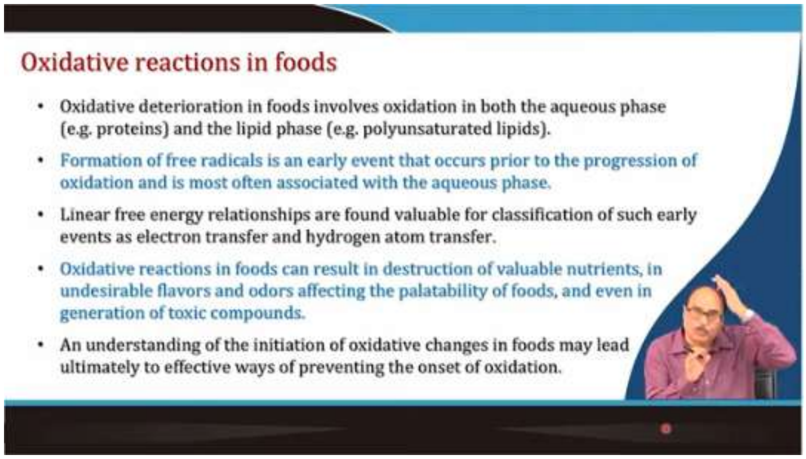
FOOD SCIENCE AND TECHNOLOGY

Lecture12

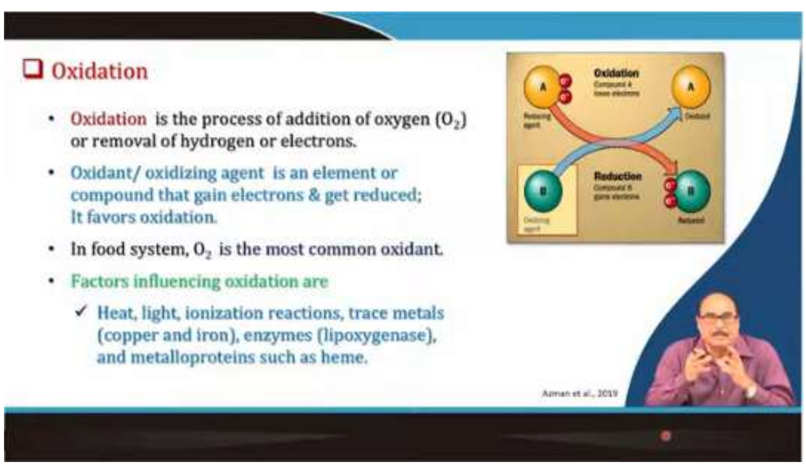
Lecture 12: Oxidative Reactions in Foods

Hello everyone, Namaskar. In this 12th lecture today, we shall study the oxidative reactions in foods.

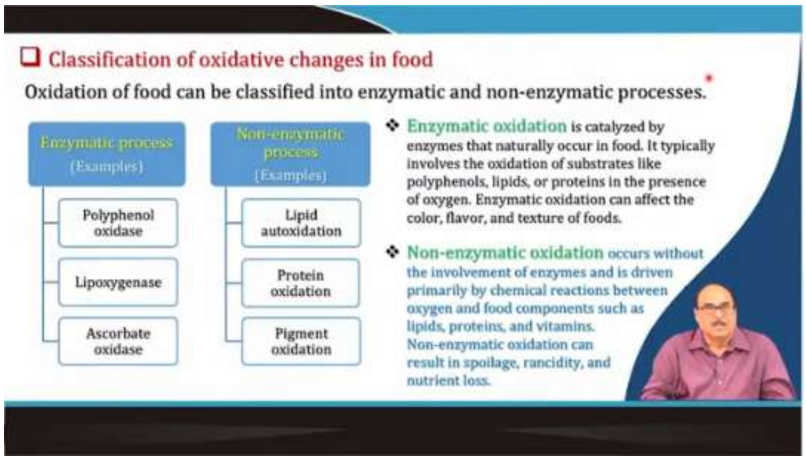
Here we will talk about what oxidation is, what the different oxidative reactions in food are, protein denaturation, lipid oxidation, enzymatic browning, ascorbic acid oxidation, and also the oxidation reactions leading to the discolouration or decolouration of pigments.



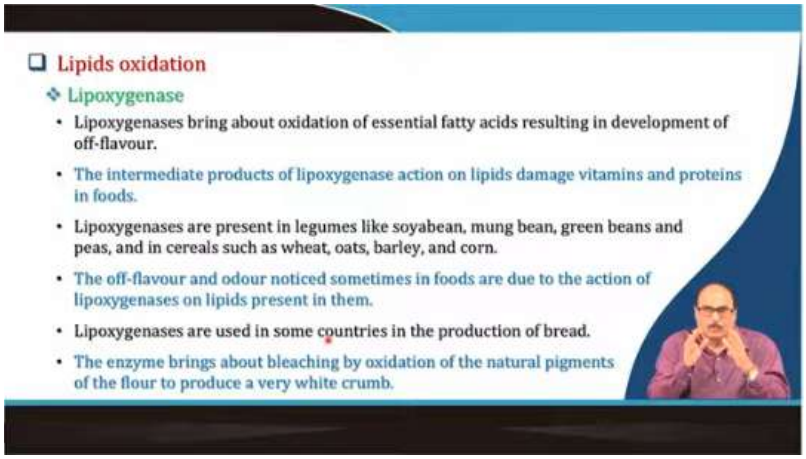
So, you know, these oxidative deteriorations in food involve oxidation in both the aqueous phase, like in the case of proteins, etc., as well as in the lipid phase, like polyunsaturated lipids. The formation of free radicals is the early event that occurs prior to the progression of oxidation. And it is more often associated with the aqueous phase. So, linear free energy relationships are found valuable for the classification of such early events as electron transfer and hydrogen atom transfer. So, oxidative reactions in food can result in the destruction of valuable nutrients, undesirable flavours, or other factors affecting the palatability of food, or they can also lead to the generation of toxic compounds. So, an understanding of the initiation of these oxidative changes in foods and, finally, what the end products of oxidation are, etc., may ultimately lead to effective ways of preventing the onset of oxidation reactions.



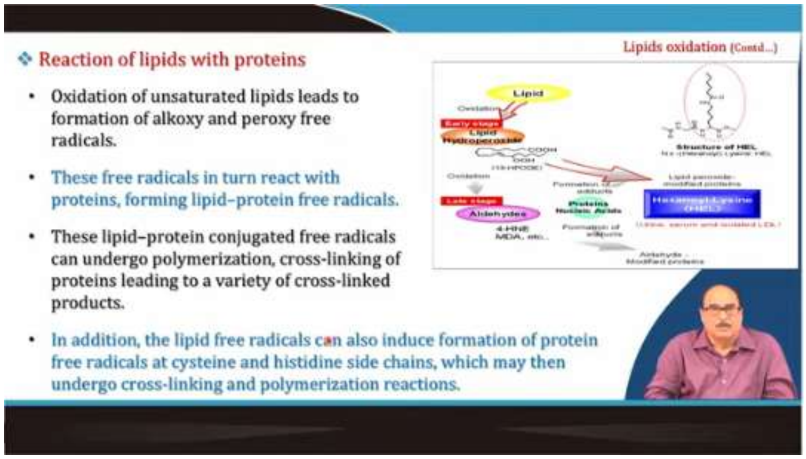
So, let us understand what oxidation is. As you can see here in this process, it has been said that oxidation is the process of the addition of oxygen or the removal of hydrogen from a product. The oxidant or oxidizing agent is an element or compound that gains electrons and gets reduced. And it favors the oxidation process, that is, the oxidation of compound A, which loses the electron and gets oxidized. In the food system, O2 is the most common oxidant. The various factors that lead to the oxidation process or encourage oxidation include heat, light, ionization, radiation, then trace metals like copper and iron, even enzymes like lipoxygenases, and metalloproteins such as heme, etc. They influence the rate of oxidation reactions in foods.



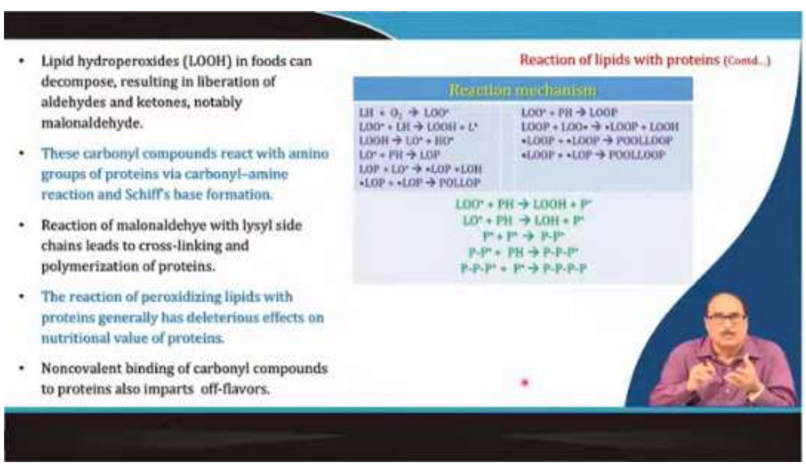
The oxidative changes in the food may be of two types. One may be enzymatic oxidation, and the other may be non-enzymatic oxidation processes. The enzymatic oxidation is catalyzed by the enzymes that naturally occur in food. It typically involves the oxidation of substrates like polyphenols by polyphenol oxidase enzymes, lipids by lipoxygenase enzymes, or ascorbic acid by ascorbic acid oxidase, etc. So, enzymatic oxidation can affect the color, flavor, and texture of foods. The other type of oxidation may be non-enzymatic oxidation, which occurs without the involvement of enzymes and is driven primarily by chemical reactions between oxygen and food components such as lipids, proteins, and vitamins. These non-enzymatic oxidation reactions can result in the spoilage of the food. It may result in the generation of rancidity, that is, auto-oxidation, etc., and also in the nutrient loss of the food. So, lipid auto-oxidation, protein oxidation, pigment oxidation, etc., are examples of non-enzymatic oxidation processes.



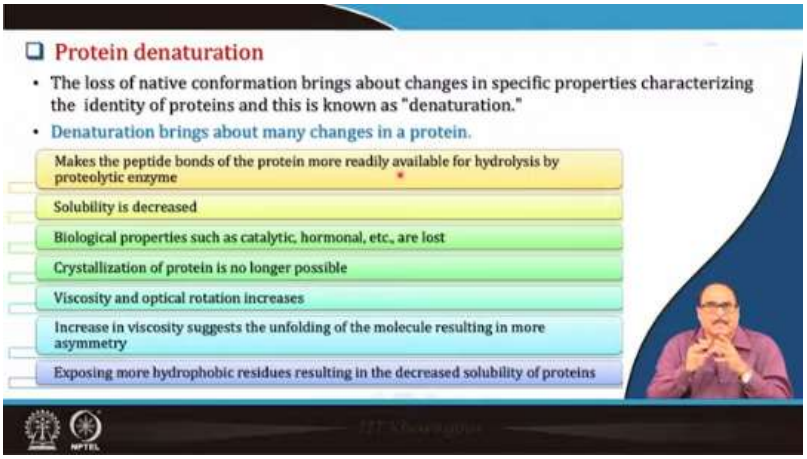
So, let us understand a little bit deeper about one of them. First, we will talk about lipid oxidation and lipid oxidation by means of lipoxygenase. So, what does lipoxygenase do? Lipoxygenase adds oxygen to the lipid moiety, particularly where there is an unsaturated linkage. It brings about the oxidation of unsaturated fatty acids, resulting in the development of off-flavours. So, intermediate products of lipoxygenase action on lipids damage the vitamins and proteins in foods. Lipoxygenase is present in legumes like soybean, mung bean, green beans, peas, and other cereals like wheat, oat, barley, corn, and so on. So, they add oxygen and then promote the oxidation of these lipids. The off-flavours and colours sometimes noticed in foods are due to the action of lipoxygenase on lipids present in them, and in such a case, the development of any flavour in soybeans is mainly because of the action of the lipoxygenase enzyme. So, lipoxygenase is used in some countries in the production of bread, alright. So, the enzyme brings about bleaching by oxidation of natural pigments in the flour to produce a very white crumb. So, that is also useful. Sometimes, lipoxygenase is used for useful purposes to produce white crumb bread.



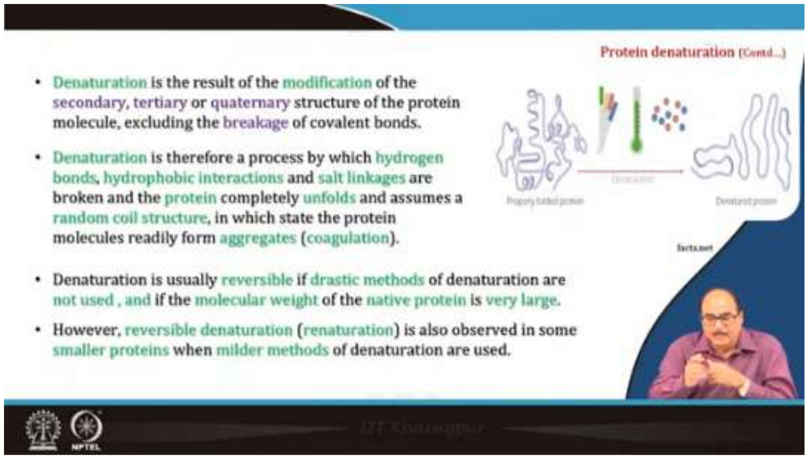
So, then the reactions of lipids with proteins. Oxidation of unsaturated lipids leads to the formation of alkoxy and peroxy free radicals, as you can see here, it is shown in this picture, and these free radicals, in turn, react with the protein, forming lipid-protein free radicals. These lipid-protein conjugated free radicals can undergo polymerization and cross-linking of proteins, leading to a variety of cross-linked products. And in addition, the lipid-free radicals can also induce the formation of protein-free radicals at cysteine and histidine side chains, which may then undergo cross-linking and polymerization reactions. So, a series of reactions may take place here.



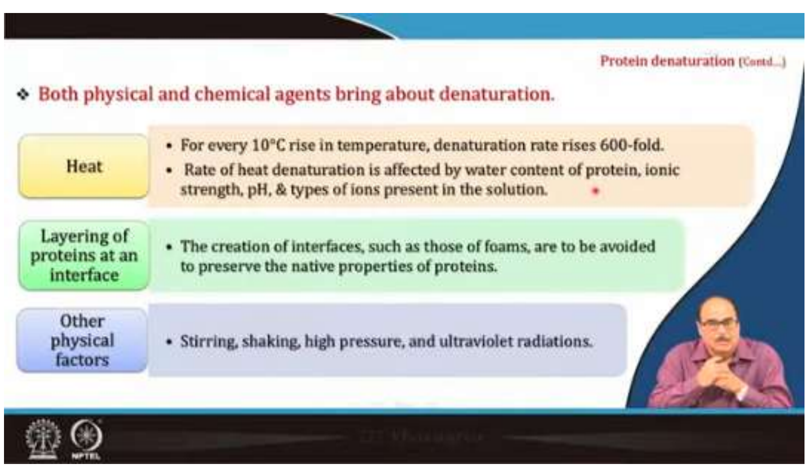
So, lipid hydroperoxide, which is the result of autoperoxidation in this process, is formed. These hydroperoxides in food can decompose, resulting in the liberation of aldehydes, ketones, notably malonaldehyde, etc. The auto-oxidation process is shown here in this picture, the reaction mechanism, etc. You can see that the lipid molecule combines with oxygen, giving a LO•, and again the lipid molecule forms lipid hydroperoxide. A lipid moiety free radical is formed, and then these reactions continue in a series. Once initiated, it proceeds through auto-oxidation, propagation, and finally termination. In the end, various compounds are formed, and these carbonyl compounds can react with the amino group of proteins through carbonyl-amine reactions or Schiff's base formation, etc. So, reactions of malonaldehyde with lysyl side chains lead to the cross-linking and polymerization of proteins. The reactions of peroxidizing lipids with proteins generally have a deleterious effect on the nutritional value of proteins. Non-covalent binding of carbonyl compounds to proteins also imparts flavor. So, these are the various ways that lipoxygenase mediates autoxidation and oxidation of lipids.



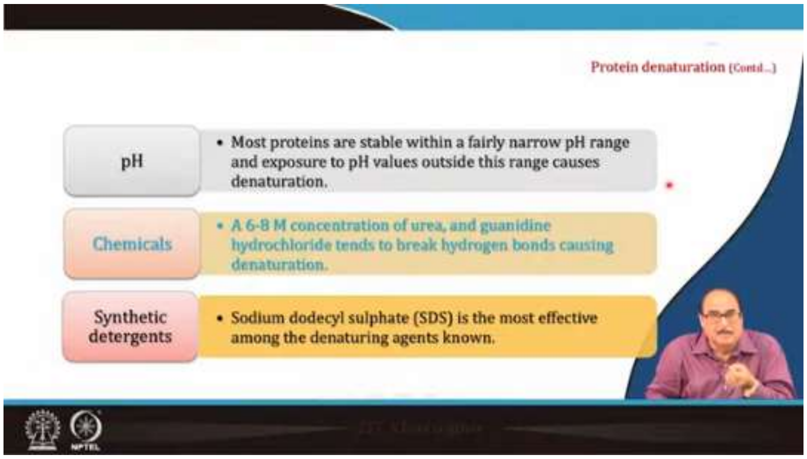
The other important oxidation reaction is the denaturation of proteins. The loss of native conformation brings about changes in the specific properties that characterize the protein's identity. This change in the protein's original network structure is known as denaturation. Denaturation means the organized three-dimensional structure of the protein is disturbed. But mind it, there is no breakage of the covalent linkage. There is no breakage of the peptide linkage. So, denaturation brings about many changes in a protein. For example, it makes the peptide bond of the protein more readily available for hydrolysis by proteolytic enzymes. The solubility of the protein is decreased as a result of denaturation. Biological properties such as catalytic and hormonal properties of the protein are destroyed when it is denatured. Crystalline properties are lost like crystallization of the protein is no longer possible. Viscosity and optical rotation increase. An increase in the viscosity suggests the unfolding of the molecule, resulting in more asymmetry and exposing more hydrophobic residue, resulting in the decreased solubility of proteins.



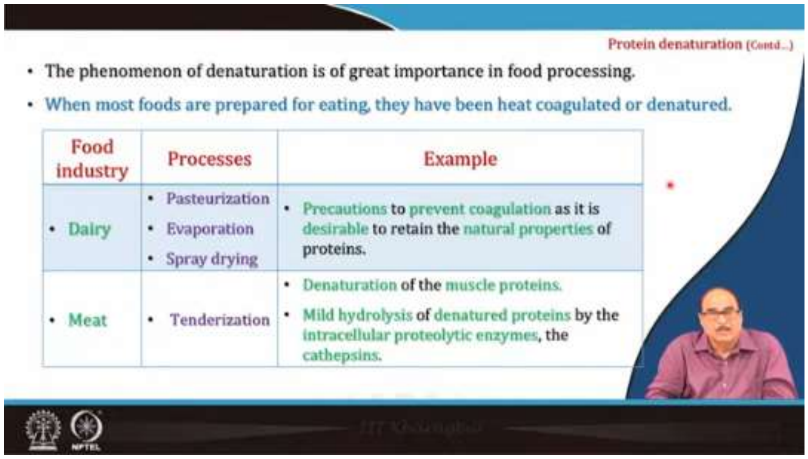
Denaturation, as you can see, is any protein that has an organized, purely organized, three-dimensional structure, and then when it is denatured that is the organized structure is disturbed. It is a result of modification of the secondary, tertiary or quaternary structure of the protein molecule, extending the breakage of covalent bonds, not the peptide bonds. Denaturation is, therefore, a process by which hydrogen bonds, hydrophobic interactions, salt linkages, etc., are broken, and the protein completely unfolds and assumes a random coil structure in which the protein molecules readily form aggregate or coagulate. As you can see, when you boil an egg, then that is it becomes solid, that is, protein denatures. Denaturation is usually reversible if drastic methods of denaturation are not used and if the molecular weight of the native protein is very large. However, reversible denaturation, which is also sometimes known as renaturation, is also observed in some smaller proteins when milder methods of denaturation are used.



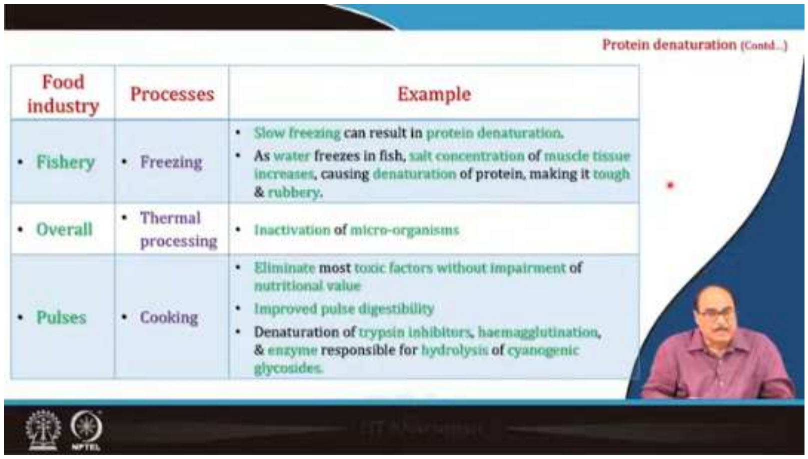
So, denaturation is influenced by many agents, like both chemical and physical agents, which affect the denaturation. Heat is one of the very important reactions. For every 10-degree Celsius rise in temperature, the denaturation rate increases by almost 600-fold. The rate of heat denaturation is affected by the water content of the protein, ionic strength, pH, and types of ions present in the solution. The layering of protein at an interface, such as the creation of foams, should be avoided to preserve the native properties of the protein. Other physical factors, like stirring, shaking, high pressure, ultraviolet radiation, etc., influence the denaturation process.



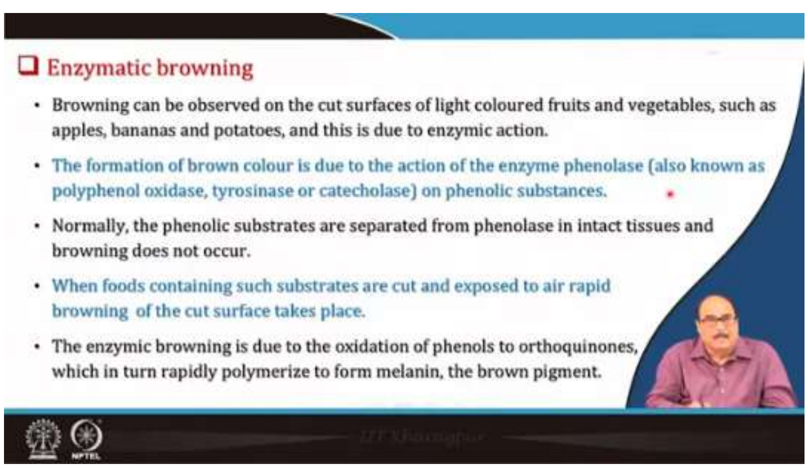
The pH is another important factor. That is, most proteins are stable within a fairly narrow pH range. Exposure to pH values outside this range causes their denaturation. Among the chemicals, even a 6 to 8 molar concentration of urea and guanidine hydrochloride tends to break the hydrogen bonds, causing denaturation of the protein. Because, in this organized three-dimensional structure, the structure is held in place by various hydrogen bonds, covalent bonds, and other bonds. So, these are broken down in the denaturation. And then, among the synthetic detergents, sodium dodecyl sulfate, SDS, is considered to be the most effective. synthetic chemicals, which brings about the denaturation of proteins.



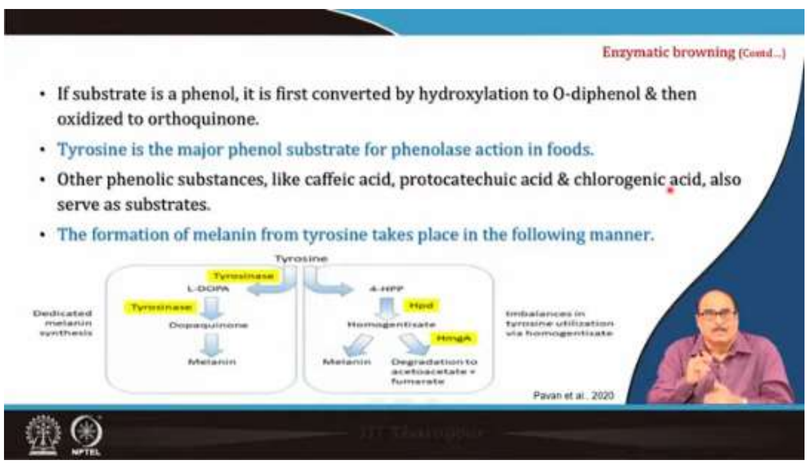
So again, this phenomenon of denaturation is of great importance in food processing. Many a time desirable. Many a time undesirable. Sometimes we control. The process that is denatured is like in the egg. Well, that is a raw egg. If you see that the protein is present in nature, and the same egg, when it is boiled or when it is fried or heated, the protein denatures, and you get the characteristic flavour, etcetera, and that people like it. So, in many products, like in the dairy industry, when you give the heat during pasteurization, evaporation, spray drying, etc. So, here, our aim is to prevent coagulation or denaturation because it is desirable to retain the natural properties of the protein, but in that curd is made or in many fermented products when we make it. So, sometimes there is protein that is from milk when it is converted into the protein. So, milk coagulates, which means the protein also has some extent of denaturation, etcetera. So, that is there it is done. In the case of meat, that is, you see, when the fresh animal is slaughtered, then what is the quality of the meat or what is how the texture, etcetera of the meat? So, the denaturation results in the tenderization of the meat and the denaturation of the muscle protein. There is a mild hydrolysis of the denatured protein by the intracellular proteolytic enzymes, the cathepsins.



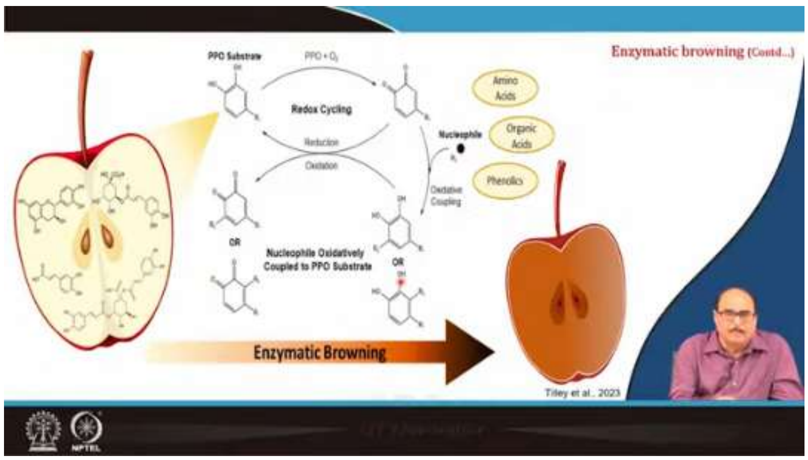
In the fishery industries, during the freezing process, slow freezing can result in protein denaturation. As water freezes in fish, the salt concentration of muscle tissues increases, causing the denaturation of protein and making it tough and rubbery. That fish becomes tough and rubbery while eating. Then, overall, you can say during thermal processing, this results in the denaturation of the body enzyme, microbial enzyme, protein enzyme, and protein, and it also results in the inactivation of microorganisms. In the pulses when you cook, cook the pulses, it eliminates most of the toxic factors without impairing the nutritional value. That is, there are many anti-nutritional biological, anti-nutritional there which are proteins, etc. They get denatured, they are detoxified. It improves pulse digestibility, denaturation of trypsin inhibitor, haemagglutinin and enzymes responsible for hydrolysis of cyanogenic glycosides, etc. They are very important examples of the denaturation of protein during heating or heat processing.



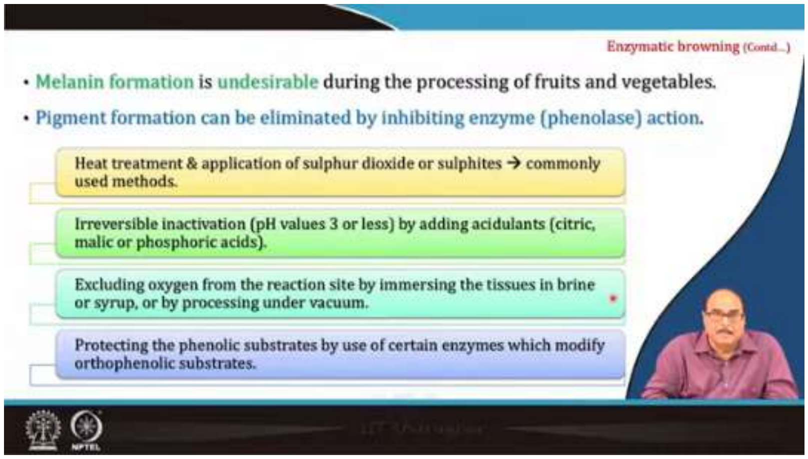
Then, enzymatic browning reactions occur. That is, browning can be observed on the cut surfaces of light-colored fruits and vegetables, such as apples, bananas, and potatoes, and this is mainly due to enzymatic oxidation. This can be described as enzymatic oxidation and enzyme reaction, and the formation of brown colour is due to the action of the enzyme phenolase, also known as polyphenol oxidase, tyrosine, catechol oxidase, or other phenolic substances. Normally, these phenolic substrates are separated from phenolases in the intact tissues, such as in a whole apple or potato, etc. Inside the cell, they are compartmentalized. So, when you peel a potato, cut an apple, or peel a banana, etc., these tissues are damaged. As a result, the substrate gets released, the enzyme gets released, and these substrates and enzymes, in the presence of oxygen, react, and the enzymatic oxidation reaction process takes place, leading to a series of reactions. Thus, we observe a rapid browning of the cut surface taking place.



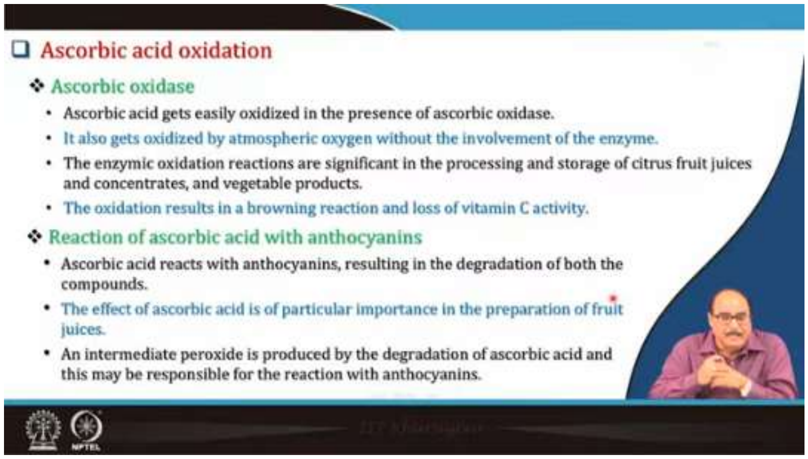
The enzymatic browning is due to the oxidation of phenols to ortho-quinones, which, in turn, rapidly polymerize to form melanins, which are essentially brown products. In an earlier lecture, we saw that the Maillard browning reaction's end product is melanoidin. Here, in the enzymatic browning reaction, the end products are normally called melanins. So, if the substrate is phenol, it is first converted by hydroxylation to ortho-diphenol and then oxidized to orthoquinone, which you can see here in this reaction. Tyrosine is the major phenol substrate for phenolase action in foods and other phenolic substances. Like caffeic acid, protocatechuic acid, and chlorogenic acid also serve as substrates. So, the formation of melanin from tyrosine takes place in the following manner: the tyrosinase enzyme produces L-DOPA, which is then converted into DOPA-quinone, and finally, it yields melanin. Alternatively, it may take another pathway, the 4-hydroxyphenylpyruvic acid pathway, which leads to hydrolysis to homogentisate, which then produces melanin or degrades into acetoacetate and fumarate. So, both pathways are involved: one is dedicated to melanin synthesis, and the other involves imbalances in tyrosine utilization via homogentisate. So, in both cases, you can say that initially, it is an enzymatic reaction, and afterwards, it follows a non-enzymatic process, leading to a series of polymers, brown polymers, etc., for melanins or compounds that give a brown colour.



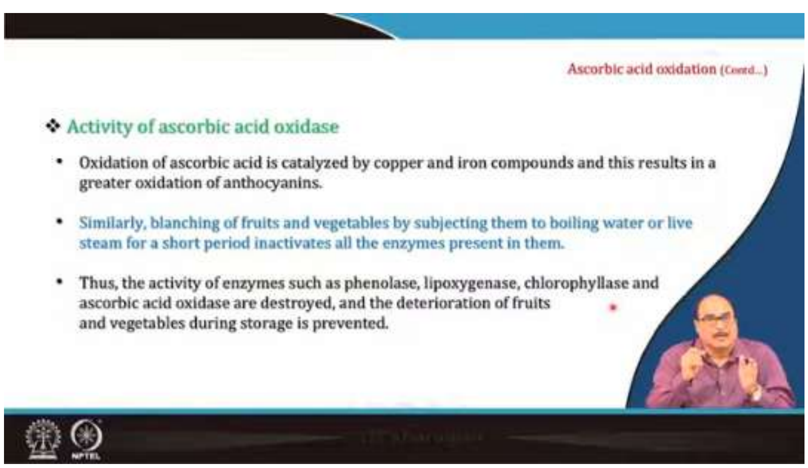
You can see here in the picture of the apple that these polyphenols and enzymes are present in different compartments when it is cut. Then, the PPO enzyme, which is the polyphenol oxidase enzyme, in the presence of oxygen, acts on the polyphenolic compound substrate, such as catechol, etc. And then, this oxidation reaction takes place, producing different compounds, which we discussed earlier. They are formed and give a brown color. It may be an organic acid, a phenolic compound, or an amino acid. Many other compounds might be formed, and the surface of the apple essentially turns brown.



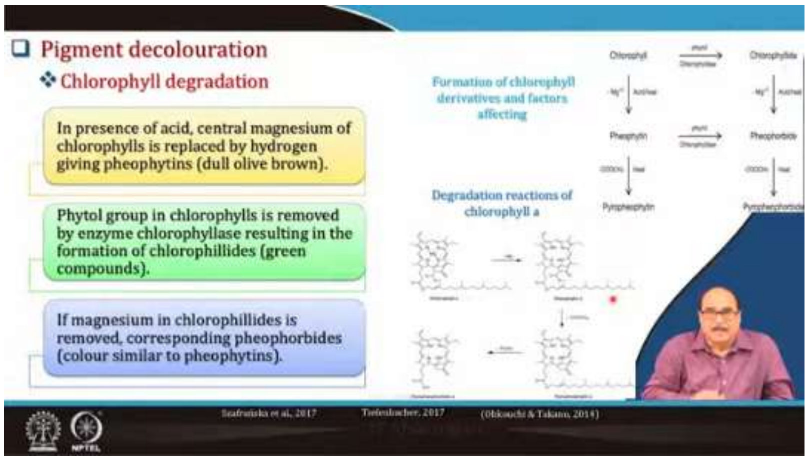
Then, melanin formation is often undesirable during the processing of fruits and vegetables. Pigment formation can, therefore, be eliminated by inhibiting the action of enzymes or phenolases. Because in many fruits and vegetables, when they are handled post-harvest, this enzymatic oxidation may result in the darkening of the product, which may not be liked by consumers. So, we need to. check it. So, one is the heat treatment, and the application of sulfur dioxide or sulfite is commonly used, either by blanching or by using a KMS solution, etc., so that enzyme activity is checked. Then also irreversible inactivation, that is, the pH value is made less than 3 or less by adding acidulants like citric acid, malic acid, phosphoric acid, etc. However, the addition of acid may have an effect on the sensory characteristics. So, these are used with care. ok. Then, exclude oxygen from the reaction because oxygen is very important in enzymatic oxidation. So, oxygen is necessary for oxidation. So, if you completely exclude the oxygen from the environment or surroundings, then it can be done either by immersing the tissues in brine or syrup or by processing them under a vacuum. Even in homemade potato chip making, etc. After peeling, the slices are dipped into a brine solution. So, the purpose here is to just prevent the enzymatic oxidation process. by protecting the phenolic substrates by using certain enzymes which modify orthophenolic substrates. So, these are the various ways by which enzymatic oxidation can be prevented.



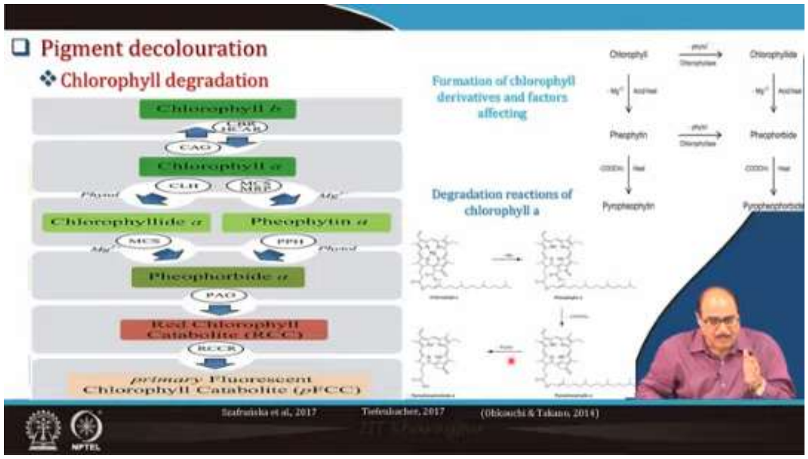
Then we talk about ascorbic acid oxidation, another important phenomenon that takes place in fruits and vegetables and changes the nature and quality of the product. there is one ascorbic acid oxidase. Ascorbic acid gets easily oxidized in the presence of ascorbic oxidase. It also gets oxidized by the atmospheric oxygen even without the involvement of the enzyme. The enzymatic oxidation reactions are significant in the presence of storage processing and storage of citrus fruit juices and concentrates and vegetable products. Oxidation results in the browning reaction, and it also loses its vitamin C activity. This ascorbic acid is converted into dehydroascorbic acid, and, finally, alpha-ketogenic acid and its enzymatic activity are completely lost. The reactions that ascorbic acid with anthocyanins. Ascorbic acid reacts with anthocyanins resulting in the degradation of both the compounds. The effect of ascorbic acid is of particular importance in the preparation of fruit juices. The intermediate peroxide is produced by the degradation of ascorbic acid and this may be responsible for the reaction with anthocyanins.



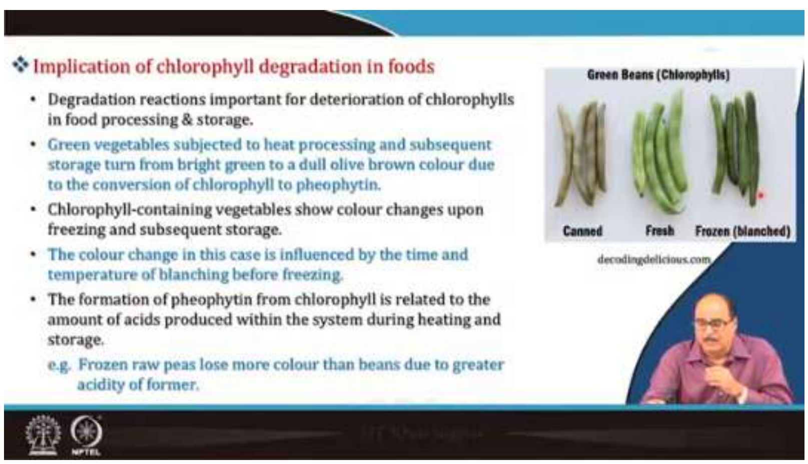
Even the activity of ascorbic acid oxidase, oxidation of ascorbic acid oxidase is catalyzed by copper and iron compounds and this results in a greater oxidation of anthocyanins. Similarly, blanching of fruits and vegetables by subjecting them to boiling or even appropriate heat process or live steam for a short period inactivates all the enzymes present in them. Thus, the activity of enzymes such as phenolases, lipoxygenases, chlorophyllase, and ascorbic acid oxidase, etc. are destroyed, and the deterioration of fruits and vegetables during storage is prevented. That is, enzymatic oxidation is prevented.



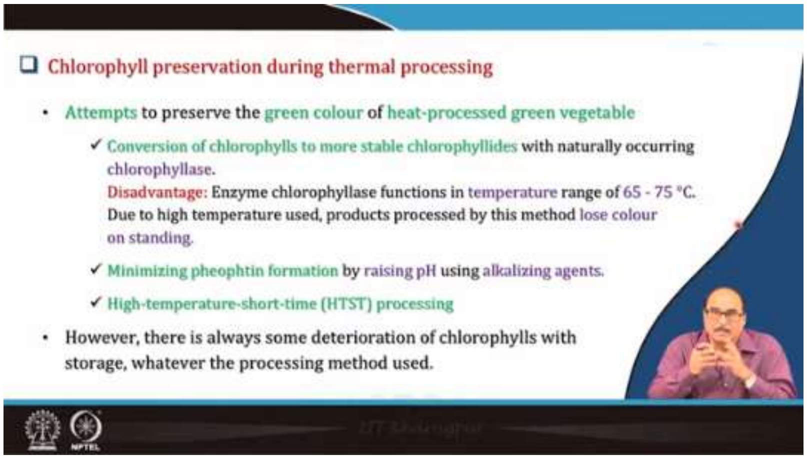
Then, we talk about pigment discoloration. In the pigment, chlorophyll is one of the important pigments in plant species. So, this chlorophyll degradation is very. So, if you see the chlorophyll structure, it contains magnesium in its centre, magnesium. And there is a side chain, a phytol group side chain. So, in fruits and vegetables, in the presence of acid, even the central magnesium of the chlorophyll is replaced by hydrogen, giving rise to the pheophytins and pheophytins are of a dull brown color. That is, pheophytins are of a dull brown color. Then, even in certain cases, this phytol group, the side chain phytol group, gets detached. And it gives rise to chlorophyllides, which are green compounds. And then, if magnesium is removed from the chlorophyllides, like you get chlorophyllides and the chlorophyllides from magnesium, then it gives the corresponding pheophorbides, and their color is similar to that of pheophytin.



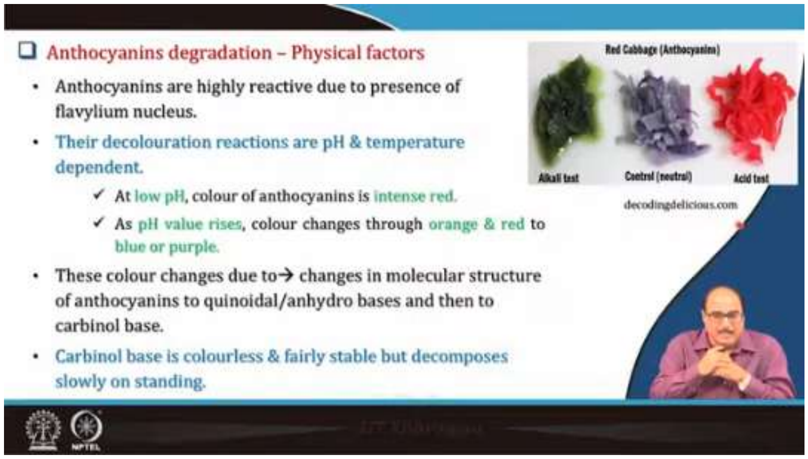
So, these are actually the reactions, either replacement of the central magnesium by hydrogen or removal of the pheophytin by the enzymes, etcetera, which results in a dynamic change in the color of these materials. That is, the green color changes to a dull olive brown color and then finally to a deep red-green color and all those things during processing. So, these reactions do take place, particularly when the material that contains chlorophyll, such as green leafy vegetables, is heated. These reactions take place.



That is, the implication of chlorophyll degradation is very important. You can see here that the fresh green beans' colour is there, and then these colour changes occur even during the canning process or even after the freezing process; the colour changes and these changes occur even when the vegetables are cooked. So, during the cooking of the vegetables, the green colour changes to brown or dull olive-brown colour, etcetera. These are mainly because of the changes in the pigment, that is, chlorophyll. Green vegetables subjected to heat cooking are due to the conversion of chlorophyll to pheophytins in chlorophyllcontaining vegetables. So, changes occur even upon freezing, as well as subsequent storage. In freezing, the colour change mainly occurs before freezing. These vegetables are kept in frozen storage and blanched. So, during blanching, that is, the heat, the color change is influenced by the time as well as the temperature of blanching before freezing. Even the formation of pheophytin from chlorophyll is related to the amount of acids present or produced within the system during heating and storage. And it has been seen that frozen raw peas lose more color than beans because, in raw peas, the acidity is more than in beans. So, these are the various factors: temperature, heating time, as well as acid content, etcetera. The cause, or even the enzymes present in them, are the various causes of degradation.

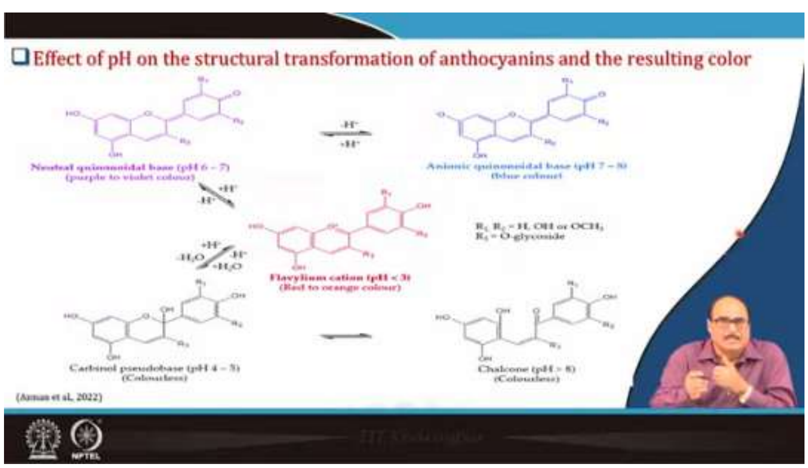


Preservation of chlorophyll, obviously, the green color during thermal processing, etcetera, what should be done? Conversion of chlorophylls to more stable chlorophyllides with naturally occurring chlorophyllase enzyme can be used. However, using chlorophyllase enzyme might have disadvantages because the enzyme functions in a temperature range of 65 to 75 degrees Celsius. Due to this high temperature, it might sometimes be too much for the vegetables and their processed products, as they may lose even color over time, or other bioactive components may get destroyed. Also, minimizing pheophytin formation by raising the pH using alkaline reagents or following the HTST process (high-temperature short time process) may result in the stabilization of colour or less reduction in the colour. However, there is always some deterioration of chlorophyll with storage, whatever processing method, etc. is used, and it becomes a very dynamic process.

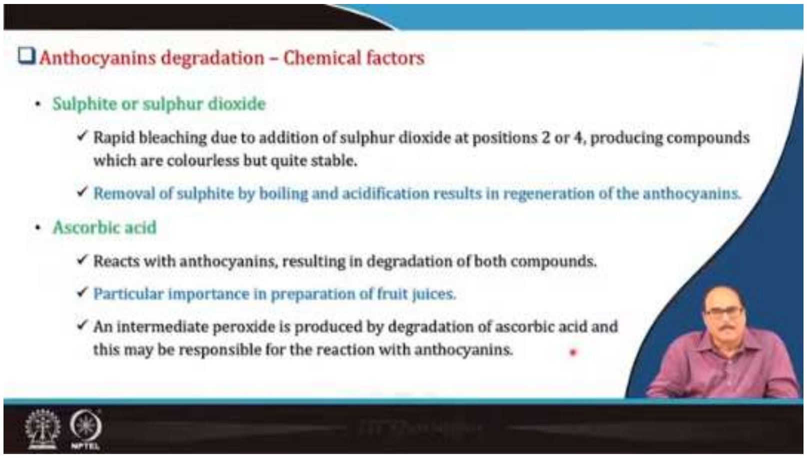


Then, anthocyanin degradation. You know, the anthocyanins are very black, deep, yellow, orange, different colours present in the flowers, etc., and they are very important. They also get degraded subject to various conditions, etc. There are various physical factors as well as chemical factors that result in the degradation of the anthocyanins. They are obviously highly reactive compounds due to the presence of a flavylium nucleus in their structure, and their discoloration reactions are pH and temperature dependent. At low pH, the color of anthocyanin is an intense red.

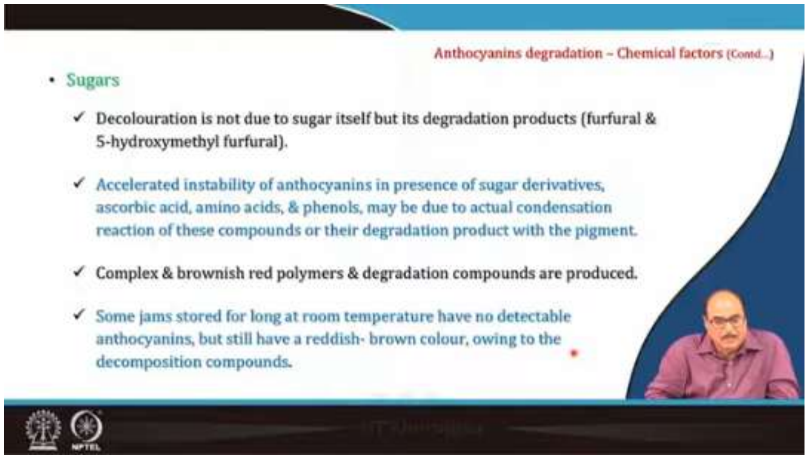
As the pH value rises, the color changes from orange to red to blue or purple and these color changes are due to the changes in the molecular structure of anthocyanins to quinoidal or anhydrobases and then to carbinol base. So, the carbinol base is colourless and fairly stable, but it decomposes upon standing.



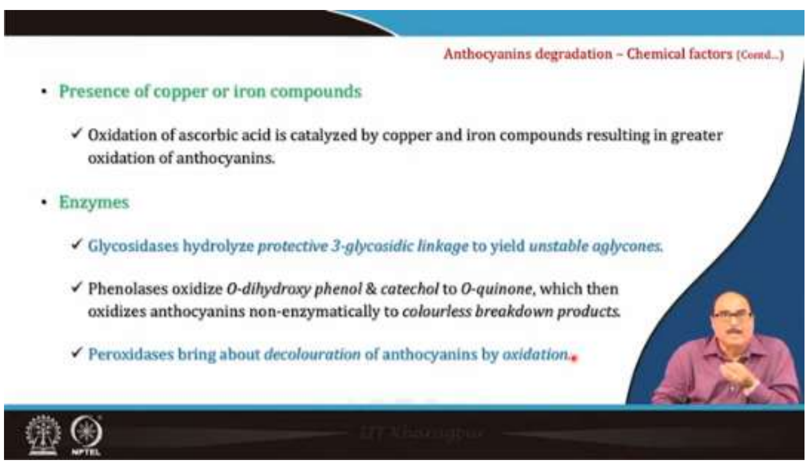
You can see here in this figure how the changes occur when the pH is changing, specifically in the pH range of 6 to 7. This is the natural quinoidal base and the color is purple to violet. And with changing pH, that is, as the hydrogen ion concentration decreases or increases—if the pH changes from 6 to 7 to 7 to 8—it becomes an anionic quinoidal base, and its colour is blue. And then again, if the pH is changed, it is a reversible reaction. Similarly, if the pH drops to less than 3, it becomes a flavylium cation, and it is red to orange in colour and very active. And then from this, if the pH is again increased or decreased when it comes in the range of 4 to 5, it is a carbinol-based pseudo base, and its colour is colourless. This carbinol base may again become a chalcone compound if the pH goes above 8. So, these changes in anthocyanin color, etc., are highly susceptible to changes in pH.



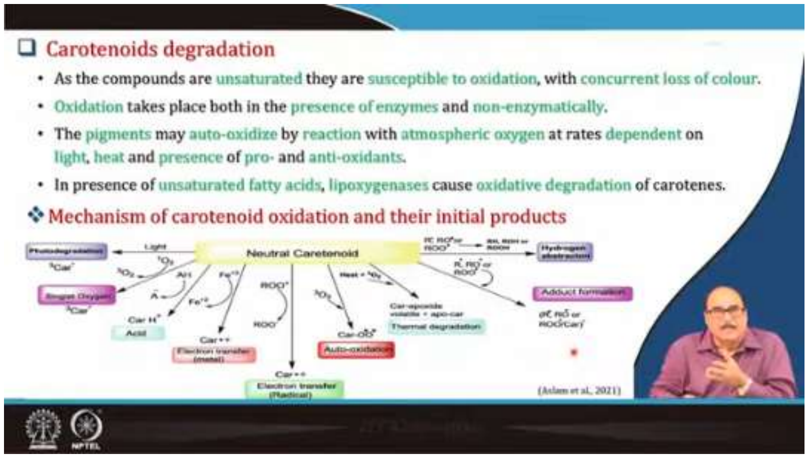
Then, the chemical factors causing the degradation of anthocyanins, etc. Number one is the presence of sulfite or sulfur dioxide. Many times, sulfur dioxide, KMS, etc., is used to preserve fruits, vegetables, flowers, etc. So, the rapid bleaching due to the addition of sulfur dioxide at position 2 or 4 produces compounds which are colorless, are quite stable, but quite stable. The removal of sulfite by boiling and acidification results in the regeneration of the anthocyanins' colour, which may sometimes be regenerated. Then ascorbic acid. Ascorbic acid also reacts with anthocyanins, resulting in the degradation of both compounds. Particularly important in the preparation of fruit juices and intermediates. Peroxide is produced by the degradation of ascorbic acid, and this may be responsible for the reaction with anthocyanins.



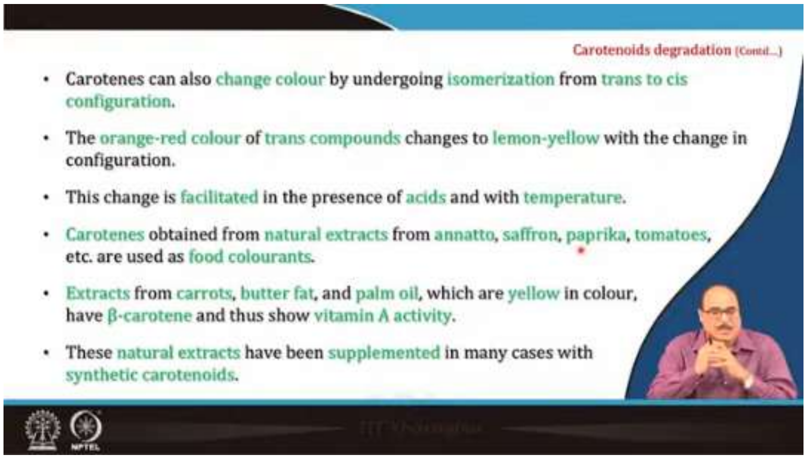
Then also the sugars. The discoloration is not due to the sugar itself, but it is due to degradation products like furfurals and 5-hydroxymethyl furfural, etc., and the accelerated instability of anthocyanins in the presence of furfural or 5-hydroxymethyl, etc., ascorbic acid, amino acids, and phenols may be due to the actual condensation reaction of these compounds or their degradation products with the pigment. Complex and browning red polymers and degradation compounds are produced, and some jams stored for long periods at room temperature have been, have no detectable anthocyanin but still have a reddish-brown color, which owes to the decomposition color of the decomposition compounds.



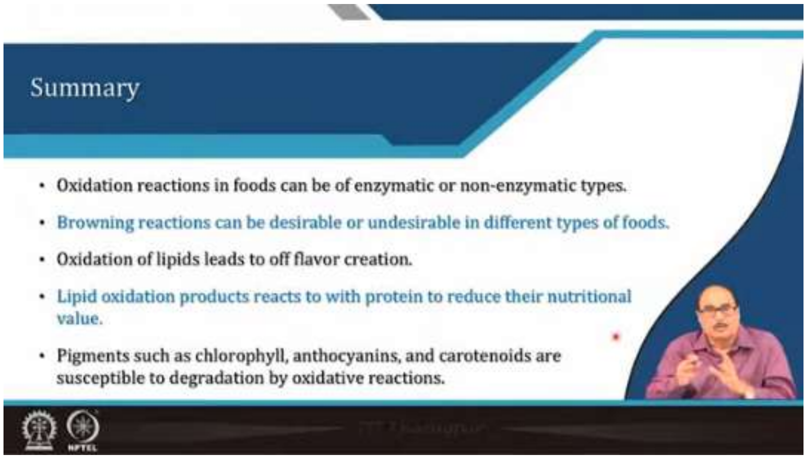
Also, the presence of copper or iron compounds causes the degradation of anthocyanin. The addition of ascorbic acid is catalyzed by copper and iron compounds, resulting in greater oxidation of anthocyanins. Even enzymes like glycosidase hydrolyze the protective 3-glycosidic linkage to yield unstable aglycones. Phenolases oxidize O-dihydroxyphenol and catechol to orthoquinone, which then oxidizes anthocyanins non-enzymatically to colorless breakdown products. Also, peroxidases bring about decoloration of anthocyanins by oxidation. So, there are many other reactions by which these colors, etc., get oxidized.



Then, other yellow pigments and carotenoids, etc., are also present in fruits, vegetables, and many other materials, and they also get degraded and oxidized. So, as the compounds are unsaturated, they are susceptible to oxidation with the concurrent loss of color. Oxidation takes place both in the presence of enzymes, as well as non-enzymatic carotenoid oxidation and degradation. Pigment may auto-oxidize by reaction with atmospheric oxidation at rates dependent on the availability of heat, light, the presence of pro-oxidants or antioxidants, and so on. So, in the presence of unsaturated fatty acids, lipoxygenases also cause oxidative degradation of carotene. Because these carotenes are also a category of lipids. So, the same general actions, such as lipid oxidation, auto-oxidation, hydrolysis, and all those things, may take place. So, here you can see in this figure, shows the mechanism of carotenoid oxidation and their initial products, such as photodegradation or singlet oxygen can react, and it may be the effect of acid or electron transfer reactions in the presence of metal ions, etcetera, electron transfer radical, or even auto-oxidation process. It may be thermal degradation or adduct formation because of either hydrogen abstraction. So, different processes may take place, and ultimately, the colour of these carotenoid compounds may change.



Carotenes can also change color by undergoing isomerization from trans to cis form. The orange-red color of the trans compound changes to lemon-yellow with the change in the configuration. This change is facilitated in the presence of acid and with temperature. Carotenes are obtained from natural extracts from annatto, saffron, paprika, even tomato, lycopene, etc. They are used as food colourants, but again, there may be changes in this colour depending upon various conditions; if proper care is not taken, there may be changes in this colour. Extracts from carrots, butterfat, and palm oil, which are yellow in color, contain beta carotene and thus exhibit vitamin A activity. These natural extracts have been supplemented, in many cases, with synthetic carotenoids as well.



So, finally, I would like to summarize this lecture by saying that oxidation reactions are very important reactions. They can lead to the deterioration of food and, sometimes, undesirable or even toxic compounds may be formed. These oxidations may be enzymatic as well as non-enzymatic reactions. Oxidation of sugars, proteins, lipids, etc., is a concern in foods for food engineers and food technologists. Pigments such as chlorophyll, anthocyanins, and carotenoids are also susceptible to degradation by oxidative reactions. Every effort, wherever necessary, should be taken to prevent these oxidative reactions in order to prevent quality changes or to maintain the product in good quality and to ensure its safety, as many times these oxidation products might be carcinogenic or adversely affect health.

So, with this, these are the references used in this lecture.

Thank you very much for your patience.