THE PROPERTIES OF A SPECIALLY PREPARED ENZYMIC EXTRACT, POLYZIME, COMPARING ITS STARCH LIQUEFYING POWER WITH MALT DIASTASE.

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The aim of this study and article is to compare certain properties of Polyzime, an enzymic extract of *Aspergillus Oryzae* prepared by special processes, with those of standard malt extract.

A. Literature.

"Polyzime," recently invented by Dr. J. Takamine, is an aqueous extract of diastatic enzymes, containing many other enzymes, made by a specially prepared culture of the fungus *Aspergillus Oryzae* on media consisting mainly of wheat bran. As many reports have been published about the enzymes produced by this fungus (generally using Taka-Diastase as sample) a brief abstract will therefore first be given of the important literature directly pertaining to this subject.

J. Wohlgemuth[1] found in a sample of Taka-Diastase amylase, maltase, trypsin, lab. erepsin, lipase and haemolysin. This amylase can resist stronger acid than can pancreatin diastase.

H. C. Sherman and A. P. Tanberg[2] showed that the amylase of *Aspergillus Oryzae* exerts its maximum activity, both amyloclastic and saccharogenic, in a very slightly acid medium; and as prepared by them it has higher amyloclastic, but lower saccharogenic power than the most active malt amylase preparations yet recorded.

H. C. Sherman, A. W. Thomas and M. E. Baldwin[3] reported that the amylase of *Aspergillus Oryzae* showed activity from P_H 2.6 with optimum at P_H 4.8. Pancreatic amylase was active between the limits of P_H 4 and 10 with greatest activity at about 7, the solutions commonly considered neutral showing under similar conditions a P_H value of 5.8. Malt amylase was active between P_H 2.5 and 9 with optimum activity at 4.4 to 4.5. The influence of the concentration of electrolytes, as distinguished from concentration of hydrogen alone, appeared greatest in the case of amylase of pancreatin, and least in the case of the amylase of *Aspergillus Oryzae*.

Experimental.

The results of our numerous experiments on Polyzime preparations and malt extract are as follows.

[1] "Zur Kenntnis der Taka-Diastase," *Biochem. Z.*, 39, 324 (1912).

[2] "Experiments upon the Amylase of *Aspergillus Oryzae*," This Journal, 38, 1638 (1916).

[3] Influence of hydrogen concentration upon enzymic activity of 3 typical amylases: This Journal, 61, 231 (1919).

B. General Properties of Polyzime.

a. Sp. gr., 1.03–1.06.

b. Amyloclastic (starch liquefying) power by Wohlgemuth's method: $D \; {}^{40°}_{30 \text{ min.}} \; =$ 3,000; $D \; {}^{40°}_{24 \text{ hours}} \; = 115,000$.

c. Saccharogenic (starch saccharifying) power by Lintner's method: Lintner's Value (21°) = 43. Lintner's Value (50°) = 150.

d. Chemical Composition:

Water	87.5%
Solid Matter	12.5
Mineral Matter	1.5
Acidity with rosolic acid as indicator:	
10 cc. Polyzime required 5 cc. 0.1 N acid to neutralize	
Total nitrogen	0.5
Protein precipitated with Stutzer's reagent	0.2%
Reducing sugar as *d*-glucose	2.0%
Amino acid as glycocoll by formaldehyde method	1.5
Dextrin	1.3

e. Destructive influence of heat.

By placing Polyzime on a water bath the following decreased percentages of diastatic power after 3 hours were obtained:

Temperature of Water Bath. °C.	Decreased Diastatic Power. %.
15	0
40	0
50	55.0
60	95.0
70	98.0

From the above experiments and many others, it is evidently necessary to preserve Polyzime at a temperature lower than 40° (104° F.); if the temperature is higher, the diastatic power is decreased proportionately. It is also shown that the optimum temperature for diastase conversion has a destructive influence upon Polyzime itself during time period of 3 hours or over.

f. Preservation of strength: If Polyzime is kept in a closed barrel at ordinary temperature it can be preserved for half a year with practically no change of the diastatic power.

C. Amyloclastic Power of Polyzime Compared with Malt Diastase.

I. Method of Experimentation.—There are quite a number of methods for this purpose, such as, for example, Dr. J. Takamine's simple quantitative method of diastatic power determination;[1] William A. Johnson's;[2] J. Wohlgemuth's;[3] S. A. Waksman's.[4] Among these, the most accurate appears to be that of Wohlgemuth, and to make subsequent data clear, we give here a condensed description of it, as follows.

[1] *J. Soc. Chem. Ind.*, **17**, 437 (1898).

[2] "A Proposed Method for Routine Valuation of Diastase Preparation," THIS JOURNAL, **30**, 801 (1908).

[3] *Biochem. Z.*, **9**, 1 (1908).

[4] THIS JOURNAL, **42**, 293 (1920).

Wohlgemuth's Method and Scale.—Place in a series of test-tubes diminishing amounts of the enzyme solution to be tested. Introduce into each tube 5 cc. of a 1% solution of soluble starch and place each tube immediately in a bath of ice-water. Transfer the tubes one at a time to a water bath at 40° for 30 minutes (or 24 hours with toluol). At the end of this time transfer again immediately to the ice-water bath to stop the action. Dilute the contents with 20 cc. of water, add one cc. of 0.01 N solution of iodine and shake well. One usually obtains colors from blue through violet, red and yellow to colorless, indicative of starch, erythrodextrin, achrodextrin, etc. Take the tube in which the blue or violet has entirely disappeared, giving place to either red or orange-red color as judged by Mulliken's color standard sheet C; note the amount of enzyme solution in this tube and calculate the power of the enzyme as the number of cubic centimeters of one per cent. starch solution which is digested to this stage in a given time by one cc. of enzyme solution.

Thus if 0.2 cc. (or gram) of the substance completely digests 5 cc. of starch solution in 30 minutes at 40°, then one cc. of that solution will be able to digest 250 cc. of one per cent. starch solution; or its power is then stated:

$$D\,_{30\,\text{min.}}^{40°} = 250 \text{ (Wohlgemuth Scale)}$$

Here the time of reaction is brought into the final expression of diastatic powers that the method may be applied to substances whose power is very slight by allowing them to act a long time.

II. Optimum Reaction of Starch Solution.

—We tried to find the best degree of acidity or alkalinity of starch solution for liquefaction with Polyzime. For this purpose we always used Wohlgemuth's method with one per cent. souble starch solution, but with different concentrations of acid or alkali and at different temperatures. The soluble starch used was a standard preparation and the acidity or alkalinity of the solution was regulated by adding hydrochloric acid or sodium hydroxide, with rosolic acid as indicator. The malt diastase used was one of the

$a.$ 20° and 2 hours $D_{2h.}^{20°} =$

Reaction of starch soln.	Malt Extract.	Polyzime.
0.0004 M HCl	430	5600
0.0003	660	6000
0.0002	930	6000
0.0001	930	6240
0.0000 (neutral)	930	5000
0.001 M NaOH	730	4200
0.0002	660	3600
0.0003	530	2500

$b.$ 50° and 2 hours. $D_{2h.}^{50°} =$

Reaction of starch soln.	Malt Extract.	Polyzime.
0.0004 M HCl	960	3320
0.0003	1700	16660
0.0002	2800	16660
0.0001	2800	16660
0.0000 (neutral)	2800	16660
0.0001 M NaOH	2800	14000
0.0002	1870	12400
0.0003	1670	11200

best malt extracts on the market with a Lintner's value (saccharifying power) of 380 (50°). The Polyzime used was a standard strength factory product, neutralized with acid. Lintner's value = 150 (50°).

These results indicate that Polyzime and malt diastase show a parallel behavior with changing acidity of a starch solution. Their maximum activities are shown in neutral or very slightly acid solutions. However, Polyzime exhibits a 3 to 5 times greater acidity.

III. Optimum Temperature of Liquefaction.—High temperature accelerates diastatic action, at the same time destroying the diastase itself. Therefore, to determine the optimum temperature, it is necessary to consider the duration of digestion. Wohlgemuth's method was used with one per cent. soluble starch solution, the reaction of which was that of 0.0001 M hydrochloric acid, with rosolic acid as indicator. The malt extract and Polyzime were the same as before.

$a.$ 2 Hours and Different Temperature. $D_{2h.}^{10°}$

Temperature. °C.	Malt Extract.	Polyzime.
20	660	4540
40	2700	15000
50	2700	15000
60	1870	6250
70	833	1000
80	200	357

$b.$ Different Times and Temperatures. $D_{10h.}^{10°} =$

Duration.	Temperature. °C.	Malt Extract.	Polyzime.
30 min.......................	40	700	3000
	55	1070	4820
	40	2700	16000
2 hours.....................	58	2800	16660
	55	2770	16000
	60	1870	6250
	40	6600	115000
24 hours....................	55	3700	31600

The conclusion to be drawn from the above is that the optimum temperature is about 55° for 30 minutes to 2 hours digestion and 40° for 24 hours. Here also the same relation exists between malt extract and Polyzime diastase.

IV. Comparison of the Amyloclastic Powers of Polyzime and Malt Diastase Samples.

The following results were obtained with Polyzime (dry Taka-Koji and Taka-Koji extract) and malt diastases (the best malt flours and extracts) with the Wohlgemuth method and for saccharogenic power tested by Lintner's method:[1]

[1] *J. prakt. Chem.*, **34,** 386 (1886).

The samples tested (all lately manufactured).

a. Polyzime "D" (dried Taka-Koji flour, Takamine Laboratory manufactured).
b. Polyzime (Taka-Koji extract, Takamine Laboratory manufactured).
c. Malt flour I.
d. Malt flour II.
e. Malt flour III.
f. Malt extract I.
g. Malt extract II.

	D $\frac{40°}{30 \text{ min.}}$ =	D $\frac{40°}{2 \text{ h.}}$ =	D $\frac{40°}{24 \text{ h.}}$ =	Lintner's value. 20°.	Lintner's value. 50°.
a..........	4700	24000	170000	96	250
b..........	3000	16000	115000	43	150
c..........	1053	4000	10000	156	550
d..........	1000	...	14000	139	410
e..........	850	...	15000	128	400
f..........	700	2700	6600	100	380
g..........	400	1000	8632	98	340

Summary.

1. The diastatic power of Polyzime does not decrease at temperatures lower than 40°. Below that temperature it preserves its enzymic activities for more than half a year with practically no change.

2. The optimum reaction of starch solution for liquefaction by Polyzime is neutral or very faintly acid.

3. Polyzime is 3 to 5 times stronger than ordinary malt extract in its amyloclastic power as indicated by testing according to Wohlgemuth's method.

4. The optimum temperature of starch liquefaction by Polyzime is 50° for a 30 minute to 2 hours digestion and 40° for 24 hours digestion, although it shows weaker saccharifying power than malt extract tested by Lintner's method.

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THE ESTERIFICATION OF ALPHA AMINO ACIDS.

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In the preparation of amino acids, or in the analysis of proteins by Fischer's ester method, some means of measuring the extent and rate of esterification would be of value. The rate of hydrolysis of a protein can be accurately followed by the Van Slyke amino nitrogen determination (as well as by other excellent methods), but in so far as we are aware, no accurate method has been devised for following quantitatively the subsequent esterification. Such a method would permit a direct determination of the relative merits of the different methods of esterification and