

MICROBIAL BIOTECHNOLOGY

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Lecture-29

Lec 29: Fermented Food - Foundations and Principles

Hello everyone, welcome to my course on microbial biotechnology. We are starting a new module today, which is on food production involving microorganisms and their products. So, in this lecture, we will be discussing fermented foods, their foundations, and the principles involved in the process. So, we have broadly two sections. The first section deals with the historical and cultural perspective of fermented foods.

Then, we have the second section, which deals with the scientific principles of fermentation. So, we begin with a definition of fermented foods. So, here you can see fermented radish, which is displayed in a store. So, we are using the definition provided by Campbell Platt in his book, *The Fermented Foods of the World: A Dictionary and Guide*. So, where he has defined fermented foods as

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those foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification in the food. So, if you look at the biological and chemical perspective of fermented foods, biologically, fermented foods result from the action of beneficial microorganisms, as defined by Campbell. So, these include the bacteria, yeast, or molds that metabolize nutrients in the food, enhancing preservation, flavor, and nutritional value while promoting probiotic activity. Fermented

foods are defined by the chemical transformation of organic compounds, primarily sugars, into acids,

Campbell-Platt in his book "Fermented Foods of the World. A Dictionary and Guide" defined fermented foods as *'those foods that have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification in the food'*.



Displayed Fermented Radish on a Stall in Kyoto, Japan.
Source: Brunini, E. (2020, November 3) Free Stock Photo: Pexels/ Pexels

Alcohols and gases are produced through microbial metabolism. This process alters the food's flavor, texture, and preservation properties. Extending on this definition, we have certain raw materials on which specific organisms and enzymes act to produce fermented food. We may have enzymes like chymosin, and microorganisms can be sourced from natural spontaneous cultures or commercial pure cultures. Certain conditions, such as aerobic and anaerobic environments, and chemicals like salt, nitrates, phytates, and sugars, interact with one another.

Biological and Chemical Perspective of Fermented Foods



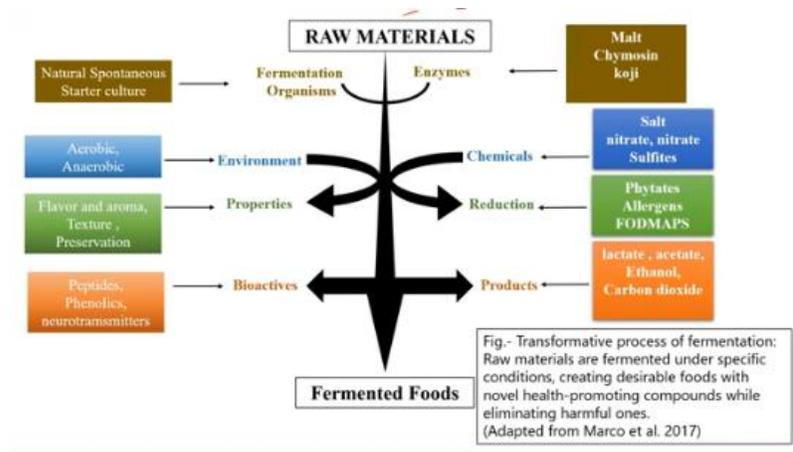
- Biologically, fermented foods result from the action of beneficial microorganisms (like bacteria, yeast, or molds) that metabolize nutrients in the food, enhancing preservation, flavor, and nutritional value while promoting probiotic activity.



- Fermented foods are defined by the chemical transformation of organic compounds, primarily sugars, into acids, alcohol, and gases through microbial metabolism. This process alters the food's flavor, texture, and preservation properties.

They change the nature of these raw materials, resulting in transformed products with improved flavor, aroma, texture, and shelf life, while also requiring caution regarding allergens and phytates. The food produced through this process not only provides nutrition but also yields bioactive molecules like peptides and phenolics. It may also produce other valuable compounds like lactate, acetate, ethanol, and carbon dioxide. Overall, this is a

transformative process of fermentation. Raw materials are fermented under specific conditions, creating desirable foods with novel health-promoting compounds while eliminating harmful ones, as shown in this diagram.



Let us now explore the history of fermented food and its origins. It has been known for thousands of years, with various civilizations using traditional fermentation techniques. For example, in Mesopotamia, near the Tigris and Euphrates rivers, inhabitants of ancient civilizations practiced fermentation. Evidence from 6000 BCE shows lactic acid fermentation for seed production. This is likely the first known use of fermentation, coinciding with animal domestication and agricultural development.

Historical significance and cultural importance across different civilizations

Year BCE/CE	Event	Civilization/ Location	Significance
6000 BCE	Evidence of lactic acid fermentation for cheese production	Mesopotamia (Tigris and Euphrates rivers, now Iraq)	First known use of fermentation, coinciding with animal domestication and agricultural development
5000 BCE	First alcoholic fermented beverage made of rice, fruit, and honey	China and Georgia	Earliest known alcoholic fermentation
4000 BCE	Development of wine production from grape juice	Egypt	Advancement in alcoholic beverage production

Then we have the earliest known alcoholic fermentation reported from China and Georgia, which is a beverage made of rice, fruit, and honey, dating back to around 5000 BCE. And then we have the development of wine production from grape juice in Egypt around 4000 BCE. So, there is an advancement in alcoholic beverage production, as you can see in this

case. Then we have evidence of fermented beverages from Babylon and pre-Columbian Mexico. And then Sudan, ranging from around 3000 to 1500 BCE.

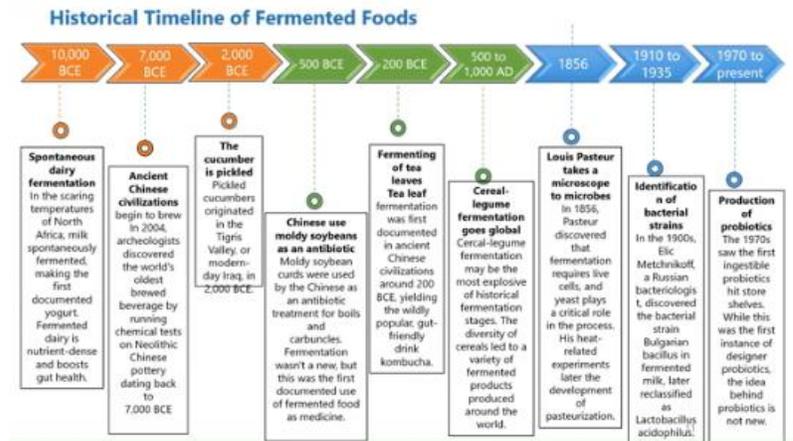
And then, of course, the elaboration of beer using barley in Sumeria. This will actually move a little bit up over here, around 1800 BCE. And this has been supposed to be the first beverage distinct from the alcoholic beverages mentioned earlier. Then, in modern times, roughly around 1857 AD, we have Louis Pasteur, who for the first time chemically explained fermentation. And provided the scientific understanding that fermentation is a living process.

Year (BCE/CE)	Event	Civilization/ Location	Significance
1857 CE	Louis Pasteur explains fermentation chemically	France	Scientific understanding of fermentation as a living process
1897 CE	Eduard Buchner explains enzyme role in fermentation	Germany	Further advancement in understanding fermentation mechanisms
1919 CE	First industrialized production of yogurt by Isaac Carasso	Barcelona, Spain	Beginning of large-scale commercial fermented product manufacturing
1928 CE	Discovery of nisin, an antimicrobial product of lactic acid bacteria	Rogers and Whittier	Important for food safety in fermented products
2012 CE	Publication of list of microbial food cultures recognized as safe (GRAS)	Global	Establishment of safety standards for fermentation cultures in food and beverages

Then we have others, like Edward Buchner, who advanced the understanding of fermentation mechanisms. Then we have the first industrial production of yogurt by Isaac Carasso. In 1919, then the discovery of nisin, an antimicrobial product of lactic acid bacteria, and then the publication of a list of microbial food cultures recognized as safe by 2012, which is important from the point of view of establishing safety standards for fermentation cultures in food and beverages. So, over a historical timeline, we can see around 10,000 BCE the spontaneous dairy fermentation, and then around 7,000 BCE, ancient Chinese civilization begins to brew, and then 2000 BCE, the cucumber is pickled in the Tigris Valley. The Chinese used moldy soybeans as an antibiotic around 500 BCE.

The fermentation of tea leaves was first documented in Chinese civilization around 200 BCE. Then, between 500 to 1000 AD, cereal legume fermentation became globally known. Later, in modern times, Lupecia worked as we have already discussed. Then, the identification of bacterial strains in the 1900s by Alek Machkinov, a Russian bacteriologist, who discovered the bacterial strain Bulgarian bacillus in fermented milk, later classified as lactobacillus. This is a very important microorganism from the point of view of probiotics,

and by the 1970s, the first ingestible probiotic was commercialized, and the field has been progressing quite rapidly over the years.



Some of the notable figures and their contributions to fermentation, both in food and beverages, include Louis Pasteur, whom we already mentioned. He advanced the understanding of fermentation, including ethanolic, lactic, butyric, and acetic types, by isolating lactic bacillus and proving it converts glucose to lactic acid. He introduced the concepts of aerobic and anaerobic fermentation. Revealing that some microorganisms can thrive without oxygen, challenging previous fermentation theories. He was the first to have a patent on purified yeast in 1873.

NOTABLE HISTORICAL FIGURES AND THEIR CONTRIBUTIONS

Louis Pasteur (1822 - 1895)

Foundational Research: Louis Pasteur advanced the understanding of fermentation, including ethanolic, lactic, butyric, and acetic types, by isolating lactic bacillus and proving it converts glucose to lactic acid.

Key Concepts: Pasteur introduced the concepts of "aerobic" and "anaerobic" life, revealing that some microorganisms can thrive without oxygen, challenging previous fermentation theories.



Louis Pasteur, French microbiologist and chemist in his laboratory
CC BY 4.0 via Wikimedia Commons

(Bordenave 2003)

The patent included new fermentation methods for beer and described the yeast as free from disease-causing germs. Significant in debates about patenting natural products, As the US patent office cited this patent to justify isolating genes, Pesce's patents helped control his biotechnological inventions, while his vaccines were compensated by the state

rather than patented. Pesce's brewing process, basically, he studied beer and winemaking, patented a process that Improved beer quality.

Previously, wort was boiled and cooled by exposure to air. Besser's method involved cooling wort in closed vessels by spraying water on the outside. Introducing special yeast post-cooling prevents contamination from wild yeast. Then we have Emil Christian Hansen. He developed isolation techniques to isolate single yeast cells, enabling pure yeast strain propagation and preventing contamination in brewing.

NOTABLE HISTORICAL FIGURES AND THEIR CONTRIBUTIONS

Louis Pasteur (1822 - 1895)

Patent on Purified Yeast (1873):

Louis Pasteur received a patent on purified yeast, the first known patent covering a microorganism. The patent included new fermentation methods for beer and described the yeast as free from disease-causing germs. Significant in debates about patenting natural products, as the U.S. Patent Office cited this patent to justify isolating genes. Pasteur's patents helped control his biotechnological inventions, while his vaccines were compensated by the state rather than patented.

Pasteur's Brewing Process:

Studied beer and wine making; patented a process improving beer quality. Previously, wort was boiled and cooled by exposure to air. Pasteur's method involved cooling wort in closed vessels by spraying water on the outside, introducing special yeast post-cooling to prevent contamination from wild yeasts. (Cassier 2016)

This had a huge impact on brewing and revolutionized the industry, improving beer quality and consistency. Major breweries like Carlsberg quickly adopted his methods, which remain very successful today. He aimed to reduce spoilage and make brewing more reliable by eliminating the unpredictability of mixed cultures. His techniques still influence modern brewing, with ongoing advancements in yeast fermentation technology. Now, what is the importance of self-fermented food?

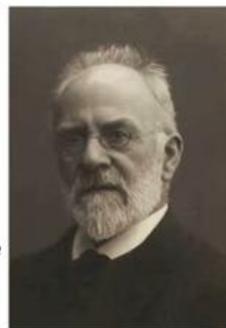
Emil Christian Hansen (1842-1909)

Isolation Techniques: In 1883, Emil Christian Hansen developed methods to isolate single yeast cells, enabling pure yeast strain propagation and preventing contamination in brewing.

Impact on Brewing: Hansen's methods revolutionized brewing by improving beer quality and consistency, quickly adopted by major breweries like Carlsberg.

Brewing Challenges: Hansen's methods reduced spoilage and made brewing more reliable by eliminating the unpredictability of mixed cultures.

Current Practices: Hansen's techniques still influence modern brewing, with ongoing advancements in yeast and fermentation technology.



Emil Christian Hansen (1842-1909), Danish microbiologist and mycologist

via Wikimedia Commons

(Raines-Casselmann, 2005)

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The question is: why fermented food when fresh food is available? First, it helps in preservation. There are times of the year in certain regions when extreme cold, heat, or heavy rainfall may make food unavailable. So, it is important to preserve food for long-term use. Historically, food fermentation extended shelf life by creating acidic environments and compounds that inhibit harmful bacteria, yeast, and molds, preventing spoilage and foodborne illnesses without modern refrigeration.

Enhanced flavor and aroma was also one of the reasons why people preferred certain food in fermented form. Fermentation breaks down the organic complex carbohydrates and proteins, releasing organic acids, alcohols and esters that create unique flavors and aromas. As seen in the tangy and umami rich profiles of foods like cheese, yogurt, sauerkraut and kimchi. Then fermentation food also provides nutritional benefits. For example, certain food like yogurt, kefir provides probiotics while certain fermentation microorganisms break down nutrients for better absorption by the body.

Why fermented foods?



- **Preservation** - Historically, food fermentation extended shelf life by creating acidic environments and compounds that inhibit harmful bacteria, yeasts, and molds, preventing spoilage and foodborne illnesses without modern refrigeration.



- **Enhanced Flavor and Aroma**- Fermentation breaks down complex carbohydrates and proteins, releasing organic acids, alcohols, and esters that create unique flavors and aromas, as seen in the tangy and umami-rich profiles of foods like cheese, yogurt, sauerkraut, and kimchi.



- **Nutritional Benefits** - Some fermented foods, such as yogurt and kefir, provide probiotics while certain fermentation microorganisms break down nutrients for better absorption by the body.

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Then fermented food also have reduced anti-nutrients, compounds that hinder nutrient absorption by breaking them down and increasing the bioavailability of nutrients. And another thing is the reduction of toxins. Fermentation can partially break down cyanogenic glycosides. For example, in cassava, which releases toxic cyanide when consumed and reduce or eliminate some of the food toxins. And it has huge significance from a point of view of culture and culinary traditions.

Fermented foods are integral to the culinary traditions of many cultures, essential to regional cuisines, connecting people to their heritage and enhancing the diversity of global culinary experiences. And one of the important thing is waste reduction. So, the food, if fresh food is not consumed, I mean, the food will be wasted. So, fermentation can transform

surplus or imperfect produce. In quality control, certain foods may be sorted out to be of not high quality.

Why fermented foods?



- **Reduced Anti-Nutrients-** Fermentation reduces anti-nutrients, compounds that hinder nutrient absorption, by breaking them down and increasing the bioavailability of nutrients.



- **Toxin Reduction-** Fermentation can partially break down cyanogenic glycosides in cassava, which release toxic cyanide when consumed, and reduce or eliminate some food toxins.



- **Cultural and Culinary Traditions-** Fermented foods are integral to the culinary traditions of many cultures, essential to regional cuisines, connecting people to their heritage and enhancing the diversity of global culinary experiences.

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Those can actually be transformed into fermented products. Food as well as fermented beverages, and it produces flavorful and nutritious food, thereby reducing waste. So, all these help us in sustainability. Fermentation is a sustainable food preservation method that requires minimal equipment and energy, unlike energy-intensive processes like canning or freezing, making it eco-friendly. Then there is the significance of artisanal and creative foods.

Fermentation encourages experimentation and creativity in the kitchen, allowing unique flavors and textures to emerge through variations in fermentation times, ingredients, and techniques. Let us now briefly look into the role microbes play in the fermentation process. The fermented food microbial ecosystem is actually very, very complex. This involves not only lactic acid bacteria, and *Saccharomyces cerevisiae*, but also other microorganisms like coagulase-negative cocci, corniform bacteria, and non-saccharomyces yeasts.

Why fermented foods?



- **Waste Reduction-** Fermentation can transform surplus or imperfect produce into flavorful and nutritious foods, reducing waste.



- **Sustainability-** Fermentation is a sustainable food preservation method that requires minimal equipment and energy, unlike energy-intensive processes like canning or freezing, making it eco-friendly.



- **Artisanal and Creative Foods-** Fermentation encourages experimentation and creativity in the kitchen, allowing unique flavors and textures to emerge through variations in fermentation times, ingredients, and techniques.

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These organisms interact and sometimes compete with the primary fermenting microbes, adding complexity to the fermentation process. Traditionally, microbiology relied on culture-dependent methods, which involve isolating and cultivating microorganisms. However, these methods have limitations as they do not always reflect the true diversity of microbial communities. Many species, especially those in low numbers or with specific growth conditions, may not be represented accurately.

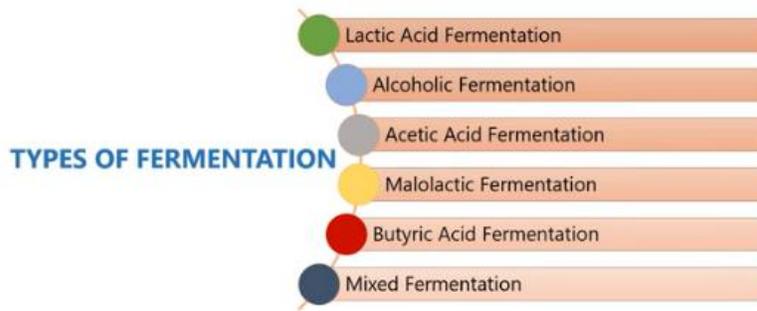
Culturing conditions in the lab often fail to replicate the natural habitat of these microbes. So, we have culture-independent methods which have emerged, offering a more comprehensive view by analyzing microbial DNA and RNA directly from the food matrix. These methods do not require culturing, allowing for the detection of all microorganisms present, even those that cannot be grown in vitro. This shift has revolutionized the study of microbial ecosystems in food, particularly with the advent of next-generation sequencing technologies like pyrosequencing, which allows for the detailed analysis of microbial communities, including rare species. Recent studies using both culture-dependent and independent approaches have expanded the understanding of fermented products from dairy, meat, and vegetable sources, revealing new insights into microbial dynamics.

The Fermented Food Microbial Ecosystem

- **Culture-independent methods** have since emerged, offering a more comprehensive view by analyzing microbial DNA and RNA directly from the food matrix. These methods do not require culturing, allowing for the detection of all microorganisms present, even those that cannot be grown in-vitro.
- This shift has revolutionized the study of microbial ecosystems in food, particularly with the advent of next-generation sequencing technologies like pyrosequencing, which allow for the detailed analysis of microbial communities, including rare species (OTUs).

Recent studies using both culture-dependent and independent approaches have expanded the understanding of fermented products from dairy, meat, and vegetable sources, revealing new insights into microbial dynamics.

Let us now discuss the various types of fermentation that occur in the production of food as well as beverages. Briefly, they can be listed as shown in this figure: lactic acid fermentation, alcoholic, acetic acid, malolactic, butyric acid, and mixed fermentation. We will discuss some of these in a little detail here and some of the others in the lecture where we will deal with fermented beverages. Lactic acid fermentation is a metabolic process where sugars are converted into lactic acid by lactic acid bacteria, essential for producing various fermented foods fermented food.



It includes acid-fermented vegetables like kimchi and chowakrod, acid-fermented cereals and beverages, traditionally cereal beers, and acid-fermented seafood and meat mixtures. The microorganisms involved in lactic acid fermentation are primarily species of LAB, including Lactococcus, Lactobacillus, and Streptococcus. They provide health benefits, enhance the digestibility of food, improve gut health through probiotics, and can extend the shelf life of products by inhibiting spoilage organisms. And they have culinary uses as well. These fermentation methods enhance the flavor of savory dishes, rice, wines, and plant-based beverages.

So, here we can see various fermented foods on the left side of the table and the particular lactic acid bacteria involved in their production. We will not go into complete details here. The information is available. You can consult this table at any point in time.

Lactic acid bacteria (LAB) used for fermented food production

Fermented foods	Lactic acid bacteria
Dairy products (butter and buttermilk yogurt and chesses)	<i>L. Lactis subsp. lactis</i> <i>L. Lactis subsp. Lactis var. diacetylactis</i> <i>Lb. delbruekii subsp. Bulgaricus</i> <i>Lb. acidophilus</i> <i>Ln. lactis</i> <i>Ln. mesenteroides subsp. Cremoris</i> <i>Streptococcus thermophilus</i>
Meat and fist products (fermented sausages, many indigenous products)	<i>Lb. sake</i> <i>Lb. plantarum</i> <i>P. pentosaceus</i> Various undefined LAB
Plant products (sauerkraut, olives, cassava products, soy sauce, and many indigenous products)	<i>Lb. ssp.</i> <i>Ln. mesenteroides</i> <i>P. Pentosaceus</i> Various undefined LAB
Bakery products (sourdough products, crackers)	<i>Lb. sanfrancisco</i> <i>Lb. fermentum</i> <i>Lb. reuteri, Lb. amylolyticus</i>

("Genetically Engineered Food" 2003)
 Abbreviations: L: Lactococcus; Lb: Lactobacillus; Ln: Leuconostoc; P: Pediococcus

So, basically, for dairy products like butter, buttermilk, yogurt, and cheeses, you have L-Lactis, then you have subspecies Lactis, then you have Delbruckii subspecies Bulgaricus, then you have Acidophilus, then you have Mesenteroides, and also Streptococcus

thermophilus. And for fermented meat and fish products, we have the LB Sake and then LB Plantarum. Then we have here the *P. pentosaceus* and various undefined LABs. So, in plant products like sauerkraut, olives, cassava products, and many indigenous products, we have the LN mesenteroides. And then for bakery products like sourdough products and crackers, we have LB San Francisco, LB fermentum, and so on.

So, let us move on to the second type of fermentation, which is alcoholic fermentation. This is the biological process where sugars are converted into ethanol and carbon dioxide by yeast, mainly *Saccharomyces cerevisiae*. This process is essential in making alcoholic beverages. We will discuss these in a little more detail in the next lecture. Similarly, we have acetic acid fermentation.

This is a process where ethanol is converted into acetic acid by acetobacter bacteria in the presence of oxygen, essential for vinegar production. Then we have malolactic fermentation, which is a process where malic acid in wine is converted into lactic acid and carbon dioxide by lactic acid bacteria, primarily *E. nococcus* only. This fermentation typically occurs after the primary alcoholic fermentation. Then you have butyric acid fermentation, which is an anaerobic process where bacteria, mainly *Clostridium*, convert carbohydrates into butyric acid, carbon dioxide, and hydrogen in low-oxygen environments like animal intestines and certain foods. Human health importance is associated with this butyric acid fermentation process.

Butyric acid is vital for gut health, providing energy for colonocytes. It supports gut barrier function and prevents inflammatory bowel disease. It also enhances flavor and nutrition in foods like seeds and fermented dairy products and improves nutrient bioavailability by breaking down complex carbohydrates and aiding in absorption. It provides anti-inflammatory effects and reduces colon cancer risk, emphasizing the importance of dietary fibers for gut health. Apart from these, we have mixed fermentations, which involve the simultaneous or sequential fermentation by different microorganisms, such as yeast and bacteria.

Butyric Acid Fermentation

- **Definition:** An anaerobic process where bacteria, mainly Clostridium, convert carbohydrates into butyric acid, CO₂, and hydrogen in low-oxygen environments like animal intestines and certain foods.
- **Human Health Importance:** Butyric acid is vital for gut health, providing energy for colonocytes, supporting gut barrier function, and preventing inflammatory bowel disease.
- **Role in Fermented Foods:** Enhances flavor and nutrition in foods like cheese and fermented dairy products.
- **Nutritional Impact:** Improves nutrient bioavailability by breaking down complex carbohydrates, aiding absorption.
- **Potential Health Benefits:** May have anti-inflammatory effects and reduce colon cancer risk, emphasizing the importance of dietary fibers for gut health.

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This method is widely used in producing various fermented foods and beverages. This mixed fermentation enhances flavor complexity. For example, in winemaking, the combination of wild yeasts and lactic acid bacteria can create unique flavor profiles that are more nuanced than those produced by single-strain fermentation strategies. As this is mixed, this process promotes microbial diversity, leading to unique characteristics in fermented products. For instance, traditional sourdough bread often uses a mix of wild yeast and lactic acid bacteria, resulting in distinct taste and texture.

Mixed fermentations improve the stability of the fermentation process. In beer production, the use of both yeasts and bacteria can help inhibit spoilage organisms, enhancing the safety and shelf life of the beer. Mixed fermentations also produce beneficial compounds such as probiotics. For example, in yogurt production, the combination of Lactobacillus and Streptococcus species contributes to the health benefits associated with consuming yogurt. Now, let us look into some of the traditional fermented foods around the world.

Mixed Fermentation

- **Microbial Diversity:** This process promotes microbial diversity, leading to unique characteristics in fermented products. For instance, traditional sourdough bread often uses a mix of wild yeasts and lactic acid bacteria, resulting in a distinct taste and texture.
- **Improved Stability:** Mixed fermentation can improve the stability of the fermentation process. In beer production, the use of both yeast and bacteria can help inhibit spoilage organisms, enhancing the safety and shelf-life of the beer.
- **Health Benefits:** Mixed fermentation can produce beneficial compounds, such as probiotics. For example, in yogurt production, the combination of Lactobacillus and Streptococcus species contributes to the health benefits associated with consuming yogurt.

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Human civilization has a long history of fermentation, dating back to 6,000–10,000 BCE. Now, let us look at the various kinds of traditional fermented foods available around the world. Here, you can see kimchi, which is basically a Korean-style pickle. Its common ingredients include napa cabbage, radishes, garlic, ginger, and chili pepper, often combined with fish sauce or fermented seafood. It is made through a fermentation process where vegetables are salted, mixed with spices, and stored in jars to ferment at cool temperatures.

There are also regional variations within Korea. These have unique recipes, varying in spice levels and additional ingredients, reflecting local tastes and available raw materials. Kimchi has many health benefits, including probiotic effects, improved gut health, and potential anti-carcinogenic properties. Another popular fermented food is soy sauce and miso in Japan, produced by fermenting soybeans and wheat with specific molds and bacteria, followed by pressing and aging. Modern methods may use chemical hydrolysis for faster production, while miso is produced by fermenting soybeans with salt and the mold *Aspergillus oryzae*, varying in fermentation time and ingredients for different flavors and types.

TRADITIONAL FERMENTED FOODS AROUND THE WORLD

Asian Fermented Foods

1. Kimchi (Korea)

- **Ingredients:** Common ingredients include napa cabbage, radishes, garlic, ginger, and chili pepper, often combined with fish sauce or fermented seafood.
- **Preparation Methods:** Kimchi is made through a fermentation process where vegetables are salted and mixed with spices, then stored in jars to ferment at cool temperatures.
- **Regional Variations:** Different regions in Korea have unique recipes, varying in spice levels and additional ingredients, reflecting local tastes and available produce.
- **Health Benefits:** Kimchi is associated with probiotic effects, improved gut health, and potential anti-carcinogenic properties.



Clear Glass Jar with Kimchi

(Antoni Shkraba 2021)
Free Stock Photo. Pexels: pexels.com

(Eirini Dimidi et al. 2019)

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Both soy sauce and miso are essential flavoring agents in Japanese cooking. So, they impact Japanese cooking to a large extent. They enhance umami and contribute to dishes like soups, marinades, and dressings. In Indonesia, there is a popular fermented food called tempeh. You can see this being offered on a plate.

2. Soy Sauce and Miso (Japan)

Production Processes:

- **Soy Sauce:** Traditionally made by fermenting soybeans and wheat with specific molds and bacteria, followed by pressing and aging. Modern methods may use chemical hydrolysis for faster production.
- **Miso:** Produced by fermenting soybeans with salt and the mold *Aspergillus oryzae*, varying in fermentation time and ingredients for different flavors and types.
- **Impact on Japanese Cuisine:** Both soy sauce and miso are essential flavoring agents in Japanese cooking, enhancing umami and contributing to dishes like soups, marinades, and dressings



Round Blue Saucer Filled With Soy Sauce
Free Stock Photo. Pexels: pexels.com

(Eirini Dimidi et al. 2019)

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It is made by fermenting boiled, dehulled soybeans with *Rhizopus oligosporus* for 35 to 37 hours at room temperature, creating a soft, chewy cake with a distinctive flavor. It is high in protein, contains beneficial lactic acid bacteria, and has reduced anti-nutritional factors, improving its digestibility and nutritional value compared to raw soybeans. It is a traditional Indonesian food, a staple protein source deeply rooted in local diets and the cultural heritage of Indonesia. In European countries, many interesting fermented foods are made.

3. Tempeh (Indonesia)

Production Process: Tempeh is made by fermenting boiled, dehulled soybeans with *Rhizopus oligosporus* for 35-37 hours at room temperature, creating a soft, chewy cake with a distinctive flavor.

Nutritional Profile: Tempeh is high in protein, contains beneficial lactic acid bacteria, and has reduced antinutritional factors, improving its digestibility and nutritional value compared to raw soybeans.

Cultural Relevance: tempeh, a traditional Indonesian food, is a staple protein source, deeply rooted in local diets and cultural heritage.



Tempeh on a Plate
Free Stock Photo. Pexels: pexels.com

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For example, sauerkraut in Germany is made by shredding cabbage, fermenting it with 2 to 3 percent salt, and utilizing natural microbial processes with *Leuconostoc* and *Lactobacillus* species, creating a tangy preserved product. This originated in Germany. It has been valued for centuries for its preservation qualities and nutritional benefits, especially during winter months when fresh vegetables are not available. It offers health benefits, including improved gastrointestinal health, richness in vitamins and probiotics, although its benefits may not solely depend on live microbes. Then we have seeds with many regional variations, not only in Europe but also in other parts of the globe.

So, you have numerous varieties like soft, semi-soft, hard, and then blue cheese, each with unique flavors and textures. So, here you can see feta cheese in this picture. So, this is white cheese made from sheep's milk or from a mixture of sheep and goat's milk. So, cheese production starts with selecting high-quality milk, often pasteurized. Though raw milk is used for traditional varieties, starter cultures and rennet form curds, which are cut, cooked, and drained of whey.

The curds are then salted for flavor and preservation before aging, where the cheese's flavor, texture, and aroma develop. Microorganisms are crucial for cheese characteristics. *Penicillium roqueforti* is the key for blue cheese, while *Lactobacillus* species contribute to acidity and preservation. So, there are many other regional fermented foods; for example, here you can see salami, which is cured sausage consisting of fermented and air-dried meat. Then we have, you know, these fermented meat products, which typically include either pork or beef or any other meat source, salt, spices, and starter cultures, often involving lactic acid bacteria for fermentation. Common methods include

2. Cheese (Various Regions)

Types of Cheese: Varieties include soft (e.g., Brie), semi-soft (e.g., Gouda), hard (e.g., Cheddar), and blue cheeses, each with unique flavors and textures.

Production Process: Cheese production starts with selecting high-quality milk, often pasteurized, though raw milk is used for traditional varieties. **Starter cultures** and **rennet** form curds, which are cut, cooked, and drained of whey. The curds are then salted for flavor and preservation before aging, where the cheese's flavor, texture, and aroma develop.

Role of Microorganisms: Microorganisms are crucial for cheese characteristics—*Penicillium roqueforti* is key for blue cheeses, while *Lactobacillus* species contribute to acidity and preservation.



Close Up Photo of Feta Cheese
(Greek brined white cheese made from sheep's milk or from a mixture of sheep and goat's milk)

Free Stock Photo. Pixels: pixels.com

(Eirini Dimidi et al. 2019)

curing, smoking, and drying, which not only preserve the meat but also enhance flavor and texture. Different cultures have unique fermented meat products, such as salami in Italy, chorizo in Spain, and bresaola in Switzerland, reflecting local tastes and traditions. These fermented meats can offer benefits like improved digestion and enhanced flavor, although moderation is advised due to potential health risks associated with high salt and fat content. So, like fermented fish, we have fermented fish products, especially traditional ones like garum. So, here we can see the fermented fish product from Manipur.

Other Regional Fermented Foods

1. Fermented meat products:

Ingredients: Fermented meat products typically include meat (such as pork or beef), salt, spices, and starter cultures, often involving lactic acid bacteria for fermentation .

Preparation Methods: Common methods include curing, smoking, and drying, which not only preserve the meat but also enhance flavor and texture .

Regional Variations: Different cultures have unique fermented meat products, such as *salami* in Italy, *chorizo* in Spain, and *bresaola* in Switzerland, reflecting local tastes and traditions .

Health Benefits: Fermented meats can offer benefits like improved digestion and enhanced flavor, although moderation is advised due to potential health risks associated with high salt and fat content .



Salami (cured sausage consisting of fermented and air-dried meat, typically pork)

Free Stock Photo: Pexels.com

(Sharma et al. 2020)

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Fermentation is driven by microorganisms or enzymes, involving acidification, protein isolation, and lipid degradation, often taking several months. Fermented fish is significant in cultures across Southeast Asia and Northern Europe. Each region offers distinct products and methods based on local traditions and available fish species. Fermentation enhances the nutritional profile of fish, improves digestibility, and produces antimicrobial substances that extend shelf life and reduce contamination risk. So, we have here the grass carp, the process for fermented zhayu product.

2. Fermented Fish products:

Ingredients: Fermented fish products commonly use fresh fish like mackerel and herring, especially for traditional sauces like garum.

Preparation Methods: Fermentation, driven by microorganisms or enzymes, involves acidification, protein gelation, and lipid degradation, often taking several months.

Regional Variations: Fermented fish is significant in cultures across Southeast Asia and Northern Europe, with each region offering distinct products and methods based on local traditions and fish species.

Health Benefits: Fermentation enhances the nutritional profile of fish, improves digestibility, and produces antimicrobial substances that extend shelf life and reduce contamination risks.



Fermented fish product from Manipur

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It is a traditional fermented fish product in China. Here, this is a grass carp fillet which is cut and then finally divided into cubes. These are mixed with rice powder, salt, hot pepper, ginger, and water, then allowed for anaerobic fermentation by filling with nitrogen, thereby making oxygen unavailable, and the final product is fermented jayu. In India, there are also many fermented foods. We are unable to mention each and every one here.



Fig.- Schematic illustration for the process of fermented *Zhayu* products (a traditional fermented fish product in China) (An et al. 2022)

But this book, *Ethnic Fermented Foods and Beverages of India: Science, History, and Culture* (2020), edited by Professor Tamang, documents many different kinds of fermented foods from India—just mentioning here. For example, bamboo shoots, which, when grown into adulthood, become very woody, but the soft shoots are fermented into a product named Khorisa, with many different names, predominantly used by communities in northeastern India and some South Asian nations. These can be directly used as chutney or as an ingredient in mashed vegetables, onions, mustard, or as a flavor enhancer in meat, fish, curries, and pickles. There are various microbes involved. Lactic acid bacteria.

Traditional Fermented Foods from India.

Adapted from ("Ethnic Fermented Foods and Beverages of India: Science History and Culture," 2020)

Product Name	Type	Culinary Uses	Microbes Involved	Tribes/Communities
Khorisa	Fermented Bamboo Shoot	<ul style="list-style-type: none"> - Used as chutney. - Ingredient in pitika (mashed vegetables with onions, mustard oil, chillies). - Enhances flavors in meat/fish curries, pickles, etc. 	Lactic Acid Bacteria (LAB): <ul style="list-style-type: none"> - Lactobacillus brevis, L. plantarum, L. paracasei subsp. paracasei, L. pentosus, L. collinoides - Produce bacteriocins inhibiting <i>Listeria monocytogenes</i>, <i>Staphylococcus aureus</i>. 	Predominantly communities from North Eastern States.
Kharoli	Fermented Oil Seed	- Served with rice meals or fermented rice (poita bhat/korkora bhat).	Microbes Involved: <ul style="list-style-type: none"> - Unknown. 	Various Assamese ethnic groups.

Then we have Listeria. It produces bacteriocins inhibiting Listeria, monocytosins, staphylococcus aureus. Then another is the fermented oil seed, basically mustard seeds. So the production is called as the currently served with rice meals or fermented rice, which again is a fermented, you know, fermentation of the leftover rice at night and it's an overnight fermentation. It is a very complex microbial product so microbes involved are still to be characterized and it is produced by various ethnic groups in Assam and adjoining areas.

Let us now have a small discussion on the scientific principles of fermentation. Many of these are already mentioned to you in some way or the other. We will discuss about the microorganisms involved in the fermentation, the biochemical reactions that happens in the environmental conditions that are required for fermentation. So, one of the important microorganisms that is involved in fermentation is the bacteria. For example, lactic acid

bacteria which is common in fermented foods and it includes various general like lactobacillus, lactococcus and streptococcus which help in the fermentation process by converting sugars into lactic acid enhancing flavor and preservation. Bacillus species is another bacteria. This is found in alkaline fermented foods, especially in Asia and Africa. Species like Bacillus subtilis contribute to fermentation of legumes and produce L-polyglutamic acid affecting its texture. So, you can see here this Bacillus subtilis forming a visible structure with white dendrites in a nutrient gel dish within two days of inoculation here.

And then here on the top, you can see the Lactobacillus, which is a gram-positive aerobic bacillus and used as a probiotic. Then we have the yeast, for example, Saccharomyces cerevisiae, which is very common. Then we have Candida and Brettanomyces, which are prevalent in alcoholic beverages, bread fermentation, etc. And they play a role in alcohol fermentation; particularly, Saccharomyces cerevisiae converts sugars into alcohol and carbon dioxide, essential for producing beer, wine, and spirits. And, of course, solid food fermentation like bread is also a product of these yeasts.

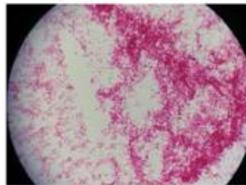
SCIENTIFIC PRINCIPLES OF FERMENTATION

Microorganisms Involved in Fermentation

1. Bacteria

Lactic Acid Bacteria (LAB): Common in fermented foods; genera include *Lactobacillus*, *Lactococcus*, and *Streptococcus*, which help in the fermentation process by converting sugars into lactic acid, enhancing flavor and preservation.

Bacillus Species: Found in alkaline-fermented foods, especially in Asia and Africa; species like *Bacillus subtilis* contribute to the fermentation of legumes and produce l-polyglutamic acid, affecting texture.



Lactobacillus is a Gram +ve aerobic Bacillus - Used as a probiotic.
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Bacillus subtilis formed visible white dendrites in a nutrient gel dish within two days of inoculation.

(Tamang, Watanabe, and Holzpfel 2016)

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So, it ferments the dough, produces gases that cause the dough to rise, contributing to the texture and flavor of sourdough and other breads. So, here we have the *Candida albicans* fungi observed with an optical microscope, and here we have the *Saccharomyces cerevisiae* observed under a scanning electron microscope. Then come the molds, and common genera

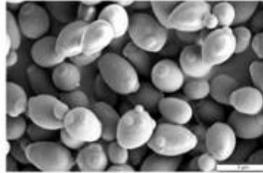
include *Aspergillus*, *Mucor*, and *Rhizopus*, which are significant in the fermentation of various foods, including seeds and soy sauce. Molds contribute to the development of flavor and texture in seeds, aiding in the breakdown of fats and proteins, which enhances the overall quality of the seeds. Molds like *Aspergillus* are crucial in

2. Yeasts

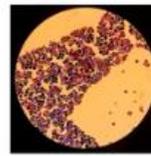
Types of Yeasts: Common genera include *Saccharomyces*, *Candida*, and *Brettanomyces*, which are prevalent in alcoholic beverages, bread fermentation etc.

Role in Alcohol Fermentation: Yeasts, particularly *Saccharomyces cerevisiae*, convert sugars into alcohol and carbon dioxide, essential for producing beer, wine, and spirits.

Role in Bread Fermentation: In bread-making, yeasts ferment dough, producing gases that cause the dough to rise, contributing to the texture and flavor of sourdough and other breads



Saccharomyces cerevisiae, SEM image
Mogana Das Murthy and Pichanathu Ramasamy, CC BY 3.0
via Wikimedia Commons



Candida albicans fungi
observed with an optic
microscope.

(Tamang, Watanabe, and Holzapfel
2016)

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the initial fermentation stage of soy sauce, where they help convert soybeans and wheat into sugars, which are then fermented by yeast and bacteria to produce the final product. So, here we can see an image of an *Aspergillus* species, and this is a *Mucor*, a genus of about 40 mold species commonly found on seeds like Tomidi Savoy. Then we have certain biochemical reactions happening when the food is getting fermented. For example, there is the conversion of carbohydrates. So, glycolysis is the initial pathway in carbohydrate fermentation, where glucose is broken down into two molecules of pyruvate.

3. Molds

Types of Molds: Common genera include *Aspergillus*, *Mucor*, and *Rhizopus*, which are significant in the fermentation of various foods, including cheese and soy sauce.

Role in Cheese Fermentation: Molds contribute to the development of flavor and texture in cheeses, aiding in the breakdown of fats and proteins, which enhances the overall quality of the cheese.

Role in Soy Sauce Fermentation: Molds like *Aspergillus* are crucial in the initial fermentation stage of soy sauce, where they help convert soybeans and wheat into sugars, which are then fermented by yeasts and bacteria to produce the final product.



Aspergillus spp.
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Mucor, a genus of
about 40 mold
species, is commonly
found on cheeses like
Tomme de Savoie.
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via Wikimedia Commons

This process occurs in the cytoplasm and generates a net gain of two ATP and two NADH molecules, which are essential for energy production. In the absence of oxygen, pyruvate undergoes fermentation to produce ethanol and carbon dioxide. This process involves two

key steps. One is decarboxylation. Pyruvate is converted into acetaldehyde by releasing carbon dioxide, and then the reduction of acetaldehyde to ethanol uses NADH, which generates NAD⁺ needed for glycolysis to continue.

Then we have the lactic acid fermentation of carbohydrate conversion, where pyruvate is directly converted into lactic acid. This process is common in muscle cells and certain bacteria, allowing for energy production without oxygen. So, here we can see sugar metabolism by *Lactobacillus* and *Saccharomyces* as representative of lab and yeasts. So, you can see in Figure A the fermentation carried out via the homofermentative pathway and the heterofermentative pathway, where in the first, glucose is converted to lactic acid. Acid, and then in the heterofermentative pathway, glucose is converted into ethanol.

BIOCHEMICAL REACTIONS

1. Carbohydrate Conversion

Glycolysis: This is the initial pathway in carbohydrate fermentation, where glucose is broken down into two molecules of pyruvate. This process occurs in the cytoplasm and generates a net gain of 2 ATP and 2 NADH molecules, which are essential for energy production.

Ethanol Fermentation: In the absence of oxygen, pyruvate undergoes fermentation to produce ethanol and carbon dioxide. This process involves two key steps:

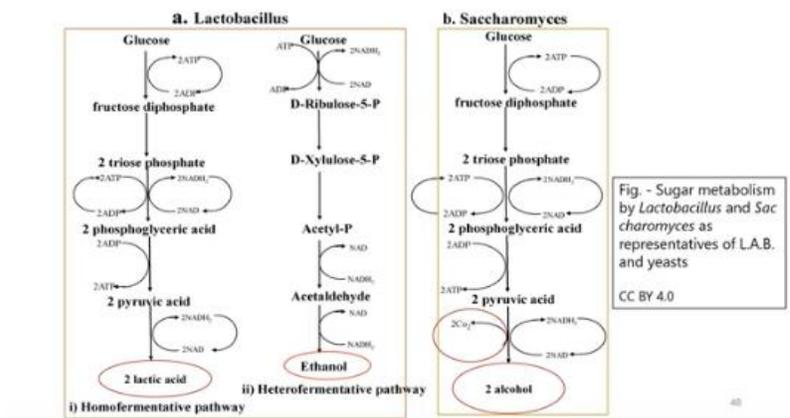
Decarboxylation: Pyruvate is converted into acetaldehyde by releasing carbon dioxide.

Reduction: Acetaldehyde is then reduced to ethanol using NADH, which regenerates NAD⁺ needed for glycolysis to continue.

Lactic Acid Fermentation: Another pathway for carbohydrate conversion is lactic acid fermentation, where pyruvate is directly converted into lactic acid. This process is common in muscle cells and certain bacteria, allowing for energy production without oxygen.

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And in the case of *Saccharomyces*, you can also see how glucose gets converted into two molecules of alcohol. Let us have a small discussion on the production of byproducts, like the production of acids. Fermentation produces various acids like lactic and acetic acid, which contribute to the sour flavor of fermented foods and aid in preservation by lowering pH, thereby inhibiting spoilage organisms and pathogens. It also produces gases like carbon dioxide, which is a key product and plays a crucial role in bread-making by causing dough to rise and adding effervescence to beverages, enhancing texture and mouthfeel. Then we have alcohol production.



Ethanol is primarily produced during the fermentation of beer and wine. It adds flavor and acts as a preservative due to its antimicrobial properties adding the shelf life of fermented products where it is produced alongside. Then we have the acids, gases and alcohols generated during fermentation which enhances the flavor profile of foods and beverages creating a range of flavors from fruity and floral to sour and tangy. Now let us have a small discussion on the impact of environmental conditions on fermentation and product quality. Mostly temperature, PAs, salt, oxygen plays a very crucial role.

2. Production of By-products

Acid Production: Fermentation produces organic acids like lactic and acetic acid, which contribute to the sour flavor of fermented foods and aid in preservation by lowering pH, thereby inhibiting spoilage organisms and pathogens.

Gas Production: Carbon dioxide, a key by-product, plays a crucial role in bread-making by causing dough to rise and adding effervescence to beverages, enhancing texture and mouthfeel.

Alcohol Production: Ethanol, primarily produced during the fermentation of beer and wine, adds flavor and acts as a preservative due to its antimicrobial properties, extending the shelf life of fermented products.

Flavor Enhancement: The acids, gases, and alcohols generated during fermentation enhance the flavor profile of foods and beverages, creating a range of flavors from fruity and floral to sour and tangy.

For example, most microorganisms thrive at 20 to 35 degrees centigrade, but higher temperature may cause off flavors or harm them. And again, lower temperatures may actually slow the fermentation process. Then we have under pH variations something like say a slight acidic pH will support fermentative bacteria and it will also prevent spoilage so because it will not allow those spoilage microorganisms to grow at a slightly acidic pH. Salt is very crucial it enhances flavor and inhibits harmful bacteria but excessive salt can slow down fermentation. The presence and absence of oxygen is also very very important environmental condition.

Anaerobic conditions are essential for alcohol fermentation. Some lactic acid bacteria can ferment with or without oxygen. So, with this we come to end of this lecture. Thank you for your kind attention. Amen.