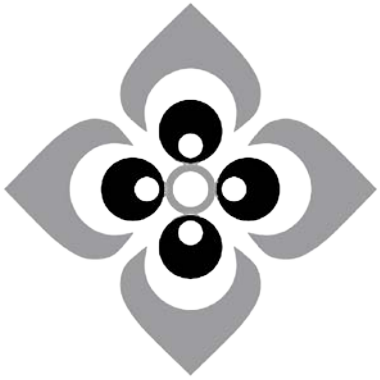

Consortium for Educational Communication

Module
on
# Food Spoilage Enzymes-PPO, Cell Wall Degrading Enzymes

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**TEXT**

**FOOD SPOILAGE**

Every food item that we eat is biological in origin, i.e. it comes from living organisms, thus it is bound to contain proteins , carbohydrates and fats and several metabolic activities would be carried out in them with the help of enzymes. These carbohydrates, fats and proteins are a source of energy. Since every living organism requires energy for survival, we consume food for procuring energy and similarly even microorganisms require energy, thus food becomes the target for the growth of some microorganisms. Food spoilage means the original nutritional value, texture, flavour of the food are damaged, the food becomes harmful to people and unsuitable to consume.

Various microorganisms may cause changes in the character of food, which may be classed as “positive” or “negative”. Products of “positive” microbial transformations include cheese, yoghurt, and wine, which can be seen as increasing the nutritional value. “Negative” aspects of microbial growth include food deterioration and spoilage by decay, and food poisoning, mainly caused by different and less widespread bacteria. As they grow, micro-organisms release their own enzymes into the liquid surrounding them, and absorb the products of external digestion. This is the main basis of microbial food spoilage, which lowers the nutritional value of the medium they are growing in. Bacteria and moulds may also produce waste products which act as poisons or toxins, thus causing the renowned ill-effects.

## Factors of Food spoilage

Nutrients in food, their kind and proportions determine the type of organism that will grow. Also, microorganisms vary in their ability to use nutrients. The presence of easily utilizable nutrients will encourage faster growth and quicker damage. For example, a food with easily utilizable sugars will allow better growth than one which contains polysaccharides. Most foods contain enough peptides and amino acids that they can meet the nitrogen requirement of most organisms found in foods. Some organisms are also proteolytic and can grow on proteins found in the food. The mineral requirement of microorganisms is generally met by the food and this is not a limiting factor. Some foods may contain antibacterial substances which may prevent bacterial growth and food spoilage. Other than the nutrients present in food, factors such as the pH, moisture content, oxidation reduction potential, etc., also influence microbial activity in foods. For example, each organism has an optimum pH for growth. Thus, both the growth as well as their survival in foods depends on the pH of the food material. Both yeasts and moulds can thrive in high acid foods like fruit, tomatoes, jams, jellies and pickles. Both

are easily destroyed by heat. Processing high acid foods at a temperature of 100°C (212°F) in a boiling water canner for the appropriate length of time destroys yeasts and moulds.

Also, the concentration of the sugars will determine the type and extent of growth, since it affects both the osmotic pressure and the $a_w$. Generally, yeasts and molds are more resistant to high concentrations of sugar than bacteria. Bacteria generally prefer low acid foods like vegetable and meat. When the conditions for bacterial cell growth are unfavourable (e.g., low or high temperatures or low moisture content), several species of bacteria can produce resistant cells called endospores. Endospores are highly resistant to heat, chemicals, desiccation (drying out), and ultraviolet light. The endospores may remain dormant for long periods of time. When conditions become favourable for growth (e.g., thawing of meats), the endospores germinate and produce viable cells that can begin exponential growth.

Microorganisms have an absolute demand for water and the optimum level of moisture required for growth varies with the organisms. The water requirement is expressed in terms of available water or water activity ($a_w$), which is the vapour pressure of the solution divided by the vapour pressure of the solvent. This is equal to the vapour pressure of the solutions in water divided by vapour pressure of the water. Each organism has a maximal, optimal and a minimal aw for growth. Most bacteria grow well in a medium of aw activity around 0.995 to 0.998. Molds differ considerably in the optimal aw. For example, Rhizopus sp., has an optimal aw of 0.995-0.980, while Penicillium sp., and has an optimal aw of 0.9935. The $a_w$ value of a food is affected by the vapour pressure of solutes such as sugars, salts, hydrophilic colloids or gels. An increase in the concentration of sugars and salts allows the water to be tied up and also causes the removal of water from the microbial cells. The $a_w$ value of the food therefore, determines to considerable extent the type of organism that can grow in it.

The oxygen tension or partial pressure of oxygen and the reducing and oxidizing power of the food (O-R potential) influences the growth of organisms. In relation to oxygen, bacteria can be aerobic, anaerobic or facultative, while fungi are mostly aerobic, yeast are aerobic or facultatively anaerobic. A high O-R potential favours the growth of aerobic and facultative organisms. Most fresh animal and plant foods have a low O-R potential in their interior but have a higher O-R outside. Thus, a fresh piece of meat could support the growth of aerobic organisms in the exterior and the growth of anaerobic organisms inside.

Other than Microbial growth and destruction, food is spoiled by enzymes present within them. This self destruction is termed as autolysis. The most important mode of destruction is by enzymes .Enzymes are proteins found in all plants and animals. If uncooked foods are not used while fresh, enzymes cause undesirable changes in colour, texture and flavour. This is because,

even though the vegetables and fruits have been plucked from the plants, their cells are still alive and continue the basic life process i.e. respiration.

## Chemical changes causing Food Spoilage

Food spoilage occurs in food products due to reaction or breakdown of the chemical components of the food, including its proteins, lipids, and carbohydrates. The rate at which the chemical reactions takes place depends on many factors, which are, water activity, temperature, pH, exposure to light or oxygen. Protein degradation can involve reactions with protein and other ingredients brought about by enzymatic activity. Protein hydrolysis is achieved by enzymes collectively called proteases. Proteases bring about the cleavage of long protein chains and form fragments of amino acids. Enzymes hydrolyzing peptide bonds in the interior of the amino acid chain are called endo-peptidases whereas proteases hydrolyzing peptide bonds at either the amino- or carboxy- terminal end of the protein are called exo-peptidases. One of the spoilage causing protease is 'Protease plasmin'. Plasmin can survive pasteurization temperature and can cause degradation of dairy proteins in milk and cause coagulation and gelatinization. Other protease can act on the proteins in meat and cause the meats to become mushy. Degradation of meat protein is also brought about by the oxidation of proteins; overexposure to oxygen can cause myoglobin and oxymyoglobin to oxidize into metmyoglobin, causing the change in meat colour from bright red to brown which renders the meat not appealing to the consumer.

Putrefaction is also protein degradation, where decomposition of animal proteins is carried out especially by anaerobic microorganisms described as putrefying bacteria. Putrefaction usually results in the formation of amines such as putrescine and cadaverine.

Enzymatic activities in fruits and vegetables can cause browning and softening of tissues. Typically these reactions are catalyzed by phenol oxidase enzymes, which react with phenol compounds and oxygen to form undesirable brown pigments. Another form of browning which happens due to non enzymatic activity is Millard Browning. This non enzymatic browning occurs due to reaction between proteins (amino acids) and reducing sugars. This is associated with loss in nutritional value along with the browning and change in the texture of food products. The essential amino acid lysine, which readily reacts with reducing sugars, is quickly lost. Carbohydrates makeup the largest proportion of any fruits and vegetables and so a larger percentage of food spoilage is due to the degradation of the carbohydrate content of these foods. Vegetable cells, as plant cells, have rigid cell walls and are glued together by various polysaccharides such as cellulose, hemicellulose, and pectin. Once

vegetables are harvested from the fields, the cells, now deprived of nutrient supplies normally obtained from soils and the air, go into senescence, or aging. The most noticeable structural change in senescent vegetables is softening, or loss of texture. Softening is caused by natural enzymatic reactions that degrade the plant cell walls. A large group of enzymes is involved in the senescence stage, including cellulase, pectinase, hemicellulase, proteinase, and others. After these enzymes break open the cells, chemical oxidation reactions take place and the vegetables develop off-flavours and loss of nutritional value. Broken cells are also much more easily subject to microbial attacks, which quickly lead to spoilage. In addition, even though the vegetables may be packaged or bagged, the plant cells continue to respire, or break down carbohydrates for energy needs. Lipid spoilage most often occurs due to oxidation reactions or action of lipolytic enzymes and other hydrolytic reactions .Lipid oxidation is the most important degradation method in fats and oils and occurs in many foods containing fats and oils or in fried foods. During this reactions, oxygen attacks unsaturated fats to form colour changes, off-flavour and sometime toxic substances. The number and location of double bonds on the fatty acids and triglycerides is one factor that affects the rate and extent of oxidation. Light and heat are other important factors as they increase the rate of oxidation. Catalase and peroxidase are the two most important oxidizing enzymes that can cause darkening in diced and sliced vegetables. A simple heat treatment (Blanching) is used to inactivate these enzymes. Atmospheric oxygen reacts with food components and cause rancidity. Rancidity is the term used for the deteriorative changes of fat with time and it results in undesirable flavour and odour. Hydrolytic rancidity occurs in foods when the lipid (fat) is hydrolyzed by the water contained in food to fatty acids. Some of the liberated fatty acids are volatile and some have very unpleasant odours and flavours. When rancidity occurs due to air, it is termed as Oxidative rancidity. The oxidation of acylglycerols which occurs in air, without the presence of enzymes, is called autoxidation. Among the products of autoxidation are hydroperoxides, ROOH. These have no taste, but they decompose easily to form aldehydes, ketones and acids, which give oxidised fats and oils their rancid flavours. It can be slow down by addition of antioxidants.

## ENZYMATIC BROWNING

Polyphenol oxidase (PPO) is a generic term for the group of enzymes that catalyze the oxidation of phenolic compounds to produce brown color on cut surfaces of fruits and vegetables. The action of PPO leads to major economic losses in some fresh fruits and vegetables, such as Irish potatoes, lettuce and some other leafy vegetables, apples, apricots, bananas, grapes, peaches

and strawberries. In some tropical fresh fruits, up to 50% can be lost due to the enzyme-caused browning. Browning also leads to off flavors and losses in nutritional quality. Therefore, the consumer will not select fruits and vegetables that have undergone browning. Black spots in shrimp are caused by PPO-catalyzed browning; the "browned" shrimp are not acceptable to the consumer and/or they are down-graded in quality. PPO activity in plants is desirable in processing of prunes, black raisins, black figs, zapote, tea, coffee and cocoa and it probably protects plants against attack by insects and microorganisms. PPO was first discovered by Schoenbein in 1856 in mushrooms. Subsequent investigations showed that the substrates for the enzyme are $O_2$ and certain phenols that are hydroxylated in the o-position adjacent to an existing -OH group further oxidized to o-benzoquinones (Equation 2) and then

nonenzymatically to melanins (brown pigments). Millions of dollars are spent each year on attempts to control PPO oxidation; to date none of the control methods are entirely successful.

## Chemistry of Enzymatic Browning

Control of enzymatic browning in fruits and vegetables and in juices and wines requires chemical knowledge of the types of phenolic substrates present in a particular plant, the level of reducing compounds, such as ascorbic acid and sulfhydryl compounds, the level of $O_2$ accessibility, nature of co-oxidizable compounds present and the pathways of polymerization and degradation of the **0**-benzoquinones. It is also essential to understand the level of PPO and substrates available at different stages of plant development. Above all, it is important to distinguish between enzyme-caused browning and non-enzyme-caused browning (the Maillard reaction) in foods. Some PPO's hydroxylate monophenols to give **0**-dihydroxyphenols, which are then further oxidized enzymatically to o-benzoquinones. The yellowish o-benzoquinones are very reactive and unstable. Further nonenzymatic reactions with $O_2$ lead to additional reactions to give complex products such as indole- 5,6-quinone from tyrosine for example with further polymerization to melanin and reaction with nucleophiles, such as amino groups of proteins. The o-benzoquinones can react covalently with other phenolic compounds by Michael addition, to give intensely colored products that range from yellow, red, blue, green and black. *o*-Benzoquinones also react with aromatic amines and thiol compounds, including those in proteins, to give a great variety of products, including higher molecular weight protein polymers.

## Inhibition of Enzymatic Browning

In theory, PPO-catalyzed browning of fruits and vegetables can be prevented by heat inactivation of the enzyme, exclusion or removal of one or both of the substrates ($O_2$ and phenols), lowering the pH to 2 or more units below the pH optimum, by reaction inactivation of the enzyme or by adding compounds that inhibit PPO or prevent melanin formation. Hundreds of compounds have been tested as inhibitors of enzymatic Browning. Exclusion and/or separation of $O_2$ and phenols from PPO prevents browning of intact tissues; commercial utilization of these methods are being examined by numerous researchers. Fruits and vegetables have "skins" (waxes, and other surface layers) that exclude $O_2$ as long as there is no damage to the skins. PPO is physically compartmentalized from phenols in the intact cell. Commerically, $O_2$ can be excluded from or reduced in concentration in fruits and vegetables by controlled atmospheric storage, packaging techniques, etc. Phenols can be removed from fruit and vegetable juices by cyclodextrins or by treatment of cut surfaces with $O_2$-impermeable coatings.

PPO activity can be decreased by modifying the pH; the pH optima of most PPO's are near 6, although there are some exceptions. Reducing compounds, such as ascorbate, sodium bisulfite and thiol compounds, decrease browning by reducing the *O*-benzoquinones back to *O*-dihydroxyphenols or by irreversible inactivation of PPO. Maltol does not inhibit PPO, but it prevents browning by its ability to conjugate with *O*-benzoquinones, while kojic acid is effective in preventing browning by both reacting with PPO and with O-benzoquinones. Competitive inhibitors, such as benzoic acid and 4-hexyl-resorcinol, are useful in controlling browning in some food products. 4-Hexylresorcinol is a very good inhibitor of enzymatic browning of shrimp, apples and Irish potatoes.

## CELL WALL DEGRADING ENZYMES

Plant cell walls, consist primarily of polysaccharides, the most abundant of which are the cellulose and pectic materials.The hemicelluloses too constitute a sizable portion of the plant cell wall. Cellulose also has the importance of being the most abundant organic matter on earth whose degradation is indispensable for the recycling of elements in the biosphere. Microbial degradation of cellulose, thus, is of paramount importance. The recalcitrance of cellulose has

been a major barrier to the hordes of microorganisms from a career in cellulose degradation plants. Efforts are under way to obtain better and more active cellulolytic microbes by discovering them in nature, developing mutants and the new possibility of genetically-engineered organisms for hyper enzyme production. Plant Pathologists have, since the beginning of this century, looked into the production of plant cell wall degrading enzymes as these are indispensable for making growth in host tissues. The search for pathogenicity in these enzymes constituted thrust area in physiology of parasitism. Other areas where these enzymes have been studied are the wood and fabric technology, for cellulases, and food industry for pectic enzymes. Interest in hemicellulases have been comparatively of recent origin. The plant cell wall is both chemically and structurally complex, containing cellulose, hemicellulose (including xylans, arabinans and mannans), and pectin. Cellulose is a homopolymer of β-1, 4 linked D-glucose. Cellulose microfibrils act as the structural backbone of the plant cell wall. During cellulose biosynthesis, chains of poly-β-1,4-D-glucose self associate through hydrogen bonding and hydrophobic interactions to form cellulose microfibrils which further self-associate to form larger fibrils. Cellulose microfibrils are somewhat irregular and contain regions of varying crystallinity. The degree of crystallinity of cellulose fibrils depends on how tightly ordered the hydrogen bonding is between its component cellulose chains. Areas with less-ordered bonding, and therefore more accessible glucose chains, are referred to as amorphous regions. The relative crystallinity and fibril diameter are characteristic of the biological source of the cellulose. The irregularity of cellulose fibrils results in a great variety of altered bond angles and steric effects which hinder enzymatic access and subsequent degradation. Because of this variability, cellulose degradation requires a variety of enzymes, presumably with wide variations in the shape of the substrate-binding pockets and/or active sites

## Cellulose:

This basic structural or skeletal material of cell walls of all higher land plants, is also present in some sea-weeds, and is synthesized by a few fungi and bacteria. Cellulose is the most abundant natural polymer and it has been estimated that $10^{10}$ and $10^{11}$ tons of cellulose are synthesized each year. The same amount is also destroyed including that which is microbially degraded.

### Cellulolytic Enzymes: The Cellulase Complex

There are three types of enzymes in the cellulase complex:

1. **β-glucosidases or cellobiases**. These enzymes hydrolyse cellobiose and other cellodextrins, also other **β**-linked glucose dimers and B-glucosides such as p-nitrophenyl-**β**-glucoside (PNPG) yielding glucose. They do not act on cellulose.

2. **Exo-β-Glucanases.** These enzymes attack cellulose molecules from the non-reducing end. The best known ones are the cellobiohydrolases of Trichoderma. Which remove cellobiose units, analogous to **β**-amylase. Exoenzymes removing glucose, analogous to glucoamylase, areapparently less common. Exo-enzymes barely hydrolyze CMC or other substituted cellulose derivatives because the substituents stop the action.

3. **Endo-β-glucanases.** These enzymes attack the cellulose molecule in a random fashion. These are the CX enzymes of Reese (1956). They are usually measured

on CMC by production of reducing groups or reduction in viscosity.

**Pectic Substances**

Pectic substances consist of a closely associated polysaccharides, the major components of which are made up of a linear chain of D-galacturonic acids in which varying proportion of the acid groups are present as methyl esters. The term pectic substances is commonly used for pectin (the methyl ester), pectic acid (de-esterified pectin) and its salts, pectates (e.g. sodiumpolypectate) and certain neutral polysaccharides lacking the galacturonan back bone which are often found in association with pectin viz. 1) arabinans 2) galactans and arabinogalactans, and

3) galacturonans and rhamnogalacturonans.

**Pectolytic Enzymes:**

Enzymes which acts on pectins have been studied in more detail than those acting on most polysaccharides, other than starch and cellulose. This is because of their involvement in plant tissue maceration which they cause by dissolving the middle-lamella (the. inter-cellular cement which holds the cells together and is chemically pure exclusively pectic material). The other reason for interest in these enzymes is in processing of fruit and vegetable products.

Enzymes acting on pectins are of three main types:

**a) Pectin methyl esterase (pectic pectyl hydrolase)**

**b) Polygalacturonases (poly-CC -1 -4-D-galacturonide glycanhydrolase)**

**c) Pectin methyl-trans-eliminases and polygalacturonate trans-eliminases (poly OC-1-4-D-galacturonoide lyase).**

Enzymes of the first type hydrolyze methoxyl ester groups and presumably plays a role in modifying pectin properties during growth of the cell wall. Pectin depolymerizing enzymes of the second and third types, and especially of the latter, are commonly elaborated by plant pathogens involved in the maceration and rotting of plant tissues, and in the decay of infected fruits and vegetables. In common with other glycan hydrolases, enzymes of second type catalyze glycoside hydrolysis with cleavage of the C—1 —0 bond, and show a preference for de-estrified pectic acids. In contrast, the lyases or trans-eliminases cleave the O-C-4 bond with formation of unsaturated uronic acid units (as in base catalyzed β elimination).

**Xylans:**

Xylans are the most abundant non-cellulosic polysaccharides in majority of angiosperms where they account for 20-30% of the dry weight of woody tissues. They are mainly secondary wall components but in monocotyledons they are also found in primary cell walls. These are less common in gymnosperms where the galactoglucomannans and glucomannans form the major hemicelluloses.

**Xylolytic enzymes:**

At present two enzymes are known to be associated with the xylolytic activity of microorganisms, both of which are hydrolases: endo-xylanase, which attacks (1-4)-β-D-Xylan at random, giving rise to xylooligosaccharides, and exo-xylanase or β-xylosidase, cleaving the same substrate from the nonreducing end leading to the production of monosaccharide xylose.