Enzyme Science and Technology
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Module - XI
Enzyme Applications (Part-I)
Lecture - 44
Application of Enzyme (Part-I Food Industry)

Hello everyone, this is Doctor Vishal Trivedi from Department of Biosciences and Bioengineering, IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course Enzyme Science and Technology.

And so far, we have discussed diversified topics related to this particular subject. And now, we come to the place where we should understand the importance of these enzyme in you know in the applications or in other kinds of utilization of these enzymes. Because ultimately, what we are doing is, we are actually producing these enzymes for you know for facilitating the process, right.

So, as you know that the enzymes are actually being required or actually being utilized for you know for catalyzing a particular type of reactions, right. In our under normal circumstances, when you do not have the enzyme, the reaction mechanisms or the reaction kinetics is very slow. So, to expedite and to make them more product, you have to use these enzymes.

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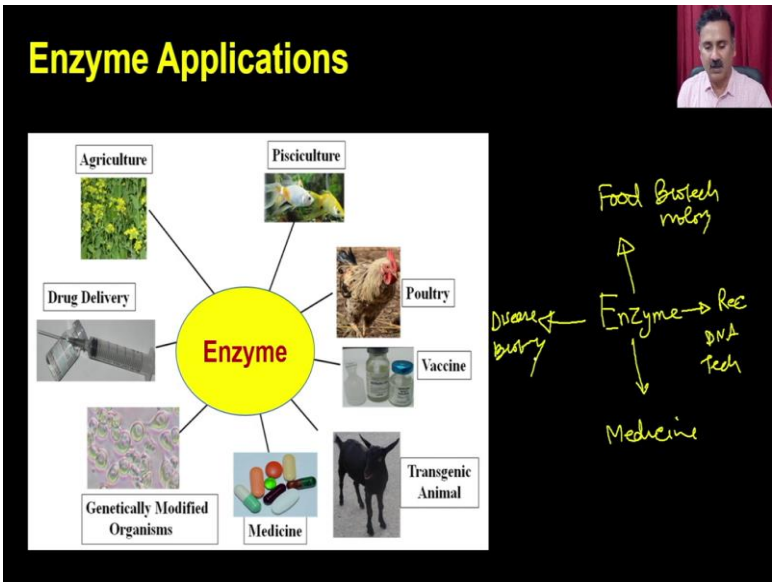

Now, if you see that the enzymes are actually having the applications in different fields. And what we are going to discuss in this particular course is very limited application for these enzymes. So, as you can see that enzymes are actually having a role in the agriculture feed, piscicultures, poultry, the enzymes are also being utilized in some or other way to produce the vaccines.

Then, it will also be utilized for many types of genetic recombinations and other kinds of genetic engineering related applications. And that is actually being resulted into producing genetically modified organisms or the transgenic animals. And then also, other hand, the enzymes are very big source of medicines.

Either the medicines are being the inhibitor for inhibiting a particular enzyme or the enzyme itself could be a you know therapeutic molecules for catalyzing some reactions. So, in this particular course, what we are going to do is, we are actually going to discuss some of these aspects.

So, what we have done is that we have categorized these applications into the some of the related fields, right. So, what we have is, we have the enzymes application of the enzyme in the phase of food biotechnology, application of enzyme in the medicine, application of enzyme in the recombinant DNA technology. And then, we are also going to discuss about the application of enzyme in the other kinds of applications such as the disease biology.

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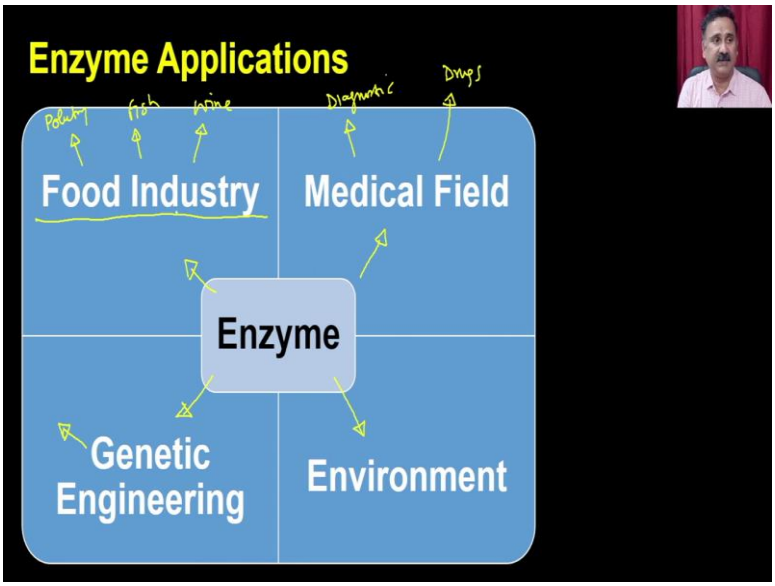

So, these are the things what we are going to discuss, right. We are going to discuss about the application of enzyme in the food industry. Food industry is also very big. So, for example, you can actually be able to discuss about how the within the food industry you can have the poultry, right and you can have the fish, you can have the wine and other kinds of products, right.

Then, within the medical field, you can have the utilization of enzyme for diagnostics. And you can also have the application of the enzyme in the case of the development of drugs. Then, similarly for the enzyme, we are also going to discuss about the application of enzyme in the case of environments. And then, lastly, we are also going to discuss about the application of enzyme in the field of genetic engineering.

So, this anyway we have discussed in detail about how you can be able to perform the genetic engineering and how you can be able to utilize the different types of enzyme. But even then, we are actually going to summarize you what are the things you have discussed so far. So, now let us start discussing about the application of enzyme in the food industry.

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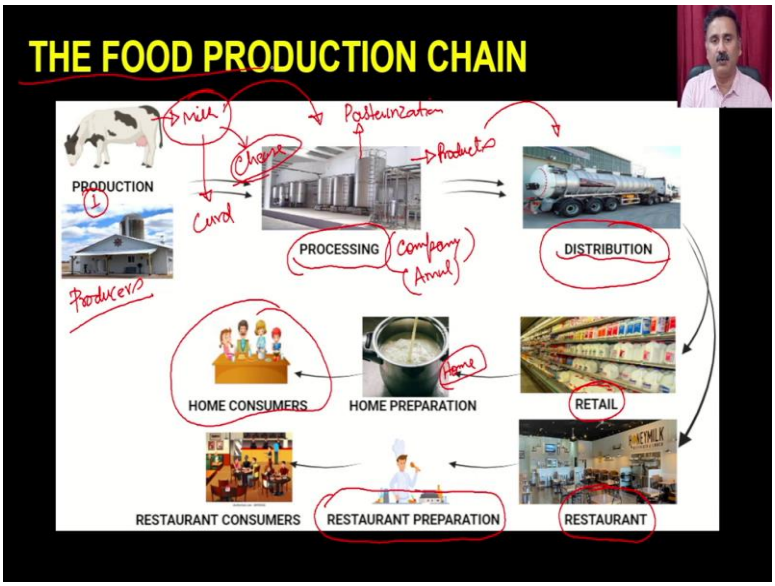

When we talk about the food, the food industry is a very very well developed and you know well developed industry where you have the multiple types of products, right. You can have the dairy products, you can have the meat, you can have the poultries, you can have the other kinds of products like the wine and other kinds of fruit juices and other things, right.

So, the in a general production of the food production chain is that you are actually going to have the producers, right. So, even within this you can also have the agriculture. So, you first you are going to have the producers. Then, the producer could be the cow, which is actually going to give you the milk or you can actually be able to have the other kinds of producers like the egg, and the within the poultry you can have the egg, and meat, and other kind of thing.

So, it will start from the producers, right. Then, the producer, whatever the produce they are going to generate that is actually going to be in a crude state. So, it is actually going to go into a processing unit. Within this processing unit for example, if we take an example of milk, then it is actually going to go into the pasteurization, right.

And or the milk is actually going to be get converted into a curd, right or milk is actually going to use for production of the cheese so, all that is actually going to occur in this processing unit, ok.

So, in the step 1 you are going to have the producers. In the step 2 you are going to have the processing unit. So, processing unit will be different. If you are trying to develop the cheese then processing unit processing process and other things are going to be different. If you want to distribute the milk and use the milk as such, then you are actually going to do the pasteurizations.

And then, if you want to convert the milk into curd or other kinds of products, then also the processing is going to be different. So, within the processing unit you are actually going to produce the different types of products, right. So, this is actually going to be the raw product, this is going to be the derived product.

Then, these derived products are actually going to get into the distributions, which means depending upon the longevity of these products, shelf life of these product, the distribution is going to happen. Either the distribution could be on room temperature or distribution could be into the cold, right.

Then, from the distribution what is the role of distribution it is actually going to take the things from the processing unit or I will say the company, right. And from the company for example, Amul, right. So, if you are going to go to the Amul processing unit from they will take the milk from all the farmers and then the milk will go into the processing unit. And then, from the processing unit you can have the different types of products like the butter, cheese, curd and all those kind of things.

And then, they will enter into the distribution. And what the distributors, distribution unit is going to do? It is actually going to give you the distribution, it will distribute the material to the either to the shopping malls like the retail shops or it is actually going to give it to the restaurant, right. Both are the places; it is actually going to use.

And from these places it is actually going to come to your home, right. So, it is actually going to come to your home, and then, you are actually going to do the home preparations. For example, if you bring the milk, you can actually be able to produce a curd in your home also and the other kinds of milk product what you can actually be able to use, right.

From the restaurant, within the restaurant it is actually will go to the kitchen of the restaurant and then the chef is actually going to use these products for making the

different types of recipes. For example, when you go to a restaurant, it may give you some type of dishes and then the ultimately it is actually going to go to the final consumer.

So, final consumer in the case of home preparation, which is going to be your family whereas, in the case of restaurant it is going to be the customers of that particular restaurant. So, in a food chain, you have you what you see is it is very very protocol based and it is actually going to be well defined processes what you are supposed to do, then only the final product, the final thing is actually going to reach to the consumer.

And that is why the food processing is a very very very systematic and complicated process. And that has to follow, right. There are certain rules there are certain ISI rules which are has to be follow while you are doing all these processing.

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So, the first is the food processing, ok. So, in the food processing, you are actually going to take the raw material, raw material could be vegetables, it could be milk, it could be meat, it could be anything, right. So, for the raw material, you are actually going to do the food processing and then you are actually going to generate the different types of products.

For example, you can actually be able to make the roti, you can actually be able to make different types of products. So, the food processing combines the raw material in the end

to produce the marketable food product that can be easily prepared and served to the customer, because ultimately you know that the role of the food is role of the industry is that they will actually going to make the product which is serviceable or which is actually going to be good for the customers. Now, what are the different types of enzyme what you are going to use?

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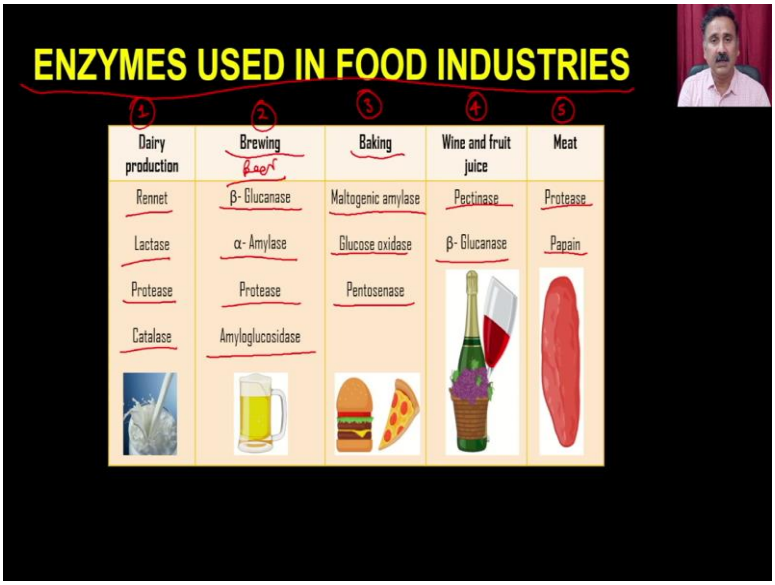

So, you are going to use the different types of enzyme in the food industry. So, you can have the dairy industry, you can have the brewing industry, brewing industry is actually going to produce alcohol, you can have the baking industry, like the industry which is actually going to the produce the cakes and pastries and all that. And then you can also have the wine or the fruit juices, and then you also have the industry for the meat.

So, when it is a dairy production, you are actually going to use the different types of enzyme like the rennet, lactase, protease and catalase. All these enzymes are having their own specific and exclusive roles that anyway we are going to discuss. When we talking about the brewing industry, where you are actually going to produce the beer, it is actually going to be utilized the different types of enzymes like the beta glucanase, alpha amylase, protease and amyloglucosidase.

Then, for the baking industry, it is actually going to use the maltogenic amylase, glucose oxidase and pentosenase. Then, for the wine and the fruit juice industry, you can use the pectinase and beta glucanase. And for the meat, you are actually going to use the

protease and the papain. And all of these enzymes have the specific role at a particular step to facilitate or to make the product better, so that it will be consumed by the customer. So, let us start first with the dairy products.

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So, in the dairy industry, which will start from the milk from the cow and it will end up into the dairy products like butters, cheese and all that, right; so, these are seen what you see here, right, different types of dairy products like the milk, cheese, cakes, ice creams and all that. So, there are 4 enzymes what you are going to use. You are going to use an enzyme which is called rennet, lactase, protease and catalase, and all these enzymes are there, have their own specific functions.

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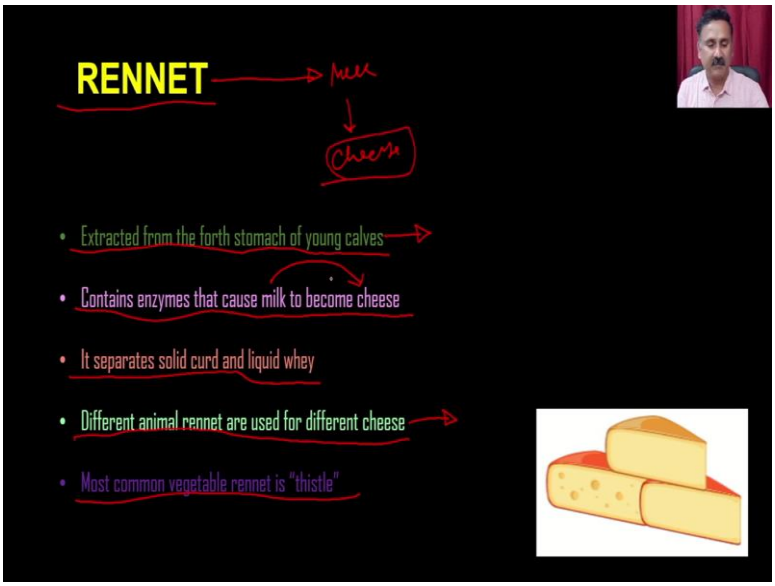

So, the first enzyme is the rennet. So, it is extracted from the forth stomach of the young calves. So, it is actually going to be an enzyme which is present only into the young calves. And it contains the enzyme that caused the milk to become the cheese. So, it actually converts the milk into the cheese.

It separates the solid curd and the liquid whey, right. So, when you treat the milk with the rennet, it is actually going to convert the milk into cheese and in this process, the solid curd and the liquid whey is actually going to be get separated. Different animal rennet's are used for a different types of cheese. So, you know that there are different types of cheese you have mozzarella cheese, you have other kinds of cheese.

So, you can actually be able to use the different animal sources, the rennet from the different sources to produce the different types of cheese. And the most common vegetable rennet is thistle. So, this is actually going to be used for production of the cheese actually. So, rennet is actually going to be used for converting the milk into cheese, ok.

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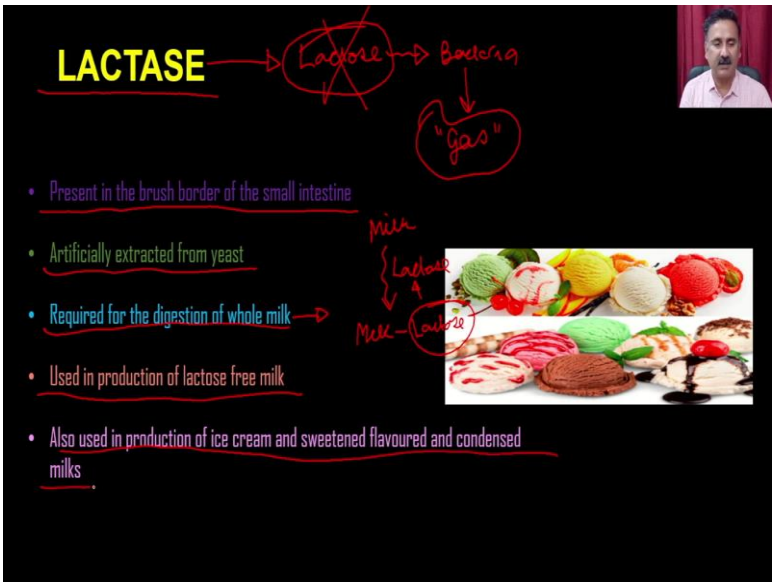

Then, we have the lactase as a name suggest the lactase is actually going to work on the sugar which is called as lactose, ok. And you know when lactose is a problematic sugar for some people who are actually having the lactose tolerance. So, the lactase is present in the brush border of the small intestine of and it is also can artificially been extracted from the yeast.

It is required for the digestion of the whole milk, so it is actually going to reduce the sugar level, right. And it is actually been used for the production of lactose free milk, ok. Because when you treat the milk with the lactase enzyme what it says the going to do is it is actually going to degrade the lactase and that is how the milk is getting converted into lactose free.

Because many of the people have the lactose intolerant because if the lactose is present in the milk and they do not have this particular enzyme, then the lactase is actually going to be utilized by the bacteria, right. And that is how it is actually going to cause the production of gas and that is actually being responsible for the lactose intolerance.

So, for those people the what the companies are doing is they are actually treating the milk, right. So, milk contains the lactose and when they it actually been treated with the enzyme called lactase then the milk is getting converted into milk without lactose because the lactose is actually going to be consumed by the lactase. It is also used in the production of ice cream and the sweetened flavoured and the condensed milk, right.

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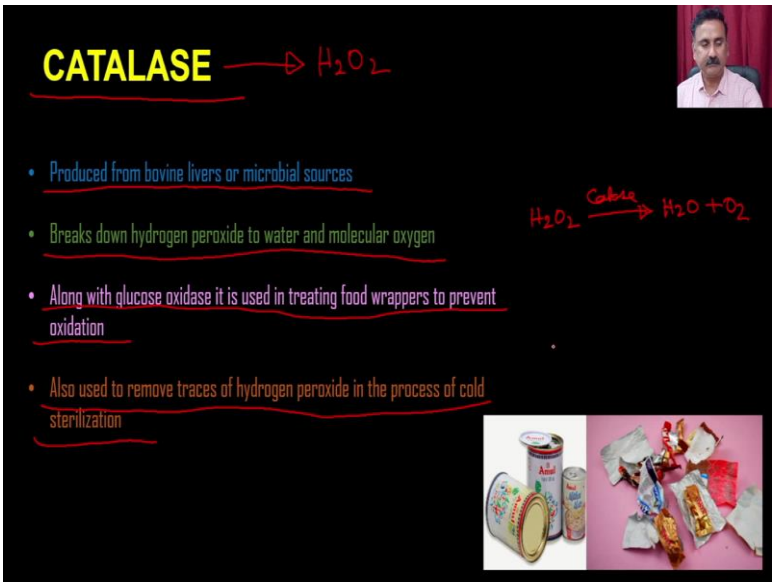

Then, we have the third enzyme which is called as catalase, right. You know that the catalase is an enzyme which is degrading the hydrogen peroxide, right. So, it is produced from the bovine livers or the microbial sources. It breakdown the hydrogen peroxide to water and the molecular oxygen. So, the reaction what the catalase is actually going to catalyze is this hydrogen peroxide, catalase, it going to produce the water plus oxygen, ok.

So, it is actually going to produce this. Along with the glucose oxidase, it is used for treating the food wrappers to prevent the oxidation. So, it is actually going to protect us food to get the bad actually also, used to remove the traces of hydrogen peroxide in the process of cold sterilization so, basically the catalase this being used for preserving the food materials.

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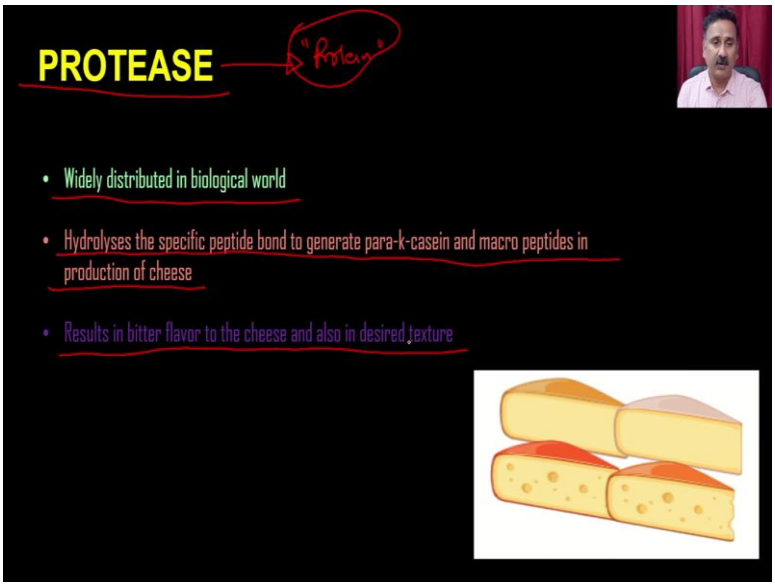

Then, we have the protease. So, protease you know that the role of the protease, it is actually going to require for degradation of the protein, right. So, the enzyme which degrades the protein are called proteases. It is widely distributed into the biological world. Hydrolyses the specific peptide bond to generate the para-k-casein, and macro peptide in the production of cheese and it results in the bitter flavor to the cheese and also in a desired textures. You can have the different types of proteases.

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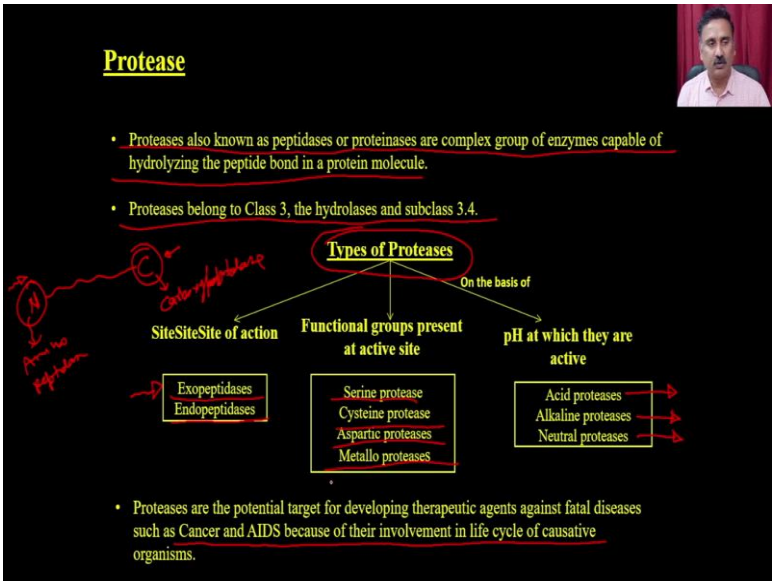

You can have the you know exopeptidases or the endopeptidases. So, proteases are known as the proteinases are complex group of enzyme, capable of hydrolyzing the peptide bond in a protein molecule. Proteases belong to the class 3, the hydrolases and the subclass of 3.4. And you can have the different types of proteases, depending upon the site of actions, functional group which is present at the active site or the pH at which they are active.

So, as per the site of the action, you can have the exopeptidase or the endopeptidase exopeptidase, which are actually going to work on the one end of the protein. So, you can have the carboxy peptidase or the amino peptidase which means if you see the protein, right the protein has two ends, right.

You have the n-terminus end, you have the carboxy end. So, exopeptidase are either going to work from this side or its going to work on this side. If it works on this side, then it is going to be called as amino peptidase, right. And if it is going to work on this side, then it is going to be called as carboxy peptidase, ok.

Similarly, at the functional group, the amino acids what are present at the active site and they will have a crucial role in the catalysis. Accordingly, they can be serene protease, cysteine protease, aspartic protease or the metallic protease. Similarly, the pH at which they are active it would be acid proteases, alkaline proteases or the neutral pH.

So, acidic pH acidic proteases would be active at pH less than 7, there will be active pH that is the more than 7 and these are actually going to work at the neutral pH. Proteases are the potential target for developing the therapeutic agent against the fatal diseases such as the cancer and the aids because of their involvement in the life cycle of the positive organisms.

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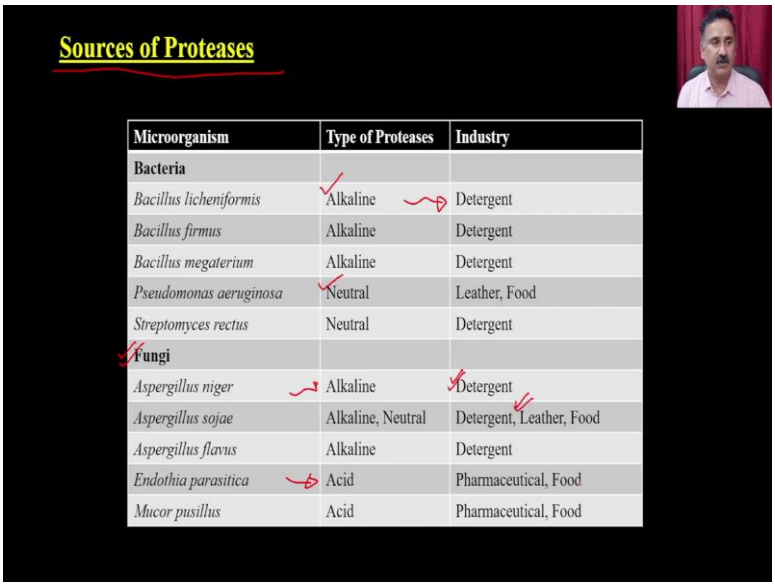

Microorganism	Type of Proteases	Industry
Bacteria		
Bacillus licheniformis	Alkaline	Detergent
Bacillus firmus	Alkaline	Detergent
Bacillus megaterium	Alkaline	Detergent
Pseudomonas aeruginosa	Neutral	Leather, Food
Streptomyces rectus	Neutral	Detergent
Fungi		
Aspergillus niger	Alkaline	Detergent
Aspergillus sojae	Alkaline, Neutral	Detergent, Leather, Food
Aspergillus flavus	Alkaline	Detergent
Endothia parasitica	Acid	Pharmaceutical, Food
Mucor pusillus	Acid	Pharmaceutical, Food

Then, you can have the sources of the proteases. You can have the sources from the different types of bacterias. Mostly the microorganisms are the good source of the proteases. You can have the different types of bacteria like bacillus, licheniformis, bacillus firmus, bacillus, megaterium, pseudomonas, streptomyces rectus and all these are actually going to give you the different types of proteases like the alkaline proteases or neutral proteases.

And they all have the role either in the detergent industry or the food industry. Then, you can also have the proteases from the fungi sources, you can have the aspergillus, you can have the endothia, and you can have mucor and all they are also going to give you the acid proteases or the alkaline proteases and they also have the role in the detergent industry, leather industry, pharmaceutical and food industry and so on.

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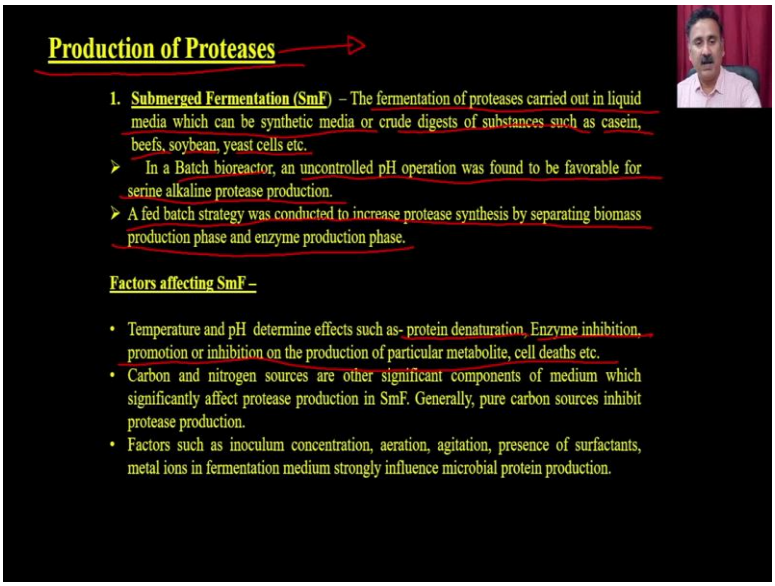

How you are going to produce the proteases? Because what you require is you require a very huge quantity of protease for your applications. So, you have two choices. One you can have the submerged fermentations. So, the fermentation of the protease carried out in the liquid media which can be synthetic media or the crude digest of the substances such as casein, beefs, soyabean yeast etcetera.

In a batch reactor, an uncontrolled pH operation was found to be favourable for the serine alkaline protease productions. And a fed batch strategy was used to increase the protease synthesis by the separating the biomass production and the enzyme production phase. There are multiple factors which are actually going to affect the fermentation based you know the protease productions, like the submerged fermentations.

You can have the temperature and pH, which is going to determine the protein denaturations and enzyme inhibitions, promotion or inhibition on the production of particular metabolites and so on. So, these are the different types of factors what are going to affect the protein production when you are going to do the submerged fermentations.

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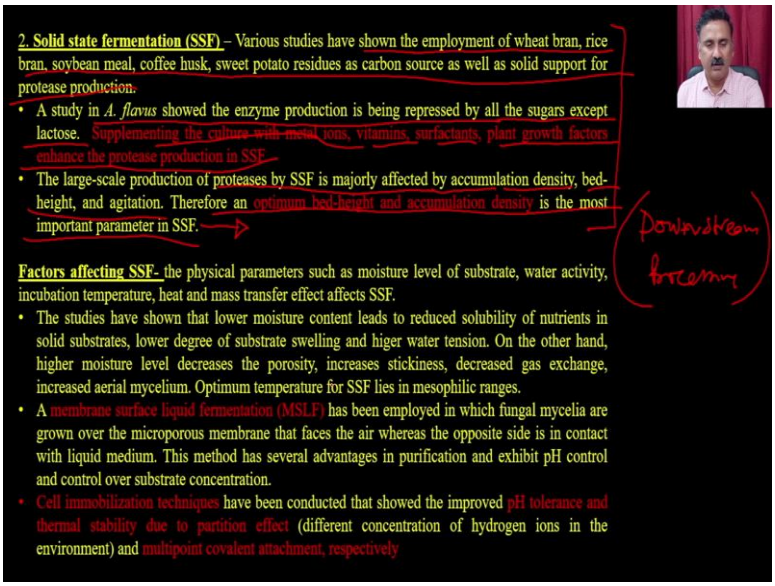

And what will be the solution? You can actually have the other method. You can have the solid state fermentations or SSF. So, various studies have shown that the employment of wheat, bran, rice bran, soybean, coffee and all these are actually good support for the protease production.

A study in the A flavus, showed that the enzyme production is being repressed by all the sugar except lactose. So, supplementing the culture with the metal ions, vitamins, surfactants, plant growth factors enhance to the protein production, protease production into the solid state fermentations. A large scale production of protease by the solid state fermentation is majorly been affected by the accumulation density, bed height and agitation.

Therefore, an optimal bed height is it is the most important parameter into the solid state fermentation. So, the purpose of this whole discussion is not to give you the detail about the fermentations, that anyway you can actually be able to you know get the more detail about if you go through some of the MOOCs courses related to the downstream processing and so on, ok.

So, there are excellent courses on the downstream processing and that actually is going to give you the more in depth inside about how the different types of factors, surfactants and all that, it actually going to affect the protease productions. So, we are not going to discuss any of these. This is just for your information's.

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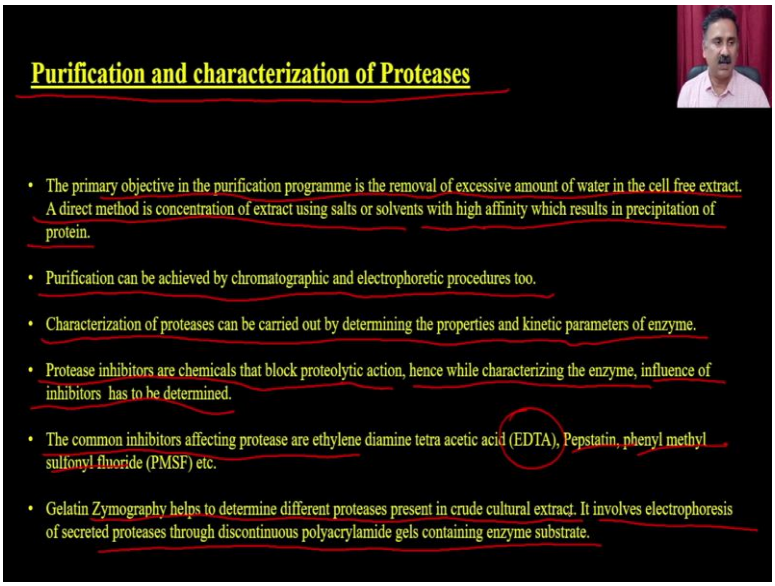

Then, the third is, once you have produced this, you are actually going to do the purification and the characterizations. So, the primary objective in the purification programme is the removal of excess amount of water in the cell free extract. A direct method is the concentration of extract using the salt or the solvent with the high density, which result in the precipitation of the protein.

Then, purification can be achieved by the chromatography and electrophoretic procedures. The characterization of protease can be carried out by determining the properties and the kinetic parameters of the enzyme. The protease inhibitors are chemical that blocks the proteolytic action. Hence, while characterizing the enzyme, the influence of the inhibitor has to be determined.

The common inhibitor affecting the protease are ethylene diamine tetra acetic acid or EDTA, pepstatin, phenyl methyl, sulfonyl fluoride and so on. So, Gelatin Zymography helps to determine the different protease present in the crude culture extract. It involves the electrophoresis of secreted protease through discontinuous polyacrylamide gels containing the enzyme substrates.

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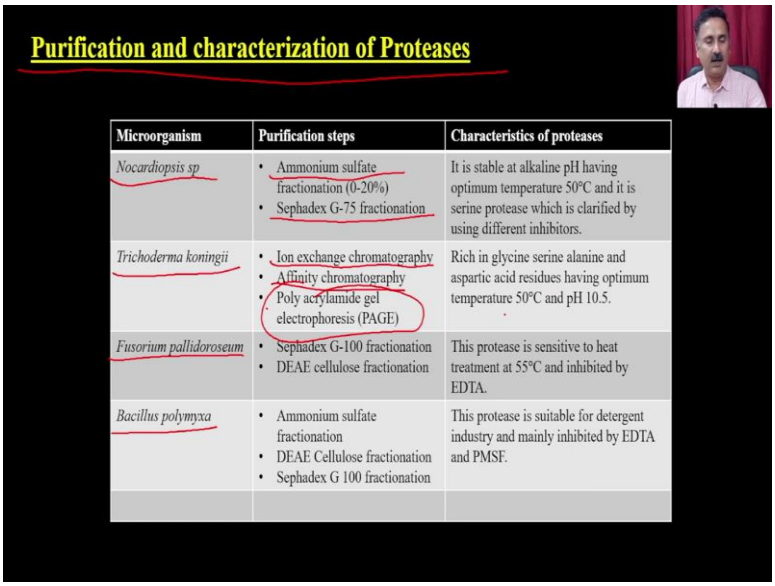

Purification and the characterization of the proteases. So, once you have actually produced the protease, you can actually be able to get the protease from the different types of microorganisms like the nocardiopsis, trichoderma, fusorium, bacillus.

You can actually do the purification steps like when do the first ammonium sulfate fractionations, and then you can do the gel filtration chromatography, and then you can do the characterization. So, when you produce the protease, you can actually be able to do the enzyme assays and so on.

Similarly, from the protease from the trichoderma, you can do the ion exchange chromatography, affinity chromatography and then you can do the page to purify the protease. And you can actually be able to do the characterizations using this. Now, going to the further step ahead, apart from the this industry, you can actually have the different types of enzymes which are also working in the brewing industries.

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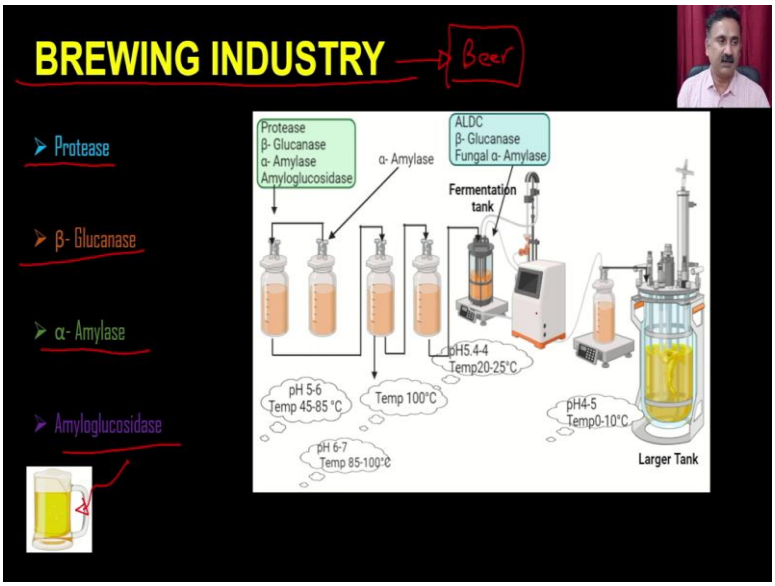

So, in the brewing industry, which is actually going to be responsible for the beer and the related product, you can actually have the in-depth procedures and the different steps. And at different steps, you are actually going to use the these enzymes like the proteases, beta-glucanases, alpha amylases and the amyloglucosidase. And all these are required for producing an excellent beer, so that it will be actually be consumed by the customers.

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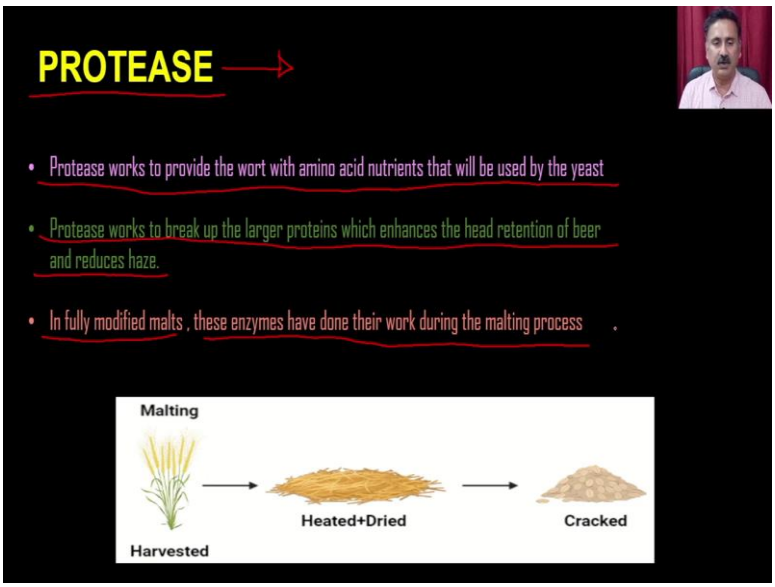

So, the first is proteases. And we have already discussed in detail how you can actually be able to produce a protease in the microbial sources, how you can actually be able to

purify, and how you can be able to do the characterizations. So, protease works to provide the wort with amino acid nutrient that will be used by the yeast. The protease works to break up the larger proteins which enhance the head retention of the beer and reduces the haze.

In fully modified malt, these enzymes have done their work during the malting process. So, in a malting process, what you are going to do is you are going to first do the harvesting of the crop, then you are actually going to dry and then you are actually going to make it the haze and the proteases are actually going to have the function in this particular process.

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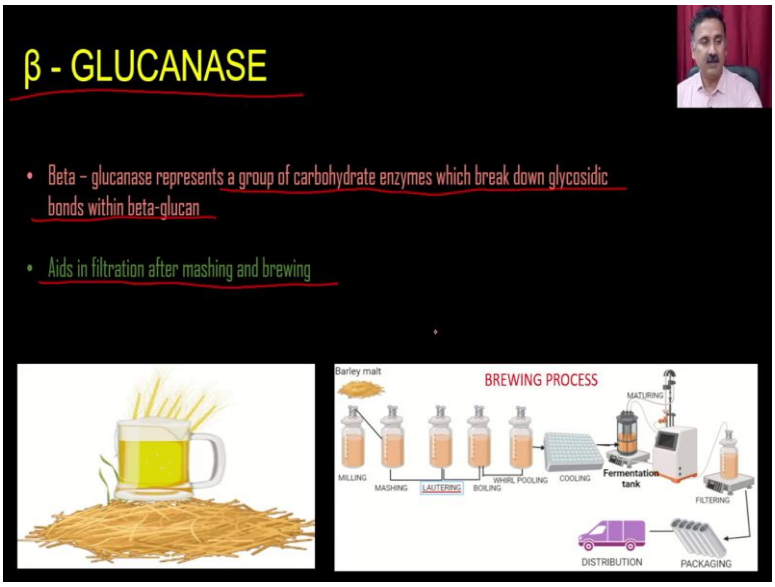

Then, the second enzyme is the beta-glucanases. The beta-glucanases is represent a large group of carbohydrate enzyme which breakdown the glycosidic bond with the beta-glucan. It aids in the filtration after the mashing and the brewing.

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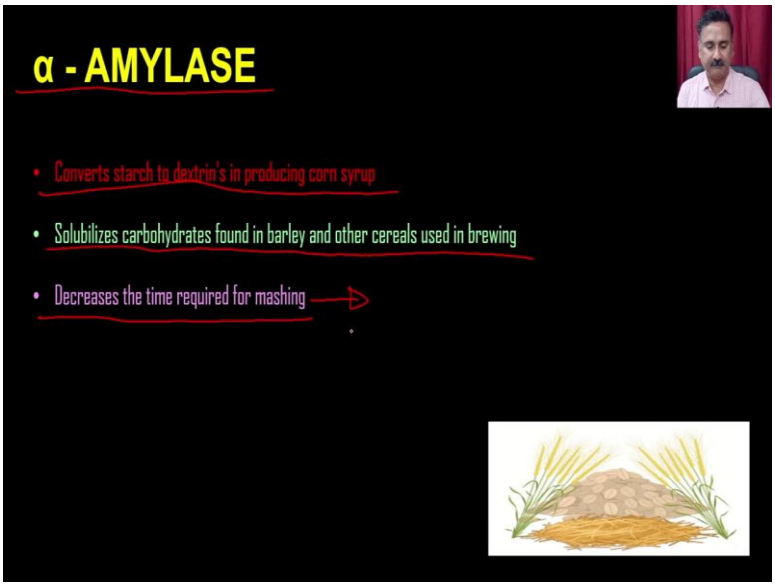

Then, we have the alpha-amylase. So, alpha-amylase converts a starch to the dextrin in producing the corn syrup. And it solubilizes the carbohydrate found in the barley and other cereals used in the brewing. And it decreases the time required for the mashing.

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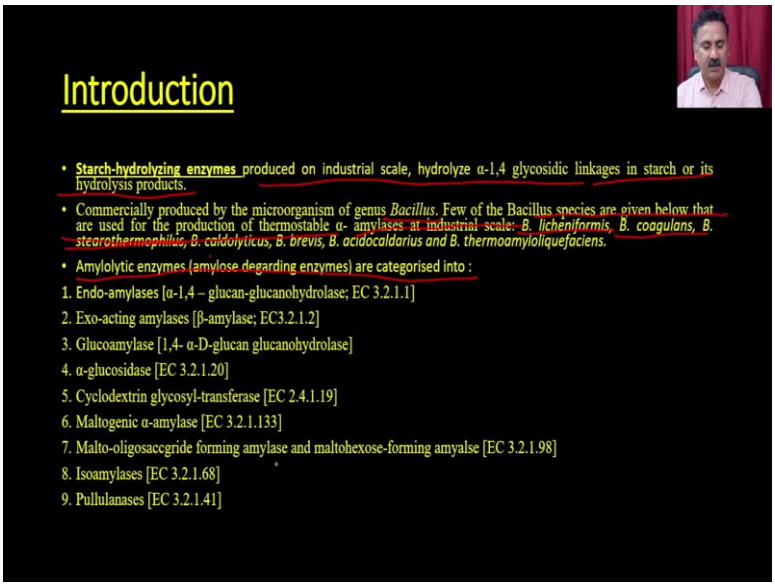

Alpha-amylase, it is a starch-hydrolyzing enzyme produced on the industrial scale, hydrolysis, the 1-4, beta 4, 1-4, glycosidic linkage in the starch and its hydrolysis products. This is commercially being produced by the microorganisms like the bacillus. Few of the bacillus species have given below that are used for the production of

thermostable alpha-amylase at industrial scale like the bacillus licheniformis, bacillus coagulans, bacillus stearothermophilus and so on. Amylolytic enzyme are categorized into these are the different types of the classes of the amylo amylases, and you can actually be able to use any of these for the brewing industry.

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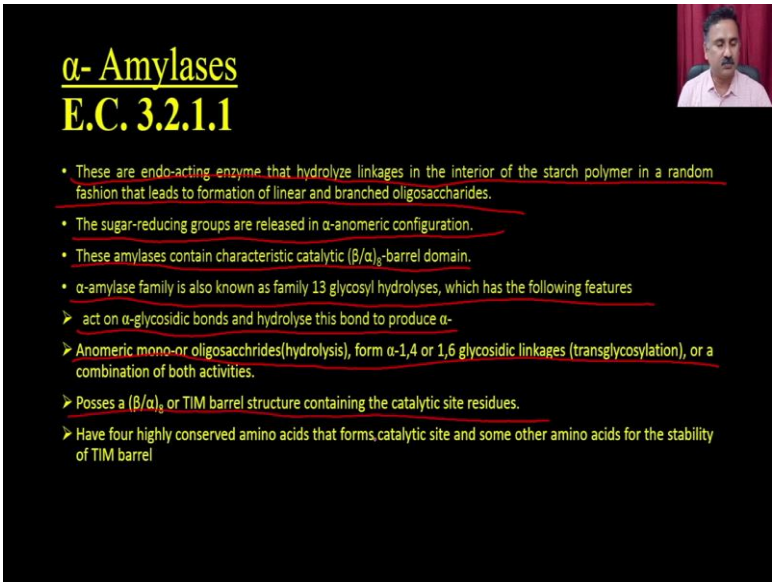

These are endo-acting enzyme that act linkage in the interior of the starch polymer in a random fashion that leads to the formation of linear and a branch oligosaccharides. The sugar-reducing groups are released in the alpha-anomeric configurations. These amylase contains characteristic catalytic barrel domain.

Alpha-amylase family is also known as family 13 glycosyl hydrolyses, which has the following features. It acts onto the alpha-glycosidic bond and hydrolysis, this bond to produce the alpha chains. And you can have the anomeric mono or disaccharides from the this, and it posses the TIM barrel structure containing the catalytic site residues.

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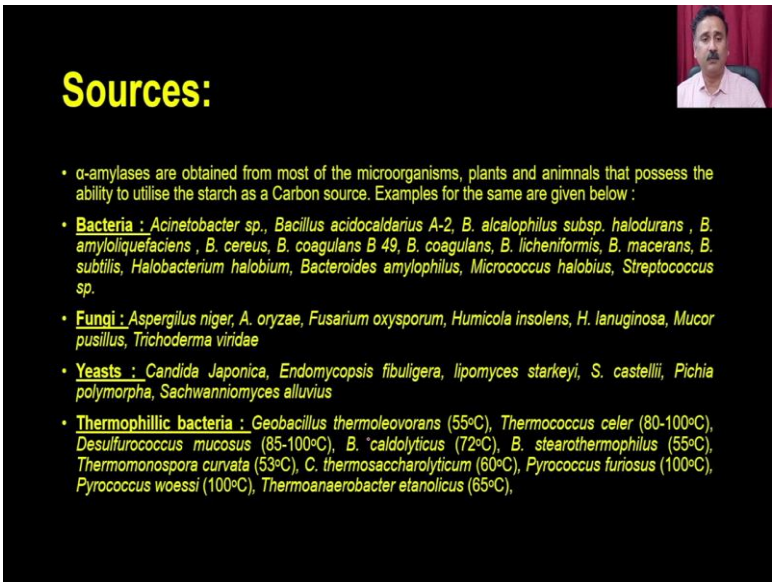

What are different types of sources for the amylases? Amylases are mostly being found from the microorganism plants and animals. From the bacteria you can have the deep sources of bacteria. So, you can actually be able to get the amylases from the different bacterias. You can actually have the fungi; you can have yeast or you can actually be able to have the thermophilic bacteria.

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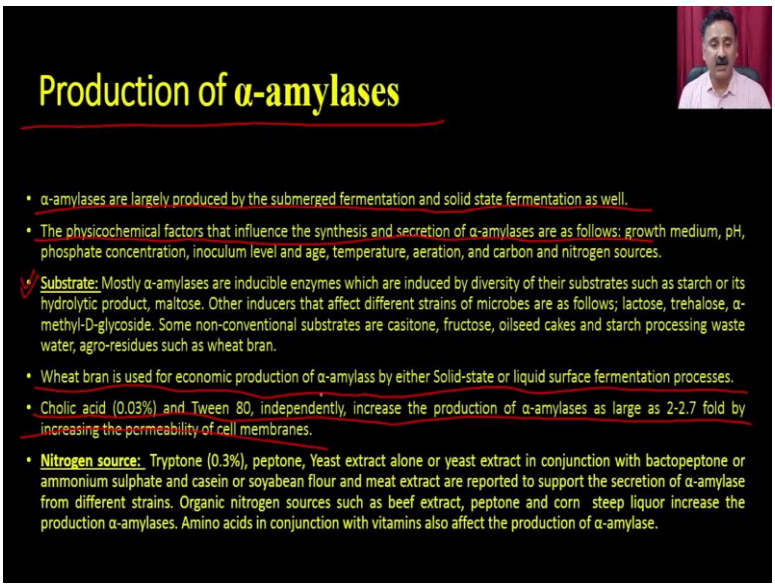

Once you have the amylases, you can actually be able to do the production of amylases. So, amylases are largely being produced by the submerged fermentation and solid state

fermentations. The physiochemical factor that influences the synthesis and secretion of amylases are as follows like the growth medium, pH, phosphate concentration, inoculum levels, age, temperature, aeration and carbohydrate, carbon and nitrogen sources.

You can actually be able to use the different types of substrates because you know that the substrate is going to stabilize the enzyme. And that is how they can also be able to use in the enzyme productions. Wheat bran is used for the economic production of alpha amylase-by the solid-state or the liquid surface fermentation processes.

Cholic acid and the Tween 80, independently, increase the production of alpha-amylases as large as 2 to 2.7 fold by increasing the permeability of the cell membrane. So, they will actually going to increase the recovery of the enzyme. And then, you also can use the different types of nitrogen sources into the fermentation process and that also is actually going to enhance the alpha amylases.

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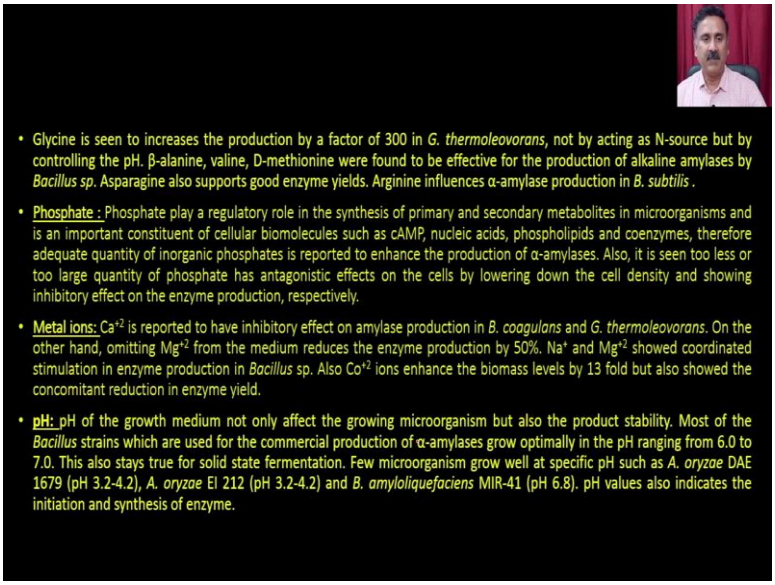

Then, glycine is seems to increase the production by a factor of 300. Then we also have the phosphate, metal ions, the pH and all these are actually going to affect the alpha amylase production because they are actually going to affect the fermentations.

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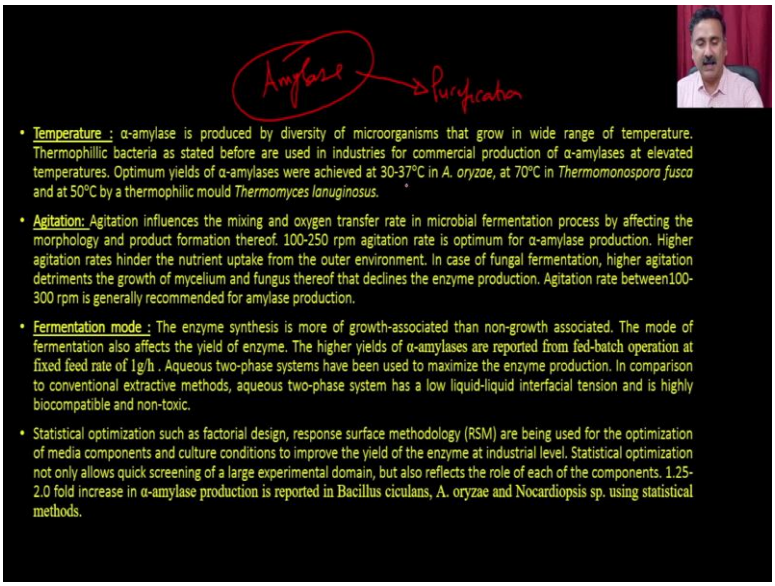

Then, we also have the other factors like the temperature, agitations, fermentation mode, statistical optimizations and all these things are, we are not going to discuss, I have just written, so that you can actually be able to follow the content. And all these are going to discuss in detail when you are actually going to go through with any of the MOOCs courses where they have discussed about downstream processing or fermentation technologies.

Once you have produced the amylase, then the next task is that you are actually going to do the purification, right. You have to isolate this. And that you are going to use do with the help of the different types of chromatography system or fractionations.

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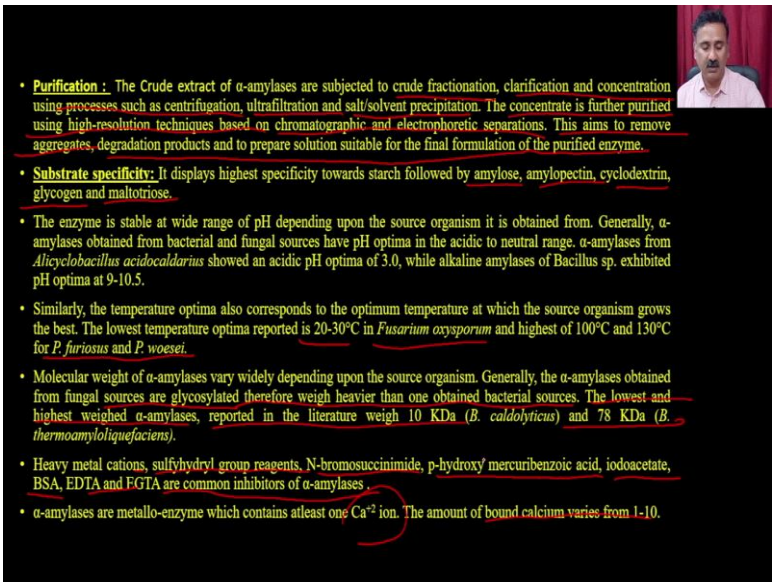

So, the crude extract of alpha amylases are subject to crude fractionations, clarifications, concentrations, such as concentrations using the process such as centrifugations, ultra centrifugations or the salt solvent precipitation. The concentrate is further purified using the high-resolution techniques such as chromatographic and electrophoretic separation. This aim to remove the aggregates, degradation product and to separate solution suitable for the final formulation of the purified enzyme.

Then, you can also have the substrate specificity, so alpha amylase could be specific towards the amylose, amylopectin, cyclodextrins, glycogens and the maltotriose. The enzyme is stable at a wide range of pH depending upon the source of the organisms. So, you can have the pH optima in the acidic to neutral range or you can also have the pH optima of 3 and so on.

Similarly, you can also have the temperature optima. So, temperature would actually vary in the range of 20 to 30 in the case of Fusarium. The maltose, the alpha amylase what you are going to purify the from the Fusarium. And it can be thermostable in the case of the these two organisms such as 100 degree and 130 degrees.

So, molecular weight of alpha amylase very widely depending upon the organism source organism. Genetically, generally, the alpha amylase obtained from the fungal sources are glycosylated therefore, being heavier than the one obtained from the bacterial sources.

The lowest and the highest molecular weight alpha amylase, reported in the literature weighing from the 10 KDa to 78 KDa, ok.

Heavy metal cations such as sulfhydryl groups reagents, N-bromosuccinimide, para-hydroxy mercuribenzoic acid, iodoacetate, BSA, EDTA and EGTA are common inhibitor of the alpha amylases. So, these are the things you can actually be able to use to stop the reactions of the process of the that particular alpha amylase is done. Alpha amylases are metalloenzyme which contains at least the calcium ions. And the amount of bound calcium varies from the 1 to 10.

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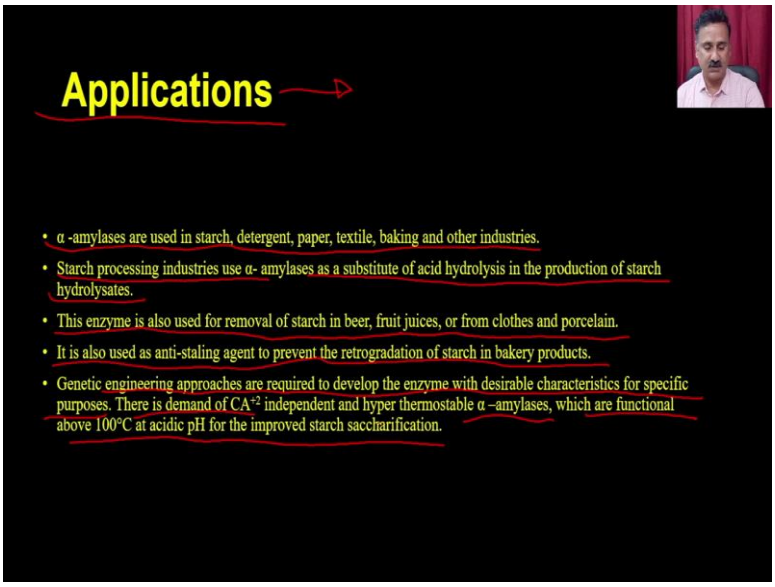

Then, what are the applications of the alpha amylase? One role is anyway in the brewing industry where it is actually going to you know make the sugar soft and it actually going to extract the sugar from the hay and other kinds of mal products. So, that the fermentation is going to be better.

So, alpha amylases are used in the starch, detergent, paper, textile, baking and other industries. Starch processing industries use alpha amylase as a substitute of acid hydrolysis in the production of starch hydrolysis. So, you can actually be able to use the acid hydrolysis or you can actually be able to use this particular enzyme.

Remember, the classical experiment where the people have used the plant extract and they will actually be able to convert the you know the sugar into a polymeric sugar into

the starch hydrolysis, right. This enzyme is also been used for removal of starch in the beer, fruit juices or from the cloths and the porcelain. It is also used as the anti-staling agent to prevent the retardation of starch in the bakery products.

And genetic engineering approaches are required to develop the enzyme with desirable characteristic for a specific purpose. There is a demand of CA, CA calcium depend independent and hyper thermostable alpha enzyme which are functional above 100 degree Celsius at acidic pH for the improved starch saccharifications.

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Now, let us talk about the baking industry, the industry which is going to give you the cake and the pastry and other kinds of baking products. So, in the baking industry, you can have the maltogenic amylase, glucose oxidase and pentosanase.

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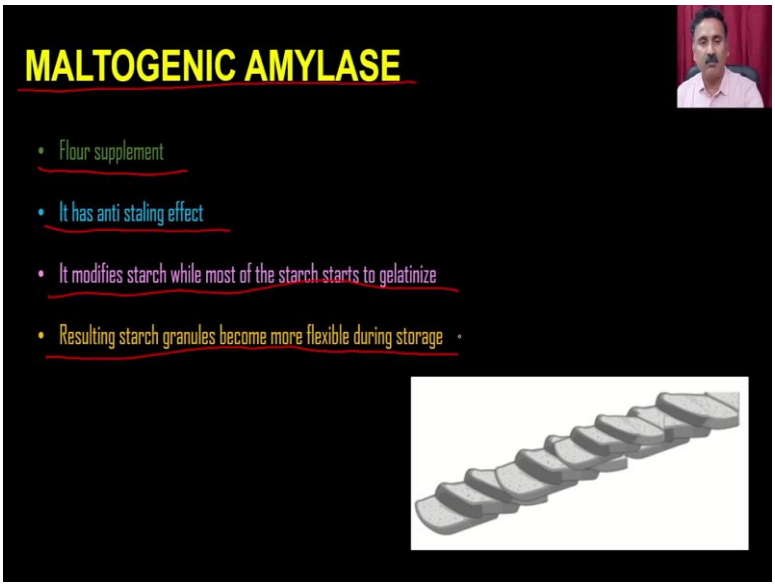

Maltogenic amylase, so this is different from the alpha amylase. It is actually going to produce the malt, right. So, it is a flour supplement, right. You remember that when you are going to make bread, cake and all that, you are actually going to first make the flour, right. So, it is actually going to be added into that.

And it has the anti-staling effects. It modifies the starch while most of the starch start to gelatinize, resulting the starch granule become more flexible during the storage. So, it is actually going to make the cake and other things little more porous and fluffy.

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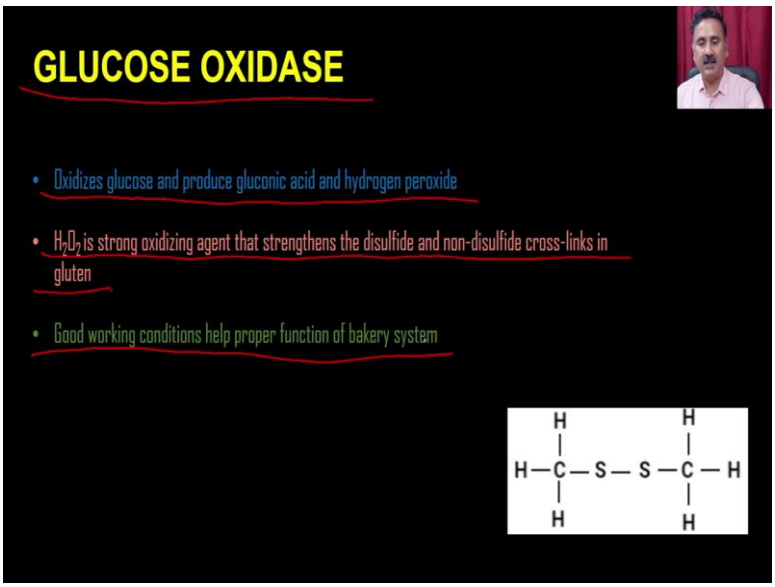

Then, we have the glucose oxidase. So, glucose oxidase oxidizes the glucose and produces the gluconic acid and hydrogen peroxide. H 2 O 2 is a strong oxidizing agent that strengthen the disulfide and non-disulfide cross link in the gluten. And it is good working conditions help the proper functioning of the baker in the baker system.

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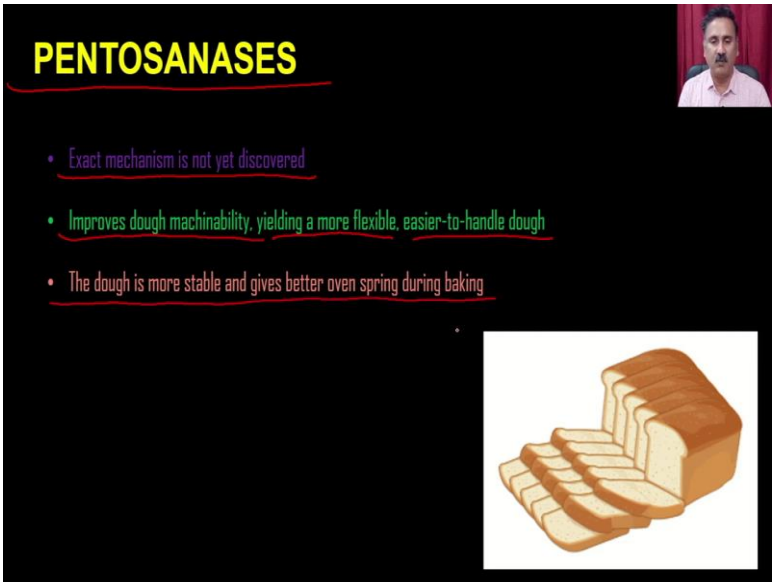

And then, you also have the pentosanases. So, pentosanases are exact mechanism of not being discovered how the pentosanases are actually going to facilitate the baking industries. But it improves the dough's machinability, yielded a more flexible easier to handle dough, right. And the dough is more stable and gives better oven spring during the baking.

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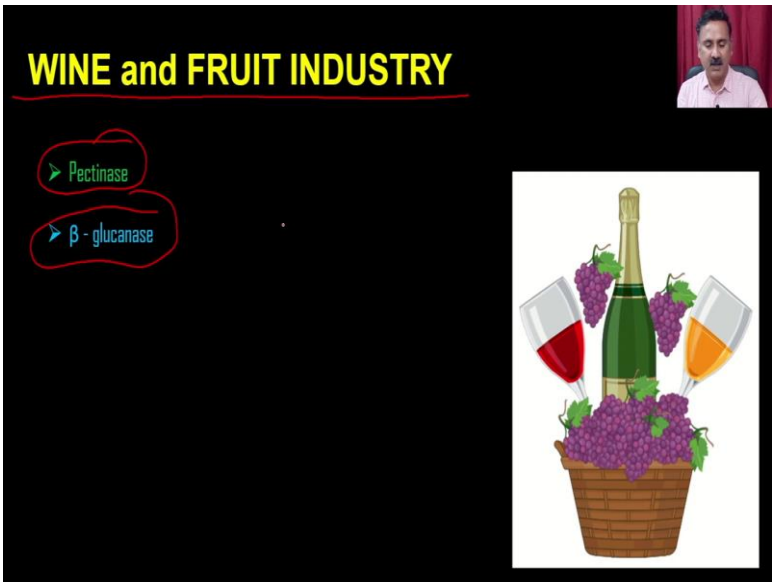

And then lastly, we are also going to discuss about the wine and the fruit industry. So, in the wine and the fruit industry, you can actually be able to use the pectinases and the beta glucanases.

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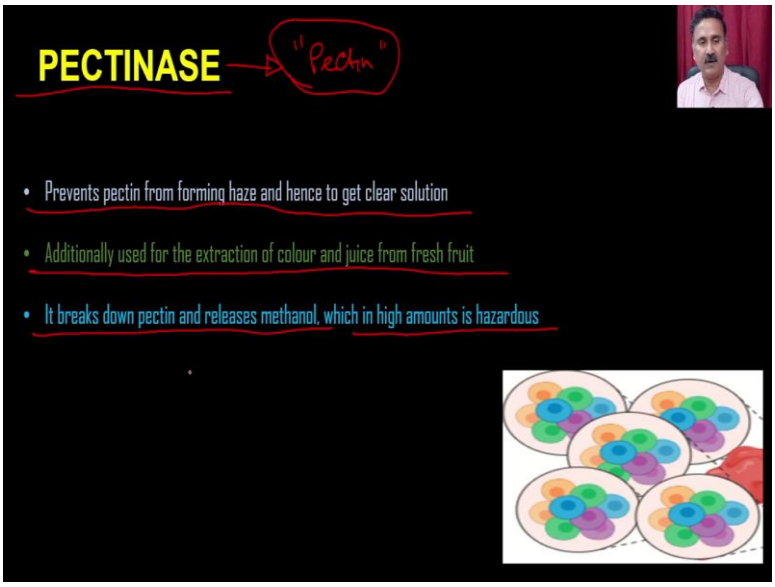

Pectinases, as the name suggests, the pectinases are actually the enzyme which is actually going to work on the pectin. So, pectin is a cell valve component which are present into the plant. So, it prevents the pectin from forming the haze and hence to get the clear solutions.

Additionally, used for the extraction of colour and juice from the fruit juices. And it break down the pectin and release the methanol, which is high amount is hazardous. So, it actually break downs the pectin and it releases the methanol which is actually a toxic product.

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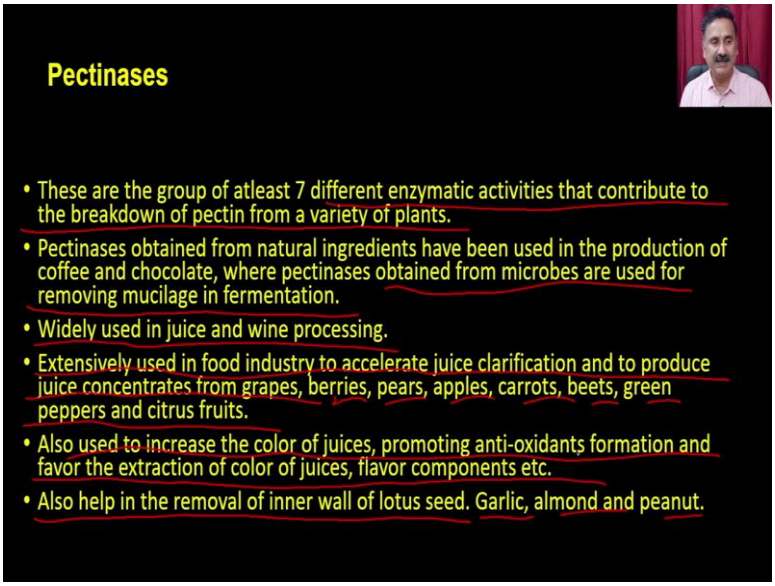

So, what are the pectinases? These are group of at least 7 different enzymatic activities that contribute to the breakdown of the pectin from a variety of plants. Pectinases obtained from the natural ingredients have been used in the production of coffee and chocolate, where pectinase obtained from the microbes are used for removing the mucilage in the fermentations. It is widely used in the juice and wine industry.

Extensively being used in the food industry to accelerate the juice clarification and to produce the juice concentrate from the grape, berries, pears, apples, carrot, beets, green peppers and citrus fruits. It is also used to increase the color of juices, promoting the antioxidant formations and favor the extraction of the color of juices, flavors, component, etcetera. It also helps in the removal of inner wall of lotus seed, garlic, almonds and peanuts.

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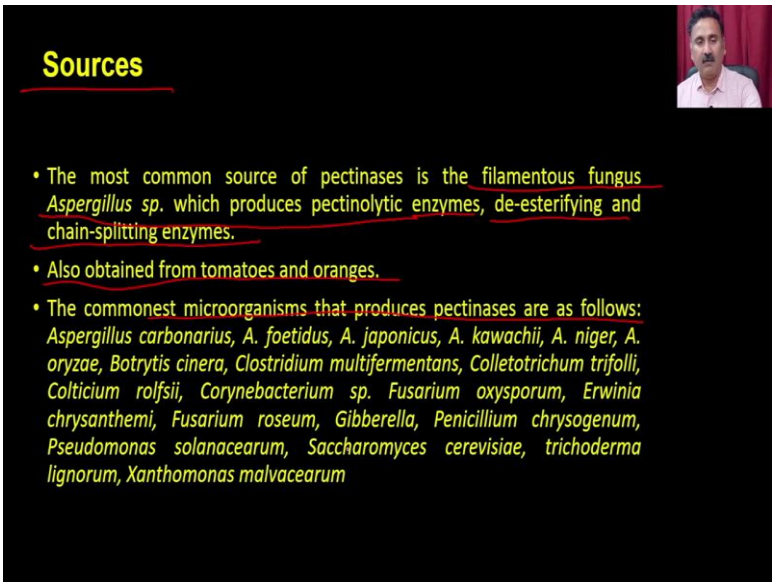

What are different sources? The most common source of pectinase is the filamentous fungus aspergillus, which produces the pectinolytic enzyme, de-esterifying and the chain splitting enzymes; also obtained from the tomato and orange. The common organism that produce the pectinases are aspergillus and so on. So, these are the clostridium and all that. So, these are the different types of microorganism what you can actually be able to use or they can be actually a good source of pectinases.

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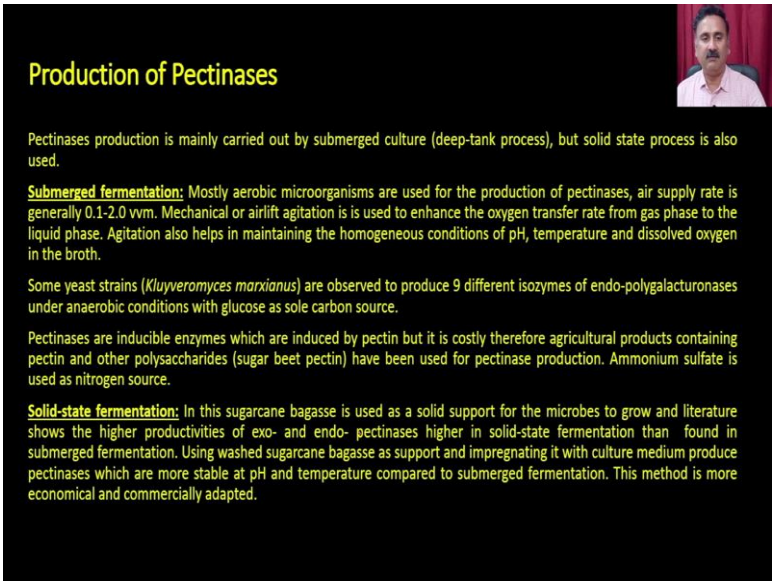

Once you have the pectinases, you can actually be able to do the production. So, pectinase production is mainly carried out by the submerged cultures, but solid state process is also being used. So, this is a submerged fermentation and the solid state fermentation conditions, what you can actually be able to use the different types of pectinases.

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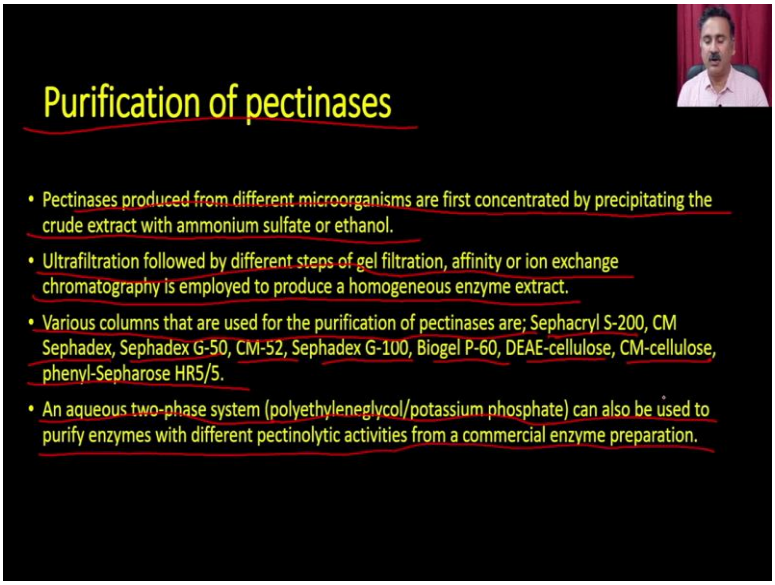

And once you have produced the pectinases you are actually going to do the purification of the pectinases. So, the pectinase production, the different microorganisms are first concentrated by precipitating the crude extract with the ammonium sulfate or the ethanol. Then, we have the ultra-filtration followed by the different steps of gel filtration, affinity or ion exchange chromatography is employed to produce a homogeneous enzyme extract.

Various columns that are used for the purification of pectinases are, Sephacryl S-200, CM Sephadex, Sephadex G-50, CM-52, Sephadex G-100, Biogel P-60, DEAE-cellulose, CM-cellulose, phenyl-Sepharose and so on. An aqueous two-phase system, the polyethylene glycol or potassium phosphate can also be used to purify the enzyme with different pectinolytic activities from a common enzyme preparations.

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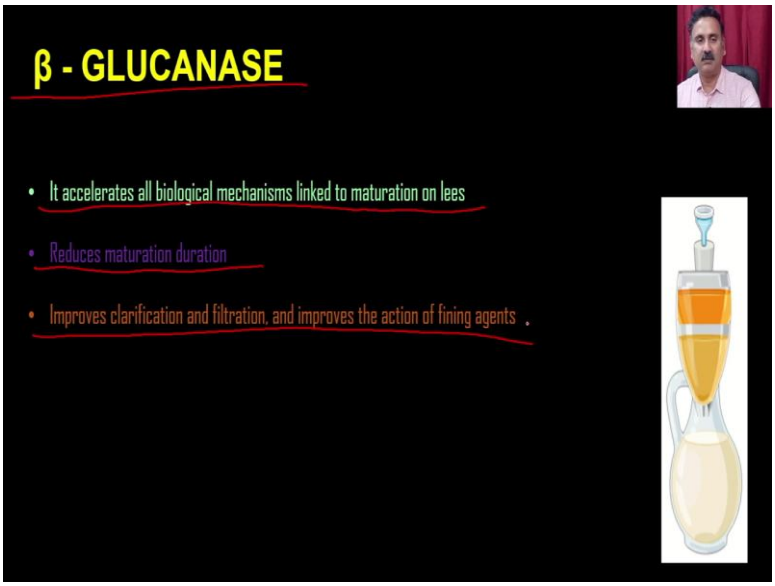

Now, the next enzyme is the beta-glucanases. So, it accelerates all biological mechanisms linked to the maturation on lees. It reduces the maturation durations. And it improves the clarification and filtration and improve the action of the fining agents.

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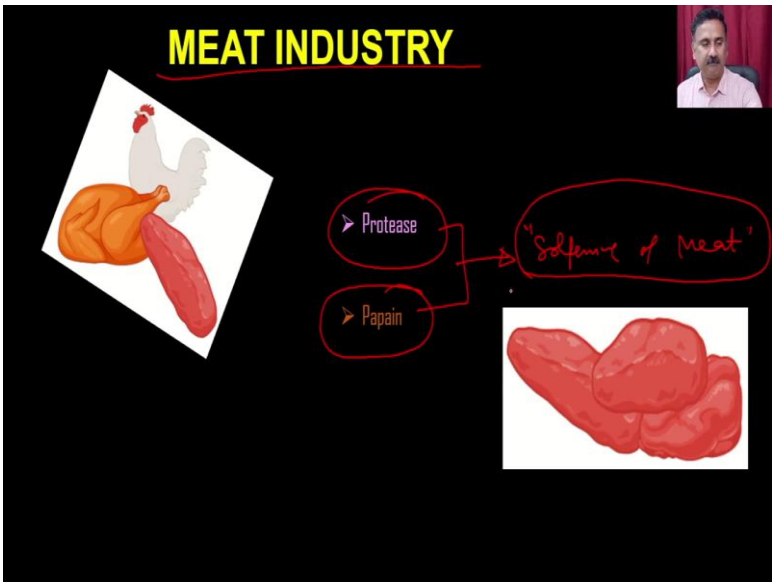

Now, let us move on to the next industry and that is called as the meat industry. So, meat industry is where you are actually going to get the chicken meat or the beef meats. And the enzyme what you are going to use in the proteases and the papain, and both of these

enzymes are actually going to use for the softening of the meat, right. Because it is actually going to make the meat little more soft, so that it will be easy to digest.

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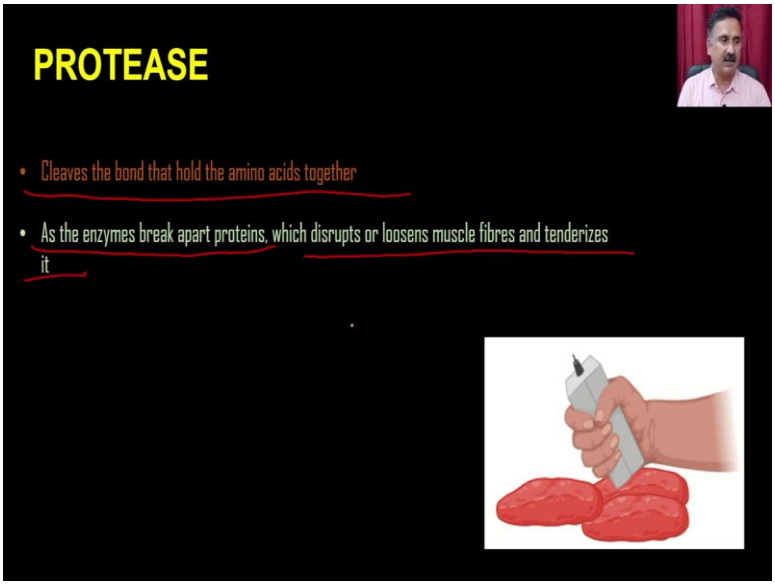

So, the protease it cleaves the bond that hold the amino acid together as the enzyme break apart protein which disrupts or loosens, muscle fibre and tenderize it. So, it is actually going to be used for the tenderizations.

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Then, also have the papain. So, papain is also a protease which is exclusively being found into the papaya. So, it is found in papaya. 90 percent of meat tenderizations

available in the grocery stores are made from the papain. It is extracted from the latex in the papaya fruits. And these enzymes are purified and sold in powder or liquid form.

So, papaya is a papain is a very very important enzyme which is present in the papaya and it is actually being used for softening of the meat. So, that it actually become you know, it enhances the taste of the meat and as well as it is easy to digest. So, what we have discussed?

We have discussed about the application of the enzyme into the food industry, and we have discussed about the different types of enzyme which will require for the brewing industries, baking industries, meat industry and the milk industry. So, with this brief discussion about the application of enzyme into the different food industry, we would like to conclude the lecture here. In our subsequent lecture, we will discuss some more applications of the enzyme.

Thank you.