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PROTEOLYTIC ENZYMES USED FOR RESTORING

This title, which I have been given and which you have seen in the programme for today, is a little difficult for me because we here at NOVO do not know much about restoring; but as we do know something about enzymes I shall stick to this subject and try to emphasize factors, that I would expect of importance when used in restoring. The little we do know about this subject we have learned from our cooperation with dr. Wendelboe and this deals mainly with the opening up of books glued together by the paper-size (be it either starch or proteine) but also in the separation of papyri from cartonage, enzymes have played a role.

There may be a number of other uses that we here at NOVO do not see at all so the best is now to present you with a little knowledge about enzymes and how they work.

First, enzymes are proteines.

Second, they are proteines with catalytic activity which means that they, by their mere presence in a reaction mixture, speed up the chemical reaction.

Third, this catalytic activity is exercised with very great specificity which is extremely contrary to the inorganic catalysts. As an example it can be mentioned that hydrochloric acid will break down both protein and starch, whereas an enzyme that will break down starch, a so called amylase, will not break down the protein and vise versa. The specificity is even

greater than that, as an alpha-amylase that will break down the alpha 1-4 linkage between the glucose molekules in the starch molekule, see the attached figure 1, will not touch the beta 1-4 link between the glucose molekules in cellulose.

Fourth, the enzymes exercise their catalytic activity under very mild reaction conditions. If we again use the example of the breakdown of starch with hydrochloric acid this is possible only at pH of around 1.5 and a temperature of 140°C for a time period of some minutes, but the alpha-amylase will do the same job at a pH of 6-7 and a temperature of around 80°C also in minutes. The same is the case for the protein breakdown.

Working with enzymes there are certain factors that are of importance and that should be considered during the use.

The first of these is the influence of the temperature. As in any other chemical reaction the reaction velocity increases when the temperature is raised. This is the same as saying that the enzyme activity increases with increasing temperature, but when increasing the temperature the heat stability of the enzyme decreases. The overall result is a curve like the one in figure 2 where the activity of an enzyme is plotted as a function of the temperature and you can see that with raising temperature the activity increases up to a certain point and after that point the heat inactivation of the enzyme - heat denaturation of the enzyme - plays a larger and larger role. A curve like this one is only valid for a certain reaction time. If we namely prolong the reaction time the heat stability of the enzyme plays a greater role.

Fig. 1. The alpha 1-4 linkage in amylose (starch) and pectin are similar.

Fig. 2
Activity of Bacterial Amylase Novo at different temperatures
Concentration of enzyme: 1.75–3.50 Novo units/ml
Substrate: Soluble starch
pH: 5.7
Buffer: Phosphate

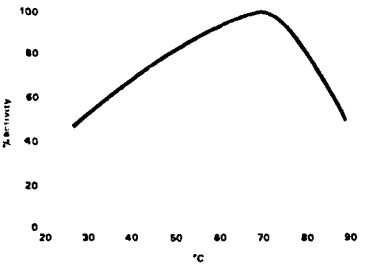


Fig. 3
Stability of Bacterial Amylase Novo at different temperatures
Concentration of enzyme (Bacterial Amylase Novo 240): 0.4 gm/l
pH: 7.0 (tris-maleate buffer)
Stabilizers: 0.5 gm $CaCl_2$+ 6.0 gm NaCl per litre.

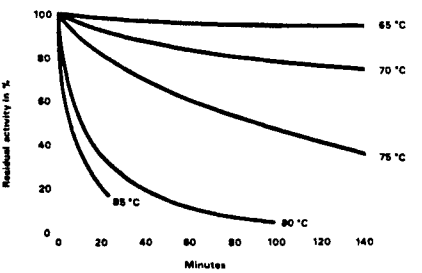


Fig. 4
Activity of Bacterial Amylase Novo at different pH-values
Concentration of enzyme: 1.75–17.5 Novo units/ml
Temperature: 37°C
Substrate: Soluble starch
Buffer: Tris-maleate

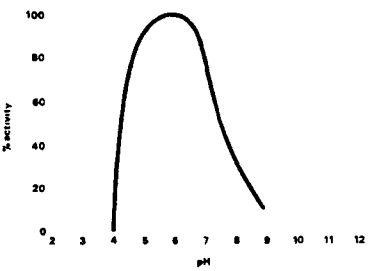
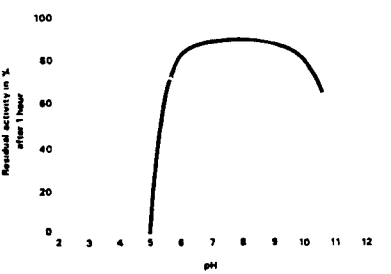


Fig. 5
Stability of Bacterial Amylase Novo at different pH-values
Concentration of enzyme (Bacterial Amylase Novo 240): 0.4 gm/l
Temperature: 70°C
Stabilizers: 0.5 gm $CaCl_2$+ 6.0 gm NaCl per litre.



In figure 3 we can get an impression of how storage at different temperatures influences the residual activity.

The other important factor is the pH. Each individual enzyme has its own optimum pH where the activity of this special enzyme is highest. On both sides of this pH optimum the activity of the enzyme will be lower as it can be seen at figure 4 where we have the pH activity curve for bacterial alpha-amylase. Again this of course also involves the stability at different pH values and the influence of pH on a bacterial amylase stability can be seen from figure 5.

After this brief survey of factors, which are of importance, I suggest that we have a look at some useful enzymes. I have listed here in figure 6 a number of amylases, that is enzymes that break down starch, potato flour, corn starch, dextrins etc. I have in this table also included the optimum working conditions for these enzymes. I have also indicated what knowledge we have on the inactivation conditions as I understood that this is or can be of importance in restoring techniques. In the last column of the figure we have included a brief mentioning of the side activities that you may find in commercial amylase preparations.

And in figure 7 we have a similar table including a number of proteinases. All the enzymes mentioned in these tables are available as technical preparations. As you can see they do contain some side activities but they have the advantage that they are cheap to buy. Small quantities may however be difficult to buy as we supply these enzymes in hundred kilo quantities. However in most cases I would expect that companies producing these enzymes will be willing to supply you with small samples. If more special en-

Fig. 6

Amylases (break down potatoflour, cornstarch, dextrins)						
Name	Opt. working conditions		Inactivation			Side activities
	pH	t °C	pH	t °C +	time min.	
Pancreatic Amylase	6,5-7	40-50		60	few	Proteinase, Lipase?
Bacterial Amylase	6-7	60	3	95-100	5	Proteinase, Glucanase Hemicellulase
Termamyl	6-7	70	4	95	few	"
Fungal-α-amylase	5	40	2	60-70		Proteinase
Amyloglucosidase	4-5	45		80	20	

Fig. 7

Proteinases (break down: gelatine, casein, hemoglobin etc.)						
Name	Opt. working conditions		Inactivation			Side activities
	pH	t °C	pH	t °C + time min.		
Pancreatic Trypsin	8,0	40	12	80	30	
Bacterial Proteinase	6,5	50	3	70	10	Amylase, Glucanase
Alcalase	8-10	50-60	2			
Esperase	8-12	60-70	difficult			
Fungal Proteinase	4,5	40	(2)	60		Amylase
Papain	5-6	40-50		(90)		?

zymes are needed or if very pure preparations are necessary one must often turn to other companies and then one could in this connection mention companies like Sigma, Worthington and Koch Light; but then the prices are quite different. A direct comparison of prices is very difficult because the units that these various enzyme producers use are not easily comparable, but as a guide-line you will find that a technical enzyme preparation often cost in the range of 20 dkr. for an amylase up to 250 dkr. for pancreatic trypsin or 400 dkr. for other special preparations whereas prices of 5-100 dkr. per gram not are unusual for enzymes from the above mentioned companies.