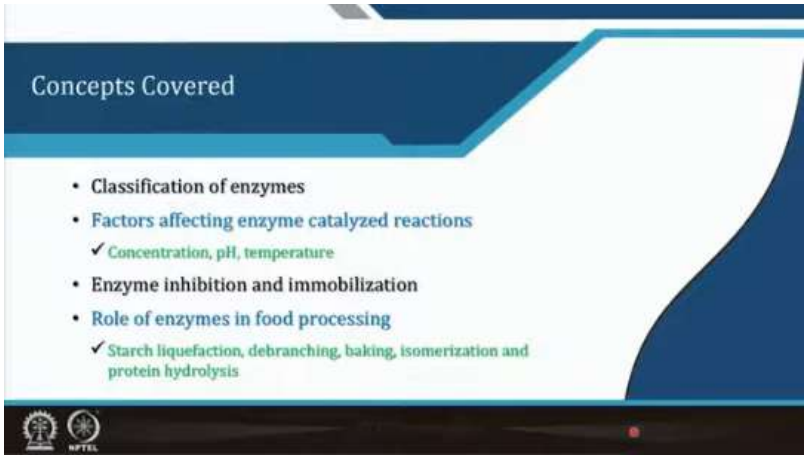
FOOD SCIENCE AND TECHNOLOGY

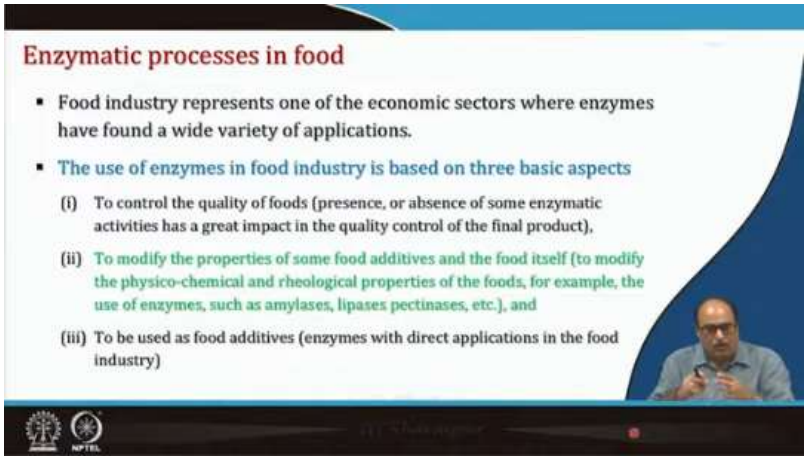
Lecture15

Lecture 15: Enzymatic processes in food

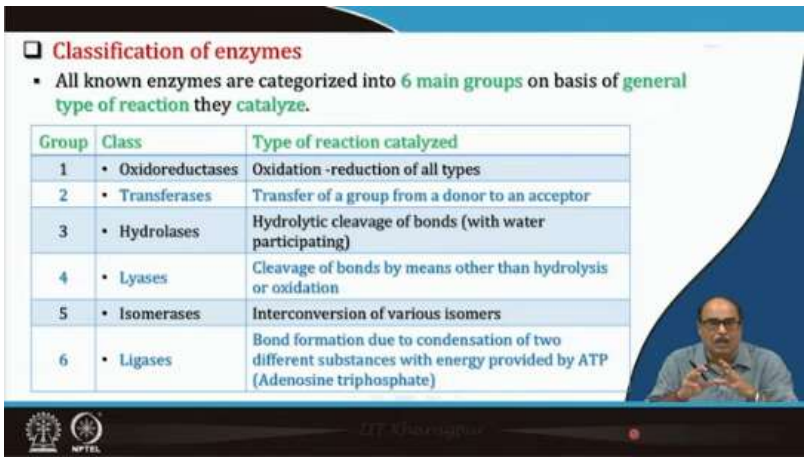
Hello everyone. Namaskar. Now, in this last lecture of module 3 that is lecture 15, we will talk about enzymatic processes in food.



We will discuss: what are enzymes, the classification of enzymes, factors affecting enzyme-catalyzed reactions like substrate concentration, effect of pH, effect of temperature, enzyme inhibition and immobilization. And finally, we will also take up some examples and applications of enzymes in food processing like starch liquefaction, branch debranching, baking, isomerization, protein hydrolysis etcetera.

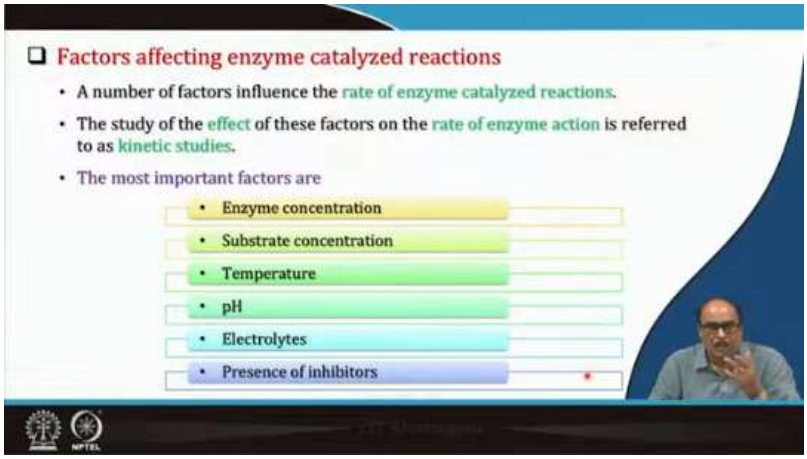


So, you know that food industry represents one of the economic sectors where enzymes have found a wide variety of applications. The use of enzymes in the food industry is based on three major principles: to control the quality of foods, like the presence or absence of some enzymatic activities, has a greater impact on the quality control of the final product. Also, we use the enzyme in industry to modify the properties of some food additives and the food itself, like to modify the physicochemical and rheological properties of food, for example, the use of enzymes like amylases, lipases, pectinases, etcetera. Then finally, the enzymes are used in the food industry as a food additive, many times, as a, they are used as food processing aids, many times, like enzymes with direct application in the food industry. There are many manufacturing processes where enzymes are used to facilitate the production of different types of foods.

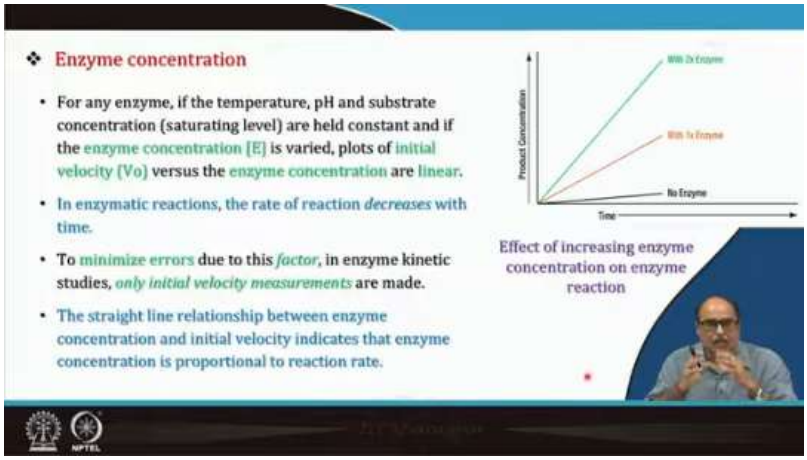


So, the all the enzymes which are available, that is they, on the basis of their activity they can be broadly into 6 main group and those are oxidoreductases, transferases, hydrolyses,

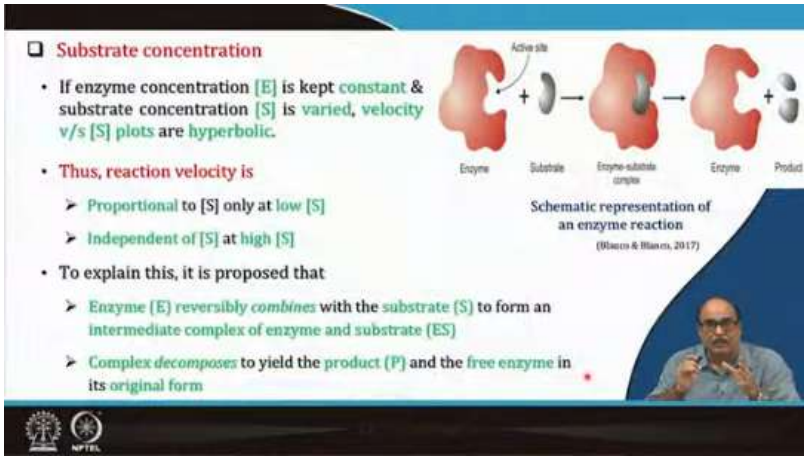
lyases, isomerases, and ligases. Oxidoreductases are those which catalyze the oxidation reduction type of, all types of oxidation reduction reactions. Whereas transferases they transfer a group from a donor to an acceptor. Hydrolases, obviously, they are involved in the hydrolytic cleavage of the bond, where the water participate in that cleavage process. Again they are cleavage of the bond by means other than hydrolysis or oxidation. There is no water involved in the cleavage. That is, it is lyases. Isomerases are involved in interconversion of various isomers and ligases: they result in bond formation due to the condensation of two different substances where energy is provided by ATP, which is adenosine triphosphate.



So, the various factors which influence the rate of enzyme catalyzed reactions include enzyme concentration, substrate concentration, temperature, pH, electrolytes and the presence of certain inhibitors. So, the study of these factors and the effect of these factors on the rate of enzyme action is generally referred to as enzyme kinetics. So, in enzyme kinetics, we consider all these aspects and all these effects.

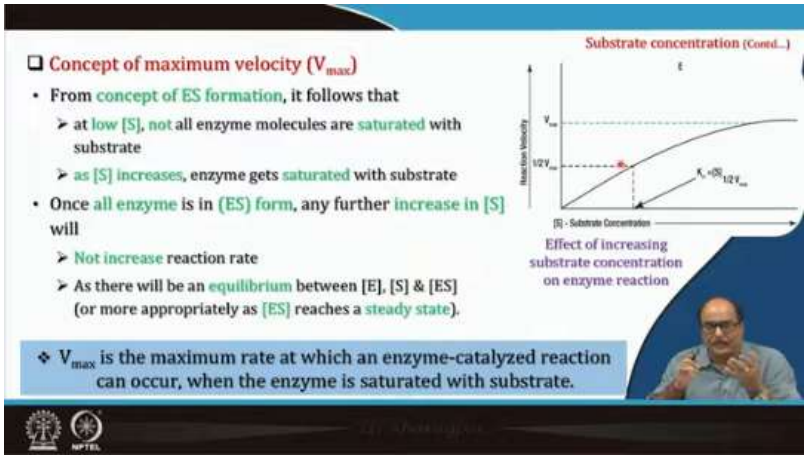


So, let us say, one by one. First, we will take up the effect of enzyme concentration on the enzyme-catalyzed reaction rate, and reaction process. So, for any enzyme if the temperature, pH, and substrate concentration, the substrate concentration at the saturating level and if they are all held constant, and if the enzyme concentration is varied, then you can see that the relationship, that is, the reaction initial velocity versus the enzyme concentration are the linear relationship. In enzymatic reactions, the rate of reaction decreases with time and therefore, to minimize errors due to this factor in enzymatic kinetic study only initial velocity measurements are made. The straight-line relationship between the enzyme concentration and initial velocity indicates that the concentration is proportional to the reaction rate.

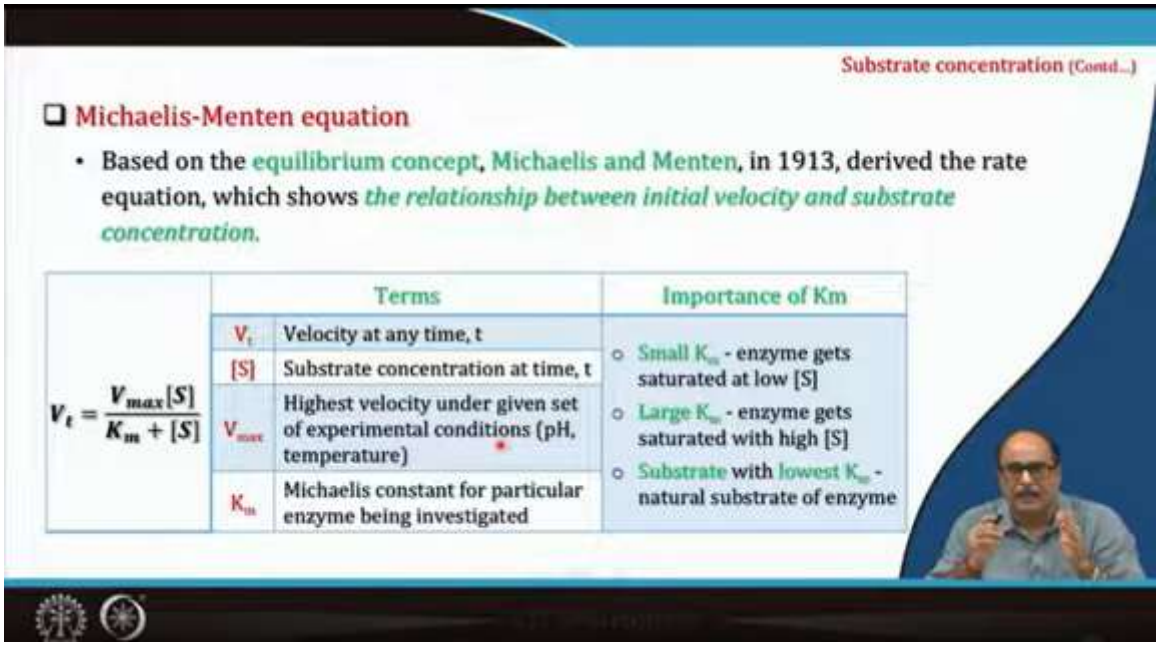


Then substrate concentration, you see, you see that enzymatic enzyme catalyzed reactions, what happens, that is, enzyme, first it forms, reacts with the substrate; then the enzyme substrate complex is formed and then further this enzyme substrate complex is

broken into enzyme and product; and enzyme remains intact in the reaction process. So, if enzyme concentration is kept constant and substrate concentration is varied, velocity v versus substrate concentration plots are hyperbolic. This reaction velocity is proportional to the substrate concentration only at low substrate concentrations, and it is independent of the substrate concentration when the concentration is very high. So, concentration of substrate is very high in the reaction medium. To explain this, it is proposed that enzyme concentration or enzyme reversibly combines with the substrate to form an intermediate complex of the enzyme and substrate that is enzyme-substrate complex, and the complex decomposes to yield the product and free enzyme is obtained in its original form.



So, let us see the concept of maximum velocity, that is Vmax, in the enzyme reaction kinetics. From the concept of enzyme-substrate formation, it follows that at low substrate concentration, not all enzyme molecules are saturated with substrate. And as the substrate concentration increases, enzyme gets saturated with the substrate. And once all the enzymes are present in the enzyme substrate complex form, any further increase in the substrate concentration will not have any influence on the reaction rate and as there will be an equilibrium between enzyme concentration, substrate concentration and the concentration of enzyme substrate complex or more appropriate as enzyme-substrate complex reaches a steady state.

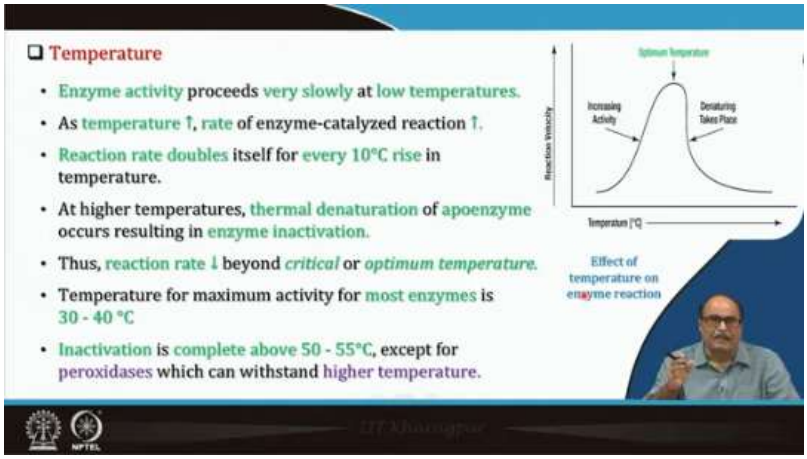


So, once it has reached a saturating level, then a further increase in the concentration may not occur. So, the V_{max}, you can say, is the maximum rate at which enzyme-catalyzed reaction can occur when the enzyme is saturated with the, means you can say here, that is, when there is an always, that is the enzyme and substrate, and also, that is, there is saturated enzyme-substrate complex. What is the maximum reaction rate you can find that is called V_{max}. And from this concept of V_{max}, we go for the Michaelis-Menten equation in the enzyme reaction kinetics, that is, it is based on the equilibrium constant and that is the Michaelis-Menten derived the rate equation which shows the relationship between initial velocity and the substrate concentration like V_t is equal to V_{max} S substrate concentration divided by K_m plus substrate concentration.

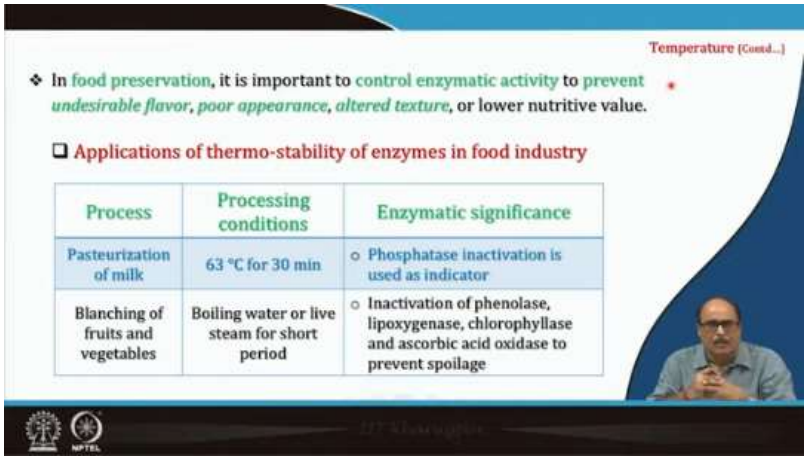
$$V_t = \frac{V_{max}[S]}{K_m + [S]}$$

V_t is the velocity at any time t. S is the concentration of substrate at time t, V_{max} is the highest velocity under a given set of experimental conditions, that is, the pH temperatures, etcetera, where they are optimum, and then Km is the Michaelis constant for the particular enzyme being investigated. So, here it has an importance that is what the importance of K_m in the enzyme reactant kinetics is. That is, if the K_m is small, the enzyme gets saturated at a low substrate concentration. If the K_m is large, the enzyme gets

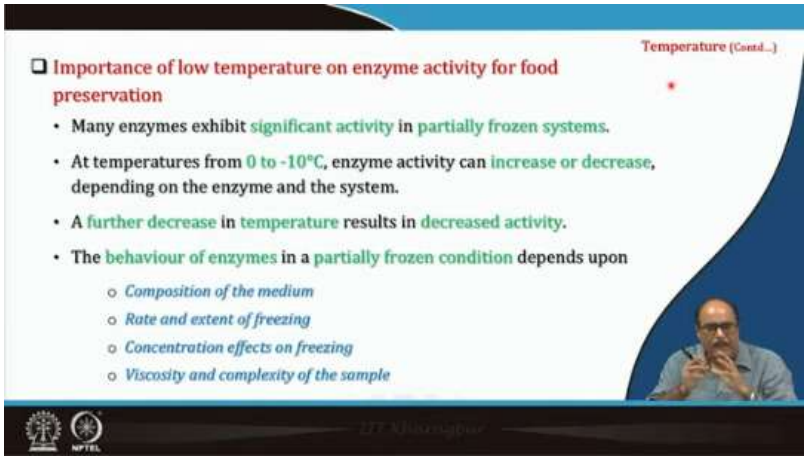
saturated with high substrate concentration. And substrate with lowest K_m, that is the, there is a means, it is the natural substrate enzyme.



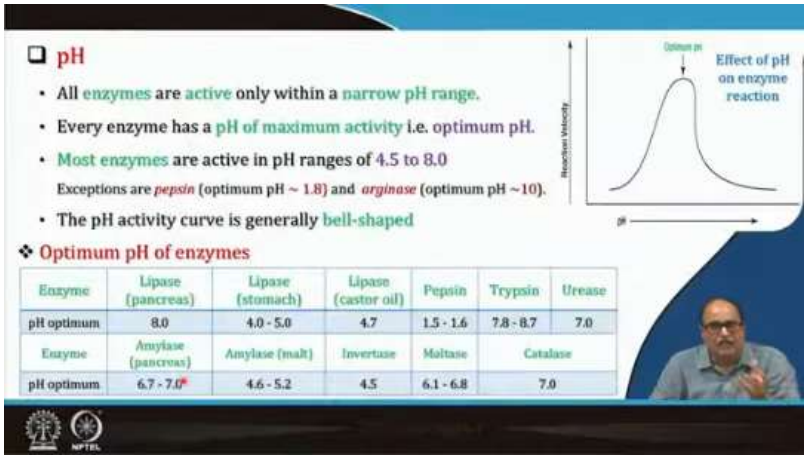
Then let us see the effect of temperature on enzyme reaction kinetics. That is, you can see here, in this figure, that is, the enzyme activity proceeds very slowly at low temperature, and as the temperature increases, the rate of enzyme catalyzed reaction also increases. And it, in general, it has been said, that the reaction rate doubles for every 10 °C rise in temperature, and as the temperature, at higher temperature may be thermal denaturation of proteins may set into and, that is, which may result into the enzyme inactivation and the enzyme-catalyzed reaction rate decreases, that is, the rate will decrease beyond the critical temperature. So, therefore, enzymes; normally. they have an optimum temperature for their maximum activity, even lower temperature side or if you go on higher temperature side, there is a less, that is, enzyme reaction will reduce, rate of the enzyme reaction will reduce, and where it is the optimum temperature, the rate will be maximum. So, the temperature of maximum activity for most of the enzymes, that is the optimum temperature for most of the enzymes, is 30 to 40 °C; normally, the 37 °C, is the biological cell temperature or human body temperature. Inactivation, that is, if you further increase the temperature above 40, 45 °C, then inactivation of the denaturation of the protein starts. And which is almost complete at around 50, 55 degrees or 55, 60 °C except which can be sustained a little further high temperature maybe 70, 75 or 80 °C temperature. Otherwise, in most of the cases, the enzyme activity will stop above 55 °C.



So, in the food preservation, it is important to control enzymatic activity to prevent undesirable flavor, poor appearance, altered texture, or even the lower nutritional value due to one or the other reason. So, application of thermostability of enzymes in food industry is very important and there are, we can take the few process, like the pasteurization of milk is done at 63 °C for 30 minutes in the LTLT process and here, that is, the inactivation of the enzyme, that is, phosphatase inactivation is used as an indicator, that is, whether the pasteurization process had been done properly or not, it is sufficient or not, time temperature combination that is indicated by: if phosphatase is inactivated means pasteurization process is ok. Similarly, in the blanching of fruits and vegetables, normally either by boiling water for or by use of live steam for short period, the enzymes are inactivated in the fruits and vegetables so as to discourage the enzymatic browning reactions, etcetera. And here, the efficacy of the blanching process is judged by the inactivation of phenolases, lipoxidases or chlorophyllase and ascorbic acid oxidase, etcetera because these are the, mainly the enzymes which are responsible for the spoilage or oxidation of fruits and vegetables, etcetera.



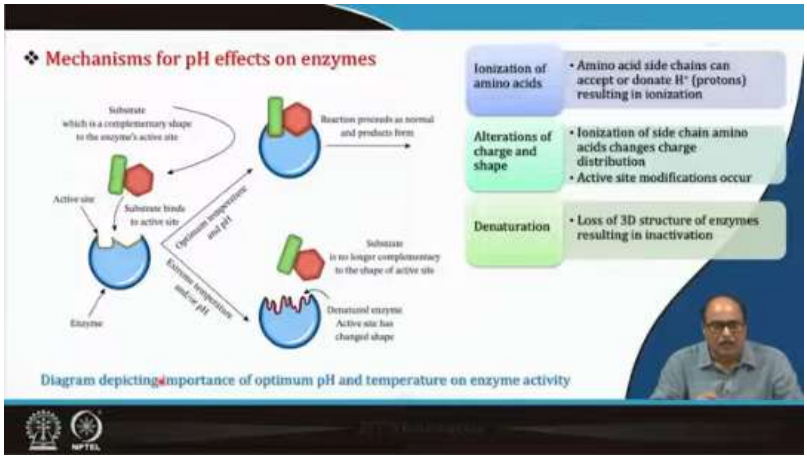
Then let us talk about importance of low temperature on enzyme activity for food preservation. Many enzymes, as I told you earlier also, exhibit significant activity in partially frozen system. At temperatures from 0 to 10 °C, enzyme activity can increase or decrease depending on the enzyme and the system. A further decrease in temperature results in decreased activity and the behavior of enzyme in a partially frozen condition depends mainly on composition of the medium, rate and extent of freezing, concentration effects on freezing and viscosity and complexity of the sample.



So, now let us discuss the effect of pH. Like temperature also, the effect of pH on enzyme activity, that is, it is a bell shaped curve, you can see here, in the figure. So, every enzyme, here also, it has a optimum pH for its maximum activity, and it may be, in general, that is 4.5 to 8, that is, the most of the enzymes, they are active maximally in the range of 4.5. However, there are certain exceptions like pepsin, it maximum activity has

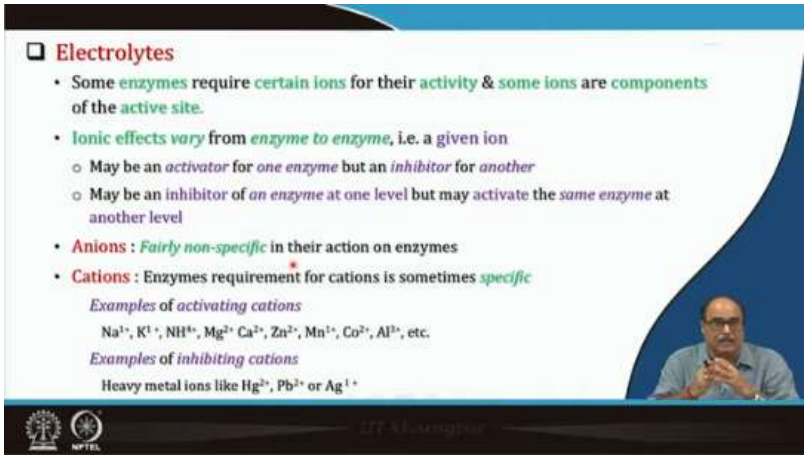
at pH at around 1.8, that is, in highly acidic medium, whereas the arginase, it has maximum activity pH at around 10, it is in alkaline medium.

So, otherwise, you go either side, whether decreasing pH side or increasing pH side, activity of the enzyme will reduce. So, in this table, I have provided the optimum pH of certain enzymes like lipases, that is, the they have maximum activity at 8, pH 8 whereas lipase in the pancreas; where the same lipase, when it is in the stomach, it will have a maximum activity in the range of pH 4 to 5. Lipase in the castor oil has a pH about say 4.7 to 8. Trypsin it has a pH of optimum pH for 7.8 to 8.7. In the malt, it has a optimum pH for 4.6 to 5.2. Amylase in the pancreas has optimum pH of 6.7 to 7.0, maltase has optimum pH of 6.7 to 6.8, catalase about 7 optimum pH.

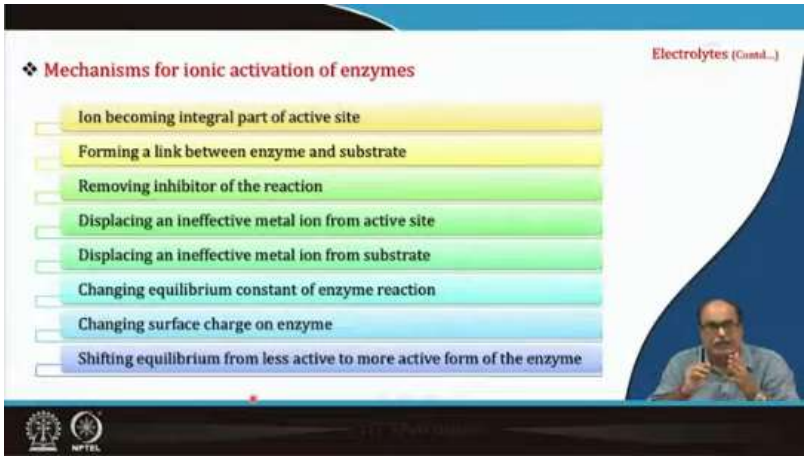


Then you see here mechanism for pH effects on the enzymes, like you can say, that is, here in general, enzyme reactions, that is pictorial, in the diagram, you can see, every enzyme has certain active sites, alright. And then the substrate, that is, it has certain functional group on this active site and its binds in the enzyme. So, when there is optimum temperature, optimum pH, etcetera, then there is a functionality of the, at the active site of the enzyme as well as substrate, their interaction, their bonding, etcetera is at a maximum rate. And this reaction proceeds as normal, and it products, etcetera, are formed, alright. And it is found like ionization of amino acid, there is amino acid side chains can accept or donate protons resulting in ionization. There may be alteration of the charge and shape; there is ionization of side chain amino acid changes large distribution; active side modification, etcetera, suitably, there is depending upon the functional group.

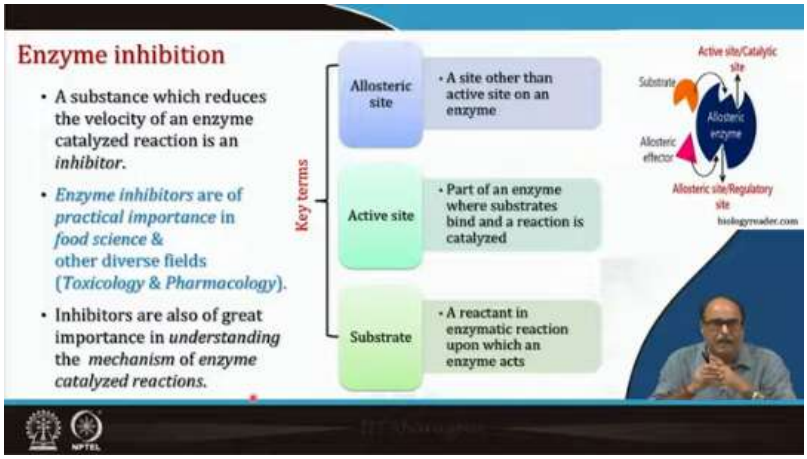
And also sometimes, that is, there may be denaturation, loss of 3D structure of enzyme, resulting in inactivation, particularly, where the extreme temperature and pH conditions, etcetera are there. Then denatured enzyme active size has changed its shape, etcetera, as you can see here. So, it is not able to combine with that. So, these are the, either ionization of amino acids, alterations of the charge and shape, or denaturation, etcetera. These are the causes which may increase the rate of the enzyme activity at a maximum rate, or its rate may be reduced, or there will be no reaction.



Then effects of electrolytes, you see, some enzyme require certain ions for their activity, and some ions are components of active sites of enzyme, as I told you earlier, that is, every active site, it has some functional groups, ions, etcetera. So, these ionic effects may vary from enzyme to enzyme; that is, a given ion may be an activator for one enzyme but an inhibitor for another enzyme, an inhibitor of an enzyme at one level, but it may activate the same enzyme at another level. So, generally anions are fairly nonspecific in their action on enzymes, whereas cations, that is, the requirement is sometimes very specific. So, examples of activating cations are sodium, potassium, ammonium or magnesium, this is something magnesium ions, calcium ions or zinc, manganese, cobalt, aluminium and all those things. Examples of inhibiting cations include heavy metal ions like silver, lead and mercury and so on.

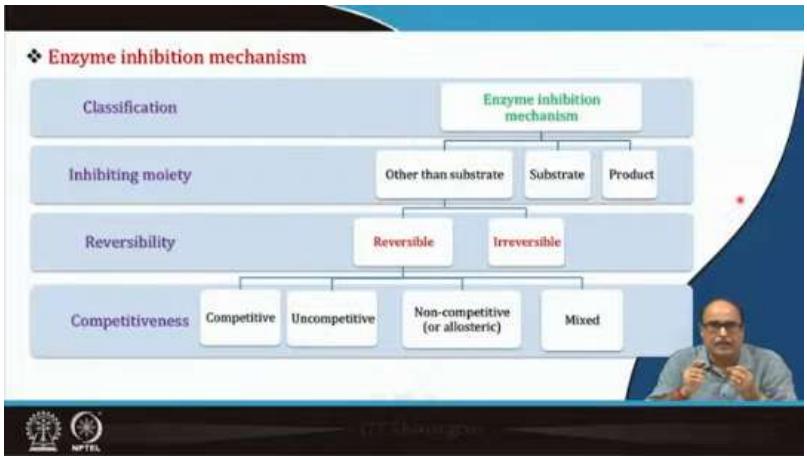


Then, if you see that mechanism for ionic activation, how they activate, that is, ions activate the enzyme or deactivate enzyme, that is, either they become integral part of the active site, they form a link between enzyme and the substrate. These ions remove inhibitor of the reaction or displace an ineffective metal ion from the active site. They may displace an effective metal from the substrate, or they may change equilibrium constant for enzyme reaction. They may change the surface charge of the enzyme, or they may shift equilibrium from less active to more active sides of the enzyme. And these are the various ways, how these ions may activate the enzyme.



So, after having studied different enzyme kinetics and enzyme reaction kinetics, now let us talk about enzyme inhibitions. Then, inhibition, from the name itself, you can find out a substrate which reduces the velocity of an enzyme-catalyzed reaction is an inhibitor. Enzyme inhibitors are of practical importance in food science and another diverse field, like toxicology, pharmacology, etcetera. inhibitors are also of great importance in

understanding the mechanism of enzyme-catalyzed reactions. So, the key term here is the allosteric effect, like a site other than the active site of an enzyme. The active site means that is the part of the enzyme where the substrate binds a reaction and a reaction is catalyzed, and the substrate is, means a reactant in enzyme reaction upon where the enzyme reacts; that, you can see here, allosteric enzyme substrate allosteric effect.

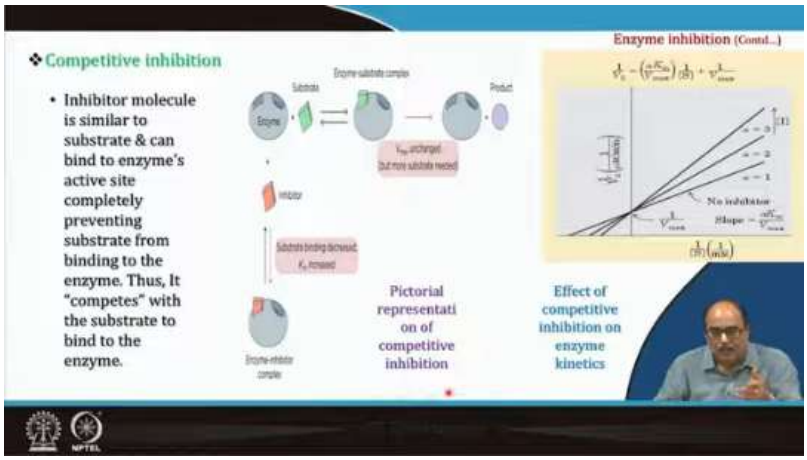


So, the mechanism of the enzyme inhibition, you can say, it may be, that, either by substrate inhibiting moiety may be substrate, or like similar product or product or other substances. Enzyme inhibition may be either two types, either reversible or irreversible. This reversible inhibition is further, competitive inhibition, non-competitive, or uncompetitive, or mixed type of reactions, and so on.

So, let us see reversible inhibition, which means that the reaction, that is reversible inhibition, is one in which the enzyme or enzyme reacts with the inhibitors or inhibitor

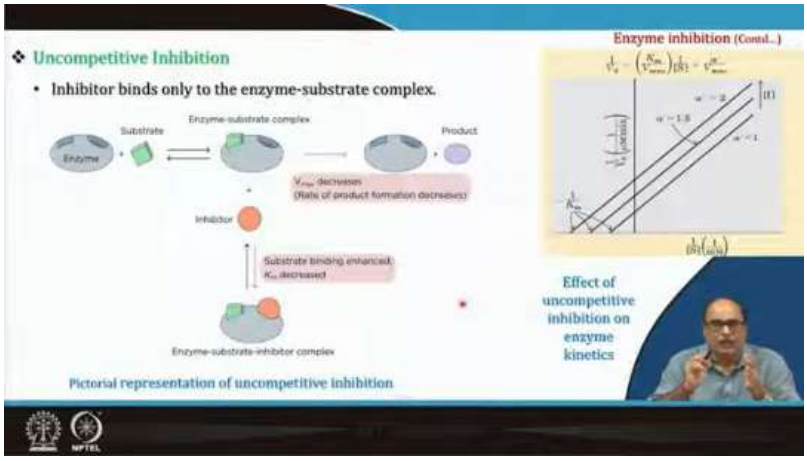
reacts with the enzyme in a reversible manner; here, we are talking about reversible inhibition. So, you can see E plus I is equal to E I, but if the factor is removed, then the E and I, again, may get separated. So, the enzyme activity is restored when the inhibitor is removed. So, these reversible inhibitions are of two types: competitive inhibition and non-competitive inhibition. Like a competitive inhibitor, competes with the substrate for the active site of the enzyme; there is a competition, and this means that the inhibitor has a structure similar to that of the substrate.

So, in the presence of an excess substrate concentration, the competitive inhibitor can be eliminated because the substrate dominates the inhibitor, and the degree of inhibition depends upon the relative concentration of the inhibitor and the substrate. What is the difference? It generally affects the Km, apparent Km. Non-competitive inhibition, that is, these interact with the enzyme in a number of ways. For example, heavy metals and heavy metal ions react reversibly with the enzyme's thiol group and chelating agents, which combine with the essential metal ions of the enzyme. And the inhibitory effect cannot be reversed by increasing the substrate concentration. The degree of inhibition is affected by inhibitor concentration and it largely affects the V max.

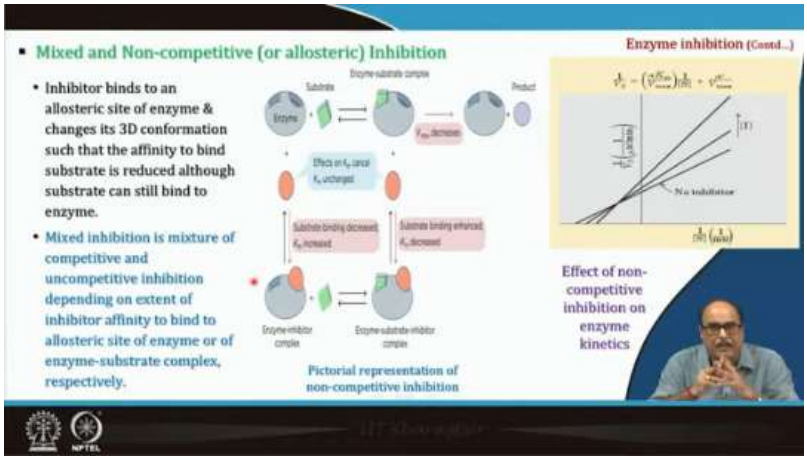


So, here you can say again, the competitive inhibition, it has been shown even the pictorially also, that is, inhibitor molecule is similar to the substrate and it can bind the enzyme's active site completely and therefore, it prevents the substrate from binding to it and thus it competes with the substrate to bind the enzyme reactions, here, you can see that. The inhibitor molecule, again, that is, typically it is shown, that is, similar to the

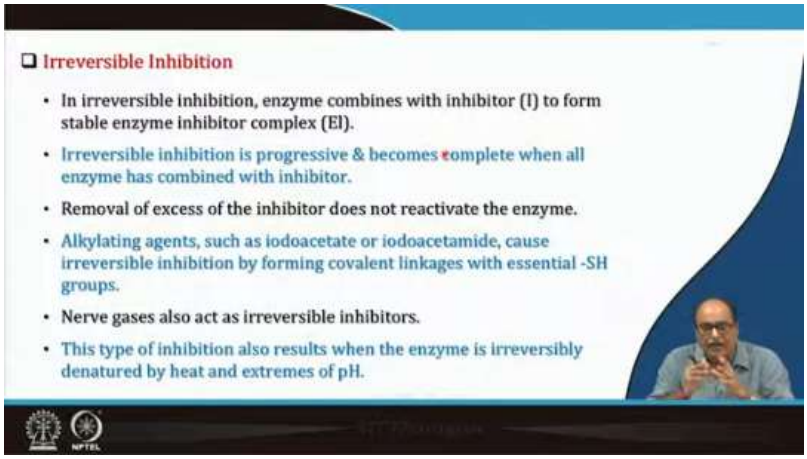
substrate, and can bind the enzyme's active site completely preventing the substrate from binding it. That, it competes with the substrate to bind the enzyme. And as example, you can take here, which is shown in this slide. It is the malonic acid. Malonic acid is competitive inhibitor of enzyme succinic dehydrogenase which converts succinic acid to fumaric acid because both succinic acid and malonic acids have two carbonyl group each. which are separated by an equal distance. And you can see here that, it is enzymes actually succinate enzyme complex and fumarate and enzyme is formed all right. And then the enzyme, when the malonate is there, almost malonate and succinate, these are the structurally almost similar, then this malonate, it will form the, and it will not allow the succinate to react and therefore, the product will not form. So, that is the basic concept in the competitive enzyme inhibitor.



So, uncompetitive inhibition means the inhibitor binds only to the enzyme-substrate complex. You can see here that enzyme and substrate, enzyme-substrate complex, and the Vmax decrease, the rate of product formation also decreases, and the inhibitor is there. So, you see inhibitor, it binds with the enzyme substrate complex. K_m is therefore, the K_m is decreased here, and the same effect is shown here by this.

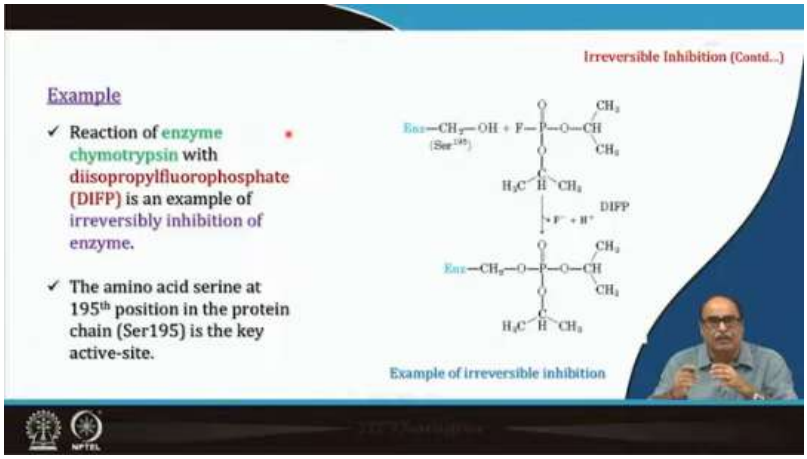


So, mixed or non-competitive or allosteric inhibition is that, here, as you can see, in this picture, the inhibitor binds to an allosteric site of enzyme, and changes its three dimensional conformation, such that the affinity to bind substrate is reduced, although substrate can still bind the enzyme. Mixed inhibition is a mixture of competitive and uncompetitive inhibition depending on the extent of inhibitor affinity to bind to allosteric sites of enzyme or of the enzyme substrate complex, respectively.

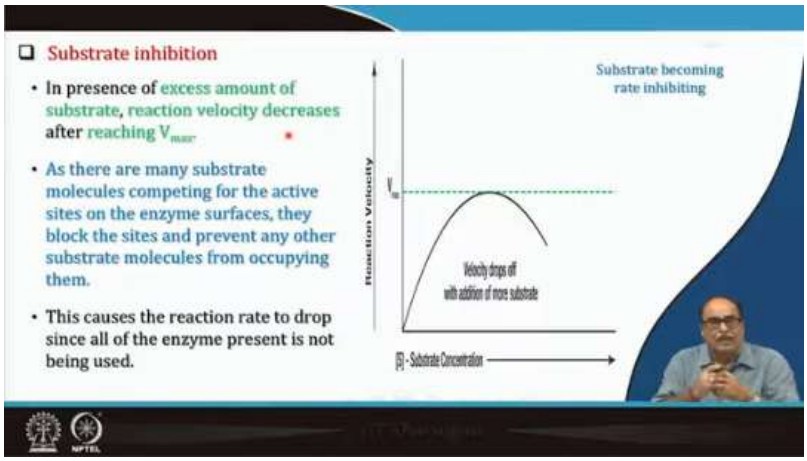


Then irreversible inhibition means, here, the enzyme can combine with the inhibitor to form a stable enzyme-inhibitor complex, that is, EI, in an irreversible manner. Irreversible inhibition is progressive and becomes complete when all the enzymes have combined with the inhibitor. Removal of excess of inhibitor does not reactivate the enzyme. Alkaline agents such as iodoacetate or iodoacetamide causes irreversible inhibition by forming covalent linkages with the essential SH groups. Nerve gases also

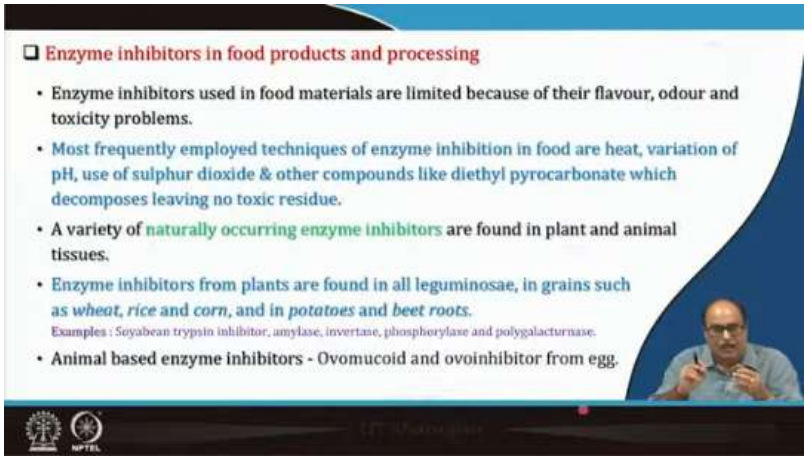
act as irreversible inhibitors of enzymes. And this type of inhibition also results when the enzyme is irreversibly denatured by heat or extremes of pH, etc.



Examples of irreversible inhibitions you can see here that the reaction of the enzyme chymotrypsin with diisopropyl fluorophosphate is an example of irreversible inhibition of the enzyme. The amino acid serine at 195th position is in the protein chain, like serine 195 is the key active site, and which get, inhibited irreversibly.

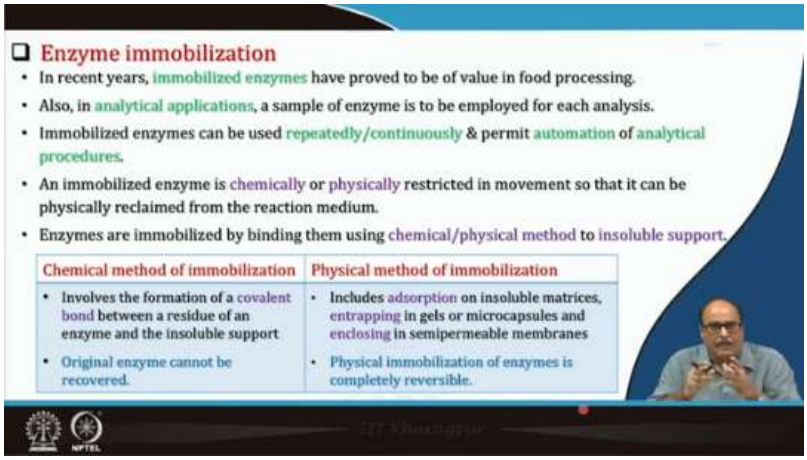


Then substrate inhibition: you can see here, in the figure, in the presence of excess amount of substrate reaction, velocity decreases after reaching the Vmax. As there are many substrate molecules competing for the active site of the enzyme surfaces, they block the site and prevent any other substrate molecules from occupying them.

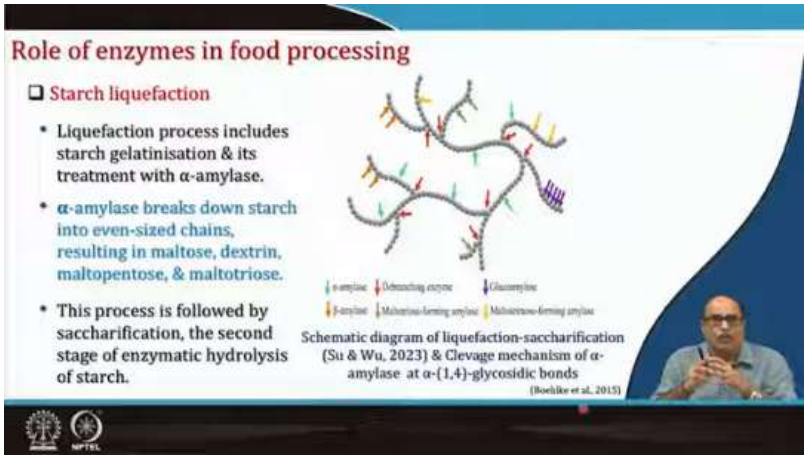


Then enzyme's inhibitors are of important significance in the food products or their processing, like enzyme inhibitors used in the food materials are limited because of their flavour, odour and toxicity problems. Most frequent employed techniques of enzyme inhibition in the foods are heat, variables of pH, use of sulphur dioxide, and other compounds like diethyl polycarbonate, which decomposes leaving no toxic residue. A variety of naturally occurring enzyme's inhibitors are found in plants and animals. Enzymes, that is, enzyme inhibitors from plant are found in almost all legumes, in grains such as wheat, rice, corn, in potatoes, beet roots, etcetera. Like soybean trypsin inhibitor is the one enzyme which has been highly understood, studied, etcetera. And, that is why, it is has to be, soybeans are normally require heat treatment before its consumption.

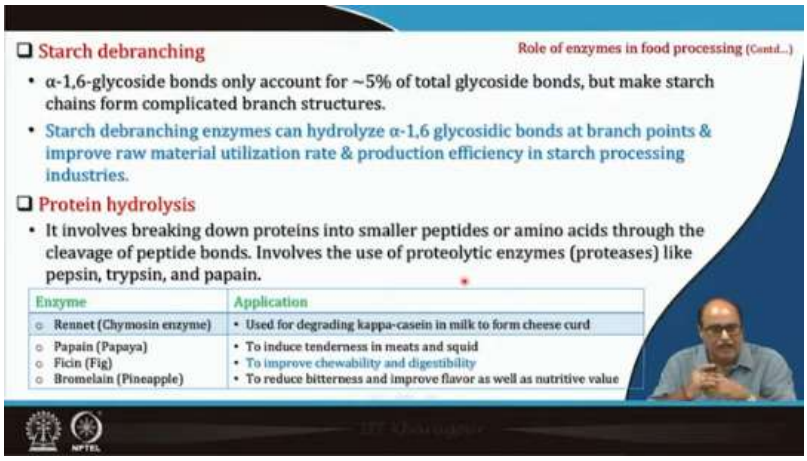
Otherwise, this trypsin inhibitor will bind the trypsin, which is a proteolytic enzyme in our system, and then, it will adversely affect the protein digestion. Similarly, amylase, invertase, phosphorylase, polygalacturonase, etcetera, are very many such types of inhibitors. So, animal-based enzyme inhibitors are ovomucoid and ovoinhibitors from eggs, etcetera. So, again, that is, there are many more of this. So, but many of these enzymes they are heat sensitive either by heating or by, like in the egg, this ovomucoid or ovoinhibitor. So, if you half fry the egg or half boil or heat it, then it gets inactivated.



Then let us talk about enzyme immobilization. In the recent years, immobilized enzymes have proved to be of great value in food processing. Also in the analytical application, a sample of enzyme is to be employed for each analysis. So, immobilized enzymes can be used repeatedly or continuously and permit automation of the analytical procedures. And immobilized enzyme is chemically or physically restricted in movement, so that it can be physically reclaimed from the reaction medium. Enzymes are immobilized by binding them using chemical or physical methods or to insoluble support. Like chemical and physical. Chemical method of immobilization involves the formation of a covalent bond between a residue of an enzyme and the insoluble support. And, original enzyme, however, in this, cannot be recovered. Physical method of immobilization includes adsorption on insoluble matrices, entrapping in gel or microcapsules, and enclosing it in semipermeable membranes. So, the physical immobilization of enzyme is completely reversible.

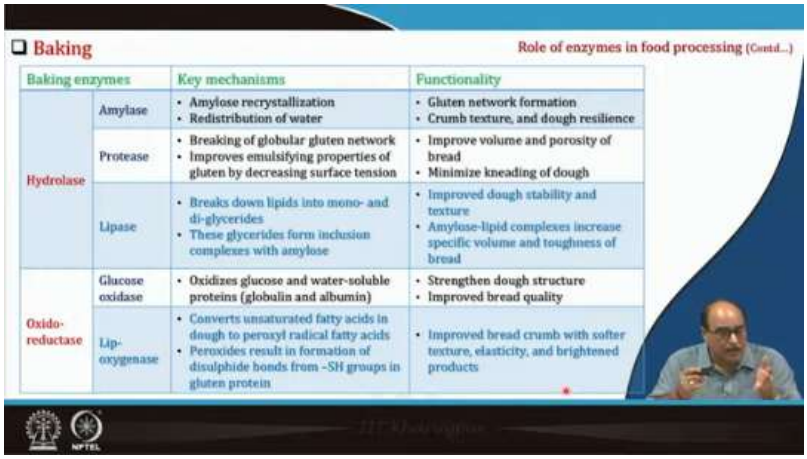


Now, we will talk about roles of enzymes in food processing industry. As I told you earlier, that is there are very widespread uses of food processing in the food manufacture as they are, enzymes are processing aid and all those things. So, we will take one or two example like you can say starch liquefaction. The liquefaction process includes starch gelatinization and its treatment with amylase, particularly alpha-amylase. So, alpha-amylase breakdown, that you can see here, that is alpha-amylase, debranching enzyme, glucoamylase, different enzymes are shown with different colours, and this is in the starch liquefaction and saccharification processes. So, the alpha-amylase breaks down the starch into even-sized chains resulting in maltose, dextrins, maltopentose, and maltotriose. And this process is followed by saccharification, that is the second stage of enzyme hydrolysis of the starch. And these different products which are formed like this maltose, dextrin, etcetera, they are formed; they have widespread applications in the food processing, that is, different ones which are broken down, here, you can see.



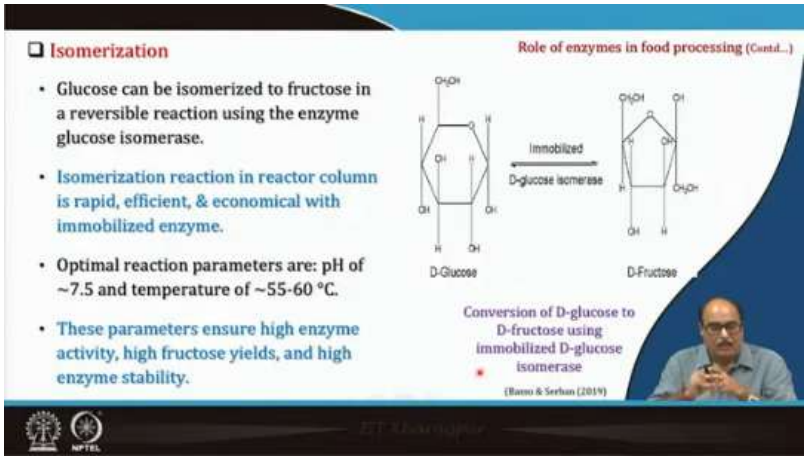
Then, starch debranching, you have seen in the debranching enzyme also in the earlier figure, that alpha 1 6 glycosidic linkages bonds account for about 5 per cent of the total glycosidic bond, but makes starch chain form complicated branch structures. So, starch debranching enzymes can hydrolyze alpha 1 6 glycosidic bonds at the branch point and improve the raw material utilization and production efficiency in such processes. Similarly, protein hydrolysis involves the breaking down of protein into smaller peptides. Earlier also, we discussed like rennet, papain, ficin, bromelain, etcetera, bromelain from pineapple, alright.

And so, they are all used, like rennet is used for degrading kappa casein in milk to form cheese curd. The bromelain is used to reduce the bitterness and improve flavor as well as nutritional value. Ficin in the fig improves chewability and digestibility. So, there are various functional applications of enzymes in food process.

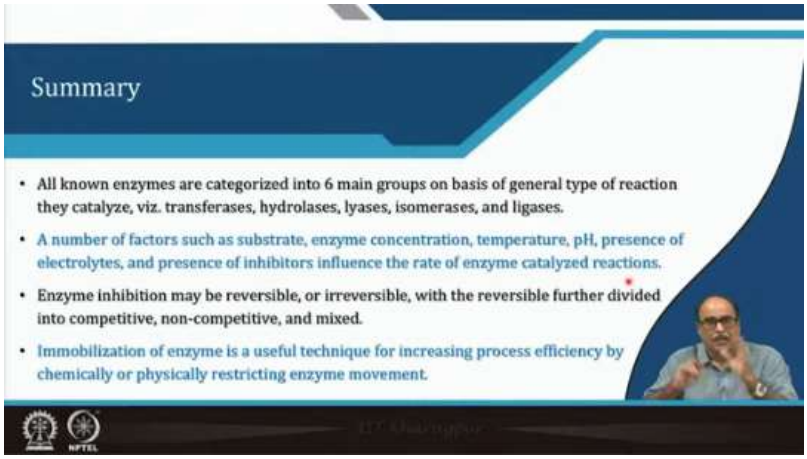


In the baking, there is many enzymes are used. There are many a times, they are used as a processing aid, and in baking, the enzyme improves the characteristics, digestibility, and other functionalities of the baked product. Like amylases, that is, they are the key mechanism with the amylose recrystallization or redistribution of water, and they have the functionality of the gluten network formation. They improve the gluten network formation and crumb crust structure, crumb texture, dough resilience properties, etcetera are improved by these amyloses. Similarly, proteases break the gluten network and improve the emulsifying properties of gluten by decreasing surface tension. And therefore, they improve the volume and porosity of the bread and minimize kneading of the dough.

Similarly, lipases are also; they have very important; they improve the dough's stability and texture. Amyloise lipid complexes increase the specific volume and toughness of the bread. Then oxidoreductases enzymes like glucose oxidase strengthen the dough structure, improve the bread quality. Lipoxygenases improve bread crumb with softer texture, elasticity and brightness of the product. This lipoxygenase converts unsaturated fatty acids in the dough to peroxide radical and free fatty acids etcetera and by which it improves the bread crumb.



Then isomerization reaction facilitated by the enzymes like glucose can be isomerized to fructose in reversible reaction using the enzyme glucose isomerase. You can see here D glucose is converted into D fructose. So isomerization reaction in reactor column is rapid, efficient, and economical with immobilized enzymes. Optimum reaction parameter is pH around 7.5 and temperature around 55 to 60 °C. And these parameters ensure high enzyme activity, high fructose yields and high enzyme stability.



Then finally, now I will like to summarize these lectures. Yes, enzymes are very important in food processing, in this, in biochemistry, in digestion of the food, and other physiological processes. Most of the enzymes are protein in nature. They are very specific in their action. They are, on the basis of their action, they are categorized into 6 major classes. And the enzyme kinetics, that is, the rate of the enzyme catalyzed reactions depends on the substrate concentration, enzyme concentration, temperature, pH, whether

there is any presence of electrolytes or presence of inhibitors, etcetera. Enzyme inhibition, also, they are of great nutritional significance.

Many the agricultural materials, plant materials, animal-based materials, have naturally present that biologically active agents, which inhibit, many times, the activity of several enzymes. So, we need to understand the roles of a mechanism of how these inhibitors act, like in a competitive manner or in competitive manner, and then accordingly, proper care has to be taken. And then, these enzymes, because they are the catalyst, normally catalyze the reactions; they do not take part in the reaction process.

Therefore, this immobilization is the method by which, after the reaction is over, then these either physically they are retrieved, reclaimed from the reaction medium or chemically combined with some other component. So that they can be reused in the process, their efficiency is increased, and the number of times they can used in the food is called immobilization.

So, with this, these are the references that are used.

Thank you very much for your patience.