Chapter 1
Role of Microorganisms in the Food Industry

Navneet Kaur and Anjuvan Singh

Abstract Microorganisms play a crucial role in the food industry as they contribute to the production, spoilage, and preservation of various food products. Dairy products like cheese, yogurt, and fermented milk rely on microorganisms to transform milk into delicious and nutritious foods. Similarly, fermented foods like sourdough bread and meats like salami also require microorganisms for production. In addition to these traditional uses, microorganisms are now being utilized to make wine, beer, and other beverages. They are also used to produce chocolate, culinary colors, and probiotics that promote human health. With the world's population increasing, it is crucial to find innovative ways to use microbes to generate wholesome food and deal with the current food supply crisis. However, while microorganisms are essential for food production, they can also cause spoilage, rendering the food unfit for human consumption. This is particularly true for bakery goods, which are prone to physical, chemical, and microbiological deterioration. Chemical and physical deterioration can decrease the shelf life of intermediate and low-moisture bread goods, while high-moisture products are more susceptible to microbiological spoilage by molds, bacteria, and yeasts. Therefore, it is vital to understand the role of microorganisms in food production, spoilage, and preservation. This chapter will discuss the significance of microorganisms in dairy and bakery goods and how they benefit the food industry. By studying their role in food production, we can develop effective strategies to preserve and extend the shelf life of bakery products, ensuring they remain safe and healthy for human consumption.

Keywords Food industry · Probiotics · Spoilage · Bakery products · Bacteria · Yeast · Mold · Biopreservatives

N. Kaur · A. Singh (✉)
Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

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1.1 Introduction

Since the beginning of time, the actions of microorganisms in food have either harmed or benefited human civilization. The development of human civilization is a continuous process. Around 8000 BC, the ancient human society discovered agriculture and animal husbandry. As a result, there was an excess of food, making the storage, preservation, and prevention of food deterioration became their crucial duties. Ancient people were unaware of using microbes for food preservation, so they used ice and fire to preserve the food. Ancient people adopted different methods for food preservation, i.e., sugaring, sun drying, freezing, cooking, salting, and smoking. History illustrates that ancient people were aware of using microbes in food processing but not in food preservation. During ancient civilizations, the production of alcoholic beverages, fermented food, and bread was practiced. However, ancient people were unaware of using microorganisms to preserve food. In 1837, Louis Pasteur became the first person to understand the role of microorganisms in food. He used heat to destroy the unwanted microorganisms in beer and wine; around 1860, this method was known as pasteurization. Since his invention, food microbiology has significantly developed (Mishra, 2013). The ancient civilization used the production of fermented food. From ancient times onward, the methods for producing fermented meat, vegetables, and milk were known to society (Fox, 1993). Although these methods were artisanal, no importance was given to the microorganisms' role. However, cultures developed that processing and storing particular raw materials in a particular way led to forming foods with desirable and organoleptically attractive properties and preserving qualities far better than those of the original substrate. Most of the time, small communities, monasteries, and feudal estates passed on their manufacturing techniques and knowledge from generation to generation. These communities typically produced small amounts of the product sold in or in their immediate region (Caplice & Fitzgerald, 1999).

Nowadays, there are two main techniques used for the fermentation of food. First of all, the technology used is "wild fermentation" or "spontaneous fermentation" in this, foods are naturally fermented, and it generally occurs when the microorganisms are present in the processing environment naturally, or they are present in raw food. Some of the foods are sauerkraut, Kimchi, and fermented soy products. Secondly, some fermented meals such as yogurt, Kombucha, and natto can be made by adding starter cultures that are used to start the fermentation process, and this type of fermentation is known as culture-dependent ferments (Rezac et al., 2018). Similarly, back-slopping is a technique for carrying out a culture-dependent ferment in which a small quantity of a previously fermented batch is introduced to uncooked food, such as sourdough bread (Marco et al., 2017). Starters can be used as commercial or natural starters to standardize the finished product's organoleptic properties (such as back-slopping) (Yann & Pauline, 2014).

Fermented foods are nowadays widely used in every cuisine in some or the other form. Fermented foods have seen a rise in popularity in the West in recent years, primarily due to claims that they provide health benefits and the growing interest in gastrointestinal health. Fermented food can exhibit positive effects on health through several processes. First, they contain microorganisms that may be probiotic, like lactic acid bacteria (Marco et al., 2017). According to research, most fermented products contain at least 10^6 microbial cells per gram, with concentrations varied based on the region, age, and time of analysis and consumption of the product (Rezac et al., 2018). Through its buffering and protective action against intestinal circumstances (such as low pH and bile acids), the surrounding food matrix appears to significantly influence the survival of probiotic strains (Bove et al., 2013). Several investigations have demonstrated that bacteria from fermented foods can enter the gastrointestinal tract; however, this is likely to vary among products, and their presence in the gut appears temporary (Zhang et al., 2016). First, these microorganisms may exert a physiological advantage in the gut by competing with pathogenic bacteria and producing immune-regulatory and neurogenic fermentation byproducts (Derrien & van Hylckama Vlieg, 2015). Second, a positive effect on health can be exerted by metabolites during fermentation. For instance, lactic acid bacteria generally produce polyamines and peptides that may impact immunological and cardiovascular health and metabolites applicable to dairy and non-dairy products (Pessione & Cirrincione, 2016). Third, some substances may further undergo fermentation and become physiologically active metabolites. For instance, phenolic substances (like flavonoids) may undergo fermentation by lactic acid bacteria to active metabolites (Filannino et al., 2015). Fourth, positive health effects can be gained from fermented foods because it includes probiotics and vitamins (Salazar et al., 2016). Last but not least, the level of toxins and anti-nutrients can be lowered by fermentation. For example, the fermentation of sourdough may lower the levels of fermentable carbohydrates (Abu-Salem et al., 2014), e.g., fermentable oligosaccharides, disaccharides, monosaccharides, and polyols or FODMAPs, the tolerance of these products can be increased in people having functional bowel disorders like irritable bowel syndrome (Laatikainen et al., 2016).

The role of microorganisms in the fermentation process is crucial, as is their role in preserving the finished product. From the beginning of time, food preservation has been essential for human survival by improving the stability and safety of different types of food. The food industry still uses several traditional methods to preserve food, including fermenting, salting, drying, and heating. Since then, there has been a significant gain in knowledge regarding the primary causes of degradation, and losses from food spoilage and contamination have decreased (Gram et al., 2002).

Understanding the composition, pH, origin, water activity (a_w), and storage conditions (such as pressure, atmosphere, and temperature) of the food as well as the characteristics of the most prevalent and resistant microorganisms can help predict the composition of the microflora during processing and storage. The proper application of technology (such as pasteurization and sterilization), sanitary measures (such as good hygiene practices and good manufacturing practices), and traceability (to prevent and reduce the distribution of poor quality and unsafe food) that can stop and delay the colonization of spoilage and pathogenic microorganisms in food. However, food is still susceptible to degradation throughout the food chain, including in grocery stores, restaurants, and consumers' homes, and hazardous microbial infection may occur (Aung & Chang, 2014; Gram et al., 2002).

Food deterioration and contamination harm consumers and the food industry, resulting in financial loss, reputational damage, and legal Punishments (Akkerman et al., 2010). Customers reject the food when its sensory qualities decline due to several intricate mechanisms involved in food deterioration caused by microbial activity. Microorganisms (such as bacteria and molds) may have spoiled food if there is visible development in the form of slime and colonies, changes in texture brought on by the breakdown of carbohydrates, lipids, and proteins, and the sense of off-flavors and off-odors (Gram et al., 2002).

Conversely, the spread of pathogenic microbes, cross-contamination, and toxin production endanger consumer health and threaten food safety. The ailment known as gastroenteritis, marked by intestinal cramps, diarrhea, nausea, fever, and vomiting, is typically brought on by the ingestion and colonization of pathogen microorganisms in the human digestive tract (Flint et al., 2005). Although bacteria are the primary cause of gastroenteritis, other agents such as parasites, viruses, yeasts, and molds are also responsible and have become more common during the past 10 years.

This chapter mainly focuses on the role of different microorganisms in fermented food and the use of different microorganisms as probiotics. The use of microorganisms produces different types of dairy and bakery products. Spoilage is caused to bakery products due to the action of microorganisms and the effect of different physical factors that affect the growth of microorganisms and last different preservation technique to reduce the growth of microorganisms that causes spoilage.

1.2 Role of Microorganisms

1.2.1 Fermented Food

Since ancient times, people have ingested fermented foods, and numerous health advantages have been linked to them. However, it was not until the 1970s that considerable scientific interest was generated regarding the potential probiotic effects associated with ingesting fermented items (Pessione & Cirrincione, 2016). In most of the world, fermented products from animal and plant sources are permissible and crucial to the diet. They use various raw materials, such as substrates, and technologies ranging from the most basic to the most sophisticated, producing finished goods with excellent sensory and textural properties (Bove et al., 2013).

1.2.1.1 Wide Use of Fermented Food

Food preservation improved nutritional value and increased flavor and aroma. The improvement of materials for relatively high-quality products and improved health benefits are the key sources behind the development and use of fermented foods. Nowadays, originality and trendiness play an essential role. These characteristics are brought about by the activity of a group of bacterial strains, especially lactose bacteria or microbes mixed with probiotics and probiotic potential in different organic substrates (Gram et al., 2002).

Because it enables us to select and use new sources, boost the yield of already-existing sources, enhance the bioavailability of nutrients, nutritional value, and flavor, and introduce specific functional characteristics in raw materials, biotechnology has made a significant contribution in recent years. Aerobic or semi-aerobic Lactobacillus strains comprise most of the microflora in these goods (Korhonen, 1994).

1.2.1.2 Advantages Related to Health

The nutritional value of a food depends on how easily it can be digested and how much of the necessary nutrients it contains. Fermentation can potentially improve both the nutrient density and the digestibility of food. Fermentation is a process in which the microbial culture's enzymatic activity may predigest the macronutrients. In healthy people having normal gastrointestinal function, predigesting proteins have a negligible positive effect. It is known that the curd clots that form in the stomach after ingesting yogurt are much smaller than those that form after ingesting milk which increases proteolysis in the stomach (Gram et al., 2002).

Carbohydrate predigesting can help people tolerate certain foods much better. For example, it is widely known that yogurt is more tolerable than milk due to its low lactose content and the lactase activity of the microbial culture for people who are lactose intolerant. Lactase activity in *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains prevents lactose from passage through the stomach and aids the digestion of lactose in the intestine. *Lactobacilli* strains include *Lactobacillus fermentum*, *Lactobacillus salivarius, Lactobacillus buchneri, Lactobacillus brevis,* and *Lactobacillus cellobiosis*, produce alpha-galactosidase activity, which can then be used to break down alpha-galactosides limits in raffinose and stachyose. Both soybean milk and legumes can be processed to remove the carbohydrates that cause flatulence. Older people those are having low stomach acid output, the acid produced during carbohydrate fermentation may help them lower the gastric fluid's pH (Filannino et al., 2015).

Although many molds, bacteria, and yeasts used in fermentation exhibit lipolytic activity when it comes to fats, the nutritional importance of this is probably minimal. Plant foods containing non-digestible substances, including cellulose, hemicelluloses, polygalacturonic acid, and glucuronic acid, may have less lipolytic activity after fermentation. Mineral and trace element bioavailability may be increased due to the breakdown of these molecules. Additional vital nutrients, including vitamins, amino acids, and proteins, may be produced during fermentation. Many physiologically active substances are said to be present in fermented milk, which may benefit human health. These substances include elements originating from milk, fermentation-related microorganisms, and their metabolic byproducts. While some lactose bacteria strains have been identified as probiotics in vivo, fermented milk's probiotic qualities may also be attributed to peptides made from hydrolyzed milk proteins. On proteolysis using starter bacteria or digestive enzymes, several milk proteins have been found to include peptides of varied functions (Caplice & Fitzgerald, 1999). These peptides contain mineral carriers, opioids, antibacterial, anti-cancer peptides, and anti-hypertensive. It is known that whey proteins and casein fractions serve as precursors for the fore mentioned bioactive peptides (Perdigon et al., 1988).

The *Lactobacillus* GG strain has been demonstrated in numerous clinical investigations to have positive health effects, such as the ability to treat gastrointestinal illnesses. Although the variables or mechanisms causing these effects are not entirely understood, mucosal immune system stimulation has been suggested. Therefore, it was intriguing to find out if milk fermentation with *Lactobacillus* GG would result in the formation of immunoreactive peptides (Gram et al., 2002). These preliminary findings imply that lactic acid bacteria in fermented milk alter the immunogenic characteristics of milk proteins. The creation of functional foods and probiotic lactic acid bacteria strains, among other things, may be impacted by this discovery (Perdigon et al., 1988).

1.2.2 Probiotics

In recent years, probiotics have attracted both consumer and academic attention. A growing body of clinical evidence supports some potential health benefits of probiotic supplementation, notably in treating specific diarrheal illnesses. Probiotics are generally "Live microorganisms that, when administered sufficiently, provide a health benefit to the host." The term "probiotic," derived from a Greek phrase meaning "for life," was first used in the 1960s. Despite being a relatively recent concept, some meals' health benefits contain live bacteria that have been understood for ages. However, it was not until the early twentieth century that researchers proposed that the gut flora may be altered by swapping out the harmful bacteria for beneficial ones (Huis in't Veld et al., 1989).

1.2.2.1 Role of Probiotics in the Food Industry

Probiotics are yeast or bacteria permitted as dietary supplements and food additives. They come in capsules, tablets, packets, powders, or other forms and are found in various fermented foods, most frequently yogurt or dairy products (Valerio et al., 2009). Probiotic products may contain single species of bacteria or a blend of several. The bacteria, specifically the *Bifidobacterium* and *Lactobacillus* species, are the most commonly utilized probiotics. *Saccharomyces boulardii*, yeast, also seems to offer health advantages. It is important to remember that probiotic benefits often only apply to a specific strain, so even within a single species, a health benefit attributed to one strain may not necessarily apply to another. Therefore, it is best to avoid broad generalizations regarding prospective health advantages (Korhonen, 1994).

1.2.2.2 Some Properties of Probiotic-Rich Fermented Milks

Only a few fermented dairy products have been linked to a probiotic effect on people in Europe (yogurt). Milk with probiotic characteristics is referred to as "fermented milk," which benefits people by restoring the balance of the microbes in the intestines and the healthy microbial ecology of the gut. The effect of cultured products on the ecology of the gastrointestinal system in humans and other animals has been extensively studied. Probiotic solutions are currently available in a variety of *Lactobacillus* and *Bifidobacterium* species. Lactobacilli that are currently used as probiotics include (Adams & Hall, 1988) *L. brevis, L. cellibiosus, L. acidophilus, L. lactis, L. fermentum, L. casei, L. plantarum,* and *L. reuteri* and *B. infantis, B. thermophilum, B. longum, B. animalis,* and *B. adolescentis* are the *Bifidobacteria* now used as probiotics.

1.2.2.3 The Following Is the List of the Ideal Qualities of a Good Probiotic Strain (Valerio et al., 2009)

1. Simple in vitro proliferation
2. Surviving during storage, processing, and incorporation into feed or food
3. Proliferation and colonization of the gastric-intestinal tract by forming lactose and bacteriocins
4. Noninvasive, nonpoisonous, nonpathogenic, non-tissue residues, and non-absorbed in the digestive tract
5. Genetic stability, the absence of mutations, and the incapacity to produce harmful germs
6. Fighting off harmful bacteria and preventing intestinal infections
7. Lactose intolerance (pre-digestion)
8. Effects on cholesterol levels
9. Antitumor action

Probiotics' positive effects are primarily related to their direct antagonistic action against particular subgroups of microbes (Enteropathogens), their impact on gut metabolism, or their promotion of systemic or mucosal immunity.

1.2.2.4 List of Microorganisms Used as Probiotics in the Food Industry (Valerio et al., 2009)

	Species of *Bifidobacterium*	Species of *Lactobacillus*	Others
1.	*B. infantis*	*L. reuteri*	*Clostridium butyricum*
2.	*B. breve*	*L. acidophilus*	*Saccharomyces boulardii*
3.	*B. lactis*	*L. bulgaricus*	*Escherichia Coli*
4.	*B. longum*	*L. gasseri*	*Streptococcus thermophilus*

1.3 Diary and Bakery Products (Fig. 1.1)

1.3.1 Fermented Milk Made with Bifidobacteria

Several *Bifidobacterium* strains can live in the intestine and enter the colon. *Bifidobacteria*, one of the primary species in the colon, can alter the native intestinal microflora, which has physiological implications such as regulating gut transit and changing the microbiota's enzyme activity (Corthier et al., 1985).

In some circumstances, *Bifidobacteria* can treat constipation and diarrhea. *Bifidobacteria* have been demonstrated in the improvement of parameters of digestion and have a minor anti-pathogen impact in humans (Kotz et al., 1990). They also have an impact on some elements of the immune system. Different strains inhibit the activity of colon enzymes necessary for transforming procarcinogens into human carcinogens (Corthier et al., 1985). In vitro studies with viable *L. helveticus* or *Bifidobacterium* sp. yogurt strains and fermented milk but not *L. acidophilus* revealed a positive effect on colon cancer differentiation and cell differentiation proliferation. *Bifidobacterium* sp. and *L. helveticus* inhibited the development of human HT-29 cells, and the activity of dipeptidyl peptidase IV increased, a particular marker of cell differentiation. These results imply that various lactic acid bacteria exhibit some degree of selection (Duval-Hflah et al., 1983). *Bifidobacteria* struggle in the presence of oxygen because they are not well suited to fermented milk. Growth and survival in acidified and partially aerobic environments are thus crucial selection criteria for particular strains. In certain investigations, milk that has

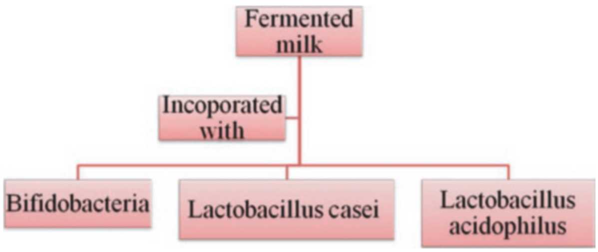

Fig. 1.1 Figure showing different strains of bacteria used in fermented milk

been fermented with both *L. acidophilus* and *Bifidobacterium* is used. It is unknown whether this pairing has different or additional health advantages.

1.3.2 Fermented Milk Made with **Lactobacillus casei**

The most frequent location of *Lactobacillus casei* in humans is the small intestine. Certain strains of *L. casei* can pass through the ileum and stomach in sufficient numbers to trigger a physical response. According to a recent in vivo study (Dewitt, 1985) survival rate in the ileum of many strains of *L. casei* was about 5–10% (except one strain at 0.5%) if *L. casei* was combined with *Bifidobacteria* around (10–30%) or *S. thermophilus* (0.5%).

The most researched lactic acid bacterium is *L. casei,* which has been shown to impact the frequency and length of different types of diarrhea and the immune system. It can help prevent and treat diarrhea brought on by antibiotics and traveler's diarrhea and can significantly impact infantile diarrhea. The ability of *Lactobacillus casei* to treat or prevent the potential of some types of diarrhea to change the activity of the intestinal microflora appears to be related, hence limiting the growth of potentially harmful bacteria. However, the actual methods for particular activities are intricate and poorly understood. Animal studies have shown that the general and specialized immune systems are stimulated. When children with acute diarrhea were treated, it was discovered that many immunoglobulin levels had increased using the *L. casei* strain. The result was not seen in fermented milk that had been heated (Denariaz, 1994).

A recent research program examined the impact of *L. casei* on *Salmonella.* According to the results of the random investigation, milk only had a 10% survival yield after *Salmonella* infection, while milk had a 90% survival yield after it was fermented with bacteria of yogurt and multiple *L. casei* strains. This finding suggests some strain specificity and that combining different strains can produce positive effects. It has been hypothesized but not proven that adherence of bacteria cells is required for diarrhea or immune system control. It has been demonstrated that *Lactobacillus casei* inhibits mutagenicity and reduces the enzyme activity linked to colon cancer risk (Ballongue et al., 1993). It was found to lower human superficial bladder cancer recurrence. The production of carcinogenic chemicals in the intestines and the immune-modulating impact may be part of the mechanism of action. Further research is required to learn how *L. casei*, *Bifidobacteria*, and lactose bacteria reduce the cancer risk (Yasui et al., 1992).

1.3.3 Using **Lactobacillus acidophilus** *to Ferment Milk*

One strain of *Lactobacillus* that does well in the gastrointestinal tract is *Lactobacillus acidophilus.* Similar *to L. casei*, it usually lives in the small intestine. Additionally, it makes up most of the flora in women's urinary tracts. *L. acidophilus* may play a role in human health in several ways, including lowering blood cholesterol and preventing vaginal Candida infections.

1.3.4 Yogurt

The most tangible health benefit of consuming yogurt is reducing lactose malabsorption. Clinical and observational studies support this effect, but the exact mechanism remains unknown. There are some organisms among *Lactobacillus,* with *L. bulgaricus* showing the most activity and *Bifidobacteria* showing the least. Yogurt with heat treatment will not have the same digestion of lactose action as yogurt with live bacteria. Isaac Carasso, a pharmacist, started the first large-scale yogurt production to try to treat intestinal illnesses. The common knowledge of the Balkan populations was the basis for this concept. Several research findings (Attaie et al., 1987) provide statistics on the yogurt effect on male diarrhea. A recent data test was conducted with *Bifidobacterium bifidum* and *Streptococcus thermophilus*, and the analysis reveals that it is impossible to predict which bacteria if any caused the observed effect. Yogurt microorganisms influence a variety of immunological variables. Yogurt consumption has been linked to reduced allergy symptoms in old age people. According to present research, yogurt and a particular *L. casei* strain found in products after fermentation have an adjuvant effect on mice's anti-choleric immune reaction to vaccination. Unlike milk, heat-treated yogurt had the same effect, implying that cell constituents or byproducts of fermentation, other than living organisms, are more important for this function.

1.3.5 Cheese

Cheese is produced by introducing a microbial culture containing a specific strain of bacteria into milk. Cheese is a food that can be made with the milk of many different animals, but the milk of the cow is typically chosen. We can also use milk from goats, reindeer, sheep, and water buffalo. Lactic acid is produced during milk fermentation due to the inoculation of bacterial cultures, and this acid gives the milk its sour flavor. Casein, or milk protein, coagulates as a result of it. The liquid portion of coagulated milk is known as whey, while the solid portion is known as cheese (Marteau et al., 1990). The cheese becomes ripe by adding bacteria, fungi, or both inoculums. The pH is lowered, the texture changes and a flavor develops when these fungal or bacterial inoculums are poured (De Simone et al., 1992). The renin enzyme, which is present in rennet pills, can be used to regulate coagulation. Calve's stomachs contain the renin enzyme. However, genetically modified microorganisms are used today to produce them (Romond et al., 1989). Lemon juice and vinegar can also be used to create coagulation.

The cheese has various flavors (Zoppi et al., 1982). Cheese comes in the following varieties, depending on the bacteria that are added:

(a) To enhance the flavor and texture of cheddar cheese, bacteria are used in the preparation process.
(b) Mold fungi are used to make Roquefort and blue cheese.
(c) Bacteria and fungus are used in conjunction to create Camembert cheese.
(d) *Propionibacterium shermanii* is used in the production of Swiss cheese.

Most cheeses range in natural form from off-white to yellow. The cheese can also be flavored with herbs and spices (Havenaar et al., 1994). Different milk fat concentrations, processing methods, aging duration variations, and mammal breeds are other factors that greatly influence the flavors and styles of cheese.

1.3.6 Bread

Yeast is a saprophytic, single-celled fungus. By secreting enzymes, yeast cells may digest foods, including sugar and minerals. In order to make bread, yeast is used (Kaila et al., 1992). When flour is mixed with yeast culture, water, and other ingredients, carbon dioxide is produced. This carbon dioxide is then trapped in the flour-based dough. CO_2 raises the dough to create the bread. Most often, starch-containing wheat flour is used. Starch serves as the yeast's energy source. Additionally, the gluten protein in the flour causes the stretchy and sticky threads to form as the inoculum of yeast reacts with the starch. The dough is lifted by these threads, which capture CO_2 (Ling et al., 1994).

Yeast has been used as a leavening agent in baking for a long time. *Saccharomyces cerevisiae* is the yeast species most frequently used because of its capacity to develop quickly and ferment sugar in the dough quickly (Aso et al., 1992). The rising of the dough is caused by the CO_2 produced during fermentation. Baker's yeast is produced in large quantities under controlled pH, humidity, and temperature conditions (Hilton et al., 1992).

1.3.7 Chocolate

Microbes are used in the production of chocolate. Cacao tree seeds are used to make chocolate. The cacao tree's white, fleshy pods contain these seeds (Valerio et al., 2009). To extract the seeds from the pods, with naturally occurring microbes, they are first fermented, primarily yeasts and bacteria, specifically *Acetobacter* and *Lactobacilli*. The compounds created by these microorganisms are the ethanol made by yeast, which causes a rise in temperature during the fermentation process, killing the beans and contributing to the chocolate's flavor. The fermentation process in chocolate is the process that creates fragrances, flavors, and vibrant colors. Acetic acid fermentation and alcoholic fermentation are the two steps that make up this process. In the first step of this procedure, yeast activity in the cocoa pulp transforms the sugar into alcohol. Acetic acid is then created by microorganisms oxidizing the alcohol.

1.4 Benefits of Microorganisms in the Food Industry (Mazhar et al., 2022)

(a) Microbes grow quickly and require less space.
(b) Microbes have a lot of protein in their cells. They have a high content of protein of roughly 40–50% in microbes and 20–40% in algae, high yielding capacities, and are less impacted by environmental factors, such as weather which does not impact them.
(c) The microorganism's protein contains all the essential amino acids.
(d) Among different microorganisms, yeast generally contains more vitamin content.

Despite the benefits of microbes in the food industry, they may also have many side effects, as mentioned below,

1.5 Deterioration of Bakery Products by Microbes

Microbiological deterioration is the most common cause of baked goods' shelf-life reduction. Manufacturers and consumers suffer financial losses due to spoilage brought on by microbial development. These losses may result from various specific circumstances, including product packaging, sanitary manufacturing procedures, storage, product turnover, and storage conditions.

Investigated whether lipoxygenase-induced enzymatic spoiling and microbiological deterioration caused by yeast, fungi, and bacteria could be discriminated against each other and unspoiled bread counterparts 48 h after baking (Fig. 1.2).

The volatile compounds produced by the various types of bread decomposition and their analogues were identified using gas chromatography-mass spectrometry. A microbial study revealed that the concentrations of each microbe used increased over time. *Leuconostoc mesenteroides, Leuconostoc citreum, Weissella cibaria, Lactococcus lactis,* and *Weissella confusa* were identified as the species by Valerio et al., (2009) with the use of repeated extragenic palindromic PCR and sequencing

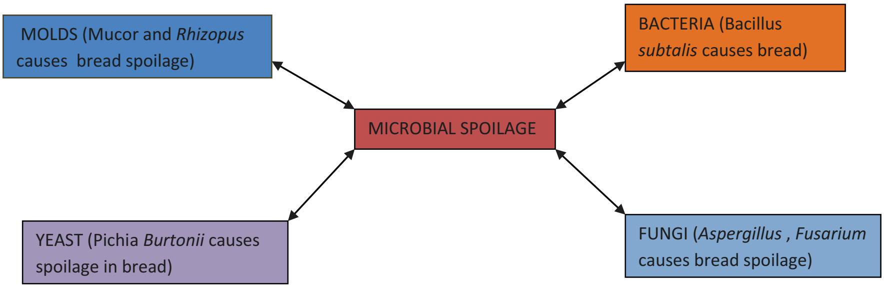

Fig. 1.2 Figure showing spoilage of bread by different Microorganisms

of the 16S rRNA gene analysis. The REP-PCR results identified 17 different patterns.

1.5.1 Bacteria

Moreover, the growth of bacteria is constrained by lowering the water activity and pH, and bacteria can contaminate baked goods. *Bacillus subtilis* spores are a good example of heat-resistant organisms; after 20 min at 65 °C, 55% are still active in amylase. This microbe, found in raw yeast, flour, sugar, and other substances, creates rope in bread. Ropey bread is distinguished by a stringy, incredibly wet crumb, a coloration ranging from brown to black, and a rotten fruit stench discharge. Typically, this issue arises during the summer when the weather is warm and muggy. Under warm, muggy conditions, ripeness can appear exceptionally quickly. Consequently, it is a widespread issue in the climate that is warm of Africa, the Mediterranean region, and Australia (Rosenkvist & Hansen, 1995).

It will be advantageous for bakers to employ ingredients with low contamination levels because raw ingredients are a primary source of *Bacillus* contamination. *Bacillus subtilis* is the principal reason behind the ropey bread; however, other Bacillus species, such as *Bacillus licheniformis, Bacillus megaterium,* and *Bacillus cereus,* are also responsible (Voysey & Hommond, 1993). The first signs of rottenness in bread include a smell of pineapple, and later on, the crumb turns discolored, squishy, and touchable sticky, rendering the bread inedible. The degradation of bread texture is caused by the development of slime, which is caused by the interaction of the amylolytic and photolytic enzymes generated by a few *Bacillus* strains. Strict hygienic standards and suitable production methods are needed to prevent problems with ropes caused by *Bacillus* spp. spores.

One sort of bacterium that might contaminate pie fillings is *Staphylococcus aureus*. Food poisoning incidents linked to cream-filled pastries have also been reported. In addition, cakes and pastries were responsible for 3% of Britain's 323 food poisoning incidents between 1969 and 1972. Other substances used in baking, including desiccated coconut, cocoa powder, and chocolate, have been discovered to contain Salmonella. For instance, *Salmonella typhimurium* significantly impacts frozen pizza (Dickson, 2001).

1.5.2 Yeast

Bakery items can have yeast issues. Species of *Trichosporon, Saccharomyces, Pichia*, and *Zygosaccharomyces* are examples of wild yeast. "chalk bread" originated from the white blotches *Saccharomyces* sp. produces on bread. According to research by Legan & Voysey, (1981), yeast issues in products of a bakery can also be divided into two categories: (a) visible yeast that appears as white or pinkish patches on the surface of the bread and (b) fermentative spoilage linked to essence odors and alcoholic and thus osmophilic yeasts. Yeasts, primarily *Pichia burtonii*, cause bread to become surface-spoiled ("Chalk Mold"). Osmophilic yeast contamination of products typically happens due to dirty tools and equipment. Therefore, osmophilic yeast contamination will be reduced by maintaining proper manufacturing methods.

1.5.3 Mold

Using preservatives is a desirable technique to lessen spoilage and ensure the food is safe because spoilage by mold is a large and expensive bakery problem. Because modern consumers dislike chemicals as preservatives, the baking industry is pressured to use fewer of them (Membre et al., 2001). Mold development is the most significant factor affecting how long high and intermediate-quality bread products will last. Although most molds require high a_w values (>0.8), some xerophilic molds can grow at a_w values as low as 0.65. Mold growth in bakery products is a serious problem that costs businesses money. Additionally, based on the product type, season, and processing method, losses from mold deterioration range from 1 to 5% (Malkki & Rauha, 2000). According to Hickey (1998), the food industry loses an average of 200 million pounds of production annually owing to mold degradation. Mold spores are frequently destroyed when fresh bread and other prepared foods are baked (Knight & Menlove, 2006). Therefore, for bread to develop mold after baking, contamination from the bakery surfaces, food handlers, equipment, the air, or raw materials must happen while chilling, slicing, or wrapping procedures. This implies that any mold-related degradation issues must manifest themselves after baking. The mold particle counts are higher in the summer than in the winter due to airborne particles from warmer temperatures and more humid storage conditions. Additionally, moisture condensed from the packaging before a product has wholly cooled on its surface may promote mold growth. Unpleasant odors are produced by mold rotting, which is frequently seen on a product's surface (Jarvis, 2001). The molds most frequently found in baked goods are *Rhizopus* sp., *Monilia* sp., *Mucor* sp., *Aspergillus* sp., *Penicillium* sp., *and Eurotium* sp.

1.6 Microbial Growth Is Affected by Physical Factors (Effects of pH, Temperature, and Activity of Water)

The key aspect affects how long bakery products may be stored without developing microbial contamination. It is critical when molds and bacteria compete to contaminate high-moisture foods (Ponte et al., 1993). Unlike bacteria, molds are typically less particular about how pH affects them. Molders prefer an acidic pH and tolerate acidic environments (3.5–5.5). Therefore, mold is more likely than bacteria to destroy meals with a pH of 4.5. Abellana et al., (1999) established a technique to determine the effect of water activity (a_w) and the growth of xerophilic fungi on baked goods and isolates, temperature, and their interactions on the mycelial growth of *Eurotium* sp. Water activity (a_w), isolate, temperature, and two or three-way interaction all affected intra-isolate variation. All isolates performed best in the water activity (a_w) temperature range at 0.90 (a_w) and 30 °C, with an interval of the growth rate of 3.8–5.1 mm day^{-1} at 0.75 (a_w), while growth was less than 0.15 mm day^{-1}. The temperature has a significant impact on how spores germinate and how mold grows. The temperature range between 18.3 and 29.4 °C is ideal for most molds. By lowering the storage temperature from 27 °C to 21 °C, (Chamberlain, 1993) discovered that the shelf life of cake free of mold doubled. He also stressed the significance of exercising prudence when distributing and keeping food.

Abellana et al., (2001) investigated how temperature and water activity affected mycelial growth rates in *Penicillium chrysogenum*, *Penicillium aurantiogriseum*, *Aspergillus flavus,* and *Penicillium corylophilum* (Chamberlain, 1993). Growth rates exhibited the expected a_w and temperature dependence on an analogous sponge cake. However, no discernible variations in the growth rates of various isolates were found. The minimum a_w values required for *Penicillium* sp. growth ranged from 0.85 to 0.90.

At 0.90 a_w, *Aspergillus flavus* could grow when the temperature was higher than 15.8 °C. They demonstrated that if the a_w is maintained at 0.85, fungal development by these species on sponge cake analogues with a composition similar to typical bakery goods is avoided. The xerophilic fungi *Eurotium chevalieri, Eurotium rubrum, Eurotium herbariorum, Eurotium amstelodami,* and *Wallemia sebi* were discovered, isolated, and described by Vytrasova et al., (2002). Investigations were made into the fungi's tolerance to high temperatures and preservatives. *Eurotium* species were discovered to be more resistant than *Wallemia sebi*. Sorbic acid was more effective than calcium propionate for preservation against xerophilic fungus. A sponge cake analogues was used by Guynot et al., (2003) to study the effects of pH, water activity (a_w), and carbon dioxide (CO_2) levels on the growth of seven fungal species that frequently lead to the spoilage of baked goods, i.e., *Eurotium* *amstelodami, Eurotium herbariorum, Eurotium repens, Eurotium rubrum, Aspergillus niger, Aspergillus flavus,* and *Penicillium.* The primary elements significantly influencing fungal development were water activity and CO_2. The concentration of CO_2 required to avoid analogues cake deterioration was determined by the effect of water activity at levels of 0.80–0.90 on fungal growth. When the amount of CO_2 in the headspace grew from 0 to 70%, the lag phase level of a_w was doubled to 0.85.

In general, samples packaged with 100% CO_2 did not exhibit any fungal growth up to 28 days of incubation at 25 °C, regardless of the a_w level. Seven molds that are frequently involved in the spoilage of bakery goods were used in an investigation by Elena into the growth of mold on the fermented bakery product analogues (FBPA) of two different pHs (4.5 and 5.5), different water activity (a_w) levels (0.80–0.90), and different potassium sorbate concentrations (0.0–0.3%). Ten-term polynomial models were developed to explain how the variables a_w, pH, potassium sorbate concentration, and fungal growth interacted. It is helpful to simulate storage spoilage concerning the factors influencing fungal development. Small amounts of a_w could only slightly diminish potassium sorbate concentrations at pH 4.5; however, fungal growths were still detectable at pH 5.5 despite adding 0.3% of potassium sorbate. In a 2008 study by Mariona Arroyo et al., four food spoilage fungi (*Penicillium roqueforti, Eurotium repens, Cladosporium herbarum, Penicillium corylophilum*) were examined concerning biotic factors like pH, water availability, and temperature and the presence or absence of a preservative. These included two mycotoxigenic food spoilage fungi, *Penicillium.* These studies aimed to get the potential for nutritional partitioning, niche exclusion, and relative coexistence in bread-based matrices. The niche's size significantly decreased as water availability, pH, and temperature decreased. There were some noticeable relationships between the preservative and pH. The data determined the relative niche overlap indices between the ochratoxigenic species and competing fungi. These showed that *Aspergillus ochraceus* and *Penicillium verrucosum* performed better than other species in terms of nutrition.

1.6.1 Salt Tolerance

At 22 °C, *Penicillium roqueforti* and *Aspergillus niger* were cultivated with NaCl and other NaCl replacers ($MgCl_2$, $CaCl_2$, $MgSO_4$, and KCl). Challenge tests on white bread were also carried out to determine the effects of a reduction in NaCl with or without replacement in partial on the growth of *Penicillium roqueforti*. With NaCl and $MgCl_2$ having the most substantial inhibitory effects on the growth of *Penicillium roqueforti* and *Aspergillus niger,* respectively, the obtained data demonstrated that the isolates displayed varying sensitivities to the salts investigated at equivalent water phase concentrations. $MgSO_4$ was the least effective antifungal. At comparable molalities, $CaCl_2$ showed the most excellent antifungal action overall. $MgCl_2$ was found to have a more negligible inhibitory effect on *Aspergillus niger* at equivalent water phase concentrations than expected based on its a_w depressing effect. This is even though the chemicals studied have significant effects that reduce water activity (a_w), which are essential in explaining the observed trends. *Penicillium roqueforti* growth on conventional white bread, bread with 30% less sodium chloride, and bread with 30% sodium chloride partially replaced by a potassium chloride and sodium chloride solution did not differ according to the challenge tests.

1.7 Microbial Growth Control in Bakery Products

1.7.1 Product Reformulation to Lower the Average a_w

Reformulation involves lowering the amount of readily available water to increase the bread product's shelf life. The a_w of the foodstuff can be decreased through evaporation or freeze-drying, dehydration, or by adding highly osmotically active ingredients such as sugars and salts directly into the food. The amount of a_w decrease practically impacts whether a food is considered non-perishable. The way that microorganisms respond to a particular level of a_w varies significantly depending on their surroundings (Guynot et al., 2003); the increasing concentration of crystalloids renders water in solutions of carbohydrates and salts inaccessible to microorganisms. Furthermore, large quantities of these chemicals directly harm bacteria osmotically. Since all metabolic functions require an aqueous environment for chemical reactions to occur, this effect may be caused by the negative impact of decreased water availability on all of these activities. Maintaining a sufficiently low a_w level is typically necessary to control mold growth in bakery items. For instance, a_w of 0.75 can extend the shelf life of food without mold by 6 months. Higher a_w values, such as those above 0.77, will only briefly extend the shelf life. However, caution must be exercised while reducing product a_w because low levels can have a negative impact on the product's quality and result in changes to its shape and texture (Arroyo et al., 2008).

1.7.2 Freezing

The long-term preservation of bakery goods, particularly those with cream fillings, has been accomplished via freezing. Controlling the growth of ice crystals requires rapid freezing. When the rate of freezing is slower, huge ice crystals are generated, which have the potential to damage internal cellular structures and membranes (Samapundo et al., 2010). Pancakes, shortcakes, cookies, and other baked goods are frequently frozen and sold frozen. Bread can be stored at −22 °C and kept fresh for several months (Kyzlink, 2001). Frozen bread ages relatively slowly compared to fresh bread, which does so in less than a week. Therefore, it ages more slowly at lower temperatures. Desrosier, (2006) claimed that bread preserved at −18 °C for a year after being frozen shortly after baking was as soft as fresh bread kept at 20 °C for 2 days.

1.7.3 Preservatives

Most frequently, preservatives are employed to prevent mold formation in baked foods. Preservatives are “an antimicrobial agent used to preserve food by reducing the growth of germs and consequent decomposition” by the Code of Federal Regulations (CFR). Preservatives can be divided into two categories: chemical and natural. Chemical mold inhibitors allowed in bread include sorbic, propionic, and acetic acids and their salts. On the ingredient statement, natural food preservers such as raisins, cultured products, and vinegar are known by their common names.

1.7.4 Biopreservatives Effect

Due to growing customer demand, biopreservatives using microbes and their metabolites to stop food from spoiling and increase its shelf life have recently attracted more attention. Particularly interesting as biopreservation organisms are lactic acid bacteria (LAB).They can produce various bioactive compounds, including organic acids, fatty acids, hydrogen peroxide, and bacteriocins and have been employed for millennia as starter cultures in the food business. It is known that LAB has antifungal properties.

Mentes et al., (2005) investigated the effects of two sourdoughs created with *Lactobacillus alimentarius* and *Lactobacillus plantarum*, which have antibacterial activity on inhibiting Bacillus strains that form ropes in wheat bread. By adding 15% or 20% low pH (pH 3.5–4.0) sourdough starter to bread dough manufactured separately using two strains of *Bacillus, Bacillus subtilis* and *Bacillus licheniformis*, visible rope production was prevented *(Lactobacillus plantarum* and *Lactobacillus alimentarius*). A 10% sourdough addition, though, was insufficient to prevent the development of visual rope. When the experiment was performed with sourdoughs with a pH higher than 4 (pH > 4), additives at 10% or 15% did not prevent the production of rope, but additions at 20% prevented the production of visible rope caused by both *Bacillus licheniformis* and *Bacillus subtilis.*

Thirty fermented wheat dough microflora samples were gathered from various Tunisian bakeries (M’hir et al., 2007). Mesophilic aerobic bacteria accounted for roughly 10^6 cfu/g in 40% of the samples (MAB). The microbiota of these samples was dominated by yeasts and lactic acid bacteria (LAB). The differences were from 10^5 to 10^8 cfu/g. From microbial counts, the LAB/yeast ratio ranged from 1:1 to 200:1. More than 50% of the analyzed samples lacked *Enterococcus* sp. They had 10^2 and 10^4 cfu/g of contaminating micrflora, including coliforms and mesophilic bacteria. About 26% of the examined samples were judged to contain LAB and coliforms. The ratio between LAB and mesophilic Bacillus is more significant. Approximately 10^4 was the LAB/mesophilic Bacillus ratio for 45% of the samples that underwent analysis. However, no Micrococcaceae were found in any of the samples. Discovered that sourdough fermentation with antifungal strains of *Lactobacillus plantarum* inhibits ordinary bread-spoiling fungus growth. In both the sourdough wheat bread and in vitro systems, the antifungal sourdoughs primarily inhibited the formation of *Penicillium expansum, Fusarium culmorum,* and *Aspergillus niger* spores. On wheat bread, however, there was no change in the proliferation of *Penicillium roqueforti* spores. Calcium propionate (CAP) was coupled with antifungal sourdoughs to limit the quantity of chemical additives in bread, and potential mutual effects were evaluated. Including 3000 ppm CAP in the bread did not affect the development of *Penicillium roqueforti,* but it slowed down the growth of the other fungi.

When antifungal sourdoughs and CAP were added to the bread recipe, a potent synergistic effect inhibited *Penicillium roqueforti* expansion. The effectiveness of lactic acid bacteria in preventing the principal bread contaminants, *Aspergillus, Fusarium,* and *Penicillium*, was assessed by Carla Luciana. Of 95 examined strains, only three (*Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus brevis*) exhibited antifungal activity. Acetic and phenyl lactic acids were the main antifungal substances. Sourdough containing *Lactobacillus brevis,* and yeasts had higher fermentation quotients (FQ = 2.0) and leave volumes (80 cm) than dough without *Lactobacillus brevis*. A reduction of 50% in the content of the calcium propionate chemical propionate was made possible by the starter culture's addition of antifungal LAB strains.

1.8 Conclusion

The current chapter emphasizes the significance of microorganisms in the food industry, as they play a crucial role in the production of various food products. However, the presence of microorganisms also leads to food spoilage, which poses a significant challenge to the food industry. Therefore, various techniques and methods are adopted to overcome this challenge and ensure the safety and longevity of food products. One of the primary techniques used to prevent spoilage is the use of preservatives in bakery foods. These include freezing, chemical preservatives, and biopreservatives, which help to extend the shelf life of high and intermediate-moisture bread items. Despite these measures, however, mold deterioration remains a severe issue that impacts the shelf life of many bakery products, resulting in significant financial losses for baking businesses. To combat this problem, it is essential to implement strategies to prevent mold growth and extend the shelf life of baked goods. This is especially important given the growing demand for baked goods globally. These strategies include maintaining high standards of bakery hygiene, which can help to prevent the growth of harmful microorganisms. Additionally, post-packing or modified environment or heat treatment packaging may be necessary to ensure that the food remains safe from spoilage by microbes. In conclusion, while microorganisms are beneficial in the food industry, they also pose a significant risk to food safety and longevity. To ensure the safety and longevity of bakery products, it is crucial to adopt effective strategies to prevent spoilage, including the use of preservatives, maintaining bakery hygiene, and implementing post-packing or modified environment or heat treatment packaging techniques as necessary. These efforts can help to minimize financial losses for baking businesses and meet the rising demand for baked goods globally.

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