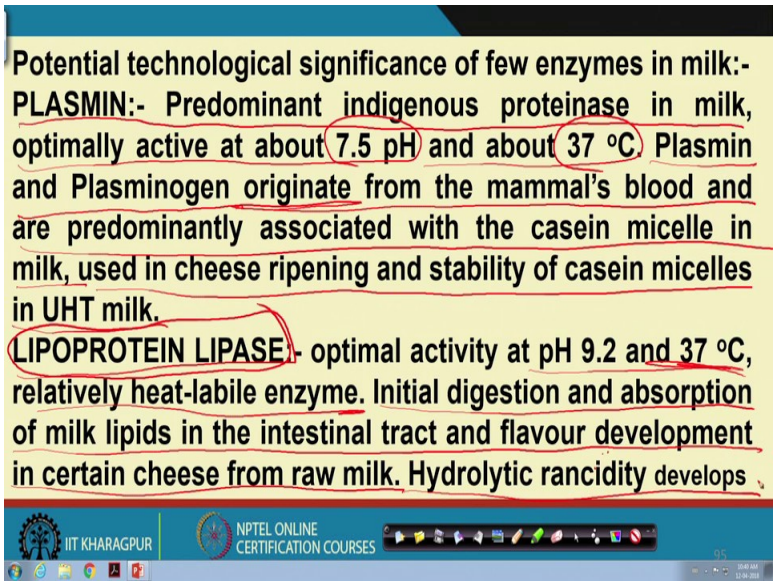
Dairy and Food Process & Products Technology
Prof. Tridib Kumar Goswami
Department of Agricultural and Food Engineering
Indian Institute of Technology, Kharagpur

Lecture - 36
Enzymes in Milk

So, we have finish not finished, we were discussing about the milk enzymes, right. So, now, in this Dairy and Food Process and Products Technology, lecture number 36 we are discussing about the enzymes in milk, right.



So, we have already said there are many enzymes present in milk and some more will tell in detail, right. So, there are would there some few enzymes have potentially technologically potential rather few enzymes where we still that plasmin is sorry one such enzyme, plasmin is one such enzyme which predominant in indigenous proteins in milk and optimally active at about 7.5 pH and also at around 37 degree centigrade. It is both in combination that pH and temperature they do act on the enzyme accordingly, right. So, some enzymes are active at lower pH some enzymes are active at higher pH depending on that that also the temperature and pH this combination that helps the enzymes to act. So, this is pH as well as the temperature both are mentioned accordingly, right.

So, this plasmin is such an enzyme which is this plasmin and plasminogen or this plasminogen originate from the mammals blood and that predominantly associated with

the casein micelle in milk used in cheese ripening and stability of casein micelle in UHT milk. So, many words we have said, right. Casein micelle we have already said. So, there is no further explanation for that.

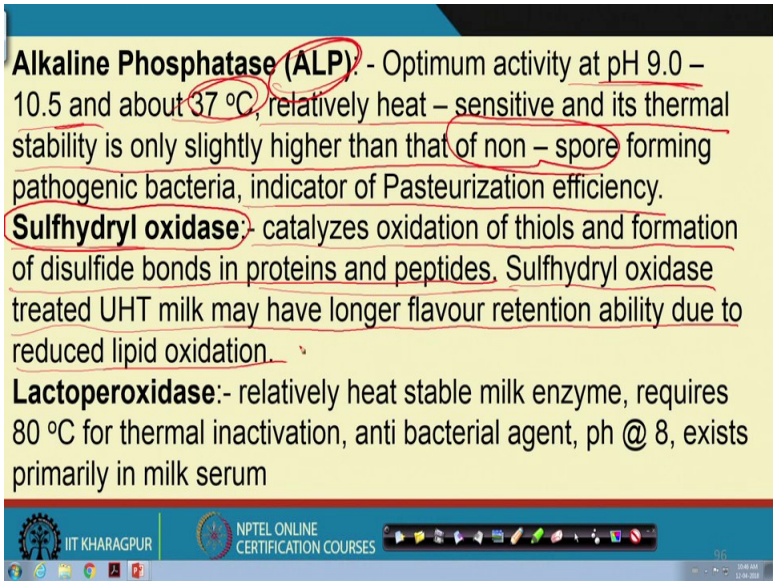
Then cheese ripening also a little we have said in the previous class that when you are going into cheese manufacturing then you will see that there are some unripened and ripened cheese and the meaning of ripening also we have said in the previous class that ripening is one where you develop lot of flavour and odour in the in the prepared cheese. So, this helps this plasmin helps to develop such. And these are originated from the mammals blood and it come from the mammals blood and it is associated with the casein micelle, right. It also tells about the UHT another term which you are coming across that is ultra high temperature, right.

We said the other class that pasteurization, right. That is decomposition of the pathogen or destruction of the pathogen pathogenic organisms, right. So, and we said it is a time temperature combination. So, always when you are you are heating it is always a time temperature combination, ultra high temperature is such a combination where temperature is raised to very high around say 140 plus degree centigrade and it is for absolutely fraction of second or second, right. It is called hold no time, it is hold at that temperature and over so that means, high temperature very very short time ultra high temperature that is also a way of pasteurization, a mean of pasteurization.

Another protein called another rather enzyme called lipoprotein lipase, right, obviously, from the word name lipase we have already said, but lipoprotein lipase. That means, it is acting on the lipoprotein, right. Lipoprotein is that lipid and protein together where there it is that is called lipoprotein, right. And is optimal activity is around pH 9.2 at 37 degree centigrade and relatively heat stable enzyme initial digestion and absorption of milk lipids in the intestinal tract and flavour development in certain cheese from raw milk is used, right. And this develops hydrolytic rancidity.

The rancid flavour, the other day we said that the rancid flavour in milk is many times associated with fishy flavour that is the degradation of the of the your lipoprotein that is or phosphoprotein, phospholipid, that is your lecithin, right, so lecithin where it is there. So, if that is degraded if it is that is decomposed then it produces triethylamine or its

derivatives, right which causes the off flavour. So, this lipoprotein lipase acts also on the lipoprotein and it may cause hydrolytic rancidity, right.



So, we then get alkaline phosphatise, this we have already said a little alkaline phosphataase. Phosphatase is one enzyme which is used to detect whether the heat treatment was sufficient for pasteurization or not, where there is a phosphatase because phosphatase we said is a heat stable enzyme or comparative other enzymes which much heat stabler and at high temperature when you are pasteurising whether it is all organisms have been killed or not is all pathogenic organisms are killed or not.

That is detected by the presence of this enzyme called phosphatase. So, alkaline phosphatase in short alp is such one where the activity is around 9 to 10.5 pH and it was around 37 degree centigrade. It is relatively heat sensitive and its thermal stability is only slightly higher than that of the non spore forming thermal stability is a little higher than non spore forming or pathogenic organisms. Normally pasteurization which you are referring all the time is killing of the pathogenic organisms which are not spore former.

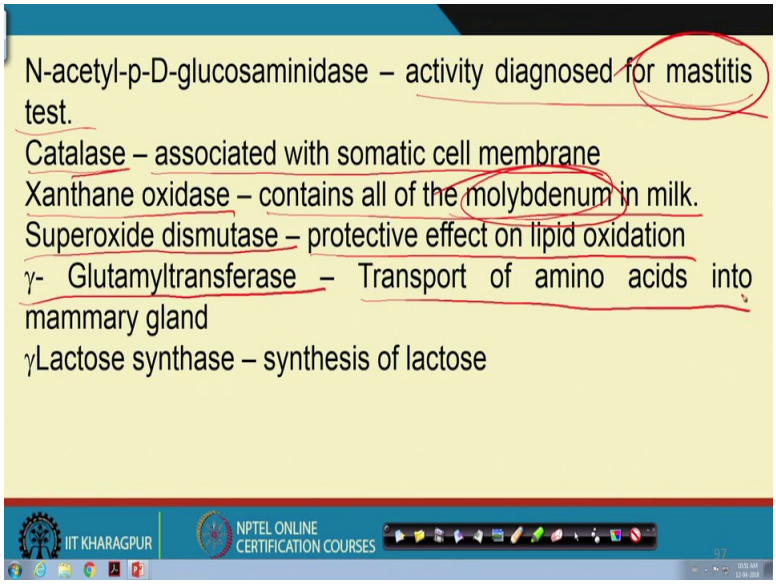
Because spore if you go into microbiology you will see that the bacteria which surrounds it like a cocoon, right. Like a cocoon is there which protects it this bacteria is also having some membrane arounding some protective cell surrounding it. So, that this is becoming very very stable that spore formers are very very then dangerous. So, we are referring to all the time is the pathogenic organisms which are not spore former, so not; this enzyme is little more resistant to heat than the non spore former pathogenic organisms, right.

That is why it is choosen as one of the indicator whether you are this heat treatment was sufficient or not, right. So, this is the indicator of pasteurization efficiency.

Then another enzyme called sulfhydryl oxidase, sulfhydryl oxidase, right. This is catalyzes oxidation of thioles and formation of disulfide bonds in proteins peptides in proteins and peptides. We have already said this is not new that proteins what peptides what that we have already discussed, so no more. Sulfhydryl oxidase treated UHT milk may have longer flavour retention ability due to reduced lipid as rather lipid oxidation, right. So, if you have sulfhydryl oxidase it helps in developing flavour in UHT the reason being the lipids which are degraded by oxidation that is not there, right, so that is reduced. So, that is the reason why some good flavour can be written in the UHT when this sulphide oxidase catalyze oxidation of thiols for forming disulfide bonds, right. So, that is forming some flavour producing components not the oxidation of the lipid.

Then lactoperoxidase, right, peroxidise we had said earlier that is the oxidation oxidative enzyme which catalyzes and by the by enzymes are catalyst, right. That we know that enzymes are catalyst, it acts as a catalyst for any reaction whether good or bad in a material. So, it acts as a catalyst, so in lactoperoxidase it is relatively heat stable milk enzyme it requires 80 degree centigrade for thermal inactivation and antibacterial agent which works around pH 8 and exists primarily in milk serum, milk serum already we have said where that distinction between serum then your plasma all this things we have said earlier, right.

So, it requires 80 degree centigrade for thermal inactivation and it is a antibacterial agent, right, lipo, lipo sorry lactoperoxidase there is anti antibacterial agent, right. So, it is more heat stable that is deactivated around 80 degree centigrade. So, high temperature it can withstand, right. That is why it is in UHT also.



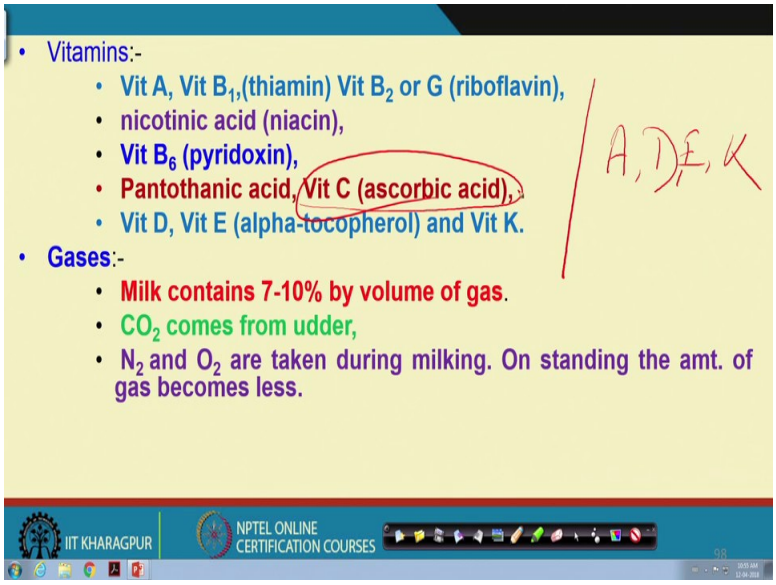
Then N-acetyl-p-D-glucosaminide glucosaminidase, right, glucosaminidase N-acetyl-p-D-glucose glucosaminidase, right; glucosaminidase, that is activity diagnosed for for mastitis rather mastitis that doctors do tell mastitis, right. That is nothing but, that that is nothing but that the infection at the udder, right, infection at the udder of the animal that is called mastitis, right, by the doctors. So, this is one term which we have not come across the mastitis it is the infection of the udder. So, that infection of the other can be can be identified by the presence of this N-acetyl-D-glucosaminidase, right.

Then catalase another enzyme which is associated with somatic cell membrane somatic cell we have already said somatic cell membrane this is associated with that catalyse, right. So, somatic cell membrane can be that catalyse we have said earlier is the oxidative enzyme. Xanthine oxidase it contains all of the molybdenum in milk xanthine oxidase, whatever molybdenum present in milk it is there in xanthine oxidase. So, this is all molybdenum in milk is available in xanthine oxidase then superoxide dismutase superoxide, dismutase not di sorry dismutase superoxide dismutase this is a protein, this is a protective effect on lipid oxidation. So, on lipid oxidation this acts as a protection that superoxide dismutase.

Then gamma glutamyltransferase, gamma glutamyltransferase it transports amino acids into memory gland. Amino acids are transferred to memory glands or that glands from where the milk is secreted, that transport of amino acid that is being done by this enzyme called gamma glutamyl glutamyltransferase. Then gamma lactose synthase gamma lactose synthase it acts for synthesis of the lactose that is gamma lactose synthase. So, all

these are intrinsic in milk it is there and we have discussed mostly that these enzymes help differently in different formation or destruction of some good or some bad of the milk, right.

So, enzymes do have lot of activities in milk and there are as we said 20-20 enzymes have been characterized from the milk and 40 other have been identified as their activity or from their activity in milk the result indicates that these are these enzymes those are under that 40 category. So, so many enzymes are present in milk which directly or indirectly helping or destroying depending on the enzyme, depending on the use for the milks which we have come across, right.



Then we come to vitamins there are lot many vitamins are there, right. From vitamin A vitamin B 2 or vitamin B's, right. Vitamin B's that is vitamin B 1 as thiamine, vitamin B 2 or G as riboflavin nicotinic acid as niacin vitamin B 6 as pyridoxin pantothenic acid, vitamin C as ascorbic acid, vitamin D vitamin E as alpha tocopherol and vitamin K all these vitamins are present in milk, all these vitamins are present in milk and we get vitamins out of that, right.

All these vitamins are present in milk that is vitamin A vitamin, B 1 as thiamine, vitamin B 2 or G as riboflavin nicotinic acid as niacin, vitamin B 6 as pyridoxin pantothanic acid, vitamin C ascorbic acid, vitamin D vitamin E that is as alpha tocopherol, and vitamin K out of which some are fat soluble and some are water soluble. Fat solubles are vitamin A, D, E and K and rest of the vitamins are water soluble, right. So, these are all vitamins

which are available in milk. But it is poor in vitamin C content, right. It is poor in vitamin C content. You see it could have been more, but nature this is a natural products nature has made in such way vitamin C normally it is this to ascorbic acid.

So, this is acid component. So, had it been high then that would have disturbed the pH of the milk and thereby that the coagulation or the stability of the casein in colloidal form that would have been disturbed. So, nature has not given that ascorbic acid so much in vitamin in milk, right. So, nature is such a beautiful thing that it controls everything and with all reasoning with all scientific reasoning it is there and I really appreciate I am amazed that how nature takes care of everything, right. That is whether it is good or whether it is bad doesn't matter, but accordingly nature takes care.

So, here that example we have given that vitamin E or tocopherol or sorry vitamin C not tocopherol vitamin C or ascorbic acid tocopherol is another which is known as the antioxidant, right. We have lot of fats in vitamin. So, antioxidants as antioxidant this tocopherol is used, right. And many many drugs do have this vitamin E and is tocopherol as antioxidant and that is why milk also do have lot of vitamin E and it is good to protect the fat as antioxidant, right.

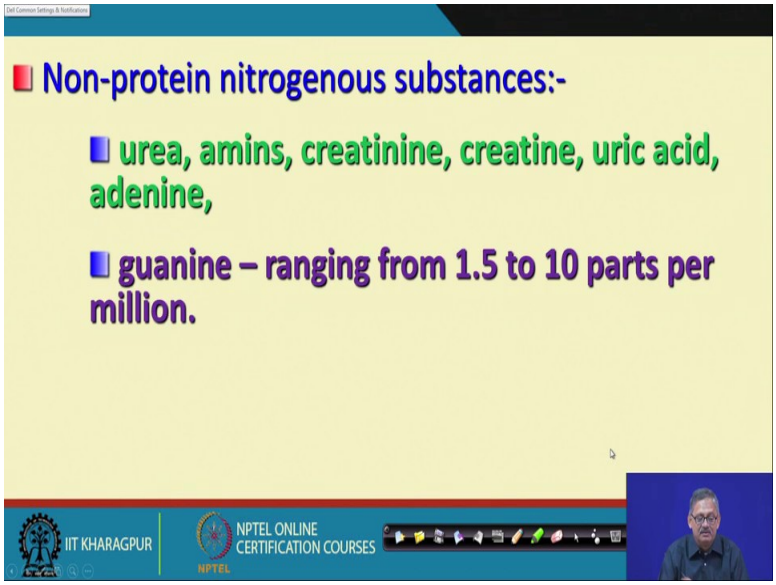
Whereas, the other one vitamin C is very poor in milk as well iron I am not sure about the why iron is so poor in milk that why nature has made such, but definitely there is some reasoning for that. Yes, iron makes the heme or it can act with haemoglobin and may come out I am not sure about that, but definitely some reasoning is also there why iron is so low in milk whereas, so many other things are so high but iron is also low, right. So, must have been some reasoning good reasoning for nature to substantiate the reason why it is not present, ok.

Some gases are also there like a milk contains around 7 to 10 percent by volume of gas, where on 7 to 10 percent by volume of gas around that carbon dioxide comes from udder, the udder when it is being milked carbon dioxide do come from there. Nitrogen and oxygen are taking during milking on standing the amount of gas becomes less, right. So, when milking is being done that time lot of incorporation of either air from there maybe carbon dioxide, maybe nitro dissolve nitrogen or oxygen gases are there is dissolved oxygen concentration for amount or quantity is diluted when you are standing

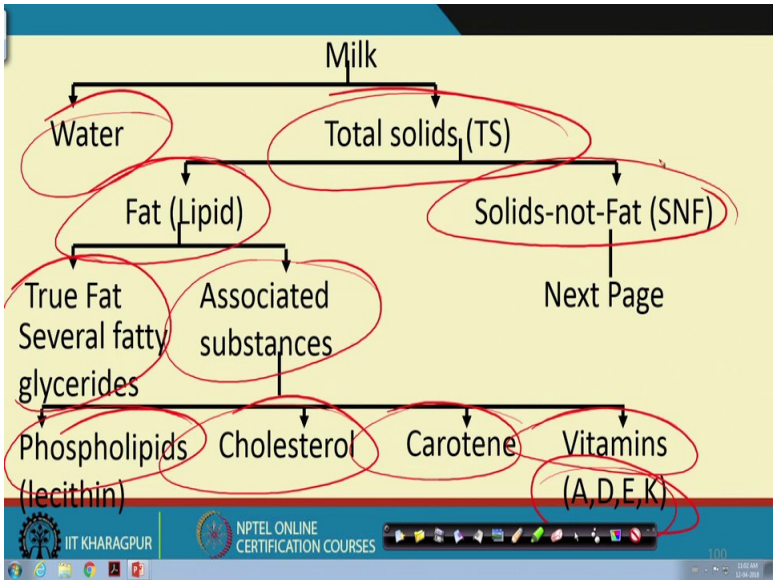
from some time then it comes out, right. As we know that the this solution of or rather the solution of the gases is a function of temperature also, right.

So, the lower the temperature, lower is the solubility the higher the temperature more is the solubility. Is it true that with high temperature within the derive oxygen or gases do enter no it is the other way, right. It is the other way, right. That is why it is the other way that is why in drinks cold drinks typically when you are putting that cold drink you see you are putting in refrigerator. Why? Because those carbon dioxide which are put into this cold drink, if at high temperature that has come though it is sealed. But it at high temperature it has come up and because of the temperature dissolution has happen it is of the surface of this content or container, and when you are putting back in the in the refrigerator this goes back into the solution and then you get the flavour you get the you get the pleasure of carbon dioxide being inside, right. So, it is that the lower the temperature solubility is more the higher the temperature now the solubility of the gas is less this is by enlarge exceptions are also there, right.

Now we come to the absolutely concluding part of the milk constituents that what milk is made of. Vitamins we have already said, gases we have already said.

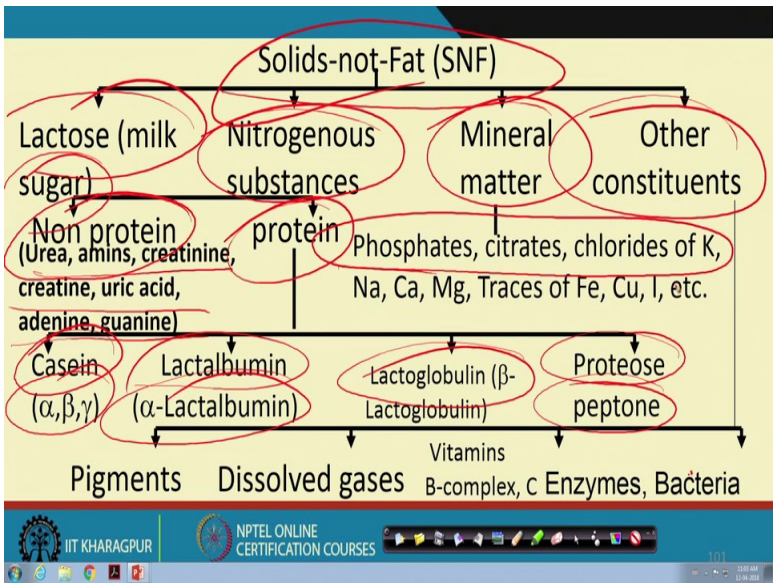


Some non protein nitrogen substances are also there like urea, amins, creatinine, creatine, uric acid, adenine, guanine ranging from 1.5 to 10 parts per million, right.



So, we come to this level that milk comprised of one side it is water and another side it is total solids, out of that total solids it is part is fat which is lipid and out of that some are true fat that is several fatty acids, are fatty glycerides and some associated substance. Some associated substances are like phospholipids or lecithin cholesterol then carotene then vitamins like A D E and K, right. This is one part.

And in total solid another part is total solid is not fat that is solid not fat, so these solid not fat includes all rest of the other material.



And that is the solid not fat is this that this solid not fat is lactose that is sugar or nitrogenous substances or mineral matters or some other constituents. In lactose it is only the milk sugar then nitrogenous substances out of which it is non protein substances like

urea, amines, creatine, creatinine, uric acid, adenine, guanine etcetera. In protein that is nitrogenous substances it is casein alpha beta gamma, this casein then lactalbumin or alpha lactalbumin, right, then lactoglobullin, that is beta lactoglobullin and proteose or peptone, right, proteose or peptone.

And in mineral matters we get phosphoric phosphates citrates chlorides of sodium, potassium, calcium, magnesium and traces of iron copper and iodine. Whereas, in some other constituents we get some pigments, some dissolved gases, some beta B complexes and vitamin C some enzymes and bacteria. So, it contains everything it contains everything. So, that is why milk if it is studied, if there is that liquid food is studied you come across with lot of chemistry and physics and associated things of food, ok.

So, hopefully we have covered lot in, we have covered lot in milk constituents and next we will follow as we have given the outline.

Thank you.