VOL. XX. [SEPTEMBER, 1898.] No. 9.

THE JOURNAL

OF THE

AMERICAN CHEMICAL SOCIETY.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF PURDUE UNIVERSITY.]

NOTES ON TAKA-DIASTASE.

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Received July 8, 1898.

THERE has recently come into notice, chiefly through its industrial applications, a starch-saccharifying enzyme of apparently unusual value. This substance, called taka-diastase, has been in use in Japan for an indefinite time in the production of alcoholic beverages in much the same capacity as that for which we employ malt. Its introduction to America is due to Mr. Jokichi Takamine, who for some years has been occupied in furthering its application in the distilling industry in the United States. Pharmaceutical preparations of this enzyme intended for the relief of amylaceous indigestion, are also on the market. So far as the writers are informed neither the material itself nor its action on starch has been much studied from the purely scientific side or, if such investigations have been made, they have not been generally published. It seemed a matter of interest, therefore, to endeavor to secure definite information regarding such a valuable and interesting material.

Mr. Takamine has recently described[1] the patented method for preparing the material, a résumé of which will be appropriate here. Taka-diastase is, so far as known, somewhat similar to malt-diastase in its chemical character; *viz.*, a highly

[1] *Am. J. Pharm.*, 70, No. 3; *J. Soc. Chem. Ind.*, 17, No. 2.

nitrogenous substance, readily soluble in water and dependent upon certain conditions of temperature for its maximum activity. Its action is also affected by alkalies and acids. It is produced as the result of the growth of a species of mould (*Eurotium Oryzae* Ahlberg), upon rice, maize, wheat bran, etc. For its production, as at present practiced in this country, wheat bran is steamed and, after cooling, is sown with the spores of the fungus. After twenty-four hours in culture-rooms at a temperature of about 25° C. the fungus growth becomes visible. In forty or fifty hours the content in diastatic material has reached the maximum and further growth of the fungus is checked by cooling. The material, now consisting of the bran felted together with fungus mycelium, is called "taka-koji."

It may be mixed with grain or starchy materials in the same manner as malt is used, and, like malt, will speedily convert the starch into fermentable sugars. An aqueous extract of the mass may be used for a similar purpose. For the preparation of a pure product, which however is not necessary for ordinary industrial purposes, the aqueous extract is concentrated by evaporation and on the addition of alcohol the diastatic substance may be precipitated as a yellowish powder, easily soluble in water, of stable keeping qualities and possessed of an unusual power of converting starch into sugar. The medicinal preparation above mentioned is obtained in this way and represents a fairly pure form of the diastatic principle. This bears the name of "taka-diastase."

This paper presents the results of some observations upon the action of taka-diastase upon starch as compared with the ordinary diastase of malt.

For the purposes of this comparison, taka-diastase was employed which was kindly furnished by the makers, Messrs. Parke, Davis & Co., of Detroit, Mich. The malt-diastase was prepared in the laboratory by digesting fresh pale malt with water at room temperature during three or four hours, filtering and adding to the clear filtrate two volumes of strong alcohol. The flocculent precipitate was collected, pressed dry, and finally dried in desiccators over sulphuric acid.

A series of experiments was carried out in which pure potato starch, which had been changed to paste by boiling, was sub-

jected to the action of the two sorts of diastase. Three methods were employed to determine the degree to which the starch had been changed; *viz.*, first, the iodine test; second, the optical behavior of the solution; third, the determination of the actual amounts of sugar produced.

In every case care was taken to preserve uniform conditions except as specified below.

1. Comparative action of the two kinds of diastase on potato starch as indicated by the iodine test.

a. Four samples of starch of one-half gram each were boiled with fifty cc. of water each. To two were added 0.05 gram malt-diastase each and they were kept at 60° C. throughout the test. To the other two were added 0.05 gram taka-diastase and these were kept at 40° C. throughout the test. At fixed intervals the solutions were tested with iodine for the presence of starch, in the following manner: Three drops of the solution were removed to a white test plate and were mixed with one drop of standard aqueous iodine solution containing one and a half grams potassium iodide and eight-tenths gram iodine in one liter. The least trace of starch is indicated under these conditions by a blue or greenish tint or as the starch is passing through some of the preliminary stages of conversion the tint will be more or less violet or purple. In this and other experiments the duplicate samples were found to agree very closely in their behavior.

The results of the observations were as follows:

End of six minutes: Malt-diastase, no perceptible change; taka-diastase, deep violet color.

End of twelve minutes: Malt-diastase, reddish brown; taka-diastase, violet to reddish.

End of eighteen minutes: Malt-diastase, light brown or yellow due to iodine solution; starch reaction had disappeared; taka-diastase, light violet or reddish.

End of forty-five minutes: Taka-diastase, light violet tint.

End of sixty minutes: Taka-diastase, light greenish-blue tint, evidently containing small traces of unchanged starch.

b. The proportions of starch to diastase were increased, in other respects the conditions remaining the same as in "*a*". Four samples of starch of one gram each, converted to paste by

boiling with fifty cc. of water, received diastase of each kind as above.

End of six minutes: Malt-diastase, deep violet; taka-diastase, deep reddish violet.

End of twelve minutes: Malt-diastase, reddish violet; taka-diastase, deep reddish violet.

End of eighteen minutes: Malt-diastase, reddish brown; taka-diastase, light reddish violet.

End of twenty-seven minutes: Malt-diastase, light brown, no starch reaction; taka-diastase, slightly reddish violet.

End of six hours: Taka-diastase, still a bluish green color showing small amounts of unchanged starch.

c. The proportions of starch to diastase were again increased. Four samples of two and a half grams each of starch were converted to paste in 250 cc. of water and received respectively 0.05 gram malt- or taka-diastase.

End of six minutes: Malt-diastase, no perceptible change; taka-diastase, deep reddish violet.

End of twelve minutes: Malt-diastase, deep reddish violet; taka-diastase, deep reddish violet.

End of forty-five minutes: Malt-diastase, brownish yellow color, no further starch reaction; taka-diastase, light violet or greenish tint.

End of twelve hours: Taka-diastase, still showed the bluish-green tint.

d. The amounts of diastase were diminished. Four samples of starch of 1.875 grams each in forty cc. of water, received respectively 0.025 gram of taka- or malt-diastase.

End of six minutes: Malt-diastase, deep blue, little or no change; taka-diastase, deep reddish violet.

End of twelve minutes: Malt-diastase, reddish brown; taka-diastase, deep reddish violet.

End of twenty-four minutes: Malt-diastase, yellowish brown color, no starch reacting; taka-diastase, reddish violet.

End of forty-five minutes: Taka-diastase, still gives a violet tint.

e. Four samples of starch of two and a half grams each in 250 cc. water with 0.025 gram respectively of malt- or taka-diastase.

End of six minutes: Malt-diastase, no change; taka-diastase, deep reddish-violet.

End of twelve minutes: Malt-diastase, deep reddish brown; taka-diastase, deep reddish brown.

End of twenty-four minutes: Malt-diastase, yellowish brown, no starch reaction; taka-diastase, deep reddish brown.

End of sixteen hours: Taka-diastase, still gave a bluish drab color.

In every case it was apparent to the observer that the effect of the taka-diastase was more rapid at the outset, as indicated by the almost immediate change from the typical blue of the starch iodine compound to reddish and violet tints. It was generally noticeable also that the taka-diastase liquefied the starch paste very rapidly, more so than the malt-diastase. On the other hand, the complete conversion of the starch into forms which no longer gave color reactions with iodine was effected much earlier by the malt-diastase, while the taka-diastase scarcely attained this result after several hours.

2. Comparative action of the two kinds of diastase on potato starch as shown by optical tests.

The different conversion products of starch exhibit varying degrees of optical activity. The dextrines possess a high specific rotation usually stated as approximately 190°. Maltose has the specific rotation 130°–140°, while the ultimate conversion product, dextrose, has the specific rotation 52.5°.

In considering the action of diastase, however, maltose may be regarded as the ultimate product, and Brown and Morris have pointed out[1] that the optical behavior of a solution of the products of diastatic action on starch paste, is that of a mixture of maltose and dextrose. A determination, therefore, of the specific rotation of such a solution gives information regarding the degree of conversion of the starch. The degree of completeness of the change from starch through dextrines of high specific rotation to maltose of a lower rotatory power will be indicated by a diminution of the specific rotation. A few qualitative observations along this line were made as follows:

Eight samples of starch of equal weight were converted to paste by boiling in equal amounts of water; to each of four were added

[1] *Chem. News*, 71, 123.

0.05 gram malt-diastase and to the other four 0.05 gram taka-diastase each. These samples were kept respectively at 60° and 40° C. At regular intervals one of each series was withdrawn, heated quickly to boiling to check further action of the diastase, cooled, and made up to a volume of 100 cc. The specific rotation was then determined in a Laurent polarimeter with sodium light. Two series of experiments were made in this way, using different amounts of starch.

a.

One-half gram starch in 100 cc. water.
0.05 gram of diastase.

	Malt-diastase at 60° C.		Taka-diastase at 40° C.	
Time. Hours.	Observed rotation.	Specific rotation.	Observed rotation.	Specific rotation.
¼	1°20′	133°20′	1°15′	125°
½	1°19′	131°40′	1°13′	121°40′
1	1°19′	131°40′	1°9′	115°
2	1°19′	131°40′	1°7′	111°40′

b.

One gram starch in 100 cc. water.
0.05 gram of diastase.

	Malt-diastase at 60° C.		Taka-diastase at 40° C.	
Time. Hours.	Observed rotation.	Specific rotation.	Observed rotation.	Specific rotation.
¼			2°47′	139°20′
½	2°57′	147°30′	2°43′	135°50′
1	2°45′	137°30′	2°29′	124°10′
2	2°47′	139°20′	2°27′	122°30′

The products of the action of taka-diastase were in all cases of lower specific rotation than those from malt-diastase, indicating a more rapid change to maltose.

3. Comparative action of the two kinds of diastase on potato starch as indicated by the amount of sugar formed.

The most practical as well as most conclusive test of the relative degree to which starch has been converted by diastatic action is that of actually determining the amount of sugar formed under comparative conditions. This has been done as follows:

Several equal samples of the starch were boiled with equal volumes of water to change into paste, were cooled to the proper temperature, and received respectively given amounts of one or

the other diastase. At the expiration of a given time one sample of each kind was withdrawn, boiled to check the action of the diastase, cooled, and filled up to definite volume. The amount of sugar present was determined by the method described by Defren.[1]

It is believed that these results form a very good basis for comparing the actual efficiency of the two enzymes for a given time.

a.

One-half gram starch. 100 cc. water. 0.05 gram diastase.

	Malt-diastase at 60° C.			Taka-diastase at 40° C.		
Time.	Cupric oxide from 10 cc.	Maltose.	Total maltose from 5 grams starch.	Cupric oxide from 10 cc.	Maltose.	Total maltose from 5 grams starch.
Hours.		Mg.	Mg.		Mg.	Mg.
¼	39.5	29.0	290	61.4	44.3	443
½	41.1	29.7	297	63.9	46.5	465
1	40.7	29.7	297	65.8	47.9	479
2	40.2	29.0	290	73.0	53.0	530

b.

One gram starch. 100 cc. water. 0.05 gram diastase.

	Malt-diastase at 60° C.			Taka-diastase at 40° C.		
Time.	Cupric oxide from 10 cc.	Maltose.	Total maltose from 1 gram starch.	Cupric oxide from 10 cc.	Maltose.	Total maltose from 1 gram starch.
Hours.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
¼	71.4	51.6	516	84.5	61.8	618
½	84.7	61.8	618	99.6	72.8	728
1	75.5	55.2	552	120.1	87.4	874
2	85.5	61.8	618	143.6	105.0	1050

These results show that for a given short time the actual saccharifying power of the taka-diastase is decidedly superior to the malt-diastase.

4. The use of taka-diastase as an analytical reagent for the determination of starch.

Malt-diastase has come to be most favorably looked upon as an agent for the selective removal and analytical determination of starch occurring in grains and vegetable materials, associated with other carbohydrates. One drawback to its common use is its unstable character in the isolated form, since it is well known

[1] This Journal, 18, 749.

that preparations of diastase rapidly deteriorate and lose their value. On this account the practice is to freshly prepare aqueous infusions of malt for immediate use in starch determination. Such infusions, however, always contain sugar, which being added to the analytical sample necessitates a subsequent correction. The use of malt-diastase in any form therefore as an analytical reagent is more or less tedious and renders welcome any substitute which would avoid these objections and at the same time act as an efficient starch solvent. Taka-diastase has seemed a promising substance for this purpose, although the qualitative tests described under "1" indicate that it does not readily effect complete conversion of starch under the conditions stated.

The following experiment upon materials commonly presented for starch determinations is instructive:

One gram each of air-dried, finely ground wheat, maize, and potato were heated to the boiling-point during thirty minutes with fifty cc. of water. After cooling to 60° C., ten cc. of malt infusion were added to each and the temperature was carefully maintained as above. To each of a similar series prepared in the same way was added 0.05 gram and to each of another similar series 0.1 gram of taka-diastase: these samples were kept at 40° C. At the expiration of four and one-half hours all were subjected to the iodine test for starch. In those treated with malt infusion all traces of starch had disappeared from the potato while the wheat and maize still showed slight traces of starch under the microscope. In those treated with taka-diastase, all, without exception, showed an abundance of unchanged starch, nor was there apparently any sharp distinction between the samples of the same material receiving different amounts of the diastase.

After seven hours the samples treated with malt-diastase gave no further indication of starch. But all of those treated with taka-diastase gave pronounced starch reactions. Additional treatment during twelve hours at ordinary temperature did not suffice to remove all of the starch from the latter samples.

Evidently these results were unfavorable to the employment of taka-diastase as a reagent but a quantitative comparison was

made as follows: Duplicate, two-gram samples of air-dried, ground wheat were weighed and gelatinized by boiling thirty minutes with 100 cc. of water. To one was added twenty cc. of a malt infusion made by digesting five grams of malt in fifty cc. of water three hours at ordinary temperature; this sample was then kept at 60° C. To the other duplicate was added 0.05 gram taka-diastase and the temperature kept at 40° C.

After four hours the solutions were filtered. The residue from the wheat treated with malt infusion proved to be free from starch when examined under the microscope after treatment with iodine solution. The wheat treated with taka-diastase was slightly stained blue when treated with iodine. The filtrates received each ten cc. concentrated hydrochloric acid and were heated in boiling water during one hour. After cooling, neutralizing, and making up to volume, the amount of sugar in each was determined by titration with Fehling's solution and the results calculated to starch in the original samples of wheat.

The treatment with malt infusion gave 55.46 per cent. of starch. The treatment with taka-diastase gave 52.94 per cent. of starch.

This result agrees wholly with the qualitative experiments previously made and indicates that under the stated conditions taka-diastase is not adapted for use in the quantitative determination of starch. It is nevertheless possible that some modification of these conditions or of the material itself may lead to more satisfactory conclusions as the result of some future investigation.

It is certain, however, that in gross industrial operations the cheapness and stability of taka-koji and taka-diastase will commend it while its ability to convert a very large proportion of the starch present in a grain into sugar within a very short time should render it a very valuable substitute for malt. The application of taka-diastase as an aid to starch digestion is doubtless of high value by reason of its convenience, its notable saccharifying power, and its permanent keeping qualities.