CCXVIII.—*Amylases of the Cereal Grains—Oats.*

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In a previous paper by one of us (J., 1902, **81**, 1177) an account was given of the properties of the amylase of barley. This enzyme, which has only a slight liquefying action on starch paste, yields 60% of crystalline maltose and 40% of α-amylodextrin when allowed to act on soluble starch. We found (J., 1921, **119**, 805) that the amylase of ungerminated rye liquefied starch paste readily, the products of the reaction being similar to those from barley amylase. When, however, this grain (rye) is germinated (malted) the enzymes produced yield from starch crystalline maltose and a non-hygroscopic unfermentable reducing dextrin (R 10·8; $[\alpha]_D$ + 181·9°). Malted rye differs from malted barley in that there is no production of the intermediate maltodextrins degradable by malt amylase.

A botanical interest attaches to the degradation products of starch by different cereal amylases, and a technical interest to the behaviour of the amylases of these germinated cereals on starch in

the fermentation industries. Since the amylases of rye differ somewhat from those of barley, we considered we should extend the work to other commonly occurring cereals.

Some years ago, Klempin (*Biochem. Z.*, 1908, **10**, 202) found that ground oats (*Avena sativa*) possessed amylolytic activity, but he did not attempt to isolate the products.

Vine (*J. Inst. Brewing*, 1911, **17**, 335) records that oats have marked liquefying power towards starch paste. Other authors have referred to the use of oats and malted oats in the brewing and distilling industries, but nothing definite appears to be known of the nature of the products formed when starch is degraded by the amylase of the ungerminated and germinated grain. As there is so little information available, it may be of interest to give a brief account of our work on the subject, and to record in what respects the oat amylases differ from those found in the botanically closely related barley and rye.

Experimental.

Ungerminated Oats.—For the purpose of this work two separate lots of oats were used, one grown in England, the other of Chilian origin. The diastatic power as measured by the Lintner method was 2° in both cases. This is a low figure as compared with those for other cereals; for instance, an average diastatic power for barley is 15—20° and for rye 26°. As the rapidity and extent of conversion is a function of the proportion of enzyme to substrate, we had to have this in mind in determining the quantity of enzyme to be used.

A filtered aqueous infusion of oats liquefied starch paste (potato) somewhat slowly, and for this reason Lintner's soluble starch was used, as we were satisfied by experimental evidence that it gave the same products as starch paste. It was found convenient to separate the amylase from a filtered 20% alcoholic infusion by precipitation with alcohol and to allow it to act on a 3% solution of soluble starch at 50°. At different times portions were withdrawn for analysis and pipetted into a volume of boiling water sufficient to stop further diastatic action.

The extent to which hydrolysis of the starch is carried depends, as is shown in the following table, on the time and the ratio of amylase to substrate. The precipitated (by alcohol) amylase from a weight of oats equal to the weight of starch used effects in 24 hours a production of 45·5% of maltose, but if the amount of precipitated amylase is increased to two and a half and to five times, practically the whole of the starch is converted into maltose.

Effect of Time and the Amount of Enzyme on Extent of Conversion of Starch.

Ratio of enzyme to solids in substrate.	4 Hours.		24 Hours.	
	$R_{3\cdot93}$.	$[\alpha]_{D3\cdot93}$.	$R_{3\cdot93}$.	$[\alpha]_{D3\cdot93}$.
1 : 1	—	—	45·5	162·2°
2·5 : 1	67·3	149·7°	91·8	138·2
5 : 1	73·0	146·9	96·6	135·0

To show the course of the reaction in more detail the results of a typical conversion are set out in the following table.

Action of Ungerminated Oat Amylase on Soluble Starch at 50°.

(Ratio of enzyme to substrate, 5 : 1.)

Time.		$R_{3\cdot93}$. Reducing power as maltose.	$[\alpha]_{D3\cdot93}$.	Iodine reaction.
Hrs.	Mins.			
—	6	10·6	183·6°	—
—	12	16·5	177·8	—
—	20	24·0	174·3	Violet
—	40	39·9	163·0	—
1	5	51·2	157·5	Violet-purple
2	0	62·6	152·7	—
4	0	73·0	146·9	Red
10	0	84·8	141·3	—
24	0	96·6	135·0	Nil

The conversion products were evaporated and poured into alcohol and a very small amount of precipitate, mostly nitrogenous matter, was filtered off. The sugar portion when concentrated set to a solid crystalline magma, which had the constants of maltose and yielded maltosazone but no insoluble osazone.

Under the conditions of the experiment it is evident that maltose is the only product formed, the action differing in this respect markedly from that of barley and rye amylases on starch (*loc. cit.*). Moreover there is no resting stage in the neighbourhood of R 60—65 as in the case of barley and rye.

It was necessary to examine the dextrinous substances present in the conversion before its completion and to compare them with those formed by the action of barley and rye amylases on starch.

The precipitated amylase from ungerminated oats (200 g.) was used for the conversion of 100 g. of soluble starch in 4% solution at 50°. When the reaction had continued for 4 hours, the iodine reaction was purple and the constants were $R_{3\cdot93}$ 58 and $[\alpha]_{D\,3\cdot93}$ 155·8°. After evaporation the products of the conversion were fractionated with alcohol. A dextrin separated which had the constants $R_{3\cdot93}$ 2·6 and $[\alpha]_{D\,3\cdot93}$ 186·9°. The portion soluble in alcohol, when isolated, was found to be pure maltose and free from any trace of dextrose.

The dextrin was submitted to the action of precipitated amylase from (*a*) barley, (*b*) malted barley for 4 hours at 55°, the ratio of amylase to substrate being 1 : 1, with the following results :—

	Barley amylase.	Malt amylase.
$R_{3\cdot93}$	25·6	58·4
$[\alpha]_{D\ 3\cdot93}$	170·4°	151·6°

The results of the action of the amylase of barley and of malt on this dextrin closely resemble those obtained when α-amylodextrin is the substrate (*loc. cit.*), and we consider on this evidence that we are justified in stating that the substance present is that dextrin.

Action of Ungerminated Oat Amylase on Oat Starch Granules.—A suspension of oat starch was digested for 6 days with a filtered aqueous extract of oats at 43° in presence of toluene. About 18% by weight of the granules was dissolved, the soluble matter having a reducing power closely approximating that of glucose; the presence of this sugar was confirmed by the osazone test (compare J., 1914, **105**, 1529).

Action of the Amylase of Malted Oats on Starch.—A bulk of oats of the same origin as used in the preceding experiments was kindly malted for us on the technical scale by Messrs. Brookes & Co. of Mistley, Essex. The malt possessed a diastatic power of 6° (Lintner). The amylase from it was prepared in the manner previously described (*loc. cit.*). It liquefied starch paste readily. Unlike those of the amylase of ungerminated oats, the reaction constants were fairly similar, whether the material was used at the rate of 1, 2, or 4·5 parts of precipitated enzyme per unit of starch. With the high amylase ratio the 1-hour figure shows, as might be expected, an acceleration, though the final constants were not far apart.

Ratio of enzyme to substrate.	1 Hour.		4 Hours.		24 Hours.	
	$R_{3\cdot93}$.	$[\alpha]_{D\ 3\cdot93}$.	$R_{3\cdot93}$.	$[\alpha]_{D\ 3\cdot93}$.	$R_{3\cdot93}$.	$[\alpha]_{D\ 3\cdot93}$.
1 : 1	40·8	169·4°	66·2	152·6°	73·2	151·2°
2 : 1	40·6	165·8	65·0	151·1	73·1	150·3
4·5 : 1	49·2	159·6	68·5	146·4	75·6	146·2

A time conversion was made with the amylase of germinated oats on lines similar to those for the ungerminated (*vide supra*) at a temperature of 50°, enzyme to substrate in the ratio 2 : 1 being used.

Time.				
Hrs.	Mins.	$R_{3\cdot93}$.	$[\alpha]_{D\ 3\cdot93}$.	Iodine reaction.
—	10	13·4	182·2°	Purple-brown
—	20	23·6	177·2	,,
—	30	29·5	171·4	Red-brown
1	0	40·6	165·8	Faint brown
2	0	53·8	158·6	Nil
4	0	65·0	151·1	—
12	0	70·0	149·0	—
24	0	73·1	150·3	—

At the end of the conversion there was no evidence of the presence of glucose (osazone test). 31% of the solids were fermentable by *S. cerevisiæ*, the constants of the unfermentable residue being $R_{3\cdot93}$ 57·5 and $[\alpha]_{D\ 3\cdot93}$ 153·6°. From these results it is evident that a considerable amount of unfermentable matter is produced in the conversion, differing entirely in this respect from the conversion products obtained by the action of ungerminated oat amylase.

A conversion was made on a scale to obtain sufficient products for fractionation. Starch (100 g.) was made into a paste with 3 litres of water, and when the temperature had fallen to 50° the precipitated amylase from 100 g. of malted oats was added. The p_H of the conversion was 7·5. At the end of 4 hours the iodine reaction was light brown.

Conversion products (70 g.).

$R_{3\cdot93}$ 54·3; $[\alpha]_{D\ 3\cdot93}$ 162·6°.

Precipitated with alcohol (92%).

Insoluble 33%. $R_{3\cdot93}$ 9·3. $[\alpha]_{D\ 3\cdot93}$ 185·9°.
Soluble 66%. $R_{3\cdot93}$ 76·5. $[\alpha]_{D\ 3\cdot93}$ 152·5°.

Insoluble portion. This was degraded with precipitated malted barley amylase and precipitated malted oat amylase for 4 hours at 55°, the constants then being $R_{3\cdot93}$ 44, $[\alpha]_{D\ 3\cdot93}$ 161·8° and $R_{3\cdot93}$ 35, $[\alpha]_{D\ 3\cdot93}$ 165·6° respectively. In neither case was glucose present. The composition of these products has not been determined, but they appear to resemble those obtained from the degradation of the dextrin precipitated by alcohol from a malted barley amylase conversion.

Soluble portion. This was purified by a further precipitation with alcohol, a trace of insoluble dextrin being removed. The constants were now $R_{3\cdot93}$ 80·9, $[\alpha]_{D\ 3\cdot93}$ 149·2°. This was fermented with a pure culture of *S. cerevisiæ* for 16 days with the result that 50% disappeared, having the constants of maltose; the remaining 50% had $R_{3\cdot93}$ 57 and $[\alpha]_{D\ 3\cdot93}$ 158·4°. This substance, the greater part of which was soluble in alcohol and formed only a little osazone, soluble in water, is being further investigated.

Action of the Amylase from Ungerminated and Germinated Oats on Oat Starch Paste.—In the work hitherto described, the substrate employed was potato either in the form of Lintner's soluble starch or as paste. It was considered desirable to ascertain if oat starch paste behaved similarly, and some was prepared by squeezing wet ground oats through bolting silk into water and washing with very dilute alkali solution, with water and finally with alcohol. The air-dry starch contained 0·2% of mineral matter and 0·35% of nitrogen.

A 3% paste of this starch was prepared and the oat enzymes were allowed to convert it for 24 hours at 50°. The constants then were :—

	$[\alpha]_{D\ 3\cdot93}$.	$R_{3\cdot93}$.
Ungerminated oat amylase :		
Ratio of enzyme to substrate 2·5 : 1	140·4°	88
Germinated oat amylase :		
Ratio of enzyme to substrate 1 : 1	148	79

It will be seen from these results that the action of the amylase of ungerminated oats and germinated oats is the same whether oat or potato starch paste is used as a substrate.

Conclusions.

(1) The amylase precipitated by alcohol from ungerminated oats, when allowed to act in sufficient amount on soluble potato starch at 50°, yields only crystalline maltose, differing in this respect from the amylases of other ungerminated cereals which have hitherto been examined. If the conversion be stopped before completion, a substance practically identical with α-amylodextrin, previously described by one of us, and crystalline maltose are formed. The amylase liquefies starch paste slowly and less easily than the amylase of ungerminated rye.

(2) Ungerminated oat amylase has a solvent action on oat starch granules, producing glucose.

(3) The amylase from germinated oats yields (1) a dextrin, $R_{3\cdot93}$ 9·3 and $[\alpha]_{D\ 3\cdot93}$ 185·9°, (2) a maltodextrin-like substance soluble in alcohol, the composition of which has yet to be determined, and (3) a sugar having the constants of maltose.

The particular interest of this work lies in the fact that we have shown that starch when hydrolysed with ungerminated oat amylase produces only maltose. We see no way of reconciling this with the theory put forward by Maquenne and Roux and elaborated by Ling and Nanji, that the starch molecule is composed of amylose and amylopectin.* Our results support the older and more simple hypothesis that starch consists of condensed maltose residues.

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* Ling and Davis (*J. Inst. Brewing*, 1902, **8**, 481) showed that when unrestricted malt diastase, *i.e.*, diastase obtained from malt dried at 32°, was allowed to act on potato starch paste for 95 hours at 55° the conversion had the constants $R_{3\cdot93}$ 96·7 and $[\alpha]_{D\ 3\cdot93}$ 138·5°, and after one purification with alcohol yielded crystalline maltose.