Course Name: Canning Technology and Value Addition in Seafood
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Microbiology and spoilage of canned food P1.

In the previous sessions, we have already discussed this particular technology by using three CCPs. One is the concept, the next one is the container and then the process. Now in this session, we will be dealing with the microbiology and spoilage which is involved in canned food. Microbiology especially in thermal processing is very important because, actually the process itself is dependent upon the initial bacterial load. It is scientifically designed to target certain microorganisms which can survive high temperature and pressure conditions.

So, in-depth study on the microbiological changes happening during the canning process is very important. Before approaching any food processing technology, main objective is to limit the growth of microorganisms in that food. So, as we discussed the concept of this technology, if you present a nutritionally rich food to the environment, many organisms are interested in consuming that food. Food is generally a source of nutrients for all the living organisms, since we are preparing this for the consumption of human beings; we are considered as the consumers of this particular product.

So, any other organisms which are interested in consuming this product is considered as a hazard. So, our objective is to prevent the entry or the availability of this particular nutrients to any other macro or microorganisms other than human beings. In the case of macroorganisms, the advantage is that we can restrict their presence or their approach or the availability of these nutrients by creating certain physical barriers around the food. For eg: -, enclosed areas are there, the container itself is there, and other biological hazard or macro hazard control systems mechanisms are in place. So, we can control them.

But in the case of microorganisms, no physical barriers may be able to restrain them for approaching the food. So, we need to calculate certain processes, we need to introduce different kinds of physiochemical mechanisms or parameters into the food so that the entry of the microorganisms or the proliferation of microorganism is constantly checked within the container or within the food packet. We already discussed that our main target is to control the microorganism since microorganisms are the primary reason for our food getting spoiled. So, just like any other food, canned food products are also prone to spoilage, given that this microorganism come in contact with the enclosed food. We need to prevent that.

If you want to control any particular group of organisms, we need to understand that their basic nature, their classification, the basic characteristic, their physiology, their anatomy, and the conditions which promote their growth and conditions which is going to limit their growth. So, all these informations are required. So, first approach will be to classify the microorganisms or to classify the target organisms according to the various parameters. So, one of the basic classification category or classification criteria is nutrition degradation capacity. So, what kind of nutrients they are interested in, for eg: -, there are a group of organisms called proteolytic microorganisms. Proteolytic organisms are microorganisms which are capable of protein degradation because of extracellular protein is produced. They have proteolytic enzymes which is produced by those microorganisms which make them capable of feeding on the protein source or utilizing the protein source available in the food. So, there are protein-loving microorganisms or proteolytic microorganisms.

There are another group of lipolytic microorganisms. Lipolytic organisms are microbes which catalyze the hydrolysis of fats to fatty acids and glycerol. They can break down the fat into fatty acids and glycerol and utilize that for their energy purposes. So, there is also sacchrolytic microorganisms which can hydrolyze disaccharides or polysaccharides to simple sugars. Sugar-loving microorganisms are sacchrolytic. There is also pectinolytic organisms which are the microorganisms which can hydrolyze pectin, a component of plants. So, different kinds of nutrition dependent microorganisms are there. Also, there are other categories that on basis of tolerance to different kinds of hurdles of processing. There also we can classify the microorganisms like microorganism which can withstand the pH conditions, water activity, low temperature, high temperature, certain gas preferences are there. These are also the classification criteria available. Based on socio-economic significance also, we can classify them as either spoilage microorganisms, pathogenic microorganism or toxic microorganisms.

In the case of canning what are the processing conditions? Because we are going to decide the target microorganisms based on the processing or preservation conditions that we are going to follow in a particular technology. Every processing or preservation technology is going to have a group of target microorganisms. Target microorganism just means that any microorganism which can overcome or which can tolerate or withstand the current preservation conditions. Our primary objective for this is to limit the presence or limit or nullify the presence of these target microorganisms in our food products. Because the principle is that once we target the target microorganism, we are able to control the target microorganisms. Obviously other kind of microorganisms will be controlled because they are incapable of surviving the preservation conditions. That is the principle behind this concept. In the case of canning, what are the conditions available? According to the process, we know that there is vacuum in the headspace. A controlled atmospheric condition or vacuum is there and also it is pH regulated food and different

kinds of pH conditions are there and also high temperature and pressure. These are the processing conditions which is going to prevail in a canned food.

The type of microorganisms which can survive a canning technology or canning conditions are going to be those microorganisms which can survive in a vacuum atmosphere, which can survive in a pH regulated atmosphere, which can also survive high temperature and pressure conditions. Depending on the pH preference, microorganisms can be classified like either acidophiles, neutrophiles or alkaliphiles. As the name indicates acidophiles are the group of microorganisms which can survive or which prefer a pH range of 1 to 5 pH range and neutrophiles are microorganism which prefer a neutral pH range 6 to 8 i.e., 7.5 range and alkaliphiles are organisms from 8 and above that alkaline conditions. So, according to the pH preference we can classify organisms as acidophiles, neutrophiles and alkalophiles.

And also, temperature, as we discussed, temperature is one of the major processing parameters that we are going to apply in the case of canning. So, in the case temperature preference also, different kinds of microorganism are present. For eg: -, there are psychrophilic microorganisms or psychrophiles which prefer a lower temperature like zero, subzero temperatures -5 to around 20˚C. They are the target microorganisms in the case of chilled and frozen preservation technologies. They are called psychrophiles and there are also mesophiles which prefer a higher temperature from 15 to 45˚C. Then there are thermophilic microorganisms which prefer a range of temperature from 45 to 80˚C and also hyper thermophiles which prefer a temperature from 65 to 150˚C. These are the group of microorganism or classification of microorganism based on their thermal preferences. So, psychrophiles prefer a low temperature, mesophilic thermophiles and hyper thermophiles. Hyper thermophiles are basically organisms that we find in thermal vents and such kind of high temperature but natural thermal vents are there in those temperatures those kinds of microorganisms present. The canning technology is concerned or thermal pasteurization or thermal sterilization technology. Both two groups such as mesophilic microorganisms and thermophilic microorganisms are going to be our point of focus.

And also, in the case of canning technology, a vacuum is there inside the sealed container. So, there is a restriction in the amount of oxygen present. There are different kinds of microorganism based on the preference of oxygen as well. For example, there are obligate aerobic organisms, which require oxygen to survive and there are also obligate anaerobes are there where they do not require oxygen to survive or presence of oxygen. Then there are also facultative anaerobic organisms are basically aerobic organisms but which can tolerate anaerobic condition to an extent. And also, aerotolerant anaerobes are there. Aerotolerant anaerobes are basically anaerobic microorganism but they can also tolerate aerobic conditions and there are also microaerophiles. Microaerophiles are the particular kind of organism they prefer a particular level of

presence of oxygen; very low levels of oxygen are required for their survival. So, these are the various classification of organisms based on oxygen preferences. So, in the case of canning, conditions it is going to be vacuum. So facultative anaerobes and obligate anaerobes are going to be the organism that we need to be targeting in the case of canning technology.

The can products are also classified on the basis of pH. As we know that there are different kinds of pH ranges available - alkaline pH is there from 8 to 11 and also neutral pH is their range is 7 to 7.5 range and also acidic pH is there from 2 to around 6 range. So, different kinds of foods are there in different range. Generally, in canning, we classify the foods according to the pH and there are four categories of pH classifications for the canned products. One is group one is low acid foods. Low acid foods mean they are having a pH less than 5.3. So, example they are called low acid foods. Example- meat, fish, milk and vegetables and some of the vegetables comes under this category. Group two is medium acid foods which has a pH range between 4.5 to 5.3. For example, some meat preparations and vegetable mixes, soups, sauces etc. Group three is acid foods which have a pH range of 3.7 to 4.5 example, canned tomato, syrups, tomato, ketchup, pineapple, figs, pears, fruits etc. Group four is high acid foods where pH is less than 3.7. Example, pickles, grapefruit, citrus, juices etc. So, these are the classification of canned foods based on the pH.

What are the significances of a pH-based classification? Why this classification is important is that it is proven in the case of high acid foods no spore forming pathogens can survive. When we are canning high acid foods, we are sure that no spore forming pathogens can survive. So, severe heat processing is not required in the case of high acid foods. For example, the temperature can be less than 100˚C because any mild heat tolerant microorganisms are generally destroyed at a temperature range of 90 to 95 ˚C. So around 100 ˚C or just below 100 ˚C can be used for canning or preserving high acid foods.

There are also different kinds of acid tolerant bacteria which can survive acidic conditions like lactobacillus and their spores. But what happens is, lactobacillus are only spoilage bacteria, they can survive temperatures of 100 ˚C or more than 100 ˚C. But the advantage of high acid foods is that there are no pathogenic microorganisms which can survive at that low pH. So, when we are processing a particular canned food according to their pH, we can design the severity of the processes which is going to significantly reduce the heat or energy requirements as well as the processing time. That is the significance of pH.

So, only in the case of low acid foods we require to concentrate on the most severe heat processing or thermal processing conditions. In the case of low acid foods, there is going to be certain target organisms. Main target organism is *Clostridium botulinum*; it is

one of the most heat assistant of food poisoning organisms and but it is inhibited below pH of 4.5. So, if you reduce the pH to 4.5, this *Clostridium botulinum* cannot survive. So high acid and acid foods they are free from *Clostridium botulinum*. So, no pressure canning is required and water bath pasteurization will do. In the case of high acid foods, we need not go to thermal sterilization or pasteurization conditions will do that. So that is why we should be clear about the target microorganisms and also another target organism is *Lactobacillus stearothermophilus (Geobacillus stearothermophilus)*. It is a target spoilage microorganism. It causes a particular spoilage known as flat sour spoilage but it is more heat resistant than *Clostridium botulinum.* But targeted heat treatment for *Lactobacillus stearothermophilus* will result in over cooking followed by nutrient loss of the food. Since, it is only a spoilage microorganism, less importance is given to *Lactobacillus stearothermophilus* because the presence of this organism could be limited or controlled by maintaining proper hygienic conditions, raw material quality, etc. So, Geobacillus is avoided by hygienic handling practices and rapid cooling.

In previous session, we discussed about the significance of the rapid cooling process up to 35 ˚C. It is to control *Geobacillus stearothermophilus* which is thermophilic in nature while the *Clostridium botulinum* is mesophilic. Common spoilage bacteria found in canned food or canning technology can be generally classified based on the spore forming capacity such as non-spore forming bacteria both anaerobic and aerobic and also non-spore forming bacteria such as yeast, molds and others. Many kinds of thermophilic and mesophilic microorganisms have the capacity to form spores, thermo tolerant spores or endospores which can tolerate a higher level of temperature than their normal tolerance limit or normal ideal range of temperature. So, bacteria with this condition are called a spore forming bacteria.

The spore formation like, when the organism confronted with extreme environmental conditions like high heat, pressure, pH differences etc., some of the mesophilic or thermophilic spore forming bacteria can convert and transform into a spore form. If they transform into a spore form, it's a non-vegetative form. So, it will have more resistance because they will expel all the water because in the spore there is no water present. So, water is not available for participating in the coagulation of cell protein. So, in order to affect the protein or the bacterial cell protein, water is required. Water is an essential component for the heat induced proteolysis or denaturation. So, when water is expelled from the bacterial cells during spore formation, it comes not available for participating in coagulation of cell protein which gives the bacterial spore a higher heat resistance and also heat resistance is also due to the presence of dipicolinic acid or DPA. A normal endospore will have around 5 to 15 % of DPA. Which will also have 2 to 10 times more Calcium than that in vegetative cells. Calcium also is very important in the case of spore formation. A normal endospore will have around 5 to 15 % DPA and also 2 to 10 times more calcium than vegetative form. It will increase the

heat resistance. So, it is basically depending upon calcium and DPA ratio. As the calcium and DPA ratio increases the heat resistance also increases. So, once the favorable condition is reached, what happens is that calcium and DPA is released and this calcium and DPA ratio falls down and eventually heat resistance also comes down and bacterial spore will convert into a vegetative cell which is capable of reproduction. So, this is the the mechanism behind the spore formation is various spore forming bacteria. This spore forming bacteria can also be classified as thermophilic spore forming bacteria, mesophilic spore forming bacteria, non-spore forming bacteria and then yeast and molds. In the case of thermophilic spore forming bacteria, they result in three types of spoilage in canned foods in the thermophilic range. Thermophilic range temperature is around 43 ˚C and above. It is basically flat sour spoilage, that there is thermophilic anaerobic spoilage and thermophilic anaerobic sulfide spoilage. These are the different kinds of spoilage caused by the thermophilic spore forming bacteria. In the case of flat sour spoilage, different class of organisms are involved. The main one is *Geobacillus stearothermophilus* which causes flat source spoilage and there is also *Bacillus coagulans*, *Bacillus thermoacidurans* and other microorganisms.

And also, thermophilic anaerobic sulfide spoilage microorganism like the D-*Sulfotomaculum nigrificans* microorganisms are also present. Basically, there are three categories of thermophilic spore forming bacteria that we need to be concerned about. It is flat sour spoilage microorganisms, thermophilic anaerobic spoilage microorganism and thermophilic anaerobic sulfide spoilage microorganisms. Besides, Thermophilic aerobic bacteria are there for example bacilli is one of the thermophilic aerobic bacterias. Bacilli is a gram-positive rod-shaped bacterium. They are a group of bacteria that can cause flat sour spoilage. The main characteristic of the flat sour spoilage as we discussed is that there is no gas is produced and also different kinds of microorganisms such as proteolytic and saccharolytic microorganisms are present. So, carbohydrate degradation also is there in the case of bacilli and which is degraded into acid without the production of gases. For example, stereo thermophilus was there. Thermophilic anaerobic spore formers in low acid food are also present but they can cannot grow less than 5 pH and around 65˚C. These are the various characteristics of thermophilic aerobic bacteria and also contain lactic acid form of a bacterial coagulanses is there. There are different kinds of bacillus coagulans. Bacteria is gram positive, rod shaped and spore forming. This organism targets acid foods with pH less than 4.2. For example, *bacillus coagulans* is a main organism which cause spoilage in the case of chromatomatous source. It can survive around ideal temperatures around 30 to 50 ˚C and also there is bacillus *thermo acidurans* which is also targeting acid foods is also aerobic in nature. In the case of thermophilic anaerobic spoilage bacteria, they are characterized with the production of hydrogen and carbon dioxide that is why they are also called as sore fermentation in which sore fermentation bacteria produce acid plus gas. They are targeting medium acid

canned foods and also, they are a category of obligate anaerobes like all and thermophiles in nature and there is *also thermoneurobacterium, thermosaccharolyticum* which was previously called *as clostridium thermosaccharolyticum* which can survive in a pH range of 6 to 8.5 and a temperature of 35 to 69 ˚C. They can produce saccharolytic enzymes and they are basically sugar reducing bacteria. Apart from this, thermo anaerobacter, cytrophyllis and other thermophilic anaerobic sulfide spoilage bacterias are also there, which is gram negative and also obligate anaerobe spore forming bacterias and targets low acid foods which spores are less resistant to heat compared to other organisms. Even though it produces hydrogen sulfide, it is generally absorbed by the food. So, generally the containers are flat but it produces rotten egg odor. Also, the hydrogen sulfide and iron can react to form iron sulfide blackening and there is also *desulfotamaculum nigrificans* which produce sulfide stinger spoilage it is also a sulfide producing bacteria.

Another category of microorganisms targeting is mesophilic spore forming bacteria. Mesophilic spoilage can occur due to under processing because insufficient heat treatment results in the survival of mesophilic clostridium and bacillus spores. They can germinate and grow to cause spoilage after processing. So, this needs to be in considered. Different kinds of mesophilic clostridium species are there. Clostridium species are basically of two types; proteolytic bacteria and saccharolytic bacteria. So, mesophilic clostridium species which come under proteolytic or putrefactive anaerobes are C*lostridium botulinum, Clostridium putrefaciens*, *Clostridium bifermentans* and *Clostridium sporogenes*. In the case of the saccharolytic clostridium which is *Clostridium butyricum* and *Clostridium pasteurianum* are two kinds of bacteria which are saccharolytic in nature. In the case of mesophilic bacillus species, there are different species like *Bacillus subtilis*, *Bacillus mesentericus, Bacillus polymyxa* and *Bacillus macerans.*

In the case of mesophilic spore forming bacteria, their ideal temperature range is around 30 to 40 ˚C. There are also proteolytic microorganisms, which are producing hydrogen sulfate, ammonia, etc. and also saccharolytic microorganisms are there which reduces sugar and carbohydrates producing volatile acids and also such as butyric acid hydrogen and carbon dioxide as well. There are other microorganisms such as *Clostridium bifermentans* which are proteolytic and putrefactive. *Clostridium sporogenes* are there and in the case of *C. sporogenes*, there is a strain called PA3679. It is used for the thermal studies because the *C. sporogenes* PA3679 has the same characteristic similarities to the *C.botulinum*. So that is why it can be used but it does not have the toxic nature of *C.botulinum.* It is comparatively safe to handle the *C. sporogenes* but it has all the thermobacterial properties or thermobacterial characteristics of *C.botulinum.* So, thermobacterial studies involving *C.botulinum* this PA3679 strain of *C. sporogenes* is used in the case of such studies because of the safety concerns. Why the PA3679 is, basically it is putrefactive anaerobic strain and mesophilic bacillus species is also there.

They are targeting low acid canned food such as *B. subtilis* and *B. mesentericus* they target seafood, meat, milk etc. *B.polymyxa* and *B.macerans* target fruits, vegetables etc.

Mesophilic spores are less resistant than thermophilic spores. In the case of thermal resistance also, this characteristic difference is there. Mesophilic spores are generally considered as less resistant than the thermophilic spores. That is why the thermophilic microorganisms which are mostly spoilage microorganisms are not targeted but mesophilic like pathogenic microorganisms are more targeted. There are also non-spore forming bacteria, thermoduric non-spore forming bacteria are enterococcus for example. *Enterococcus faecalis* and *Enterococcus faecium* are few of the examples and it's also micro bacterium. There's also *Streptococcus thermophilus* and some species of micrococcus and lactobacillus are also coming into this category and pseudomonas are the micrococcus bacteria they're *Flavobacterium proteus* and there are also other kinds of microorganisms that can cause spoilage in leaked canned foods.

The orange juice can be spoiled by *Lactobacillus plantarum* is there, then *Lactobacillus brevis,* etc. Canned vegetables and fruits are spoiled by different kinds of other bacteria which is able to grow under high acid conditions. So, these are some of the non-spores forming bacteria which probably affects the canned food as the post-process spoilage and there is also yeast and molds in the part of post-process spoilage which can affect the can. Mold species like aspergillus species or penicillium etc., different kinds of mold species are also there which can grow in jellies and canned fruits with sugar concentration up to 67 %. The most common genera of heat resistant molds are *Byssochlamys nivea* and Talaromyces *eupenicillium* these are some of the most common general heat resistant molds. Torillopsis species of yeast are also present which can cause blowing or different kinds of bulging or gaseous spoilage on sweetened condensed milk which is not properly heat processed and also different species can cause this kind of yeast can cause spoilage in canned lemon and which grows at a pH of 2. 5. So even on high acid foods this kind of yeast can grow. These non-spore forming bacteria survive due to very mild heat treatment or pasteurization and leakage of the cans like post-process spoilage and these kind of bacteria or non-spore forming bacteria surviving at mild heat treatments are known as thermodurics. Acid forming like lactobacillus can be present in under processed tomato products it can be present in peas and other fruits and also can cause spoilage.

Also, heterofermentative lactobacillus which produce carbon dioxide can also spoil these kind of food products and swell the cans. So, basically the presence of non-spore forming bacteria indicates either the presence of leakage or some kind of post-process spoilage. In the case of orange juice, this kind of *Lactobacillus plantarum*, Lactobacillus *mobilis, Lactobacillus brevis,* etc. are also found. In canned vegetables and fruits,

different kinds of bacterias or yeast and molds are present and this kind of non-spore forming bacteria can spoil this kind of canned vegetables, fruits, etc.

The presence of yeast and molds generally indicates post-process spoilage. Post-process spoilage generally means under processing leakage, recontamination, poor evacuation. Fermentative yeast is some of the common organisms found in the canned foods. In the case of canned fruits and milk. etc. and they can cause can swelling because some of these fermented yeasts can produce carbon dioxide. Some film forming yeast are also there they can produce spoilage of the surface of the pickled meat. Some film forming yeast and molds are also most common in home canned foods and they can survive high sugar content of up to 70 % and also aspergillus are there cause formation of different kinds of button like structures on the surface of the condensed milk and also fulva species are also there they can survive up to 90˚C for one minute and this kind of pasteurization condition they can survive they are commonly found in canned fruit because they can actively participate in pectin degradation. Heat resistant molds are there which can produce aero spores survive up to 85 ˚C for five minutes and also, they can produce mycotoxins. So, some of these yeast and molds can also produce mycotoxins which can eventually result in food poisoning.