

LX.—*Note on Diastatic Action.*

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IN the course of some technical investigations by one of us (*Brewing Trade Review*, 1891, 6), it was found that brewers' worts, as they issue from the mash-tun, remain constant in respect to their starch-transformation products when digested for two hours at the same temperature as that at which the conversion was conducted. The diastase in these solutions therefore exercises (within the above period) no action upon the starch products contained in it. But if fresh starch be added to these solutions, the diastase (which we may term "residual" diastase) is found to possess considerable energy in liquefying and saccharifying it, although it appeared from these experiments that the energy of this residual diastase is distinctly inferior to that of the original malt. The comparison between the energy of the original and of the residual diastase was estimated, in the investigation referred to, by rough volumetric methods, and it appeared to us of interest to determine more accurately the extent to which the added starch is acted upon by the residual diastase. The following experiments were made to elucidate the question:—The preparation of the malt extract (diastase solution) and of the starch paste, as well as the analyses of the transformation products, were carried out exactly as laid down in the researches of Brown and Heron (*Trans.*, 1879, 596). The plan of the first experiment was as follows:—

A conversion was made by digesting 5 grams of starch made into paste with 5 c.c. of normal malt extract at a given temperature for two hours. The volume of the solution during conversion was 175 to 180 c.c. At the expiration of the converting period, the conversion was quickly cooled and made up to 200 c.c. at 15.5°; a portion was abstracted for analysis, further diastatic action being arrested in it by the addition of salicylic acid. Half the volume of the conversion was used as the converting agent for a fresh lot of starch paste (2.5 grams).

This second conversion was conducted at the same temperature as the first, and for the same time. A portion of this second conversion was abstracted and analysed as before, and one-half the volume now used as the converting agent for a third lot of starch paste (1.25 grams), which was again digested at the previous temperature, and for the same time, and then analysed. In the second and third transformations, the amount of starch paste and malt extract clearly bear the same relation to each other as in the first.

The transformation products are all expressed as $K_{3.86}$, that is, in terms of the cupric reducing power of the solid matter in solution referred to pure dextrose taken as 100; the volumes taken for analysis are referred to weights from determinations of their sp. gr. at 15.5°. The first conversion will, besides maltose, contain reducing sugars, due to the malt extract; the total reducing power of the conversion is therefore corrected for this by determining the reducing matters in the malt extract used, after digesting it for the same time, and under the same conditions as the first conversion. The second and third conversions will contain (besides some of the reducing sugars from the malt extract) an abundance of maltose, formed, in the case of the second conversion, from the first, in the case of the third conversion, from the second. The second and third conversions are, therefore, corrected by determining and deducting the reducing matters in portions of the first and second transformations, after digestion for the same time, and under the same conditions, as the second and third transformations respectively. In the second conversion, the total reducing power is therefore corrected by that of the first conversion added, *plus* that due to any increase in it incident to a further digestion for two hours. Similarly with the third conversion. The solid matter of the first conversion is corrected by that of the malt extract used; that of the second by the solids of the first, and so on. The following figures give our results, after making the various corrections above described:—

Series I.—Digestions for two hours; ratio of malt extract to starch, 1 c.c. to 1 gram; this ratio maintained constant. Temperature of transformations, 52°* throughout.

* The above temperature was selected for this series as being the optimum one for diastatic energy. In some of the later conversions, the malt extract was momentarily heated at the transformation temperature before use. This was not done throughout Series I, as at the temperature employed there is no weakening of the diastase by subjection to that temperature beforehand. Thus, even when heated at 52° for three hours previous to employment, malt extract converted starch at 52° (ratio 1 c.c. to 1 gram) to a K of 48.6, and to a K of 48.0 when the ratio was 1 c.c. to 3 grams. These figures agree so closely with those of Series I, in which malt extract was unheated previous to transformation, that the influence of

	K ₃₋₈₆ .
A. First conversion	48·7
Second conversion (transformed with first).....	48·6
Third conversion (transformed with second).....	48·4
B. First conversion	48·5
Second conversion (transformed with first).....	48·6
Third conversion (transformed with second).....	48·4
C. First conversion	48·5
Second conversion (transformed with first).....	48·0
Third conversion (transformed with second).....	48·2

Under the conditions of the above experiments, the "residual" diastase is not only very vigorous, but its vigour is exactly equal to that of the original diastase. Later experiments show that this is not invariably the case; in this instance, the equality in vigour between original and residual diastases is due to the relatively high ratio of malt extract to starch, and to the employment of an optimum temperature. Of these favouring causes, the former is found to be the less important. This is in some measure indicated by three independent transformations, carried out, as before, at 52°, but where the whole of the starch paste previously used (15 grams) is converted at once (instead of being previously added) by 5 c.c. of malt extract. The ratio of malt extract to starch is now 1 : 3. The digestions are two hours, as before, and the corrections for malt extract, &c., applied as previously described. The following are the results :—

	K ₃₋₈₆ .
D	48·4
D ₁	48·5
D ₂	48·1

The products of these conversions are identical with those of A, B, and C, in spite of the lowering of the malt extract by two-thirds of the previous amounts.

Such influence as is exercised by the ratio of malt extract is more plainly seen in Series II. The transformations were carried out again at the optimum temperature (52°), and under the same conditions as in Series I, excepting that in the second and third transformations of each set, the starch is transformed by progressively diminishing portions of first conversion.

Series II.—Transformations at 52° for two hours.

such preparatory treatment, when subsequently converting at optimum temperatures, may be neglected.

692 MORITZ AND GLENDINNING ON DIASTATIC ACTION.

	Ratio of malt extract to starch.	K ₃₋₈₆ .
A. First conversion.....	1 c.c. : 1 gram.	48·4
Second conversion (trans- formed with first)	1 c.c. : 2 grams.	48·5
Third conversion (trans- formed with first)	1 c.c. : 4 grams.	47·7
Fourth conversion (trans- formed with first)	1 c.c. : 10 grams.	40·1
B. First conversion.....	1 c.c. : 1 gram.	48·7
Second conversion (trans- formed with first)	1 c.c. : 2 grams.	48·7
Third conversion (trans- formed with first)	1 c.c. : 4 grams.	46·6
C. First conversion.....	1 c.c. : 1 gram.	48·3
Second conversion (trans- formed with first)	1 c.c. : 4 grams.	46·8
Third conversion (trans- formed with first)	1 c.c. : 10 grams.	41·8

From these experiments, it is clear that diminishing the relative amount of malt extract to starch does to some extent reduce the vigour of the residual as compared with that of the original diastase, but not very markedly, and certainly not to an extent proportional to the diminution. The influence of temperature upon the energy of residual diastase is far more marked, and is shown in Series III and IV. In these series, the same conditions are adopted as in Series I, the ratio of malt extract to starch being high, and maintained constant throughout; but the temperatures of transformation adopted throughout being such as to restrict the full vigour of the diastase.

Series III.—Ratio of malt extract to starch 1 c.c. to 1 gram; maintained constant throughout. Transformations conducted throughout at 61°, malt extract previously heated to 63°.* Other details exactly as in Series I.

	K ₃₋₈₆ .
A. First conversion	45·3
Second conversion (transformed with first).....	36·9
Third conversion (transformed with second).....	24·3
B. First conversion	46·4
Second conversion (transformed with first).....	37·8
Third conversion (transformed with second).....	28·2

* The difference of 2° between the temperature of transformation and that to which the malt extract is previously heated, permits of a slight accidental rise during

Series IV.—As above, but conversions at 66°; malt extract previously heated to 68°.*

	K_{3-86}
First conversion	38·4
Second conversion (transformed with first)	28·8
Third conversion (transformed with second)	22·5

In these series there is a very marked discrepancy between the vigour of the "residual" and of the original diastase. Previous experiments indicated that this is rather attributable to the restrictions of the diastase by the higher temperatures than to any deficiency in the quantity of malt extract used. But to place the cause of this diminution of vigour of residual diastase beyond doubt, the following experiments were made. A restricted conversion was conducted at 61° with malt extract previously heated to 63°; the second conversion was, however, conducted with a *larger* amount of malt extract than the first. The following results were obtained:—

	Ratio of malt extract to starch.	K_{3-86}
First conversion	1 c.c. : 1 gram.	46·4
Second conversion (transformed with first)	2 c.c. : 1 gram.	35·6

The decrease in the vigour of the residual as compared with original diastase is therefore as marked as in Series III, where the temperatures adopted were the same as here, but where the amount of malt extract was not increased. It is a reasonable presumption, therefore, that the cause of the decrease in diastatic vigour must be sought in the temperature. That this is so is clearly shown by the following experiment; the starch paste was transformed at 61° by malt extract previously heated to 63°, *and kept at that temperature for three hours*. Ratio of malt extract to starch, 1 c.c. to 1 gram; period of digestion, two hours. The K_{3-86} of this transformation was 38·5. The K_{3-86} of two similar transformations at similar temperatures, but where malt extract was only momentarily taken to 63° and then quickly cooled, was 45·3 and 46·4 respectively. The marked decrease in the vigour of the diastase in those experiments where a decrease is found is therefore essentially to be attributed to the influence of a *protracted* subjection to a temperature in excess of the optimum; such influence being more pronounced where malt extract *alone* is exposed to temperatures exceeding the optimum than where (as during

transformation without interference with results. The malt extract is momentarily heated to the required temperature, and is then quickly cooled to 15·5°.

* See previous note.

transformations) it is exposed to them when mixed together with starch or starch products.

Incidentally it may be added that in conducting transformations with different kinds of starch, the source of the starch was found to be an important factor in determining the extent to which it becomes hydrolysed by a given volume of malt extract in a given time under given conditions. With the three kinds of starch experimented upon, we found that the starch which gelatinises at the lowest temperature is hydrolysed to the greatest extent. The following results bear this out.

Temperature, 52°; 5 grams starch transformed with 10 c.c. of normal malt extract.

	K ₃₋₈₆		Gelatinising temperatures (approximate).
	Series I.	Series II.	
Potato starch	49·6	49·6	65°
Maize „	45·9	46·4	75°
Rice „	43·2	43·3	80°

Note.—Some of the above results are of some importance in regard to the operations of brewing. During the “mashing” or conversion of malted grain, a certain amount of starch is only rendered available for conversion some time after the attainment of the resting stage in the transformation of the main bulk of starch which is converted during the early stages of the process. Were the transformations carried out at the optimum temperatures in respect to diastatic vigour, there is reason to believe, from the results of Series I, that the “later” starch (if it may be so termed) would be converted by the “residual” diastase to the point reached by the early transformation. But practical operations being always conducted at temperatures restrictive of the full activity of the diastase, it is clear, from the results of Series III and IV, that the residual diastase is incompetent to hydrolyse the “later” starch to the same point to which the original starch is transformed. Occasionally the difference in the conversion products in the early and late stages of conversion gives rise to practical difficulties, and it is sought to overcome them by reserving some of the early worts for intermixture and digestion with the later worts, with the object of transforming the latter to the same point as the former, by means of its residual diastase.

The results of Series III and IV indicate that this action would be only an incomplete one, the residual diastase of the early wort having been weakened by subjection for some time to a temperature adverse to its continued vigour. That this is actually so is shown by the following experiments. The weak later worts were digested with strong first worts for two hours; the digestions were carried out at a

lower temperature than that at which either wort was prepared. The reducing power of both strong and weak worts was determined after digesting for the same time and at the same temperature as the mixture; the results given in the last column are corrected for the reducing power of the strong wort added:—

	Ratio of strong to weak wort.	Approximate temperature of strong wort on leaving mash-tun.	Approximate temperature of weak wort on leaving mash-tun.	K ₃₈₆		
				Strong.	Weak.	After mixture and digestion.
<i>a</i>	1 : 5	67·7	67·2	40·6	36·7	39·6
<i>b</i>	1 : 10	67·2	66·6	38·2	29·9	33·2
<i>c</i>	1 : 10	67·2	66·6	39·1	30·6	35·6
<i>d</i>	1 : 10	68·3	68·8	43·3	30·7	34·8

The solid matters in the weak worts ranged from 1·5 to 3 grams per 100 c.c.; those in the strong from 21 to 24 grams per 100 c.c.; the ratio of 1 vol. strong to 10 vols. weak wort is therefore a high one. In spite of the relatively large amount of strong wort (and therefore of "residual" diastase), the starch products of the weak wort are not in any case carried to the same point as that to which the original starch was converted. These experiments have a special bearing upon the operations of brewers and distillers who employ unmalted as well as malted grain, but these considerations are of too technical a nature to be entered into here.

We consider that our results entitle us to form the following conclusions:—

The attainment of a resting stage in the transformation of starch by diastase by no means shows that the energy of the diastase is exhausted. The energy of this "residual" diastase is, in fact, very considerable, even under conditions adverse to its activity. It is somewhat weakened by increasing the amount of starch it has to convert, but it is weakened to a marked extent by subjecting it for some time to a temperature exceeding the optimum one for saccharification, especially when it is subjected alone, and unmixed with starch or starch products. When, however, it is not exposed for any length of time, whether previous to the first or to subsequent transformations, to a temperature exceeding the optimum, it appears capable, after transforming a considerable amount of starch, to transform further quantities to nearly the same point, when such further quantities are added successively and subsequent to the attainment of the resting stage in the preceding transformation.