Fungus and Enzyme.
Preparation of Aspergillus Oryzae Amylase

By Taichi HARADA.

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The role of fungi in nature is of very great importance to our daily life.

Pathogenic fungi are those which cause a very annoying disease "Eczema" in the human body[1] and also plant diseases in plants. Non-pathogenic species are those which cause deterioration and destruction of timbers, textiles and foods, and those which produce many organic acids,[2] e. g. citric, lactic, gluconic, kojic, etc., arsine compounds, penicillin,[3] etc. and also those which produce many important enzymes, e. g. amylase, protease, tannase, invertase, etc. This is, of course, speaking in a broad sense of the difference between the pathogenic and the non-pathogenic species.

The amount of the enzymes produced by fungi depends upon the composition of culture medium in which they are grown. According to Takada[4] wheat bran increases the production of Asp. oryzae enzyme as much as two times as that of embryo of rice bud and five times as that of oil removed bean. In this connection the author refers to his previous paper[5] in which very important precautions of making Koji were pointed out.

In the preservation of enzyme in the Koji cake the cake may be dried to prevent bacterial infections or after crushing the cake loosely the enzyme may be extracted at once with cold water and then filtered and preserved in a manner suitable for the purpose for which the preparation is to be used. Sodium chloride at the saturation point gives the most satisfactory results as an antiseptic. This solution may be used for most purposes; oral administration, food preparation or for the desizing of textiles. The amylase prepared from the water extract by alcohol precipitation is not a pure single enzyme but a mixture of many enzymes.[6]

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(1) T. Harada, This Bulletin, **9** (1934), 186, 192.

(2) Prescott and Dunn, "Industrial Microbiology", McGraw-Hill Book Co., New York (1940).

(3) Fleming, *Brit. J. Exp. Path*, **10** (1929), 266; "Penicillin", Hekiso Soc., Kawade Book Store, Tokyo (1946); also Sumiki's "Penicillin", Sangyo Tosho Co., Tokyo (1946).

(4) *J. Brew. Asstn. Japan*, **22** (1944), 232; also Takada, Katsui and others, *J. Appl. Micology*, **2** (1948), 88.

(5) T. Harada, *Ind. Eng. Chem.*, **23** (1931), 1424.

(6) Inouye, "Useful Molds", Hokko Book Store, Tokyo (1948).

(7) T. Harada, *Ind. Eng. Chem. Anal. Ed.*, **3** (1931).

In the factory, in precipitating the enzyme with alcohol we often encounter great trouble when the Koji water extract becomes unusual in nature giving a colloidal (light) or a gummy (heavy) precipitate which can not be thoroughly dehydrated. In other words, it does not give a flocculent precipitate. When a gummy precipitate is obtained, it must be dissolved in water and then the precipitation must be carried out again. Such a colloidal or a gum-like precipitate could not be easily dried or if it is dried a dark lump may be obtained. In both cases not only is it very difficult to make it in powder form after drying, but the diastatic strength becomes greatly lost. Of course the trouble of gummy formation may be removed by cutting down the amount of alcohol but this decreases the enzyme. It was found that, in case the precipitate tends to become colloidal, an addition of a small amount of sodium phosphate, or potassium phosphate, or the like to the extract not only solves the trouble but also gives an increase of diastatic power to the preparation in its total strength. However, in the case of gummy formation the dibasic halogen compound will solve the trouble but it will decrease the total Lintner's value (L. V.). This is due to a destruction of the enzyme.

Experimental

Influence of Inorganic Salts upon Amylase Precipitation. Twenty five cubic centimeters of the water extract of Koji were mixed with a small amount of inorganic salts to which 75 c.c. of alcohol were added for the precipitation. It was mixed well, filtred, dried and finally the strength determined by the author's[7] method for L.V.

Table 1.

Salts (0.05 g.)	Yield (g.)	Tested L.V.	Total L.V.
$(NH_4)_2SO_4$	0.6370	3816	2431
KH_2PO_4	0.6211	4133	2567
$NaH_2 \cdot PO_4 \cdot H_2O$	0.6377	4133	2636
K_2HPO_4	0.6475	3933	2547
$Na_2HPO_4 \cdot 5H_2O$	0.6595	4033	2660
Alcohol alone	0.6361	3617	2301

Original total L.V. of 25 c.c. of the extract $(111 \times 25) = 2775$

Table 2.

Salts	(g.)	Yield (g.)	Tested L.V.	Total L.V.
$MgCl_2 \cdot 6H_2O$	0.05	0.6330	2875	1820
"	0.10	0.6635	2450	1626
"	0.20	0.6910	1500	1037
$CaCl_2 \cdot 2H_2O$	0.05	0.5710	3075	1756
"	0.10	0.6480	1825	1183
"	0.20	0.7680	1000	768
Alcohol alone		0.6190	3075	1903

Original total L.V. of 25 c.c. of the extract $(159 \times 25) = 3975$

Table 3.

Salts (0.10 g.)	Yield (g.)	Tested L.V.	Total L.V.
NaCl	0.6714	4775	3206
$NaHCO_3$	0.8250	3975	3280
$Na(NH_4)_2PO_4 \cdot 4H_2O$	0.6925	4450	3082
$MgSO_4 \cdot 7H_2O$	0.7580	4175	3165
$(NH_4)_2HPO_4$	0.6817	3773	2573
K_2HPO_4	0.6965	4175	2908
Alcohol alone	0.6905	3975	2745
Original total L.V. of 25 c.c. of the extract		$(159 \times 25) = 3975$	

Table 4.

Salts (g.)		Yield (g.)	Tested L.V.	Total L.V.
$MgCl_2 \cdot 6H_2O$	0.10	0.6765	5725	3873
"	0.20	0.7145	3975	2840
$CaCl_2 \cdot 2H_2O$	0.10	0.6625	5100	3379
"	0.20	0.8045	1525	1227
Alcohol alone		0.6560	6350	4166
Original total L.V. of 25 c.c. of the extract			$(191 \times 25) = 4775$	

Preparation of Dry Amylase without Alcohol for Oral Purpose. In preparing the amylase in tablet form for oral administration, the Koji extract was mixed with magnesium or calcium carbonate in the ratio of about three to one and placed in a large flat container and while slowly mixing it, it was dried by means of a dry air current at low temperature (30–35°C). L.V. of the mixture was obtained up to 750. This amylase-carbonate mixture may be easily made into tablets.

Summary

1. The influence of inorganic salts upon the precipitation of amylase with alcohol under given conditions has been studied.

2. The preparation of dry amylase without alcohol for oral purpose has been described.

Scientific Research Institute,
Tokyo