535. *Physicochemical Studies on Starches. Part XIII.* The Fractionation of Oat and Wheat Starches.*

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The behaviour of laboratory-prepared oat and wheat starches on fractionation by dispersion and aqueous leaching has been critically examined. Aqueous leaching at various temperatures resulted in sub-fractionation of the amylose. The physical properties of the various starch-products have been determined and are discussed.

Satisfactory fractionation of laboratory-prepared cereal starches into their component amylose and amylopectin appears to be more difficult [1,2] than is the case for potato starch. This paper deals with the problems for oat and wheat. The effect of variations in methods of fractionation on molecular size and β-amylolysis limits of the amylose has been examined. Results are discussed with regard to the structure of amylose and the nature of the granule.

Experimental Methods

Preparation of the Starches.—Starch was isolated from oats (var. Milford) and wheat (var. Victor II) by aqueous extraction of the defatted grain, and was purified from protein by Anderson and Greenwood's method.[3] The starches were then exhaustively defatted with boiling 80% methanol. A commercial sample of wheat starch was also studied.

Leaching and Dispersing Procedures.—The methods employed to fractionate the starches in a nitrogen atmosphere by (i) aqueous leaching at 70° and 98°, and (ii) complete dispersion of the granular structure with and without pretreatment with M-potassium hydroxide at 0° were as described elsewhere,[4] with the exception that some dispersions were carried out in the presence of phosphate buffer (pH 6·5; 2 ml./100 ml. of dispersion).

* Part XII, *J.*, 1958, 711.

[1] MacWilliam and Percival, *J.*, 1951, 2259.
[2] Greenwood and Das Gupta, *J.*, 1958, 707.
[3] Anderson and Greenwood, *J. Sci. Food Agric.*, 1955, **6**, 587.
[4] Cowie and Greenwood, *J.*, 1957, 2862, 4640.

Characterization of Starch Products.—Measurements were made of (i) iodine affinity, (ii) limiting viscosity number $[\eta]$ in M-potassium hydroxide, and (iii) β-amylolysis conversion limit. (See earlier papers in this Series.)

Properties of Whole Starches.—The properties of the whole starches were:

Starch	Protein (%) [a]	I.A.[b]	Amylose (%) [c]	$\overline{R}$ [d]	C.L. for amylopectin
Oat	0·24	5·13	27·0	26·8	19·6
Wheat	0·33	5·00	26·3	25·6	18·9

[a] % N × 6·25. [b] Iodine affinity. For both starches (I.A./19·0) × 100. [d] Ratio of terminal to non-terminal groups from HIO_4 oxidation (see ref. 5). [e] Conversion limit, calc. from previous column.

Results and Discussion

For both starches, the purely physical methods of purification used did not reduce contaminating protein to below about 0·3%, a value about ten times greater than that for potato starch. Johnston's method [6] involving extraction with 1% ammonium oxalate was also not satisfactory. Tightly bound protein appears to be, in fact, a characteristic of these laboratory-prepared granular starches. Its significance with regard to granular structure is not known, but it might well hinder the swelling properties and so influence its dispersive properties. The salient features of the results of various fractionation

Table 1. *Properties of the components from oat starch.*

		Amylopectin		Amylose				
Expt.	Method of fractionation	Purity (%) [a]	% of total amylose retained [b]	I.A.[c]	$[\eta]$ in M-KOH	D.P.[d]	Conversion limit (i)	Conversion limit (ii)
F1	70° Aqueous leach	82	67	19·1	160	1190	95	100
F2	98° Aqueous leach	94	23	18·0	340	2500	79	97
F3	Thymol–Bu^{n}OH dispersion without buffer	92	31	13·7	180	1330	71	86
F4	Thymol–Bu^{n}OH dispersion with buffer (pH 6·47)	93	27	14·7	212	1570	—	—
F5	KOH-pretreatment	99	<4	16·8	263	1950	72	91

[a] Calc. from (iodine affinity/19·0) × 100. [b] % of total amylose in starch retained as impurity in the amylopectin. Calc. by assuming original starch to contain 27% of amylose. [c] Iodine affinity. [d] Approx. degree of polymerization. Calc. from $\overline{D.P.} = 7{\cdot}4\,[\eta]$ (see text). [e] β-Amylolysis limits for (i) pure β-amylase, and (ii) β-amylase + Z-enzyme. Expressed as % conversion into maltose. Accuracy ± 2%.

Table 2. *Properties of the components from wheat starch.*

		Amylopectin		Amylose				
Expt.	Method of fractionation	Purity (%) [a]	% of total amylose retained [b]	I.A.[c]	$[\eta]$ in M-KOH	D.P.[c]	Conversion limit [e] (i)	Conversion limit [e] (ii)
F6	70° aqueous leach	81	74	17·8	145	1070	98	—
F7	98° aqueous leach	94	23	15·8	300	2240	66	89
F8	Thymol–Bu^{n}OH dispersion without buffer	96	12	19·0	260	1920	65	96
F9 *	Thymol–Bu^{n}OH dispersion without buffer	95	18	17·2	133	980	—	—
F10	Thymol–Bu^{n}OH dispersion with buffer (pH 6·47)	96	12	17·6	280	2060	—	—
F11	KOH-pretreatment	96	12	18·0	258	1910	—	—

[a] Calc. from (iodine affinity/19·0) × 100. [b] Calc. as for Table 1 by assuming original starch to contain 26·3% of amylose. [c] As for Table 1. * Commercial starch.

experiments are summarized in Tables 1 and 2. Values for the average degree of polymerization of the amylose ($\overline{D.P.}$) were calculated by using the relation previously obtained for potato amylose.[4] Although this may not be extremely accurate, the calculated values are likely to be of the correct order of magnitude.

[5] Anderson and Greenwood, *J.*, 1955, 3016.
[6] Johnston, *Nature*, 1956, **178**, 370.

Whilst sub-fractionation of the amylose component by aqueous leaching of the granules at various temperatures was satisfactory, both starches proved extremely difficult to disperse before conventional fractionation by precipitants. Resulting dispersions were turbid even after two hours' boiling, and addition of either sodium chloride or phosphate buffer (pH 6·47) made no improvement. Preliminary experiments with oat starch made in collaboration with Mr. J. M. G. Cowie had shown thymol to be a more suitable initial precipitant than *cyclo*hexanol, butan-1-ol, pentanol, or pyridine. This reagent was used therefore for wheat. Difficulty was found in recrystallizing both amyloses from butan-1-ol solutions; in some instances, an iodine affinity of 19·0% (the maximum value found for these amyloses: cf. ref. 5) could not be achieved.

The limiting viscosity numbers of the 98°-leached products (F2 and F7) were higher than for the other amylose fractions. This suggests that degradation must have occurred during the dispersion—even in a nitrogen atmosphere—and the two amyloses appear to be more susceptible to hydrolysis than potato amylose. (For example, when amylose F2 was heated in boiling water in the presence of nitrogen for 1 hr., $[\eta]$ decreased from 340 to 260, whilst for amylose F7 under similar conditions $[\eta]$ decreased from 300 to 280.)

Fractionation Conditions.—In view of the apparent lability of the amylose components of oat and wheat starch, fractionation by aqueous leaching at 98° (cf. ref. 7) yields amylose of higher purity and limiting viscosity number than does a conventional dispersion. However, the purity of the amylopectin obtained by this method may not be high (cf. also refs. 4 and 8). For laboratory-prepared cereal starches, as has already been found for *Zea mays* starch,[2] the method [9] involving pretreatment with M-potassium hydroxide at 0° appears to be most satisfactory; it yields purer amylopectin, and amylose which is relatively little degraded.

Fractionation of Commercial Wheat Starch.—The sample of commercial wheat starch gave an amylose with a low limiting viscosity number. The decrease was relatively much larger than between laboratory- and commercially-prepared potato starch.[4] This is related perhaps to the more vigorous purification necessary to remove protein in the manufacture of cereal starches.

Uniformity of Structure of Amylose.—To investigate whether the different amylose fractions were linear or contained some branch-point or other anomaly, β-amylolysis experiments were carried out (see Tables 1 and 2). The concurrent action of β-amylase and Z-enzyme [10] enabled another calculation to be made of the amount of amylopectin impurity in some fractions; this value agreed well with that from iodine affinity measurements. Table 3 summarises the results and previous values obtained for potato amylose.[4]

TABLE 3. *Comparison of β-amylolysis limits for various fractions of amylose from laboratory-prepared starches.*

	Potato †			Oat			Wheat		
Prep. of sample in N_2	% of total amylose	$[\eta]$	β-limit *	% of total amylose	$[\eta]$	β-limit *	% of total amylose	$[\eta]$	β-limit *
Aq. leaching at 70°	40	240	100	33	160	95	28	145	98
Aq. leaching at 98°	80	370	86	77	340	80	77	300	69
Dispersion of granule ...	100	440	77	100	180	77	100	260	66

† Results from ref. 4. * Expressed as % conversion into maltose. Calculated by assuming 56% conversion of any amylopectin impurity into maltose.

The same general trend for the β-amylolysis limits of the different fractions is observed as for potato starch.[4] Leaching at 70° caused limited swelling of the granules and enabled short-chain, essentially linear amylose (as shown by its high conversion into maltose) to

[7] Baum, Gilbert, and Wood, *J.*, 1955, 4047.
[8] Bengough, Stanwix, and Steedman, *Chem. and Ind.*, 1957, 1241.
[9] Potter, Silveira, and McCready, *J. Amer. Chem. Soc.*, 1953, **75**, 1335.
[10] See Cowie, Fleming, Greenwood, and Manners, *J.*, 1958, 697.

diffuse out. Extraction at higher temperatures gave amylose which was incompletely hydrolysed, the amount of resistant material increasing with increase in temperature and consequent swelling and disruption of the granule. The difficulties in determining the nature of the barrier to β-amylolysis have been discussed elsewhere,[11] but it is thought that since disruption of the granule is involved, branching in the amylose is not improbable.[4]

For oat amylose, if about 35% is linear, and the whole is hydrolysed to 77% with β-amylase, then the portion containing an anomaly must be hydrolysed to about 65%. For wheat amylose, similar calculations show that the portion containing an anomaly must be hydrolysed to about 50%. These results suggest that the barrier to β-amylolysis in these amyloses is again essentially randomly situated (cf. ref. 10).

Although Peat,[12] Hopkins,[13] and Hassid,[14] with their collaborators, have suggested that pure β-amylase converts only about 70% of amylose into maltose, our experiments on amylose sub-fractions indicate that amyloses obtained by dispersive methods consist of two types of molecule, some being linear and others having a randomly situated barrier to this enzymic hydrolysis. On this basis, the percentage of linear material (L) in any amylose fraction can be calculated to the first approximation from the expression: $(T - L)/(100 - L) = 1/2$, where T = the percentage conversion into maltose for the total fraction. Values of L from calculation and experimental determination may not agree, since the aqueous leaching is not necessarily quantitative. The results from such calculations (*i.e.*, potato, *ca.* 55; oat, *ca.* 55; wheat, *ca.* 30%) suggest that the amount of linear material may vary from starch to starch.

The authors thank Professor E. L. Hirst, F.R.S., for his interest in this work and the Rockefeller Foundation for financial support.

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The University, Edinburgh, 9. [*Received, February 5th*, 1958.]

[11] Cowie, Fleming, Greenwood, and Manners, *J.*, 1957, 4430.
[12] Peat, Pirt, and Whelan, *J.*, 1952, 705, 714.
[13] Hopkins and Bird, *Nature*, 1953, **172**, 492.
[14] Neufeld and Hassid, *Arch. Biochem. Biophys.*, 1955, **59**, 405.