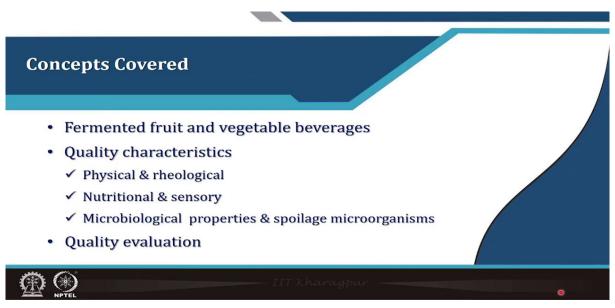
Post Harvest Operations and Processing of Fruits, Vegetables, Spices and Plantation Crop Products Professor H. N. Mishra Agricultural and Food Engineering Department Indian Institute of Technology, Kharagpur Lecture 50 Quality Characteristics



This lecture discusses the quality characteristics of plant based fermented foods and beverages. It includes different fermented fruit and vegetable beverages and its quality characteristics such as physical and rheological qualities, nutritional and sensory properties, microbiological properties and spoilage microorganisms. It also discusses about evaluation of these properties and quality characteristics.

# **Fermented Fruits and Vegetables**

# <text><list-item><list-item><list-item> **Demented fruit and vegetables are usually fermented to improve** the palatability and shelf life. **Many forms of juice either raw, centrifuged and filtered are** used for fermentation. However, it is important to kill the harmful spoilage microorganism thus heat treatments like pasteurization, sterilization are usually done before fermentation. Various types of fermentation depending upon the need is done by making different conditions favorable for enzyme activities. The fermented beverage is stored at low temperature for higher shelf life.

The fruits and vegetables are usually fermented to improve the palatability and shelf life. Many forms of juice either raw, centrifuged and filtered are used for fermentation. However, it is important to kill the harmful spoilage microorganism thus heat treatments like pasteurization, sterilization are usually done before fermentation. Various types of fermentation depending upon the need is done by making different conditions favorable for enzyme activities. The fermented beverage is stored at low temperature for higher shelf life.

Beverage	Origin	Substrate	Microorganisms isolated	
Hardaliye	Turkey	Red grapes	Lactobacillus paracasei, L. casei, L. brevis, L. pontis, L. acctotolerans, L. sanfrancisco. L. vaccinostereus	
Kombucha	China	Tea	Glucomacctobacter spp. (G. xylinus), Acctobacter spp., Lactobacillus spp., Zygosaccharomyces spp., Hanseniaspora spp., Torulaspora spp., Pichia spp., Dekkera spp., Saccharomyees spp.	
Gefilus	Finland	Fruit juice	Lactobacillus rhamnosus GG, Propionibacterium freudenreichii ssp. shermanii JS	

**Different Beverages and Microorganisms Used in Fermentation** 

The table provided shows different fermented beverages from different countries and the microorganism used for the fermentation or that has been isolated from the fermented beverages. Hardaliye is a beverage originated from Turkey that has red grapes as its substrate. Microorganism that are isolated from the beverage are Lactobacillus paracasei, L. casei, L. brevis, L. pontis, L. acctotolerans, L. sanfrancisco. L. vaccinostereus. Kombucha is another feremented beverage from China. It is fermented tea and microorganisms used are Glucomacctobacter spp. (*G*. xylinus), Acctobacter spp., Lactobacillus spp., Zygosaccharomyces spp., Hanseniaspora spp., Torulaspora spp., Pichia spp., Dekkera spp., Saccharomyees spp. Gefilus is a fermented juice from Finland and organisms used are Lactobacillus rhamnosus GG, Propionibacterium freudenreichii ssp. shermanii JS.

Beverage	Origin	Substrate	Probiotic microorganisms	
Proviva	Sweden	Orange, strawberry, or blackeurrant juice	Lactobacillus plantarum 299v	
GoodBelly	U.S.A.	Mango, blueberry acai, pomegranate, blackberry, tropical green, cranberry, watermelon, tropical orange, and coconut water juices	Lactobacillus plantarum 299V	
Biola	Norway, Finland	Orange-mango and apple- pear flavors and several vegetable juices	Lactobacillus rhamnosus GG	

In Sweden, Proviva is prepared from orange, strawberry or black currant juices. Microorganisms involved is *Lactobacillus plantarum 299v*. Goodbelly is a fermented beverage from USA prepared from Mango, blueberry acai, pomegranate, blackberry, tropical green, cranberry, watermelon, tropical orange, and coconut water juices, fermented by *Lactobacillus plantarum 299v*. Norway and Finland has Biola prepared from orange, mango, apple-pear flavour and several vegetable juices. *Lactobacillus rhamnosus GG* is the organism involved.

Beverage	Origin	Substrate	Probiotic microorganisms	
Kevika	U.S.A.	Sparkling lemon ginger probiotic drink	Bacillus coagulans, L. rhamnosus, L. plantarum, L. paracasei	
Rela	Sweden	Fruit juice	Lactobacillus reuteri MM <sub>53</sub>	
Healthy life probiotic	Australia	Apple and mango juice	Lactobacillus paracasei and Lb. plantarum	
Malee probiotic juices	Thailand	White grape and orange juice	Lactobacillus paracasei	
fermentation	of fruit and v	the common microorg vegetable beverage. depending upon the ty		

Kevika is from USA, which is a sparkling lemon or ginger probiotic drink. Microorganisms used are *Bacillus coagulans, L. rhamnosus, L. plantarum, L. paracasei*. In Sweden Rela it is prepared from fruit juices and *Lactobacillus reuteri MM*<sub>53</sub>. Healthy life probiotic is a drink from Australia made from apple and mango juices and *Lactobacillus paracasei and Lb. plantarum*. In Thailand, Malee probiotic juice is prepared from white grape and orange juice. *Lactobacillus* 

*paracasei* is the microorganism used. It could be summarized from the table that lactobacillus plantarum is the most commonly used microorganism for the fermentation of food and beverages, which is a probiotic bacterium. Substrate can be different depending upon the type of beverage prepared.

# **Quality Characteristics**

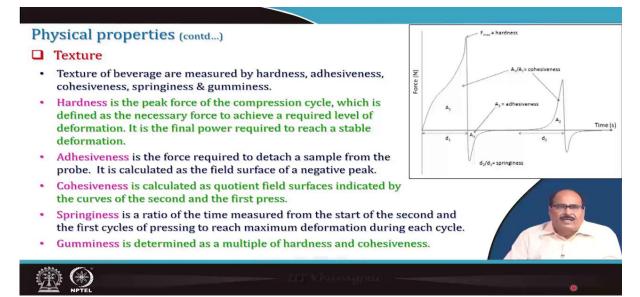




Quality characteristics includes the physical, chemical, and microbiological properties of the beverage. The physical properties of fermented beverage are of great importance for their positive perception by consumers. The plant based fermented beverage must provide the required texture liked by consumer. The consumer do their first judgment based on the physical properties of the fermented beverage. Color and appearance are the most important for quick judgment. However, taste, texture, aroma, etc. also contributed equal role in acceptance of final products. The physical properties of the beverages that are considered in quality analysis are texture, total soluble solids (TSS), color, pH, titratable acidity and aroma.

# Texture

The texture is measured as hardness, adhesiveness, cohesiveness, springiness and gumminess. The texture profile curve provided shows that hardness is the highest force, followed by cohesiveness and adhesiveness as the negative peak.



Hardness is the peak force of the compression cycle, which is defined as the necessary force to achieve a required level of deformation. It is the final power required to reach a stable deformation. Adhesiveness is the force required to detach a sample from the probe. It is calculated as the field surface of a negative peak. Cohesiveness is calculated as quotient field surfaces indicated by the curves of the second and the first press. Springiness is a ratio of the time measured from the start of the second and the first cycles of pressing to reach maximum deformation during each cycle. Gumminess is determined as a multiple of hardness and cohesiveness.

#### Physical properties (contd...)

- Total soluble solids
- Total soluble solids (TSS) are measured by refractometer either hand and abbe's refractometer.
- The measured TSS or sugar content includes the carbohydrates, organic acids, proteins, fats and minerals of the fruit.
- It represents from 10-20% of the fruit's fresh weight which increased as fruit matured making the product less acidic, sweeter fruit.

#### Color measurement

- The color of the fruit juice and concentrate was determined using a colorimeter.
- This is very important property as it defines the first appearance of fermented fruits and vegetable beverages.

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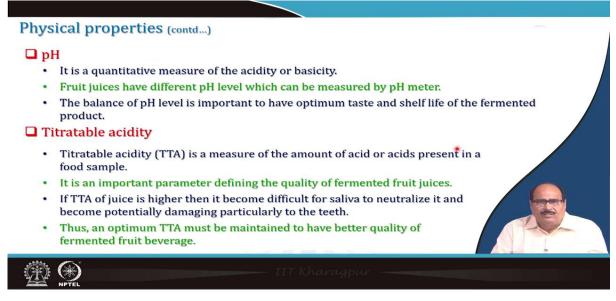
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## **Total Soluble Solids**

Total soluble solids (TSS) are measured by refractometer either hand and abbe's refractometer. The measured TSS or sugar content includes the carbohydrates, organic acids, proteins, fats and minerals of the fruit. It represents from 10-20% of the fruit's fresh weight which increased as fruit matured making the product less acidic, sweeter fruit.

# **Color Measurement**

The color of the fruit juice and concentrate was determined using a colorimeter. This is very important property as it defines the first appearance of fermented fruits and vegetable beverages. For measurement photometric methods or Hunter lab colorimeter is used.

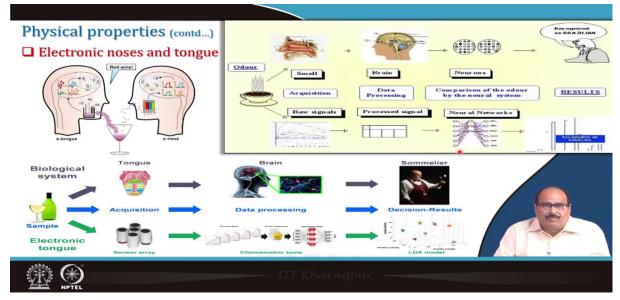


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pH is a quantitative measure of the acidity or basicity. Fruit juices have different pH levels which can be measured by pH meter. The balance of pH level is important to have optimum taste and shelf life of the fermented products.

# **Titratable acidity**

Titratable acidity is a measure of the amount of acid or acid present in a food sample. It is an important parameter defining the quality of the fermented fruit juices. If titratable acidity of juice is higher, then it becomes difficult for saliva to neutralize it and becomes potentially damaging particularly to the teeth. Thus an optimum TTA must be maintained to have a better quality of fermented fruit beverage.



## **Electronic Noses and Tongue**

Electronic noses and tongue could be used to determine the taste and smell of the fermented beverages. The slide shows the sensory perception of the red wine via human tongue and nose, and electronic tongue and nose. Wine tasting requires high skill and sensory perception.

The mouth has tongue which has taste buds, where acquisition of taste occurs. Similarly, etongue has sensory arrays that works as taste buds. They collect the information as like in our actual system. Then information acquired by tongue is immediately sent to our brain for processing. The brain analyses the information sent by the tongue and record it as the taste of that product. The e-tongue send the information to the chemometric tools, process it by comparing the pre-recorded data sets and models developed to give the decision.

Similarly, the olfactory cells in the human nose acquires the information on the smell and send it to the brain by the nerves. The neurons in the brain compares the odor and provides the decision. The e-nose acquires the smell using sensors and the information is processed using neural networking and decision is provided.

In summary, similar to the human nose and tongue these electronic devices use sensors and processing model for brain to provide results. The food matters are volatilized and the volatiles are sensed by sensors like metal oxide sensors. The data collected is sent to the instrument to analyze using neural network or PCA. In discriminative sensory analysis the system is first provided with fresh samples or standard samples and the data generated is compared with the given samples.

#### **Rheological properties**

- The rheological measurement of the fermented beverage is important for knowing about its consistency.
- In general, the controlled viscometer having coaxial geometry or cone and plate geometry is used for carrying out steady shear experiment.
- In this experiment, the shear rate is varied from 0 to 100 Hz and the temperature is remained fixed.
- The viscosity vs. shear rate graph reveal flow property of juice which is either the pseudoplastic or dilatant.
- The consistency coefficient of juice sample is measured by evaluation of Power Law model.

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0.90

0.7

0.3

c (1/s)

(mPa.s)

liscosity

pparent

#### **Rheological Properties**

The rheological measurement of the fermented beverage is important for knowing about its consistency. In general, the controlled viscometer having coaxial geometry or cone and plate geometry is used for carrying out steady shear experiment. In this experiment, the shear rate is varied from 0 to 100 Hz and the temperature is remained fixed. The the viscosity versus shear rate graph this revealed the flow property of the juices which is either a pseudoplastic or dilatant. The figure shows the curve plotted with apparent viscosity (mPa.s) as Y-axis and shear rate (1/s) as X-axis at different temperatures. The apparent viscosity decreases with increase in shear rate and temperature. The consistency coefficient of juice sample is measured by evaluation of Power Law model.

#### Rheological properties (Contd.) Power law model The consistency coefficient and flow behaviour index are used for explaining the $\eta = K \gamma^{n-1}$ rheological properties of fermented beverage. Temperature and total soluble solids of Where, beverage affect consistency coefficient and $\eta$ is viscosity of juice (Pas) viscosity. K is the consistency coefficient (Pas<sup>n</sup>) The consistency coefficient and viscosity γ is shear rate (Hz) of beverage follows Arrhenius concept. n is flow behavior index $K = A_K \exp\left(\frac{E_K}{RT}\right)$ Where, $A_{K}$ , $A_{\eta}$ are the pre-exponential constants $E_{\kappa}$ , $E_{n}$ are the activation energy (KJ/mol) $\eta = A_{\eta} \exp\left(\frac{E_{\eta}}{RT}\right)$ R is the universal gas constant (KJ/K.mol) T is absolute temperature (K)

The power law model is given by the equation,

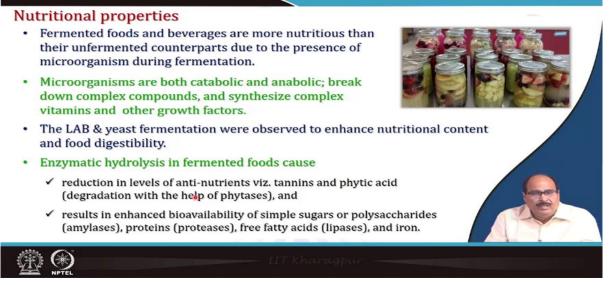
$$\eta = K\gamma^n$$

Where,  $\eta$  is viscosity of juice (Pas); K is the consistency coefficient (Pas<sup>n</sup>);  $\gamma$  is shear rate (Hz); n is flow behavior index.

$$K = A_K \exp \frac{E_K}{RT}$$
$$\eta = A_\eta \exp \frac{E_\eta}{RT}$$

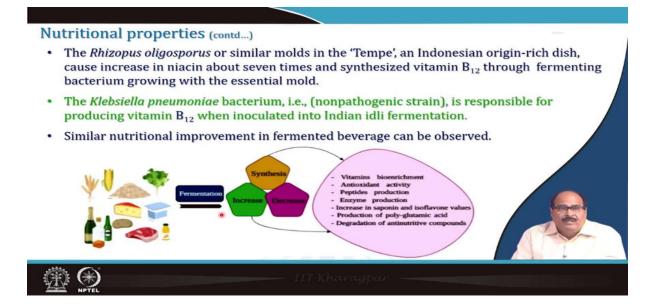
Where,  $A_K$ ,  $A_\eta$  are the pre-exponential constants;  $E_K$ ,  $E_\eta$  are the activation energy (KJ/mol); R is the universal gas constant (KJ/K.mol); T is absolute temperature (K).

The consistency coefficient and flow behaviour index are used for explaining the rheological properties of fermented beverage. Temperature and total soluble solids of beverage affect consistency coefficient and viscosity. The consistency coefficient and viscosity of beverage follows Arrhenius concept.



## **Nutritional Properties**

During the fermentation process, various new primary and secondary metabolites are produced and compounds like vitamins, minerals are synthesized. Thus, fermented beverages are considered more nutritive then their unfermented counterparts. Fermented foods and beverages are more nutritious than their unfermented counterparts due to the presence of microorganism during fermentation. Microorganisms are both catabolic and anabolic; break down complex compounds, and synthesize complex vitamins and other growth factors. The LAB & yeast fermentation were observed to enhance nutritional content and food digestibility. Enzymatic hydrolysis in the fermented food cause reduction in the levels of anti-nutrients like tannins and phytic acids et cetera, degradation with the help of phytases, and results in enhanced availability of simple sugar or polysaccharides (amylases), proteins (protease), free fatty acids (lipases), and iron.



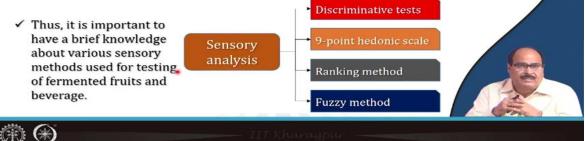
The *Rhizopus oligosporus* or similar molds in the 'Tempe', an Indonesian origin-rich dish, cause increase in niacin about seven times and synthesized vitamin  $B_{12}$  through fermenting bacterium growing with the essential mold. Thus, the fermented food has B12 which is not normally present in plant food sources. Similarly, *Klebsiella pneumoniae* bacterium, i.e., (nonpathogenic strain), is responsible for producing vitamin B12 when inoculated into Indian idli fermentation. Similar nutritional improvement in fermented beverages can be observed. Fermentation process synthesis vitamins resulting in bio-enrichment, produces peptides, enzymes, polyglutamic acid, increases antioxidant activity, saponin and isoflavone values, and causes degradation of antinutritive compounds.

## **Sensory Properties**

Sensory properties is crucial as most of the fermented beverages are accepted based on their sensory characteristics by the consumers. The parameters analyzed through sensory studies include taste, aroma, flavour, colour, appearance, aftertaste, texture et cetera.

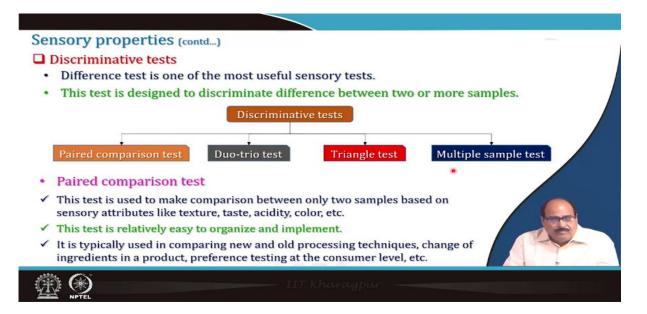
#### Sensory properties

- The parameters analysed through sensory studies include taste, aroma, flavour, colour/appearance, aftertaste, texture, etc.
- Various statistics based methods are used for analysing the sensory data and draw conclusion from it.
- The acceptability of fermented fruits and beverages is largely depend upon its sensory properties.



The acceptability of the fermented food and beverage is largely dependent upon its sensory properties. Thus, it is important to have a brief knowledge about the sensory method used for testing of fermented foods and beverages. In sensory analysis, the panel of judges or even consumers are given the beverage and asked to record their perception about the beverage into a datasheet provider and then the data are analyzed to make decisions. The different tests for sensory analysis are discriminative tests, 9-point hedonic scale testing, ranking method and fuzzy method.

#### **Discriminative Tests**



Discriminative or difference tests is one of the most useful sensory tests. This test is designed to discriminate difference between two or more samples. The types of discriminative tests are paired comparison test, duo-trio test, triangle test and multiple sample test.

# **Paired Comparison Test**

Paired comparison test is used to make comparison between only two samples based on sensory attributes like texture, taste, acidity, colour, et cetera. This test is relatively easy to organize and implement. It is typically used in comparing new and old processing techniques, change of ingredients in the product, preference testing at the consumer level et cetera. If already a standard reference beverage exists in the market, this test is used to compare any newly developed beverage with it.

## **Duo-trio Test**

		Duo-Trio Test	
Sensory properties (contd)	Assessor Name:	Assessor No.:	Date:
Duo-trio test		ith an identified reference sample e and then the two coded samples	
<ul> <li>It is a modified paired comparison test. One sample is identified as reference (R), first given to panelists for evaluation. Subsequently two coded samples, one of which is identical to R, are presented.</li> </ul>	Explain why the other san slight, moderate, obvious,	or very obvious). hich the matched sample is, please	e intensity of difference (very slight
<ul> <li>The panelist is asked to indicate, which of the two samples is the same as 'R'.</li> </ul>	Comments:	. <u> </u>	
<ul> <li>The test is suitable for products that have taste and/or kinesthetic effects such that s significantly reduced.</li> </ul>			A Contraction of the second se
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It is a modified paired comparison test. One sample is identified as a reference (R), first given to panelists for evaluation. Subsequently two coded samples, one of which is identical to R are presented. The panelists are asked to indicate which of the two samples is same as the reference R. The test is suitable for products that have relatively intense odor, taste and/or kinesthetic effects such that sensitivity of evaluator is significantly reduced. The duo trio assessment sheet is provided in the slide. It has column for assessor name, number and date and the instructions to perform the test. The sample codes along with column for the comments are also provided.

# **Triangle Test**

#### Sensory properties (contd...)

- Triangle test
  - ✓ Triangle test is most well known and more frequently used out of the three difference tests.
  - As its name implies, it is a three-product test in which all the samples are coded and the panelist's task is to determine which two are most similar or which one is most different from the other two.
  - This test is more difficult test because the panelist must recall the sensory characteristics of two products before evaluating the third and then make a decision.



Triangle test is the most well-known and more frequently used out of the three different tests. As its name implies, it is a three-product test in which all the samples are coded and the panelist's task is to determine which two are most similar, or which one is most different from the other two. The test is more difficult test because the panelists must recall the sensory characteristics of two products before evaluating the third and then make a decision.

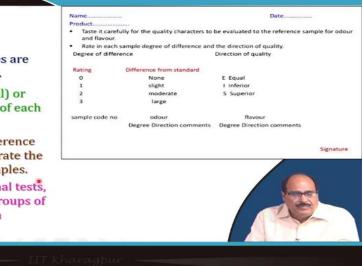
An example of the test is provided in the slide. There are three samples here coded as 212, 347, and 652. The panelists are provided with the samples and the assessment sheet. They have to record which sample is different from other two. If no difference is perceived, the panelists are forced to guess one samples. The data obtained is analyzed statistically to come to a conclusion. As shown in the slide, all panelist except one selected 347 as the most different. Thus, the probability of 347 being a different sample is higher.

## **Multiple Sample Test**

Test involving more than 3 samples are classified as multiple sample tests. They may have equal (symmetrical) or unequal (asymmetrical) numbers of each sample. When they are applied as true difference tests, the judge is required to separate the sample into two groups of like samples. When they are applied as directional tests, the judge is asked to identify the groups of higher or lower intensity of a given criterion. The assessment sheet provides the information to group the samples. The panelists have to taste the samples and evaluate it to the reference sample of odor and flavor. They have to rate in each sample degree of difference and direction of quality.

#### Sensory properties (contd...)

- Multiple sample test
  - Test involving more than 3 samples are classified as multiple sample tests.
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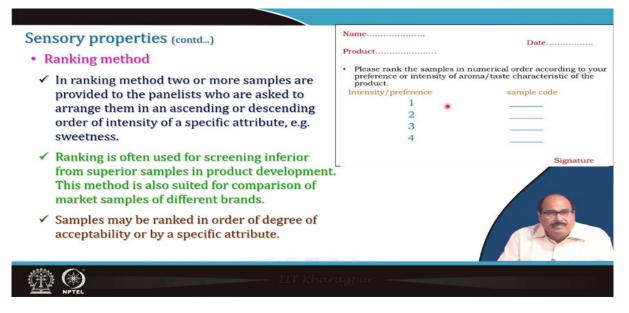


## 9 Point Hedonic Scale

Sensory properties (contd)	Participant no.	9.Poir	nt Hedonic scal	e method for	sensory analysis Tester	I on
<ul> <li>9-point hedonic test</li> </ul>	You are request basis on the 9-p				s of characteristi	es mentioned on the
<ul> <li>✓ Hedonic relates to the psychology of pleasurable and non pleasant states of consciousness.</li> </ul>	Scale Liked Extrem Liked very m Liked modera Liked alightly Neither liked J Disliked sligh Disliked very Extremely dis	och tely nor disliked tly erately much	Score 9 8 7 6 5 4 3 2 1			
<ul> <li>In hedonic method, psychological states of like and dislike are measured on a rating scale.</li> </ul>	Sample Somple 1 Somple 2 Somple 3	Color	Texture	Aroma	Breakability	Overall acceptability
<ul> <li>Normally rating scale has been categorized into five forms, viz. numerical, graphic, standard, cumulated points and forced choice forms.</li> </ul>	Sample 4				Age group	e of the participant
<ul> <li>The nine points numerical scale as given in figure has been most extensively used for new product development &amp; consumer studies.</li> </ul>					F	9

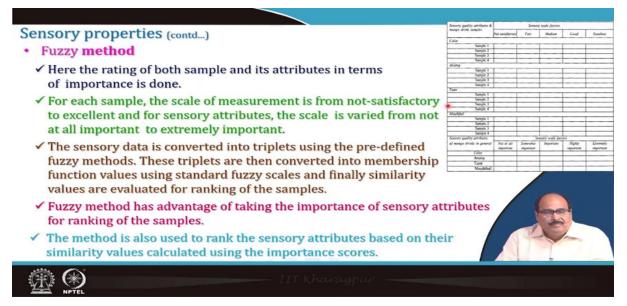
Hedonic relates to the psychology of pleasurable and non-pleasant states of consciousness. In hedonic method, psychological states of like and dislike are measured on a rating scale. Normally rating scale has been categorized into five forms, viz. numerical, graphic, standard, cumulated points and forced choice forms. The nine points numerical scale as given in figure has been most extensively used for new product development & consumer studies. The samples are rated on a 9-point hedonic scale ranging from 1 to 9 with 1 being extreme dislike and 9 being liked extremely. The samples are rated for their color, texture, aroma, breakability, and overall acceptability.

# **Ranking Method**



In ranking method two or more samples are provided to the panelists who are asked to arrange them in an ascending or descending order of intensity of a specific attribute, e.g. sweetness. Ranking is often used for screening inferior from superior samples in product development. This method is also suited for comparison of market samples of different brands. Samples may be ranked in order of degree of acceptability or by a specific attribute. The assessment sheet for ranking the samples based on aroma/taste characteristic of the product is provided in the slide.

# **Fuzzy Method**



In fuzzy method the rating of both sample and its attributes in terms of importance is done.

For each sample, the scale of measurement is from not-satisfactory to excellent and for sensory attributes, the scale is varied from not at all important to extremely important. The sensory data is converted into triplets using the pre-defined fuzzy methods. These triplets are then converted into membership function values using standard fuzzy scales and finally similarity values are evaluated for ranking of the samples. Fuzzy method has advantage of taking the importance of sensory attributes for ranking of the samples. The assessment sheet for fuzzy method is provided.

#### **Microbiological Properties**

#### Microbiological properties

- In fermented beverages, various kind of microorganisms like yeasts, molds, algae, viruses, bacteria, pathogens, spoilage, beneficial, inert, etc. may be present.
- Early destruction of spoilage causing microorganism (MO) are generally done before fermentation. However, further safe GMP practices must be followed to have spoilage MO free fermented beverage at the end.

Fermented Foods/Beverages	Substrates Used	Microorganisms Involved in Fermentation				
Dairy products Curd, Yogurt, Cheese, Yakult, Kefir	Milk and milk casein	Lactobacillus bulgaricus, Lactococcus lactis, L. acidophilus, L. cremoris L. casei, L. pancasei, L. thermophilus, L. kefiri, L. caucasicus, Penicillium camemberti, P. roqueforti, Acetobacter Iovaniensis, Klugveromyces lactis, Saccharomyces cerevisiae				
Vegetable products	Soybean, cabbage, ginger,	Leuconostoc mexenteroides, Aspergillus sp., Rhizopus oligosporus, R. oryzze, J. sakei, L. plantiarum, Thermologa sp., L. kokkaidonensis, L. rhammosus, Rhodorarla rubar, Leuconobec currossam, Bifidobacterium ilentium, Enteroxoccus faccults, Weissellu confusa, Candula sake				
Kimchi, Tempeh, Natto, Miso, Sauerkraut	cucumber, broccoli, radish					
Cereals Bahtura, Ambali, Chilra, Dosa, Kunu-Zaki, Marchu	Wheat, maize, sorghum, millet, rice	<ul> <li>L. pantheris, L. plantaruan, Penicillium sp., S. cerevisiae, L. mesenteroides, F. Jaccalis, Trichosporon pullulans, Pedieevecus scidilactici, P. cerevisiae, Delbrueckii hansenii, Deb. tamari</li> </ul>				
Beverages		Aspergillus oryzae, Zygosaccharomyces bailii, S. cerevisiae,				
Wine, Beer, Kombucha, Sake	Grapes, rice, cereals	Acetobacter pasteurianus, Gluconacetobacter, Acetobacter xylinus, Komagataeibacter xylinus				
Meat Products		1. sakei, 1. curvatus, 1. plantarum, Leuconostoc carnosum,				
Sucuk, Salami, Arjia, Jama, Nham	Meat	Leuconostoc gelidium, B. licheniformis, E. faccalis, E. hirae, E. durans, Bacillus subtilis, L. divergens, L. carnis, E. cecorum, B. lentus				

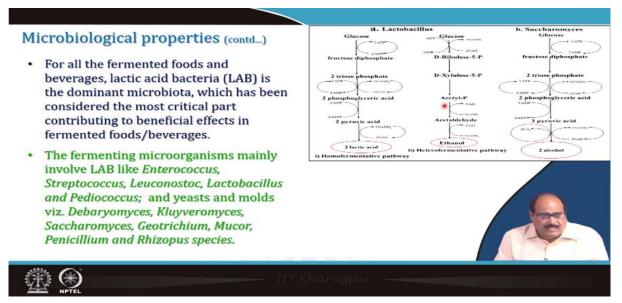
 However, the thermal processing should be done in such a way so that the spoilage organism get killed but beneficial organism must remain preserved.

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In fermented beverages, various kind of microorganisms like yeasts, molds, algae, viruses, bacteria, pathogens, spoilage, beneficial, inert, etc. may be present. Early destruction of spoilage causing microorganism (MO) are generally done before fermentation. However, further safe GMP practices must be followed to have spoilage MO free fermented beverage at the end. However, the thermal processing should be done in such a way so that the spoilage organism gets killed but beneficial organism must remain preserved. The table provides information about the fermented food and beverages, substrate used and the microorganism involved in the fermentation. Dairy based fermented products are curd, yoghurt, cheese, yakult, kefir, and et cetra. The substrate used are milk and milk casein. Microorganisms *involved are L. bulgaricus, L. lactis, L. acidophilus, L. cremoris, L. casei, L. paracasei, L. thermophilus, L. Kefir, L. caucasicus, Penicillium camemberti, P. roqueforti, Acetobacter lovaniensis, Kluyveromyces lactis, and Saccharomyces cerevisiae. In case of vegetable-based products like kimchi, tempeh, natto, miso and sauerkraut, the substrate are soybean, cabbage, ginger, cucumber, broccoli, and radish, the fermenting microorganisms are <i>Leuconostoc* 

mesenteroids, Aspergillus sp., Rhizopus oligosporus, R. oryzae, L sakei, L. plantarum, Thermotoga sp., L. hokkaidonensis, L. rhamnosus, Rhodotorula rubra, Leuconostoc carnosum, Bifidobacterium dentium, Enterococcus faecalis, Weissella confuse, and Candida sake. For the cereals based fermented foods like bhatura, ambali, chilra, dosa, kunu-zaki, and marchu, the substrates are wheat, maize, sorghum, millet and rice. The microorganisms involved are L. pantheris, L. plantarum, Penicillium sp., S. cerevisiae, L. mesenteroids, E. faecalis, Trichosporon pullulans, Pediococcus acidilactici, P. cerevisiae, Delbruekii hansenii, and Deb. Tamari. For the beverages like wine, beer, kombucha and sake, the substrate are grapes, rice and cereals. Aspergillus oryzae, Zygosaccharomyces bailii, S. cerevisiae, Acetobacter pateurianus, Gluconacetobacter, Acetobacter Xylinus, and Komagataeibacter xylinus are the fermenting organisms. Meat products like sucuk, salami, arija, jama and nham has meat as substrate, while L. sakei, L. curvatus, L. plantarum, Leuconostoc carnosum, Leuconostoc gelidium, B. licheniformis, E. faecalis, E. hirae, E. durans, Bacillus subtilis, L. divergens, L. carnis, E. cecorum, and B. lentus as fermenting microorganism.



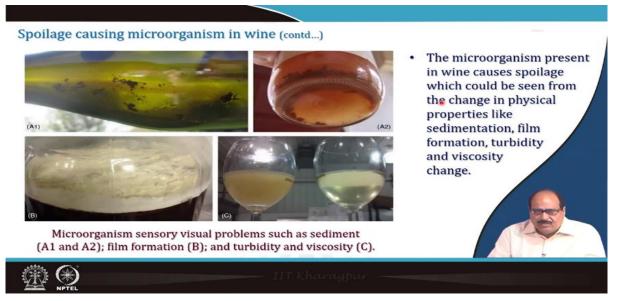
For all the fermented foods and beverages, lactic acid bacteria (LAB) is the dominant microbiota, which has been considered the most critical part contributing to beneficial effects in fermented foods/beverages. The fermenting microorganisms mainly involve LAB like *Enterococcus, Streptococcus, Leuconostoc, Lactobacillus and Pediococcus;* and yeasts and molds viz. *Debaryomyces, Kluyveromyces, Saccharomyces, Geotrichium, Mucor, Penicillium and Rhizopus species*. In homofermentative pathway, Lactobacillus converts glucose to fructose diphosphate and to 2 triose phosphate, to 2 phosphoglyceric acid, to 2 pyruvic acid and finally to 2 lactic acids. In the process it uses phosphate from ATP to produce ADP and

takes hydrogen from NADH<sub>2</sub> to give out NAD. In heterofermentative pathway glucose is converted to D-Ribulose -5 - P and it involves one ATP and 2 NAD. It is converted to D-Xylulose-5-P and to Acetyl-P. It takes up H<sub>2</sub> from NADH<sub>2</sub> to produce Acetaldehyde and produces ethanol with NADH<sub>2</sub>. *Sacharomyces* converts glucose to ethanol with fructose diphosphate, triose phosphate, phosphoglyceric acid, and pyruvic acid as the intermediate metabolites.

Microbiological properties (contd) <ul> <li>Spoilage causing microorganism in wine</li> </ul>	
<ul> <li>Several microbial contaminants appear to survive on walls and other interior surfaces of wineries, including those of presses and fermentation tanks, and within wooden barrels.</li> </ul>	- Aleres
<ul> <li>Zygosaccharomyces, Dekkera, Saccharomyces, and Saccharomycodes causes spoilage of bulk and bottled wines.</li> </ul>	s Summe
✓ D./B. bruxellensis and Zygosaccharomyces bailii are spoilage yeasts.	
✓ S. cerevisiae appears to be more problematic than indicated, as some strains isolated from dry white wines seem to be more of a potential spoilage yeast than Z. bailii, due to its sorbic acid and sulfite tolerance at high ethanol levels.	
✓ Both Z. bailii and S. cerevisiae can grow at low pH in the presence of acid concentrations near the legal limits.	
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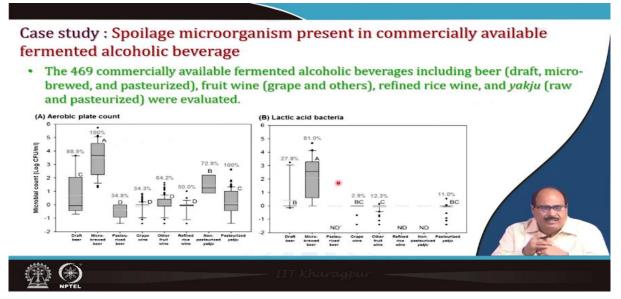
#### **Spoilage Causing Microorganism in Wine**

Several microbial contaminants appear to survive on walls and other interior surfaces of wineries, including those of presses and fermentation tanks, and within wooden barrels. *Zygosaccharomyces, Dekkera, Saccharomyces, and Saccharomycodes* causes spoilage of bulk and bottled wines. *D./B. bruxellensis and Zygosaccharomyces bailii* are spoilage yeasts. *S. cerevisiae* appears to be more problematic than indicated, as some strains isolated from dry white wines seem to be more of a potential spoilage yeast than *Z. bailii*, due to its sorbic acid and sulfite tolerance at high ethanol levels. Both *Z. bailii* and *S. cerevisiae* can grow at low pH in the presence of acid concentrations near the legal limits. The microorganism present in wine causes spoilage which could be seen from the change in physical properties like sedimentation, film formation, turbidity and viscosity change.



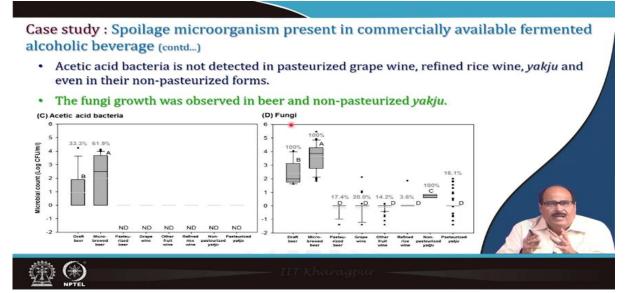
The picture shows the microorganisms sensory visual problems such as sedimentation, film formation, turbidity and change in viscosity.

## **Case Study**



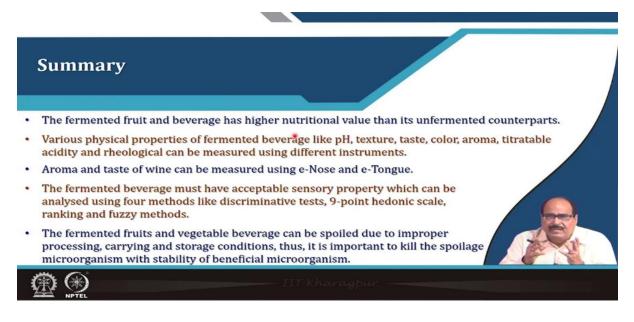
Spoilage microorganism present in commercially available fermented alcoholic beverage were studied and presented as the case study. About 469 commercially available fermented alcoholic beverages including beer (draft, micro-brewed, and pasteurized), fruit wine (grape and others), refined rice wine, and *yakju* (raw and pasteurized) were evaluated. The graph shows that Microbrewed beer contained the highest number of microorganisms (average aerobic plate count, 3.5; lactic acid bacteria, 2.1; acetic acid bacteria, 2.0; and fungi, 3.6 log CFU/ml), followed by draft beer and yakju (P < 0.05), whereas the other FABs contained , 25 CFU/25 ml microorganisms. Unexpectedly, neither microbial diversity nor microbial count correlated

with the alcohol content (4.7 to 14.1%) or pH (3.4 to 4.2) of the product. Despite the harsh conditions, coliforms (detected in 23.8% of microbrewed beer samples) and B. cereus (detected in all FABs) were present in some products. B. cereus was detected most frequently in microbrewed beer (54.8% of samples) and nonpasteurized yakju (50.0%), followed by pasteurized yakju (28.8%), refined rice wine (25.0%), other fruit wines (12.3%), grape wine (8.6%), draft beer (5.6%), and pasteurized beer (2.2%)



Acetic acid bacteria are not detected in pasteurized grape wine, refined rice wine, *yakju* and even in their non-pasteurized forms. The fungi growth was observed in beer and non-pasteurized *yakju*.

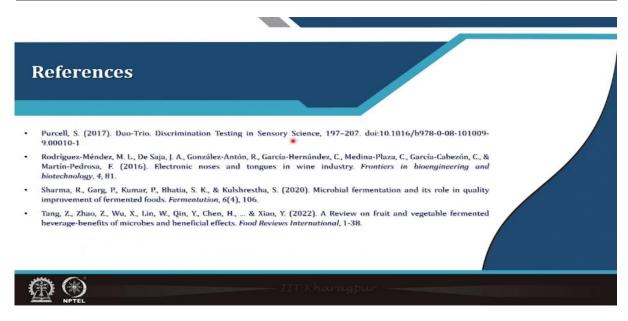
#### **Summary**



The fermented fruit and beverage have higher nutritional value than its unfermented counterparts. Various physical properties of fermented beverage like pH, texture, taste, color, aroma, titratable acidity and rheological can be measured using different instruments. Aroma and taste of wine can be measured using e-Nose and e-Tongue. The fermented beverage must have acceptable sensory property which can be analysed using four methods like discriminative tests, 9-point hedonic scale, ranking and fuzzy methods. The fermented fruits and vegetable beverage can be spoiled due to improper processing, carrying and storage conditions, thus, it is important to kill the spoilage microorganism with stability of beneficial microorganism.

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References for further reading are provided in the slide.