Fungus and Enzyme. Preparation of Aspergillus Oryzae Amylase and its Use in Bread Making

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Introduction

The degree of panary ferment which is caused by yeast depends largely upon the presence of fermentable sugar produced by the action of diastatic enzyme in flour. The amount of diastase in the flour is, therefore, one of the important factors in bread making. Flour deficient in diastatic activity can not be baked into a desired large loaf of bread unless a sufficient amount of sugar is added to the dough. It is known that to supplement the deficiency, wheat is mixed with a little sprouting one[1] and is milled in the usual manner or the flour is mixed with corn flour[2] having a high diastatic activity or with malt preparation.

In the experiment, it is observed that the use of fungal amylase preparation, taka-diastase, in bread making gives an undesirable effect, caused not by the true amylase of such preparation but by the proteolytic enzyme accompaning the amylase. That is to say the proteolytic enzyme is not only responsible for a reduction of gas retaining power of the dough but also for the characteristic bread disease "Ropiness".[3]

Therefore, it is desirable to prepare the diastatic enzyme preparation as free as possible from the proteolytic enzyme.

Discussion

The present author[4] has shown that the diastatic enzyme solution passes through a colloidal membrance, indicating that it possesses a relatively small molecule. The enzyme preparation prepared by this method was tried in bread making but this was abandoned since it was not economical. There are many methods for the separation or purification of amylase but they are not applicable to the industry from the same stand point.

Although it was thought that alkaline or acidic of the culture medium, bran, might

(1) Sherwood and Bailey, *Cereal Chem.* **3**, 163 (1926).
(2) Sapozhnikova, *C. A.* **43**, 7569 (1949).
(3) Unpublished observation by the author at Takamine Laboratory, Inc., Clifton, N. J., U. S. A. (1930).
(4) T. Harada, *Ind. Eng. Chem.* **23**, 1424 (1931).

influence the production of proteolytic enzyme, a few experiments has proved otherwise. The best method is to find the special strain of the fungus which does not produce any of the enzyme in question in its growth, but such is almost impossible at present.

In the previous paper[5] the present author reported that in precipitating the enzyme with 70 volume percent alcohol when the precipitate becomes gum-like it may be adjusted to a desired precipitate by cutting down the amount of alcohol. However, in this case this will cause a loss of diastatic enzyme preparation. When the precipitating diastase preparation of this dilute alcohol was determined for diastatic and proteolytic (casein-digesting) enzymes, a relatively large amount, in other words, a strong proteolytic enzyme (Fuld-Gross' method) was present in comparison to that of diastatic enzyme in the control.

Therefore, it is interesting to note here that the proteolytic enzyme could be removed by this simple fractional precipitating process, and that the diastase preparation, free or almost free from the enzyme in question is possible to be used in the bread making industry instead of malt preparation not only more economically but in producing fine loaves of bread. It is seen that the amount of diastase and protease in the flour or the dough in bread making becomes a real problem.

Fraction

It was shown that a fractional precipitation by alcohol serves to a certain degree as separation or purification of the amylase; that is, in the first fraction, with around 57 volume

Table 1

The Influence of Fractional Alcoholic Precipitation upon Diastase and Proteolytic Enzyme

Origin of fungus strain*		Yield, g.	L. V.	% L. V.**	Total L. V., %	T. V.	Total T. V.	T. V. in 100% L. V.
A	1. Koji extract 100 +alc. 150 (61%)	1.40	1100	73.3	102.6	111.1	155.5	151.6
	2. The filtrate of No. 1 225+alc. 215 (77.6%)	2.99	5651	376.7	1126.3	3.2	9.6	0.9
	3. Koji extract 100 +alc. 375 (75%)	4.12	4100	273.3	1126.0	41.7	171.8	15.3
B	1. Koji extract 100 +alc. 115 (51%)	0.9	125	8.3	7.5	47.6	42.8	573.5
	2. The filtrate of No. 1 205+alc. 230 (74%)	1.2	1967	131.1	157.3	37.0	44.4	28.2
	3. Koji extract 100 +alc. 350 (74%)	2.0	1283	85.5	171.0	47.6	95.2	55.7
	1. Koji extract 125 +alc. 188 (57%)	1.2	125	8.4	10.1	25.7	30.8	306.0
	2. The filtrate of No. 1 290+alc. 270 (75%)	1.1	2117	141.1	155.2	8.9	9.8	6.3
	3. Koji extract 125 +alc. 438 (74%)	2.2	1034	68.9	155.1	22.2	48.8	32.2
	1. Koji extract 110 +alc. 190 (60%)	1.4	190	12.7	17.8	111.1	155.4	874.8
	2. The filtrate of No. 1 275+alc. 230 (72%)	2.0	535	35.7	71.4	15.4	30.8	43.1
	3. Koji extract 110 +alc. 350 (72%)	3.0	480	320	96.0	66.7	200.1	208.4
C	1. Koji extract 100 +alc. 137 (55%)	0.6	230	8.7	5.2	8	4.8	92.0
	2. Whole filtrate of No. 1+alc. 270 (79%)	0.7	4600	306.7	214.7	6	4.2	2.0
	3. Koji extract 100 +alc. 375 (75%)	1.1	3240	216.0	237.6	8	8.8	3.7

* Orgin of fungus strain A, Asp. oryzae strain of Takamine Laboratory; B, Asp. oryzae, a mixed strain; C, Asp. oryzae B. strain of the Sakaguchi Laboratory of this institute.

** 100% = 1500 L. V.

(5) T. Harada, This Bulletin, **22**, 197 (1949).

percent alcohol of Koji water extract, it gives a relatively strong tryptic but weak diastatic enzyme preparation, and in the second fraction, with 72 percent alcohol or over, it gives a preparation, fairly free from the proteolytic enzyme with a strong diastatic power, in comparison to the first fraction and the control.

It is seen that as total L. V. (Table 1), the diastase preparation may greatly be lost in the first fraction but this loss may be collected in the second fraction to make it up to about 70 volume percent or over. Further purification might be carried out by dissolving the precipitate and by repeating the process of the fraction but it is industrially not economical. The degree of purity, of course, depends largely upon the strength of the extract and the origin of the fungus strain as shown in the experiment.

Selective Use of the Fraction

The rich proteolytic but poor diastatic enzyme content preparation in the first fraction may be superior than that of an ordinarily prepared taka-diastase or the control when used internally such as for stomach disturbance but the proteolytic freed rich diastatic enzyme preparation is superior than malt preparation in the bread making industry.

Experiment

(a) One hundred cc. (Table 1, A) of the water extract of Asp. oryzae fungus Koji (free from spore) No. 1 and 3 were treated with 150 and 375 cc. of about 95 percent alcohol to give about 61 and 75 volume percent respectively in a 500 cc. capacity Erlenmayer flask, mixed well, left standing one hour or so, then supernatant liquid decantated to another flask. The bottom liquid mixture with precipitate was transferred a to precipitation tube, centrifugalized and separated. The precipitate was washed with 95 percent alcohol and filtered rapidly by suction, washed with ether and finally it was dried in a calcium desiccator. On the other hand (No. 2), the whole (225 cc.) alcoholic liquid part, the filtrate of No. 1, was treated with further addition of alcohol (215 cc.), mixed, precipitated again, and this second precipitation was washed with 95 percent alcohol, finally dried in a calcium desiccator. Table 1, B and C are the results of the similar treatment for the samples of different origins.

(b) When the amylase, fairly free from proteolytic enzyme, was tried number of times in bread making (flour, 350 : diastase, 50 L. V. in total) by the method[6] of Bailey, the result was quite a satisfactory bread in every respect. The characteristic fermentation (bread-disease) does not easily set in comparison with that prepared by the Takamine's method. This publication will soon follow.

Summary

1. The fungal amylase preparation containing rich proteolytic but poor diastatic enzyme has been prepared by precipitating Koji water extract with the amount of alcohol which gives around fifty to sixty volume percent solution.

2. A strong fungal amylase but very weak proteolytic enzyme preparation has been prepared by fractional precipitation of the filtrate of 1 by adding further alcohol up to seventy percent or over.

3. The possibility of utilizing fungal diastase preparation in the bread making industry has been pointed out.

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(6) Bailey, *J. Ind. Eng. Chem.*, **8**, 53 (1916).