Post-Harvest Operations and Processing of Fruits, Vegetables, Spices and Plantation Crop Products

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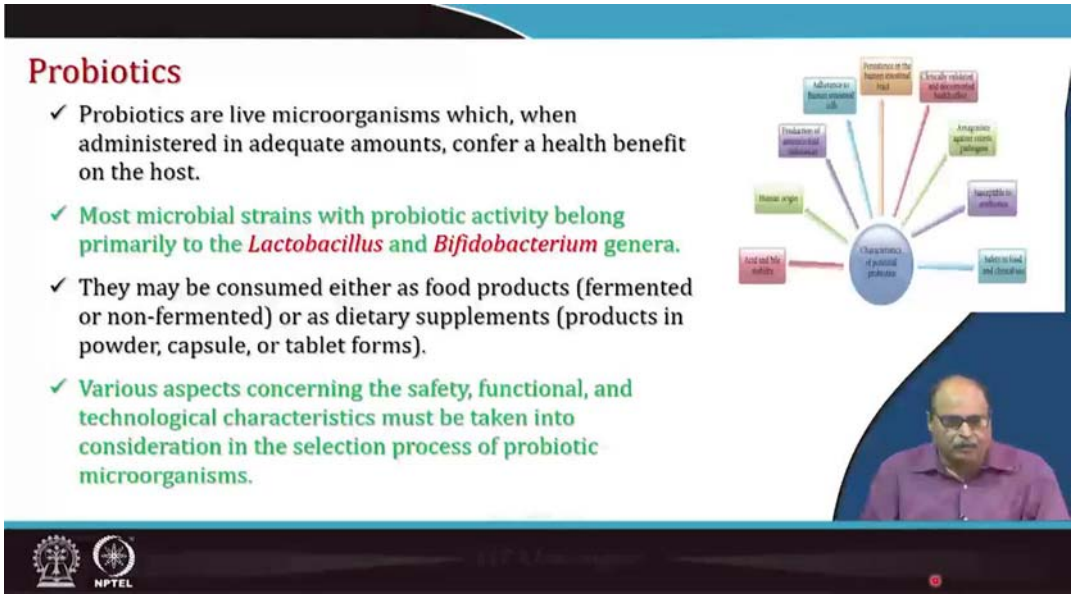
Lecture 48

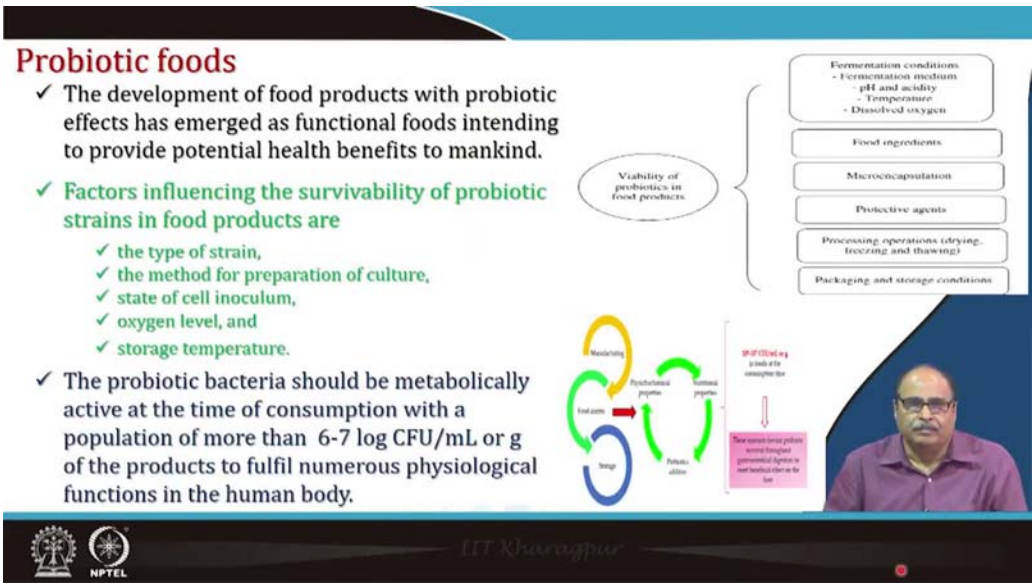
Probiotic / Fermented Vegetable Products

This lecture provides a discussion about probiotic and fermented vegetable products. The topics, which are covered that are the probiotics and their health benefits, fermentation of vegetables, fermented vegetable products, the bioactive compounds in fermented vegetable products, and finally, the microbial spoilage of fermented vegetables.



Probiotics

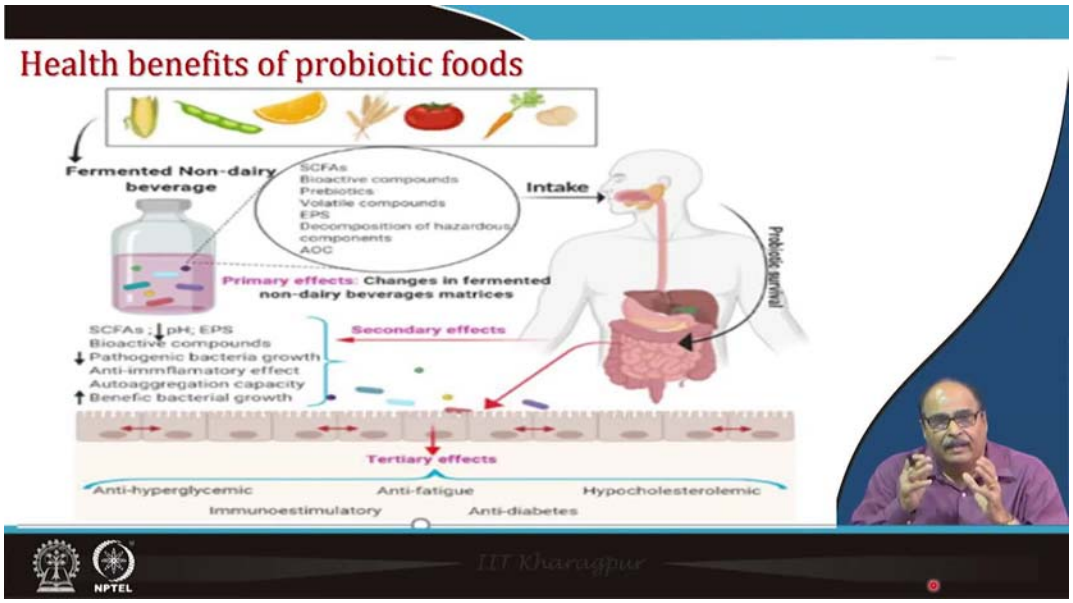
The probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host. Most of the microbial strains which have probiotic activity belong primarily to the *Lactobacillus* and *Bifidobacterium* genera. They may be consumed either as a food product like in the case of fermented food products (fermented or non-fermented) or as a dietary supplement i.e., the products in powder, capsules, or tablets forms. Various aspects concerning the safety, functional, and technological characteristics must be taken into consideration while selecting the probiotic microorganism.



Probiotic foods

The development of a food product with a probiotic effect has emerged as functional foods intending to provide health benefits to the mankind. When the foods are added with the probiotic microorganism, and when the foods are processed, various aspects of the viability of this probiotic bacterium in the food products during the processing and even during the

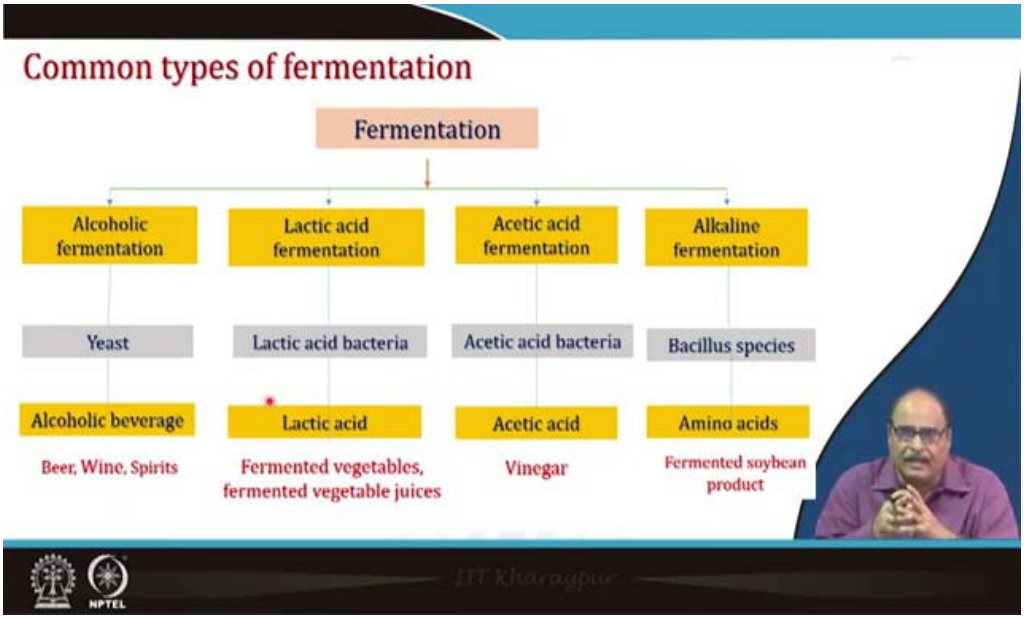
post processing like during the storage etc. has to be considered properly. Various factors, which influence the probiotic strains in the food product include type of the strain, type of the bacteria, the method for preparation of the bacterial culture, state of the cell inoculum, oxygen level, storage temperature, processing conditions, factors during processing like temperature etc. The substrate condition in the food, various nutrients concentrations are also very important because the probiotic bacteria should be metabolically active at the time of consumption of the food with a population of more than 6-7 log CFU/mL or g of the products to fulfil numerous physiological functions in human body.



Health benefits of probiotic foods

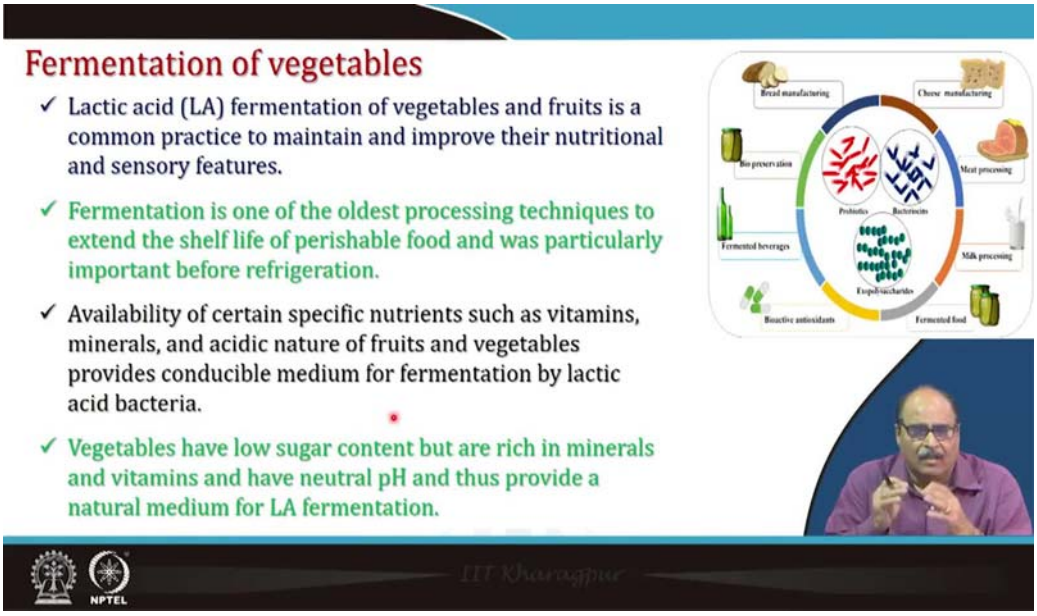
There are various reports on the health benefits of the probiotic foods. In this lecture, mainly nondairy probiotic foods will be focused. Earlier it was stated that only dairy products like milk, and milk retail products will support the growth of probiotic bacteria because milk contain beneficial microbe lactobacillus, and it was thought that milk was a good substrate for probiotic bacteria. But now, various researches have conducted, which has proved that even nondairy materials like fruits, vegetables, etc. also constitute a good substrate for the growth of probiotic bacteria. Various probiotic bacteria are selected properly and added in the nondairy beverages like fruit juice, vegetable juice or even concentrate pulp etc. When this bacterium is added in the nondairy beverages, then there maybe changes in the component i.e., in the fermented nondairy beverage matrix. Therefore, this bacterium brings about some desirable changes into the nondairy beverage. When this food containing the probiotic bacterium is consumed, the probiotic bacteria in live form is going into the system and the material, which are being consumed and its components characteristics are changed. The material becomes beneficial. it gives a double effect inside our gut. This bacterium gets established inside our gut, where they modulate, and regulate the functions. It has been proved that there is a secondary effect. The growth of pathogenic bacteria, anti-inflammatory group are suppressed and desirable microflora grow. Various desirable effects are produced inside our system, which helps in digestion. This produces various beneficial effects inside human body like suppression of different undesirable effects like anti-hyperglycemic, anti-

fatigue, hypercholesterolemic, immunomodulatory, anti-diabetic, etc. and beneficial effects are promoted. Accordingly, conjunction of probiotic bacteria and probiotic food becomes a very good healthy ingredient for human body.



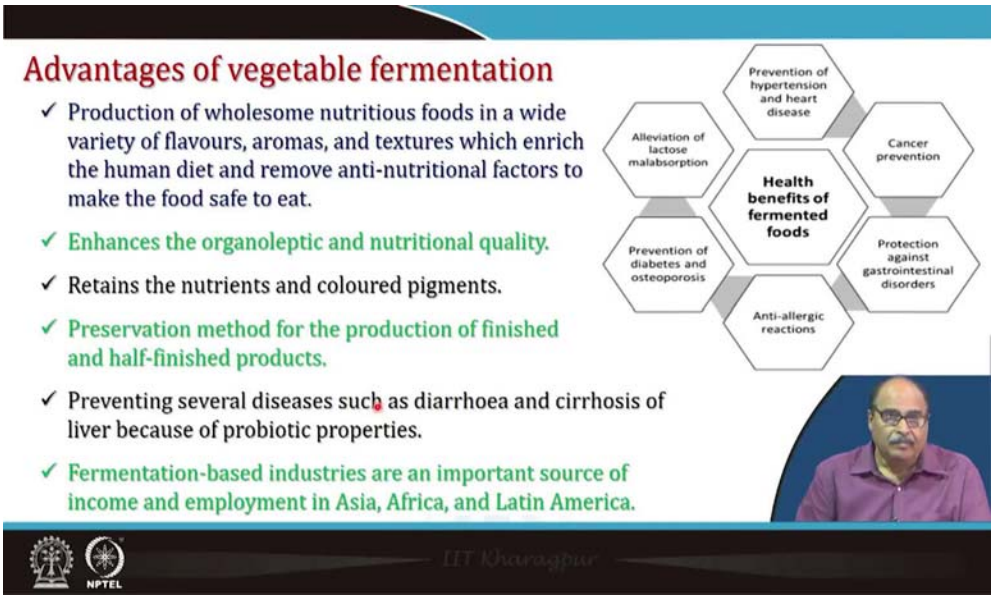
Common types of fermentation

Different types of fermentation can occur. Fermentation may be either alcoholic fermentation, which is generally encouraged by yeast and it is knowingly prominent in alcoholic beverages like wines, beers etc. Another type of fermentation is lactic acid fermentation, which is mostly done by lactic acid bacteria and here major product with the lactic acid and this type of fermentation occurs mainly in the fermented vegetables, fermented vegetable juices, etc. Acetic acid fermentation is another type of fermentation, where acetic acid bacteria play a major role, which converts sugar into acetic acid in the sugarcane juice and here, vinegar is the product. Alkaline fermentation is occurred by bacillus species and these products are known for their amino acid content and this is mainly fermented soybean products. In case of fermented vegetable products, lactic acid fermentation becomes a dominating type of the fermentation process.



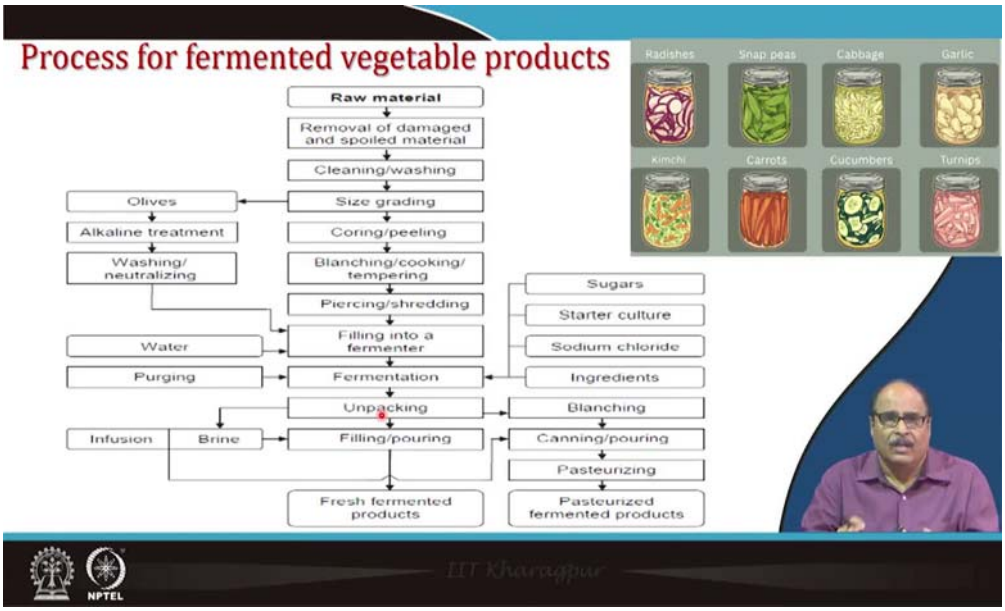
Fermentation of vegetables

Lactic acid (LA) fermentation of vegetables and fruits is a common practice to maintain and improve their nutritional and sensory characteristics. Fermentation is one of the oldest processing techniques, which extends the shelf life of the food. Availability of certain specific nutrients like vitamins, minerals, and acidic nature of the fruits and vegetables provides very conducible medium for fermentation by lactic acid bacteria. Vegetables, which contain oligosaccharides becomes a very good food for of the probiotic and they are considered prebiotic. Fruits and vegetables provide a good substrate for the growth of both probiotic bacteria and other bacteria. Vegetables have low sugar content, but are rich in minerals, and vitamins and have neutral pH and thus provide a natural medium for lactic acid fermentation process.



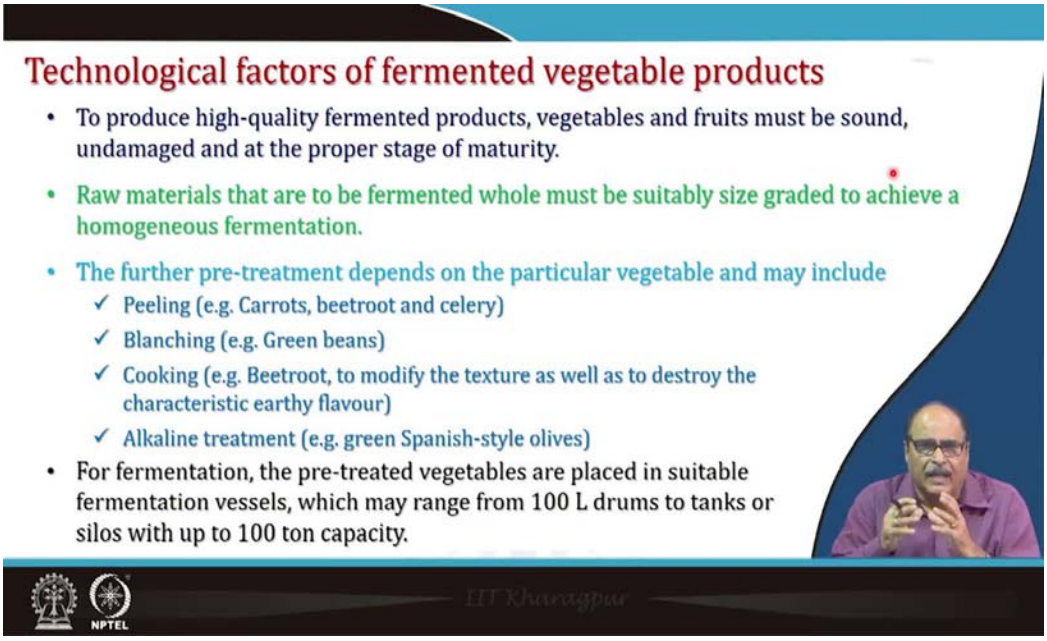
Advantages of vegetable fermentation

Vegetable fermentation helps in making the vegetable both nutritious, and wholesome. It removes some of the anti-nutritional factors. It enhances the organoleptic and nutritional quality, retains the nutrients and colored pigments in the vegetable to some extent. There is a prevention method to produce finished and half-finished product. It prevents several diseases such as diarrhea, cirrhosis, liver because of the probiotic properties. Fermentation based industries are important source of the income and employment in several countries in Asia, Africa, Latin America etc. During the fermentation process, various metabolic and bioactive compounds are generated, which further increases the nutritional characteristics.



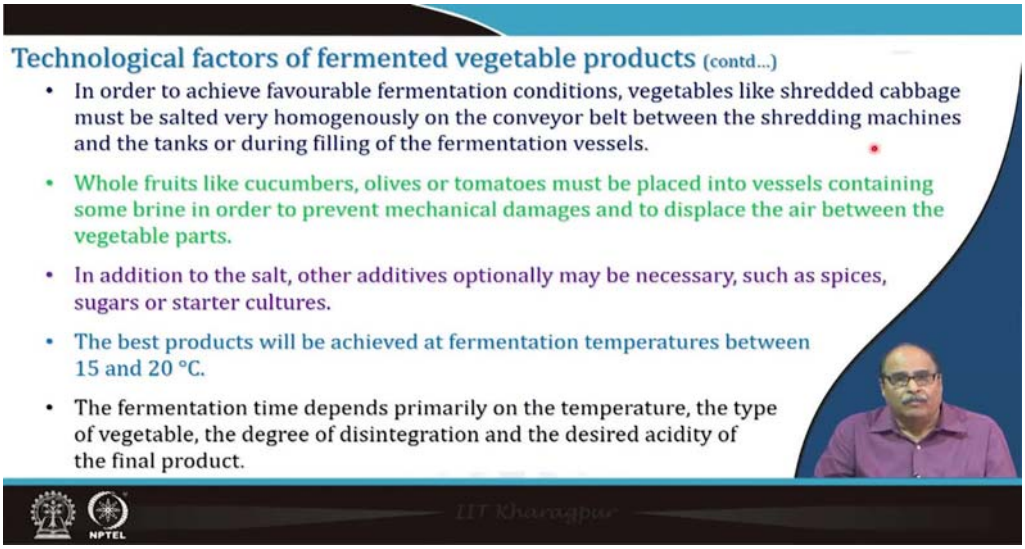
Process for fermented vegetable products

Here, a general process flowchart is given for making the fermented vegetable products. The fruits or vegetables are subjected to the treatments like cleaning, washing, cutting, grading, sizing, etc. The whole fruit or whole vegetable can be peeled or unpeeled. They can be fermented. According to the case, the fruits or vegetables are properly prepared. In the case of olives, there is size grading. These olives are given alkaline treatment, then washing and neutralization are performed, which facilitate the fermentation or growth of the bacteria. Then blanching, cooking, tempering, etc. are done and finally, juicing is performed. In case of cabbage, shredding will be performed. These prepared materials are added with some time with water, or other ingredients like sugars, starter cultures, sodium chloride, other ingredients like spices, cardamom, flavoring ingredients etc. All these together are properly mixed or smeared. In some cases, nitrogen purging is also done to avoid the oxidation related problems. Finally, it is subjected to the fermentation in suitable environment, suitable container. After the fermentation is over, the material is unpacked, maybe some times, even if required some brine can be added to infusion or even this it can be filled or poured directly as a fresh consume product or it can be subjected to the treatment like blanching, canning, pasteurization. Pasteurized and fermented product packed in the proper glass containers or other proper now pet pouches etc. are also used for its packaging.



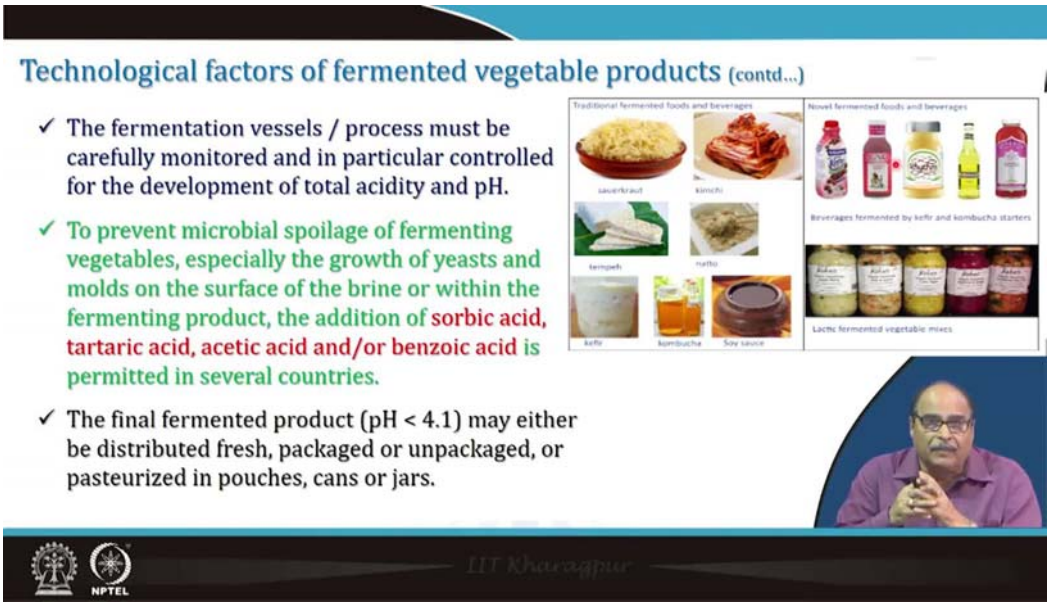
Technological factors of fermented vegetable products

To produce high quality fermented products, the fruits and vegetables must be sound, undamaged and at the proper stage of maturity. Raw materials that are to be fermented whole, must be suitably size graded to achieve a homogeneous fermentation. The further pre-treatment depends on the particular vegetable and may include peeling, blanching, cooking etc. In the case of green beans blanching becomes important. In case of carrots, beetroot, celery etc., peeling is the better fermentation process. Here, for microbial action peeling is necessary. Alkaline treatment is appropriate fermentation process for green Spanish-style, olives. For fermentation, the pre-treated vegetables are placed in suitable fermentation vessels, which may range from 100 L drums to tanks or up to silos up to 100-ton capacity.



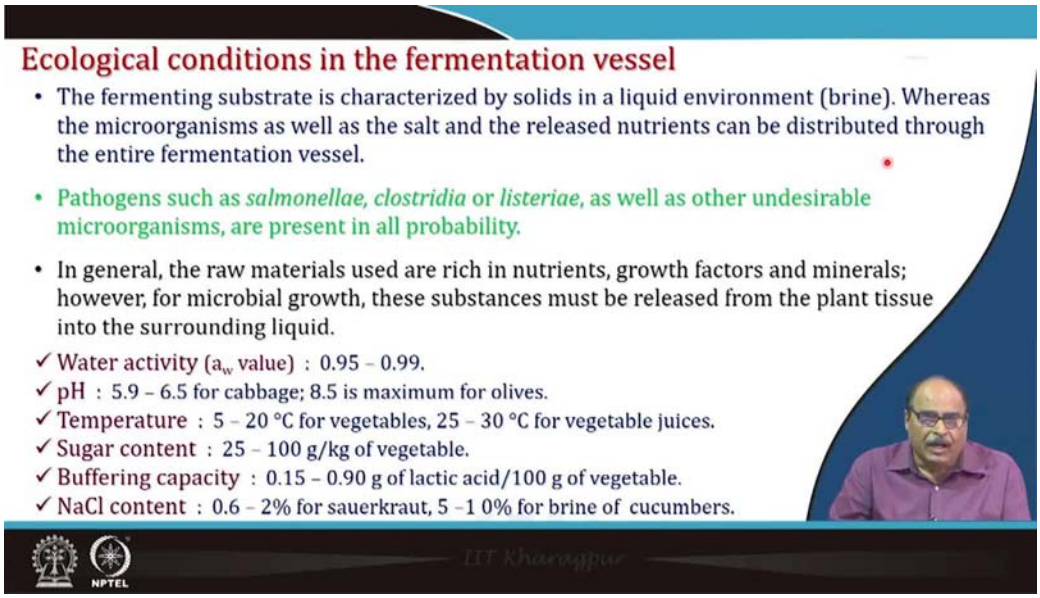
In order to achieve favorable fermentation conditions, vegetables like cabbage should be shredded and must be salted very homogeneously on the conveyor belt between the shredding machines and the tank or during filling of the fermentation vessels. Whole fruits like

cucumbers, olives or tomatoes must be placed into the vessels containing some brine in order to prevent a mechanical damage and to displace the air between the vegetable parts. In addition to the salt, other additives optionally may as necessary, such as spices, sugars, or starter cultures etc. The best products will be achieved at fermentation temperatures between 15 and 20 °C. The fermentation time depends primarily on the temperature, the type of vegetable, the degree of disintegration, and the desired acidity of the final product.



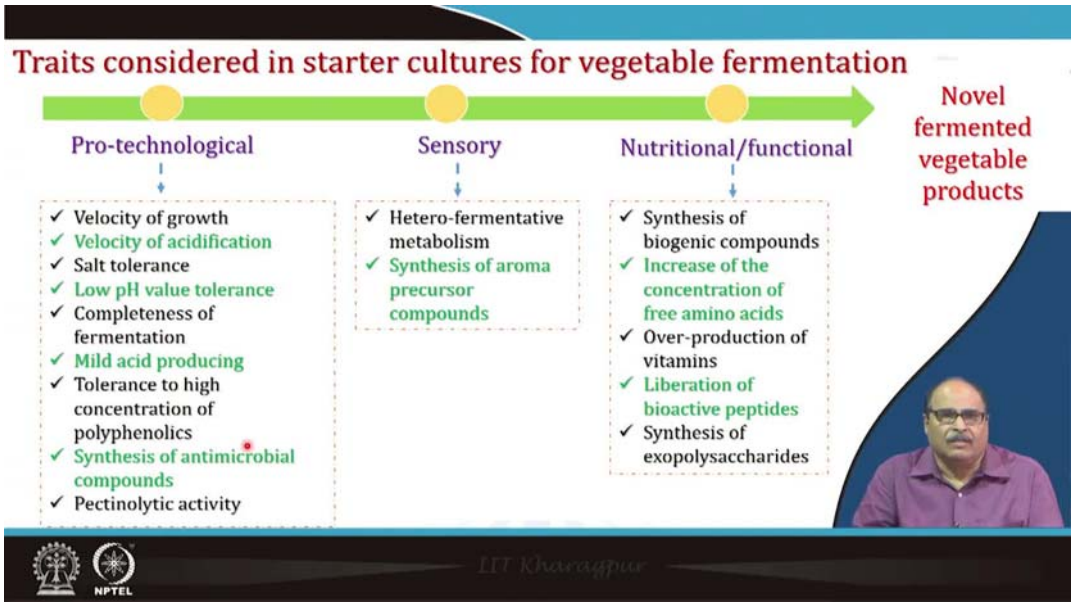
The fermentation vessel or process must be carefully monitored and in particular controlled for the development of total acidity and pH. The growth of yeasts and molds on the surface of the brine or within the fermenting products should of be taken care of to prevent microbial spoilage of fermenting vegetables.

The addition of sorbic acid, tartaric acid, acetic acid and or benzoic acid is permitted in several countries. The final fermented product (pH < 4.1) may either be distributed fresh, packaged, or unpackaged, or pasteurized in pouches, cans or jars for later consumption.



Ecological conditions in the fermentation vessel

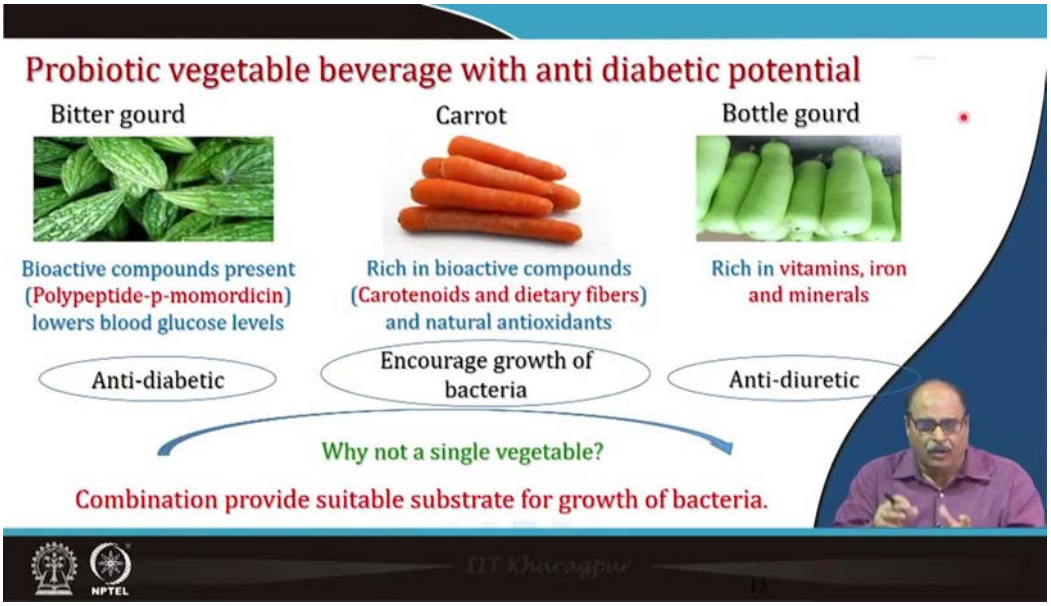
The fermenting substrate is characterized by solids in a liquid environment (brine). Whereas the microorganism as well as the salt and the released nutrients can be distributed through the entire fermentation vessel. Pathogens such as *salmonella*, *clostridia* or *listeriae*, as well as other undesirable microorganisms, are present in all probability. In general, the raw materials used are rich in nutrients, growth factors and minerals; however, for microbial growth these substances must be released from the plant tissue into the surrounding liquid. The environment should be maintained as per the requirements maybe water activity: 0.95 to 0.99, pH: 5.9 to 6.5 for cabbage; 8.5 is maximum for olives. Temperature: 5 to 20 °C for vegetable, 35 to 30 °C for vegetable juices, sugar content: maybe 25 to 100 g/kg of vegetables. Buffering capacity: 0.15 to 0.90 g of lactic acid/100 g of vegetable. Sodium chloride content: 0.6 to 2% for sauerkraut, 5 to 10% for brine of cucumbers. These conditions will be properly adjusted depending upon the materials, and the requirements.



Traits considered in a starter culture for vegetable fermentation

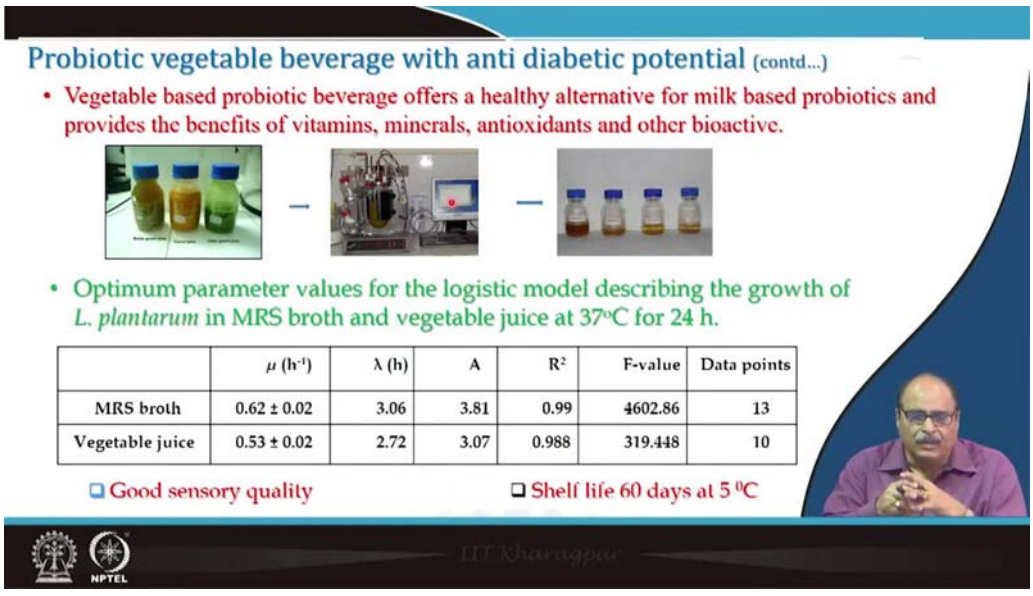
There are three aspects. One is the pro-technological aspect, i.e., the starter culture. All the possible technological efforts should be considered for selecting the starter culture. The velocity of the growth, velocity of acidification, salt tolerance, low pH value tolerance, completeness of fermentation process, whether it is mild acid producing or high acid producing, its tolerance to high concentration of polyphenolics, because most of these fruits and vegetables contain high number of polyphenolics, all are to be taken care of. Synthesis of antimicrobial compounds, pectinolytic activity etc. are also to be taken care of. All these things are the pre-technological aspects, which are taken into consideration while selecting the starter culture. The second aspect is the sensory characteristics. This may be the hetero-fermentative metabolism. Then it produces various products or synthetics of aroma precursor compounds take place. Therefore, the various secondary metabolites, which are produced and how do they influence the sensory characteristics of the product, are another very important considerations. The third aspect is the synthesis of nutritional and functional components like

biogenic compounds. Increase of the concentration of free amino acid, over production of vitamins, liberation of bioactive peptides, synthesis of the exopolysaccharide etc. are also considered properly. If the starter culture, raw material, proper conditions are maintained during fermentation, it will give a novel fermented vegetable product.



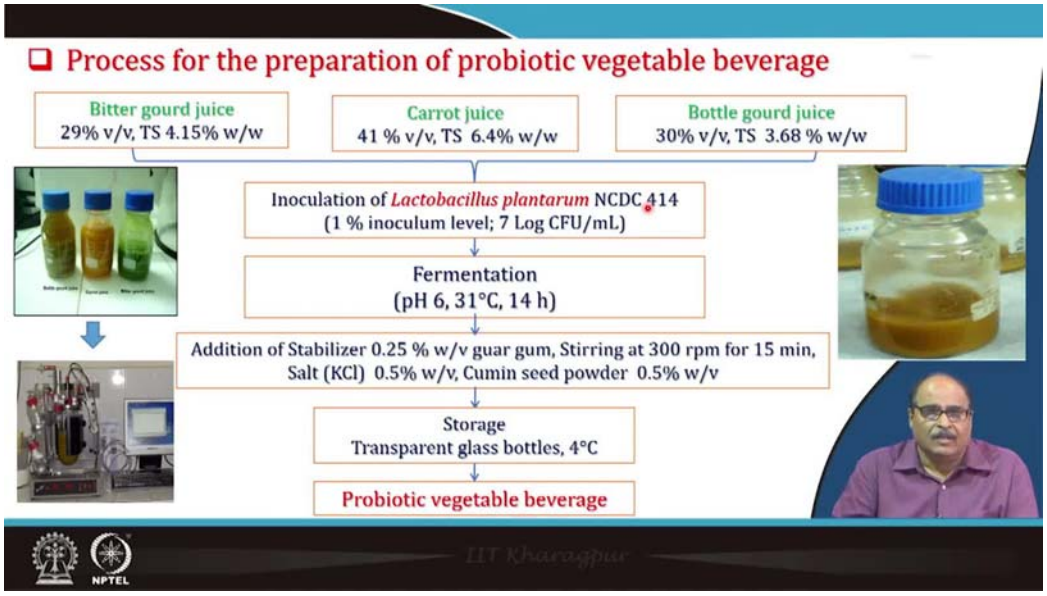
Probiotic vegetable beverage with anti-diabetic potential

Bitter gourd, carrot, and bottle gourd have been taken. Bitter gourd has bioactive compounds present in it i.e., Polypeptide-p-momordicin and it is known to lower the blood glucose levels. Bitter gourd is a anti diabetic material. Carrot is also rich in bioactive compounds like carotenoids and dietary fibers as well as natural antioxidants. It encourages the growth of the probiotic bacteria, it provides a good substrate i.e., micronutrients for the bacterial growth. The bottle gourd is rich in vitamins and irons and minerals. It is anti-diuretic. Combination of vegetables always provide suitable substrate for growth of bacteria.



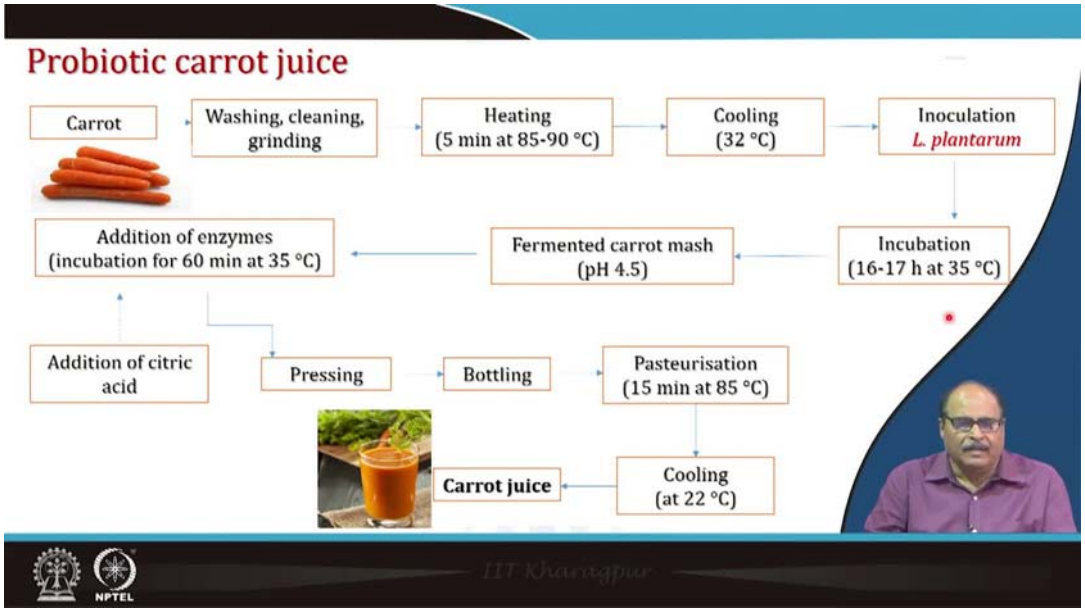
	μ (h^{-1})	λ (h)	A	R^2	F-value	Data points
MRS broth	0.62 ± 0.02	3.06	3.81	0.99	4602.86	13
Vegetable juice	0.53 ± 0.02	2.72	3.07	0.988	319.448	10

Optimum parameter value for the logistic model describing the growth of *lactobacillus planetarum* in MRS broth and vegetable juice at 37 °C for 24 hours are given in the table. It can be seen from the table that the vegetable juices provide good substrate for the growth of the probiotic bacteria. The final product has a good sensory quality and its self-life is found to be 60 days at 5 °C temperature.



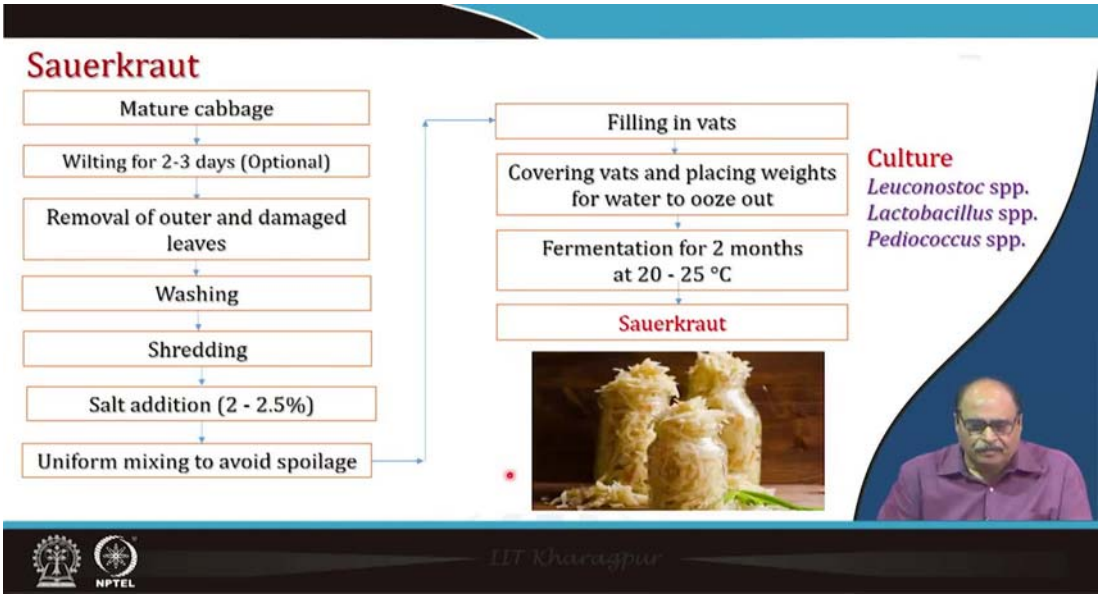
Process technology for the preparation of probiotic vegetable beverage

Bitter gourd juice, carrot juice and bottle gourd juice are taken first. 29% v/v bitter gourd juice, 41% v/v carrot juice, 30% v/v bottle gourd juice are taken. The total solid (TS) content for bitter gourd is about 4.1% w/w, for carrot juice the TS content is 6.4% w/w and for bottle gourd juice it is about 3.68% w/w. These 3 juices are mixed first and finally inoculated with the *Lactobacillus plantarum* NCDC 414. The inoculation level is 1% and initially the concentration is 7 log CFU per ml. it is then fermented. The fermentation is carried at pH 6, and temperature is 31 °C for 14 hours. Sometimes, there is addition of stabilizer like 0.25% w/v guar gum. After that, stirring is performed at 300 rpm for 15 minutes. Then salt and cumin seed powder are added in order to improve the flavor. The storage is done into transparent glass bottles and kept at 4 °C. In this way, a healthy and tasty probiotic vegetable beverage is produced.



Probiotic carrot juice

In this case, the ground carrot is heated for 5 minutes at 85-90 °C. Cooling is performed then. After that, inoculation is done with *Lactobacillus plantarum* and it is incubated for 16-17 hours at 35 °C. The fermented carrot mash is obtained whose pH is about 4.5. Then the addition of enzymes is done and it is incubated for 60 minutes at 35 °C and citric acid is added here. Then this is pressed for getting the fermented juice. The juice is bottled and finally pasteurized. Pasteurization is done for 15 minutes at 85 °C. It is cooled then and finally the pasteurized carrot juice containing probiotic is obtained.



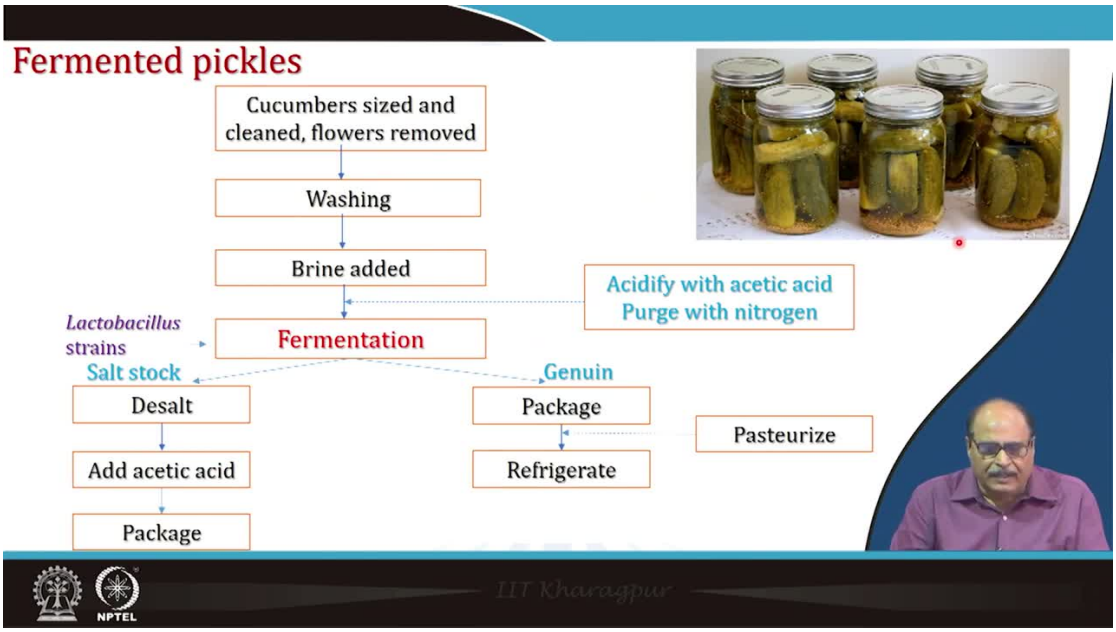
Sauerkraut

Sauerkraut is fermented cabbage. Mature cabbage is taken first. Wilting is done for 2 to 3 days, which is an optional step. Then removal of the outer and damaged leaves is performed. Washing and shredding are done. Then salt is added to 2 to 2.5% and it is ensured that this

salt should be mixed uniformly in order to avoid spoilage. Then it is filled in vats and covered. The vats are covered with suitable cover and the cover is placed by placing weights for water to ooze out. Then the fermentation process is carried out for about 2 months at 20-25 °C. After the sauerkraut is obtained. The cultures, which are used here are *Leuconostoc spp.*, *Lactobacillus spp.*, *Pediococcus spp*.

Fermented beetroot wine

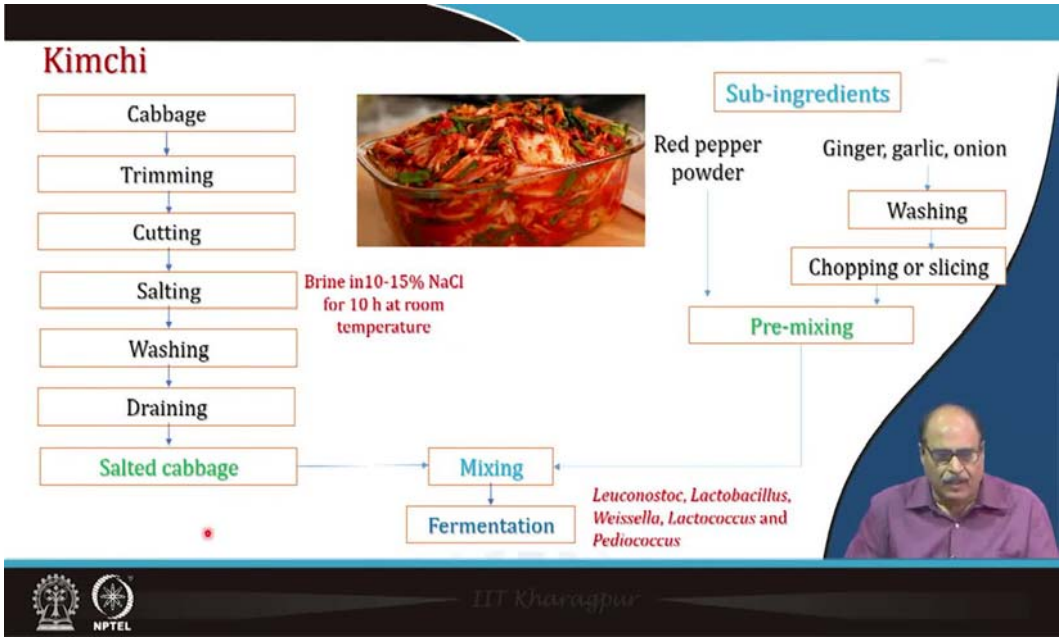
The beetroot juice is taken first and it is subjected to the fermentation process like the same steps e.g., fermentation, clarification, packaging, etc.



Fermented pickles

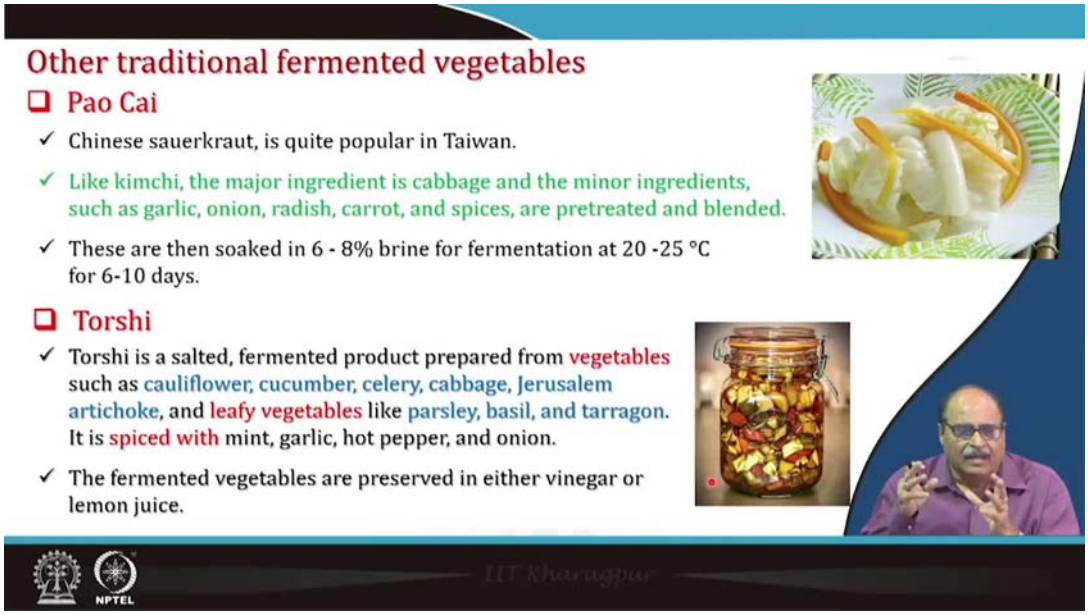
Cucumbers are sized and cleaned. The flowers are removed. The cucumbers are washed. Then brine is added. Sometimes the whole mixture is acidified with acetic acid and purged

with nitrogen. Fermentation is done now. Salt stock is obtained which is desalted. Acetic acid is added then and packaging is done. Even genuine as such package and refrigerate or even pasteurized.



Kimchi

Cabbage is used in this process. It is the salted then. The cabbage is brine in 10-15% NaCl for 10 hours at room temperature. The sub-ingredients like red pepper powder, ginger, garlic, onions, etc. are chopped and these are all prepared. The cabbage and the other sub-ingredients are mixed, and the fermentation is done. It is allowed with the fermenting bacteria like *Leuconostoc*, *Lactobacillus*, *Weissella*, *Lactococcus*, etc.



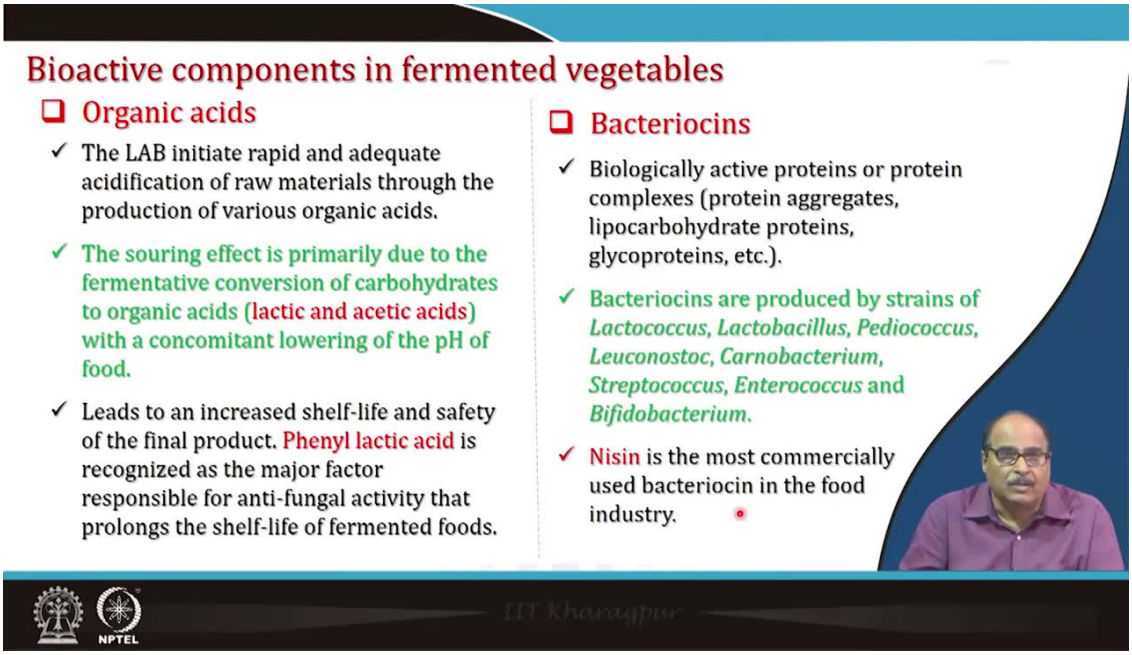
Other traditional fermented vegetables

Pao cai

Chinese sauerkraut, is quite popular in Taiwan. Like Kimchi, the major ingredient is cabbage and the minor ingredients are garlic, onion, radish, carrot, and spices. They are pretreated and blended. They are then soaked in 6 to 8% brine for fermentation at 20-25 °C for 6 to 10 days.

Torshi

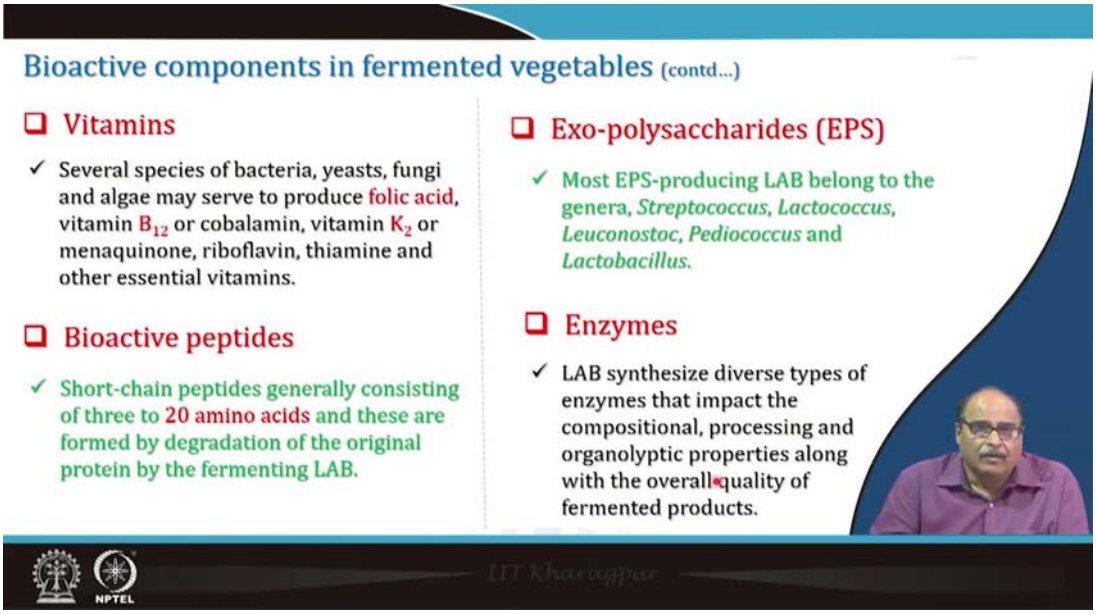
Torshi is a salted, fermented product prepared from vegetables such as cauliflower, cucumber, celery, cabbage, Jerusalem artichoke, and leafy vegetables like parsley, basil, and tarragon. It is a spiced with mint, garlic, hot pepper, and onion. The fermented vegetables are preserved in either vinegar or in the lime juice.



Bioactive components in the fermented vegetables

Several organic acids are produced. The lactic acid bacteria (LAB) initiate a rapid and adequate acidification of raw materials through the production of various organic juice.

The lactic acid and acetic acid are the commonly produced organic acids during this process that also lead to the increased shelf-life of the final product. Phenyl lactic acid is recognized as the major factor which is responsible for the antifungal activity that prolongs the shelf-life of fermented foods. Bacteriocins another very important bioactive component, that it produced during this bacterial growth with various lactic acid bacteria etc. They produce various biologically active compound, biologically active proteins, or protein complexes like protein aggregates, lipocarbohydrate proteins, glycoproteins, etc. *Lactococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Streptococcus*, *Enterococcus*, *Bifidobacterium* are known to produce bacteriocins. Nisin is one such bacteriocins which is the commercially used bacteriocin in several food industry.



Vitamins

Several species of bacteria, yeasts, fungi and algae may serve to produce folic acid, vitamin B_{12}, vitamin K_2, menaquinone, riboflavin, thiamine and other essential vitamins.

Bioactive peptides

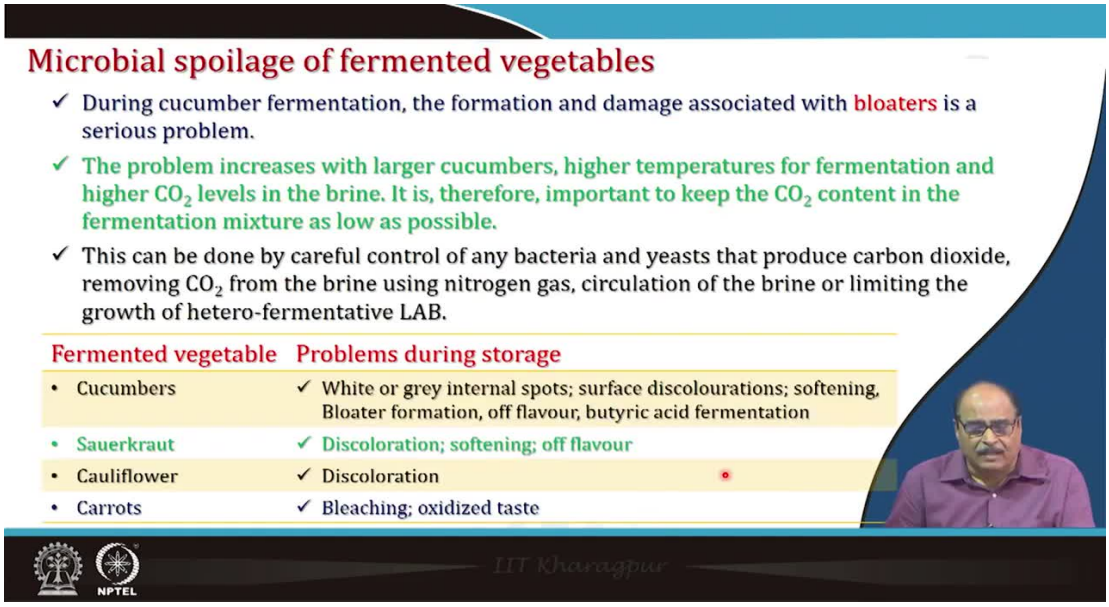
Short-chain peptides generally consisting three to 20 amino acids and these are also formed by degradation of the original protein by fermenting LAB. They have the health benefits.

Exo-polysaccharides (EPS)

Most exo-polysaccharide producing lactic acid bacteria belong to the genera, *Streptococcus Lactococcus*, *Leuconostoc*, *Pediococcus*, and Lactobacillus. These exo-polysaccharides (EPS) are also present in their fermented products and which act as a prebiotic food for the probiotic bacteria.

Enzymes

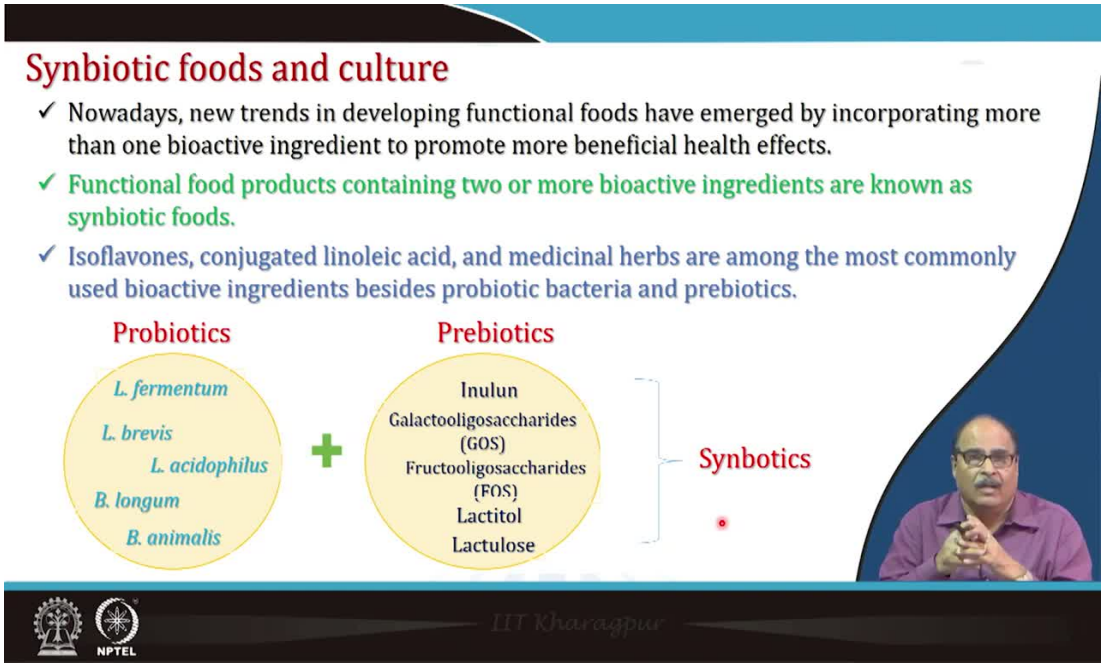
Lactic acid bacteria synthesizes diverse types of enzymes that impact the compositional processing and organoleptic properties along with the overall quality of the fermented products.



Microbial spoilage of the fermented vegetables

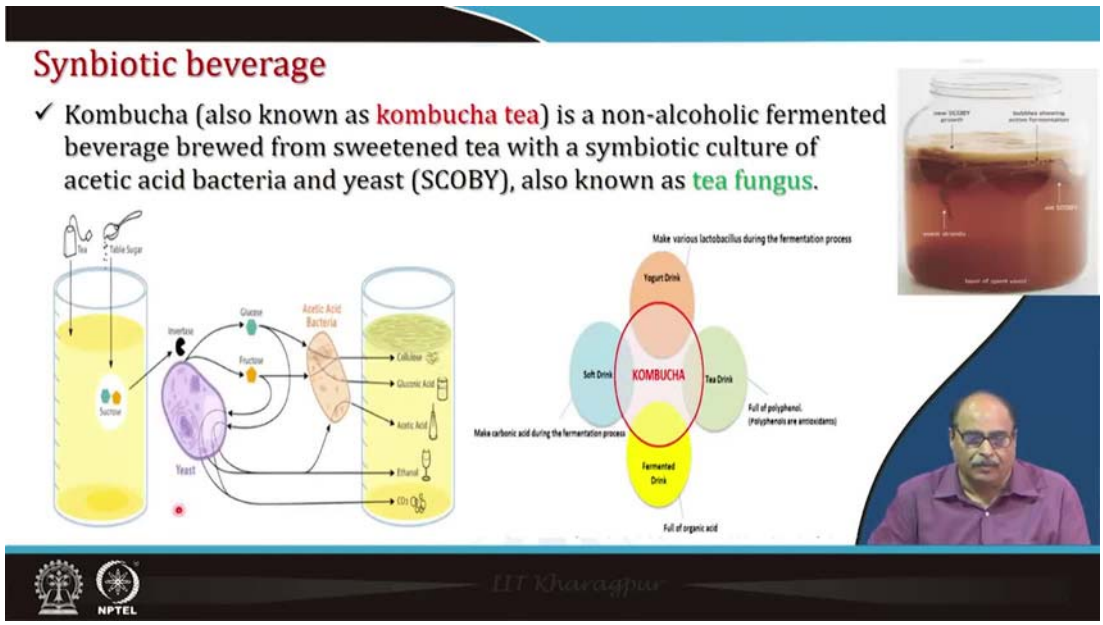
During cucumber fermentation, the formation and damage associated with the bloaters is a serious problem. The problem increases with larger cucumbers higher temperatures for fermentation and higher CO_2 level in the brine. It is therefore important to keep the CO_2 content in the fermentation mixture as low as possible. This can be done by careful control of any bacteria and yeasts that produce CO_2, and removing the CO_2 from the brine using nitrogen gas, circulation of the brine or limiting the growth of hetero-fermentative bacteria, which produces more and more carbon dioxide.

From the Table, it can be seen that in case of the fermented vegetable cucumber, there will be problems during the storage like white or grey internal spots, surface discolorations, softening, bloater formation, off flavor, butyric acid fermentation. In case of sauerkraut, discoloration, softening, off flavor are there. In case of cauliflower, discoloration will be the result. For carrots, the bleaching, oxidized taste are the problems arise during the storage. Therefore, proper care should be taken to ensure that there is no microbial spoilage during storage.



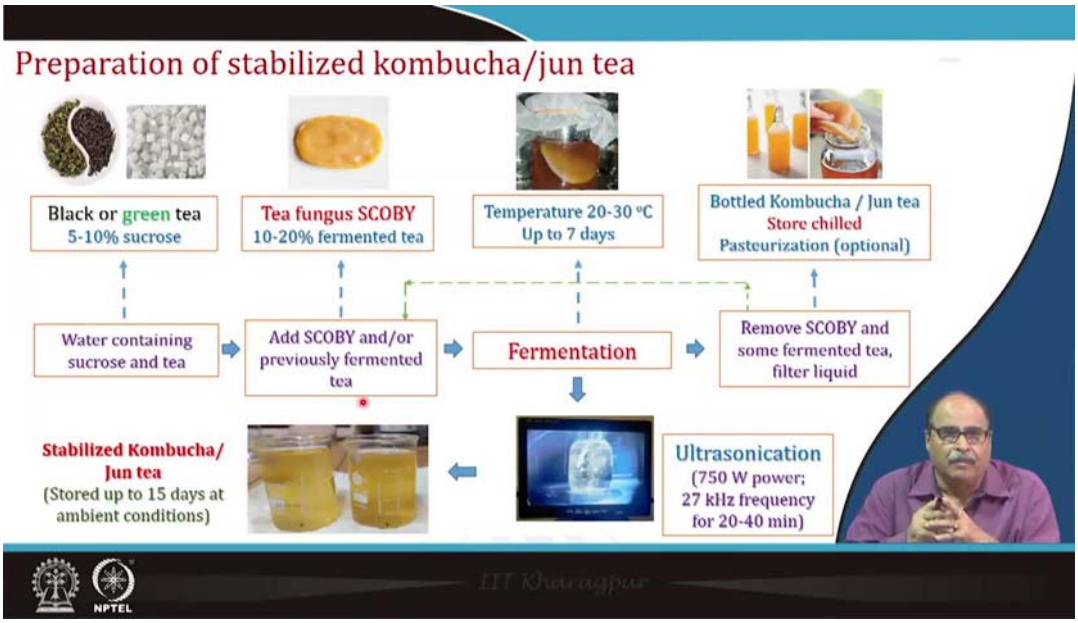
Synbiotic foods and culture

Nowadays, new trends in developing functional foods have emerged by incorporating more than one bioactive ingredient to promote more beneficial health effects. The probiotic bacteria like *L. farmentum*, *L. brevis*, *L. acidophilus*, *B. longum* and the prebiotic like oligosaccharides, inulin, galactooligosaccharide, fructooligosaccharide, lactitol, lactulose is mixed to produce symbiotic food. While selecting a prebiotic, the effort must be taken that it should not adversely affect the sensory characteristics. When this synbiotic foods are consumed it means that the food is consumed along with the live bacteria. Inside human gut, this microorganisms grow and provide a beneficial effect.



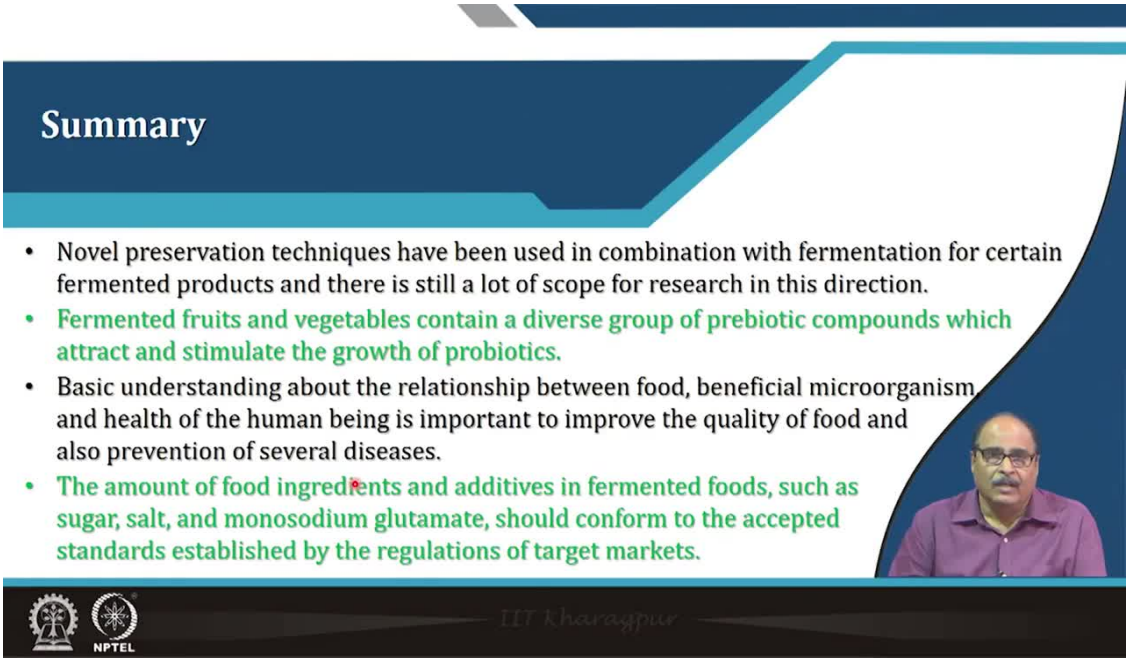
Synbiotic beverage

Kombucha (also known as Kombucha tea) is a nonalcoholic fermented beverage brewed from sweetened tea and with a symbiotic culture of acetic acid bacteria and yeast, that is known as SCOBY, also known as tea fungus. The sucrose infused green tea or black tea is taken first. This sucrose and this yeast enzyme act. This converts it into acetic acid bacteria. It has some action. The cellulose, gluconic acid, acetic acid, ethanol, carbon dioxide, which are present inside the microbe, will be present in the beverage and they give various effect.



Preparation of stabilized kombucha/jun tea

This technology has been developed. The water containing sucrose and tea i.e., either black tea or green tea, which contains around 5 to 10 % sucrose has been taken first. SCOBY is added then. SCOBY is a previously fermented tea. The tea fungus SCOBY is 10 to 20 % fermented tea. These two are fermented then. The temperature is around 20 to 30 °C. The fermentation is allowed to continue up to 7 days. Then Kombucha or Jun tea is produced. Removal of SCOBY, fermented tea, filter liquid etc. has been done. It can be bottled. Bottled Kombucha or Jun tea is stored, chilled, or pasteurized. After fermentation, ultrasonication treatment maybe done at 750 W power, 27 kHz frequency for about 20-40 minutes. This process gives a stabilized Kombucha or Jun tea. It can be stored up to 15 days at ambient condition. It is a very good healthy and tasty tea drink i.e., fermented tea drink.



Summary

In summary it can be stated that novel preservation technologies have been used in combination with fermentation for certain fermented products and there is still a lot of scope for research and development in this area. Fermented fruits and vegetables contain a wide group of prebiotic components, which attract and stimulate the growth of probiotic etc. Basic understanding about the relationship between food, and beneficial microorganisms, the beneficial effects of its constituents, the processing conditions, during processing their behavior, the influence in sensory characteristics by the post processing conditions of the probiotic containing food, its storability etc. should be there. The amount of food ingredients and additives in fermented foods, such as sugar, salt, and monosodium glutamate, should conform to the accepted standards established by the target markets.

The references used in this lecture are mentioned above.