CLXXXVI. AMYLASE DURING THE GROWTH AND RIPENING OF GRAINS.

BY TADEUSZ CHRZĄSZCZ AND JÓZEF JANICKI.

From the Laboratory of Agricultural Technology, Poznań University.

(Received 1 June 1936.)

RESEARCH conducted by us on the amylase of forming and ripening rye grain [Chrzaszcz & Janicki, 1936, 2] has shown that in the forming grain immediately after blossoming amylase exists with all its functions intact, i.e. not only with the saccharifying but also with the liquefying and dextrinizing functions. Hence it does not differ from malt amylase. As the grain develops and ripens, the liquefying and dextrinizing functions dwindle and finally disappear; saccharifying power remains, but lessened. This drop in amylase content or in the power of its various functions could, up to a certain stage of ripening of the grain, be counteracted by means of an eleuto-substance, in the shape of peptone nullifying the inactivating influence of the sisto-substance [Chrzaszcz & Janicki, 1933].

Our experiments on the relation of increase in the quantity of active amylase in cereals to the decomposition of protein by the action of added proteases furnished evidence against the views of Ford & Guthrie [1908] and of a number of other workers who stated that corn amylase is bound to protein, and that its liberation and activation can be effected only by means of the decomposition of the protein by the action of proteases. Experiments on the influence of hydrogen sulphide on increase of active amylase in cereals have also definitely shown that these views were mistaken [Chrzaszcz & Janicki, 1936, 1].

Since the observation that amylase with its three functions appears in rye grain immediately after blossoming conclusively contradicts the view that whole amylase only appears later, during germination, it was considered of interest to investigate whether this phenomenon likewise appears in other cereals, viz. oats, wheat and barley.

EXPERIMENTAL.

Rye grain was taken immediately after formation and at certain intervals during the process of ripening. The procedure followed was the same as in the research on rye [Chrzaszcz & Janicki, 1936, 2]. The grain was ground in a mortar, covered with water to form a 10% extract, which after 20 min. was centrifuged. Amylolytic power was then determined. Peptone was added and extracts prepared; in the case of barley, papain was used as the eleuto-substance and, in that of wheat, NaCl also. The tables give the percentage content of water in the cereals and the percentage of each extract used.

Amylolytic power was examined as in the previous research: thus, the saccharifying power was expressed in ml. *N*/20 iodine, computed for 1 g. of dry grain; the dextrinizing power was given without conversion, and the liquefying power was determined by the method of Lintner & Sollied, and expressed as g. of starch liquefied by 1 g. of dry corn.

Barley.

The outcome of the experiment indicates that during the period of ripening, barley grain has not only saccharifying but also dextrinizing and liquefying powers, although in a much less degree than ripening rye grain. It is also striking

Table I. *Amylolytic power of ripening barley grain.*

Days after blossoming	H ₂ O content of grain %	Extract %	Peptone added %	Saccharifying power		Dextrinizing power. Min. to change colour of starch with iodine to			Liquefying power	
				ml. N/20 iodine per 1 g. of grain	Change %	Violet-blue	Brownish red	Achro. stage	g. of starch liquefied by 1 g. of grain	Change %
10	63.8	3.62	—	150	—	100	405	Not obtained after 840 min.	6.9	—
			1	150	0.0	100	405	„	8.1	+17.4
			2	143	— 4.6	100	345	„	8.1	+17.4
			4	150	0.0	90	340	„	9.2	+33.3
11	62.5	3.75	—	190	+26.6	100	425	„	7.0	+ 1.5
			1	192	+28.1	90	425	„	7.3	+ 5.8
			2	178.1	+18.7	90	425	„	7.3	+ 5.8
			4	169.1	+12.7	90	425	„	7.3	+ 5.8
			8	169.1	+12.7	85	365	„	7.3	+ 5.8
			—	165.3	+ 7.5	135	Not obtained after 840 min.	„	0.0	—100.0
14	41.7	5.83	2	175.7	+17.1	135	„	„	0.0	—100.0
			4	165.3	+ 7.5	135	„	„	0.0	—100.0
			8	143.8	— 4.1	140	„	„	0.0	—100.0
			—	—	—	—	—	—	—	—

that the dextrinizing and liquefying powers diminish and disappear in barley much more quickly than in rye, the loss in barley taking place during the “milk” stage.

The following experiment was carried out in order to ascertain whether papain helps to increase the amylolytic power of an extract of barley grain taken 14 days after blossoming.

Table II. *Influence of papain on the amylolytic power of ripening barley grain.*

Days after blossoming	H ₂ O content of grain %	Extract %	Papain added g.	Saccharifying power		Dextrinizing power. Min. to change colour of starch with iodine to			Liquefying power	
				ml. N/20 iodine per 1 g. of grain	Change %	Violet blue	Brownish red	Achro. stage	g. of starch liquefied by 1 g. of grain	Change %
14	42.0	5.8	—	165	—	135	Not obtained after 840 min.	„	0.0	—
			1	181	+9.7	135	„	„	0.0	—
			2	165	0.0	135	„	„	0.0	—

Table II shows that papain had no effect on the dextrinizing and liquefying powers of barley grain in the phase of initial lactic maturity: the outcome was the same as with the action of proteolytic enzymes in general on ripe barley grain.

Wheat.

Experiments were then made on wheat grains (Table III).

It follows from Table III that wheat behaves similarly to rye, i.e. it is only in the later phases of ripening that the liquefying power dwindles and disappears. The dextrinizing power, however, remains, but only in very moderate strength.

In order to ascertain whether the dextrinizing powers of other types of wheat are greater or not, an experiment was made with two other varieties, the eleuto-substance used being, in addition to peptone, sodium chloride (Table IV).

Table IV shows that wheat No. 3 has high liquefying and dextrinizing powers accompanied by a considerable content of sisto-substances, whose action can be excluded by using peptone and sodium chloride as eleuto-substances. The last-named acts best when in concentrations up to 1 %; in concentrations of 2 % or

Table III. *Amylolytic power of ripening grains of wheat No. 1.*

Days after blossoming	H ₂ O content of grain %	Extract %	Peptone added %	Saccharifying power		Dextrinizing power. Min. to change colour of starch with iodine to			Liquefying power	
				ml. N/20 iodine per 1 g. of grain	Change %	Violet-blue	Brownish red	Achro. stage	g. of starch liquefied by 1 g. of grain	Change %
10	40.7	5.93	—	227.6	—	30	90	Not obtained after 840 min.	8.0	—
			1	221.0	— 2.9	28	90	"	11.2	+ 40.0
			2	223.9	— 1.6	28	85	"	11.2	+ 40.0
			4	225.4	— 0.9	28	85	"	11.2	+ 40.0
12	46.4	5.36	—	262.1	+ 15.2	30	100	"	7.8	— 2.5
			1	259.1	+ 13.9	28	100	"	10.9	+ 36.2
			2	256.0	+ 12.5	28	95	"	10.9	+ 36.2
			4	231.6	+ 1.8	28	90	800	10.9	+ 36.2
16	38.9	6.11	—	203.1	— 12.0	60	265	Not obtained after 840 min.	5.4	— 32.5
			2	210.2	— 7.5	50	180	"	6.0	— 25.0
			4	203.1	— 12.0	50	170	"	6.0	— 25.0
			6	200.0	— 12.1	50	165	"	6.0	— 25.0
23	35.5	6.45	—	192.4	— 15.4	60	400	"	0.0	— 100.0
			2	199.2	— 12.4	50	205	"	4.4	— 45.0
			4	199.2	— 12.4	52	175	"	4.4	— 45.0
			6	183.6	— 19.3	55	205	"	4.4	— 45.0

Table IV. *Influence of peptone and NaCl on ripening wheats Nos. 2 and 3.*

Days after blos- soming	H ₂ O content of grain %	Extract %	% added		Saccharifying power		Dextrinizing power. Min. to change colour of starch with iodine to			Liquefying power	
			Pep- tone	NaCl	ml. N/20 iodine per 1 g. of grain	Change %	Violet- blue	Brownish red	Achro. stage	g. of starch liquefied by 1 g. of grain	Change %
Wheat No. 2.											
23	48.0	5.2	—	—	238.6	—	55	140	Not obtained after 840 min.	7.7	—
			2	—	250.0	+4.8	50	130		9.1	+18.2
			4	—	258.7	+8.0	45	120		11.3	+46.7
			—	2	250.0	+4.8	56	175		7.1	— 7.9
			—	4	247.9	+3.9	60	265		5.5	—28.7
Wheat No. 3.											
25	38.5	6.15	—	—	216.0	—	20	65	400	13.5	—
			2	—	226.2	+1.9	15	50	320	20.3	+50.4
			4	—	220.2	+1.9	15	50	320	23.3	+72.6
			—	0.5	222.0	+2.7	13	50	350	20.3	+50.4
			—	1.0	218.2	+1.0	12	55	370	23.3	+72.6

more, it decreases the power of the dextrinizing and liquefying functions, at least in the case of wheat No. 2. The dextrinizing power of wheat No. 3 was so great that starch was easily dextrinized to the achro-stage with an extract of this wheat.

Oats.

Oats during germination have high dextrinizing and liquefying but relatively low saccharifying powers. It was therefore of interest to examine these three functions in the ripening grain (Table V).

It can be seen from Table V that in ripening oat grain there is a more or less similar relation between the various amylolytic functions to that in malt, viz. relatively high liquefying and dextrinizing and only moderate saccharifying powers. In the case of ripening oat grain, the solutions of dextrinized starch likewise have a greenish tinge just as is often the case with germinating oats (malt of oats).

Table V. *Amylolytic power in ripening oats.*

Days after blos- soming	H ₂ O content of grain %	Extract %	Added peptone %	Saccharifying power		Dextrinizing power. Min. to change colour of starch with iodine to			Liquefying power	
				ml. N/20 iodine per 1 g. of grain	Change %	Violet- blue	Brownish- red	Other stages	g. of starch liquefied by 1 g. grain	Change %
9	40.9	5.91	—	142.2	—	34	150	375 (green)	11.3	—
			2	145.2	+ 2.1	34	90	300 (375, blue)	13.0	+15.0
12	40.8	5.92	—	114.4	−24.3	85	220	Achro. not obtained after 840 min.	10.0	−11.5
			3	115.5	−23.1	40	100	365 (green)	10.6	− 6.2
			6	134.5	− 5.4	30	90	840 (achro. not ob- tained)	10.0	−11.5
14	40.0	6.0	—	121.0	−15.5	63	145	840 (achro. not ob- tained)	9.2	−18.6
			3	118.6	−16.6	38	100	400 (achro.)	10.4	− 7.9
			6	114.2	−19.7	35	100	840 (achro. not ob- tained)	11.0	− 2.7
Oats No. 2.										
19	55.5	4.45	—	140.4	—	43	140	840 (achro. not ob- tained)	11.8	—
			3	197.0	+40.3	35	80	325 (achro.)	17.3	+46.6
			6	187.2	+33.4	30	80	840 (achro. not ob- tained)	22.0	+86.5
27	42.0	5.8	—	94.3	−32.9	62	225	840 (achro. not ob- tained)	5.8	−50.9
			3	103.2	−26.6	68	200	700 (achro.)	6.1	−48.3

DISCUSSION.

The results of these experiments on barley, wheat and oats confirm and greatly extend the research carried out with forming and ripening rye grain. It appears that after the blossoming of the cereal, all three amylolytic functions (liquefying, dextrinizing and saccharifying) appear in the forming grain, that is to say exactly as in germinating barley (in malt). This phenomenon is therefore a general one, appearing in all species of cereal. These three amylolytic functions are not maintained, however, at a constant level, but on the contrary, as the grain develops and ripens, the various functions gradually decrease in activity (dextrinizing and liquefying powers particularly, as these almost completely disappear in the later phases of ripening). This decrease in the strength of the various functions is a reversible feature up to a given phase of development of the grain since the primary activity can be restored with the help of eleuto-substances (peptone or weak solutions of salt). Evidently sisto-substances accumulate in the ripening grain and act with steadily increasing effect. Their activity can be counteracted by the influence of the eleuto-substances mentioned, but only up to a certain stage of development of the grain and in a great measure individually in dependence on the type of cereal and its variety. Evidently at a certain stage of development these sisto-substances collect in such large quantity, or their ability to bind amylase increases to such an extent, that their activity cannot be nullified by the eleuto-substances; this in turn evokes an almost complete loss of dextrinizing and liquefying powers.

It is of interest that in forming barley grain the loss of these two functions takes place during the "milk" stage, but in other cereals this happens much more slowly and the dextrinizing and liquefying functions finally disappear only when the grain is almost fully ripe. This phenomenon is in agreement with our former observations, viz. that barley and buckwheat have the highest content of sisto-substance, evidently produced in great quantity concurrently with the beginning of the grain formation.

These experiments confirm the view expressed when investigating rye, viz. that Nordh & Ohlsson's [1932] hypothesis, affirming that the so-called α -amylase forms only during the germination of grains, is erroneous.

Purr's [1934] view that the disappearance of dextrinizing power of amylase is due to the oxidation of ascorbic acid during ripening, likewise appears to be untenable. The experiments on ripening grains, those on rye and those on wheat, oats and barley, fail to confirm this author's suppositions. The gradual diminution of the dextrinizing function can be counteracted with peptone and even with NaCl acting as eleuto-substances. This fact points to the adsorptive inactivation of this function, as it is difficult to suppose that by such means it is possible to reverse the process of oxidation of ascorbic acid during the preparation of the grain extract. Further research will elucidate whether the disappearance or decrease of the dextrinizing and liquefying functions is evoked exclusively by the adsorption-elution of amylase, so often indicated by us, or whether apart from this, still other factors enter into consideration.

SUMMARY.

1. In wheats, oats and barley, as is the case with rye, whole amylase is found with its three functions intact (liquefying, dextrinizing and saccharifying) immediately after blossoming from the moment the grain is formed.

2. As the grain ripens, the liquefying and dextrinizing functions gradually disappear, and the saccharifying function undergoes partial decrease.

3. This disappearance of the liquefying and dextrinizing functions of amylase is above all of an adsorptive nature and can be counteracted by eleuto-substances (peptone or NaCl). The latter give better results with wheat at concentrations of 0.5–1.0 %. The disappearance of dextrinizing and liquefying functions could not be counteracted by the action of papain.

4. The activities of the various functions of amylase and the speed of their diminution and disappearance, as also their liberation by the action of eleuto-substances, are an individual trait of cereal and its various species. With barley, which possesses the highest quantity of sisto-substances, the diminution of the various functions of amylase commences earliest, i.e. during the early stages of ripening.

5. Nordh and Ohlsson's view that α -amylase is formed only during the germination of grains, as also Purr's statement that the gradual total loss of dextrinizing power of amylase is due to the oxidation of ascorbic acid, were not confirmed by our previous experiments on rye or by the present ones on the development of amylase in ripening barley, wheat and oats.

REFERENCES.

- Chrząszcz & Janicki (1933). *Biochem. Z.* **263**, 250.
— — (1936, 1). *Biochem. J.* **30**, 342; *Biochem. Z.* **285**, 47.
— — (1936, 2). *Biochem. Z.* in press.
Ford & Guthrie (1908). *J. Inst. Brew.* **14**, 61.
Nordh & Ohlsson (1932). *Hoppe-Seyl. Z.* **204**, 89.
Purr (1934). *Biochem. J.* **28**, 1141.