in evaluating various modified starches, and in elucidating the action of β-amylase on the starch fractions. A study of the reducing values of the A-fraction toward hypoiodite, bromine, alkaline copper, ferricyanide and alkaline dinitrosalicylate indicates that none of these reagents is specific for terminal aldehyde groups.

A technique has been devised for subfractionating the A-fraction by successive partial precipitations with *n*-octyl alcohol. The A-fraction appears to consist of a continuous series of homologous linear polymers, rather than a limited number of discrete components. The linear material of longest chain length (as indicated by intrinsic viscosity and reducing value) is precipitated first by octyl alcohol, followed successively by subfractions of progressively shorter chain length. The ease of retrogradation of a linear starch substance is inversely related to its chain length. Linear subfractions of equal intrinsic viscosity and reducing value have the same retrogradation tendency, irrespective of their source. Thus the lower retrogradation of tapioca and potato starches must be attributed to the longer chain length of their A-fractions and not to anomalous branching.

However, indirect evidence from iodine affinities suggests the presence of a material intermediate between the strictly linear and the highly branched fractions, possibly amounting to 5–7% of the total starch substance in the case of corn starch. This material is precipitated by Pentasol but not by *n*-butyl alcohol. To minimize contamination of the fractions by this intermediate material, it is recommended that Pentasol be used for the primary separation and *n*-butyl alcohol for recrystallization.

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[CONTRIBUTION FROM THE DIVISION OF PLANT NUTRITION AND FOOD TECHNOLOGY, COLLEGE OF AGRICULTURE, UNIVERSITY OF CALIFORNIA]

Starch. III. Structure of Apple Starch

BY A. L. POTTER, W. Z. HASSID AND M. A. JOSLYN

While all starches possess certain fundamental common structural features, it is now recognized that starches from different sources vary in the following respects: (1) proportion of the two constituents, amylose and amylopectin, (2) average length of the amylose chains, (3) average chain length of the amylopectin branches in the molecule, (4) molecular size of amylose and of amylopectin.

Practically all the available information regarding starch structure has been derived from work on cereal and tuber starches, chiefly corn and potato. It was therefore of interest to examine a fruit starch in order to ascertain whether or not its structure differs from that of the cereal and tuber starches previously studied.

The starch was isolated from apples and separated by Schoch's "Pentasol" precipitation method[1] into amylose and amylopectin. The yield of amylose was 24.8% of the total starch. Analysis of the whole starch by Schoch's[2] modification of Bates and collaborators'[3] potentiometric iodine titration method showed an amylose content of 26.5%. The intrinsic viscosity of the amylose was 0.99 and that of the amylopectin was 0.96.

Treatment with crystalline β-amylase hydrolyzed the amylose to the extent of 90% maltose. With amylopectin the hydrolysis ceased when 63.5% was degraded to maltose.

Upon acetylation of the two fractions with acetic anhydride at room temperature and the determination of their osmotic pressures, a number-average molecular weight of 160,000 (560 glucose residues) was obtained for the acetylated amylose and 1,200,000 (4200 glucose residues) for the acetylated amylopectin.

In a previous study[4] the number-average molecular weights of six acetylated amylose components from starches of six different plant sources ranged from 180,000 to 370,000. The acetylated amylopectins from the same sources ranged from 2,000,000 to 10,000,000.

The same osmotic pressure–concentration relationship was used as previously reported for the other acetylated amyloses and amylopectins.[4] Using the values $n = 1.39$ and 2.25 for acetylated apple amylose and acetylated amylopectin, respectively, and plotting π/C against C^n, straight lines were obtained. Employing this method, the intercept of the coördinate could be determined with greater accuracy, thus resulting in more reliable molecular weight determinations.

End-group determination by the periodate oxidation method showed an average of 24 glucose residues per end-group for the amylopectin. For the amylose an average chain-length of 530 glucose residues was obtained. The latter value is in fair agreement with 560 obtained by osmotic pressure measurements, indicating that, like po-

(1) T. J. Schoch, "Advances in Carbohydrate Chemistry," edited by Pigman and Wolfrom, Academic Press, Inc., New York, N. Y., Vol. I, 1945, pp. 258–261.

(2) T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).

(3) F. L. Bates, D. French and R. E. Rundle, *ibid.*, **65**, 142 (1943).

(4) A. L. Potter and W. Z. Hassid, *ibid.*, **70**, 3774 (1948).

tato and Easter lily amylose,[4] a single chain represents one amylose molecule. The apple amylopectin data are in accord with the data obtained for other amylopectins, showing that the molecule consists of a great number of branched chains averaging approximately 24 glucose residues per end-group.

The study of the apple starch did not reveal any anomalous features concerning its structure. It appears to be similar to that of the cereal and tuber starches previously examined, except that the molecular weights of the apple starch are lower.

Experimental

Preparation of Apple Starch.—The starch was prepared from Newtown Pippin apples grown in Willow Glen district of San Jose, California. Approximately 25 lb. of apples were harvested early in August, stored for a week at 0°, then crushed in an apple grater and pressed in a hydraulic press, using wooden racks and canvas cloths. The press juice was collected and treated with 5 g. of potassium metabisulfite ($K_2S_2O_5$) to inhibit browning. The press cake was mixed with an equal weight of water and pressed again. The juice was stored at room temperature until the starch settled, the supernatant liquid was decanted and the sediment washed with water to remove pulp particles by flotation. The starch was then resuspended in water and the washing was repeated several times until all the pulp particles and soluble material was removed. The starch was filtered on a Buchner funnel, washed with ethanol and dried *in vacuo* at 60°. The fatty acids were removed from the dry material according to Schoch's[5] method by means of five successive twenty-four-hour extractions with hot 85% methanol. The yield was 23 g. When viewed under the microscope the granules appear spherical or sometimes slightly irregular. Their size varies from 2.5 to 10 μ.

Separation of the Starch into Amylose and Amylopectin.—The two fractions were separated according to Schoch's method.[1] A 2% suspension was made, using 20 g. of defatted apple starch, gelatinized on a steam-bath and autoclaved for three hours at 18 lb. pressure. After the addition to the hot solution of 10% (by volume) "Pentasol" and cooling, the amylose precipitated in the form of spherocrystals. The crude material was purified by reprecipitating four times with butanol.

The amylopectin was isolated from the first mother liquor by the addition of methanol. The precipitate was dissolved in water and the amylose impurities removed by the addition of butanol. A yield of 4.96 g. of amylose and 12.4 g. of amylopectin was obtained. The amylose yield constitutes 24.8%.

The proportion of amylose and amylopectin was determined in the apple starch by using the potentiometric iodine titration method of Bates, French and Rundle as modified by Schoch.[2] The iodine bound by the amylose was found to be 19.0% whereas that of the amylopectin was 0.1%. The unfractionated starch absorbed 5.1% iodine, showing a 26.5% amylose content.

Intrinsic Viscosity.—In evaluating the intrinsic viscosities of the fractions, the relative viscosity was determined in an Oswald type viscosimeter, using 1 N potassium hydroxide solution as a solvent. Determination of four concentrations between 0.1 and 0.4% were made in a constant temperature bath at 35 ± 0.01°. The log of the relative viscosity was plotted against concentration and the intrinsic viscosity was evaluated by multiplying the value of the slope of the line by 2.3.[6] The intrinsic viscosity $[\eta]$ of the amylose was found to be 0.98 and that of amylopectin 0.95.

(5) T. J. Schoch, THIS JOURNAL, **64**, 2954 (1942).

(6) S. Arrhenius, *Z. physik. Chem.*, **1**, 285 (1887); E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 153 (1935); *Ind. Eng. Chem.*, **30**, 1200 (1938).

Acetylation.—Two-gram samples of the starch fractions were dispersed in formamide and acetylated at room temperature with acetic anhydride in the presence of pyridine as previously described.[4] The yield of acetylated amylopectin was 88% and that of the acetylated amylose 90%. An acetyl value of 43.6% was obtained for the acetylated amylose and 44% of the acetylated amylopectin. The calculated $COCH_3$ content for the triacetate $(C_6H_7O_5(CH_3CO)_3)_n$ is 44.8%. The specific rotation of both the acetylated amylopectin and amylose in chloroform (c, 2) was $[\alpha]_D$ +170°.

TABLE I

OSMOTIC PRESSURE DATA FOR ACETYLATED APPLE STARCH FRACTIONS

Concn., C, g. per l.	$C^{1.39}$	Osmotic pressure, π (g. per sq. cm.)	π/C
	Amylose		
2.83	4.2	0.350	0.182
5.74	11.4	0.920	.235
7.17	15.4	1.30	.266
10.05	24.7	2.18	.319
	Amylopectin		
	$C^{2.25}$		
12.3	280	0.325	0.0389
15.1	450	0.540	.0525
17.6	630	0.760	.0634
20.0	840	1.06	.0778

Determination of Molecular Weights.—The molecular weights of the acetylated apple amylopectin and amylose were determined by osmotic pressure measurements, using chloroform as a solvent. The method employed was the same as that previously used for the determination of a number of acetylated starch fractions from various plant sources.[4] The osmotic pressure was measured for each acetylated starch fraction at several different concentrations (Table I).

The intercept of the coördinate was determined by plotting π/C against C^n and the molecular weight was calculated using the van't Hoff equation. From the intercepts of the ordinates values of π/C were obtained equal to 0.157 and 0.0210 for acetylated amylose and amylopectin, respectively. These values corresponded to a number-average molecular weight of 160,000 for acetylated amylose of 1,200,000 for acetylated amylopectin.

End-Group Determination of Amylopectin and Amylose by Periodate Oxidation.—A series of five 0.2-g. samples of amylopectin were oxidized with 0.37 M sodium periodate at 2° according to the method previously described.[7] After ten hours the samples were taken at five-hour intervals and analyzed for formic acid by titrating with 0.01 N barium hydroxide. The number of ml. of base (5.2) at twenty-five hours was taken as the end-point for the amylopectin. The average number of glucose residues per end-group for amylopectin, calculated on the basis of one mole of formic acid produced per chain, was found to be 24.

Duplicate 0.5-g. amylose samples were similarly oxidized with sodium periodate for twenty-five hours. The acid produced required 1.75 ml. of 0.01 N barium hydroxide for neutralization. The average chain-length of amylose, calculated on the basis of 3 moles of formic acid produced per chain, was 530 glucose residues.

Hydrolysis with β-Amylase.—A 0.02-g. sample of polysaccharide was dissolved in 1 ml. of 1 N potassium hydroxide, diluted to 35 ml. and mixed with 5 ml. of 2 M acetate buffer at pH 4.7. One drop of crystalline β-amylase[8] solution, containing approximately 500 Schwimmer units, and a drop of toluene were added to the mixture

(7) A. L. Potter and W. Z. Hassid, THIS JOURNAL, **70**, 3488 (1948).

(8) A. K. Balls, M. K. Walden and R. R. Thompson, *J. Biol. Chem.*, **173**, 9 (1948).

and the hydrolysis was allowed to proceed for twenty-four hours. Another drop of β-amylase was then added and the mixture was allowed to remain at room temperature for another twenty-four hours. The reducing value was then determined by oxidation with ferricyanide[9,10] and calculated as maltose.

The amount of polysaccharide originally present in the sample was found by determining the glucose obtained when a 2-ml. aliquot portion was treated with 1 *N* sulfuric acid for two and one-half hours at 100°. On this basis, the extent of the amylopectin hydrolyzed with β-amylase to maltose was estimated to be 63.5%. The limit of amylose hydrolysis with this enzyme was 90%.

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Summary

Apple starch was separated into amylose and amylopectin, the amylose content being 24.8% of the total. Osmotic pressure measurements of the acetylated fractions gave a number-average molecular weight of 160,000 (560 glucose residues) for the acetylated amylose and 1,200,000 (4200 glucose residues) for the acetylated amylopectin.

End-group determination by periodate oxidation showed an average of 24 glucose residues per end-group for the amylopectin and a chain-length of 530 glucose residues for the amylose. Since the end-group value for amylose is in fair agreement with the value of 560 obtained from osmotic pressure measurements, it is assumed that a single chain represents one amylose molecule.

The data indicate that apple starch is similar in structure to the cereal and tuber starches, with the difference that the molecular weights of its components are smaller.

(9) W. Z. Hassid, *Ind. Eng. Chem., Anal. Ed.*, **9**, 228 (1937).

(10) W. Z. Hassid, R. M. McCready and R. S. Rosenfels, *ibid.*, **12**, 142 (1940).

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Chain Initiation in Styrene Emulsion Polymerization[1]

BY WENDELL V. SMITH

Introduction

The chain length and rate of polymerization in emulsion are controlled principally by the relationship between two quantities, the rate of chain initiation and the rate of chain propagation. In contrast with bulk polymerization, the rate constant for the termination step does not play a significant role in the mechanism of emulsion polymerization.[1a] The rate of chain propagation in styrene polymerization has already been considered[1a] and this paper discusses the rate of the initiation step in styrene polymerization.

Three methods are used for investigating the rate of initiation. The first is based on the observation that fragments of the chain initiator, persulfate, are chemically combined with the polymer.[2] It consists in determining how rapidly these fragments become combined by measuring the rate of polymerization and the radioactivity of polystyrene prepared using radioactive persulfate as initiator. The second consists in determining the initial rate of polymer particle formation from measurements of the size of the particles and the rates of growth of the particles, making use of a previously developed theory relating these.[1a] The third consists in determining the rate of molecule formation from the molecular weight of polymer and the rate of polymerization.

Experimental Procedures

Radioactive Potassium Persulfate.—The radioactive S^{35} was received as a trace constituent of potassium chloride from Clinton Laboratories (now Oak Ridge National Laboratory) on allocation by the U. S. Atomic Energy Commission.

The active sulfur in the irradiated unit was isolated as barium sulfate by boiling with bromine in water solution then precipitating with barium ion. The barium sulfate (1.3 mg.) was converted to sulfate free potassium persulfate by dissolving in concentrated sulfuric acid, adding potassium sulfate and electrolyzing cold. The precipitated potassium persulfate after washing and drying (0.35 g.) contained 12% of the total sulfur; it was made up to a 1.81% solution. After the preliminary measurements the persulfate was regenerated by a new electrolysis at which time it was further diluted with inactive sulfur.

Determination of Sulfur Content of Polystyrene from its Radioactivity.—All radioactivities were determined using a Radiation Counter Laboratories thin mica window Geiger–Müller counter tube and scaling circuit.

The resolving time[3] of 2.5×10^{-4} sec. requires a correction of only 0.25% for a counting rate of 10 per sec.

The range of the β-radiation from S^{35} is quite

(1) Presented at the North Jersey Section Meeting-in-Miniature, January 10, 1949, Newark, N. J.

(1a) W. V. Smith and R. H. Ewart, *J. Chem. Phys.*, **16**, 592 (1948); W. V. Smith, THIS JOURNAL, **70**, 3695 (1948).

(2) W. V. Smith and H. N. Campbell, *J. Chem. Phys.*, **15**, 338 (1947); W. E. Mochel and J. H. Peterson, THIS JOURNAL, **71**, 1426 (1949).

(3) A. F. Reid, A. S. Weil and J. R. Dunning, *Anal. Chem.*, **10**, 824 (1947).