.70	8127	8.48	.93	-	.93	14.64	14.47	14.55
T	-	-	-	-	-	-	-	-	-
SC	8.35	8.50	8.42	1.04	-	1.04	14.51	14.88	14.69
L	8.79	8.70	8.74	.76	-	.76	12.72	12.89	12.80
Average	7.67	7.68		.79	-		15.14	15.28	
Food Sample									
Oranges	1.36	1.37	1.36	.073	-	.073	.65	.75	.70
Apples	1.46	1.33	1.39	.42	-	.42	.86	.96	.91

TABLE XVI

Average of all Samples on the Various Methods for Cellulose

Samples	Ave. % by Olmstead & Williams Method	Ave. % by Crampton and Maynard Method	Ave. % by Davis and Miller Method
1st week (fecal)	2.42	3.70	3.70
2nd week (fecal)	9.23	12.80	12.80
4th week (fecal)	2.96	5.00	5.00
6th week (fecal)	1.92	5.87	5.87
8th week (fecal)	3.61	7.67	7.67
1st week (basal)	1.16	1.17	1.17
2nd week (bran)	3.27	1.88	1.88
4th week (celery)	-	1.24	1.24
4th week (prunes	-	1.32	1.32
6th week (cabbage)	1.64	1.95	1.95
8th week (lettuce)	1.33	1.24	1.24
8th week (oranges)	1.19	1.36	1.36
8th week (apples)	1.11	1.39	1.39

TABLE XVII

Average of all Samples on the Various Methods for Hemicellulose

Samples	Ave. % by Olmstead and Williams Method	Ave. % by Crampton and Maynard Method	Ave. % by Davis and Miller Method
1st week (fecal)	2.05	-	2.71
2nd week (fecal)	11.51	-	10.45
4th week (fecal)	1.43	-	2.54
6th week (fecal)	.60	-	1.42
8th week (fecal)	.60	-	.79
1st week (basal)	.031	-	-
2nd week (bran)	.45	-	.65
4th week (celery)	-	-	.17
4th week (prunes)	-	-	.19
6th week (cabbage)	.184	-	.52
6th week (lettuce)	.041	-	.037
8th week (oranges)	.070	-	.073
8th week (apples)	.353	-	.42

TABLE XVIII

Averages of All Samples on the Various Methods f or Lignin

Samples	ave. % by Olmstead and Williams Method	Ave. % by Crampton and Maynard Method	Ave. % by Davis and Miller Method
1st week (fecal)	4.36	27.98	13.27
2nd week (fecal)	5.85	26.04	11.19
4th week (fecal)	6.71	27.38	18.01
6th week (fecal)	4.73	24.47	12.36
8th week (fecal)	4.53	24.12	15.22
1st week (basal)	.218	6.01	.49
2nd week (bran)	.60	11.97	3.63
4th week (celery)	0	4.23	.74
4th week (prunes)	-	3.02	.95
6th week (cabbage)	.273	4.16	1.05
6th week (lettuce)	.222	2.74	1.00
8th w eek (oranges)	.217	7.86	.70
8th week (apples)	.308	6.02	.91

TABLE XIX

Nitrogen on Original Samples -- Micro Kjeldahls

Sample	Sample No.	cc. H_2SO_4 used	Weight in grams	Per cent nitrogen
Food Sample 1st week Basal	R_1	6.50	.0522	3.41
Food Sample 2nd week All-Brann	R_2	8.10	.0504	4.40
Food Sample 4th week Prunes	R_3	4.82	.0520	2.54
Fecal Sample first week (L. O.)	R_4	12.23	.0538	6.22
Fecal Sample 2nd week (L. O.)	R_5	6.95	.0522	3.79
Fecal Sample 4th week (L. O.)	R_6	3.05	.0549	1.58

TABLE XX

Nitrogen on Olmstead and Williams Residues

(Micro-Kjeldahls)

Sample	Sample No.	H_2SO_4 cc. used	Weight in grams	Nitrogen %	
				Based on Digested Residue	Based on Undigested Residue
Food Sample 1st week (Basal)	A_1	2.76	.0532	1.50	6.81
Food Sample 2nd week (All-Bran)	A_2	6.57	.0504	3.71	26.50
Food Sample 4th week (Prunes)	A_3	3.21	.0552	1.66	16.43
Fecal Samples 1st week (L. O.)	A_4	3.76	.0514	2.08	4.16
Fecal Sample 2nd week (L. O.)	A_5	4.47	.0504	2.52	3.31
Fecal Samples 4th week (L. O.)	A_6	3.80	.0511	2.11	4.39

TABLE XXI

Nitrogen on Crampton and Maynard Residues

(Micro-Kjelkahls)

Samples	Sample No.	H_2SO_4 cc. used	Weight in grams	Nitrogen % Based on Digested Residue	Nitrogen % Based on Undigested Residue
Food Sample 1st week (Basal)	B_1	7.13	.0506	4.00	13.79
Food Sample 2nd week (All-Bran)	B_2	5.78	.0513	3.21	9.72
Food Sample 4th week (Prunes)	B_3	3.20	.0505	1.68	6.00
Fecal Samples 1st week (L.O.)	B_4	15.83	.0512	8.82	4.41
Fecal Samples 2nd week (L.O.)	B_5	8.18	.0508	4.58	9.54
Fecal Samples 4th week (L.O.)	B_6	15.26	.0494	4.18	8.70

TABLE XXII

Nitrogen on Miller and Davis Residues

(Micro-Kjeldahls)

Sample	Sample No.	H_2SO_4 cc. used	Weight in grams	Nitrogen % Based on Digested Residue	Nitrogen % Based on Undigested Residue
Food Sample 1st week (Basal)	C_1	-	-	-	-
Food Sample 2nd week (All-Bran)	C_2	.73	.0116	1.82	14.3
Food Sample 4th week (Prunes)	C_3	.66	.0074	2.54	34.3
Fecal Samples 1st week (L.O.)	C_4	9.37	.0504	5.10	10.0
Fecal Samples 2nd week (L.O.)	C_5	1.18	.0505	.667	6.01
Fecal Samples 4th week (L.O.)	C_6	7.07	.0505	1.93	19.3

TABLE XXIII

Summary of Tables XIX-XXII

Samples	Olmstead & Williams			Crampton & Maynard			Davis & Miller			% Nitrogen based on Orig. Undigested Samples
	% N. based on dig. residue		% Lignin	% N. based on dig. residue		% Lignin	% N. based on dig. residue		%Lignin	
		undig.			undig.			undig.		
Food Sample 1st week	1.50	6.81	.218	4.00	13.79	6.02	-	-	.50	3.41
Food Samples 2nd week	3.71	26.50	.60	3.21	9.72	11.95	1.72	14.3	3.75	4.40
Food Sample 4th week	1.66	16.43	-	1.68	6.00	3.00	2.54	34.3	.95	2.54
Fecal Sample 1st week	2.08	4.16	4.20	8.82	4.41	24.50	5.10	10.00	17.30	6.22
Fecal Sample 2nd week	2.52	3.31	6.60	4.58	9.54	24.00	.667	6.01	10.60	3.79
Fecal Sample 4th week	2.11	4.39	10.50	4.18	8.70	31.80	1.93	19.3	21.35	1.58

TABLE XXIV

Crude Fiber in Relation to Particle Size

Sample	Non-Heat-treated Ball-mill grind Grams Crude Fiber	Weight	%	Heat-treated Ball-mill grind Grams Crude Fiber	Weight	%	Non-heat-treated Coffee-mill grind Grams Crude Fiber	Weight	%	Heat-treated Coffee-mill grind Grams Crude Fiber	Weight	%
Sample 1	.1476	2.0006	7.38	.1890	2.0038	7.10 9.43*	.1516	2.0022	7.57	.1634	2.0004	8.17
Sample 2	.1480	2.0012	7.39	.1908	2.0004	7.71 9.53*	.1518	2.0010	7.58	.1683	2.0004	8.41
Sample 3	.1468	2.0004	7.34	.1862	2.0012	7.60 9.30*	.1532	2.0010	7.65	.1656	2.0002	8.28
Sample 4	.1490	2.0010	7.44	.1885	2.0004	6.95 9.42*	.1492	2.0010	7.45	.1718	2.0004	8.59
Average			7.38			7.34 9.42*			7.56			8.36
Sample 5	.1526	2.0005	7.62	.1342	2.0002	6.71	.1532	2.0003	7.65	.1588	2.0002	7.94
Sample 6	.1463	2.0000	7.31	.1436	2.0007	7.17	.1598	2.0006	7.99	.1620	2.0000	8.10
Sample 7	.1522	2.0003	7.60	.1482	2.0008	7.41	.1498	2.0001	7.49	.1644	2.0001	8.22
Sample 8	.1505	2.0007	7.52	.1478	2.0006	7.39	.1540	2.0000	7.70	.1631,	2.0001	8.15
Average			7.51			7.17			7.70			8.10
Average of 1st and 2nd sets			7.44			7.25			7.63			8.23

*Determined without asbestos.
Not averaged in.

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Pringle

A comparative study of methods for the quantitative estimation of cellulose, hemicellulose & lignin.