LXXV. ON THE PRESENCE OF MALTASE IN GERMINATED AND UNGERMINATED BARLEY.

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(*Received July 13th, 1923.*)

It has long been known that when starch is hydrolysed in presence of malt diastase under certain conditions, glucose is one of the products. Some chemists have attributed this production of glucose to the presence of the enzyme, maltase, in the malt. Thus Effront in 1899, dealing with the conversion of maltose into glucose by enzyme action, remarks [1899]: "L'infusion de malt agit très peu sur le maltose, mais le malt concassé agit energiquement sur les sirops de maltose, qui se transforment en sirop de dextrose."

It is, however, on record that, when starch is hydrolysed in presence of enzyme preparations which do not contain maltase, glucose is, under certain conditions, produced. It was stated by Ling and Baker [1895] in 1895 that glucose is one of the hydrolytic products of the action on potato starch paste of the diastase prepared from kilned malt. In a subsequent paper [1897] these authors observed that they had now satisfied themselves that the production of glucose in the manner indicated was in no way connected with the kilning of the malt. However, Ling and Davis later [1902] showed that when an aqueous solution of precipitated diastase is heated at 65°, or at a few degrees above this temperature, and the solution allowed to act on starch paste, glucose is invariably present among the final products of hydrolysis. Here we have a distinct confirmation of the original observation of Ling and Baker.

Brown and Millar [1899] showed that the so-called stable dextrin can be partially hydrolysed in presence of malt diastase at 55° and that glucose is among the products. Ling [1903] showed that the malto-dextrin-α of Ling and Baker also yields about 10 % of glucose when hydrolysed in the presence of malt diastase at 55°.

Davis and Ling [1904], who measured the quantity of glucose formed from heated diastase solutions, showed that the maximum quantity of the sugar just mentioned is produced when the diastase solution is heated at 68–70°, a temperature at which maltase is inactive. Ling and Rendle [1904] showed that commercial concentrated malt extracts contain from 17–22 % of glucose. It will be seen therefore that glucose may be produced from starch and some of its hydrolytic products by diastase preparations not containing maltase.

We have indeed proved that no glucose is produced when precipitated malt diastase is used as the hydrolysing agent for starch unless the diastase has been heated to 65° in aqueous solution. Moreover, under all conditions, whether its aqueous solution has been heated or not, no glucose is produced by its action on maltose.

It appears to be otherwise with malt extract, Maquenne having recently shown [1923] that glucose is produced when maltose is treated with malt extract. As a result of our present experiments we find that maltase is present in the extract of both green and kilned malt and also in ungerminated barley. In the latter case, however, the enzyme is present in a form in which it cannot be extracted by water, for barley extract is without action on maltose whilst barley grist when added to maltose solution causes hydrolysis to glucose. Maltase is an endogenous enzyme which is very sensitive to reagents and very difficult to isolate. It is also thermo-labile, being destroyed below 70°.

The method adopted in the experiments we have carried out to demonstrate the presence of maltase was in the case of malt to act on a solution of maltose with an extract of the malt, or in the case of barley, where the enzyme cannot be extracted with water, with the barley grist. After a convenient period of incubation the percentage of maltose hydrolysed was determined by estimating gravimetrically the glucose formed as phenylosazone according to the method described by Davis and Ling [1904].

Dealing first with the malt experiments, five different kinds of malt were employed. No 1 was a green malt made from English two-rowed barley. No. 2 a green malt made from a foreign six-rowed barley, and Nos. 3, 4 and 5, were all kilned malts with diastatic powers, 23°, 52° and 102° (Lintner) respectively. The malt extract used was prepared by digesting one part of the finely ground malt with ten parts of water containing a little toluene at the ordinary temperature for 24 hours.

To 100 cc. of the filtered extracts thus obtained from the above-mentioned malts were added 2·12 g. of maltose and the solutions were incubated at 50°. A convenient volume of each of the malt extracts with a little toluene was also incubated side by side without the addition of maltose to serve as blanks. After a period of 45 hours the amount of glucose in each of the solutions was estimated gravimetrically as follows:

20 cc. of each of the solutions containing about 3 % carbohydrates were measured out into clean 25 cc. test-tubes and 1 cc. of phenylhydrazine and 1·5 cc. of 50 % acetic acid added to each. The solutions were then heated in a boiling water-bath for exactly one hour. At the end of this period the liquid was carefully decanted on to a tared Gooch crucible which was previously warmed with boiling water. Care was taken to decant the supernatant liquid on to the filter before transferring the glucosazone. The precipitate of glucosazone was then washed with 25 cc. of boiling water and then dried in a steam oven until the weight was constant. In the following results the difference in weight of the glucosazone between the blanks and actual experiments repre-

sents the amount of glucose due to the hydrolysis of maltose. The amount of glucose corresponding to the glucosazone was obtained by multiplying the weight of the glucosazone by the factor 1·98.

Table I.

Malts used	Wt. of the osazone from 20 cc. With maltose g.	Wt. of the osazone from 20 cc. Without maltose g.	Difference for 20 cc. g.	Wt. of maltose hydrolysed per 100 cc. g.	% of maltose hydrolysed
(1) English green malt	0·1990	0·1808	0·0182	0·1712	8·07
(2) Foreign green malt	0·1970	0·1596	0·0374	0·3518	16·59
(3) Kilned malt (Californian). D.P. 23°	0·2135	0·2017	0·0118	0·1110	5·23
(4) Kilned malt (English). D.P. 52°	0·3016	0·2768	0·0248	0·2332	11·00
(5) Kilned malt (Danubian). D.P. 102°	0·2818	0·2675	0·0141	0·1310	6·18

These results show conclusively that a soluble maltase is present in malts made under different conditions. The power of the enzyme appears however not to vary directly with the diastatic power of the malt expressed on the Lintner scale, but according to the temperature and rate at which the moisture has been expelled on the kiln. To account for the presence of a soluble maltase three possibilities suggested themselves.

The enzyme may exist in the barley before germination; or it may be elaborated during the processes of germination; or it may exist in the barley in an insoluble form before germination by which it may be rendered soluble. Further experiments were carried out to settle these points and the results show that the last view is the most probable. In the barley experiments starch paste was used as the substrate instead of maltose as it is known that barley diastase does not give rise to any other sugar than maltose [cp. Baker, 1902]. Our own experience, moreover, indicates that no maltase is present in barley extract. Accordingly, barley grist was employed for converting the starch paste and under these circumstances a considerable amount of glucose was formed.

The experiments were carried out as follows. To 20 cc. of 2–3 % starch paste 3 g. of the finely ground barley grist were added and the mixture with the addition of a little toluene incubated at 50° for 24 hours. At the end of this period the liquid was filtered and the glucose estimated as before in 20 cc. portions of the filtrate. Blank experiments were also carried out without the addition of starch, but no glucosazone was obtained from these.

In the following table are given the results of five such experiments.

Table II.

Specific gravity of the conversion liquid	Wts. of the osazone from 20 cc. g. With starch	Wts. of the osazone from 20 cc. g. Blank	Difference g.	Glucose per 100 cc. g.	% of maltose hydrolysed on the total solids
1008·65	0·015	Nil	0·015	0·1485	6·41
1010·30	0·053	,,	0·053	0·5248	19·00
1008·10	0·020	,,	0·020	0·1980	9·13
1007·50	0·012	,,	0·012	0·1188	5·94
1009·90	0·022	,,	0·022	0·2178	8·24

From these experiments we can safely infer that there is an insoluble maltase present in the raw barley, and that this undergoes modification during germination and is rendered partially soluble. The soluble maltase content of malt varies according as it is low dried or high dried. Green malts especially contain considerably more maltase, as the enzyme is probably partially destroyed during the kilning process.

Conclusions.

1. It is shown that malts, whether green or kilned, obtained by germinating barley contain an enzyme capable of hydrolysing maltose. The power of the enzyme depends, *caeteris paribus*, on the temperature and the way in which the malt has been heated on the kiln.

2. Diastase preparations obtained by precipitating a cold water extract of malt with alcohol do not contain maltase, as this enzyme is destroyed by the alcohol.

3. Ungerminated barley contains an enzyme capable of converting maltose into glucose. It cannot be extracted with water, but its activity is demonstrated by allowing ground barley (grist) to act on maltose.

Our thanks are due to W. J. Harper (Assistant in the Department) for carrying out some of the analyses connected with this work.

REFERENCES.

Baker (1902). *J. Chem. Soc.* **81**, 1177.
Brown and Millar (1899). *J. Chem. Soc.* **75**, 315.
Davis and Ling (1904). *J. Chem. Soc.* **85**, 16.
Effront (1899). Les Enzymes, p. 358.
Ling (1903). *J. Inst. Brewing*, **9**, 448.
Ling and Baker (1895). *J. Chem. Soc.* **67**, 707.
—— —— (1897). *J. Chem. Soc.* **71**, 502.
Ling and Davis (1902). *J. Inst. Brewing*, **8**, 491.
Ling and Rendle (1904). *Analyst*, **29**, 243.
Maquenne (1923). *Compt. Rend. Acad. Sci.* **176**, 804.