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METAGENOMIC INSIGHTS INTO FOOD MICROBIOTA DYNAMICS: A COMPREHENSIVE REVIEW OF STORAGE-INDUCED MICROBIAL SHIFTS

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ABSTRACT

Food microbiome has a big influence on food safety, standards, and shelf life. Microbial dynamics also change while food is being stored for a variety of reasons. The use of metagenomic techniques to decipher the complicated relationships between food microbiota and storage conditions is examined in this review, offering fresh perspectives on microbial interactions and community dynamics. The limitations of traditional culturing techniques have led to the development of metagenomic methodologies, which provide unmatched insights into food storage microbiology by exposing complete microbial communities, their interactions, and functional alterations. The paper compares traditional and metagenomic techniques and highlights important aspects influencing microbial dynamics during storage, it also emphasises the need of taking food microbiome and microbial alterations during storage into account. This review provides insights into microbial behaviour and its impact on food safety and quality by demonstrating the application of metagenomic analysis to these food categories. The review also addresses the effects of microbial changes brought on by storage on the nutritional value, sensory qualities, spoiling hazards, and shelf-life estimation of food. Finally, it looks at new developments that could enhance storage procedures and guarantee food safety in the future, like real-time monitoring, predictive modelling, and combining metagenomics with other omics technologies.

Keywords: Dynamics, Food storage, Metagenomics, Microbiome, Omics modelling, Spoilage

INTRODUCTION

Communities of microorganisms that present in a specific environment, like the human body; specifically in gut, skin or mouth are known as Microbiota. It plays an important role for a healthy body (**Valdes *et al.*, 2018**). Research explored through culture-independent methods shows that foods are dynamic ecosystems, emerging as vital sources of microbes to our gastrointestinal tract. Every bite we take contain a mix of beneficial and harmless microorganisms introduced in our body, originating from the food itself (food microbiota) (**Hauptmann *et al.*, 2020**) and also dispense nutrients (substrates) for the microbes living in our gut to improve overall gut health (**Zhang, 2022**), the intrinsic microbiota of various food products is shaped by a series of factors across the entire food supply chain, including manufacturing protocols and food processing, agricultural practices, environmental conditions (**Jarvis *et al.*, 2018**). A healthy gut microbiome is crucial for immune function, metabolism, and neurological wellbeing. Dietary influences, counting of specific foods, lifestyles and eating patterns, can misbalance the gut bacteria, ultimately exert influence on human health (**Valdes *et al.*, 2018**). Shifts in lifestyle, characterized by a reliance on ready to eat and convenience foods, have created new challenges in ensuring food safety, particularly with regards to microbial contamination **(Kotzekidou, 2016)**.

Even after continuous advancement of detection of microorganisms in food science and technology, foodborne pathogen infections resume to be an unrelenting global problem, resulting in 548 million illness and 230,000 causalities annually. It's necessary to have speedy and precise pathogenic detection and identification capabilities in order for food processors, regulators, and other stakeholders to productively cope up with foodborne (**Billington *et al.*, 2022a**). With the growing demands and public awareness towards the dietary preference, currently there are various ways to recognize microbes as follows, culture methods, polymerase chain reaction (PCR), microarrays and High-Throughput Sequencing (HTS) (Hilton et al., 2016a). Morphological and phenotypic traits have historically been essential for species distinction and classification, with many species described solely on these characteristics, therefore culture technique is widely used (**Wijayawardene *et al.*, 2021**). Traditionally, over 100 years microbial culture remains the cornerstone of diagnostic techniques in microbiology. Culture dependent technique involve the use of microbial culture for growing pathogens on specific media, followed by biochemical tests for further species level identification and antibiotic susceptibility testing (**Hilton *et al.*, 2016b**).

This technique restricted to identify limited microbes based on their structural and biochemical trait and are failing to point out uncultured microorganism in food, innovations in Next-generation sequencing (NGS) have democratized microbial whole-genome sequencing more affordable, efficient and accessible tool for identification (**Wijayawardene *et al.*, 2021; Billington *et al.*, 2022a; Sadurski *et al.*, 2024**). New emerging techniques like culture-independent techniques do not require any specific microorganism culture and its limitations. Metagenomic approaches, encompassing, metabarcoding, shotgun metagenomics and long-read sequencing are DNA and RNA based sequencing technologies empower direct recognition of complex food microbial ecosystem **(Billington *et al.*, 2022a; Sadurski *et al.*, 2024)**.Next-generation sequencing (NGS), also known as high-throughput sequencing (HTS) or deep sequencing, is a revolutionary DNA sequencing technique which dramatically enhance diagnostic capabilities that allows for rapid and extensive analysis. The integration of high-throughput screening (HTS) enables swift and comprehensive analysis, processing large datasets with unprecedented speed. Compared to the traditional gold standard, Sanger sequencing, NGS, powered by HTS has significantly increased throughput, affordability, velocity of sequencing, and improved result quality **(Chen *et al.*, 2022; Sadurski *et al.*, 2024)**.

Inherently carry diverse microbial loads in food and food products poses spoilage and health risk when consumed, as per Food and Agriculture Organization of the United Nations (FAO) report, $1/^{3rd}$ of food production was lost to spoilage globally before reaching to consumers. Eliminating or inhibiting microbial growth is crucial to mitigate the risk so there must be better understanding of microbial shift during storage of food to increase the shelf life for safety of food. There are predictive microbiology modelling, leveraging quantitative studies which enables the prediction of microbial growth factors, informing risk assessments **(Stavropoulou & Bezirtzoglou, 2019a)**. In this review, the value of food microbiota for healthy gut and various approaches for diagnosis of microbiota in food include traditional and culture independent techniques and their recent innovations are described. It also highlights different metagenomic-based approaches for food safety along with factors affecting the microbial dynamic and significance of microbial shift with emerging and future trends.

Metagenomic Approaches in Food Microbiota Analysis

The term "metagenome," which describes a group of genes found in samples used to research functional analysis and cloning. It is defined as the study of hereditary data of microbes and it can be further classified into shotgun metagenomics and amplicon metagenomics (**Nam *et al.*, 2023**). In the process of metagenomics, DNA is extracted from any sample to be examined or from the environment, and then sequencing and genome analysis are performed. Targeted metagenomics techniques, like 16S ribosomal RNA (16S rRNA) gene metagenomics, involve a number of procedures, including collection of sample, isolation of DNA, amplification of the 16S rRNA polymerase chain reaction (PCR), clean up, and next generation sequencing (NGS), which is followed by sequence analysis using a diversity of computational tools (**Kamble *et al.*, 2020**). The technique of processing many DNA strands with NGS technologies is known as DNA metabarcoding. It permits microbial identification in complicated food and food products including numerous food microbes (**Preckel *et al.*, 2021**), a shotgun technique have the ability to discover and characterize unsanctioned genetically engineered microbes by the help of small or large read sequencing (**Buytaers *et al.*, 2021**). 16S rRNA gene sequencing targets are specific genes only in bacteria/archaea, resolutions are species and genus levels, coverage of organisms are bacteria, archaea like prokaryotics, moreover data complexity very less only. Targets of shotgun metagenomics: whole genome of all organisms, species or strain level resolutions, coverage of organisms are bacteria, fungi, virus, eukaryotics, archaea, data complexity are very high, cost effective, functional information are resistance genes, pathways.

Fermented foods, like yogurt, cultured milk, kimchi, soya curls, kombucha, and shivakraut, are among the popular food varieties today that depend on the presence of certain bacteria to produce particular flavors (**Voidarou *et al.*, 2020; Buytaers *et al.*, 2021**). Prior to now, microbiological composition in foods has been examined through the use of culture techniques, which have limitations in terms of their capacity to identify bacteria that are not culturable. The majority of microorganisms cannot be cultivated using conventional techniques. The problem of enumerating every member of the microbial community in an environmental sample has been overcome by metagenomics, which allows complete genomes to be analyzed without the need for culture. Compared to conventional methods, a culture-independent approach to metagenomics, incorporates a larger quantity of genetic data (**Nwachukwu & Babalola, 2022**). The 16S rRNA in the food industry are in bacterial communities, quick deduction of shifts, microbial community monitoring routinely, common pathogens like *Salmonella*, *Listeria* are deducted. In the probiotic characterization of *Lactobacillus*, *Bifidobacterium* confirmation analyzed.

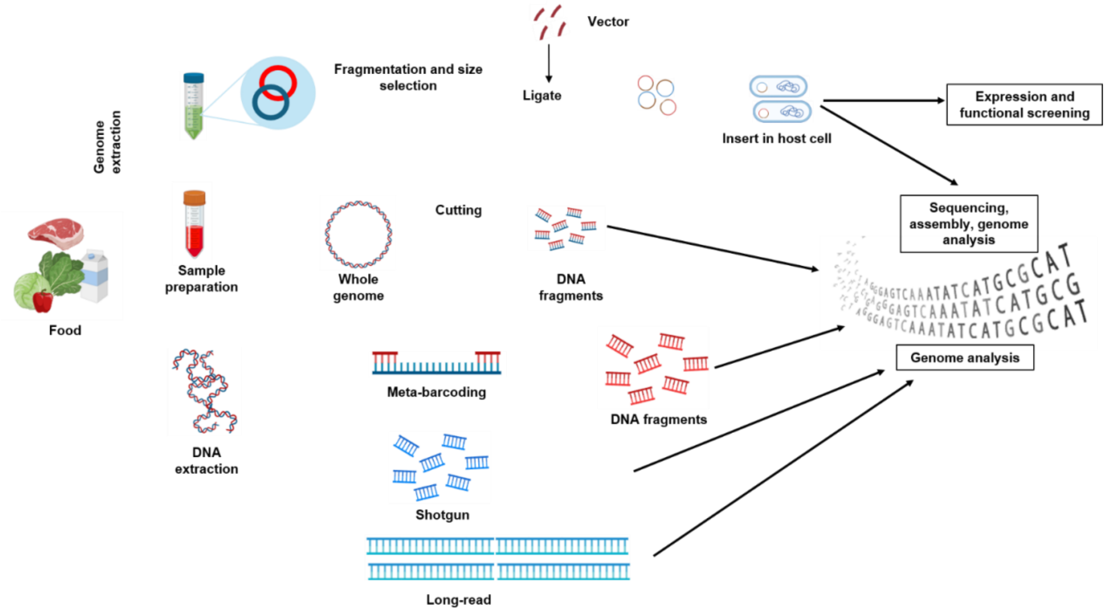


Figure 1 Metagenomic approaches in genomic analysis

In the diagnosis of food safety, the next disruptive revolution will use metagenomic-based techniques, such as shotgun and long-read metagenomics and metabarcoding. These innovative techniques provide a holistic view of microbial communities, detecting entire populations of microbes present in a single food sample, ingredients, or environmental materials.

Metabarcoding – This method focuses on specific microbial groups, such as bacteria (16S rRNA) or fungi (ITS), regardless of their abundance, allowing for precise analysis of diverse microbial communities. When sequencing depth is insufficient, uncommon, or low taxa are identifiable more affordable than gun-sequencing in this the complete genome does not need to be rebuilt (**Billington *et al.*, 2022a**).

Shotgun metagenomics- The term "shotgun metagenomics" refers to the unfocused sequencing of every microbial genome ("genomics") found in a sample, or "meta" sequencing. Shotgun sequencing can be used to recover entire genome sequences as well as assess the composition and functional diversity of microbial populations (**Quince et al., 2017**). The DNA molecules in this sequencing are randomly fragmented, producing tiny DNA fragments; this method is used in metagenomics to characterize the microbial community.

Long-read metagenomics

DNA or RNA genomic sequencing is possible. Ideal for populations with few species dominance and low diversity, avoiding amplification biases associated with metabarcoding approaches, sequencing genomic DNA or RNA is possible, no amplification bias, in contrast to sequencing using metabarcoding Phylogenetic genetic markers or targets can be deduced from collection (**Maguire *et al.*, 2021**).

Next generation sequencing

Next-generation sequencing (NGS) and pyrosequencing are examples of second-generation approaches. Read lengths for NGS and sequencing by synthesis or ligation methods range from 50 to 500 bps. Use of NGS techniques works on transcriptomics and metagenomics to identify the functional activity of undergoing fermentation food (microbial metabolite compounds). The steps involved in a typical sequencing workflow are data analysis, bioinformatics workflow, preparation of sequencing library (**Akaçin *et al.*, 2022; Chelliah *et al.*, 2022**). The two second-generation NGS technologies, 454/Roche and Illumina/Solexa platform are frequently used in metagenomic research (**Frank *et al.*, 2016**). The encounter of metagenomics and other new innovative technologies include exciting opportunities for evolution and notable challenges and risks that must be addressed. Standardization - The first challenge prior to metagenomics emerging as the new "gold standard," sample preparation, sequencing technique, and analysis must be uniform (**Forbes *et al.*, 2017**). To prepare the library, enough length and adequate volumes of high-quality DNA are needed. Satisfying these requirements includes the delicate nature of concentrated package and cleaned DNA from the digested and dephosphorylated vector for ligation continues to be difficult (**Laudadio *et al.*, 2019**). Fig. 1 summarizes the metagenomic approaches used in genomic analysis of microbial dynamics during storage of foods. The bias of the reference database that was used to annotate sequence reads. Enhancing high-throughput gene sequencing technology to sequence longer reads with greater precision and depth can help overcome these difficulties (**Lema *et al.*, 2023**).

Factors influencing microbial dynamics during food storage

Food encompasses a wide range of edible substances that may be raw, partially processed, or prepared. It includes numerous techniques such as cutting, trimming, salting, curdling, drying, fermenting, pickling, curing and smoking for preparation of food consumed by humans (**AL-Mamun *et al.*, 2018**). During storage of these foods it may spoil, because of microbes. Microbial dynamics during food storage alter the attribute of these foods, and may cause food spoilage and food poisoning as a consequence due to their metabolic activities (**Hamad, 2012**). There are several important factors that hold microbial growth in food including intrinsic factors, temperature, humidity, extrinsic factors, implicit factors, processing factors as well as combined effects of these factors shown in Figure 2 (**Erkmen & Bozoglu, 2016**). Under lab conditions microbial testing is important for *Salmonella*, *Listeria* organisms, for physical analysis colour, pH, texture, particle contaminations, in chemical testing analysis deducting chemicals like colouring agents, food additives, allergens and different types of pesticide residues.

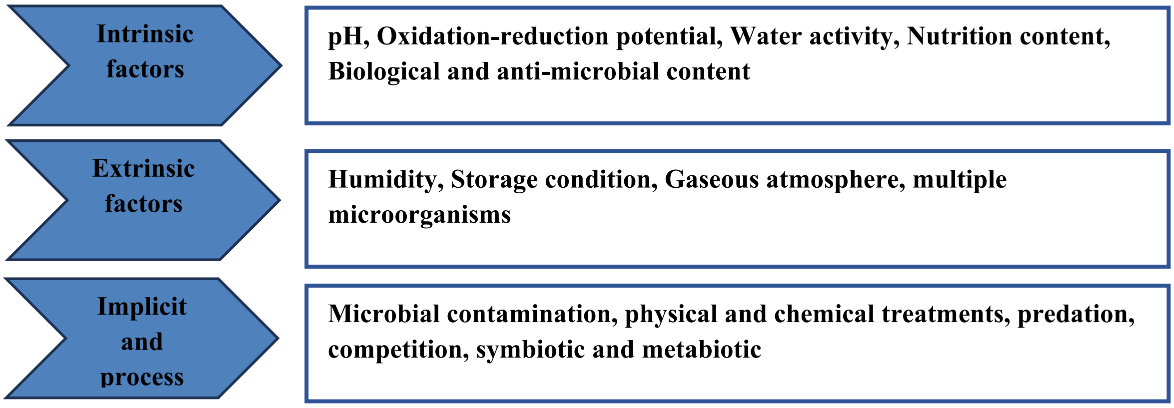


Figure 2 Several factors affecting the growth of food microbes

Nutrient content

All Microorganisms stand in need of a source of energy and electrons for growth to occur, a few basic fundamentals involve, nitrogen, water, energy source, vitamins and minerals to thrive and maintain their metabolic processes. Foodborne microbes utilize these fundamentals to obtain power from carbohydrates, alcohol and proteins which are the primary source of energy and nitrogen. Food rich in carbohydrates ruins high carbs food by carbohydrate fermentation process, mainly yeast and molds (**Preetha & Narayanan, 2020**).

pH

Each type of microbes requires appropriate minimum and maximum pH to grow in food. The concentration of hydrogen ion in food is determines the pH by equation:

$$pH= -\log_{10} [H+]$$

Basically, microbial growth in food does not occur or occur very slowly at a pH level lower than 4.6, except for a few microorganisms. Increase in pH promoting the microbes to grow leads to spoilage of food as shown in Table 1 (**Arshad & Batool, 2017**). Optimal pH ranges for microbial growth vary as follows: (**Preetha & Narayanan, 2020**).

Table 1 pH range of different microbial groups

Microbe	Minimum pH	Optimum pH	Maximum pH
Bacteria	4.5	6.5-7.5	9.0
Yeasts	Below 3.5	4.0-6.5	Up to 6.5
Moulds	Below 3.5	4.5-6.8	8.0-11.0

Water activity

Accessibility of water in sufficient amounts promotes microbial growth. The term water activity (aw) of food is the ratio of the water vapour pressure of food to the vapour pressure of pure water. Based on water activity (aw) range; High-moisture foods (>0.85), are mainly affected by the growth of pathogenic food microbes in comparison to Intermediate-moisture foods (aw range 0.60-0.85) and low-moisture food (aw < 0.60) (**Preetha & Narayanan, 2020**).

Packing condition

By controlling gas exchange, package barrier properties remarkably affect microbial growth. Modified Atmosphere Packaging (MAP) technologies, including Controlled Atmosphere Packaging (CAP), Controlled Atmosphere Storage (CAS), Direct Addition of Carbon Dioxide (DAC) and Hypobaric Storage help to enhance food quality and safety (**Hamad, 2012**).

Redox potential

Oxygen adequately influences food systems as a powerful redox couple. When food is disclosed to air, a high positive oxidation/reduction (O/R) potential develops. The O/R potential, symbolized as Eh, indicates a system's tendency to donate or accept electrons. Aerobic microorganisms thrive in positive Eh environments, whereas anaerobic microorganisms require negative Eh (reduced) conditions.

Temperature

Microorganisms grow within an acceptable temperature range, cogent generation time and lag period. Based on temperature preferences, they're categorized as: Psychrophiles (low-temperature) having optimum temperature range 10°C-15°C; Psychrotrophs (cold-tolerant) favours moderate temperature 15°C-30°C; Mesophiles, mostly include bacteria (optimum 37°C, e.g., human pathogens); Thermophiles (45°C-70°C, e.g., Bacillus, Clostridium) and Extreme thermophiles/hyperthermophiles (80°C+).Understanding microbial growth dynamics, including temperature interactions, enables adjustments to storage temperature, enhancing shelf life and reducing food spoilage and poisoning risks (**Hamad, 2012; Kotzekidou, 2016**).

Case Studies

Metagenomics, the research involving the direct analysis of genetic material from the collection and processing of environmental samples, has become a powerful tool for understanding microbial communities in food products (*Metagenomics - an Overview | ScienceDirect Topics*, n.d.). No culture-independent method rivals the efficiency of high-throughput sequencing (HTS), which enables the accurate identification of most microorganisms, offering critical insights into food safety, spoilage, and a crucial impact of microbiota in fermentation, contributing significantly to the resulting health benefits (**Billington et al., 2022b; Ercolini, 2013**). In this section, we delve into metagenomic studies across several food categories, highlighting key findings and their relevance to food quality, safety, and production processes, to highlight the importance of the metagenomic analysis which will help to screen at the stages of process till shelf life of the food. FDA/FSMA are USA based standard methods, protocols, etc., preventive controls, QA, operations collaborate to document compliance, EFSA in Europe its doing scientific data sharing for risk assessments, GFSI (BRC, SQF) are involved in integrated food safety plans, training and internal audits, Codex Alimentarius involved in global guidelines adopted across food safety programmes. Packaging methods modified atmosphere packaging methods, vacuum packaging, aseptic, sterile, smart, active packaging, sustainability, barrier properties. Storage conditions are cold storage, refrigeration, freezing, dry storage, ambient, controlled atmosphere storage. Production safety plans are Critical Control Points (CCPs), corrective actions, and preventive maintenance.

Dairy Products

Yogurt, butter, milk, and cheese are well-known dairy products, recognized for their rich microbial diversity is related to temperature, which plays a crucial role in their production, flavor, and texture; metagenomic analyses show that lactic acid bacteria (LAB), particularly *Lactobacillus* and *Streptococcus* species, dominate the microbial communities in fermented dairy products like yogurt and cheese (**Coelho *et al.*, 2022; You *et al.*, 2022**). These bacteria contribute to the fermentation process by transforming lactose into lactic acid, leading to a reduction in pH levels and degrading milk proteins into smaller peptides and lipids into free fatty acids, thereby enhancing preservation, flavor, and safety (**Wang *et al.*, 2021; Sionek *et al.*, 2023**).

Metagenomic sequencing of raw milk has revealed pathogenic and spoilage microorganisms like *Pseudomonas*, *Bacillus*, and *Staphylococcus* species, which produce exopolysaccharides that form biofilms on milk surfaces, leading to defects like proteolysis, lipolysis, off-flavors, and sweet curdling, ultimately causing quality deterioration and foodborne illnesses (**Porcellato *et al.,* 2021; Saha *et al.,* 2024**). Studies have identified microbial communities involved in cheese ripening, with species like *Propionibacterium freudenreichii* metabolizing lactate into substances like acetate and propionate, which contribute to the distinctive flavor of Swiss cheese, while *Penicillium* species enhance the unique characteristics of blue and mold-ripened cheeses (**Zheng *et al.,* 2021; Anastasiou *et al.,* 2022**). In addition to spoilage and fermentation-related microbes, dairy metagenomics reveals microbial resistance genes. *In silico* screening identified recombinant strains *of Lactococcus lactis* demonstrating enhanced resistance to β-lactam antibiotics, highlighting its role as a source of antibiotic resistance (AR) genes. This underscores the risks of antibiotic overuse in dairy farming and highlights the value of integrating functional metagenomics with next-generation sequencing for food safety; thereby it will stop the intake of antibiotic containing dairy products for lactating women.

Meat and Poultry

Metagenomic analysis of meat and poultry has shed light on the complex microbiomes present in both raw and processed meats. Raw meat products, including beef, pork, and chicken, harbor diverse microorganisms, ranging from beneficial or harmless species to harmful bacterial contaminants like *E. coli*, *Salmonella*, *S. aureus*, *L. monocytogenes*, and *Campylobacter jejuni*, which can cause spoilage or pose health risks (**Nørrung *et al.,* 2009**). Poultry meat is a known source of zoonotic diseases, highlighting the need to understand its microflora development to control the proliferation of spoilage and pathogenic bacteria, which can produce spoilage compounds like lactic acid and ethanol within four days of production due to the dominant spoilage microflora (**Marmion *et al.,* 2021**). The AR gene detection in the meat microbiome raises concerns about the transmission of antimicrobial-resistant pathogens through the food chain, as these bacteria can be transferred to humans via meat consumption (**Conceição *et al.,* 2023**). Despite on-farm interventions, microbes like *Salmonella* and *Campylobacter* can persist within broiler flocks, spreading among animals and entering the food chain (**Marmion *et al.,* 2021**).

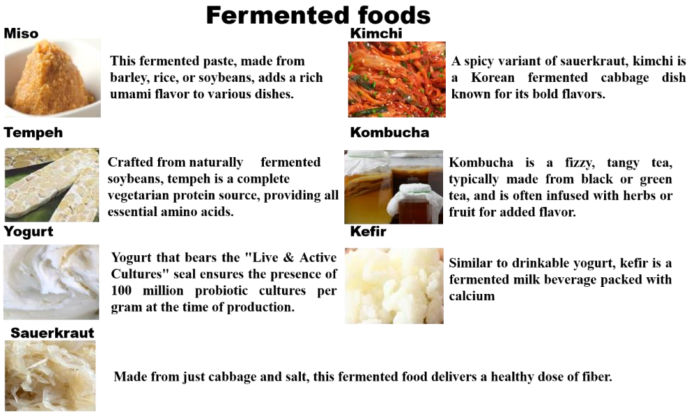
Meat fermentation, particularly in products like sausages, is significantly influenced by microbial communities, as these fermented meat products develop unique flavors and textures while benefiting from extended preservation through the Maillard reaction, oxidation of lipids, degradation of protein, and the activity of microorganisms (**Zhong *et al.,* 2021**). LAB and *coagulase-negative staphylococci* (CNS) have a part in flavor development and preservation, making metagenomic tools invaluable for optimizing fermentation processes and ensuring product quality (**Maheshwari *et al.,* 2021**). Ensuring microbial safety in meat and poultry products is critical to prevent foodborne illnesses, spoilage, and economic losses. Various pathogens and spoilage organisms can contaminate meat throughout processing, storage, and handling. Major pathogens are meat and poultry products are *Salmonella* spp. (Beef, poultry, undercooked meats), *Listeria monocytogenes* Processed meats (e.g., deli), cold storage, *Escherichia coli*, *Campylobacter* spp. *Clostridium perfringens, Clostridium botulinum, Staphylococcus aureus*. There are different testing methods: Total Plate Count (TPC), coliforms, spoilage organisms testing, *Campylobacter* testing, *Listeria monocytogenes*, *Salmonella* detection, the traditional method of microbiology analysis of meat and meat products may take time.

Fruits and Vegetables

Fresh produce, including fruits and vegetables, is often consumed raw, making microbial contamination a critical concern for food safety. Metagenomic studies of fruits and vegetables have revealed a diverse microbial population, primarily consisting of genera such as *Pseudomonas*, *Sphingomonas*, *Aeromicrobium*, and *Pantoea*, while another study identified *Lactobacillus plantarum*, *L. fermentum*, and *L. pentosaceus* as the main players in fermented vegetables (**Liu *et al.,* 2023**). The microbial composition varies based on elements such as; temperature, plant type, cultivation practices, environmental factors, and post-harvest handling (**Alegbeleye *et al.,* 2022**). Spoilage organisms in fruits and vegetables encompass fungi, Gram-positive and Gram-negative bacteria, and such as yeasts and molds, all of which can lead to soft rot, discoloration, and off-putting odors (**Alegbeleye *et al.,* 2022**). For example, *Erwinia carotovora* has been identified as a major spoilage bacterium in metagenomic analyses of vegetables, causing soft rot in products like potatoes and carrots. Additionally, microbes like *Salmonella* and *Listeria monocytogenes* have been detected on contaminated fresh produce, posing significant food safety risks. Metagenomics has also been employed to study the organic impact versus conventional farming on the microbial profile of produce, revealing that organic farming practices may promote a higher diversity of beneficial microorganisms, potentially enhancing food safety and quality (**Khoiri *et al.,* 2021**). Most of the vegetables are naturally less sugars than the fruits, particularly non starchy vegetables like kale, brococcoli, spinach, certain B vitamins, A, K are higher levels, good sources minerals like iron, Mg, K, and calcium. Higher amount of fiber content in cruciferous and leafy vegetables high insoluble fiber, which aids digestion, due to starch, lower sugar content, and most vegetables have very low calories. It contains phytochemical, antioxidants, such as glucosinolates and lutein. Fruits contain fructose, it makes fruits sweet in taste, vitamin C level is rich in kiwis, citrus fruits, strawberries, etc., and it contains polyphenols and flavonoids. Fruits are providing quicker energy than the vegetables, soluble fibers are high in apples, berries, pears, etc., watermelons; oranges are high in water contents. A healthy diet must include both fruits and vegetables, with fruits being superior providers of fast energy and certain antioxidants and veggies being more nutrient-dense per calorie. For best health, a varied combination of both is recommended.

Fermented Foods

Fermented products like kefir, miso, yogurt, sauerkraut, kimchi, tempeh, kombucha, and sourdough bread, derive their unique flavors, textures, and preservation qualities from the complex microbial communities involved in their fermentation process (Gondaliya et al., 2024). Features of the respective foods are illustrated in Fig 3. Metagenomic studies of fermented foods have demonstrated that LAB, such as *Streptococcus thermophilus* and *Lactococcus lactis*, dominate the microbial populations during fermentation, playing key roles in acid production and flavor development (**Pasolli *et al.,* 2020**). In kimchi, a traditional Korean fermented vegetable dish, metagenomic analyses have identified the primary microbial genera as *Leuconostoc*, *Lactobacillus*, and *Weissella*, revealing a high metabolic potential for heterotrophic lactic acid fermentations within the kimchi microbiome (**Jung *et al.,* 2011**). Metagenomics offers insights into the microbial dynamics and safety of fermented foods by identifying dominant and potentially harmful microorganisms while assessing the impact of fermentation conditions on microbial diversity and metabolic activity (**Bigot *et al.,* 2015**). Furthermore, using RNA-based techniques in fermented dairy products, such as kefir, provides a clearer picture of active microbial communities compared to DNA-based methods, highlighting the importance of live microbes in flavor and safety (**Bigot *et al.,* 2015**). The traditional method of microbiology analysis will be a tedious method for identifying the entire microorganism as it takes a long time to identify.



This fermented paste, made from barley, rice, or soybeans, adds a rich umami flavor to various dishes.

A spicy variant of sauerkraut, kimchi is a Korean fermented cabbage dish known for its bold flavors.

Crafted from naturally fermented soybeans, tempeh is a complete vegetarian protein source, providing all essential amino acids.

Kombucha is a fizzy, tangy tea, typically made from black or green tea, and is often infused with herbs or fruit for added flavor.

Yogurt that bears the "Live & Active Cultures" seal ensures the presence of 100 million probiotic cultures per gram at the time of production.

Similar to drinkable yogurt, kefir is a fermented milk beverage packed with calcium

Made from just cabbage and salt, this fermented food delivers a healthy dose of fiber.

Figure 3 Showcases the unique features of various fermented products

Miso adds a rich umami flavor and enzymes from fermented soybeans, barley, or rice. Tempeh is a complete vegetarian protein made from fermented soybeans. Yogurt, containing live probiotics, supports gut health, especially with "Live & Active Cultures." Kimchi, a spicy Korean cabbage dish, offers tangy flavors and probiotics. Kombucha, a fizzy fermented tea, is rich in organic acids and probiotics. Kefir is a calcium-rich, probiotic milk drink similar to yogurt but thinner. Sauerkraut, fermented cabbage, delivers fiber and gut-friendly bacteria. Probiotics, bioactive chemicals, and improved nutrient availability are the main reasons why eating fermented foods have so many health advantages. Probiotics are rich, it helps gut microbiome balance, supports digestion, reduces issues like constipation, bloating, gas, etc., kimchi, kefir, yogurt, sauerkraut. It provides a strong immune system (it helps healthy gut support in 70% immune function), fermented foods containing *lactobacillus*, fermentation can increase bioavailability of minerals and vitamins. Antinutrients breakdown, block absorption of zinc, iron and calcium. Gut brain axis links digestive health, probiotics reduce symptoms like stress, anxiety, depression, supporting blood sugar control, improving satiety, modulating fat storage hormones. Fermented foods help lower blood pressure, inflammatory markers, LDL cholesterol, it helps reduce chronic inflammation and oxidative stress (**Siddiqui *et al.,* 2024**).

Seafood

Seafood, particularly fish and shellfish, is highly perishable due to biochemical reactions and the metabolic activity of specific spoilage organisms (**Siddiqui *et al.,* 2024**). Prior to NGS, metagenomic analysis of seafood identified psychrotrophic bacteria like *Pseudomonas* and *Shewanella* as dominant; however, *Psychrobacter* and previously unrecognized bacterial taxa have also been found to contribute to seafood spoilage (Parlapani, 2021). *Pseudomonas* and *Psychrobacter* species produce proteases and lipases that degrade proteins and lipids, resulting in altered flavor and texture, and overall seafood quality (**Abril *et al.*, 2024**). A study found that *Vibrio parahaemolyticus, Staphylococcus aureus, Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. were present in seafood items, with *Staphylococcus aureus* being the most prevalent, underscoring their importance as key pathogens in public health and disease (**Zarei *et al.*, 2012**). Additionally, metagenomics has been used to study the microbial communities associated with aquaculture practices, revealing how farming methods and water quality affect the microbiome of farmed fish and shellfish (**Nogueira & Botelho, 2021**). The application of metagenomics in seafood can also extend to the study of biopreservation techniques, where beneficial microorganisms are introduced to inhibit spoilage and pathogenic bacteria (**Borges *et al.*, 2022**). For example, the use of *LAB* to produce bacteriocins has shown promise in prolonging the shelf-life of seafood products (**Cirat *et al.*, 2024**). Key Insights on microbes dominant in each food category are illustrated in Table 2. Because seafood contains a wealth of vital elements, it provides a variety of nutritional and health advantages, a great source of full protein is seafood, constructing and mending tissues, keeping up muscle mass, and boosting the immune system. Heart health: lower your chance of developing heart disease, Brain function: promote cognitive development and wellness; inflammation reduction: aid in the management of arthritis and other inflammatory diseases; mental health: may lessen anxiety and depressive symptoms. Vitamin A is essential for immunological function, eyesight, and reproduction, vitamin D is essential for healthy bones and a strong immune system, red blood cell production and nerve function are supported by vitamin B12, leaner, lower-calorie options include prawns, scallops, cod and tilapia, omega-3-rich foods include salmon, sardines, mackerel, herring, and trout.

Table 2 Summary of Metagenomic Findings in Various Food Categories

Food Category	Dominant Microbes	Key Insights	References
Dairy Products	*Lactobacillus, Streptococcus* species	Probiotics in yogurt and kefir improve gut health; Fermentation extends shelf life.	(Coelho et al., 2022; You et al., 2022).
Meat and Poultry	*Campylobacter jejuni, S. aureus, E. coli*, *Salmonella*, *L. monocytogenes*	Effective cooking and storage practices are vital to prevent harmful bacterial contamination.	(Nørrung et al., 2009)
Fruits and Vegetables	*Pseudomonas*, *Sphingomonas*, *Aeromicrobium*, *Pantoea*	Spoilage is common; fermentation can extend shelf life and enhance nutritional value.	(Liu et al., 2023)
Fermented Foods	*Streptococcus thermophilus* and *Lactococcus lactis*	Fermentation boosts beneficial microbes, adds probiotics, and enhances flavors.	(Pasolli et al., 2020)
Seafood	*Pseudomonas, Shewanella, Psychrobacter*	Spoils rapidly; need proper refrigeration; risk of foodborne pathogens if undercooked.	(Parlapani, 2021)
Grains	Bacteria and fungi	Wheat flour, wheat corn and silage stored wheat	(Nørrung et al., 2009)
Meat	Bacteria and fungi	White pudding, sausages, beef carcasses and Products, lamp	(Liu et al., 2023)
Refrigerated foods and vegetables	Bacteria, fungi, virus, plants, virulence genes	Spoilage, pathogen detection, tracking Contamination	(Pasolli et al., 2020)

Impact of Storage-Induced Microbial Shifts

The storage of food products significantly influences their quality, nutritional value, safety, and shelf life, primarily due to microbial shifts that occur over time. Understanding these shifts is essential for optimizing food preservation methods and ensuring consumer safety.

Food Quality and Sensory Attributes

Microbial dynamics shift due to temperature fluctuations during storage conditions, particularly in fermented foods like cheese, directly impact sensory attributes like aroma, texture, and flavor. For example, during cheese ripening, the initial dominance of *Streptococcus* and *Lactococcus* shifts to *Leuconostoc* and *Lactobacillus* in the core and *Weissella* in the rind, facilitating proteolysis and flavor development(**Sant'Anna et al., 2019**). Additionally, moisture content significantly influences microbial shifts in cheese, with dry environments favoring non-starter lactic acid bacteria (NSLAB) such as *Leuconostocaceae* and *Lactobacillaceae*, which thrive in low-moisture conditions (**Sant'Anna *et al.*, 2019; Camargo *et al.*, 2021**). As moisture gradually decreases and pH changes due to lactic acid production, these shifts further modulate the microbial landscape, enhancing the sensory complexity of products stored over extended periods.

Nutritional Value

The microbial shifts during storage significantly influence the nutritional value of fermented foods. As microbes like *Streptococcaceae* break down proteins during ripening, releasing essential amino acids that enhance the nutritional profile, the presence of non-starter lactic acid bacteria thriving in low-moisture conditions further influences the bioavailability of vitamins and minerals potentially boosting the product's overall nutritional value. Secondary fermentation activities by specific bacterial communities can enhance the concentrations of specific micronutrients, such as B vitamins, in stored products (**Maleke *et al.*, 2021**). However, microbial contamination with spoilage bacteria can degrade the nutritional quality by accelerating decomposition, leading to nutrient loss. As such, maintaining appropriate microbial balance is key to preserving or even enhancing the nutritional value of food during storage.

Food Safety and Spoilage

Food safety is among the most important priorities associated with microbial shifts during storage. In some cases, improper storage conditions favor the growth of opportunistic microbe and spoilage bacteria. For example, in the cheese samples from the "Entre Serra" study, spoilage organisms such as *Serratia marcescens*, *Citrobacter freundii*, and *Klebsiella species* were identified (**Camargo *et al.*, 2021**). The presence of Enterobacteriaceae, a family known to cause early blowing in cheese through gas production, highlights the risk of spoilage associated with poor hygiene practices and environmental conditions during storage (**Pasquali *et al.*, 2022**). Infectious microbes, such as *Salmonella spp., Listeria monocytogenes*, and *Vibrio parahaemolyticus*, can proliferate under improper storage conditions, posing significant health risks to consumers (**Chintagari *et al.*, 2017**). The emergence of spoilage organisms can also lead to foodborne illnesses, as certain bacteria, such as *Clostridium botulinum*, produce harmful toxins, with *C. botulinum* being one of the most potent toxins known (**Lorenzo *et al.*, 2018**). Regular monitoring of microbial populations during storage is essential for identifying potential hazards and ensuring food safety.

Shelf-Life Prediction

Accurate shelf-life prediction is essential for food waste reduction and enhancing user contentment, as microbial shifts significantly influence food product longevity. Predictive microbiology offers a scientific approach to estimating the proliferation of infectious microbes and determining the shelf life of food products by incorporating factors like pH, temperature, and water activity, enabling predictive models to anticipate the proliferation of these organisms and set guidelines for optimal storage conditions that allow manufacturers to extend product shelf life (**Tarlak, 2023**). Furthermore, advanced techniques such as metagenomic analysis and real-time PCR can monitor microbial populations and their activities, providing important perspectives on the shelf life of different food products and also for the public health.

Emerging Trends and Future Perspectives

Recent advancements in metagenomics have opened new pathways to understanding food microbiota dynamics during storage. These innovations, including integration with other -omics technologies, real-time microbial monitoring, predictive modeling, and tailored storage solutions, represent significant shifts in food microbiology research. Together, they provide a comprehensive approach to managing food spoilage and enhancing food safety.

Integration with Other-Omics Approaches

One of the most promising developments in microbiota research is the integration of metagenomics with other omics technologies like transcriptomics, metabolomics, and proteomics. Meta-transcriptomics provides an understanding of microbial gene pathways through quantification of transcript abundance to reflect bacterial gene expression, while meta-proteomics complements metagenomics and meta-transcriptomics by measuring total protein levels produced by both the host and microbiota, thereby revealing the roles that microorganisms play in supporting host health; concurrently, metabolomics enhances the understanding of metabolic processes by measuring the concentrations of small molecules (**Filardo et al., 2024**).

Real-Time Monitoring of Microbial Dynamics

Traditionally, microbial changes in food during storage were assessed through end-point analyses, but recent advancements allow for real-time monitoring using high-throughput sequencing technologies (HTS). High-throughput sequencing (HTS) generates millions of reads and billions of base pairs per sample, paving the way for future applications in real-time microbial monitoring, with direct shotgun metagenomics analysing DNA from clinical urine samples without enrichment culturing currently showing promise in achieving bacterial identifiability comparable to traditional methods (**Sekse et al., 2017**). By tracking these changes in real-time, it becomes possible to identify critical points where microbial spoilage begins, leading to timely interventions. This technology enhances food safety protocols by providing precise data on microbial succession and its impact on food quality.

Predictive Modelling of Microbial Shifts

With the advent of machine learning and artificial intelligence, predictive modelling has become an essential tool for forecasting microbial shifts in food systems by utilizing vast datasets generated through metagenomics and other -omics approaches to predict how microbial communities respond to different storage conditions (**Taiwo et al., 2024**). Recent predictive modelling studies demonstrate the efficacy of algorithms of machine learning, like gradient boosting and random forest, in forecasting microbial growth and assessing food safety risks, as shown in various applications from yogurt spoilage to Salmonella risk assessment in poultry farms. This way of modelling approach elucidates the link between food characteristics and bacterial growth, enabling the prediction of bacterial behavior within food matrices and enhancing safety, while also assessing risks related to food spoilage to ensure quality control in storage management. The most widely used predictive models and tolls used in the food industry are presented in Fig. 4. Kinetic Model: Tracks microbial growth or death rates in relation to concentration, aiding in the prediction of chemical spoilage. Probabilistic Model: Evaluates the chances of microorganisms producing toxins or undergoing sporulation (**Stavropoulou & Bezirtzoglou, 2019b**). Empirical Model: Describes the relationship between variables through a polynomial equation, linking inputs to outputs. Mechanistic Model: Predicts outcomes under modified conditions by analyzing and determining key parameters (**Stavropoulou & Bezirtzoglou, 2019b**).

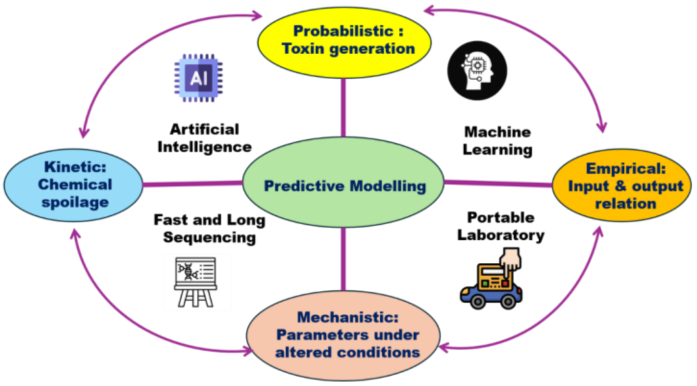


Figure 4 Common mathematical models used in the food industry

Potential for Tailored Storage Solutions

The potential for tailored storage solutions lies in understanding microbial dynamics, as different food products harbor unique microbial communities that shift during storage, enabling the design of specialized environments that selectively inhibit spoilage organisms while promoting beneficial microbes. For instance, modified atmosphere packaging (MAP) creates an optimal environment by reducing oxygen and elevating carbon dioxide levels, extending freshness and shelf life while inhibiting ethylene action, enzymatic browning, and respiration activity, thereby emphasizing its significance in tailored storage solutions (**Vakkalanka *et al.*, 2012**). To minimize food degradation during storage, kinetic models must describe the relationship between intrinsic and extrinsic influences, like moisture and temperature, enabling predictions and optimizations to enhance shelf life (**Sousa Gallagher *et al.*, 2011**).

CONCLUSION

Metagenomics has revolutionized our understanding of the microbial ecology of food systems and has provided unique insights into food quality, safety, and production. More importantly, the integration of metagenomics with other omics techniques, such as transcriptomics and metabolomics, has better described the microbial activities and their effects on foods. Future studies should, therefore, be directed at the development of real-time monitoring systems of microbial shifts in foods during storage and processing. This will lead to more targeted interventions and specific storage options for enhancing the safety and quality of foods. Besides that, predictive modeling using machine learning and AI is a promising avenue of research for forecasting the behavior of microorganisms in complex food matrices. First, standardization of metagenomic protocols and bioinformatic processing is required for comparability among different studies. Second, in order to fully develop the concept of food microbiomes, further extension of the metagenomic investigation will be required for a broader spectrum of food types and various processing conditions. Lastly, the translation of metagenomics into industrial applications, namely enhancement of the fermentation process, biopreservation, and strain-specific probiotics, is a thrilling frontier of future research and development.

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