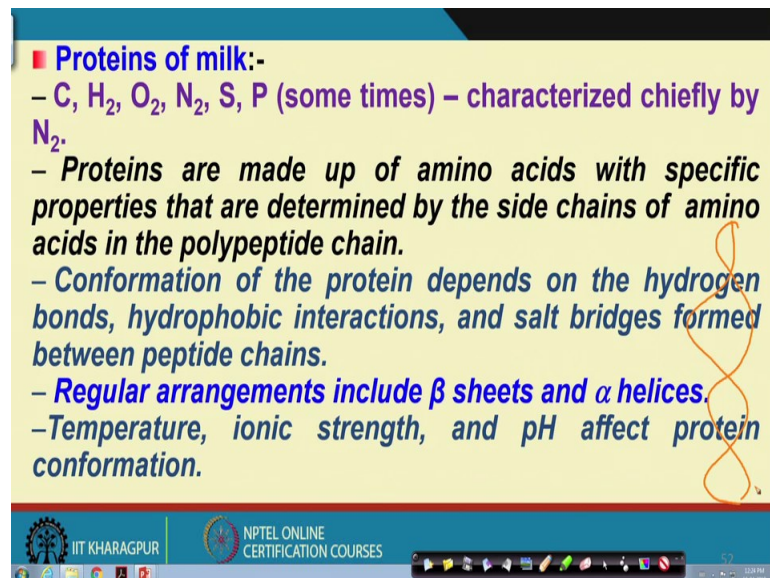


Dairy and Food Process & Products Technology
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Lecture - 29
Milk Protein

So we have finished the basics of protein and amino acids, now we come back to our normal class of that Milk Protein right, we have finished milk fat and, now we will go to milk protein. So in our this Dairy and Food Process and Products Technology class in lecture number 29 we come back to milk protein right. So in that milk protein, when we are talking so we have seen that, proteins are made of carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus right.



■ **Proteins of milk:-**

- **C, H₂, O₂, N₂, S, P (some times) – characterized chiefly by N₂.**
- **Proteins are made up of amino acids with specific properties that are determined by the side chains of amino acids in the polypeptide chain.**
- **Conformation of the protein depends on the hydrogen bonds, hydrophobic interactions, and salt bridges formed between peptide chains.**
- **Regular arrangements include β sheets and α helices.**
- **Temperature, ionic strength, and pH affect protein conformation.**

So that sulphur, phosphorus in some of the proteins it may or some of the proteins it may not be, but normally this proteins are characterized by the nitrogen content and that is why we tend the protein content with the help of the Kjeldahl method, and there we get the nitrogen and with the conversion factor we get the protein right, and we have seen that proteins are made of amino acids with specific properties, that are determined by the side chains of the amino acids in the polypeptide chain. This all are now no longer Greek to us because we have completed right, and confirmation of the protein depends on the hydrogen bonds, hydrophobic interactions and salt bridges formed between peptide chains; all these all bridge hydrophobic or hydrogen bonds or peptide bonds these also we have covered up right.

So regular arrangements include beta sheets and alpha helices right, so this alpha helices and beta sheets also we have covered in our previous classes right, so that alpha helices where it is like a helical group right. So helice means it is as helices we know, so this is like this and this is one and another is like that.

So this is like a helical group it appears right and all this depends on the temperature ionic strength pH that affects the protein conformation, and conformation we also said is nothing but the arrangement or the special arrangement in the special arrangement of the functional groups of the molecule that is the conformation of the particular molecule or atom or system right, so this we have seen.

– Normal milk – 2.8 to 4% (30 to 35 g / kg) – remains constant – influenced by lactation stage of the animal.

- Two types – Casein (80%) and Lactalbumin or whey or serum protein (18%) – derived genetically.
 - Lactoglobulin – 0.05 to 0.07%.
 - Casein --
- four main types of casein have genetic variation
- α_{s1} casein, α_{s2} casein, β casein, and κ casein.
- These are phosphorylated and hydrophobic and associate

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Now let us look into that normally milk contains around say 2.8 to 4 percent, out of which 30 to 30; 2.8 or 2 4 percent is around, say 30 to 35 grams per k g of the milk, it contains protein or nitrogenous compounds and remains constant because normally proteins do not come out as fat we have seen, if we just keep fat just like that standing for some time we have seen that fat molecules to come out and get separated from the milk, and because of that the fat component gets depleted right, but unlike that unless you are heating milk this protein does not come out and protein remains more or less constant in the milk system right.

So this is influenced by the lactation stage of the animal, so this protein content also depends on the lactation stage, that a little we have may not be a little elaborately also we have discussed earlier right. So 2 types of proteins are there, one is called casein, which

contains around 80 percent and lacto or lactalbumin right. Lactalbumin or which is known as whey protein or serum protein, now what is serum what is plasma those things also we have covered earlier right.

So this contains around 18 to 20 percent of the whey protein of the total protein right, and they some derived also proteins are also there for example, lactoglobulin which is may be around 0.05 to 0.07 percent present there right. Now out of these proteins in milk that is casein and whey protein right, casein contains 80 percent, whey proteins roughly we call it to be 20 percent 80 percent protein and 25 casein and 20 percent, we call to be whey protein right of what of the total protein present to milk itself contains protein.

We normally call it to be around 4 percent again the many factors are responsible for this variation of the protein. So we have discussed on that. So assuming out of 4, 80 percent this casein so that means, around 3 to 4, 3 to 3.5 grams of protein or casein per 100 gram of these milk, and remaining is out of 4. So remaining 0.521 gram is your whey protein right now this casein there are 4 types of casein, they are having its variation 4 types they are alpha s 1, casein alpha s 2 casein, beta casein and kappa casein. So I repeat 4 types of caseins alpha s 1 casein, alpha s 2 casein, beta casein and kappa casein these 4 types are there.

With themselves and each other.

- They represent 38%, 10%, 36%, and 13% of whole casein.
- Overall 21 variants of casein have been identified that occur by genetic mutation.
- Caseins have distinct disordered molecular structures that lack disulfide bridges.
- Caseins are very heat stable and withstand temperature about 150 °C before getting dissociated.
- Hydrophobicity is due to

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These are phosphorylated and hydrophobic these caseins are phosphorylated and hydrophobic and they are associated with themselves and each other right. So they respond they represent around 38 percent, 10 percent, 36 percent and 13 percent of the

whole casein that is alpha s 1 casein is 38 percent, alpha s 2 casein around 10 percent, beta casein is 36 percent and kappa casein is roughly 10 percent right.

So this is about the casein, 4 types which are present overall 21 variants of casein have been identified that occurred by genetic mutation right, caseins have distinct disordered molecular structure that lack disulfide bridges also caseins are very heat stable and withstand temperature about 150 degree centigrade before getting dissociated means it may get into your dissociation means denaturing right.

So denature also we said the other day so before it is did getting dissociated or denature or degraded that it is around 150 degree centigrade it can withstand right. Hydrophobicity is due to the, high ratios of apolar amino acids. So those amino acids which are not polar hydrophobicity is due to that high ratios of apolar amino acids, apolarity is even in valine, leucine, isoleucine, phenyl phenylalanine and proline.

- **high ratios of apolar amino acids**
- **apolarity is even in valine, leucine, isoleucine, phenylalanine and proline.**
- **Apolarity ranges between 35 to 45%.**

Hydrophobicity is counteracted by

- ✓ **High phosphate content**
- ✓ **Low concentration of sulphur containing amino acids such as methionine and cysteine that allow the caseins to be reasonably water soluble.**

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So they do have lot of apolarity in them and that in turn is influenced to the protein. That is this hydrophobicity of the casein hydrophobicity of the casein is due to the apolarity of the casein molecule that is the high ratios of apolar amino acids in which we have seen that valine, leucine, isoleucine, phenylalanine and proline they do call high ratios of hydro apolarity right, and apolarity ranges between 35 to 45 percent that is almost 50-50 is the polar and non polar right, and because of this non polarity or apolarity they do react or they do behave like hydrophobic in nature right.

Now this hydrophobicity can be encountered by high phosphate content if there be if there be high phosphate content because phosphate is a hydrophilic right, phosphate group is hydrophilic very much hydrophilic. So that will encounter the hydrophobicity if that is present. So to into to nullify the hydrophobicity lot of phosphate groups if can be it if lot of phosphate group it can be present in the protein then the hydrophobicity of the protein can be reduced that is you are that one way to counter the hydrophobicity or low concentration of sulphur containing amino acids such as methionine and cysteine that allow casein to be reasonably soluble in water right.

So, if low concentration of sulphur containing amino acids are also present for example, they are present we have seen in sulphur in methionine and cysteine. So if small quantity of methionine and cysteines are also there then also that can that can counter the hydrophobicity or hydrophobic nature of the protein and it will not repel water to that extent we have seen it is around 35 to 45 percent apolarity that is hydrophobic or non polar systems in protein right.

Caseins susceptibility to proteolysis is due to the

- **lack of secondary and tertiary structures of (α_{s1} casein, casein, β casein).**

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So we come to then that the caseins susceptibility to proteolysis is due to the lack of secondary and tertiary structures of alpha casein and beta casein alpha s 1 casein and beta casein because of them they do not have the secondary and tertiary structures so much. So that is one of the reason why the proteolysis that is break down of protein proteolysis is breakdown of protein that take or casein that casein is susceptible to proteolysis, that is breakdown of the casein is due to the fact that the alpha casein alpha s 1 casein rather because we have not only alpha, but alpha s 1 and alpha s 2. So out of that alpha s 1

casein and beta casein they do have less or they are lacking secondary and tertiary structure. So that is why the hydro that is why the proteolysis of casein is possible right.

α-Caseins

α _{s1} -casein	α _{s2} - casein
<ul style="list-style-type: none"> 199 amino acids and is app. 23.6 kDa. highest charge of all the casein molecules. Consists of at least eight phosphoserine units. It has 17 proline residues that ultimately disrupt the formation of secondary structures such as α - helices and β - sheets. 	<ul style="list-style-type: none"> 207 amino acids and is app 25.4 kDa. least hydrophobic casein molecule. Several genetic variants, contain between 10 and 13 phosphoserine units. Contains two cysteine residues. It exists as a dimer in milk.

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Now what is alpha s 1 casein or alpha casein right, so alpha caseins means alpha s 1 and alpha s 2 caseins. So if you look at alpha s 1 casein this alpha s 1 casein is having 199 amino acids and is approximately around 23.6 kilo dalton in length right, size molecular size is 23.6 kilo Dalton. They, now like normally we say the lengths in millimetre, metre, then micrometre, then nanometre.

But these molecules are being said in terms of Dalton and they are around 23.6 kilo Dalton in alpha casein around this is average around 199 amino acids are present highest charge of all the casein molecules. So it has maximum charge in all the casein molecules and that is the reason why we said that when you have the freshly drawn milk it may have the acidity and some acidity may be impacted by this casein molecules right. So it has high charge that is alpha s 1 casein, this consist of least 8 phosphoserine units right, least 8 phosphoserine units it has 17 proline residues that ultimately disrupt the formation of secondary structure such as alpha helices beta sheets.

So alpha helices beta sheets are secondary structure so because of the 17 proline residues, they do not allow the formation of the secondary structures like alpha helices or beta sheets, this is the reason why alpha casein is not soluble or is just we will before this slide we had said in the previous slide, that they are hydrophobic right, the hydrophobicity is because of that said they are or they can be easily that is dissociated or

degraded or denatured because of the non presence of the secondary and tertiary structures right alpha s 1 casein whereas alpha s 2 casein.

α -Caseins	
α_{s1} -casein	α_{s2} - casein
<ul style="list-style-type: none"> □ 199 amino acids and is app. 23.6 kDa. □ highest charge of all the casein molecules. □ Consists of at least eight phosphoserine units. □ It has 17 proline residues that ultimately disrupt the formation of secondary structures, such as α - helices and β - sheets. 	<ul style="list-style-type: none"> □ 207 amino acids and is app. 25.4 kDa. □ least hydrophobic casein molecule. □ Several genetic variants, contain between 10 and 13 phosphoserine units. □ Contains two cysteine residues. □ It exists as a dimer in milk.

They are consisting of 207 amino acids and their average size is around 25.4 kilo Dalton, which was 23.6 kilo Dalton in alpha s 1 here it is 25.4 kilo Dalton. So least hydrophobic casein molecule, so the hydrophobicity in casein that is beta alpha s 2 casein is the minimum hydrophobicity that is it is not that bad with the water system or polar system right.

So it has least hydrophobicity amongst all the protein amongst all the forms of the proteins that is alpha s 1 alpha s 2 beta and kappa casein right, and it has several genetic variants contain between 10 to 13 phosphoserine units it contains between 10 to 13 phosphoserine units whereas, in alpha s 1 it was around 17 phosphoserine units right and it contains 2 cysteine residues right, it exists as a dimer in milk right not as a monomer or polymer it exists in milk as a dimer that is 2 molecules are associated as dimer right.

β-Casein

- β-casein consists of 209 amino acids and is approximately 24 kDa.
- It is the most hydrophobic casein molecule.
- There are six known genetic variants that contain between zero and five phosphoserine units.
- Similar to α_{s1}-casein, β-casein has few secondary structures due to the presence of 35 proline residues.

α_{s1} β

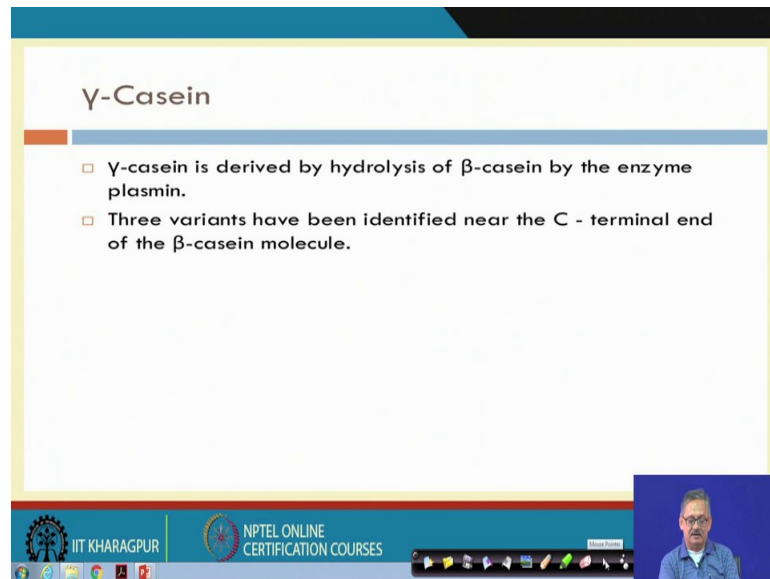
So looking at alpha s 1 and alpha s 2 casein we then say that beta casein that is other type of the casein that contains around 209 amino acids, and approximate size is around 24 kilo Dalton right, it has the most hydrophobic casein molecule it has the most hydrophobic casein molecule the hydrophobicity if you remember just sometime back we said is primarily due to alpha s 1 and beta casein right, and beta casein has the highest or most hydrophobicity amongst the caseins variety right.

So there are 6 known genetic variants that contain between 0 and 5 phosphoserine units right. So phosphoserine units are becoming less much much lower, alpha casein was 17 s alpha s 1 was 17 alpha s 2 was between 10 to 13, and this one less than around 5 or 6 around 5 6 numbers of your not around 5 0 to 5 0 to 5 phosphoserine units are there.

Similar to alpha s 1 casein beta casein has few secondary structures due to the presence of 35 proline residues right. So we said that the degradation or the proteolysis of the protein is primarily due to the lack of the presence of your secondary and tertiary structures in alpha s 1 casein and beta casein. So here also we see that it has a little beta casein has a little few secondary structures which may help to sustain which may help to resist a little against the proteolysis right.

γ-Casein

- γ-casein is derived by hydrolysis of β-casein by the enzyme plasmin.
- Three variants have been identified near the C - terminal end of the β-casein molecule.



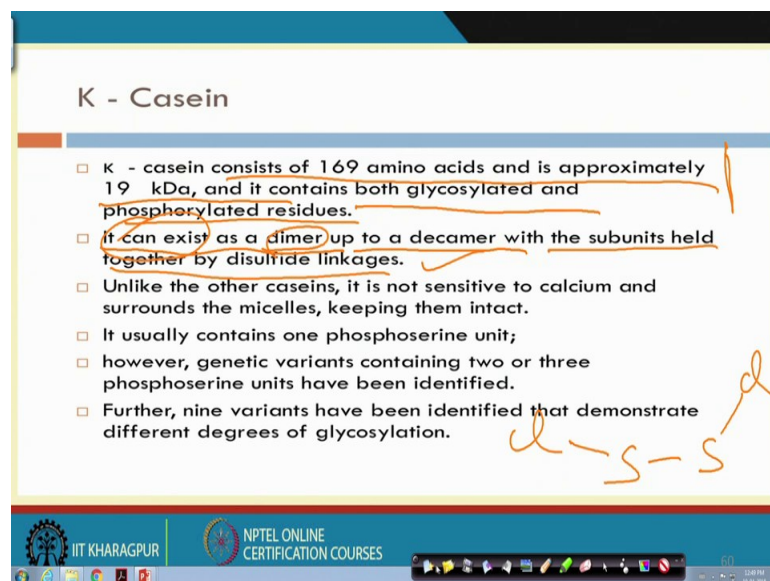
So the third one is gamma casein ok alpha s 1, alpha s 2, beta that is alpha casein beta casein gamma casein right. So gamma casein is derived if we do not take pen then it will go to the next page, so alpha casein is derived by hydrolysis of beta casein by the enzyme plasmin by the enzyme called plasmin. So alpha casein is hydrolysed 2 beta casein gamma casein is derived by hydrolysis of beta casein by the enzyme called plasmin.

So from beta casein gamma casein is produced and that is by the hydrolysis with the help of the enzyme called plasmin, and 3 variants have been identified near the carbon terminal and of the beta casein molecule right. So there could be 3 such variations of the gamma casein.

K - Casein

- K - casein consists of 169 amino acids and is approximately 19 kDa, and it contains both glycosylated and phosphorylated residues.
- It can exist as a dimer up to a decamer with the subunits held together by disulfide linkages.
- Unlike the other caseins, it is not sensitive to calcium and surrounds the micelles, keeping them intact.
- It usually contains one phosphoserine unit;
- however, genetic variants containing two or three phosphoserine units have been identified.
- Further, nine variants have been identified that demonstrate different degrees of glycosylation.

d-s-s-d









Casein Micelle

Molecules of Casein + Minerals (Calcium phosphate)

Composition:

- **93% caseins : 4 phosphoproteins**
 - α_{s1} - CN : 36%
 - α_{s2} - CN : 10%
 - β - CN : 34%
 - κ - CN : 12%
- **7% : colloidal mineral complex containing phosphate, calcium, magnesium and citrate**










Now, if we look at then we kappa casein that is what is remaining that kappa casein is what that kappa casein consists of 169 amino acids and approximately around 19 kilo Dalton, approximately around 19 kilo Dalton is the size and it contains both glycosyl and phosphoryl that is it is glycosylated and phosphorylated residues it contains right. So glycosyl and phosphoryl these 2 groups so that means, it is glycosylated as well as phosphorylated. So it contains both the glycosylated and phosphorylated residues right.

Now, this is the unit of the kappa casein it consists of it consists of it or it can exist it can exist as a dimer up to a up to a decamer with the subunits held together by disulfide linkage right disulfide linkage the other day we had said that this is like this right. So we gave it to be with chlorine and many others this is the simplest s 2 l, c l 2 right, simplest disulfide, which we can think of or which we can give example. So that disulfide linkage is subunits held together by the disulfide linkage right.

K - Casein

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- Further, nine variants have been identified that demonstrate different degrees of glycosylation.

Then unlike the other caseins it is not sensitive to calcium and surrounds the micelles keeping them intact. So micelles we have already said earlier now again it may be in the subsequent class we caseins now we are again in the milk protein. So in the casein so casein micelle may be a little we can highlight, but earlier we have said. So unlike the other caseins it is not sensitive to calcium and surrounds the micelle keeping them intact it usually contains 1 phosphoserine unit.

However, genetic variants containing 2 or 3 phosphoserine units have been identified, further 9 variants have been identified that demonstrate different degrees of glycosylation right.

So we have come across with different causes why proteins are hydrophobic in nature, which component of the protein, alpha, beta, gamma and kappa out of which alpha s 1 and alpha s 2, how they are behaving what are the reason how many number of aminoacids are present all these we have discussed right. So next we may be going to the casein micelle.

Thank you.