[CONTRIBUTION FROM THE LABORATORY OF AGRICULTURAL CHEMISTRY, UNIVERSITY OF WISCONSIN, MADISON.]

THE CARBOHYDRATE CONTENT OF THE NAVY BEAN.[1]

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Abundant data exist as to the percentage of nitrogen-free extract in all of our foodstuffs, but only in a comparatively few cases is there any information available as to the kind and amount of carbohydrates comprising it. Included in the nitrogen-free extract are a variety of substances such as sugars, dextrin, starch, inulin, mannans, galactans, mucilages, gums, organic acids, lignin, tannins, higher alcohols, alkaloids, and cellulose. The irrationality of including in a single group, compounds of such widely different chemical properties and unequal nutritive value was early recognized by different investigators.[2] More significant methods of analyses were developed by Stone,[2] Sherman[2] and others in order to separate the nitrogen-free extract into such fractions as sugars, dextrin, starch, pentosans, lignin, and cellulose. In spite of this early activity very few food materials have been subjected to such a method of analysis and, as far as the literature consulted shows, no separation of the carbohydrates of the navy bean has been published.

Recently Street and Bailey[3] have made a study of the carbohydrates of the soy bean. Sucrose, dextrin, starch, galactans, pentosans, and cellulose represented 22.4% of the 31% of nitrogen-free extract. Only 0.5% of starch was found which is in marked contrast to the starch content of other legume seeds.

Experimental.

The Carbohydrates Extracted by Various Solvents.—Preliminary to an examination of the nitrogen-free extract, an analysis was made of commercial navy beans, hereafter designated as 1917 sample, according to the methods of the Association of Official Agricultural Chemists.[4] This gave the following percentage composition: moisture 12.96, ether extract 1.83, crude fiber 3.94, ash 3.88, crude protein 18.42, nitrogen-free extract 58.97.

A dissection of the nitrogen-free extract was made by subjecting the beans to the action of various solvents, as indicated in the following table. The weight of material extracted and the carbohydrates found in

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] Tollens, *Landw. Vers. Sta.*, **39**, 401 (1891); *Exp. Sta. Rec.*, **8**, 640 (1896); Atwater, *Office Expt. Sta. Bull.*, **21**, 48 (1895); Sherman, THIS JOURNAL, **19**, 291 (1897); *Stone, ibid.*, **19**, 347 (1897); Headden, *Col. Expt. Sta. Bull.*, **39** (1897); Widtsoe and Stewart, *Utah Expt. Sta. Bull.*, **58** (1898); Schweitzer, THIS JOURNAL, **26**, 252 (1904).

[3] Street and Bailey, *J. Ind. Eng. Chem.*, **7**, 853 (1915).

[4] *J. Assoc. Official Agr. Chemists*, **1**, No. 4; **2**, Nos. 1, 2, 3 (1916).

these extracts are also given in Table I. The determinations were made according to the Official Methods[1] except in the case of the chlorine-soluble material, where a method outlined by Shorger[2] was followed. All calculations are reported on the basis of the original undried beans.

TABLE I.

CARBOHYDRATE CONTENT OF THE VARIOUS EXTRACTS.

Calculated on air dry basis.

Material.	Treatment.	Total material extracted. %.	Amount and kind of carbohydrates extracted. %.
I. Ether extract residue	Hot 95% alcohol extraction	5.46	1.59—Reducing sugars after hydrolysis
			0.45—Pentosans
II. Ether-alcohol extract residue	Cold water extraction	14.17	3.71—Dextrin
			1.90—Pentosans
III. Ether-alcohol extract residue	Malt extract digestion	48.78	35.20—Starch
			2.73—Pentosans
IV. Malt extract residue	Boiling 1% HCl	16.35	0.83—Reducing sugar as glucose
			1.44—Pentosans
V. HCl residue	Boiling 1.25% NaOH	10.60	0.00—Reducing sugar after hydrolysis
VI. NaOH residue	Chlorine treatment		1.13—Chlorine soluble

TABLE II.

SUMMARY OF ANALYSIS FOR THE 1917 SAMPLE OF NAVY BEANS.

	%.
Moisture	12.96
Ash	3.88
Ether extract	1.83
Protein	18.42
Total sugar	1.59
Starch	35.20
Pentosans	8.37
Galactans	1.33
Dextrins	3.71
Hemicelluloses	0.83
True Cellulose	3.11
Organic acids, waxes, etc., by difference	8.77
	100.00

The carbohydrate of most prominence is starch which comprises about 60% of the nitrogen-free extract in this sample. Next in order come the pentosans which are found in several of the extracts. About 30% of the total pentosans appear in the malt extract. Small amounts of pentosans are found in the alcoholic and hydrochloric acid extracts but about one-

[1] *Loc. cit.*

[2] Shorger, *J. Ind. Eng. Chem.*, 9, 556 (1917).

half remains undissolved by these solvents. These data indicate that the pentosan material is present in different forms of varying solubility. In all but one case, the pentosans were determined on the residues after the various extractions. In the case of the hydrochloric acid extract, the determination was made directly on the extract.

In Table II is summarized the work done on the 1917 sample of navy beans, and it brings out the fact that there are many carbohydrates other than starch in the nitrogen-free extract. Starch comprises somewhat more than one-half and pentosans nearly one-eighth of the total nitrogen-free extract. The small amount of celluloses is deserving of note in connection with the digestive disturbances that have been experienced with beans in the diet.[1] The production of gas that results may be due not so much to the fermentation of celluloses as to the fermentation of other more abundant carbohydrates.

On account of the high percentage of starch found in the 1917 sample, it seemed desirable to make an analysis of the starch content of beans grown during another year, as well as to repeat the determination on the 1917 sample. A new sample of commercial navy beans was procured, hereafter designated as 1919 sample. A finely ground portion, passing a 40-mesh sieve, was submitted to a general analysis, as described in connection with the 1917 sample. A somewhat lower moisture content, 8.87%, and a slightly higher percentage of nitrogen-free extract, 61.8%, was found in this sample. The analysis of the 1917 sample gave practically the same figure for starch, 35.64%, as was obtained two years earlier. A much higher percentage of starch, 50.5%, was found in the 1919 sample. As both of these samples were bought in the open market, nothing is known regarding the conditions of their growth. It is well known that length of season, temperature, and moisture affect the amount of starch stored in seeds, and this difference in the starch content of the two samples is probably related to one or more of these climatic factors.

In the course of these analyses it was noted that fineness of grinding, time of digestion, and fermentation during the filtration had a marked influence on the percentage of starch obtained. The digestibility of the starch was greatly increased by grinding the sample to an impalpable powder in a ball mill. Samples ground in an ordinary feed mill gave from 10 to 12% less starch than the finely pulverized material. The starch grains are evidently imbedded in cellulose or protein material in such a manner as to prevent the ready action of the diastase. The necessity of an impalpable powder for the action of the diastase may perhaps explain the more difficult digestion of legume starches as compared with the cereal starches, noted by Pauletig.[2] As a result of their work

[1] McCollum, Simonds and Pitz, *J. Biol. Chem.*, **29**, 523 (1917).

[2] Pauletig, *J. Chem. Soc.*, **112**, I, 670 (1917).

on the digestibility of various starches, Stone[1] and O'Sullivan[2] concluded that there is great difference in the susceptibility of starches to the action of enzymes. Some later investigators, Ford[3] and Sherman[4] report that all starches when purified are equally digestible. Sherman believed certain waxy or fatty materials are associated with the starches and interfere with enzymatic action.

Time Required for Digestion of Starch.—In the determination of starch by enzyme extracts it is customary to apply the iodine test to decide when all the starch has been digested. The legume residues were found to give a blue coloration with iodine even after 10 hours digestion with the malt extract. Whether a positive test for starch is obtained depends largely on the selection of the particles to which the iodine is applied. Particles of cellular material, such as the outside of the seed, or of the aleurone layer gave no blue color while the more flocculent and less easily removed particles usually did. If a mass of the residue was collected by filtration, a blue color was invariably obtained.

The starch-iodine reaction is very sensitive, and probably shows with such minute quantities of starch that, in work of this nature, where large percentages are concerned, the quantity of undigested starch is negligible. Some hemicelluloses[5] and hydrates of cellulose[6] give blue colorations with iodine and their presence may be responsible for this test.

Because of the difficulty cited above in determining the completion of digestion by the iodine test, a series of seven determinations was made, in which digestion was continued for various lengths of time. One sample was analyzed at the end of 2 hours, three after 4 hours, two after 6 hours, and one after 10 hours.

The 6- and 10-hour samples were boiled at the end of 4 hours and new portions, 15 and 25 cc. respectively, of malt extract were added. In each case salicylic acid was added at the end of the digestion period to check enzymatic action and to prevent fermentation during the subsequent filtration. A portion of the filtrate was hydrolyzed and the reducing sugars determined in the usual manner. The data are given in Table III.

A consideration of the results of the foregoing experiment indicates that 4 hours digestion is probably sufficient, even though the iodine test is still positive on the residues. A 10-hour digestion does not increase the amount of starch to an appreciable extent, and in view of the fact that

[1] Stone, *Office Exp. Sta. Bull.*, **34** (1896).

[2] O'Sullivan, *J. Chem. Soc.*, **85**, 616 (1904).

[3] Ford, *J. Soc. Chem. Ind.*, **23**, 414 (1904).

[4] Sherman, THIS JOURNAL, **41**, 1123 (1919).

[5] Haas and Hill, "The Chemistry of Plant Products," Longmans, Green and Co. **1917**, p. 144.

[6] Cross and Bevan, "Cellulose," Longmans, Green and Co., **1918**, p. 7.

longer manipulation increases the possibility of error, the shorter time of digestion is preferable.

In this experiment, the possibility of salicylic acid bringing about hydrolysis of some carbohydrate other than starch, was tested. The material digested with malt was left in contact with the salicylic acid for 3 days, but as may be seen from Table III, showed no increased production of reducing sugars. The addition of salicylic acid was of particular advantage in the case of digestion mixtures which filtered slowly. Considerable loss may result from the fermentation of sugars in such cases, unless the activities of the microörganisms are inhibited by the addition of a preservative.

TABLE III.

TIME REQUIRED FOR COMPLETION OF DIGESTION OF STARCH.

Sample.	Malt extract. Cc.	Time of digestion. Hours.	Reducing substances as starch. %.[a]
1	25	2	44.64
2	25	4	48.85
3	25	4	50.92
4	25	4	50.51
5	40	6	51.48
6	40	6	50.96
7	65	10	52.23
7 (after 3 days)	65	10	52.02

[a] Air dry basis.

The Starch Content of Peas.—To obtain a comparison of the starch content of navy beans with some other legume seed two varieties of peas, Alaska garden and Canada field peas, were examined. These samples were prepared and analyzed in the same manner as the 1919 sample. Table IV gives the results of the analyses. Both samples of peas showed a high content of starch, the percentage being about equal to that of the 1919 sample of navy beans. The starch comprises more than 80% of the nitrogen-free extract, and leaves only a small percentage of the latter to be accounted for by pentosans, galactans, etc. This is in agreement with the 1919 sample and in sharp contrast to the 1917 sample of navy beans. The data from all the analyses emphasize the fact that legume

TABLE IV.

STARCH AND OTHER CONSTITUENTS OF TWO VARIETIES OF PEAS.

	Alaska Garden Peas. %.	Canada Field Peas. %.
Moisture	10.36	8.60
Ether extract	1.13	0.81
Crude fiber	5.74	4.44
Protein	19.63	28.48
Ash	2.88	3.20
Nitrogen-free extract (by difference)	60.26	54.47
	100.00	100.00
Starch	51.21	45.11

seeds are not only rich in protein but also contain a large amount of starch.

Summary.

From the analyses of navy beans, Alaska garden and Canada field peas, it was found that the largest portion of the nitrogen-free extract is starch. Of the 58.97% of nitrogen-free extract in the 1917 sample of navy beans 35.20% was starch, and 15% was distributed among pentosans, dextrins, cellulose, galactans, and sugars, in the order named. Of the 61.80% of nitrogen-free extract of the 1919 sample, 50.54% was total reducing substances, calculated as starch. In the case of the Alaska garden peas, the nitrogen-free extract was 60.26%, and the starch was 51.21%. The Canada field peas had a somewhat lower content of starch, 45.11%, but the nitrogen-free extract was correspondingly low, 54.47%. The starch content of beans varies from year to year, but in general, the legume seeds investigated were found to contain a large amount of starch.

The completeness of digestion of legumes by malt diastase was greatly enhanced by fine grinding. The increase in digestible starch amounted to from 10 to 12% in the finely ground material. Interfering substances, such as cellulose or protein, are broken up and the starch is exposed to the action of the enzyme. The iodine test for the presence of starch is not a satisfactory means of determining when to stop the digestion. After 10 hours' digestion with several additions of malt extract, the residues of legumes still show a blue coloration. The amount of starch obtained by digesting for 10 hours was but little greater than that found with 4 hours' digestion. The iodine test is sensitive to extremely small quantities of starch, and shows that a trace of this may remain undigested even after 10 hours. It is also possible that some substance other than starch gives the blue coloration.

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[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO.]

PREPARATION OF 5,5′-MERCURI-BIS-3-NITRO-4-HYDROXY-PHENYL-ARSONIC ACID.[1]

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Although arsphenamine and neo-arsphenamine have proved very efficacious in the war on spirochaetes, it has been found most effective to

[1] This investigation was undertaken by Mr. Hanke, Swift Fellow of the University of Chicago, at my suggestion as Special Adviser to the Hygienic Laboratory of the U. S. P. H. S. The coöperation of Dr. Kharasch, National Research Fellow in Organic Chemistry, was invited because of his interest in the theories of the substitution and stability of mercury in aromatic nuclei. As outlined in the text, interesting questions of broader theoretical moment in this field were actually developed.—*J. S.*

[2] The material presented here is used by Martin Hanke in his dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Chicago.