

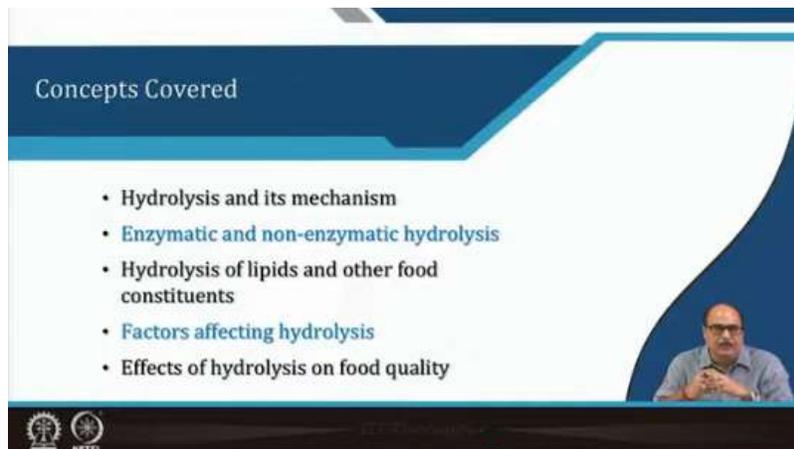
# FOOD SCIENCE AND TECHNOLOGY

## Lecture13

### Lecture 13: Hydrolytic Reactions in Foods



Hello everyone, namaskar. We are now in module 3, which is on major chemical processes in food. In lecture 13 today, we will discuss hydrolytic reactions in food.



Concepts we shall talk about include what is hydrolysis and its mechanism, then enzymatic and non-enzymatic hydrolysis. We shall also talk about hydrolysis of lipids and other constituents of food, and then what are the various factors which govern hydrolytic processes in foods and the effect of hydrolytic reactions on food quality.

## Hydrolysis

- Hydrolytic reactions in food products involve the breakdown of chemical bonds in the presence of water.
- These reactions are significant in food processing, preservation, and digestion, as they can lead to changes in texture, flavor, and nutritional value.
- Hydrolysis is a chemical reaction where a water molecule interacts with a compound, leading to the breakdown of that compound into two or more products.

$$AB + H_2O \rightarrow AH + BOH$$

- In hydrolysis, the water molecule ( $H_2O$ ) splits into two parts
  - ✓ a hydrogen ion ( $H^+$ ) and a hydroxide ion ( $OH^-$ )
- These ions are essential in breaking bonds in compound.

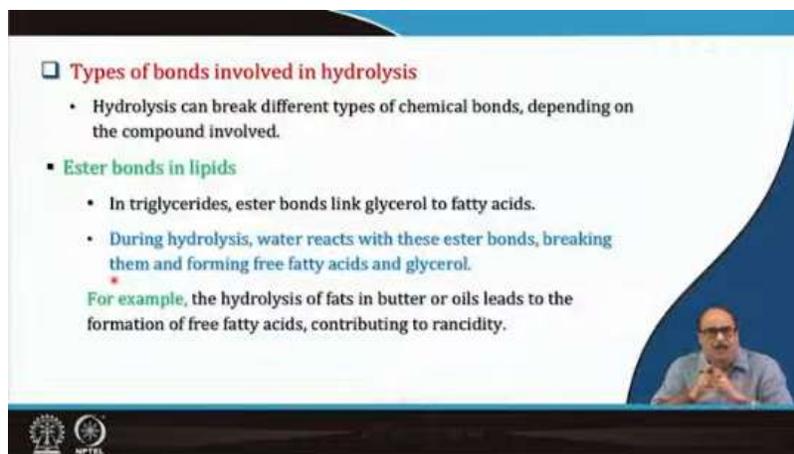



So, let us see what is hydrolysis. You know, hydrolytic reactions in food products basically involve the breakdown of chemical bonds in the presence of water. Hydro, lysis. Hydro means water and lysis means breaking, breakdown. And these reactions are significant in food processing, preservation, and digestion, as they can lead to changes in the texture, flavor, and nutritional value of the food. And many times, many food constituents during the digestion process, there are various hydrolytic bonds, the other bonds, etcetera, are broken down by hydrolysis into smaller components, etcetera. and then these help in the process of digestion, as well as they help in the processing and preservation of foods. Hydrolysis is a chemical reaction where a molecule, as you can see here in this reaction, interacts with a compound, leading to the breakdown of the compound into two major products. Like, for example, you can say here AB is a compound, you can say, and that is  $H_2O$ , ok,  $H_2O$  is a water molecule. So, when it reacts in the hydrolysis process, then  $H_2O$  is ionized, it splits into two parts like H ion (hydrogen ion) and hydroxide ion ( $OH^-$  ion). Then, this H ion and OH ions again break down the AB, and it forms that AH reacts with A, and you can say OH reacts with B. That is this. Positive and negative ions again interact with their counterparts, alright. That is, the positive ion will interact with the negative ion, the negative ion with the positive ion, and  $AH + BOH$ . These two compounds now split into  $AH + BOH$ . So, these are essential in breaking down the compound, that is, hydrogen ions and hydroxide ions.

**Mechanism of hydrolysis**

- ❖ **Water as a reactant**
  - In hydrolysis, a water molecule is added to a chemical bond, typically breaking the bond.
  - The water molecule splits into a hydrogen ion ( $H^+$ ) and a hydroxide ion ( $OH^-$ ).
  - These ions then attach to the fragments of the original molecule, leading to the breakdown of larger molecules into smaller ones.
- ❖ **Catalysis**
  - Enzymes or acidic/alkaline conditions often catalyze hydrolytic reactions in food.
  - Enzymes like proteases, amylases, and lipases specifically target proteins, carbohydrates, and fats, respectively, to facilitate hydrolysis.

So, for this reaction to take place, that is the mechanism of how this hydrolysis takes place. There are two important components: one is the presence of water; water itself acts as a reactant and the other part is the catalyst. Water as a reactant, as I told you in the earlier slide, in hydrolysis, that is, a water molecule is added to a chemical bond, typically breaking the bond. The water molecule splits into H ion and OH ion, and these ions then attach to the fragments of the original molecule, leading to the breakdown of a larger molecule into a smaller one. So, this is here that water is causing or giving the process. At the same time, in this, water also acts as a reactant in the hydrolysis process. Then there are various catalysts like enzymes, acid, alkali, and such other conditions which can catalyze hydrolytic reactions in foods. Like, for example, enzymes like proteases, amylases, and lipases significantly target respectively the protein, carbohydrate and fat molecules, and their hydrolysis is facilitated. So, these enzymes act as catalysts. Similarly, there are many others which we will take up later. That is, the other catalysts which can catalyze, increase, or allow those reactions to continue.



**Types of bonds involved in hydrolysis**

- Hydrolysis can break different types of chemical bonds, depending on the compound involved.
- Ester bonds in lipids**
  - In triglycerides, ester bonds link glycerol to fatty acids.
  - During hydrolysis, water reacts with these ester bonds, breaking them and forming free fatty acids and glycerol.

For example, the hydrolysis of fats in butter or oils leads to the formation of free fatty acids, contributing to rancidity.

The types of bonds which are involved in hydrolysis, that is, even hydrolysis can break different types of chemical bonds, depending upon the compound involved. Like, it can break ester bonds, it can break peptide bonds, it can break glycosidic bonds, or it can break other bonds as well, such as covalent bonds and so on. So, if you take the example of ester bonds in lipids, in fact, in triglycerides, this is the ester bond which links the glycerol to fatty acids. That is, a triglyceride has three fatty acids connected to one glycerol molecule, and there are three ester linkages. So, during hydrolysis, water reacts with these ester bonds and breaks them into three fatty acids. The fatty acid which was connected with the glycerol with the ester linkage, the ester linkage is broken, then fatty acids are released, and you can get in the system diglycerides, monoglycerides, or even if there is complete hydrolysis, all three free fatty acids are released, and you can get only the free fatty acids plus the glycerol in the system. That is, the hydrolysis of fats in butter or oil leads to the formation of free fatty acids, and these free fatty acids are sometimes responsible for the rancidity in butter or ghee and such products.

**Types of bonds involved in hydrolysis**

- Hydrolysis can break different types of chemical bonds, depending on the compound involved.
- Ester bonds in lipids**

Hydrolysis of triglycerides produces fatty acids which react with a base to form soap molecules.

You can see here, pictorially, these reactions; this is the formula of a triglyceride where these three fatty acids are connected to the three OH groups of glycerol, alright. Then, when water is the reactant in the hydrolysis reaction, it may be heat or pressure or other catalysts that may also be involved. So, finally, it gives glycerol plus fatty acids.

And, these fatty acids, in fact, they are present in ghee and all those things, they may be responsible for some peculiar flavour or other flavours because, after hydrolysis in the fat or ghee, the flavour of the fatty acids becomes evident. But if there is an alkaline medium present, if sodium hydroxide is the catalyst or the alkaline medium, then these fatty acids combine with sodium hydroxide, alright. And then, they form the sodium salt of the respective fatty acid. Here, NaOH is broken into Na plus OH, and Na interacts with the fatty acid, and OH interacts with H. So, you can get H<sub>2</sub>O and the sodium salt of the respective fatty acids, and that is nothing but soap. This is how soaps are formed in the industry.

**Types of bonds involved in hydrolysis (Contd...)**

- Glycosidic bonds in carbohydrates**

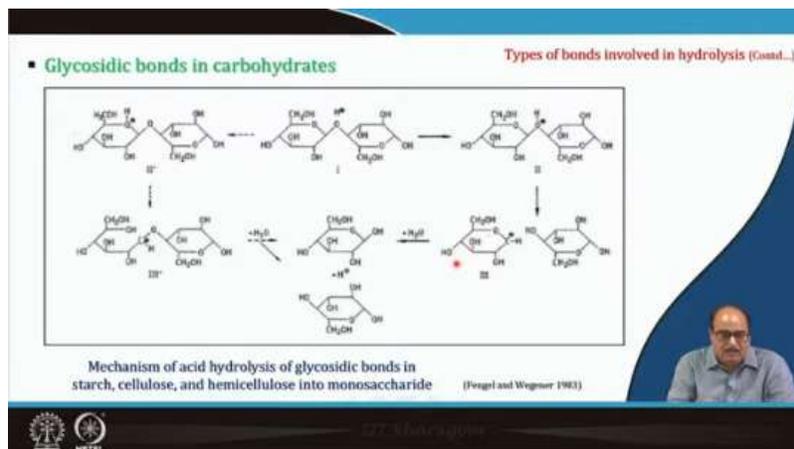
- ✓ Glycosidic bonds connect monosaccharide units in carbohydrates like starch or cellulose.
- ✓ Hydrolysis breaks these bonds, resulting in simpler sugars.
- ✓ Enzymes like amylases catalyze the hydrolysis of starch into maltose or glucose.

For example the digestion of dietary starch involves breaking it down into glucose, which the body can absorb.

(Frogs) and Wegner 1983)

Then, the breaking up of glycosidic bonds in carbohydrates during the hydrolysis process, ok. You know that it is the glycosidic bond; maybe we will discuss it when we discuss macronutrients, etcetera. Then, it is the various monosaccharide units; They are joined with the glycosidic linkages into disaccharides, trisaccharides, tetrasaccharides, finally, oligosaccharides, and then polysaccharides. Then here, now hydrolysis again breaks these glycosidic bonds, alright. Like when the two molecules of two monosaccharides join together, then again one molecule of water is released, and you get a disaccharide.

So, again when water breaks down these two, you will get two respective monosaccharides, and that water will be involved. These break down, resulting into the simpler. So, enzymes like amylases catalyze the hydrolysis of starch into maltose or glucose. Even the digestion of dietary starch, as I told you, is the hydrolysis by these enzymes, etcetera, which break down into glucose, which the body can easily absorb because the body cannot absorb starch or other forms of polysaccharides, etcetera. So, it has to be broken down by enzymes, and normally the hydrolysis reaction takes place in our system, the digestion process, and glucose is released, and then glucose is finally utilized.



So, here you can say that is the glycosidic, this pictorial presentation mechanism of hydrolysis of glycosidic bonds in starch, cellulose, hemicellulose, and its conversion finally into the monosaccharide, respective monosaccharide. Because in starch, cellulose, hemicellulose, etcetera, all these mostly the constituent monosaccharide unit is, This, glucose; and the glucose, it is linked with either alpha 1-4 linkage or beta 1-6 linkage or

alpha 1-6 linkage or different types of beta 1-4, alpha 1-6, and all those things. So, these various linkages, etcetera, are there and which Linkage may be that these different enzymes, respective enzymes like amylases, glucoamylases, and others, etcetera, that are, they interact, and water, as you see, that water is a different form; sometimes it can add to the anomeric hydroxyl group, sometimes water molecules can add directly.

With this oxygen, which is there in the cycle of that monosaccharide unit. Ultimately, that water is taking part in the reactions, and the water is splitting, and the resultant is that the glycosidic bond is broken and the resultant monosaccharide is released. So, you can see how it happens, okay. Then, similarly, peptide bonds, like monosaccharides, they are joined in the form of glycosidic bonds and give rise to disaccharides, tetrasaccharides.

Types of bonds involved in hydrolysis (Contd...)

- **Peptide bonds in proteins**
  - Peptide bonds connect amino acids in proteins.
  - Hydrolysis breaks these bonds, either into smaller peptides or individual amino acids.
  - Proteases, such as pepsin or trypsin, catalyze these reactions by cleaving specific peptide bonds.
  - ✓ For example, in food processing, protein hydrolysates are created by hydrolyzing proteins into smaller peptides or amino acids, improving their solubility and digestibility.

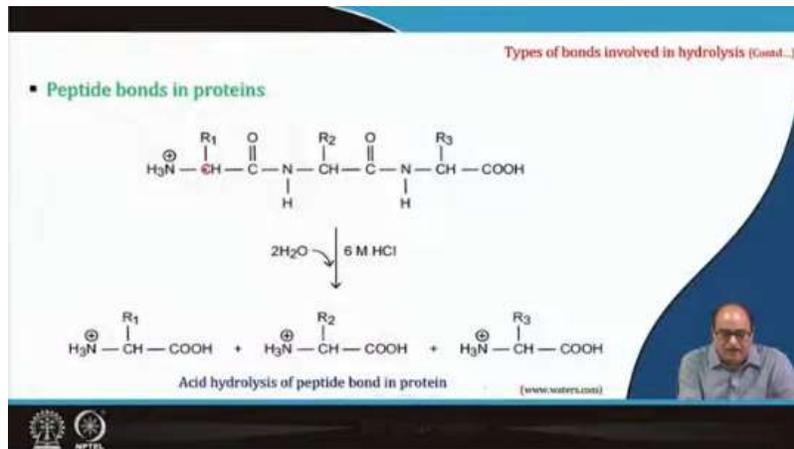
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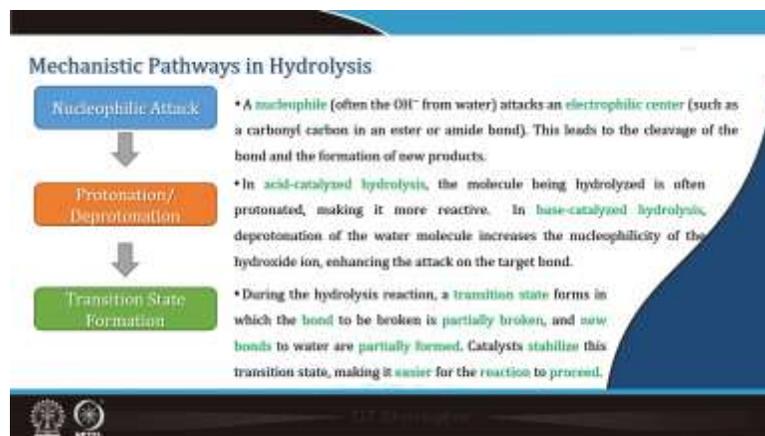
Similarly, different amino acids, these amino acids, they combine together with peptide linkages, and these amino acids are dipeptides, tripeptides, and tetrapeptides. Tetrapeptide means there are four amino acid residues, and they are joined with three peptide linkages. If there is, you see, in this manner, there are polypeptides; polypeptide means there with the N minus one number of peptide linkages.

So, hydrolysis breaks down these peptide linkages, peptide bonds, and therefore, it may convert bigger peptides into smaller peptides or even, ultimately, if there is complete hydrolysis, it may finally convert the peptide into amino acids. So, proteases, that is, the protein-splitting enzymes like pepsin, trypsin, etc., catalyze these reactions by cleaving specific peptide bonds, like, for example, in food processing. Protein hydrolysates are

created by hydrolyzing proteins into smaller peptides or amino acids, which improves the solubility as well as the digestibility of these respective peptides.



So, you can see here: this is the peptide bond; like this is one amino acid; and CONH, this is the peptide bond; there is another amino acid. So, there is another CONH, which is the peptide bond, alright. So, this is either in the alkaline solution, acidic solution, or even neutral pH, that is, water. It breaks down, and the peptide bond is broken down, that is, you can get NH<sub>2</sub> and then COOH. The OH joins here COOH, and this NH is combined here. So, you can get here COOH, and this is the additional if you already have NH<sub>2</sub> then. So, this is how peptide bonds are broken.



So, let us talk about the mechanism, that is, obviously, all these mechanistic pathways in hydrolysis, like there is a nucleophilic attack, as you could see in the mechanism diagram where the various polysaccharides, trisaccharides, or oligosaccharides, when they. So, a nucleophile, often the hydroxyl group from water, attacks an electrophilic center, such as

a carbonyl carbon in an ester or amide bond, and this leads to the cleavage of the bond and the formation of a new product. So, that is the first mechanism, and then there may also be protonation or deprotonation. So, in this protonation or deprotonation, what happens? In acid-catalyzed hydrolysis, the molecule undergoing hydrolysis is often protonated, making it more reactive. In base-catalyzed hydrolysis, deprotonation of the water molecule increases the nucleophilicity of the hydroxide ion, thereby enhancing the attack on the target molecule.

And then, the third pathway may be the transition state formation. In the transition state formation, what happens? During the hydrolysis reaction, a transition state forms, in which the bond to be broken is partially broken, and new bonds to water are partially formed. And then, catalysts, which may be various different types, stabilize this transition state, making it easier for the reaction to proceed. So, these are the three mechanistic pathways in hydrolysis.

**Enzymatic and non-enzymatic hydrolysis**

- While hydrolysis can occur spontaneously under certain conditions, it often requires a catalyst to proceed efficiently, which can be enzymes (enzymatic hydrolysis) or acid/base catalysis (non-enzymatic hydrolysis).
- **Enzymatic hydrolysis**
  - Specificity**
    - Enzymes like proteases, amylases, and lipases are highly specific, meaning they target specific bonds within a molecule. For example, lipases target ester bonds in lipids, while proteases target peptide bonds in proteins.
  - Mechanism**
    - Enzymes lower the activation energy required for hydrolysis enhancing reaction rate.
    - They often do this by stabilizing the transition state of the reaction or by bringing the reactants (water and the compound) into close proximity in an optimal orientation.

Now, let us discuss enzymatic and non-enzymatic hydrolysis, that is, while hydrolysis can occur spontaneously under certain conditions, it often requires, as I told you earlier also, a catalyst to proceed efficiently, and the catalyst may be enzymes or it may be acids, bases, or such other catalysts. Enzyme, that is, accordingly, the hydrolytic reactions may be, if the catalyst is an enzyme, it is called enzymatic hydrolysis. If the catalyst is acid, base, heat, or such other factor, it is called non-enzymatic hydrolysis.

So, the enzymatic hydrolysis, as the enzymes are the catalyst here, and enzymes are basically proteins and they are very specific in their action, and therefore, enzymes like

proteases, amylases, lipases are highly specific, meaning amylases will always break only the polysaccharide linkages, etcetera, lipases will always break only the ester linkages, proteases will only hydrolyze the peptide linkages. They target the specific bonds within a molecule, okay. Their mechanism, as discussed earlier, is that of enzymes. Basically, what they do is they lower the activation energy required for the hydrolysis, enhancing the reaction rate, and they often do this by stabilizing the transition state of the reaction or by bringing the reactants, that is, the water and that compound, into close proximity in an optimal condition or in an optimal orientation.

■ **Non-enzymatic hydrolysis**

**Acidic conditions**

- In acid-catalyzed hydrolysis, a proton ( $H^+$ ) from an acid helps to break bonds by adding to the molecule being hydrolyzed, making the bond more susceptible to nucleophilic attack by water.

For example, the hydrolysis of starch in acidic conditions can produce dextrans and maltose.

**Alkaline conditions**

- In base-catalyzed hydrolysis, the hydroxide ion ( $OH^-$ ) from a base acts as a nucleophile, attacking the bond in the molecule.

An example is the saponification of fats, where an alkali (like NaOH) hydrolyzes the ester bonds in triglycerides, forming soap (salts of fatty acids) and glycerol.

The slide features a blue and white color scheme with a small video inset of a man in a blue shirt speaking in the bottom right corner. Logos for institutions are visible in the bottom left corner.

Then, non-enzymatic hydrolysis means acid hydrolysis or alkaline hydrolysis. In acid-catalyzed hydrolysis, as you can see, a proton from an acid helps to break the bond by adding to the molecule being ionized. This proton is added to the molecule, that is, there is a protonation of the molecule which is being hydrolyzed, making the bond more susceptible to nucleophilic attack by water.

Like hydrolysis of starch in acidic conditions can produce dextrans, maltose, etcetera, by this protonation process under acidic conditions. Similarly, in the alkaline condition, non-enzymatic hydrolysis means that the hydroxide ion in the alkali acts as a catalyst. So, the hydroxide ion there, formed from a base, acts as a nucleophile, and attacks the bond in the molecule. For example, in the saponification reaction of fat, which we have seen earlier, an alkali like sodium hydroxide is involved. It hydrolyzes the ester bond into triglycerides, and then this alkali reacts with the free fatty acids, forming the sodium salt of the respective fatty acid as well as releasing glycerol into the system.

## Hydrolysis of food constituents

Enzymatic v/s Non-enzymatic hydrolysis

Aspect	Enzymatic hydrolysis	Non-enzymatic hydrolysis
▪ Catalyst	✓ Specific enzymes (protease, lipase, amylase)	✓ Acid, base, or heat
▪ Specificity	✓ Highly specific	✓ Low specificity
▪ Conditions	✓ Mild (e.g. physiological pH, moderate temperature)	✓ Harsh (e.g. extreme pH, high temperature)
▪ Control	✓ Controlled and predictable	✓ Less controlled, more random
▪ Applications	✓ Food processing, digestion, protein hydrolysates	✓ Industrial hydrolysis, amino acid analysis, flavour enhancement
▪ Product quality	✓ High, with minimal amino acid degradation	✓ Variable, with possible amino acid degradation



So, here we talk about the hydrolysis of food constituents. And then again, enzymatic versus non-enzymatic hydrolysis in this table I have given: what is the aspect of enzymatic hydrolysis and non-enzymatic hydrolysis. So, a comparison between these two. So, number one: catalyst. So, in enzymatic hydrolysis, as you could see earlier in the examples, it is very specific, involving proteases, lipases, amylases, etcetera. Whereas, in non-enzymatic hydrolysis, the catalysts are not very specific; they may be acid, base, or sometimes heat or other catalysts.

Then, as regards the specificity of the process, enzymatic hydrolysis is highly specific, whereas non-enzymatic hydrolysis may have low specificity. Conditions for enzymatic hydrolysis may be mild, like physiological pH, moderate temperature, etcetera, which are suitable for enzymatic hydrolysis. Whereas, in non-enzymatic hydrolysis, it may take place under harsh conditions like extreme pH, high temperature, etcetera. As far as the control of these reactions is concerned, enzymatic hydrolysis can be controlled and is predictable. Whereas, non-enzymatic hydrolysis is comparatively less controlled and more random in nature.

So, if you take examples like enzymatic hydrolysis in food processing, in digestion, protein hydrolysates formation, etcetera. Whereas, non-enzymatic hydrolysis is the case in many industrial processing of protein, natural hydrolysis, amino acid analysis, flavor enhancement, etcetera. Then, the product quality, if we talk about that, is the enzymatic hydrolytic: They are high in quality, good in quality, with minimal amino acid degradation, like, even if you can say, the example of the hydrolysis in which it takes,

that is, the proteins are digested in our system. So, and it is enzymes are there, given there is no change in the amino acid, etcetera, their quality: racemization, etcetera, is not there.

So, the amino acid nutritional value quality, etcetera, are there. Whereas, in acid hydrolysis or alkaline hydrolysis, there may be significant racemization of the amino acid, and their optical properties may be changed, and there may be other variations, etcetera, other changes might be there. So, this is variable and with possible amino acid degradation and those things in non-enzymatic hydrolysis.

**Enzymatic hydrolysis of proteins**

- **Specific enzymes:** Proteases or peptidases causes hydrolysis of the peptide bonds.

Protease	Examples	Site of action	Products
• Endopeptidases	✓ Trypsin, chymotrypsin	✓ Within protein chain	✓ Large peptide fragments.
• Exopeptidases	✓ Carboxypeptidase, aminopeptidase	✓ At ends of protein chain	✓ Individual amino acids or dipeptides.

The diagram illustrates the enzymatic hydrolysis of proteins. It shows a protein chain being broken down by endopeptidases into large peptide fragments, and then by exopeptidases into individual amino acids or dipeptides.

Protein → Endopeptidases → Peptides → Exopeptidases → Peptides acid from amino acids, dipeptides

So, the enzymatic hydrolysis of protein: if you see that the proteases are peptidases, cause hydrolysis of the peptide bonds, and there may be the proteases may be endopeptidases or exopeptidases. So, examples of endopeptidases are trypsin or chymotrypsin. Their action site is within the protein chain, you can say here that it is endopeptidase, this is the protein chain. So, inside the protein chain or within the protein chain, they can interact. Like these are the sites. So, here these are acting, and from this position, they are breaking down the protein and basically, their product is large, very large peptide; they are broken into again comparatively large peptide fragments. So, exopeptidases: they are normally carboxypeptidases or aminopeptidases, and they interact normally at the end of the protein chain, and individual amino acids or dipeptides, etc., are formed by their action.

❑ **Enzymatic hydrolysis of proteins**

- **Specific enzymes:** Proteases or peptidases causes hydrolysis of the peptide bonds.

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Mechanism	Action
• Nucleophilic attack	✓ Nucleophilic amino acid residues (e.g. Serine, cysteine) in active site attack carbonyl carbon of peptide bond to form tetrahedral intermediate.
• Transition state stabilization	✓ Enzyme stabilizes intermediate, facilitating breakage of peptide bond



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Then, the mechanism of these enzymes: they can again act both by nucleophilic attack as well as transition state stabilization. In the nucleophilic attack, the nucleophilic amino acid residue like serine, cysteine, etc., in the active site attacks the carbonyl carbon of the peptide bond to form the tetrahedral intermediate. Whereas, in the transition state stabilization, these enzymes, that is, they stabilize the intermediates, facilitating the breakage of the peptide bonds.

❑ **Acid hydrolysis of proteins**

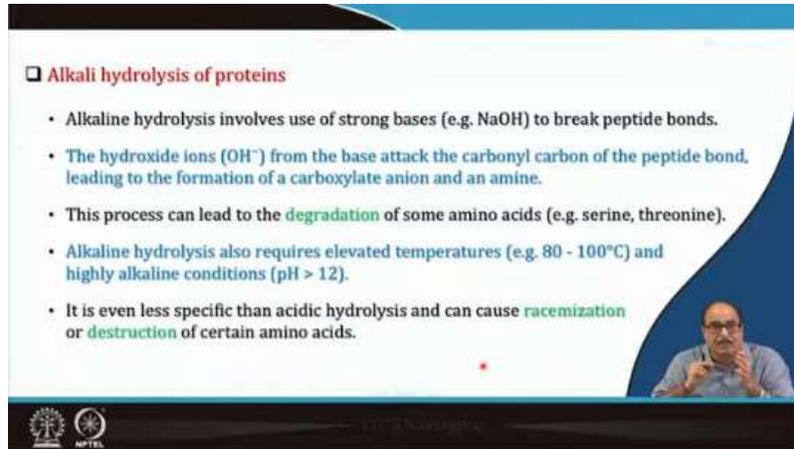
- Non-enzymatic hydrolysis occurs without involvement of enzymes & typically requires harsher conditions.
- It is less specific & often used in industrial processes or laboratory settings.
- The high  $H^+$  ion concentration protonates carbonyl oxygen of peptide bond, making it more susceptible to nucleophilic attack by water.
- Peptide bond is cleaved as water adds to protonated carbonyl carbon, forming free amino acids.
- Acidic hydrolysis usually occurs at elevated temperatures (e.g. 100-110°C) and under highly acidic conditions (pH < 2).
- The process is less specific than enzymatic hydrolysis, leading to the complete breakdown of the protein into its constituent amino acids.



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Acid hydrolysis of the protein, if we talk about it, is a non-enzymatic hydrolysis. It occurs, as we discussed earlier, without the involvement of enzymes, and it typically requires harsher conditions. It is less specific and often used in industrial processes or laboratory settings. The high hydrogen ion concentration protonates the carbonyl oxygen of peptide bonds, making it more susceptible to nucleophilic attack by water. The peptide bond is cleaved as water adds to the protonated carbonyl carbon, forming free amino acids. Acid hydrolysis usually occurs at elevated temperatures, like 100 to 110 °C, or

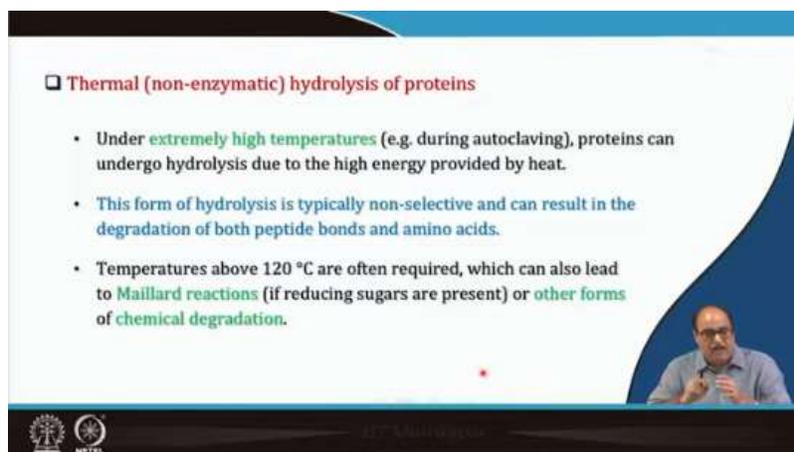
under highly acidic conditions, like a pH less than 2 or so. The process is less specific than enzymatic hydrolysis, and it leads to the complete breakdown of the protein into its constituent amino acids.



**Alkali hydrolysis of proteins**

- Alkaline hydrolysis involves use of strong bases (e.g. NaOH) to break peptide bonds.
- The hydroxide ions ( $\text{OH}^-$ ) from the base attack the carbonyl carbon of the peptide bond, leading to the formation of a carboxylate anion and an amine.
- This process can lead to the **degradation** of some amino acids (e.g. serine, threonine).
- Alkaline hydrolysis also requires elevated temperatures (e.g. 80 - 100°C) and highly alkaline conditions (pH > 12).
- It is even less specific than acidic hydrolysis and can cause **racemization** or **destruction** of certain amino acids.

Similarly, if you talk about alkaline hydrolysis of the protein, a strong base like sodium hydroxide breaks the peptide bond here. So, hydroxide ions from the base attack the carbonyl carbon atom of the peptide bond, leading to the formation of carboxylate and an amine and this process can lead to the degradation of some amino acids like serine, threonine, etc. Alkaline hydrolysis also requires elevated temperatures, maybe 80 to 100 °C, and highly alkaline conditions, with a pH of more than 12. So, it is even less specific than acid hydrolysis and causes racemization and/or destruction of certain amino acids.

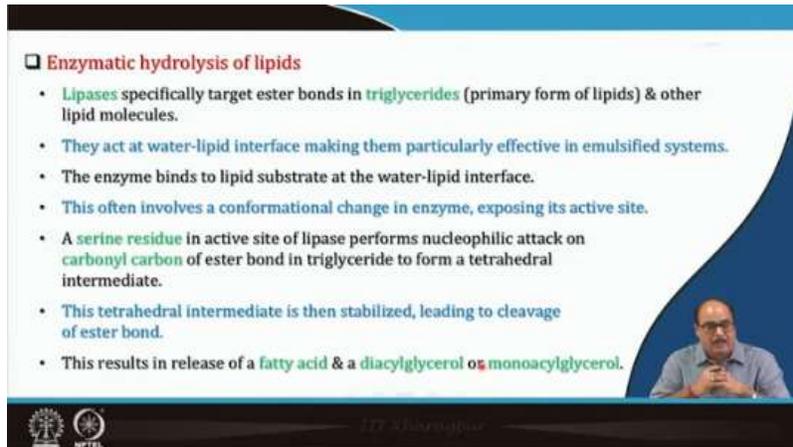


**Thermal (non-enzymatic) hydrolysis of proteins**

- Under **extremely high temperatures** (e.g. during autoclaving), proteins can undergo hydrolysis due to the high energy provided by heat.
- This form of hydrolysis is typically non-selective and can result in the degradation of both peptide bonds and amino acids.
- Temperatures above 120 °C are often required, which can also lead to **Maillard reactions** (if reducing sugars are present) or **other forms of chemical degradation**.

Then, thermal or non-enzymatic hydrolysis of protein under extreme conditions. During autoclaving, etc., the proteins can undergo hydrolysis due to the high energy provided by

heat and this form of hydrolysis is typically non-selective and can result in the degradation of both peptide bonds as well as amino acids. Temperatures above 120 °C are often required, which can also lead to Maillard reactions if reducing sugars are present in the system or other forms of chemical degradation.



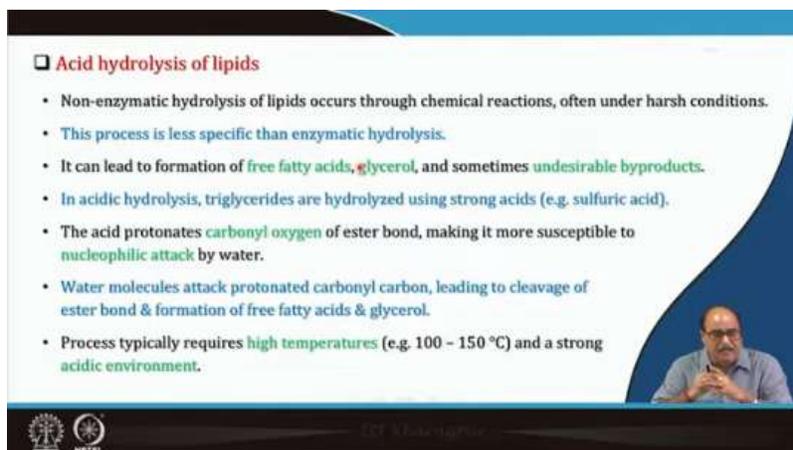
**Enzymatic hydrolysis of lipids**

- **Lipases** specifically target ester bonds in **triglycerides** (primary form of lipids) & other lipid molecules.
- They act at **water-lipid interface** making them particularly effective in emulsified systems.
- The enzyme binds to lipid substrate at the water-lipid interface.
- This often involves a conformational change in enzyme, exposing its active site.
- A **serine residue** in active site of lipase performs nucleophilic attack on **carbonyl carbon** of ester bond in triglyceride to form a tetrahedral intermediate.
- This tetrahedral intermediate is then stabilized, leading to cleavage of ester bond.
- This results in release of a **fatty acid** & a **diacylglycerol** or **monoacylglycerol**.





Then, similarly, if you take the enzymatic hydrolysis of the lipid, like triglycerides, the primary form of lipids and other lipid molecules, they act at the water-lipid interface, making them particularly effective in emulsion systems. The enzyme binds to the lipid substrate at the water-lipid interface, and this often involves a conformational change in the enzyme, exposing its active site. A certain residue in the active site of the enzyme lipase performs a nucleophilic attack on the carbonyl group of the ester bond in the triglyceride, and it forms a tetrahedral intermediate. This tetrahedral intermediate is then stabilized, leading to the cleavage of the ester bond and this results in the release of fatty acids and diacylglycerol or monoacylglycerol and so on.



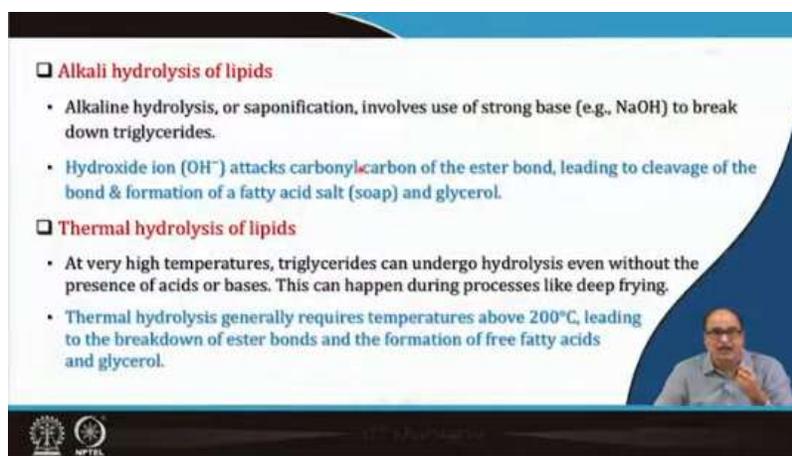
**Acid hydrolysis of lipids**

- Non-enzymatic hydrolysis of lipids occurs through chemical reactions, often under harsh conditions.
- This process is less specific than enzymatic hydrolysis.
- It can lead to formation of **free fatty acids**, **glycerol**, and sometimes **undesirable byproducts**.
- In acidic hydrolysis, triglycerides are hydrolyzed using strong acids (e.g. sulfuric acid).
- The acid protonates **carbonyl oxygen** of ester bond, making it more susceptible to **nucleophilic attack** by water.
- Water molecules attack protonated carbonyl carbon, leading to cleavage of ester bond & formation of free fatty acids & glycerol.
- Process typically requires **high temperatures** (e.g. 100 - 150 °C) and a strong **acidic environment**.





Similarly, acid hydrolysis of lipids: in the non-enzymatic hydrolysis, when through chemical reactions often under harsh conditions, the reaction is less specific; it can lead to the formation of free fatty acids, glycerol, and sometimes even undesirable byproducts may also be formed in acid hydrolysis. Triglycerides are hydrolyzed. Strong acids like sulfuric acid, etc., may be used, and the acid protonates the carbonyl oxygen of the ester bond, making it more susceptible to nucleophilic attack by water. Water molecules attack the protonated carbonyl carbon, leading to the cleavage of the ester bond and the formation of free fatty acids and glycerol. So, this process again typically requires high temperatures, sometimes even 100 to 150 °C, and strong acidic conditions.



**Alkali hydrolysis of lipids**

- Alkaline hydrolysis, or saponification, involves use of strong base (e.g. NaOH) to break down triglycerides.
- Hydroxide ion (OH<sup>-</sup>) attacks carbonyl carbon of the ester bond, leading to cleavage of the bond & formation of a fatty acid salt (soap) and glycerol.

**Thermal hydrolysis of lipids**

- At very high temperatures, triglycerides can undergo hydrolysis even without the presence of acids or bases. This can happen during processes like deep frying.
- Thermal hydrolysis generally requires temperatures above 200°C, leading to the breakdown of ester bonds and the formation of free fatty acids and glycerol.

The slide also features a small video inset of a man in a light blue shirt speaking, and logos for IIT Bombay and NPTEL at the bottom left.

Then, in alkaline hydrolysis: Hydroxide ions attack the carbonyl group of the ester bond, leading to the cleavage of the bond, the formation of free fatty acids and glycerol, or the salts of the free fatty acids like soap. Then, the thermal hydrolysis of lipids. At very high temperatures, triglycerides can undergo hydrolysis even without the presence of acids or bases, and this can happen during the process of deep fat frying. Thermal hydrolysis generally requires temperatures above 200 °C or so, leading to the breakdown of the ester bond and the formation of free fatty acids and glycerol.

**Enzymatic hydrolysis of carbohydrates**

- Amylases like  $\alpha$ -amylase &  $\beta$ -amylase break down starches into smaller units like maltose and glucose.
- $\alpha$ -Amylase randomly cleaves internal  $\alpha$ -1,4 glycosidic bonds.
- $\beta$ -amylase cleaves at the non-reducing ends, producing maltose.
- Cellulases break down cellulose (structural polysaccharide in plant cell walls), into glucose.
- Cellulases specifically target  $\beta$ -1,4 glycosidic bonds.
- The carbohydrate substrate binds to the active site of the enzyme.
- An amino acid residue in the enzyme (often a carboxyl group from aspartic or glutamic acid) acts as a nucleophile, attacking the glycosidic bond.
- The enzyme stabilizes transition state, facilitating cleavage of glycosidic bond.




Then, similarly, let us talk about the enzymatic hydrolysis of carbohydrates. Like alpha-amylase, beta-amylase, etc., they break down starches into smaller fractions like maltose and glucose. Alpha amylases randomly cleave internal alpha 1,4 glycosidic linkages, whereas beta amylases cleave at the non-reducing ends producing maltose. Cellulose breaks down; cellulase enzyme breaks down the cellulose, which is the structural polysaccharide in plant cell wall structure, into glucose. Here, the cellulases especially target the beta 1,4 glycosidic bonds. The carbohydrate substrate binds to the active site of the enzyme, and the amino acid residue in the enzyme, which is often a carboxylic group from aspartic or glutamic acid, aspartic or glutamic acid. It acts as a nucleophile by attacking the glycosidic bond, and this enzyme stabilizes the transition state, facilitating the cleavage of the glycosidic bonds.

**Acid hydrolysis of carbohydrates**

- Non-enzymatic hydrolysis occurs through chemical means, typically high temperatures or extreme pH.
- This method is less specific than enzymatic hydrolysis.
- Strong acids (e.g. hydrochloric acid) are used to hydrolyze glycosidic bonds in carbohydrates.
- The acid protonates oxygen in glycosidic bond, making it more susceptible to cleavage.
- Water acts as nucleophile, attacking carbon in glycosidic bond and breaking the bond.

**Alkal hydrolysis of carbohydrates**

- Strong bases (e.g. sodium hydroxide) can also hydrolyze glycosidic bonds.
- However, this method is less common for carbohydrate hydrolysis due to potential degradation of the sugar molecules.
- The hydroxide ion ( $\text{OH}^-$ ) attacks the glycosidic bond, leading to its cleavage.




Then, acid hydrolysis of the carbohydrates. Again, non-enzymatic hydrolysis is typically less specific than enzymatic hydrolysis. Hydrochloric acid, sulfuric acid, etc., are used to

hydrolyze the glycosidic bonds. The acid protonates the oxygen in glycosidic bonds and makes it more susceptible to cleavage. Water acts as a nucleophile, attacking the carbon in the glycosidic bond and breaking the bond. Similarly, alkali hydrolysis of the carbohydrate involves strong bases like sodium hydroxide, etc., which hydrolyze glycosidic bonds. However, this method is less common for carbohydrate hydrolysis due to the potential degradation of the sugar molecule. The hydroxide attacks the glycosidic bond, that is, particularly the OH ion, leading to its cleavage.

**Usefulness of hydrolysis in food industry**

Constituent	Applications	
	Enzymatic	Non-enzymatic
• Protein	<ul style="list-style-type: none"> <li>✓ To modify functional properties</li> <li>✓ To enhance flavor</li> <li>✓ To produce protein hydrolysates</li> </ul>	<ul style="list-style-type: none"> <li>✓ To produce amino acid mixtures</li> <li>✓ To prepare hydrolyzed vegetable proteins (HVP) (flavor enhancers)</li> </ul>
• Lipids	<ul style="list-style-type: none"> <li>✓ To modify fats &amp; oils</li> <li>✓ To enhance flavors in cheese, margarine &amp; shortening</li> </ul>	<ul style="list-style-type: none"> <li>✓ To produce soap</li> <li>✓ To produce food additives (fatty acids &amp; glycerol)</li> </ul>
• Carbohydrate	<ul style="list-style-type: none"> <li>✓ To modify starches</li> <li>✓ To produce high-fructose corn syrup</li> <li>✓ To produce fermentable sugars for brewing &amp; baking</li> </ul>	<ul style="list-style-type: none"> <li>✓ To produce soap</li> <li>✓ To produce glucose syrup, invert sugar</li> <li>✓ To break polysaccharides</li> </ul>

So, here we have discussed the usefulness of hydrolysis in the food industry, like protein hydrolysis, which is an enzymatic process. It is normally useful in modifying functional properties, enhancing flavor, or producing protein hydrolysates, etc. Whereas, non-enzymatic hydrolysis may be used to produce amino acids and mixtures from proteins or to prepare hydrolyzed vegetable protein (HVP), which is often used as a flavor enhancer. Similarly, in lipids, enzymatic hydrolysis may modify fats and oils. It may enhance the flavor of cheese and margarine, as well as shorten it. Non-enzymatic hydrolysis is used to produce soaps industrially, as well as to produce food additives like fatty acids, glycerol, etcetera. Then, in carbohydrate systems, enzymatic hydrolysis is used to modify starches to produce high fructose corn syrup and fermentable sugars for brewing, baking, etcetera. Non-enzymatic hydrolysis is used to produce soap again, to produce glucose syrup, invert sugars, etcetera, or to break down polysaccharides.

### Factors affecting hydrolysis

- **Temperature**
  - ✓ Higher temperatures generally increase the rate of hydrolytic reactions, though extreme temperatures can denature enzymes involved in catalysis.
- **pH**
  - ✓ The acidity or alkalinity of the environment can affect the activity of enzymes and the rate of hydrolysis.
  - ✓ Different enzymes have optimal pH ranges (e.g. pepsin works best in acidic conditions, while trypsin works best in alkaline conditions).
- **Water activity**
  - ✓ The availability of water is crucial for hydrolytic reactions.
  - ✓ Low water activity can slow down or prevent these reactions, which is a principle used in food preservation techniques like drying.




So, the factors which affect the hydrolytic process are temperature. High temperature generally increases the rate of hydrolytic reactions, although extreme temperatures can denature enzymes involved in catalysis. pH again; the acidity or alkalinity of the environment can affect the activity of the enzymes and the rate of hydrolysis. Different enzymes have optimal pH ranges; like pepsin works best in acidic conditions, while trypsin works best in alkaline conditions. Similarly, water activity and the availability of water are crucial for hydrolytic reactions. Low water activity can slow down or prevent these reactions, which is a principle used in preservation techniques like drying, etcetera; in that, water activity is reduced.

### Effect of hydrolysis on food quality (Contd.)

- **Texture changes**
  - ✓ Hydrolysis can alter the texture of foods. For example, hydrolyzed proteins can lead to a softer texture in meat products.
- **Flavor development**
  - ✓ Hydrolytic reactions can produce flavor compounds, such as the savory flavors from amino acids or the sweet flavors from sugars.
  - ✓ Hydrolyzed vegetable proteins (HVP): In the production of HVP, proteins are hydrolyzed into amino acids & smaller peptides, creating savory flavors used as seasonings.
- **Nutritional changes**
  - ✓ Hydrolysis can improve digestibility, making nutrients more accessible.  
For instance, the hydrolysis of starches into simpler sugars during cooking can make carbohydrates easier to digest.




Then texture changes: like, hydrolysis can alter the texture of food, such as hydrolyzed proteins leading to a softer texture in meat or a softer texture in meat. Then flavour development: hydrolytic reactions can produce flavour compounds, such as savoury flavours from amino acids or sweet flavours from sugars, etcetera. Hydrolyzed vegetable

protein, that is, in the production of HVP, proteins are hydrolyzed into amino acids and smaller peptides, which actually create savoury flavours, etcetera, and is used as a seasoning. Then there are also: hydrolysis can improve, as I told you earlier, digestibility, making nutrients more susceptible.

Effect of hydrolysis on food quality (Contd...)

- **Starch gelatinization**
  - ✓ During cooking, water and heat cause starch granules to swell and undergo hydrolysis, breaking down glycosidic bonds and forming a gel.
  - ✓ This process is key in baking and cooking rice or pasta.
- **Retrogradation**
  - ✓ Upon cooling, the hydrolyzed starch can re-associate, leading to a firm texture.
  - ✓ This is what happens when bread stales.
- **Cheese ripening**
  - ✓ *Proteolysis*: In cheese, proteolysis involves the hydrolysis of casein proteins by proteases, leading to texture changes and flavor development.
  - ✓ *Lipid hydrolysis*: Lipases break down milk fat into free fatty acids, which contribute to the characteristic flavors of aged cheese.

Hydrolysis of starch into simpler sugars during cooking can make carbohydrates easier to digest. So, there are also hydrolytic reactions that are useful, there may be some nutritional changes or good changes in the characteristics of the product. Starch gelatinization during cooking: water and heat cause starch granules to swell and undergo hydrolysis, breaking down glycosidic bonds and forming a gel. This process is key during the baking process, as well as in cooking rice or pasta, etcetera. Similarly, after cooking, when it is cooled down, it retrogrades, the starch retrogrades, like the forms which were broken in the gelatinization process. They are reformed, reassembled, and lead to giving a firm texture. And this is what happens. That is, retrogradation is the main reaction responsible for the staling of bread. Then cheese ripening: in cheese ripening, there may be proteolysis, which involves the hydrolysis of casein protein by proteases, leading to structural changes and flavor development. Lipid hydrolysis may occur; these lipases break down milk fat into free fatty acids, which contribute to the characteristic flavor of aged cheese, and so on.

Summary

- Hydrolytic reactions are fundamental to both natural processes and industrial applications in the food industry.
- They influence everything from food texture and flavor to nutritional content and shelf life.
- Both enzymatic and non-enzymatic hydrolysis of food constituents play crucial roles in different contexts.
- Enzymatic hydrolysis being the more common and controlled process in biological and industrial applications.

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So, finally, I would like to summarize this lecture by saying that yes, hydrolytic reactions are very important reactions in food. They are fundamental to both natural food and naturally processed food, or even they are exploited industrially to have very good or advantageous effects in food processing. They influence everything, from food texture, flavor, nutritional value, and shelf life, etc.; both non-enzymatic and enzymatic reactions; even the hydrolyzed food constituents play a crucial role in different contexts. Many times, they are advantageous, but mind it, sometimes they may become the hydrolytic products, etc., the products of hydrolysis of various constituents may react with other food components, etc., and sometimes they may lead to the formation of some undesirable compounds, and so on. In fact, hydrolysis reactions are a good many times. They are sometimes bad also. So, we need to take proper care; that is, it has to be properly understood. You have to see what the food composition is, what the various constituents are, what the situation, temperature, pH, and other environmental conditions are to which it is exposed or likely to be exposed during various operations, and accordingly, one should take care. The conditions should be set in such a way that if required, these reactions should be accelerated, or if not required, these reactions should be decelerated or stopped.

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So, these are the references for this lecture.



**THANK YOU !**

Thank you very much. Thank you for your presence here.