

XXI.—*On the Transformation-products of Starch.*

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THE change which starch-paste undergoes in presence of malt-extract (diastase solution) has been investigated by Musculus, Payen, and more recently by Schwarzer.

Musculus (*Ann. Ch. Phys.* [3], iv, 203] states that the solid matter dissolved from starch-paste by the action of diastase at  $70^{\circ}$  to  $75^{\circ}$ , or by dilute sulphuric acid under certain conditions, contains sugar and dextrin in the proportion of one molecule of the former to two of the latter, and that with diastase no further reaction takes place, while by continuing the action of sulphuric acid, the dextrin is slowly converted into sugar.

Payen (*Ann. Ch. Phys.* [4], iv, 286) asserts that more than 50 per cent. of the solid matter dissolved by the same reaction is sugar, and says (*Ann. Ch. Phys.* [4], vii, 382) that four samples taken from another operation in the space of  $1\frac{1}{2}$  hours contained 17.9 per cent., 20.9 per cent., 25.8 per cent., and 26.03 per cent. sugar on the total matter in solution.

Schwarzer (*J. pr. Chem.* [2], i. 212) agrees with Musculus in finding equivalent quantities of dextrin and sugar in solution, but differs from him in supposing that dextrin is formed first, then sugar, and that the reaction ceases when definite equivalent proportions are produced. He says that when the reaction takes place above  $65^{\circ}$ , the product always contains less sugar than that produced at a lower temperature; above  $65^{\circ}$ — $70^{\circ}$  the proportion of sugar to dextrin is as 1 eq. : 3 eqs.; below  $60^{\circ}$ , when the principal phase of the reaction is finished, the dextrin and sugar found in solution are in the proportion of 1 eq. : 1 eq. This experimenter considered the change completed when iodine no longer gave any colour. He determined the sugar volumetrically with Fehling's copper solution.

I repeated these experiments, and obtained results agreeing only partially with those given by the chemists named. Certainly below  $60^{\circ}$ , when the precautions recommended by Schwarzer are taken, the solid matter in solution *frequently* contains 50 per cent, or thereabouts, of sugar. The reaction, however, does not stop here. I find the reducing power becomes constant only when the cuprous oxide precipitated, *weighed* as cupric oxide, represents glucose equal to 65—66 per cent. of the solid matter in solution derived from the starch, the precipitate produced by the malt-extract added being allowed for. The discrepancies between my experiments and those of Schwarzer may be accounted for by two causes.

1st. The difficulty of obtaining, without a foregone conclusion, results nearer than 5 or 6 per cent. by employing the Fehling's solution volumetrically.

2nd. The very slow and gradual increase from 50 per cent. sugar to 65 per cent.

Now the difficulty arises—it also presented itself to Schwarzer—why should not more than 50 per cent., or even 65 per cent., of starch be converted into sugar by the action of diastase? Schwarzer refers to the partial conversion of oxygen into ozone as a parallel phenomenon. I, however, do not think that the one change has any application to the other; and as every fact connected with the transformation of starch is of importance to the manufacture in which I am engaged, I thought it worth the trouble to endeavour to account, in a more satisfactory manner, for this curious point. In pursuit of this object, no better way seemed open to me than to try to isolate the products resulting from the transformation, and study their characters separately. All agree that these products are sugar (the variety of sugar is seldom mentioned) or matter capable of reducing cupric oxide, and dextrin not having that power. The numbers expressing the percentage of sugar, in all investigations on this subject, are calculated on the supposition that the substance in solution which reduces cupric oxide exerts the same reducing power as invert sugar, or dextrose. The dextrin is determined by difference.

There are three principal methods given by which dextrin may be prepared from starch:—

1. By the action of heat on the dry substance.
2. The action of acids, and
3. The action of malt-extract (diastase) on starch-paste.

I shall confine myself to the last two methods.

#### A. *By the Action of Malt-extract.*

100 grams of air-dried starch were stirred up with 300 c.c. water at  $40^{\circ}$ , and the mixture poured with continued stirring into 2 litres of boil-

ing water. The paste thus formed was allowed to cool to  $70^{\circ}\text{C}.$ , and the cold extract from 20 grams of pale malt added to it. Disorganisation immediately set in, the liquid became almost clear; for the first 4 or 5 minutes the filtrate from the solution filtered cold, coloured iodine blue; after some time longer the solution treated in the same way coloured iodine dark reddish-brown, in fact, the colour of concentrated tincture of iodine; at last the cold filtered solution was no longer coloured by iodine. When this stage of the reaction was reached, the solution was boiled for a short time, allowed to cool, and filtered from some insoluble matter which I have invariably found to retain the power of colouring iodine blue. The filtrate was evaporated on the water-bath to 300 c.c., and then precipitated with alcohol. The precipitate was allowed to settle, and the clear supernatant liquid decanted from the insoluble, almost white, waxy mass. This, according to some authors, is dextrin. Biot and Persoz say it can be purified from sugar by dissolving it at least ten times in water and reprecipitating with alcohol, which must not be used in excess. I dissolved and reprecipitated the substance 15 times, and another portion as many as 30 times, the alcohol employed being sp. gr. 0.830,\* but I found in each case that it retained the power of reducing cupric oxide, the lowest reduction corresponding to 8 per cent. dextrose.

### *B. By the Action of Acids.*

100 grams of starch, converted into a paste as described above, were heated with 10 c.c. ordinary sulphuric acid until the solution, filtered cold, no longer coloured iodine. The acid was separated with baryta, and the filtrate evaporated to 300 c.c. The precipitate from this syrup, with alcohol, redissolved, and precipitated 30 times, still retained the power of giving a reduction with cupric oxide equal to 9 per cent. dextrose.

Starch-paste treated in the same way with oxalic acid, the acid being separated with lime, gave the same results, but the transformation was slower.

The question naturally suggested itself, is this body, even after the repeated partial precipitations, still a mixture of dextrin and sugar? or has pure dextrin a reducing power on cupric oxide? An elementary analysis would not settle the point, because the *increase* of hydrogen and *decrease* of carbon, due to the admixture of 8 or 9 per cent. dextrose, could be more or less accounted for by the errors of manipulation; the results would, therefore, not be altogether satisfactory. The action of the body on polarised light seemed to afford some means of explaining the difficulty. The numbers given for the specific rotatory power of dex-

\* This is the strength of the alcohol employed in these experiments.

trin do not agree; in all books it is put as  $[\alpha] = +138.68^\circ$ ; Béchamp says it is  $[\alpha] = +176^\circ$ . I determined the specific rotatory power\* of the three substances, the preparation of which is described above, viz., *a*, that obtained by the action of malt-extract; *b*, by the action of sulphuric acid; and *c*, by the action of oxalic acid, with following results:—

<i>a</i>	sp. rot. power	$[\alpha] = +204^\circ$ .
<i>b</i>	„	$[\alpha] = +205^\circ$ .
<i>c</i>	„	$[\alpha] = +204^\circ$ .

The three bodies gave nearly the same reduction with cupric oxide; it equalled—

In <i>a</i> .....	8.8 per cent.
In <i>b</i> .....	8.5 per cent.
And in <i>c</i> .....	9.0 per cent. glucose.

This would seem to indicate that the composition of the substance prepared by the three methods was the same. In another preparation, *d*, however, by the action of dilute sulphuric acid, in which the reaction extended over eight hours, I found the sp. rot. power to be  $[\alpha] = +198^\circ$ , and the reduction with cupric oxide to be equal to 9.0 per cent. glucose. Either the substance *d* must be a different body from *a*, *b*, and *c*, although the difference in the rotatory power is not great, or the optical activity of the constituent which reduces cupric oxide cannot be the same. This led me to prepare substances having different reducing powers on cupric oxide, by the continued action of sulphuric acid on the body described as *d*, and to determine if the specific rotatory power diminished as the reducing power increased. I found it to be so; but it was not necessary to continue the experiments in this direction, for I observed that the reducing power of the solution could be very considerably diminished by fermentation. I made use of this fact to eliminate the reducing body.

The precipitate produced by alcohol in the syrup obtained by the action of malt-extract on starch-paste, as described above, was dissolved in water; the solution boiled to drive off the alcohol, cooled to  $20^\circ\text{C}$ ., and then 2 per cent. on the matter in solution, of yeast, added to it. Fermentation began in a short time, and in about three days the solution became clear. The specific gravity before fermentation should be 1.090. The clear liquid was filtered to remove the deposited yeast and the filtrate precipitated with alcohol. The precipitate collected to a white, soft, waxy mass, with a lustre more or less silky. This substance was re-dissolved in a small quantity of water, filtered from a little insoluble

\* The optical observations were made with a Soleil by Duboscq. As the mean of six determinations, I found the specific rotatory power of cane-sugar to be  $[\alpha] = +74^\circ$ ; the extremes being  $+74.2^\circ$  and  $+73.6^\circ$ . The number usually given is  $+73.8^\circ$ .

matter, and reprecipitated; the process was repeated three or four times. The supernatant liquid of the last precipitation was nearly clear; it was poured off and fresh alcohol added; the substance then became gradually brittle, and broke up into a white, apparently semi-crystalline powder. This was washed two or three times with alcohol, thrown on a filter, pressed between folds of blotting-paper and immediately placed under a bell-jar over sulphuric acid. If this precaution be not quickly taken, moisture is absorbed, and the powder again becomes soft and waxy. The body thus obtained, dried in a current of dry air at  $100^{\circ}$ , exerted a reducing power, when boiled for 20 minutes with Fehling's copper solution, only equal to 2 per cent. glucose.

In this way I made eight preparations, viz. :—

3. *e*, *f*, and *g*, from the product of the action of diastase on starch-paste, stopping the transformation by boiling when the colour with iodine was no longer blue, but reddish-brown.

2. *h* and *i* in the same way, stopping the reaction when iodine gives no colour.

1. *j*, from the solution with sulphuric acid, the acid being separated when iodine ceased to give a blue colour, but reddish-brown.

1. *k*, in the same way, when iodine gave no coloration; and

1. *l*, obtained by the action of oxalic acid; coloured by iodine reddish-brown.

I determined the specific rotatory power in each of these preparations, with the following results. The substances were treated with a current of dry air at  $100^{\circ}$  until the weight became constant. This operation takes at least eight hours:—

Substance.	Specific rotatory power.	Per cent. reducing cupric oxide.
<i>e</i> .....	$[a] = + 213\cdot0^{\circ}$	2·03
<i>f</i> .....	+ $212\cdot0^{\circ}$	2·20
<i>g</i> .....	+ $212\cdot7^{\circ}$	1·24
<i>h</i> .....	+ $213\cdot1^{\circ}$	1·20
<i>i</i> .....	+ $212\cdot7^{\circ}$	1·40
<i>j</i> .....	+ $213\cdot0^{\circ}$	1·15
<i>k</i> .....	+ $213\cdot5^{\circ}$	0·80
<i>l</i> .....	+ $214\cdot0^{\circ}$	1·03

From these determinations I feel justified in concluding that the eight preparations are one and the same body, and coupled with the numbers obtained for *a*, *b*, and *c*, that the body in the pure state does not reduce cupric oxide.

Dextrin in this state is a brittle white powder, showing in some instances shining particles which one would suppose were the faces of crystals, but as under the microscope no trace of crystallisation can

be observed, they must be fracture surfaces. It dissolves very easily in water; if the dehydrated body be employed, with a rise of temperature. In cold alcohol, sp. gr. 0·82, it is not perceptibly soluble; at least the cold alcohol which had been for three days in contact with the dry substance, exerted no action on a ray of polarised light, passed through a layer of it 220 mm. in length. An aqueous solution containing in 100 c.c. 10 grms. dry substance, has a sp. gr. = 1·0385, more correctly, 1·03845, the mean of many determinations, varying between 1·0384 and 1·03855. Its specific rotatory power may be taken  $[\alpha] = + 213^\circ$ . This agrees closely enough with the optical activity  $[\alpha] = + 211^\circ$  of the substance described by Béchamp as *soluble starch* colouring iodine blue.

The three bodies, viz. :—

Soluble starch coloured by iodine blue,

$\alpha$  Dextrin coloured by iodine reddish-brown,

and  $\beta$  Dextrin not coloured by iodine,

are therefore alike in their action on polarised light, and in their activity they in no way correspond with the specific rotatory power  $+ 138\cdot68^\circ$  nor  $+ 176^\circ$  given by Béchamp for dextrin.

Dried over sulphuric acid, its weight becomes constant when it contains 9·5 to 10 per cent. water. This it completely loses in a current of dry air at  $100^\circ$ . Determinations in six different samples gave numbers of which the above are the extremes.  $C_6H_{10}O_5 + H_2O$  contains 10 per cent. water. That no doubt may attach to the composition of the body, I made two elementary analyses, one of  $\alpha$ -dextrin, the other of  $\beta$ -dextrin, both dry, with the following results :—

	$\alpha$	$\beta$	Theory for $C_6H_{10}O_5$ .
Carbon . . . . .	44·16	44·23	44·44
Hydrogen . . . .	6·44	6·36	6·17

It is unnecessary to remark on those numbers; they are sufficiently near when we consider that the body contains at least 2 per cent. of a substance richer in the elements of water, and consequently poorer in carbon.

Treated with malt-extract, the reducing power of a solution of dextrin gradually increases and becomes constant when the cuprous oxide precipitated, weighed as cupric oxide, is equal to 66 per cent. glucose calculated on the dextrin employed. The specific rotatory power of the matter in solution is then  $[\alpha] = 150^\circ$ , having fallen from  $+ 213^\circ$ .

So far, there is nothing in the character of this body to explain why the change should stop when the reducing power equals 66 per cent. of dextrose. We have consequently to turn to the reducing body, and study its characters.

If we suppose the substance described above as *d* to be dextrose having a specific rotatory power of  $+53^\circ$ , we get for the non-reducing body an angle  $[\alpha] = +212.3^\circ$ . The same angle would evidently be not obtained if the reducing substance in *a*, *b*, and *c* were dextrose. On the supposition that the specific rotatory power of the non-reducing body in this last case was  $+122^\circ$ , that for the reducing substance would be  $+120^\circ$  or thereabouts.

Dubrunfaut (*Ann. Chim. Phys.* [3], xxi, 178) has pointed out a sugar in the product of the action of diastase on starch-paste having a rotatory power three times as great as that of dextrose, viz.:  $[\alpha] = +159.6^\circ$  ( $53.2^\circ \times 3$ ). He says that it is identical with the sugar produced by sulphuric acid in the first stages of the reaction. He also states that it is of the same crystalline habit as dextrose, but less soluble in alcohol than that body.

As shown above by the determinations *a*, *b*, and *c*, the specific rotatory power of the substance which reduces cupric oxide, observed by me, is  $[\alpha] = +120^\circ$ , if that of the non-reducing body be  $+213^\circ$ . This is the case under all circumstances, with the substance giving the greatest amount of reduction as well as with that giving the least.

I have prepared the body described by Dubrunfaut, and have very little doubt that the same body is in my hands as that pointed out by him. Its specific rotatory power is  $[\alpha] = +150^\circ$ , Dubrunfaut's number being  $+159.6^\circ$ . It is much less soluble in alcohol than dextrose, and has apparently the same crystalline habit. I find, however, that only 65 per cent. of it is capable of reducing copper-solution in the same proportion as dextrose. If the substance without action on cupric oxide be dextrin (sp. rot. power  $+213^\circ$ ), the rotatory power of the reducing body would be  $+121^\circ$ , close to the number invariably obtained by me from the active substance. Hence, if the body mentioned by Dubrunfaut be a pure sugar, only 65 per cent. of it acts on cupric acid in the same proportion as glucose, and on the contrary, if it be a mixture of dextrin and sugar, the sugar has not the specific rotatory power given by him, namely, three times that of dextrose  $+159.6^\circ$ , but  $+121^\circ$ .

The following experiments were made with the view of ascertaining whether the substance is a mixture or a simple compound. I may add also its mode of preparation.

100 grams of air-dried malt were mixed with 300 c.c. water at  $40^\circ$ , and the mixture well stirred to diffuse as completely as possible the starch granules through the liquid, and then poured with continual stirring into 2 litres of boiling water. The paste was cooled to  $40^\circ$  C., the cold extract from 20 grams of pale malt added to it, and the mixture kept between  $40^\circ$  and  $45^\circ$  for three hours. It was then boiled for some time, cooled, filtered, and the filtrate evaporated at  $80^\circ$ , to 300 c.c. The solid matter in solution, calculated from the specific gravity taking 10

grams in 100 c.c. to be represented by 1.0385, had a specific rotatory power  $[\alpha] = +170^\circ$ . It was the same after evaporation as before boiling: hence these two operations produced no change in the substance. The syrup was boiled for a short time with 2 litres of alcohol, sp. gr. 0.820; on cooling, the clear solution was decanted from the undissolved syrup and put aside in a corked flask. At the end of six days, the sides of the vessel were covered with a crystalline crust. This substance dried at  $100^\circ$  in a current of dry air, had a specific rotatory power  $[\alpha] = +150^\circ$ , and reduced copper solution equal to 65 per cent. glucose.

I made five other preparations in the following manner:—

1. The mother-liquid from the last crystallisation was filtered and set aside. In a week the matter crystallised out was separated.

2. The mother-liquid from the last crystallisation yielded a further crop of crystals.

3. Another portion of starch-paste was treated as described above. The solid matter in solution had a specific rotatory power  $[\alpha] = +166^\circ$ , and a reducing power equal to 51 per cent. glucose. The filtrate from the cold solution was evaporated to 300 c.c., placed on a dialyser over 2 litres of water, and allowed to stand for 20 hours. The solid matter in the diffusate had a specific rotatory power  $= +150$ , and gave a reduction of cuprous oxide equal to 65 per cent. glucose. The syrup obtained by evaporating the diffusates of several experiments yielding the same results as those given here, was treated with sufficient alcohol to dissolve one-half at the boiling temperature. When cold, the clear alcoholic solution was decanted from the bright undissolved syrup and set aside to crystallise. The white crystalline substance formed was removed at the end of a week.

4. A portion of this body was treated with a quantity of alcohol insufficient to dissolve it all, the solution allowed to cool, decanted from the insoluble matter, and set aside to crystallise.

5. The first crystallisation from alcohol was dissolved in water and left to itself over sulphuric acid. In time, the solution became a pasty mass of crystals. These were pressed between filter-paper and again crystallised from alcohol.

The following table shows the numbers obtained on the analysis of the five preparations, 1, 2, 3, 4, and 5, dried at  $100^\circ$  in a current of dry air:—

Subs.	Specific rotatory power.	Per cent. reducing cupric oxide.
1 .....	+ 150·6°	64·4
2 .....	+ 150·6°	65·0
3 .....	+ 149·5°	64·4
4 .....	+ 150·0°	63·9
5 .....	+ 150·6°	65·5

These numbers leave very little doubt as to the identity of the five substances, and when I consider the various treatments they were subjected to in their preparation, I am inclined to conclude that this body is a simple compound, and not a mixture of dextrin and sugar. In further support of this opinion, I may offer the following facts, taking the evidence at present for what it is worth:—

I. The ultimate product of the action of malt-extract on starch-paste below 50°, is a substance having a rotatory power equal to that of this body, when the amount of malt-extract added is allowed for, and reduces the same amount of copper-solution, viz., a quantity equal to about 65 per cent. glucose.

II. No matter what the specific rotatory power of the substance dissolved from starch-paste by the action of malt-extract, the optical activity of the body passing through a dialyser from the syrup left on evaporation is always  $[\alpha] = +150^\circ$ , or thereabouts.

The following are only a few of many observations made, the maximum being + 152°, the minimum + 149°, for the specific rotatory power of the matter in solution in the diffusate, the quantity being calculated from the specific gravity, taking 10 grams in 100 c.c. to be represented by 1·0385:—

The substance in—

1st diffusate,	sp. rot. power	= + 150°.
2nd	„ „	= + 152°.
3rd	„ „	= + 149·8°.
4th	„ „	= + 149·8°.
5th	„ „	= + 149°.
6th	„ „	= + 151°.

III. If the substance were a mixture of one-third dextrin and two-thirds sugar, as the reduction with cupric oxide would seem to indicate, it would contain—

Carbon .....	41·47 per cent.
Hydrogen .....	6·49 „

Combustions of the preparations described as 2 and 3, above, dried in a current of dry air at 100°, gave the following results:—

	2.	3.
Carbon . . . . .	41·97 per cent.	42·02 per cent.
Hydrogen . . . . .	6·48 „	6·50 „

The theory for a sugar isomeric with cane-sugar or with lactose, viz.,  $C_{12}H_{22}O_{11}$ , is—

Carbon . . . . .	42·10 per cent.
Hydrogen . . . . .	6·43 „

I think that this, coupled with the facts before stated, leaves very little doubt as to the character and composition of the body under consideration. That it reduces only  $\frac{2}{3}$  as much cupric oxide as glucose, is no reason for considering it to be a mixture. Lactose, a well-defined crystallised body, reduces only  $\frac{7}{10}$  (a quantity not far removed from  $\frac{6}{9}$ ) as much cupric oxide as dextrose, and is isomeric with the substance here described, as far, at least, as the elementary analysis can prove it to be so.

If, then, this body be a simple sugar, and I submit that the evidence is altogether in favour of the supposition, we have at once an explanation of the difficulty which it was the object of this investigation to clear up. This sugar, which, following Dubrunfaut, we shall call *malto*se, is the end-product of the action of malt-extract on starch-paste. It is converted, by the continued action of acids, into dextrose.

There are many points of interest connected with this body; I am at present engaged in an examination of them.

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