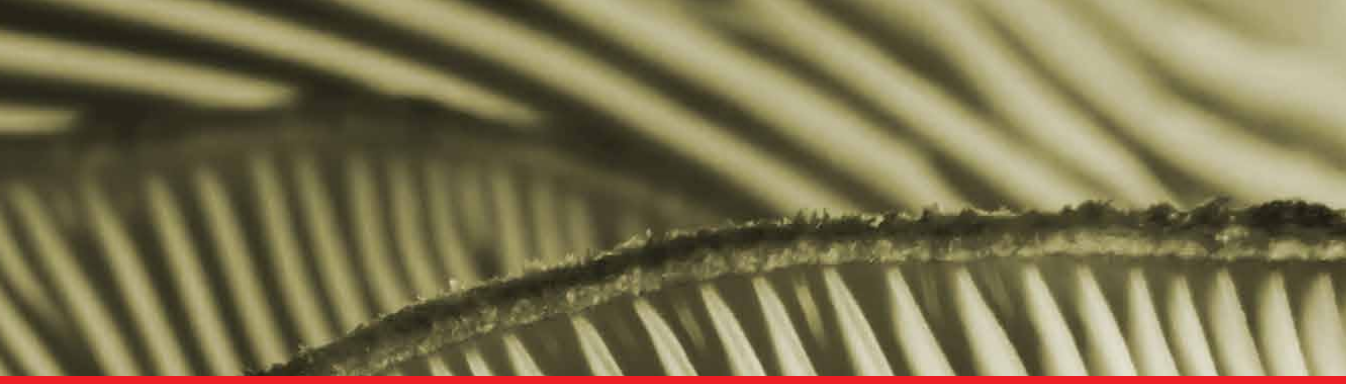

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Edited by Ruogu Tang

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Food Science and Nutrition

Volume 18

Aims and Scope of the Series

The significance of food is undeniable, especially in light of the impending challenge facing humanity: ensuring there will be enough food to meet the basic needs of a population expected to reach approximately 10 billion by 2050. These food-related challenges align with some of the United Nations' sustainable development goals, with a target to achieve them by 2030. One thing is certain: food should be not only nourishing and safe but also tailored to the diverse needs of individuals throughout their lifetimes, all while meeting consumers' sensory expectations. Understanding the diverse chemical composition of food, often referred to as biodiversity, and how these components can contribute to human health by considering factors like bioaccessibility, bioavailability, and bioactivity at the organ level, is crucial for grasping and promoting a healthy diet. Thanks to the continuous evolution of analytical methods and interdisciplinary research, significant strides have been made in the field of food science and nutrition.

Meet the Series Editor

Maria Rosário Bronze has been working in Analytical Chemistry since 1986. Her Ph.D. in 1999 contributed to the study of food products using capillary electrophoresis. The main goal of her research since 1999 has been focused on Analytical Chemistry applied mainly to the analysis of foods and by-products of food industry. She conducted research in collaboration with national and international research groups, at iBET and ITQB Technology Division. From 2017 until 2021 she was head of Food & Health Division at iBET and head of the Food Functionality and Bioactives Laboratory. MR Bronze has been an Associate Professor at the Pharmacy Faculty of Lisbon University and head of the Structural Analysis Laboratory since 2012. As a researcher, MR Bronze is a Senior Scientific Advisor at Food & Health Division at iBET and Head of Food Functionality and Bioactives Laboratory at the same Institute, Collaborator at iMED and Researcher at ITQB NOVA. Her current research is focused on quality and beneficial health effects of food components. Gas and liquid chromatography associated with mass spectrometry are used by MR Bronze in the characterization of samples. Sensory evaluation is also an important area of her research. The main food products studied by her are olive tree products (olive, olive oil, leaves), cereals such as maize, legumes (faba bean, pea, chickpea, lentils) fruits (apple, grapes, opuntia ficus), fruit juices and wine, among others. More recently her interests have also involved biodiversity, bioaccessibility, and bioavailability studies on food products and their components, mainly phytochemicals as phenolic compounds, using different analytical tools such as mass spectrometry. As a senior scientific advisor at Food & Health Division at iBET she is involved in different areas: (i) isolation, characterization and formulation of bioactive and functional compounds or extracts from natural sources and wastes from food and other related industries; (ii) pre-clinical assays to provide support to understand health claims related with the beneficial effects of food nutrients/bioactive components; (iii) establishment of analytical methodologies including mass spectrometry state-of-the-art to fully characterize different matrices, from food products, natural extracts or biological fluids (Food Functionality and Bioactives Laboratory).

Meet the Volume Editor

Ruogu Tang is a postdoctoral research scientist with an interdisciplinary background spanning polymer materials, biomedical engineering, antimicrobial and antifouling technologies, carbon materials, and food science. His research focuses on developing functional and sustainable materials that bridge the fields of biology, chemistry, and engineering for environmental and food applications. He has published over 20 peer-reviewed journal articles and contributed to numerous collaborative projects that integrate polymer design, microbial systems, and analytical modeling. Dr. Tang also serves in cross-disciplinary research centers dedicated to material characterization and food quality assessment. As the Academic Editor of *Fermentation in the Food Industry*, he is dedicated to promoting innovation that bridges the gap between materials science and biotechnology, advancing food sustainability and global health.

Contents

Preface

Fermentation represents one of the oldest biotechnological processes known to humankind and remains a cornerstone of the global food industry today. From traditional preservation methods to cutting-edge biotechnological innovations, fermentation continues to shape the quality, safety, and nutritional value of foods. In recent years, the convergence of microbiology, molecular biology, and process engineering has redefined the boundaries of food fermentation, introducing opportunities for improved product functionality, safety, and sustainability.

The edited volume, *Fermentation in the Food Industry*, provides an integrative overview of contemporary advances in food fermentation, covering microbial, biochemical, technological, and nutritional perspectives. The chapters collectively illustrate how both traditional and modern approaches contribute to the development of sustainable and health-promoting foods. This volume serves as a reference for researchers, professionals, and students interested in understanding the scientific foundations and industrial applications of fermentation.

Overview of the Chapters

Chapter 1 – *Microbial Fermentation: From Food Tradition to Cutting-Edge Biotechnology.* This chapter serves as a conceptual bridge between historical knowledge and modern biotechnology. The authors review the evolution of fermentation practices, from spontaneous microbial activity to precision-controlled bioprocesses, and outline the expanding role of fermentation in the innovation of functional foods.

Chapter 2 – *Mechanisms of Preservation by Lactic Acid Bacteria in Food Fermentation*. This chapter provides a detailed account of how lactic acid bacteria contribute to microbial safety and extended shelf life in fermented foods. The chapter elucidates the metabolic mechanisms, including organic acid production and bacteriocin synthesis, that underlie their preservative action.

Chapter 3 – *Toward the Establishment of Yeast Alcoholic Fermentation Design Technology*. This chapter concludes the volume by exploring the engineering and computational aspects of yeast-driven alcoholic fermentation. This chapter envisions the design of precision fermentation systems that integrate systems biology and bioprocess optimization for industrial scalability.

Chapter 4 – *New Advances in the Role of Yeasts in Table Olive Fermentation*. This chapter focuses on the multifaceted contribution of yeasts to flavor development, texture, and microbiological stability in table olive production. The chapter demonstrates how yeast diversity and metabolic interactions with lactic acid bacteria determine the final product quality.

Chapter 5 – *Pulque: More than an Alcoholic Beverage – History, Native Microbiota, Fermentation Process and Its Potential Health Benefits*. This chapter presents a comprehensive case study of pulque, a traditional Mexican fermented beverage. Beyond its cultural and historical significance, the chapter emphasizes the complexity of its native microbial consortia and its potential as a source of functional compounds with probiotic and antioxidant properties.

Chapter 6 – *Fermented Rice Beverages in the Food Industry: Advances in Processing, Nutrition, and Commercialization*. This chapter opens the volume with an in-depth discussion of cereal-based fermentation systems. The authors describe how rice-based beverages serve as a bridge between traditional artisanal products and industrial-scale innovations, highlighting advancements in microbial starter selection, nutritional enhancement, and consumer-driven commercialization.

Chapter 7 – *Fruit Vinegar Production*. This chapter explores the biochemistry and industrial relevance of acetic acid fermentation. This chapter highlights the role of acetic acid bacteria in converting diverse fruit substrates into high-value vinegars and discusses recent technological optimizations in fermentation control, product standardization, and sensory quality.

Chapter 8 – *Reduction of Acrylamide in Plant-Based Foods through Traditional and Innovative Fermentation Techniques.* This chapter examines an emerging topic in food safety. The authors describe how targeted fermentation processes can mitigate acrylamide formation in thermally processed foods, offering a promising strategy for reducing dietary exposure to this compound.

Editorial Perspective

Collectively, these eight chapters offer a comprehensive overview of fermentation as both a traditional craft and a frontier in modern food biotechnology. The book integrates the perspectives of microbiologists, food technologists, and bioprocess engineers, emphasizing cross-disciplinary approaches to improve food quality, safety, and sustainability. The diversity of topics—from rice beverages and fruit vinegars to functional compounds and biodesign—underscores the universality of fermentation as a transformative process in the food industry.

This volume is intended to inspire further research into microbial ecology, fermentation technology, and product development, while fostering collaboration between academic and industrial sectors. It highlights not only the technological advancements but also the cultural and nutritional significance of fermented foods across different regions and traditions.

The Academic Editor expresses sincere gratitude to all contributing authors for their dedication, scholarly rigor, and collaboration throughout the preparation of this volume. Special appreciation is extended to the reviewers and the IntechOpen

editorial team for their meticulous guidance and professional support. The editor also acknowledges the colleagues and assistants who contributed to manuscript coordination, figure preparation, and correspondence management during the editing process.

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Section 1

Fundamentals and Modernization of Food Fermentation

Chapter 1

Microbial Fermentation: From Food Tradition to Cutting-Edge Biotechnology

Raquel Gómez-Pliego, Judith Espinosa-Raya, Hulme Ríos-Guerra and Jair Alejandro Temis-Cortina

Abstract

Microbial fermentation is an anaerobic biotransformation process performed by bacteria, yeasts, and fungi, which convert sugars and other organic compounds into valuable products, including organic acids, alcohols, and gases. This process involves converting carbon-rich substrates into energy, leading to the production of secondary metabolites through pathways such as glycolysis, lactic acid fermentation, and alcoholic fermentation. The efficiency of fermentation depends on environmental factors like pH, temperature, and nutrient levels. Traditionally, microbial fermentation has been used to preserve food, produce alcoholic beverages, improve sensory qualities, and boost the nutritional value of products. Recently, it has also become an important method for clean-label food production. By replacing artificial preservatives with naturally occurring microbial metabolites, fermentation allows for the creation of healthier, more natural foods with simpler ingredient lists, meeting consumer demand for transparency and minimal processing. Lactic acid bacteria (LAB), commonly used in food fermentation, are also known for their role in environmental bioremediation. These microorganisms can break down pollutants in water, soil, and air, transforming harmful substances into non-toxic compounds through biochemical processes. Their adaptability and metabolic diversity make them ideal for eco-friendly cleanup. Additionally, fermentative microorganisms foster innovation in the food industry. They support the development of alternative proteins, functional foods enriched with probiotics, and precision-fermented products like plant-based cheeses and cultured meats. In modern industry, microbial fermentation plays a crucial role in producing antibiotics, vitamins, enzymes, hormones, biofuels, and bioplastics. It is a sustainable, low-emission, and biodegradable technology that promotes environmental conservation, advances.

Keywords: spontaneous and directed fermentation, microbial fermentation, fermentative metabolisms, lactic acid or alcoholic fermentation, biotechnology fermentation, lactic acid bacteria

1. Introduction

1.1 Definition of microbial fermentation

Microbial fermentation is an anaerobic biotransformation process that occurs in the absence of oxygen, where enzymes from bacteria, yeasts, and fungi convert sugars and other organic compounds into valuable products such as organic acids, alcohols, aromatic compounds, and gases [1]. Unlike fermentation, aerobic respiration is a catabolic process where cells completely oxidize glucose in the presence of oxygen (O_2), producing adenosine triphosphate (ATP), carbon dioxide (CO_2), and water (H_2O). This process involves glycolysis, the citric acid cycle (also known as the Krebs cycle), and oxidative phosphorylation through the electron transport chain, where oxygen serves as the ultimate electron acceptor. Although often confused by non-specialists, fermentation and aerobic respiration are fundamentally different in mechanisms, metabolic pathways, energy yield, and oxygen requirements.

Fermentation involves glycolysis followed by specific anaerobic pathways (e.g., lactic acid or alcoholic fermentation), yielding only 2 ATP per glucose molecule through substrate-level phosphorylation. In contrast, aerobic respiration can produce approximately 36–38 ATP molecules per glucose molecule, making it much more energy-efficient in oxygen-rich environments. This chapter focuses solely on microbial fermentation, especially processes involving strict anaerobes, facultative anaerobes, and microaerophiles. Aerobic respiration and strict aerobic pathways are distinct from fermentation.

1.2 Historical significance and current applications

Humans have utilized fermentation since ancient times, serving as both a method for food preservation and an enhancement of its taste and nutritional value [2]. Ancient societies employed fermentation techniques to produce staples like bread, wine, and beer, illustrating some of the earliest examples of applied fermentation biotechnology.

In the mid-nineteenth century, Louis Pasteur presented scientific evidence that supported the microbial basis of fermentation. He demonstrated that specific bacteria and yeasts generate characteristic metabolic products. Over time, fermentation has progressed from a traditional practice based on observation to a sophisticated biotechnological approach, now being utilized for the development of functional foods and sustainable innovations. Given its increasing application across biotechnological fields, it is essential to understand the significance of fermentation in nutrition, health, and sustainable production. **Figure 1** highlights significant historical milestones, tracing the evolution from ancient methods to contemporary advances such as metabolic engineering and precision fermentation [3].

An important distinction within fermentation processes lies in the choice between spontaneous and controlled approaches. Spontaneous fermentations rely on indigenous microbial communities and natural environmental factors. In contrast, controlled fermentations involve the intentional use of selected microbial strains under monitored conditions, such as specific temperatures, pH levels, and oxygen levels. This controlled approach offers greater consistency, safety, and productivity in the generation of fermented goods and value-added bioproducts.

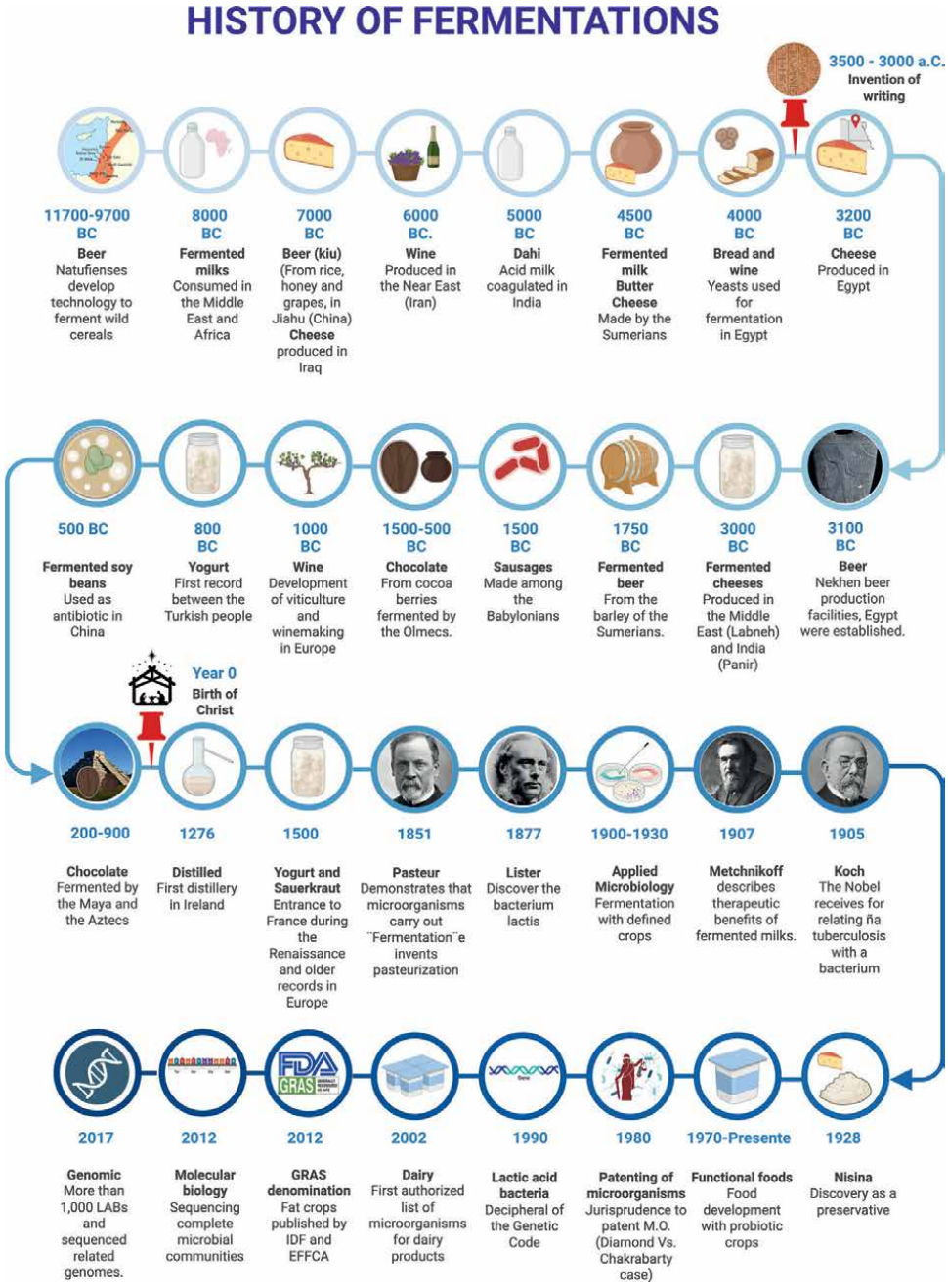

Figure 1.
Timeline traces the evolution of fermentation throughout history.

1.3 Spontaneous and directed fermentation

Fermented products are obtained through the action of microorganisms and their enzymes, which transform food components while providing preservation advantages

and potential health-promoting effects. This transformation can occur via spontaneous fermentation, where native microbes present in the raw materials drive the process, or through guided fermentation, which employs chosen starter cultures to achieve targeted characteristics in the final product [4, 5].

1.3.1 Spontaneous fermentation

Spontaneous fermentation occurs naturally without intentionally adding specific microbial strains. It starts with native microorganisms from the raw materials, equipment, or environment. This method is commonly used in preparing many foods and drinks across different cultures.

Notable examples include the following:

- Kimchi (Korea)
- Sauerkraut (Central Europe)
- Sourdough bread (global)
- Pulque, pozol, and tepache (Mexico)
- Cacao and coffee (Africa and Latin America)
- Tempeh (Indonesia)
- Injera (Ethiopia)
- Olives (Mediterranean)
- Fish sauces (Southeast Asia)
- Cassava-based foods like gari or fufu (Africa)

Microbial dynamics in these fermentations can be understood through community assembly, which is driven by two main ecological processes. Dispersal involves the introduction and spread of microbes from ingredients, tools, or the environment; and selection occurs when environmental factors such as temperature, pH, salinity, or substrate composition influence microbial survival and dominance. Advanced tools like metagenomics, amplicon sequencing, comparative genomics, and metatranscriptomics are used to study microbial behavior, interactions, and gene expression during spontaneous fermentation.

1.3.2 Directed fermentation

Controlled or directed fermentation relies on the intentional introduction of starter cultures to steer and improve the fermentation process, leading to products with greater uniformity and superior quality. This approach is widely used in the manufacturing of yogurt, beer, wine, and various fermented foods [3]. Advances in biotechnology have enabled the development of inoculated fermentation systems, where careful strain selection and optimized bioreactor conditions play a

fundamental role [4, 5]. Additionally, in some processes, the formulation of the growth medium is strategically designed to boost enzyme activity and optimize metabolite synthesis [6].

Modern fermentation heavily depends on starter culture collections, which are critical for ensuring process reproducibility, product safety, and efficiency. These collections safeguard thoroughly characterized microbial strains (primarily lactic acid bacteria, yeasts, and molds) chosen for their specific functions, such as acid production, flavor enhancement, or antimicrobial properties [7, 8].

Notable examples of these culture banks include the following:

- *ATCC* (USA)
- *DSMZ* (Germany)
- *NCIMB* (UK)
- *BCCM/LMG* (Belgium)
- *INRAE-CIRM* (France—food-grade lactic acid bacteria)

Furthermore, genetically modified microorganisms (GMMs) are engineered to enhance metabolite production, stress tolerance, and yield. Their applications include the biosynthesis of vitamins (e.g., B_{12}), enzymes (e.g., amylases, proteases), amino acids (e.g., lysine), and flavor compounds, enabled by advances in synthetic biology and metabolic engineering [9, 10]. The use of GMMs is regulated by strict frameworks that differ by region and application, particularly in food products.

2. Lactic acid bacteria and food safety: Understanding GRAS and QPS classifications

Lactic fermentation is one of the oldest biotechnological methods used in food production worldwide. Originally developed as a preservation technique, this process is powered by LAB microorganisms that convert simple carbohydrates, such as glucose or lactose, into lactic acid, as well as secondary metabolites like acetic acid, ethanol, and carbon dioxide (CO_2). This acidification prevents spoilage organisms, extends shelf life, and improves flavor, texture, nutritional value, and product stability [11].

LAB employed in food production are typically classified as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration (FDA), and many also hold Qualified Presumption of Safety (QPS) status from the European Food Safety Authority (EFSA).

The GRAS classification, established by the FDA, applies to substances (including microorganisms) that have either a well-documented history of safe consumption or are backed by robust scientific validation from expert panels. Companies can voluntarily notify the FDA about GRAS status, although self-affirmation is permitted under U.S. regulations [12].

In contrast, the QPS system is a safety assessment model used by EFSA within the European Union, which evaluates microbial safety based on four main criteria: (1) precise taxonomic classification, (2) a documented history of safe use, (3) absence of transferable antibiotic resistance, and (4) no known toxigenic properties [13].

These regulatory pathways simplify the approval and application of LAB in food fermentations by reducing the need for strain-specific evaluations when used appropriately. Consequently, LAB are broadly utilized in the fermentation of dairy products, vegetables, meats, and cereals, promoting both safety and quality. Besides their regulatory importance, LAB strains with GRAS or QPS recognition play a pivotal role in contemporary food biotechnology, aiding in microbial balance, generating bioactive substances, and improving sensory characteristics. This versatility extends to both conventional fermented foods and probiotic-enriched products, making an understanding of GRAS and QPS frameworks essential for safe innovation in food microbiology.

It is important to note, however, that not all bacteria intended for probiotic applications are inherently safe. Certain Clostridium species, such as *Clostridium botulinum*, *Clostridium perfringens*, and *Clostridioides difficile*, are associated with toxin production and health hazards. Nevertheless, non-pathogenic strains like *Clostridium butyricum* have been safely utilized as probiotics, particularly in Asian countries, under rigorous regulatory control [14]. These strains are often subjected to detailed genomic assessments to confirm the absence of virulence factors, and in some instances, inactivated spores or postbiotic forms are used to ensure safety while maintaining functional benefits [15, 16].

These examples underscore the need for strain-level safety evaluation, even within genera containing GRAS/QPS species, and highlight the importance of rigorous microbiological and toxicological assessments for emerging probiotic candidates.

2.1 Changes that occur during food fermentation

LAB constitute a diverse group of microorganisms characterized as being either strictly or facultatively anaerobic, catalase-negative, non-spore-forming, and with a primarily fermentative metabolism. The most representative genera include *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus*, and *Enterococcus*. The type of fermentation they perform depends on the specific enzymatic machinery present in each genus and species. From a metabolic perspective, LAB fermentations are classified as homofermentative or heterofermentative [17].

Figure 2(A) illustrates the metabolic pathway involved in homolactic fermentations, where lactic acid is produced at approximately 85–90% yield. The process is typical of bacteria such as *Lactococcus lactis* or some homofermentative Lactobacillus species. This high yield makes homofermentative LAB ideal for producing fermented foods with a tangy flavor and a simpler microbial profile [18].

In heterolactic fermentation, lactic acid is produced to a lesser extent, representing approximately 50% of the final products generated from glucose. In heterolactic fermentation, starting from a single molecule of glucose, heterofermentative LAB typically produce: one molecule of lactic acid, one molecule of ethanol or acetic acid, and one molecule of carbon dioxide (CO_2). This pathway is known as the pentose phosphate pathway (phosphoketolase pathway), an example of which is found in heterolactic bacteria, *Leuconostoc* spp., and *Lactobacillus fermentum*. Therefore, compared to homofermentative LAB, heterolactic LAB generates less lactic acid and a greater variety of compounds, which influence the aroma, flavor, and texture of fermented products (see **Figure 2(B)**) [19].

LAB fermentations are a natural acidification process that not only improves the shelf life of foods by reducing their pH and producing antimicrobial compounds such as bacteriocins, but also inhibits the growth of pathogenic microorganisms

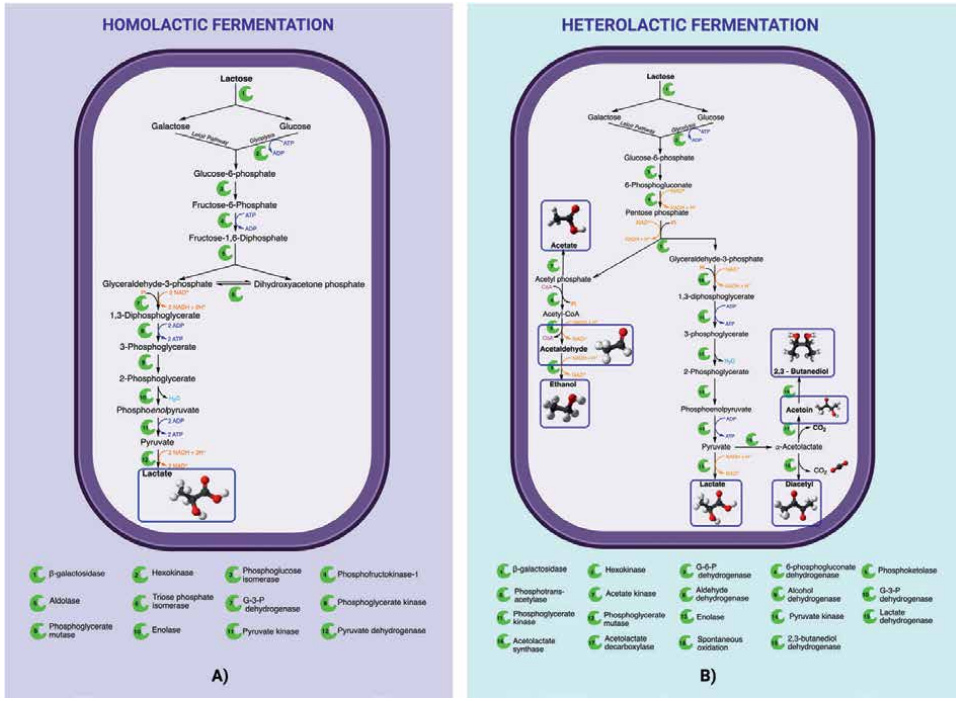

Figure 2.
Comparison of microbial fermentation pathways. (A) Homolactic fermentation pathway, where glucose or lactose is converted primarily into lactate as the main end-product through the Embden-Meyerhof-Parnas pathway [18]. (B) Heterolactic fermentation pathway, where glucose or lactose is metabolized into multiple end-products, including lactate, ethanol, acetate, CO_2, diacetyl, and other metabolites via the phosphoketolase pathway [19].

and generates compounds that provide aroma, flavor, and contribute to the balance of these, improving the sensory profile of foods, due to the production of: diacetyl (smell and taste of butter and milk), acetaldehyde (fruity flavor), ethyl acetate (fruity, floral, rum and green herbs aromas, seasonings and resinous), acetic acid, lactic acid, 2,3-butanediol (slightly sweet aroma, mild flavor, and reduces the acidity of the product), it should be noted that the predominant flavor and aroma will depend on the food matrix. Bioactive compounds are also produced, such as peptides, free amino acids, reduced phytic acid (which increases the bioavailability of minerals), γ-aminobutyric acid (GABA), with antihypertensive and anti-inflammatory effects, B vitamins (B1, B2, B9, B12), and exopolysaccharides (dextrans) or hydrocolloids that modify the viscosity and texture of foods [20].

The success of any fermentation process depends on multiple variables and factors (**Figure 3**), including the type of microorganism used, the control of physicochemical conditions (such as incubation temperature, fermentation time, substrate concentration, and salt and sugar content), as well as the characteristics of the food matrix, water availability, the presence of micronutrients, pH, the use of standardized starter cultures or native strains, and the fermentation system employed (batch, continuous, linear, or exponential, among others).

The current trend is toward the selection of native strains with specific characteristics, the design of synergistic consortia, and the development of functional fermented foods targeted at populations with specific needs (children, older adults, and people with metabolic diseases).

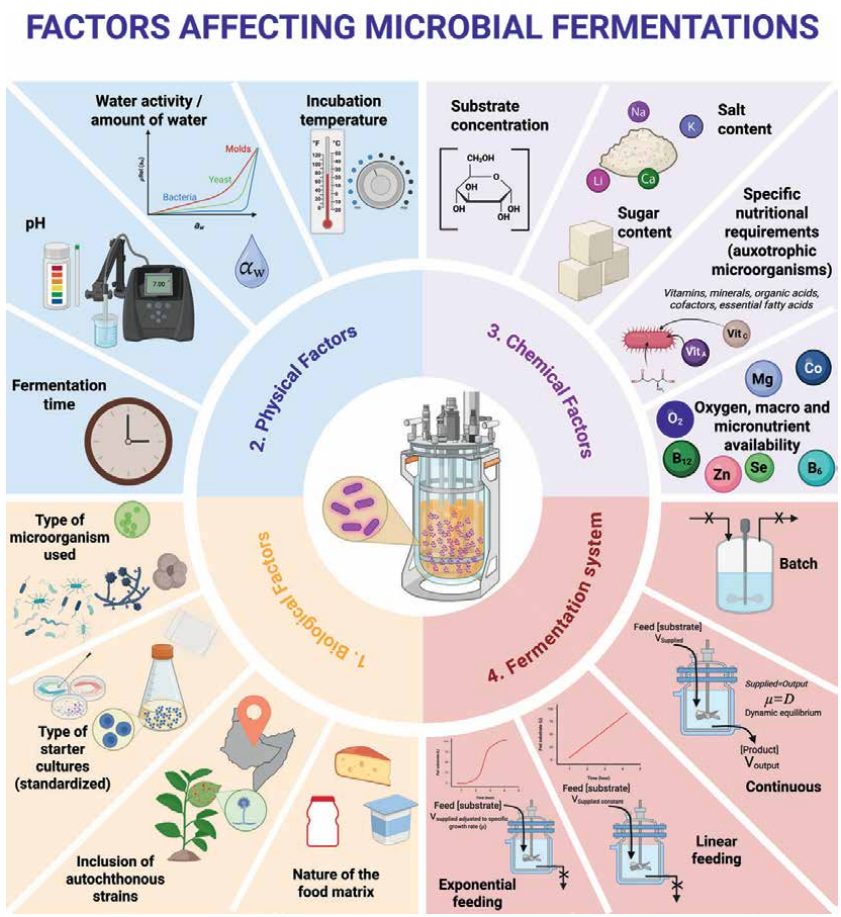

Figure 3.
Factors affecting microbial growth in a fermentation process.

These fermentations are essential in a wide variety of foods around the world, from dairy products such as yogurt and cheese to fermented vegetables, cured meats, cereals, alcoholic beverages, and traditional blends, as well as meats and vegetables. **Figure 4** illustrates a general representation of some of the most used genera in lactic acid fermentations, including the substrates utilized, metabolic degradation pathways, the types of foods in which they are employed, and the benefits of lactic acid fermentations.

Lactic acid fermentation is used in a wide variety of foods. They are employed in the production of fermented dairy products, such as yogurt, kefir, creams, and certain cheeses; in fermented meats, such as serrano ham, sausages, and cured meats; in fermented vegetables, such as sauerkraut, kimchi, and pickles; and alcoholic beverages, such as wine, or in traditional alcoholic beverages, such as pulque or some sour beers. This diversity is due to the great ecological adaptability of LAB, which can develop in various types of food matrices under both controlled and spontaneous conditions [21].

In addition to its technological impact, lactic acid fermentation has become increasingly relevant due to its functional and nutraceutical potential. Various LAB

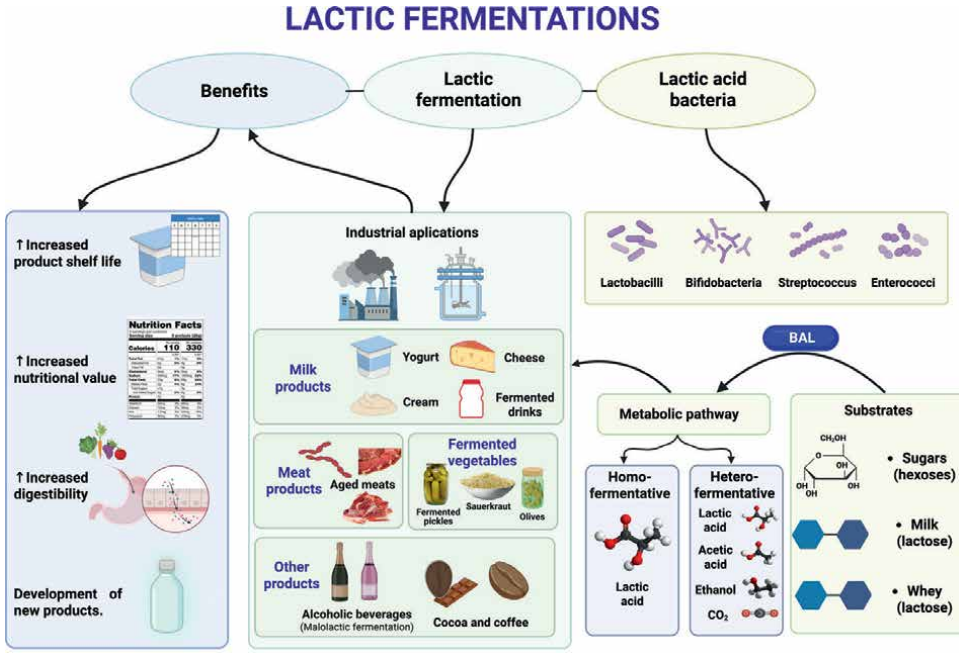

Figure 4.
General summary of lactic fermentation: microorganisms, substrates, routes, and benefits.

strains have shown beneficial effects on intestinal health, primarily by enhancing the gastrointestinal microbiota, strengthening the immune system, and increasing nutrient bioavailability. In this context, lactic acid fermentation functions not only as a method for preserving food but also as a means of developing new functional fermented foods, enhancing taste and aroma, and promoting the health of humans and other living beings [22].

2.2 Lactic fermentation in dairy products

2.2.1 Yogurt and fermented milks (kefir)

Yogurt is one of the most iconic fermented products, produced through the synergistic action of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. During the fermentation process, these microorganisms acidify the milk, coagulate the proteins, and release bioactive peptides, generating compounds responsible for their characteristic flavor.

In addition to yogurt, there are many fermented milks such as kefir, kumis, and jocoque. Kefir, for example, contains a mixed microbiota of lactic acid bacteria, yeasts, and occasionally acetic acid bacteria, which gives it remarkable sensory and functional complexity. Common probiotic strains found in kefir include *Lactobacillus kefiri*, *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus*, along with yeasts such as *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*. These microorganisms not only enhance gut health but also contribute to kefir's distinctive tangy flavor and light effervescence, the latter being a result of carbon dioxide production by yeasts during fermentation [23].

Cheeses.

For this product, LAB is essential for the initial acidification and the ripening process. In fresh cheeses, such as panela or cottage cheese, the primary role of LAB is acidification, with minimal maturation. In contrast, ripened cheeses undergo either surface ripening, as in Camembert, where microbial action (including *Lactococcus lactis* and yeasts) occurs predominantly on the rind, or internal ripening, as in Manchego, where LAB such as *Lactococcus lactis*, *Lactobacillus helveticus*, and *Leuconostoc mesenteroides* are distributed throughout the matrix. These LAB species contribute to proteolysis, lipolysis, and aroma development during aging. The metabolic diversity of LAB (recognized under GRAS/QPS classifications) ensures safe fermentation while also enhancing the sensory complexity and preservation of cheese through natural acidification and the production of bioactive metabolites.

2.3 Lactic acid fermentation in meat products

In fermented meat products such as salami, Spanish chorizo, pepperoni, or Serrano ham, LAB are used as starter cultures, in combination with *Staphylococcus* species, to ensure controlled and safe fermentation. These bacteria perform multiple technological functions: they reduce pH by producing lactic acid, stabilize meat color by reducing nitrates and nitrites, inhibit relevant pathogens such as *Listeria monocytogenes*, and contribute to the development of characteristic aroma compounds in the final product.

Lactic acid fermentation in these foods demands strict control of temperature and humidity and is crucial for the product's sensory quality and safety. The most used species include *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Pediococcus acidilactici*, which are well-known for their technological effectiveness and safety in food. During the preparation of fermented meat products, glucose or dextrose is often added to serve as a fermentable energy source for LAB. When these sugars are metabolized by LAB, they produce lactic acid, which lowers the pH and helps preserve the product and ensure microbiological safety. LAB also uses amino acids, peptides, and vitamins found in meat for growth and metabolic functions [24].

2.4 Lactic acid fermentation in vegetables

Vegetable fermentation using LAB is an ancient practice with a significant presence in various cultures worldwide. Products such as sauerkraut in Central Europe, kimchi in Korea, fermented pickles in North America and Asia, and pickled carrots in multiple regions are representative examples of this type of food. These fermentations can be spontaneous, taking advantage of the microorganisms naturally present on the surface of the vegetable and in the environment, or they can be directed by the addition of specific starter cultures to standardize the quality and safety of the final product.

The fermentation process is substantially dominated in its initial phase by *Leuconostoc mesenteroides*, a species that initiates the production of lactic acid, carbon dioxide, and aromatic compounds. As the environment becomes more acidic, this species is replaced by more acid-tolerant bacteria, such as *Lactobacillus plantarum* and *Lactobacillus brevis*, which continue the pH decrease and stabilize the fermentation process.

During this process, a highly acidic environment (low pH) is created, which inhibits the growth of pathogenic and spoilage microorganisms, thereby contributing to the food's safety and the preservation of the product. In addition, organic acids (such

as lactic acid and acetic acid), antioxidant compounds, and, in some cases, bioactive metabolites, including GABA (gamma-aminobutyric acid), are produced. GABA is known for its potential anxiolytic and neuroregulatory effects.

Fermentation also has a significant sensory and nutritional impact, as it enhances the texture and flavor of the vegetables. It can increase the bioavailability of specific nutrients, such as B vitamins and phenolic compounds. Together, these effects position fermented vegetables not only as traditional foods but as functional foods with potential benefits for human health [21, 25].

2.5 Functional and nutraceutical benefits of lactic fermentations

2.5.1 Probiotic properties

Numerous studies have demonstrated that certain Lactobacillus strains exhibit probiotic effects, particularly *Lactobacillus rhamnosus GG*, *Lactobacillus casei*, *Lactobacillus plantarum,* and *Lactobacillus reuteri*. Among the most documented benefits are as follows:

- Improved intestinal health: strengthening the epithelial barrier, mucin production, and microbiota regulation. Immunomodulation: stimulation of dendritic cells, increased secretory IgA, and reduction of proinflammatory cytokines.
- Nutrient bioavailability: LAB can release minerals trapped in phytates or improve the absorption of vitamins such as folate and B12.
- Production of bioactive compounds, such as antihypertensive peptides, antioxidants, and neuromodulatory compounds such as GABA [20, 25].

3. Other fermentation processes of food and industrial relevance

In addition to lactic acid fermentation, there are other fermentation processes of great importance in the food and biotechnology industries. These fermentations, carried out by a diverse variety of microorganisms, including bacteria, yeasts, and fungi, enable the transformation of substrates into compounds that not only preserve foods but also enhance their sensory, functional, and nutritional properties.

These processes include alcoholic fermentation, which is essential in the production of beverages such as wine, beer, and spirits. Additionally, fermentations involved in the production of cocoa and coffee utilize microbial consortia that modulate aroma and flavor. Propionic and butyric fermentations are also of great importance, as they are used both in food production and in the production of organic acids of industrial interest.

Figure 5 summarizes the key microbial groups, substrates, and products associated with these fermentation processes.

Some of these fermentations are described below, highlighting the microorganisms involved, substrates, their primary metabolic pathways, and the technological or functional impact of the resulting products.

3.1 Lactic fermentations in alcoholic beverages

Although alcoholic fermentation is typically carried out by yeast, some traditional beverages, such as pulque, pozol, and certain sour beers, feature mixed

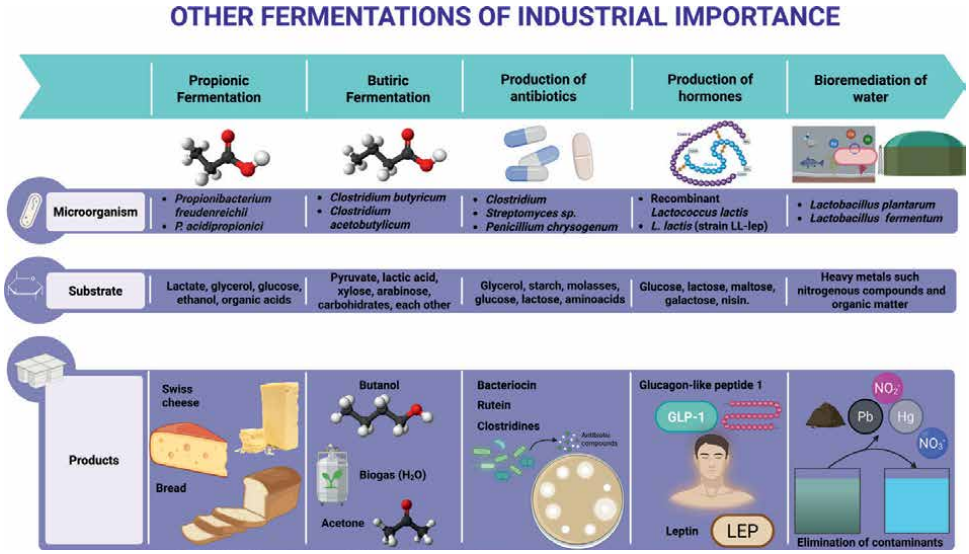

Figure 5.
Overview of other industrially relevant microbial fermentations, their substrates, and key applications.

cultures where LAB actively contribute during fermentation. In pulque, for example, *Lactobacillus*, *Leuconostoc*, and *Weissella* ferment the sugars in aguamiel (the sap of the maguey tree), alongside ethanol-producing yeasts. Their presence stabilizes the pH, generates aromatic compounds (such as diacetyl acetate), and offers potential probiotic benefits. Similarly, in lambic or Berliner Weisse beers, LAB is inoculated to acidify the wort and impart a characteristic sour note.

Several traditional fermented products exhibit complex microbial dynamics, where LAB coexist with yeasts and fulfill key metabolic functions. In pulque, a beverage made from aguamiel, a diverse microbiota has been documented. In addition to sugar-fermenting yeasts, such as *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, and *Candida zemplinina*, LAB strains like *Lactobacillus acidophilus*, *L. plantarum*, *L. mesenteroides*, and *Weissella confusa* are present. These bacteria metabolize simple sugars (glucose, fructose, and sucrose), producing lactic acid that contributes to acidification, inhibits pathogens, and generates secondary aromatic compounds, including acetate, diacetyl, and ethanol in minor amounts. Moreover, certain LAB strains isolated from pulque have demonstrated probiotic properties, such as gastrointestinal tolerance and pathogen inhibition [26, 27].

In pozol, a thick fermented beverage prepared from nixtamalized corn, LAB also participate alongside yeasts in fermenting residual carbohydrates from cooked starch. Dominant species include *L. fermentum*, *L. plantarum*, and *Pediococcus acidilactici*, which contribute to acidification, preservation, and sensory complexity [28, 29].

Researchers and brewers are increasingly exploring genetic modifications of LAB and yeast to enhance desirable characteristics in sour beers, such as tartness, mouthfeel, and complexity, while minimizing off-flavors.

3.1.1 Lactic acid bacteria (LAB)

Engineered LAB strains, including *Lactobacillus* and *Pediococcus*, have been developed to improve fermentation efficiency, regulate lactic acid production, and

reduce undesirable byproducts such as diacetyl. These modifications allow brewers to fine-tune sourness and complexity, resulting in consistent and controlled flavor profiles in sour beer styles like Berliner Weisse, Gose, and Lambic.

3.1.2 Yeast strains

Yeast engineering, particularly in *Saccharomyces cerevisiae*, has also been explored to enhance the production of flavor-active compounds, including esters, phenolics, and higher alcohols, which impart fruity, spicy, or aromatic notes. Engineered yeasts can be used in combination with LAB during mixed fermentations, generating more complex sensory profiles through microbial interaction and co-fermentation.

3.1.3 Malolactic fermentation (MLF)

MLF plays a significant role in modulating acidity and sensory properties in both alcoholic beverages and wines. In beer and cider, engineered LAB strains are applied to better control MLF, converting malic acid to lactic acid, thus reducing sharp acidity and contributing to smoother textures, particularly in barrel-aged sours and farmhouse ales.

Traditional sour beers, such as Belgian Lambic or German Berliner Weisse, depend on spontaneous or co-inoculated fermentations involving wild yeasts (e.g., *Brettanomyces*) and LAB species like *L. delbrueckii* and *L. brevis*. LAB consume sugars in the wort, lowering pH (to about 3.5), and produce lactic acid that adds acidity and improves microbiological stability [29].

In winemaking, MLF, mainly driven by *Oenococcus oeni*, but also *Lactobacillus* and *Pediococcus*, reduces the sourness and astringency derived from malic acid, yielding lactic acid, which provides a smoother mouthfeel. This process is crucial in red wines and certain aged whites, contributing to balance, stability, and flavor complexity. Additionally, secondary metabolites such as diacetyl can introduce buttery or creamy notes appreciated in specific white wines like Chardonnay.

Overall, the use of LAB in lactic fermentations goes beyond just acidification and preservation. It helps create unique sensory profiles and opens new possibilities for developing functional beverages, especially when strains with proven probiotic properties are chosen.

3.2 Fermentation of cacao, coffee, and vanilla

The fermentation of cocoa, coffee, and vanilla is a crucial process in the development of their flavor, aroma, and overall quality. Without this fermentation, chocolate, coffee, and vanilla would lack the characteristic flavor that defines them. The processes are spontaneous and depend on diverse microbial consortia acting sequentially.

3.2.1 Cacao

In the case of cacao (*Theobroma cacao*), fermentation takes place in piles or boxes where the opened pods are placed. In the first 24–48 hours, yeasts such as *Saccharomyces cerevisiae*, *Candida tropicalis*, and *Hanseniaspora uvarum* predominate, metabolizing the mucilage sugars (glucose, fructose, and sucrose), producing ethanol, CO_2, and volatile aromatic compounds [30].

Lactic acid bacteria, such as *L. plantarum* and *P. acidilactici*, subsequently intervene, producing lactic acid and modifying the microbial environment. Finally, acetic acid bacteria, such as *Acetobacter pasteurianus*, oxidize ethanol to acetic acid, which penetrates the beans and induces internal biochemical reactions, including the degradation of proteins and polysaccharides, thereby generating aroma precursors [30].

3.2.2 Coffee (Coffea arabica, Coffea canephora)

Fermentation occurs after the fruit pulp is removed, using wet or dry methods. In wet processes, the berries are fermented with their mucilage, rich in pectin, glucose, and organic acids. Initially, yeasts such as *Pichia kluyveri, Hanseniaspora spp*., and *Saccharomyces spp*. dominate, transforming sugars into ethanol and volatile compounds [31].

LAB, such as *L. fermentum*, intervene later, generating lactic acid, and, at some stages, acetic acid bacteria contribute to the acid profile. The type of fermentation (submerged, dry, or semi-dry), its duration, and environmental conditions determine key attributes such as acidity, sweetness, body, and the presence of fruity or floral notes [31].

3.2.3 Vanilla

Vanilla planifolia requires an essential post-harvest fermentation process for the development of its characteristic aroma. The fresh pods have no pleasant odor; only after undergoing blanching, sweating, drying, and curing do key enzymatic and microbial activities become active, enabling the transformation of aroma precursors.

This process involves both endogenous plant enzymes and specific microbial communities, resulting in combined biosynthesis. One of the main transformations is the conversion of phenolic glycosides, especially glucovanillin, into vanillin, the principal aromatic compound in cured vanilla [32, 33].

During fermentation, microorganisms such as *Bacillus spp*., yeasts (*Candida spp.*), and LAB (e.g., *Lactobacillus plantarum*) play a role. These microbes break down polysaccharides, release phenolic compounds, and generate organic acids, alcohols, and esters that enhance the final flavor profile. Their activity takes place in a matrix rich in sugars, amino acids, and phenolics derived from the vanilla pods [34].

In summary, vanillin biosynthesis is not exclusively microbial, but rather the result of a synergistic interaction between plant enzyme activity (initiated during curing) and microbial fermentation, both of which are essential to achieving the natural vanilla aroma.

3.3 Propionic fermentation

Propionic fermentation is a microbial process carried out by bacteria of the genus Propionibacterium, although some clinically important species have recently been reclassified as *Cutibacterium*. In the food industry, the most relevant species is *Propionibacterium freudenreichii*, known for its role in cheese ripening and the production of propionic acid [35].

This type of fermentation converts lactic acid, glycerol, or other sugars into propionic acid, acetic acid, and CO_2 via the propionic acid pathway (Wood-Werkman). This combination of products has critical technological, sensory, and functional applications [36].

Food applications. In the production of Swiss cheeses, such as Emmental, *P. freudenreichii* is responsible for the development of the characteristic sweet flavor, as well

as the well-known "eyes" or holes in the cheese, produced by the release of CO_2 during secondary fermentation. It also contributes to the texture, aroma, and ripening of the cheese, in synergy with other lactic acid microorganisms [37].

Industrial production. At the industrial level, this bacterium is used in controlled fermentation to produce propionic acid, which is used as a food preservative in bakery under the additive number E280. This compound inhibits the growth of contaminating molds and bacteria, extending the shelf life of baked goods [35].

Functional and health benefits. Beyond its technological applications, propionic acid generated by Propionibacterium has health benefits, including the inhibition of pathogens such as *Salmonella spp*. and *Listeria monocytogenes* [35]. Furthermore, it has been associated with positive metabolic effects, including the reduction of hepatic cholesterol synthesis and the modulation of blood glucose levels, making it a metabolite of interest in functional and prebiotic nutrition. Its use in combined fermentations with lactobacilli has also been proposed to enrich the sensory profile of fermented foods and enhance their nutritional functionality [36].

3.4 Butyric fermentation

Butyric fermentation is carried out by strictly anaerobic bacteria of the *Clostridium* genus, particularly *Clostridium butyricum*, a model species for this type of metabolism. These bacteria ferment various carbohydrates, including glucose, lactose, xylose, and arabinose, and produce lactic acid, glycerol, and succinate, with butyric acid being the primary metabolite. This process is accompanied by the release of gases such as CO_2 and hydrogen [38].

Applications in food. Although butyric fermentation may be undesirable in some foods due to the production of rancid or unpleasant odors, it has been used in other contexts in a controlled manner. Some strains of *Clostridium butyricum* are utilized as probiotics in Asia, particularly in Japan and China, due to their ability to survive in the gastrointestinal tract and modulate the gut microbiota [39].

Benefits of butyric acid for human health. Butyric acid is also a key metabolite in the human colon, produced by the fermentation of dietary fiber by gut bacteria such as *Faecalibacterium prausnitzii* and *Roseburia spp*. Its proven benefits include the following:

- Be the primary source of energy for colonocytes, promoting the renewal of the intestinal epithelium.
- Have anti-inflammatory and anti-cancer properties, particularly in the context of inflammatory bowel diseases.
- Maintain the integrity of the intestinal barrier and modulate the local immune response [40, 41].

3.4.1 Microbiological and industrial importance

Waste fermentation. Butyric fermentation is utilized in the recovery of organic waste, as it enables the conversion of agricultural or agro-industrial byproducts into value-added compounds. One of its most notable uses is biogas production, in anaerobic digestion processes where *Clostridium spp*. participate in the generation of hydrogen and methane [42].

Production of butyrate and biopolymers. The butyric acid produced can be used as a precursor in the synthesis of biodegradable bioplastics, such as polyhydroxybutyrate (PHB) and the copolymer PHBV poly(3-hydroxybutyrate-co-3-hydroxyvalerate), materials of growing interest in the sustainable packaging industry [43].

Solvent production (ABE fermentation). Certain Clostridium species, such as *C. acetobutylicum*, are also used in ABE (acetone-butanol-ethanol) fermentation, an industrial process with historical and emerging applications in biofuel and industrial solvent production [44].

4. Participation of LAB in environmental improvement through bioremediation of water, soil, and air

LAB are not only essential for food fermentation but also play an important role in environmental bioremediation. Through their metabolic activity, LAB contribute to the breakdown or immobilization of toxic compounds in water, soil, and air, providing an eco-friendly method of pollution control. This bioremediation is possible due to LAB's ability to degrade various contaminants via fermentative pathways, producing organic acids, bacteriocins, and enzymes that help modify pH and reduce pathogen activity, making them valuable for environmental restoration. LAB such as *Lactobacillus spp.*, *Leuconostoc spp.*, *Pediococcus spp.*, *Enterococcus spp.*, and *Lactococcus spp.* are particularly important in this context [45, 46].

Several studies have demonstrated the potential of native and genetically modified LAB for contaminant degradation or adsorption. For example, *Lactobacillus plantarum* and *L. rhamnosus* have been shown to bind mycotoxins like aflatoxins and ochratoxins, as well as heavy metals, such as lead and cadmium, through their cell wall components [47]. Genetically engineered *Lactococcus lactis* strains have been investigated for their enhanced capacity to accumulate metals.

Additionally, some LAB strains have shown limited abilities to degrade phenolic compounds and pesticide residues [48]. While their role in bioremediation is still evolving, these findings underscore the potential of LAB as safe, sustainable agents for environmental cleanup.

4.1 Water bioremediation

Water pollution, often contaminated with heavy metals, hydrocarbons, pesticides, and persistent organic compounds, can be addressed by various microbial processes. Aquatic microorganisms like *Pseudomonas putida* and *Bacillus subtilis*, along with algae and fungi, can metabolize or absorb these pollutants.

Some bioremediation strategies include:

- Bioreactors: These closed systems are designed for industrial and municipal wastewater treatment, where microorganisms degrade pollutants.
- Biosorption: In this process, live or dead microbial biomass absorbs metals like Cd, Pb, or Hg.
- Aquatic phytoremediation: Plants like *Eichhornia crassipes* (water hyacinth) and *Lemna minor* (duckweed) help absorb and accumulate pollutants like heavy metals and nutrients [49].

4.2 Air bioremediation

Air pollution from volatile organic compounds (VOCs), nitrogen oxides, sulfur, and methane can be treated using various techniques:

- Biofilters: Beds of porous materials such as peat or compost are colonized by bacteria like *Mycobacterium* and *Xanthobacter*, which degrade VOCs.
- Bioscrubbers: Contaminated gas passes through a scrubbing tower filled with liquid solutions containing degrading microorganisms.
- Methane biotraps: Methanotrophic bacteria such as *Methylosinus* and *Methylococcus* oxidize methane, converting it to CO_2 and water [50].

4.3 Soil bioremediation

Soil contamination by hydrocarbons, heavy metals, chlorinated solvents, and pesticides can be managed by various bioremediation strategies:

- Biostimulation: The addition of nutrients (N, P) or oxygen stimulates the growth of native microorganisms.
- Bioaugmentation: Introducing efficient microorganisms like *Pseudomonas fluorescens*, *Rhodococcus erythropolis*, and *Bacillus megaterium* enhances degradation capabilities.
- Phytoremediation: Plants such as *Helianthus annuus* (sunflower) and *Brassica juncea* (mustard) absorb and immobilize contaminants like Zn, Pb, and As.
- Mycoremediation: Fungi like *Pleurotus ostreatus* and *Trametes versicolor* break down persistent organic compounds [51, 52]

LAB can be used in combination with biochar or plant materials for synergistic bioremediation. Biochar enhances the adsorption of pollutants due to its high surface area and its ability to retain heavy metals and organic compounds. LAB, on the other hand, can degrade organic pollutants through metabolic processes. When combined, LAB not only degrade the pollutants but can also benefit from the nutrients provided by the plant materials, while biochar retains toxic compounds and improves the efficiency of the bioremediation process. Additionally, plant materials can facilitate the growth of LAB by providing a suitable environment for their activity, thereby creating a more integrated and effective bioremediation strategy [53].

5. Clean labeling and the role of microorganisms in food innovation

The concept of clean labeling refers to foods formulated without artificial additives, using only natural, minimally processed, and consumer-recognizable ingredients. While there is no universal legal definition, this approach responds to the growing demand for healthier, more transparent, and sustainable products.

In this context, the use of microorganisms, particularly LAB and yeasts, has become a key strategy. These microorganisms not only enable fermentation and sensory enhancement, but also serve as natural alternatives to chemical preservatives, enhancing microbial safety, nutritional functionality, and shelf life.

5.1 Applications in dairy products

In fermented products such as yogurt, cheese, and ice cream, the use of natural functional ingredients (e.g., lemon peel, beetroot, perilla oil) combined with probiotic cultures and LAB allows for the replacement of synthetic stabilizers, colorants, and preservatives. These formulations improve texture, stability, and nutritional value, supporting the development of clean-label products without compromising quality [54].

5.2 Applications in meat products

In cured meat products, sodium nitrite has traditionally been used for its antimicrobial effects (especially against *Listeria monocytogenes*) and its role in color stabilization. However, due to concerns over the potential formation of carcinogenic nitrosamines, clean-label alternatives have emerged, including:

- Vegetable extracts rich in natural nitrate (e.g., celery, beetroot).
- Nitrate-reducing starter cultures, capable of converting plant-based nitrate into nitrite in a controlled, natural manner.

Many of these bacteria—such as *Lactobacillus sakei*, *Lactobacillus curvatus*, *Pediococcus acidilactici*, and *Leuconostoc mesenteroides*—exhibit nitrate reductase activity. These LAB strains ferment the vegetable extracts, releasing nitrite gradually and safely, maintaining the preservative function without requiring the declaration of "added nitrite" on the label [55, 56].

Furthermore, natural antioxidants such as rosemary extract or vitamin C are often added to inhibit nitrosamine formation, thereby enhancing product safety within a clean-label framework.

5.3 Applications in bakery products

In the baking industry, the use of non-conventional yeasts isolated from fermented germinated cereals has shown excellent results. For instance, *Wickerhamomyces anomalus*, isolated from fermented germinated barley, was used as a starter culture in Type IV sourdough to produce enriched wheat bread. This yeast improved dough volume, bread texture, and resistance to mold development (*Aspergillus flavus*), while achieving high sensory acceptability. All of this was accomplished without artificial dough improvers or preservatives, making the bread a genuinely clean-label product [57].

6. Conclusion

Microbial fermentation stands today as one of the most powerful and versatile tools in biotechnology, driving innovation across the food, health, industrial, and

environmental sectors. Its ability to transform low-value raw materials (including agro-industrial residues and organic byproducts) into high-value products such as functional foods, bioactive compounds, biomaterials, and enzymes reflects its extraordinary potential.

Far beyond its traditional role in food preservation, fermentation fosters the development of safer, more nutritious, and functional products, while promoting production systems that are energy-efficient, low in waste, and less reliant on harmful chemicals. In doing so, it aligns closely with the goals of green biotechnology and the United Nations Sustainable Development Agenda.

Each fermentation type (lactic, acetic, propionic, or butyric) contributes unique benefits to food quality, including extended shelf life, enhanced gut health, and the generation of bioactive substances with antimicrobial and antioxidant activity. At the same time, the targeted use of specific microbial strains supports the production of compounds with environmental applications, such as in the bioremediation of polluted ecosystems.

Recent advances in synthetic biology and precision fermentation have propelled this field into a new era. Engineered microorganisms now allow for the sustainable and scalable production of high-value molecules (such as proteins, lipids, and specialty metabolites) without dependence on animal or plant sources, offering revolutionary solutions for food, pharmaceuticals, and biomaterials.

Ultimately, advancing our understanding and application of microbial fermentation represents a strategic investment in building a more sustainable, healthier, and resilient future. As both a traditional practice and a cutting-edge scientific platform, fermentation will remain central to applied microbiology's contribution to human well-being and global ecological balance.

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Conflict of interest

The authors declare that they have no conflict of interest.

Notes/thanks/other declarations

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Chapter 2

Mechanisms of Preservation by Lactic Acid Bacteria in Food Fermentation

Charles Obinwanne Okoye, Bonaventure Chidi Ezenwanne and Olufemi Olasoji

Abstract

Lactic acid bacteria (LAB) are pivotal in food fermentation, leveraging their metabolic capabilities to enhance preservation, safety, and sensory attributes of various food products. This manuscript explores the mechanisms by which LAB preserve food, focusing on their antimicrobial and antioxidant activities; their applications in dairy, meat, and vegetable fermentations; as well as emerging uses in probiotic and plant-based products. LAB, including genera like *Lactobacillus, Streptococcus, Pediococcus*, and *Leuconostoc*, produce organic acids (e.g., lactic and acetic acids) through homofermentative and heterofermentative pathways, lowering pH to inhibit spoilage organisms and pathogens. Bacteriocins, such as nisin and pediocin, offer targeted antimicrobial action, serving as natural alternatives to synthetic preservatives. Additionally, LAB synthesize bioactive compounds like riboflavin (vitamin B2) and exopolysaccharides (EPS), which enhance nutritional value and improve texture and shelf life. This chapter details LAB's role in dairy, meat, and vegetable fermentations, highlighting their contribution to flavor, safety, and probiotic functionality. Emerging applications in plant-based fermentation address the growing demand for vegan products, with LAB degrading anti-nutrients and enriching nutrient profiles. However, challenges such as strain-specific variability, environmental sensitivity, and regulatory concerns surrounding genetically modified LAB persist. Advances in omics technologies, synthetic biology, and artificial intelligence are proposed to optimize LAB performance, enabling tailored preservation strategies. This chapter underscores LAB's critical role in sustainable, clean-label food production, advocating for multidisciplinary research to unlock their full potential in addressing modern food industry demands.

Keywords: food fermentation, lactic acid bacteria, preservation mechanisms, organic acids, probiotics, omics technologies, sustainable food production

1. Introduction

Fermentation is one of humanity's oldest biotechnologies, employed for centuries to preserve food, boost nutritional value, and enhance sensory qualities [1].

Originating in early human societies as a practical method to extend shelf life and improve flavor and texture, fermentation gradually became an integral part of culinary traditions across the globe [2]. Across Europe, the Middle East, and the Indian subcontinent, diverse cultures mastered the fermentation of milk and cereals, giving rise to various fermented dairy products [3]. It is a natural process in which microorganisms break down complex organic molecules into simpler forms while simultaneously enhancing the nutritional value, bioavailability, and sensory attributes, such as vitamins, proteins, amino acids, flavors, and aroma, of food [4]. It fundamentally involves the controlled activity of microorganisms on food substrates, a process that can occur spontaneously through the native microflora present in raw ingredients [5].

In the mid-nineteenth century, Pasteur revealed that microbial activity drives fermentative transformations, which spurred the deliberate selection of environmental microorganisms to optimize fermentation efficiency [6]. By 1890, pure yeast strains were being used for winemaking, and the early twentieth century introduction of microbicidal chemicals and pasteurization further refined microbial control. These advances highlight the central role of microorganisms in altering the chemical and physical properties of fermented foods. Microbial fermentation itself is an anaerobic metabolic pathway in which carbohydrates, such as sugars or grains, are converted into alcohols, acids, or gases [7, 8]. This transformation not only preserves food but also enhances shelf life, prevents spoilage, and inhibits the growth of harmful pathogens. As a result, modern fermentation techniques continue to rely heavily on well-characterized microbial strains to ensure consistency, quality, and functionality in both traditional and industrial food production [4].

Lactic acid bacteria (LAB) are the primary microorganisms responsible for fermentation and flavor development in food [9]. LAB comprise a diverse group of non-sporulating, gram-positive cocci or rods that are nonmotile, acid-tolerant, and non-respiring [10]. They obtain energy by phosphorylating sugars and are primarily categorized as either homofermentative or heterofermentative, with some species capable of both pathways, referred to as heterogeneous bacteria [11]. Homofermentative LAB, such as *Lactobacillus plantarum* and *Pediococcus pentosaceus*, metabolize hexoses via glycolysis, producing two molecules of lactate per glucose molecule [12]. Conversely, heterofermentative LAB can ferment both hexoses and pentoses, utilizing the pentose phosphate pathway with key enzymes like ribose-5P epimerase and phosphoketolase, resulting in carbon dioxide, lactate, and either acetate or ethanol [13].

The most commonly recognized LAB belong to four main genera: *Lactobacillus*, *Streptococcus, Pediococcus, and Leuconostoc* [14, 15]. *Lactobacillus*, a key genus within the phylum Firmicutes, plays a vital role in food fermentation by enhancing microbiological safety and extending shelf life. These bacteria dominate the fermentation environment by producing organic acids that suppress spoilage and pathogenic microbes [14]. Their functional and technological activities not only improve food safety and nutrition but also contribute to desirable sensory qualities and detoxification within the food matrix [16]. *Streptococcus* is one of the primary bacterial species used in yogurt production [17]. Strains of *S. thermophilus* derived from various sources exhibit distinct phenotypic characteristics. During fermentation, *S. thermophilus* grows rapidly and generates organic acids, which in turn promote the growth of *Lactobacillus bulgaricus* [18]. The rate of acidification and extracellular polysaccharide (EPS) production by *S. thermophilus* influences the final texture and gel formation, while rapid acidification also shortens fermentation time, enhancing efficiency in yogurt production [19]. Nonetheless, several *Pediococcus* species have been identified

as adventitious cultures in certain cheeses and are occasionally involved in milk fermentation and cheese production [20]. These species exhibit a broad spectrum of distinctive morphological, physiological, nutritional, and genetic characteristics. For instance, *Pediococcus pentosaceus* is a non-motile, spherical, facultative, anaerobic gram-positive bacterium that typically appears in pairs or tetrads. Naturally found in spontaneously fermented products, it has been shown to improve product quality, enhance food safety, and increase production efficiency, especially when used in combination with other bacterial strains in mixed fermentations. [21]. Furthemore, *Pediococcus* is widely applied in the industrial fermentation of meat and vegetables and shows promise as a biopreservative, despite its known involvement in the spoilage of beer and wine [20]. *Leuconostoc* species are widely recognized as key flavor producers in dairy cheese, primarily due to their synthesis of compounds such as acetoin and diacetyl [22]. Although classified as non-starter lactic acid bacteria (NSLAB) in cheesemaking, they are also commonly found on vegetables and in various fermented foods [23]. These bacteria are often deliberately added to improve the taste and consistency of fermented foods, yet they can also emerge spontaneously as part of the natural microbiota in dairy fermentations. As heterofermentative, facultative anaerobes, *Leuconostoc* spp. play a central role in citrate metabolism, converting citrate into key flavor compounds crucial to the sensory profile of cheese [24].

Overall, LAB play a critical role in improving food safety and quality by effectively inhibiting pathogenic and spoilage microorganisms through their safe metabolic activities. They transform available sugars into organic acids and generate bioactive compounds, such as bacteriocins, vitamins, and free amino acids, during fermentation, which disrupt microbial and fungal membranes [25–27]. LAB support biopreservation by producing natural antimicrobial agents [9], extending food shelf life and ensuring safety [16]. Certain metabolites also exhibit antioxidant properties by preventing free radical formation, underscoring LAB's significant applications in the food industry beyond traditional fermentation processes [28].

The objective of this chapter is to explore the food preservation mechanisms of LAB, including their antimicrobial and antioxidant activities; review their current applications in dairy, meat, vegetable fermentations, and probiotic formulations; highlight advances in strain selection, metabolic engineering, and novel delivery systems; and assess their future potential in clean-label, functional, and sustainable food preservation strategies.

2. Mechanisms of preservation by LAB

LAB have long been recognized for their central role in the fermentation of a wide variety of food products, owing to their efficient metabolic systems that convert simple substrates into compounds beneficial for flavor development, food preservation, and nutritional enhancement [29, 30]. Although LAB possess relatively small and simple genomes with limited biosynthetic capabilities, they exhibit a wide array of metabolic activities finely tuned for survival in nutrient-rich environments like fermented foods [29]. Over the decades, multidisciplinary investigations combining genetics, physiology, and biochemistry have expanded our understanding of LAB metabolism [31], providing essential insights for their optimized application in food processing and biopreservation. The metabolic pathways of LAB not only ensure energy generation and growth but also contribute significantly to food quality through the production of lactic acid, other organic acids, antimicrobial peptides

(bacteriocins), vitamins, and exopolysaccharides (EPS). These metabolites are essential for creating favorable sensory attributes, improving shelf life, ensuring food safety, and even offering health-promoting functions when consumed as part of probiotic products [32–34]. This section outlines the major metabolic pathways in LAB and identifies promising areas for further research and development.

2.1 Acidification through organic acid production

A key characteristic of LAB is their capacity to produce diverse organic acids as metabolic byproducts during carbohydrate fermentation. These acids include lactic acid (the primary product), as well as acetic acid, formic acid, succinic acid, and others [35]. LAB mainly ferment carbohydrates to produce lactic acid, leading to substantial acidification of the food matrix. This acidification is essential as it creates unfavorable conditions for pH-sensitive spoilage microorganisms and pathogenic bacteria [36]. The acidic conditions created by these organic acids not only impart a tangy flavor and aroma to fermented foods such as yogurt, kimchi, and sourdough bread but also act as natural preservatives by suppressing pathogenic microbes. In homofermentative LAB, glucose is primarily converted via the Embden-Meyerhof-Parnas (EMP) pathway into pyruvate, which is then reduced to lactic acid through lactate dehydrogenase (LDH) activity [37]. Depending on the strain's LDH isoforms, either L-(+)-lactate or D-(−)-lactate may dominate as the major product, affecting both the sensory properties and digestibility of the final product [38]. Conversely, heterofermentative LAB employ the pentose phosphate and phosphoketolase pathways, yielding a balanced mixture of lactic acid, ethanol (or acetic acid), and carbon dioxide from sugar fermentation (**Figure 1a**). This metabolic flexibility is especially crucial in cereal-based or vegetable-based fermentations, where pentose sugars are more prevalent. LAB can also metabolize citrate and other organic acids, producing secondary metabolites like acetate, succinate, and formate through enzymes like fumarase, fumarate reductase, and pyruvate-formate lyase [39]. These metabolites contribute to the complex flavor profiles of cheese and fermented dairy products. Nonetheless, as highlighted by Wu et al. [5] and Okoye et al. [40], more research is essential to comprehensively map the interactions of these alternative metabolic pathways under various environmental and nutritional conditions relevant to food systems.

2.2 Bacteriocin production

Bacteriocins are antimicrobial peptides synthesized by many LAB strains that effectively suppress the growth of related species or foodborne pathogens. These peptides are gaining attention as natural alternatives to synthetic preservatives due to their specificity, safety, and natural origins [41]. In food systems, bacteriocins such as nisin, pediocin, and enterocin are widely used to inhibit the growth of harmful microorganisms like *Listeria monocytogenes*, *Staphylococcus aureus*, and other undesired microbes, thereby enhancing the safety of food products and extending their shelf life [42]. Bacteriocins are classified into four major groups: Class I (lantibiotics), Class II (small, heat-stable peptides), Class III (larger, heat-labile proteins), and Class IV (complex proteins with lipid or carbohydrate components) (**Figure 1b**). The production of bacteriocins follows a genetically encoded process, which involves synthesizing a pre-peptide that undergoes post-translational modifications before being transported and secreted via specialized machinery [43]. These processes are

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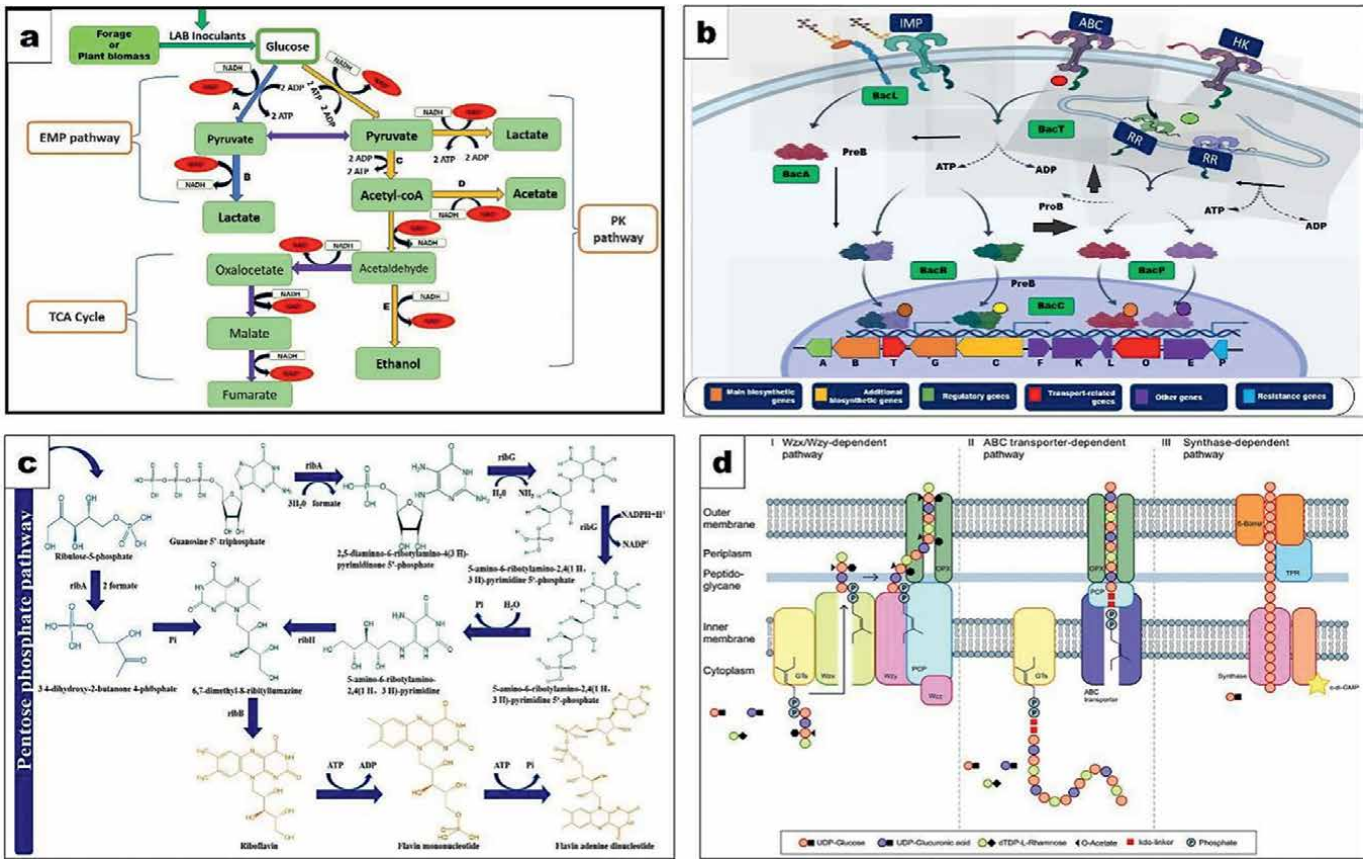

Figure 1.
Metabolic pathways associated with LAB preservation mechanisms. (a) Organic acid synthesis via homo- and heterofermentation (EMP and PK pathways); (b) Bacteriocin biosynthesis and regulation; (c) Riboflavin biosynthesis pathway from GTP and Ru5P; (d) Exopolysaccharide biosynthesis via Wzx/Wzy-dependent, ABC transporter-dependent, and synthase-dependent pathways. Reproduced with permission from Okoye et al. [30].

regulated by operons that coordinate the expression of genes encoding the structural peptide, immunity proteins, regulatory systems, and secretion apparatus [44]. A critical regulatory system is the three-component signal transduction system, consisting of the inducing peptide (IP), histidine protein kinase (HPK), and response regulator (RR). This system ensures that bacteriocin production is triggered in response to population density and environmental stress [45]. However, advances in omics technologies and synthetic biology have opened new avenues to enhance the production and broaden the spectrum of bacteriocins, making them even more relevant in the context of minimally processed foods [30].

2.3 Vitamin biosynthesis

Riboflavin, or vitamin B_2, is a critical micronutrient that acts as a precursor for flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are coenzymes involved in various oxidation-reduction reactions [46]. Some LAB strains can synthesize riboflavin *de novo*, thus enhancing the nutritional quality of fermented products without external fortification [47]. The riboflavin biosynthesis pathway (RBP) in LAB initiates with the precursors GTP and ribulose-5-phosphate (Ru5P) (**Figure 1c**). The pathway involves four main genes—*ribA*, *ribB*, *ribG*, and *ribH*—which are generally organized in an operon but not necessarily expressed in the order of the reaction sequence [48]. Specifically, *ribA* encodes GTP cyclohydrolase II, which generates a pyrimidine intermediate from GTP. This intermediate, combined with a sugar compound derived from Ru5P, is processed by *ribG*, which encodes a deaminase-reductase enzyme, to form lumazine. *RibH* encodes lumazine synthase, which converts the precursor into a lumazine intermediate, while *ribB* (riboflavin synthase) carries out the final transformation into riboflavin [44]. The ability of LAB to biosynthesize riboflavin varies among strains and is influenced by environmental conditions such as carbon source, pH, and oxygen levels. Optimizing these conditions in fermentation systems could significantly improve the nutritional output of LAB, making them attractive for the production of fortified dairy, cereal, and plant-based fermented products.

2.4 Exopolysaccharides production

EPS produced by LAB are long-chain sugar polymers that either remain bound to the cell wall or are secreted into the extracellular environment [49]. EPS plays a crucial role in improving mouthfeel, viscosity, and water retention in food systems, especially in fermented dairy and cereal-based products [50]. The biosynthesis of EPS is genetically encoded and typically organized into distinct clusters. The three main biosynthetic pathways in LAB include the Wzx/Wzy-dependent pathway, which is the most common mechanism for the production of heteropolysaccharides. In this pathway, specific glycosyltransferases sequentially add sugar residues to lipid carriers located in the inner membrane of the cell. Once the sugar units are attached to these lipid carriers, the repeating units are translocated across the membrane with the help of the flippase protein Wzx. Subsequently, the polymerization of these units into larger polysaccharide chains is facilitated by the Wzy protein, which plays an important role in the assembly and export of the final product. This pathway is essential for the biosynthesis of various heteropolysaccharides that contribute to the texture, stability, and functional properties of LAB-derived fermented food products (**Figure 1d**) [30].

Secondly, the ATP-binding cassette (ABC) transporter-dependent pathway is primarily responsible for the synthesis of capsular polysaccharides (CPS) in LAB. In this pathway, glycosyltransferases located on the cytoplasmic face of the membrane synthesize the polysaccharide repeat units, which are then translocated to the cell surface by an ABC transporter system. This transport system requires ATP hydrolysis to move the polymer across the membrane and is often implicated in contributing to the LAB's resistance to environmental stresses, such as osmotic and oxidative pressures. The synthesis of CPS via this pathway plays a significant role in enhancing the robustness of LAB in harsh food processing environments, thereby improving product stability and microbial survival during fermentation [30].

Lastly, the synthase-dependent pathway is distinct in that it involves a single membrane-bound glycosyltransferase that continuously synthesizes homopolysaccharides such as dextran or levan. Unlike the Wzx/Wzy-dependent pathway, which requires multiple enzymes for the complete synthesis and transport of the polysaccharides, the synthase-dependent pathway relies on a single enzyme to catalyze both the elongation of the polysaccharide chain and its secretion across the membrane. This mechanism of EPS production is simpler in terms of enzymatic machinery but still contributes significantly to the texture and functional properties of LAB in fermented food products. Notably, it does not require the action of the flippase (Wzx) or polymerase proteins typically involved in more complex polysaccharide biosynthetic pathways [30]. Building on these preservation mechanisms, LAB are widely applied across various food sectors, with their metabolic capabilities driving the production of safe, flavorful, and nutritious fermented products.

3. Applications of LAB in the food industry

LAB play a vital role in food biotechnology, particularly in producing a wide range of fermented products such as dairy items (like cheese, fermented milk, and yogurt) and fermented meat, fish, fruits, vegetables, and cereals. They enhance these foods' flavor, texture, and nutritional quality, which is why they are often used as adjunct cultures. LAB are the most widely utilized microorganisms for food preservation and fermentation. Their significance lies primarily in their safe metabolic processes, during which they convert sugars into organic acids and other useful by-products [14]. To fully understand the details of LAB application in the food industry, it is essential to identify and examine its uses in different production processes.

3.1 Fermented dairy products

Fermented foods are created through the fermentation of specific sugars by LAB, with their origins tracing back to ancient times. **Table 1** highlights the most commonly used LAB strains as starter cultures in dairy fermentations. A large portion of these fermented foods fall under the dairy category—such as cheese, yogurt, and fermented milk—with many variations produced using starter cultures. Historically, these products were made through a method called back-slopping, where the characteristics of the final product depended on the dominance of the most adaptable strains. Even earlier, fermentation occurred spontaneously, driven by the natural microflora resident in raw materials and their surrounding environment. In modern food biotechnology, controlled fermentation predominantly utilizes characterized starter cultures, with LAB strains chosen for their specific, desirable traits tailored

Product	LAB	References
Cheese	*Lactococcus lactis* ssp. *lactis*, *Lactobacillus paracasei*, *Leuconostoc pseudomesenteroides*, *Lactobacillus casei*	[51]
Cheese	*Lactobacillus, Lactococcus*	[52]
Milk	*Lactobacillus, Lactococcus*	[52]
Yogurt	*Lactobacilli*	[53]
Fermented milks	*Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus*	[54]
Yakult	*Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus*	[55]
Sheep-fermented milk	*Streptococcus thermophilus*, *Lactobacillus delbrueckii*	[56]
Buttermilk	*Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus casei*	[57]
Kefir	*Leuconostoc mesenteroides, Lactobacillus kefiri, Lacticaseibacillus casei*	[58]
Viili	*Lactococcus lactis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus durans*, *Enterococcus lactis*, *Lactococcus cremoris*	[59]

Table 1.
Lactic acid bacteria used as starter cultures in producing some fermented dairy products.

to individual products [60]. The milk fermentation process depends heavily on the activity of LAB, which enables the transformation of milk into high-quality fermented dairy products. LAB may be present naturally (spontaneously) or introduced as starter cultures. Both spontaneous and inoculated LAB strains offer valuable potential for use in the production of fermented milk. During fermentation, LAB produce acids that act as natural preservatives and contribute to flavor development. Additionally, they generate exopolysaccharides, which play a key role in forming the texture of the final product [61].

Milk and fermented dairy products like yogurt, kefir, and buttermilk constitute nutrient-rich matrices that support microbial growth, which can lead to spoilage. One of the most notable preservative qualities of LAB is their ability to produce acids, which have antimicrobial effects. The acidification process helps protect milk by inhibiting the growth of spoilage organisms and harmful pathogens. In addition to acid production, LAB also produce antimicrobial compounds known as bacteriocins, which further contribute to the preservation of these products [62].

3.2 Fermented vegetables

Fermentation by LAB is regarded as a straightforward and effective biotechnological method for preserving and improving the taste, nutritional value, shelf life, and safety of fruits and vegetables [63]. The integration of traditional bio-preservation techniques with modern biotechnological tools enables tailored fermentation protocols through rational starter culture selection, promoting greater consumption of fresh-like fruits and vegetables [64, 65]. LAB ferment the carbohydrates present in fruits and vegetables into lactic acid (LA), reducing the pH of the final products to around 4.0, which helps ensure their stability. This acidification effectively restricts the proliferation of spoilage organisms and harmful pathogens. Additionally, LAB support a healthy gut microbiota by suppressing the growth of harmful bacteria such as *Escherichia coli, Staphylococcus, and Salmonella*, thereby contributing to overall health [66]. LAB are commonly recognized as probiotics because of their

health-enhancing properties, including their potential to reduce serum cholesterol levels. They also enhance immune function and help prevent tumor development by neutralizing carcinogenic compounds in the gastrointestinal tract, primarily through the reduction of harmful enzyme activity produced by fecal bacteria [67] or breaking down certain enterotoxins [68].

Fruits are frequently used in alcoholic fermentation processes, such as in the production of wine and beer, due to their high content of minerals, vitamins, and sugars. Their slightly acidic nature makes fruit juices an ideal environment for yeast growth, allowing for the efficient conversion of sugars into ethanol. In contrast, vegetables contain lower levels of sugar but are abundant in vitamins and minerals and have a neutral pH, making them suitable for fermentation by LAB. Fermentation in both fruits and vegetables can occur naturally through the activity of surface-residing LAB like *Lactobacillus*, *Leuconostoc*, and *Pediococcus*. However, introducing starter cultures such as *Lactobacillus plantarum*, *L. rhamnosus, L. gasseri*, *L. acidophilus,* and all probiotic strains can enhance consistency and ensure reliable fermentation outcomes (**Table 2**). In commercial-scale production of lactic acid fermented vegetables like sauerkraut, pasteurization or the addition of preservatives after fermentation is a common practice. However, these processes eliminate most of the LAB, thereby negating any potential probiotic benefits [64]. During the fermentation of sauerkraut or kimchi, the biopreservative properties of LAB help prevent enzymatic and microbial activities that might otherwise cause the bright colors of fresh produce to deteriorate. This protective action also shields the food from oxidative processes, which are often responsible for discoloration or browning in various foods [79].

3.3 Meat products

Meat and meat products are highly perishable owing to their favorable pH, rich nutritional composition, and high moisture content [80]. Meat products serve as

Product	LAB	References
Sauerkraut	*Leuconostoc mesenteroides*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Lactobacillus casei*	[69]
Kimchi	*Lactococcus lactis, Leuconostoc citreum*	[70]
Tomato	*Leuconostoc mesenteroides* ssp. *mesenteroides* Tsc	[71]
Fermented olives	*Lactobacillus pentosus, Pediococcus parvulus, Leuconostoc pseudomesenteroides*	[72]
Pineapple (*Ananas comosus* L. Merr.)	*Lactiplantibacillus plantarum, Lactiplantibacillus rossiae*	[73]
Cabbage	*Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus paraplantarum*	[74]
Carrots	*Leuconostoc mesenteroides, Lactobacillus paracasei, Lactobacillus brevis,, Lactobacillus plantarum,*	[75]
Sweet cherry (*Prunus avium* L.)	*Lactobacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus, Leuconostoc mesenteroides subsp. mesenteroides*	[76]
Pickles	*,Lactiplantibacillus plantarum Lactiplantibacillus pentosus*, *Pediococcus parvulus, Levilactobacillus brevis*, *Lacticaseibacillus casei*, *Lentilactobacillus parabuchneri*,	[77]
Cucumbers	*Pediococcus pentosaceus*	[78]

Table 2.
Lactic acid bacteria used as starter cultures in fermented vegetables.

valuable nutritional sources for humans, offering high levels of B vitamins, proteins, essential amino acids, and minerals. However, their optimal pH, abundant nutrients, and high water activity also create ideal conditions for microbial growth [81]. Key bacterial genera associated with meat spoilage include *Enterobacter*, *Pseudomonas, Brochothrix, Moraxella*, *Acinetobacter, Proteus,* and *Leuconostoc*. Some of these, such as *Pseudomonas* and *Enterobacter*, can produce biogenic amines, which may pose food safety concerns [82].

LAB are essential in the fermentation of meat products, contributing to enhanced texture, improved flavor, preservation, and extended shelf life. Due to the strong buffering capacity and low content of carbohydrate in fresh meat, fermentation tends to be mild, maintaining the food's organoleptic qualities with minimal changes [80]. In traditional meat fermentation, carbohydrate supplementation enhances lactic acid production by LAB, leading to a reduction in pH and subsequent protein denaturation [83]. LAB can be used as a functional ingredient in meat products or as starter cultures in fermentation. When applied directly, LAB can be introduced in freeze-dried or fresh cultures using various methods, such as incorporation into meat batter formulations or fresh meat or by spraying onto the surface of fresh meat products [84]. Traditionally, LAB have been extensively employed as primary starter cultures in conventional meat fermentation processes (**Table 3**), converting carbohydrates into lactic acid and producing various bioactive metabolites including organic acids, diacetyl, flavor precursors, and antibacterial and antifungal peptides [95].

LAB produce antimicrobial and bioactive compounds, including bacteriocins and biosurfactants. Bacteriocins can inhibit the proliferation of pathogenic or spoilage microorganisms. Current research estimates that over 50% of all bacterial species are capable of producing bacteriocins [96]. Bacteriocins offer additional benefits, such as lowering the risk of pathogenic microorganism transmission and allowing for reduced usage of synthetic preservatives [97]. Biosurfactants are biodegradable, amphiphilic, and non-toxic compounds with antimicrobial properties that can effectively contribute to the preservation of meat and meat products [98]. Biosurfactants are typically produced in two forms: either cell-bound or secreted into the surrounding

Product	LAB	References
Dry-cured Salame Napoli	*Lactobacillus plantarum*	[85]
Nham	*Lactobacillus plantarum*	[86]
Traditional sausage	*Lactobacillus* spp., *Lactobacillus sakei*	[87]
Dry sausages	*Enterococcus faecium, Lactobacillus rhamnosus, Lactobacillus paracasei*, *Lactobacillus casei*,	[88]
Salami	*Lactobacillus plantarum*	[89]
Chouriço	*Lactobacillus plantarum*	[90]
Dry-fermented salchichón and chorizo	*Pediococcus acidilactici*	[91]
Wine-marinated meat	*Latilactobacillus sakei*	[92]
Frankfurter	*Pediococcus acidilactici*	[93]
Alheira	*Pediococcus acidilactici*	[94]

Table 3.
Lactic acid bacteria used as starter cultures in fermented meat products.

environment [99]. They possess complex and diverse chemical structures, including polysaccharide-protein complexes, lipoproteins, and lipopolysaccharides [100]. These molecules exhibit notable surface activity and can form micellar aggregates, resulting in a reduction of surface and interfacial tension.

3.4 Other emerging uses

LAB are now being strategically employed across novel food processing to broaden the functionality and diversity of fermented products. A major area of development is the use of these bacteria in fermenting plant-based foods, driven by the growing popularity of vegan and vegetarian diets. This approach supports the production of shelf-stable, nutritious, and flavorful products without the need for animal-derived ingredients [101]. Currently, LAB are used to ferment a wide range of plant-based substrates, including coconuts, oats, legumes, almonds, soy, and various grains. LAB fermentation not only aids in preservation but also boosts the nutritional value of these foods by producing bioactive compounds, probiotics, and vitamins while breaking down antinutritional factors such as lectins and phytates [102]. In recent years, the fermentation of plant-based products using LAB has seen continuous advancements. Notable developments include the ongoing screening of specialized strains for plant-based fermentation and the use of co-fermentation with multiple strains. These innovations provide a strong technical foundation for improving plant-based fermented products' flavor, nutritional content, and health benefits. Compared to traditional direct-pitch fermentation, immobilized bacteria fermentation offers better control over the process and enhances yield [103]. Despite the extensive applications of LAB in food production, several challenges remain in optimizing their performance and expanding their use, prompting exploration of innovative solutions to enhance their efficacy.

4. Challenges and future directions

Despite the proven efficacy of LAB in enhancing food safety and shelf life through various preservation mechanisms, several challenges hinder their full-scale application and optimization in the food industry. One major limitation is the strain-specificity of LAB functionalities. Not all strains within a species exhibit consistent capabilities in terms of acid production rates, bacteriocin synthesis efficiency, or EPS formation yields. This variability complicates standardization and may lead to inconsistent outcomes in industrial fermentation processes. Moreover, environmental factors such as temperature, pH, oxygen levels, and substrate composition significantly influence key performance metrics, including growth kinetics (e.g., doubling time, lag phase duration), metabolic activity (e.g., carbohydrate utilization rates, organic acid production), and metabolite production (e.g., bacteriocin and EPS yields). These factors can limit LAB robustness across diverse food matrices [36]. To address this, advanced strain screening using high-throughput phenotyping and genomic tools can identify strains with robust performance under varied conditions. Additionally, co-culture strategies, where complementary LAB strains are combined, could enhance consistency and functionality in fermentation processes.

Another pressing challenge is the regulatory and consumer perception landscape. While LAB are generally recognized as safe (GRAS), the use of genetically modified LAB to enhance preservation functions (e.g., increased bacteriocin yield or tailored

EPS production) raises ethical, legal, and safety concerns, particularly in regions with strict genetically modified organism (GMO) policies [26]. Potential solutions include developing non-GMO approaches, such as directed mutagenesis or adaptive laboratory evolution, to improve LAB traits while maintaining regulatory compliance. Consumer education campaigns highlighting the safety and benefits of LAB-based biopreservation could also improve acceptability. Additionally, bacteriocins, though effective against many foodborne pathogens, often exhibit a narrow antimicrobial spectrum, limiting their efficacy against a broad range of spoilage organisms or pathogens. This can compromise food safety by allowing resistant or unaffected microbes to proliferate, potentially leading to spoilage or health risks. Moreover, resistance development among target microorganisms is an emerging concern, as prolonged exposure to bacteriocins may select for resistant strains, reducing their long-term efficacy and posing risks of cross-resistance to other antimicrobials [41]. These issues underscore the need for careful monitoring of microbial populations in food systems and the development of broad-spectrum bacteriocins or synergistic antimicrobial combinations. Interactions between LAB metabolites and other food components can also lead to undesired sensory attributes, such as off-flavors or texture changes, affecting consumer acceptability of fermented products [33, 104, 105]. Reformulating food matrices or optimizing fermentation conditions (e.g., temperature, pH) could mitigate these sensory challenges.

Moving forward, future research should leverage systems biology and multi-omics approaches (genomics, transcriptomics, proteomics, metabolomics) to unravel regulatory networks and optimize LAB metabolic outputs, such as acid production efficiency and bioactive compound synthesis. Synthetic biology offers promising tools to engineer LAB with enhanced preservation traits, such as increased bacteriocin production or environmental resilience, while maintaining safety and sensory quality (**Figure 2**) [106]. For example, CRISPR-based gene editing could fine-tune metabolic pathways to broaden bacteriocin spectra or enhance EPS production.

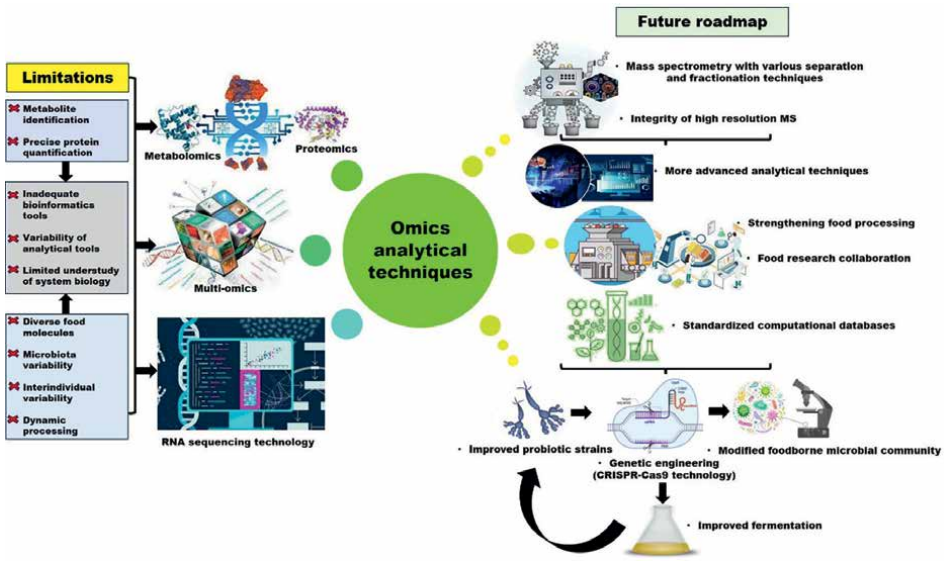

Figure 2.
Proposed roadmap for integrated omics technique application in LAB for food preservation. Reproduced with permission from Okoye et al. [106].

Integrating artificial intelligence (AI) and machine learning into fermentation monitoring can enable real-time optimization of microbial growth rates, metabolite production, and environmental conditions, improving process efficiency. To address bacteriocin limitations, research into novel antimicrobial peptides or combination therapies (e.g., bacteriocins paired with organic acids or plant-derived antimicrobials) could expand their spectrum and reduce resistance risks, enhancing food safety. Emphasis should also be placed on exploring underutilized or novel LAB strains from traditional or extreme environments, as these may harbor unique preservation potentials suitable for next-generation functional foods. For instance, LAB isolated from fermented foods in harsh climates could offer robust performance in challenging industrial settings. A multidisciplinary approach combining microbial ecology, food science, and biotechnology is essential to overcome current limitations and harness LAB's full potential in sustainable and safe food preservation [106].

5. Conclusion

LAB are indispensable in food fermentation, offering robust mechanisms for preservation, safety, and quality enhancement across diverse food matrices. Their ability to produce organic acids, bacteriocins, vitamins, and exopolysaccharides underpins their efficacy in extending shelf life, inhibiting pathogens, and enriching nutritional and sensory profiles. From traditional dairy and meat products to modern plant-based and probiotic applications, LAB demonstrate versatility and adaptability, aligning with consumer demands for clean-label, functional, and sustainable foods. However, challenges such as strain-specific functionality, environmental variability, and regulatory hurdles surrounding genetically modified strains necessitate innovative solutions. The integration of systems biology, multi-omics, and synthetic biology offers promising avenues to enhance LAB's metabolic outputs, enabling precise control over preservation traits while maintaining safety and consumer acceptability. Additionally, artificial intelligence and machine learning can optimize fermentation processes in real time, improving consistency and efficiency. Exploring novel LAB strains from traditional or extreme environments may uncover unique preservation potentials, further expanding their utility in next-generation foods. This chapter highlights the need for a multidisciplinary approach, combining microbial ecology, food science, and biotechnology, to overcome current limitations and fully harness LAB's capabilities. By addressing these challenges, LAB can continue to play a transformative role in sustainable food preservation, ensuring safe, nutritious, and appealing products that meet global food security and health demands. Future research should prioritize scalable, consumer-friendly innovations to solidify LAB's position as a cornerstone of modern food technology.

Conflict of interests

The authors declare no conflict of interest.

Abbreviations

AI	artificial intelligence
EMP	embden–meyerhof–parnas pathway

EPS	exopolysaccharides
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
GMO	genetically modified organism
GTP	guanosine triphosphate
HPK	histidine protein kinase
IMP	immunity proteins
IP	inducer peptide
LA	lactic acid
LAB	lactic acid bacteria
LDH	lactate dehydrogenase
NSLAB	non-starter lactic acid bacteria
PK	phosphoketolase pathway
RBP	riboflavin biosynthesis pathway
RR	response regulator
Ru5P	ribulose-5-phosphate
TCA	tricarboxylic acid cycle

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Chapter 3

Toward the Establishment of Yeast Alcoholic Fermentation Design Technology

Daisuke Watanabe

Abstract

Alcoholic fermentation is one of the most important microbial functions utilized by humans, yet the mechanisms that regulate its efficiency are not fully understood. This review focuses on recent progress in understanding how fermentation capacity can be modified in the yeast *Saccharomyces cerevisiae*, particularly through studies of sake yeast strains. A key discovery was the identification of *RIM15*, a gene encoding a conserved Greatwall-family protein kinase, as a major inhibitory factor in alcoholic fermentation. Remarkably, a single-step deletion of *RIM15* was found to significantly accelerate fermentation, marking a breakthrough in fermentation research. Further studies revealed that this effect is linked to downstream alterations in the synthesis of 1,3-β-glucan, a principal constituent of the yeast cell wall, that has a trade-off relationship with fermentation performance. These findings provide a clear and accessible target for designing yeast strains used in fermentation industry. Consequently, this research has opened the door to developing yeast with enhanced fermentation ability without the need for conventional genetic modification. Although still in their early stages, such alternative approaches may prove useful in contexts where regulatory or consumer concerns about genetic engineering are significant. As this approach matures, it is poised to become a technological innovation that not only enhances fermentation-based industries but also opens new frontiers in microbial biotechnology.

Keywords: alcoholic fermentation, *Saccharomyces cerevisiae*, Rim15p, 1,3-β-glucan synthesis, fermentation design

1. Introduction

Alcoholic fermentation is a metabolic process in which sugars such as glucose are broken down into ethanol and carbon dioxide *via* glycolysis, primarily by microorganisms like the yeast *Saccharomyces cerevisiae*. This process has been utilized since ancient times in the production of alcoholic beverages and fermented foods such as bread. In the mid-nineteenth century, Louis Pasteur demonstrated that alcoholic fermentation is driven by microbial activity—a discovery that profoundly shaped the emergence of microbiology and biochemistry as scientific disciplines. The elucidation

of the glycolytic pathway (Embden–Meyerhof pathway) by Gustav Embden and Otto Fritz Meyerhof in the 1930s further deepened our understanding, making alcoholic fermentation one of the best-characterized metabolic pathways to date. In *S. cerevisiae*, the enzymes involved in this process and the genes encoding them have been comprehensively identified.

With recent advances in synthetic biology, our ability to engineer microbial metabolism has progressed rapidly. Techniques such as genetic recombination and genome editing now allow us to reprogram microorganisms as living factories, unlocking new possibilities in fields ranging from food production to energy and pharmaceuticals. In this context, the idea of tailoring the fermentative capacity of yeast may appear increasingly attainable. For example, ethanol production by yeast underpins bioethanol manufacturing. Enhancing its efficiency could help accelerate the transition from fossil fuels and contribute to climate change mitigation and the realization of a more sustainable society. In the liquor industry, growing health consciousness has led to increased demand for low-alcohol beverages, which may be addressed by developing yeast strains with selectively attenuated ethanol production. Despite extensive characterization of yeast fermentation mechanisms and advances in genetic and metabolic engineering, improving fermentation efficiency remains challenging due to the complexity of regulatory networks and unintended side effects on yeast physiology and product quality. This review explores the current state of research on designing yeast strains with customized fermentation capabilities, highlighting recent advances and future directions in the field.

2. Facing the challenges of engineering alcoholic fermentation

Efforts to reprogram fermentation performance using synthetic biology have proven far more difficult than initially anticipated. In *S. cerevisiae*, alcoholic fermentation is not merely a specialized carbon metabolic pathway but forms a core part of cellular energy metabolism *via* glycolysis. Thus, this pathway includes essential genes such as *PGK1*, which encodes phosphoglycerate kinase responsible for ATP generation through substrate-level phosphorylation [1]. During the early stages of recombinant DNA technology, modifying such essential genes was highly challenging due to the lethality associated with gene disruption. Moreover, the final step of ethanol production is catalyzed by a family of alcohol dehydrogenase genes (*ADH1* to *ADH5*) [2], which exhibit functional redundancy. As a result, altering a single gene often failed to produce a clear phenotypic change, making targeted interventions even more complex.

In 1989, Schaaff et al. [3] created a series of yeast strains that overexpressed individual genes involved in alcoholic fermentation. While they succeeded in enhancing specific enzyme activities, they observed no significant increase in the ethanol production. This outcome suggested that glycolysis and subsequent alcoholic fermentation are tightly regulated, robust metabolic processes governed by finely tuned gene expression and enzyme activity. It also demonstrated the limitations of simplistic genetic modifications and led to a temporary stagnation in the application of synthetic biology to this field. A common strategy in synthetic biology is to suppress side-product pathways and redirect metabolic flux toward the desired product. However, central metabolic pathways like glycolysis and alcoholic fermentation are highly interconnected with multiple branches, making it difficult to even define which fluxes should be considered as side-pathways.

In this context, a breakthrough was reported by Alper et al. [4]. By modifying global transcriptional regulators in yeast, they successfully increased ethanol productivity under laboratory conditions. This was achieved by enhancing tolerance to high glucose concentrations and ethanol stress, thereby reducing cell death. Published in *Science*, this pioneering study demonstrated the feasibility of engineering alcoholic fermentation using synthetic biology. It also established the now widely accepted idea that improving stress tolerance contributes to enhanced fermentative performance—an approach that has influenced the breeding of industrial yeast strains. For instance, in the sake yeast industry, Kyokai no. 11 [5]—an ethanol-tolerant strain derived of Kyokai no. 7—was developed to prevent from cell death during sake fermentation. However, these advancements raised further questions: Is it sufficient to focus solely on metrics like cell viability or population size to optimize fermentation? A renewed emphasis on cellular-level metabolic capacity may enable more efficient and precisely engineered fermentative processes.

3. Alcoholic fermentation control unveiled by sake yeast genetics

We investigated characteristic features of sake yeast, which exhibits high alcoholic fermentation capacity, by conducting whole-genome sequencing analysis [6] and comparative transcriptome analysis [7], using a laboratory strain with a well-characterized genome as a reference. It should be noted that both types of yeast strains used are taxonomically classified as *S. cerevisiae*. Consequently, we revealed that a loss-of-function mutation in the *RIM15* gene, which encodes a Greatwall-family protein kinase, contributes to the enhanced fermentative ability of sake yeast [8]. A frameshift mutation caused by a single nucleotide insertion ($rim15^{5054\_5055insA}$) was found in representative sake yeast strains (Kyokai no. 6, 7, 9, and 10) and in their derived strains. Rim15p, the gene product, plays a role in inducing the expression of various stress-responsive genes during sake mash fermentation; however, in sake yeast, the expression of these genes is markedly suppressed. Moreover, deletion of a functional *RIM15* gene in laboratory yeast increased its fermentative capacity to levels approaching that of sake yeast. These findings led us to conclude that the loss-of-function mutation in *RIM15* is a major determinant of the high alcoholic fermentation ability of sake yeast.

Based on these findings, we advanced to breeding other industrial yeast strains by modifying *RIM15* (**Figure 1**). For example, yeast-driven alcoholic fermentation is used in bioethanol production, which is increasingly being adopted as a renewable energy source. Fermentation trials using sugarcane molasses at high temperature (35°C) showed that deletion of *RIM15* in the industrial yeast strain PE-2, widely used in Brazil, shortened the fermentation time by approximately 27% [9]. In lager beer production, *Saccharomyces pastorianus*, a low-temperature-adapted hybrid between *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*, is typically used. Disruption of the *S. cerevisiae*-derived *RIM15* gene in this species promoted alcoholic fermentation in high-gravity wort [10]. Importantly, the industrial strains used in these studies were already optimized for their respective fermentation environments, yet further improvements in fermentation performance were achieved by *RIM15* disruption. These results underscore the potential utility and versatility of targeting Rim15p in fermentation design.

How does Rim15p regulate cellular metabolism to inhibit alcoholic fermentation, and for what physiological purpose? (**Figure 2**). Greatwall-family protein kinases, including

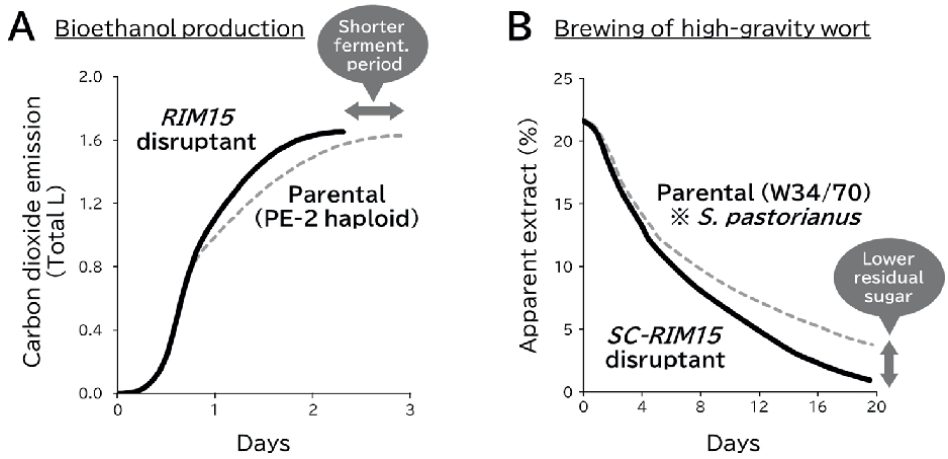


Figure 1.
Breeding of industrial yeast strains through modification of the RIM15 *gene (A) Example of bioethanol production using sugarcane molasses [9]. The rate of carbon dioxide evolution during alcoholic fermentation was increased, resulting in a shortened fermentation time. (B) Example of beer brewing using high-gravity wort [10]. The rate of decrease in apparent extract (mainly sugars in the wort) was accelerated, leading to a reduced residual sugar level.*

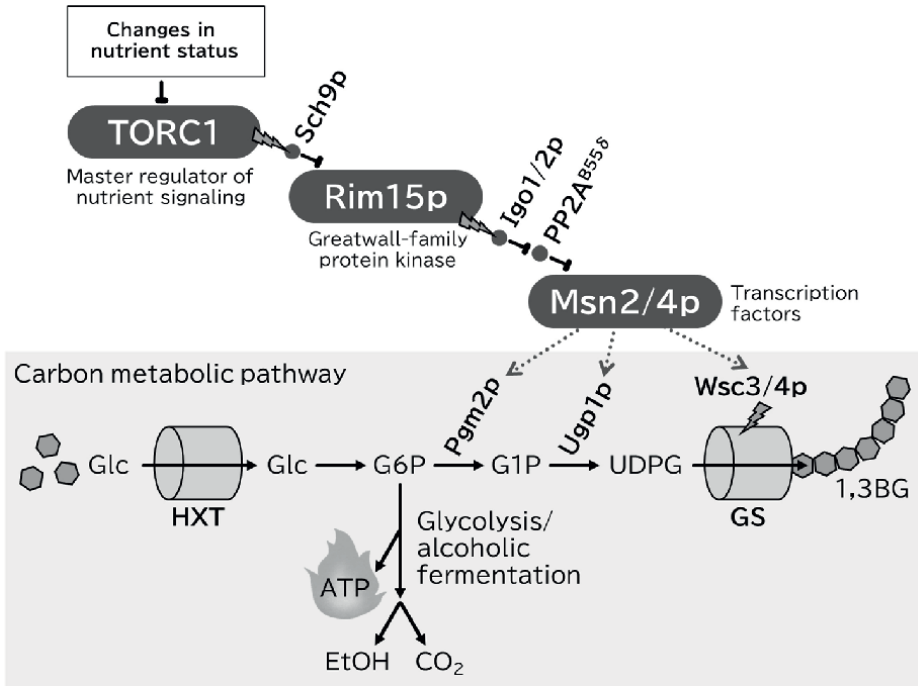


Figure 2.
Regulatory mechanism of alcoholic fermentation based on sake yeast research. Glc: glucose, G6P: glucose-6-phosphate, G1P: glucose-1-phosphate, UDPG: UDP-glucose, 1,3-BG: 1,3-β-glucan, EtOH: ethanol, HXT: hexose transporter, GS: 1,3-β-glucan synthase.

Rim15p, are conserved cell cycle regulators in eukaryotes [11, 12]. However, the full picture—including their phosphorylation targets, upstream signaling, and downstream physiological effects—remains incompletely understood. In *S. cerevisiae*, Rim15p

activity is known to be suppressed by the target of rapamycin complex 1 (TORC1), a master regulator of nutrient signaling, *via* downstream Sch9p kinase [13]. Rim15p, through its direct phosphorylation targets α-endosulfins Igo1/2p, inhibits the B55δ-bound form of protein phosphatase 2A ($PP2A^{B55\delta}$), thereby modulating various life processes *via* protein dephosphorylation. Our recent study has revealed that $PP2A^{B55\delta}$ suppresses nuclear localization of transcription factors Msn2/4p during alcoholic fermentation [14]. Thus, Rim15p is considered to mediate gene expression through Msn2/4p in response to nutrient status during alcoholic fermentation. The transcriptomic impact of Rim15p dysfunction in sake yeast can be attributed to impairment of this Msn2/4p pathway [7].

Msn2/4p broadly regulates genes involved in stress responses and cellular metabolism, but it notably induces the expression of genes related to the synthesis of 1,3-β-glucan, a major component of the yeast cell wall. 1,3-β-glucan is a polysaccharide composed of linearly linked glucose units, and its precursor UDP-glucose is derived from glucose-6-phosphate, an intermediate of glycolysis. Msn2/4p induces not only genes encoding the metabolic enzymes *PGM2* (phosphoglucomutase) and *UGP1* (UDP-glucose pyrophosphorylase), which both contribute to UDP-glucose production, but also *WSC3*/*WSC4*, which encode cell wall sensor proteins that activate 1,3-β-glucan synthase [14, 15]. This dual regulatory mechanism facilitates 1,3-β-glucan synthesis by coordinating substrate supply and signal transduction. Indeed, sake yeast strains lacking Rim15p or Msn2/4p exhibit suppressed expression of these genes and a thinner 1,3-β-glucan cell wall layer compared to laboratory yeast (**Figure 3**) [15]. Furthermore, changes in 1,3-β-glucan content were found to inversely correlate with alcoholic fermentation capacity in laboratory yeast [14, 15].

In summary, the sake yeast-specific loss-of-function mutation in *RIM15* enhances alcoholic fermentation by disrupting the Rim15p–Msn2/4p pathway, a key regulator of cell wall synthesis *via* 1,3-β-glucan production. This pathway promotes the expression of genes such as *PGM2*, *UGP1*, and *WSC* family members, which coordinate both metabolic enzyme activity and signal transduction required for 1,3-β-glucan biosynthesis. Impairment of this regulatory circuit reduces cell wall synthesis and consequently increases the availability of glucose for glycolysis and ethanol production.

Collectively, these findings suggest that glycolysis—through which glucose is catabolized for energy—and 1,3-β-glucan synthesis—through which glucose is polymerized—compete for glucose flux in a trade-off relationship, governed by

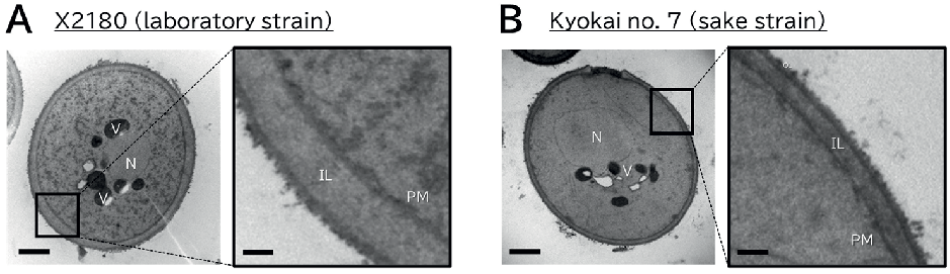


Figure 3.
Yeast cell wall in fermenting sake mash [15]. Transmission electron microscopy (TEM) images of yeast cells in fermenting sake mash (10 days after the onset of the experiment). (A) A representative cell of laboratory yeast strain X2180. (B) A representative cell of sake yeast strain Kyokai no. 7. The right panel shows a magnified view of the region outlined in black in the left panel. V: vacuole; N: nucleus; PM: plasma membrane; IL: inner layer of the cell wall (mainly composed of 1,3-β-glucan). Scale bars represent 1 μm (left) and 200 nm (right).

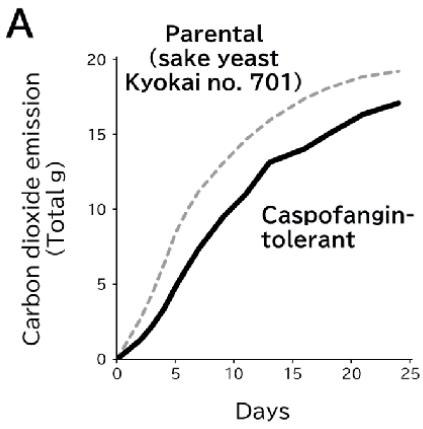
the Rim15p–Msn2/4p pathway in *S. cerevisiae*. While glycolysis and alcoholic fermentation occur in the cytoplasm, 1,3-β-glucan synthase is located on the plasma membrane, where it polymerizes UDP-glucose and exports the polymer from the cytoplasm. This spatial separation may effectively sequester the carbon source away from glycolytic enzymes. Although the yeast cell wall is well known for its role in maintaining cell shape and protecting against external stress, our study has revealed a new biological significance: its involvement in carbon metabolism regulation.

4. Alcoholic fermentation design without genetic modification

Through previous research, we identified the Rim15p-Msn2/4p pathway and its downstream 1,3-β-glucan synthesis as major factors that antagonistically interfere with alcoholic fermentation. While altering the functions of Rim15p or Msn2/4p typically requires genetic modification or genome-editing techniques, similar functional changes could potentially be achieved by targeting 1,3-β-glucan through drug screening technologies based on research on antifungal agents [16, 17]. Specifically, by exploring mutated strains or diverse strains isolated from natural environments, it is possible to efficiently identify strains with unique alcoholic fermentation properties. Among these, strains that can be directly applied in industries like the food sector, where resistance to genetic modification is strong, are particularly promising. This chapter introduces a case study [14] in which the alcoholic fermentation capability of sake yeast was lowered without using genetic modification techniques, highlighting its practical applications and potential.

We used caspofungin [18], an antifungal agent that targets 1,3-β-glucan synthase, widely used in experiments and whose mechanism of action is well understood. Using sake yeast strain Kyokai no. 701 as the parent strain, we introduced mutations into the yeast cells by ethyl methanesulfonate treatment and cultured them in the presence of caspofungin. We isolated six resistant strains based primarily on their high resistance to caspofungin; these strains were expected to commonly have enhanced 1,3-β-glucan synthase activity. At this stage, their fermentation capacity had not yet been assessed. However, subsequent fermentation tests revealed that all resistant strains exhibited significantly decreased alcoholic fermentation ability. These results demonstrate that selecting strains by antifungal resistance alone can serve as an effective screening criterion to design fermentation properties through nongenetic modification techniques.

We conducted small-scale sake fermentation tests using the most phenotypically stable resistant strain (**Figure 4**). The results showed that, under the same conditions as the parent strain, the amount of carbon dioxide produced during fermentation decreased over the fermentation period, and the final alcohol content dropped from 19.4% in the parent strain to 16.8% in the resistant strain. Similarly, the sake meter value (SMV; inversely correlated with the specific gravity of sake), decreased, suggesting that the reduction in alcohol content resulted in a sweeter sake. Interestingly, there was no significant change in key indicators of sake flavor, such as acidity, amino acid content, and major aroma compounds (i.e., isoamyl acetate and ethyl caproate). This indicates that the effects on other metabolic pathways were minimal. Typically, when mutations are introduced, unexpected changes can occur, often leading to undesirable changes in sake flavor. In contrast, our fermentation design developed in this study specifically influences alcoholic fermentation with minimal effects on other metabolic pathways, making it a notable approach.



B

	Parental	Tolerant
Ethanol (vol%)	19.4 ± 0.8	*16.8 ± 0.4 ↓
SMV	3.6 ± 0.1	*−2.9 ± 0.4 ↓
Total acid	3.2 ± 0.3	3.0 ± 0.2
Amino acid	2.3 ± 0.1	2.2 ± 0.2
Isoamyl acetate (ppm)	5.8 ± 0.2	6.3 ± 0.0
Ethyl caproate (ppm)	1.1 ± 0.1	1.2 ± 0.2

(*t test, $p < 0.05$)

Figure 4.
Sake yeast strain with low fermentation capacity developed through non-GM techniques. [14]. (A) Carbon dioxide production during alcoholic fermentation. (B) Component analysis at the end of fermentation.

However, at present, the decrease in alcohol content is still small and does not yet have the impact needed for the production of low-alcohol sake. We aim to further refine this technology and promote its application through continued research. To this end, we are working on two research topics. First, we seek to identify novel regulatory factors independent of the Rim15p–Msn2/4p pathway. For example, we have discovered that TORC1, a master regulator of nutrient signaling, may regulate alcoholic

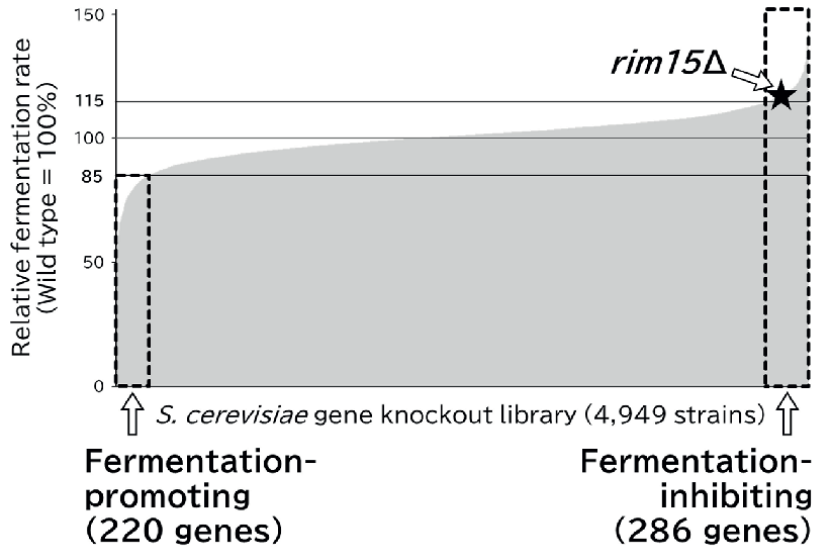


Figure 5.
Genome-wide screening of regulators of alcoholic fermentation. Alcoholic fermentation was assessed by measuring carbon dioxide production three days after the onset of fermentation in a nutrient-rich medium for yeast. The relative values are shown as percentages compared to the wild-type strain (set at 100%). Genes whose deletion reduced fermentation rate to 85% or lower were identified as candidate positive regulators of fermentation, while those whose deletion increased the rate to 115% or higher—including RIM75*—were identified as candidate negative regulators.*

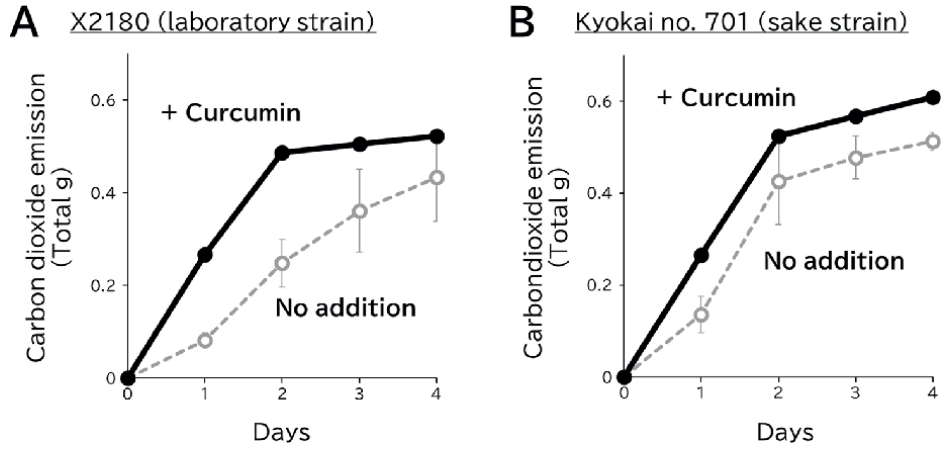


Figure 6.
Enhancement of alcoholic fermentation by curcumin addition [20]. Alcoholic fermentation was evaluated by measuring carbon dioxide production in a fermentation test using a nutrient-rich medium for yeast. The dotted lines indicate results under curcumin-free conditions, while the solid lines indicate results with curcumin added (1 mg/mL). (A) Using laboratory yeast strain X2180-1 A. (B) Using sake yeast Kyokai No. 701.

fermentation through a parallel pathway that does not rely on Rim15p–Msn2/4p as well [19]. Furthermore, using a library of gene knockout strains of *S. cerevisiae* (approximately 5000 strains), we have identified several candidate regulatory factors involved in alcoholic fermentation ability (**Figure 5**). Identifying novel regulatory factors through these studies could potentially lead to additive or synergistic effects when combined with modifications to the Rim15p-Msn2/4p pathway.

Another direction is the development of inhibitors and promoters that control alcoholic fermentation using additives. We have conducted research to identify plant-derived compounds applicable to the food industry and discovered that curcumin, a compound found in turmeric, significantly promotes alcoholic fermentation (**Figure 6**) [20]. This effect was also observed in sake yeast where the Rim15p-Msn2/4p pathway was inhibited, suggesting its potential as a new, alternative technology of fermentation design. By further developing these studies, precise control of alcoholic fermentation could become possible, leading to diverse applications in fermentation technologies.

5. Future possibilities of yeast alcoholic fermentation design technology

Recent advances in understanding and controlling yeast alcoholic fermentation, particularly through manipulation of the Rim15p–Msn2/4p pathway and 1,3-β-glucan synthesis, have opened new avenues for precise and customizable fermentation design. These innovations hold great promise for transforming various industries by enabling enhanced control over fermentation efficiency and product profiles, often without relying on direct genetic modification. The ability to fine-tune fermentation characteristics through such approaches allows for more flexible responses to market demands and regulatory constraints, potentially broadening the scope of yeast applications beyond traditional sectors.

In the realm of sustainable energy production, improvements in fermentation performance can directly translate into higher bioethanol yields and reduced processing

times, which help lower operational costs and energy consumption. This contributes to a more economically viable biofuel industry and supports global initiatives aimed at reducing greenhouse gas emissions and dependence on fossil fuels. Additionally, engineered fermentation processes could be optimized to utilize diverse and nonfood biomass feedstocks, further enhancing the sustainability and scalability of bioethanol production [21, 22].

Within the beverage industry, the capacity to precisely regulate ethanol output has significant implications. With growing health awareness worldwide, there is a rising consumer demand for low-alcohol and nonalcoholic beverages [23] that retain desirable flavors and mouthfeel. Through tailored yeast fermentation design, it becomes possible to produce such beverages without compromising sensory qualities, which often suffer when alcohol content is artificially reduced. This could expand product lines and open new markets for breweries, wineries, and sake producers alike. Furthermore, controlling fermentation parameters could also lead to innovations in flavor profile customization, enhancing the uniqueness and appeal of fermented products. Another important aspect is the ability to develop yeast strains with enhanced or attenuated fermentation capabilities using nongenetic modification techniques such as selective breeding, chemical mutagenesis, or screening for naturally occurring variants [24]. This is particularly valuable in regions or industries where regulatory frameworks are stringent or consumer acceptance of genetically modified organisms is limited. Such approaches provide practical pathways to commercialize improved yeast strains more rapidly and with fewer legal or public hurdles, facilitating innovation in fermented foods, beverages, and bioproducts.

Despite these exciting prospects, several challenges remain before fermentation design technology can be fully realized at an industrial scale. Ensuring the genetic and phenotypic stability of newly developed yeast strains during long-term use is critical to maintaining consistent fermentation performance. Moreover, scale-up from laboratory or pilot studies to commercial production often reveals unforeseen issues related to process variability, microbial interactions, and environmental stressors that must be addressed. Maintaining desirable sensory attributes, nutritional value, and product safety while modifying fermentation pathways also requires careful optimization.

Nevertheless, the continuous accumulation of knowledge about yeast physiology and regulatory networks, combined with advances in fermentation engineering and high-throughput screening, provides a robust foundation for overcoming these challenges. As these technologies mature, they are expected to foster new fermentation-based products and processes that are more efficient, sustainable, and responsive to societal needs. Ultimately, the integration of fermentation design technology into industrial practice promises to not only enhance existing fermentation industries but also catalyze the emergence of novel biotechnological applications, contributing broadly to food security, renewable energy, and sustainable manufacturing.

6. Conclusion

In this chapter, the efforts to fermentation design of yeast, along with the history of applying synthetic biology, current challenges, and future possibilities, were discussed. Our research on sake yeast has led to a breakthrough by identifying the presence of Rim15p, the major inhibitory factor of alcoholic fermentation, and shedding light on the metabolic control mechanism mediated by Rim15p. By applying

these findings, we have demonstrated the potential for altering the alcoholic fermentation capability of industrial yeast strains. Although alcoholic fermentation design technology without genetic modification is still under development, if a technology that allows for the precise modification of the most widely and historically utilized microbial function—alcoholic fermentation—can be established, such an advance would constitute a significant turning point in the application of yeast for industrial fermentation. We are confident that, with ongoing research progress, this innovative technology will continue to evolve and ultimately bring sustainable value to the field of microbial manufacturing, from fermented foods to energy production.

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Microbial Diversity and Functional Applications

Chapter 4

New Advances in the Role of Yeasts in Table Olive Fermentation

Patricia Gil-Flores, David Penco-Parra and Joaquin Bautista-Gallego

Abstract

Table olive fermentation is a very important process that renders the naturally bitter raw fruit suitable for consumption. Microorganisms, particularly yeasts, are responsible for the process and play a significant role in the development of flavor, texture, and preservation. During fermentation, yeasts contribute to the production of a range of volatile molecules, including esters, alcohols, and phenols, that are largely responsible for the impartation of the characteristic aromas and flavors to the final product. A relevant example of this process is the formation of ethyl acetate, a molecule that imparts fruity notes to olives. Besides the role in flavor development, yeasts can also produce acidification through the fermentation of the sugars present in the olives. This acidification process by yeast, though normally low, can help to preserve the olives through inhibition of spoilage microorganism growth, thus enhancing the product's shelf-life. Yeasts also engage in significant interactions with lactic acid bacteria during the fermentation. While it is established that lactic acid bacteria play a prominent role in the acidification, the metabolic processes carried out by yeasts can influence the growth and functioning of the bacteria, resulting in a more harmonious and effective fermentation. Such interactions contribute to improving the quality and safety of the olives. Recent studies have also demonstrated the potential for using new yeast species. Therefore, these yeasts provide unique fermentation characteristics that result in more complex and richer flavor profiles, creating new possibilities for high-value, artisanal olive products and addressing growing consumer demand for natural and functional foods.

Keywords: yeast, table olive, fermentation, lactic acid bacteria, technological applications, microbiology, food science

1. Introduction

The global table olive industry represents one of the oldest and most economically significant agricultural sectors, with particular relevance in Mediterranean countries. Olives have been cultivated for over 6000 years, and today, olive production continues to be central to both the economies and cultural practices of many nations. According to the Food and Agriculture Organization [1], the world's table olive production was over 2.8 million tons in 2023. Moreover, the International Olive Oil Council [2] estimates that table olives' world production reached around 2,856,000 tons in the

2024/2025 season (**Figure 1**). Thus, it can be highlighted that the main producers are European Union, Turkey and Egypt. Furthermore, focusing on the European Union, it can be detected that the three main producers are Spain, Greece and Italy (96.6% of the EU production) (**Figure 2**). In addition, the table olive sector in Spain is concentrated in Sevilla, Córdoba, Málaga, Badajoz and Cáceres (98% of the national production) and therefore 78% of the table olive producers are located in Andalusia or Extremadura [3].

These production data are global, but it must be taken into account that there are three main types of elaborations: (a) the green Spanish style, (b) directly brined green olives, and (c) black olives which includes naturally black olives (Greek style) and ripe olives by alkaline oxidation (Californian style) [4–6]. In recent years, green "seasoned" table olives have grown in popularity among consumers, driven by a growing interest in traditional and naturally processed foods [3, 5, 7].

Olives are drupes that, among other traits, contain a bitter compound (oleuropein), relatively low levels of sugars (2.6–6.0%), and a high oil content (12–30%), although these parameters vary depending on the cultivar and degree of ripeness [5]. Due to these attributes, olives are not suitable for direct consumption, which has led to the development of diverse processing methods aimed at making them palatable—methods that differ significantly across regions. Furthermore, the dual characteristic

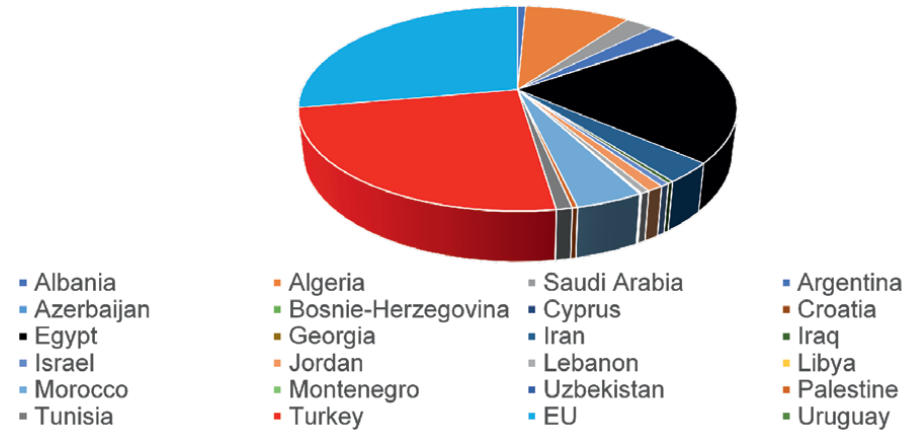

Figure 1.
Predicted world production (×1000 tonnes) for 2024/2025 season of table olives by countries/regions. Source: Adapted from IOC [2].

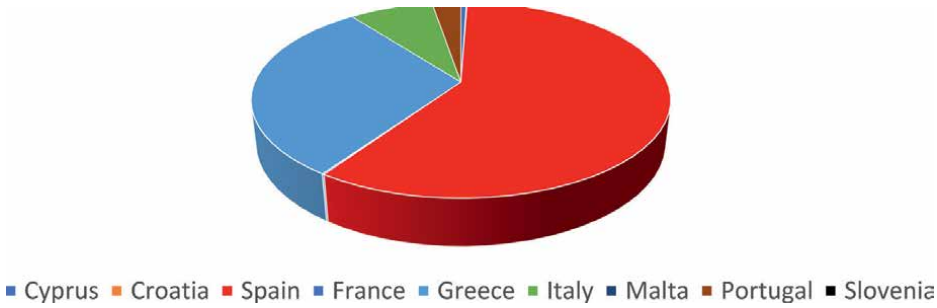

Figure 2.
Predicted EU production (×1000 tonnes) for 2024/2025 season of table olives by countries/regions. Source: Adapted from IOC [2].

of table olives, their content of both nutritionally valuable constituents (such as monounsaturated and polyunsaturated fatty acids, absence of trans fats, dietary fiber, vitamins, and minerals) and bioactive compounds [5, 8] has led to their consideration as a potential functional food. A food product is considered functional when it exerts a beneficial effect on one or more physiological functions, beyond its basic nutritional effects, and thereby contributes to an improvement in health (natural antioxidant compounds, fiber, etc.) or a reduction in the risk or progression of disease.

Another notable feature from a functional perspective is the potential of fermented table olives as carriers of probiotic microorganisms [9]. In this context, different studies clearly demonstrated the feasibility of selecting indigenous starter cultures that also exhibit potential probiotic properties [10, 11]. Many of these advances will likely require milder processing conditions than those traditionally employed. Consequently, ensuring rigorous control of food quality and safety will be essential to produce future table olive products.

However, table olives are fermented to enhance flavor, texture, and preservation, making the fermentation process a critical step in the production of this food. The processing of table olives typically involves brine fermentation, a process that allows the olives to undergo a complex transformation. This process is influenced by the microorganisms that are present, with yeasts and lactic acid bacteria playing a pivotal role in shaping the final product's characteristics [5]. These microorganisms are integral not only for flavor development but also for the product's nutritional value and stability [12]. Thus, a deep understanding of the fermentation microbiota, including the role of yeasts, is essential for improving the quality and consistency of table olives. Finally, the table olive sector is in a continuous search for new options to carry out the whole fermentation process in a stable and controlled way. The main challenges for table olive producers in the initial steps of the fermentation are: the high concentration of chloride salt; the high pH due to the NaOH treatment in the Spanish style; and the low microbial populations at the beginning of the fermentation process; among other factors. Several authors have reported the inherent risks of spoilage in spontaneous olive fermentation [5, 13, 14], focusing on the importance of the first month of fermentation, above all in the Spanish style. Bearing in mind these characteristics, nowadays producers are more open to the use of starter cultures, especially lactic acid bacteria starters. However, these microorganisms also have great problems growing under some circumstances, and sometimes it is necessary to reinoculate more than once to start the fermentation process. At this point, yeasts play a key role in table olive fermentation to get a faster and more stable fermentation, and to improve the growth of lactic acid bacteria (LAB) during the first month after brining.

This chapter aims to provide an overview of the role of yeasts in table olive processing, emphasizing their wide diversity influenced by the type of processing and olive cultivar, as well as their relevance as key fermentative agents. Additionally, we explore the potential application of yeasts in various stages of olive fermentation and storage, considering their capacity to produce aromatic compounds, enzymes, vitamins, and antioxidants, along with their ability to support the growth of lactic acid bacteria. Potential directions for future research involving these microorganisms are also discussed.

2. Yeast species involved in table olive fermentations

Yeasts are unicellular eukaryotic microorganisms belonging to the kingdom Fungi, primarily classified within the phyla *Ascomycota* and *Basidiomycota*. Taxonomic efforts

have currently identified approximately 2000–2200 accepted species, although this number represents only a fraction of the estimated total yeast diversity, which ranges from 20,000 to as many as 200,000 species [15–17]. Earlier estimates suggested around 1500 described species [18], but advances in molecular techniques and environmental sequencing have significantly expanded the understanding of yeast biodiversity [19].

Yeasts are widely distributed across natural ecosystems, being most commonly isolated from sugar-rich substrates such as fruits, nectar, and plant exudates [20, 21]. However, several species have demonstrated the capacity to colonize a broad range of ecological niches, including those shaped by anthropogenic activity (such as fermented food industries). Notably, certain yeast lineages have undergone processes of domestication—both unconscious and intentional—over thousands of years due to their desirable traits in food and beverage fermentation [22–24]. This domestication process has led to the selection of strains with improved fermentative performance, stress resistance, and other industrially beneficial phenotypes.

Regarding table olives fermentations, yeasts are usually responsible for the initial phase of fermentation and after which LAB, together with yeasts, complete the process [25]. As a result, yeasts represent a major microbial group involved in the fermentation of table olives. They contribute significantly to the development of the product's sensory attributes and can enhance the growth of lactic acid bacteria [26]. Nonetheless, uncontrolled proliferation of certain fermentative yeast species may lead to undesirable effects, including olive spoilage, gas pocket formation, and turbidity in the brines [27].

A great yeast diversity has been identified at table olive fermentations by many studies (**Table 1**) using classical molecular methods and metagenomic analysis. Overall, the repeated identification of certain yeast genera and species across diverse studies underscores their ecological importance and technological potential in table olive production. Among them, the genera *Aureobasidium*, *Candida*, *Debaryomyces*, *Pichia*, *Saccharomyces* and *Wickerhamomyces* emerge as the most frequently identified across various olive cultivars, countries, and processing methods. *Candida* is one of the most recurrent genera, with multiple species such as *C. aaseri*, *C. tropicalis*, *C. boidinii*, and *C. diddensiae*, being isolated from all processing styles. Thus, the latter two species are reported in several studies spanning diverse countries including Spain, France, Italy, Portugal and Morocco, reflecting their widespread occurrence and adaptability. *Aureobasidium*, and in particular *Aureobasidium pullulans*, is another widely distributed yeast, frequently identified in cultivars such as Aloreña de Málaga, Cypriot, Gemlik, and Halkidiki. Due to this distribution, *A. pullulans* has been detected in several studies [28–30], highlighting its consistent presence in table olive ecosystems and potential importance, possibly due to its osmotolerance and enzymatic versatility. However, its role in table olive fermentation is still not fully understood. Within the genus *Pichia*, species such as *P. membranaefaciens*, *P. manshurica* and *P. kudriavzevii* are frequently reported, especially in black olive preparations [30, 31, 35]. These yeasts are known for their contribution to flavor development and for their potential to inhibit spoilage microorganisms, which might explain their recurrent detection in fermentations. *S. cerevisiae*, a cornerstone in various fermentation systems, is also reported in several olive fermentations, particularly in black olives and mainly in Greek-style processes [28, 29, 31, 32, 35]. Its frequent identification may be attributed to its metabolic efficiency, ethanol production, and probiotic properties. However, one of the most identified yeasts is *W. anomalus* which has been detected in at least 24 different olive cultivars. This species exhibits high enzymatic

Specie	Cultivar	Country	References	Preparation type
Aureobasidium pullulans	Aloreña de Málaga, Cypriot, Gemlik, Halkidiki, Kalamata, Konservolia, Manzanilla, Nyons, Taggiasca	Cyprus, France, Greece, Italy, Spain, Turkey	[28–37]	Directly brined green olives, Spanish style, Black olives
Aureobasidium spp.	Kalamata	Greece	[38]	Black olives
Barnettozyma californica	Kalamata	Greece	[29]	Black olives
Brettanomyces custersianus	Halkidiki, Konservolia	Greece	[30, 35]	Spanish style, Black olives
Candida aaseri	Gemlik, Manzanilla, Nocellara dell'Etna, Nocellara messinese	Turkey, Italy, Greece, Spain	[39–42]	Directly brined green olives, Spanish style, Black olives
Candida albicans	Aloreña de Málaga	Spain	[34]	Directly brined green olives
Candida apicola	Aloreña de Málaga, Picual, Thassos	Cyprus, Greece, Spain	[32, 33, 43]	Directly brined green olives
Candida atlantica	Gemlik, Nyons	France, Turkey	[28, 40]	Black olives
Candida boidinii	Arbequina, Bosana, Cellina di Nardò, Cordovil, Galega, Gemlik, Hojiblanca, Istrana nera, Kalamata, Konservolia, Leccino, Leucocarpa, Manzanilla, Nocellara del Belice, Nocellara dell'Etna, Nocellara messinese, Negrinha de Freixo, Nyons, Peranzana	France, Italy, Morocco, Portugal, Spain, Turkey	[11, 25, 26, 28, 29, 39, 40, 44–53]	Directly brined green olives, Spanish style, Black olives
Candida citrea	Cordovil, Galega	Portugal	[52]	Direct brined green olives, Black olives
Candida diddensiae	Aloreña de Málaga, Arbequina, Bosana, Cypriot, Kalamata, Manzanilla, Nocellara del Belice, Nocellara dell'Etna, Nocellara messinese, Picual, Taggiasca	Cyprus, Italy, Spain	[11, 26, 32, 34, 36, 37, 39, 41, 42, 44, 45, 49, 54]	Directly brined green olives, Spanish style, Black olives
Candida etchelsii	Thassos	Greece	[43]	Black olives
Candida ethanolica	Amfissis, Kalamata	Greece	[35]	Black olives
Candida glabrata	Galega, Manzanilla	Portugal	[55, 56]	Directly brined green olives, Black olives
Candida glaebosa	Arbequina	Spain	[46]	Directly brined green olives
Candida gropengiesseri	Arbequina	Spain	[46]	Directly brined green olives

Specie	Cultivar	Country	References	Preparation type
Candida intermedia	Leucocarpa	Italy	[57]	Directly brined green olives
Candida ishiwadae	Cellina di Nardò	Italy	[53]	Black olives
Candida maris	Manzanilla	Portugal	[55]	Directly brined green olives
Candida molendinolei	Kalamata	Greece	[29]	Black olives
Candida naeodendra	Kalamata	Greece	[29]	Black olives
Candida norvegica	Cordovil, Galega, Negrinha de Freixo	Portugal	[50, 52]	Directly brined green olives, Black olives
Candida oleophila	Cordovil, Galega	Portugal	[52]	Direct brined green olives, Black olives
Candida olivae	Aloreña de Málaga, Konservolia	Greece	[34, 58]	Black olives
Candida parapsilosis	Aloreña de Málaga, Arbequina, Brandofino, Manzanilla, Nocellara del Belice, Passanulara, Taggiasca	Italy, Portugal, Spain	[34, 37, 45, 55, 59]	Directly brined green olives, Spanish style
Candida sake	Aloreña de Málaga, Cordovil, Galega	Portugal, Spain	[34, 52]	Directly brined green olives, Black olives
Candida silvae	Cordovil, Galega	Portugal	[52]	Directly brined green olives, Black olives
Candida sorbosa	Arbequina	Spain	[46]	Direct brined green olives
Candida thaimueangensis	Aloreña de Málaga, Manzanilla	Spain	[34, 41, 44]	Directly brined green olives, Spanish style
Candida tropicalis	Aloreña de Málaga, Gordal, Manzanilla, Negrinha de Freixo, Nocellara messinese, Thassos	Greece, Italy, Portugal, Spain	[34, 36, 39, 41, 43, 44, 50]	Directly brined green olives, Spanish style, Black olives
Candida utilis	Galega	Portugal	[56]	Black olives
Candida valida	Cordovil, Galega	Portugal	[52]	Directly brined green olives, Black olives
Candida versatilis	Thassos	Greece	[43]	Black olives
Candida zeylanoides	Manzanilla	Portugal	[55]	Directly brined green olives
Candida sp.	Aloreña de Málaga, Kalamata, Leccino, Nyons	France, Greece, Italy	[25, 28, 34, 38]	Black olives
Citeromyces matritensis	Aloreña de Málaga, Cordovil, Galega	Portugal, Spain	[34, 52]	Directly brined green olives, Black olives

Specie	Cultivar	Country	References	Preparation type
Citeromyces nyonsensis	Aloreña de Málaga, Nyons, Taggiasca, Thassos*	France, Greece, Italy, Spain	[28, 34, 37, 43, 60]	Directly brined green olives, Black olives
Cryptococcus albidus	Gemlik, Leucocarpa*	Greece, Italy, Turkey	[31, 57, 61]	Directly brined green olives, Black olives
Cryptococcus carnescens	Nyons	France	[28]	Black olives
Cryptococcus flavus	Arbequina	Spain	[46]	Directly brined green olives
Cryptococcus macerans	Aloreña de Málaga	Spain	[33]	Directly brined green olives
Cryptococcus magnus	Nyons	France	[28]	Black olives
Cutaneotrichosporon cutaneum	Manzanilla	Portugal	[55]	Directly brined green olives
Cyberlindnera rhodanensis	Arbequina	Spain	[45]	Directly brined green olives
Cystobasidium minutum	Manzanilla	Portugal	[55]	Directly brined green olives
Cystofilobasidium capitatum	Cordovil, Galega	Portugal	[52]	Directly brined green olives, Black olives
Debaryomyces carsonii	Cellina di Nardò	Italy	[25]	Black olives
Debaryomyces hansenii	Aloreña de Málaga, Cellina di Nardò, Cypriot, Gemlik, Gordal, Halkidiki, Kalamata, Konservolia, Leucocarpa, Manzanilla, Negrinha de Freixo, Picual, Taggiasca, Thassos	Spain, Greece, Italy, Portugal, Turkey, Cyprus	[25, 26, 31, 32, 34, 35, 37, 44, 50, 55–57, 61, 62]	Directly brined green olives, Spanish style, Black olives
Debaryomyces sp.	Cellina di Nardò, Leccino	Italy	[25]	Black olives
Dekkera bruxellensis	*	Greece	[61]	Black olives
Diutina rugosa	Manzanilla	Portugal	[55]	Directly brined green olives
Groenewaldozyma tartarivorans	Cellina di Nardò	Italy	[25]	Black olives
Hanseniaspora guilliermondii	Aloreña de Malaga, Gordal, Manzanilla	Spain	[44, 54]	Directly brined green olives, Spanish style
Hanseniaspora sp.	Manzanilla	Spain	[41]	Spanish style
Kloeckera apiculata	Cordovil, Galega, Gemlik	Portugal, Turkey	[31, 52]	Directly brined green olives, Black olives
Kloeckera spp.	Galega	Portugal	[56]	Black olives
Kluyveromyces lactis	Arbequina, Gordal, Manzanilla*	Spain	[36, 41, 44, 45, 60]	Directly brined green olives, Spanish style

Specie	Cultivar	Country	References	Preparation type
Kluyveromyces marxianus	Manzanilla	Portugal, Spain	[41, 55]	Directly brined green olives, Spanish style
Lachancea kluyveri	Gemlik	Turkey	[31]	Black olives
Lachancea thermotolerans	Manzanilla	Spain	[41]	Spanish style
Lodderomyces elongisporus	Aloreña de Málaga	Spain	[34]	Directly brined green olives
Metschnikowia pulcherrima	Cordovil, Galega, Taggiasca	Italy, Portugal	[37, 52]	Directly brined green olives, Black olives
Meyerozyma sp.	Gemlik	Turkey	[40]	Black olives
Meyerozyma guilliermondii	Aloreña de Málaga, Arbequina, Ascolana, Brandofino, Castriciana, Cypriot, Gordal, Kalamata, Manzanilla, Negrinha de Freixo, Passanulara, Picual, Taggiasca, Thassos	Cyprus, Italy, Portugal, Spain	[32, 34, 37, 41, 43, 46, 50, 55, 60]	Directly brined green olives, Spanish style, Black olives
Millerozyma farinosa	Black olives	Greece	[61]	Black olives
Naganishia globosa	Gemlik	Turkey	[31]	Black olives
Nakazawaea molendinolei	Bosana, Manzanilla	Italy, Spain	[11, 48]	Directly brined green olives, Black olives
Nakazawaea spp.	Manzanilla	Spain	[36]	Directly brined green olives
Naumovozyma dairenensis	Arbequina	Spain	[46]	Directly brined green olives
Ogataeae spp.	Halkidiki, Kalamata	Greece	[30, 38]	Spanish style, Black olives
Papiliotrema laurentii	Gemlik, Manzanilla*	Greece, Portugal, Turkey	[31, 55, 61]	Directly brined green olives, Black olives
Pichia carsonii	Arbequina	Spain	[46]	Directly brined green olives
Pichia fermentans	Aloreña de Málaga, Cordovil, Galega	Portugal, Spain	[34, 52]	Directly brined green olives, Black olives
Pichia inconspicua	Manzanilla	Portugal	[55]	Directly brined green olives
Pichia kluyveri	Arbequina, Brandofino, Castriciana, Manzanilla, Nocellara del Belice, Nocellara dell'Etna, Passanulara	Spain, Italy	[45, 46, 49, 59]	Directly brined green olives, Spanish style
Pichia kudriavzevii	Aloreña de Málaga, Carolea, Galega, Gemlik, Leucocarpa, Manzanilla, Negrinha de Freixo, Nocellara dell'Etna, Nocellara messinese*	Bulgaria, Italy, Portugal, Spain, Turkey, USA	[5, 29, 34, 39–41, 44, 56, 57, 63]	Directly brined green olives, Spanish style, Black olives

Specie	Cultivar	Country	References	Preparation type
Pichia manshurica	Aloreña de Málaga, Cellina di Nardò, Gordal, Halkidiki, Hojiblanca, Kalamata, Manzanilla, Negrinha de Freixo, Nocellara del Belice, Nocellara dell'Etna, Peranzana, Taggiasca, Thassos	Italy, Greece, Portugal, Spain	[29, 30, 33, 34, 36, 37, 41–44, 48, 50, 51, 53, 64, 65]	Directly brined green olives, Spanish style, Black olives
Pichia membranaefaciens	Aloreña de Málaga, Arbequina, Cellina di Nardò, Cordovil, Galega, Gemlik, Gordal, Halkidiki, Hojiblanca, Kalamata, Konservolia, Leccino, Manzanilla, Negrinha de Freixo, Nocellara dell'Etna, Nyons, Taggiasca, Thassos*	France, Greece, Italy, Portugal, Spain, Turkey, USA	[25, 28, 30, 31, 34, 35, 37, 41, 43–46, 49–52, 62, 64, 66]	Directly brined green olives, Spanish style, Black olives
Pichia occidentalis	Aloreña de Málaga, Arbequina	Spain	[46, 54]	Directly brined green olives
Pichia sp.	Aloreña de Málaga, Cellina di Nardò, Kalamata, Leccino	Greece, Italy, Spain	[25, 33, 34, 38]	Directly brined green olives, Black olives
Priceomyces carsonii	Nyons	France	[28]	Black olives
Rhodotolura glutinis	Aloreña de Málaga, Arbequina, Manzanilla, Negrinha de Freixo	Portugal, Spain	[45, 50, 54, 55]	Directly brined green olives, Spanish style, Black olives
Rhodotolura graminis	Negrinha de Freixo	Portugal	[50]	Directly brined green olives
Rhodotorula mucilaginosa	Manzanilla, Nocellara dell'Etna, Taggiasca	Italy, Portugal, Spain	[37, 41, 42]	Directly brined green olives, Spanish style
Saccharomyces cerevisiae	Aloreña de Málaga, Bella di Cerignola, Bosana, Cellina di Nardò, Cordovil, Galega, Gemlik, Halkidiki, Hojiblanca, Itrana bianca, Itrana nera, Kalamata, Konservolia, Leccino, Manzanilla, Negrinha de Freixo, Nocellara del Belice, Nocellara dell'Etna, Nocellara messinese, Nyons, Peranzana, Taggiasca*	Cyprus, Greece, Italy, Portugal, Spain, Turkey	[11, 25, 28, 29, 31–35, 37, 39, 41, 42, 44, 49–55, 62, 65]	Directly brined green olives, Spanish style, Black olives
Saccharomyces paradoxus	Nyons	France	[28]	Black olives
Saccharomyces sp.	Gemlik, Kalamata, Manzanilla, Peranzana Alta Daunia	Greece, Italy, Spain, Turkey	[36, 38, 40, 67]	Directly brined green olives, Spanish style, Black olives
Schwanniomyces etchellsii	Gemlik, Konservolia, Leccino, Manzanilla, Nyons, Tanche	France, Greece, Italy, Spain, Turkey	[25, 28, 30, 40, 41, 68]	Spanish style, Black olives

Specie	Cultivar	Country	References	Preparation type
Sporobolomyces roseus	*	Greece	[61]	Black olives
Starmerella etchellsii	Amfissis	Greece	[35]	Black olives
Starmerella sorbosivorans	Gordal	Spain	[44]	Spanish style
Tausonia pullulans	Cordovil, Galega, Kalamata	Greece, Portugal	[52, 62]	Directly brined green olives, Black olives
Torulaspora delbrueckii	Cordovil, Galega, Gordal, Manzanilla, Thassos*	Greece, Portugal, Spain	[41, 43, 44, 52, 55, 60, 61]	Directly brined green olives, Spanish style, Black olives
Vanrija humicola	Manzanilla	Portugal	[55]	Directly brined green olives
Wickerhamomyces anomalus	Aloreña de Málaga, Arbequina, Ascolana, Azeitera, Bella di Cerignola, Bosana, Cellina di Nardò, Cypriot, Gemlik, Gordal, Halkidiki, Kalamata, Konservolia, Manzanilla, Negrinha de Freixo, Nocellara dell'Etna, Nocellara messinese, Nyons, Picual, Taggiasca, Tanche, Thassos, Throuba Thassou	Cyprus, Spain, Turkey, Italy, Portugal, Greece	[11, 25, 31, 32, 34–37, 39, 40, 42, 44–46, 49, 50, 53, 55, 62, 66]	Directly brined green olives, Spanish style, Black olives
Wickerhamomyces subpelliculosus	Thassos	Greece	[43]	Black olives
Wickerhamomyces sydowiorum	Konservolia	Greece	[30]	Black olives
Wickerhamomyces spp.	Kalamata, Manzanilla	Greece, Spain	[38, 41]	Spanish style, Black olives
Yamadazyma diddensiae	Taggiasca	Italy	[37]	Directly brined green olives
Yamadazyma mexicana	Nocellara messinese	Italy	[39]	Directly brined green olives
Yarrowia deformans	*	Spain	[60]	Directly brined green olives
Yarrowia lipolytica	*	Spain	[60]	Directly brined green olives, Spanish style
Zygoascus hellenicus	Gemlik, Nocellara dell'Etna, Nocellara messinese	Italy, Turkey	[31, 39, 40, 49]	Directly brined green olives, Black olives
Zygoascus meyerae	Nocellara messinese	Italy	[39]	Directly brined green olives
Zygosaccharomyces bailii	Black olives	Greece	[61]	Black olives

Specie	Cultivar	Country	References	Preparation type
Zygosaccharomyces bisporus	Kalamata, Picual	Cyprus	[32]	Directly brined green olives, Black olives
Zygosaccharomyces sp.	Gemlik, Leccino	Italy, Turkey	[25, 31]	Black olives
Zygotorulaspora mrakii	Aloreña de Málaga, Bosana, Gemlik, Halkidiki, Leccino, Manzanilla, Nyons, Taggiasca*	France, Greece, Italy, Spain, Turkey	[11, 25, 28, 31, 33–35, 44, 48, 60, 65]	Directly brined green olives, Black olives

*Cultivar not identified by authors.

Table 1.
List of yeasts identified in table olive fermentations by cultivars, countries and production type.

activity, contributing to the degradation of oleuropein [26] and notable antagonistic activity against spoilage microorganisms and potential pathogens, owing to its ability to produce killer toxins and other antimicrobial compounds [55, 68]. Its resilience in high-salt, low-pH environments typical of olive brines further underscores its ecological fitness in this niche [69]. Several studies have also highlighted its potential probiotic and technological properties, making *W. anomalus* a promising candidate for use as a starter culture in controlled fermentations [44].

Concerning the number of detected yeasts for each cultivar, Manzanilla reaches the highest species diversity (**Figure 3**, left), highlighting not only the presence of the main species but also different species only detected in this cultivar, such as *D. rugosa*, *K. marxianus*, *L. thermotolerans* and *C. maris*. Furthermore, the presence of yeast depending on the country where they were isolated is closely related to the country's table olive production capacity (**Figure 3**, center). Thus, the countries with the greatest diversity are Spain, Greece, and Portugal. As previously mentioned, a higher production of this fruit leads to a greater interest in carrying out this type of study to identify the different microorganisms present. Finally, an analysis of the presence of these species related to the processing type was carried out (**Figure 3**, right). Black olives and directly brined green olives displayed the highest amount of yeast diversity, both alone and those species shared between both styles. However, Spanish style

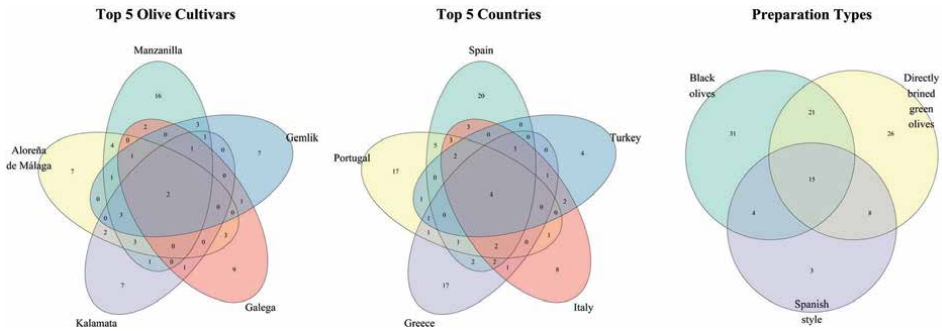

Figure 3.
Venn diagrams showing the number of yeast species detected in: the main five olive cultivars (left), top five countries (center), and the different processing styles (right). Source: Author's original work.

showed lower numbers perhaps because this style is performed mainly in Spain, while the other two styles are often used in many different countries. Another possible reason for this lower diversity could be the initial NaOH treatment (2.5%, w/v) and the high pH levels. Moreover, 15 different species were detected in all processing styles.

3. Technological and functional characterization

3.1 Technological characterization

The yeasts isolated from both brines and fermented fruits have been studied for a range of technological properties, with the aim of identifying strains suitable as starters for table olive fermentation. Thus, different enzymatic activities have been assessed by qualitative or quantitative analysis, such as pectinolytic, xylanolytic, β-glucosidase, catalase, esterase and lipase activities [53]. Furthermore, their resistance to stressful conditions of table olive fermentation has been evaluated [31].

Several works have detected a strong β-glucosidase activity in different isolates belonging to *W. anomalus* and *P. galeiformis* [53]. This is a desirable feature due to its involvement in oleuropein hydrolysis, which reduces olive bitterness [26, 70] and play an important role in directly brined olives without NaOH treatment (Spanish style). In addition, some *C. diddensiae*, *C. boidinii*, *C. oleophila*, *D. hansenii*, *P. kluyveri*, *P. manshurica* and *T. delbrueckii* strains have shown high levels of this activity [51, 70, 71]. However other species such as *S. cerevisiae* lacked this activity, confirming prior reports that β-glucosidase is rare in this genus [72].

In Ref. [55], only a few species have shown undesirable properties such as polysaccharolytic activity, that could produce fruit softening. The absence of this activity in most species is important, as it prevents fruit softening during fermentation, although some *W. anomalus* and *K. marxianus* isolates showed reduced polysaccharolytic activity [55]. Besides, fresh olive isolates belonging to *Cryptococcus* spp., *D. hansenii* and *C. minutum* species generally exhibited high pectolytic and xylanolytic activity, often linked to spoilage and textural defects [55]. In contrast, catalase activity has been detected in many species, particularly among brine-derived isolates, due to the protection that this activity provides against oxidative stress by preventing the formation of oxidative radicals and lipid oxidation [44, 71].

Regarding the lipase and esterase activity, different strains of *W. anomalus* have also displayed important levels higher than others table olive yeast. Even more, *C. parapsilosis* and *M. guilliermondii* have displayed an interesting esterase activity [53]. It can be highlighted that esterase activity is mostly detected and with higher values than lipase activity. This fact is relevant since this activity plays a role in the release of free fatty acids which are precursors of important volatile compounds [73, 74]. Higher esterase activity is particularly important in the context of table olive fermentation, as esterases play a crucial role in the hydrolysis of ester bonds, resulting in the release of volatile aroma compounds. These compounds contribute significantly to the development of the characteristic sensory profile of the final product, enhancing both its aroma and overall quality. Consequently, elevated esterase activity is considered a desirable trait in microbial strains involved in the fermentation process.

Another feature that can provide a technological advantage is the use of yeast with killer phenotype. Thus, these yeasts can inhibit or kill sensitive yeast and fungal strains, while remaining immune to their own toxins. Several yeasts, such as *W. anomalus* and *S. cerevisiae*, produce killer toxins contributing to improved microbial

control, especially under stress conditions such as high phenolic content or salinity [75]. Their application enabled fermentation with reduced salt and without chemical preservatives, promoting both product safety and sensory quality. Moreover, killer yeasts supported hydroxytyrosol formation, enhancing the nutraceutical profile of the final product. These findings underline the biotechnological value of killer yeasts in developing safer, more sustainable, and high-quality fermented olives.

Lastly, an interesting technological characteristic is the behavior of yeasts regarding the lactic acid content and the acidification which occurs during table olive fermentation. Some species such as *D. rugosa* completely degraded lactic acid, confirming that the proliferation of this yeast specie during the fermentation is undesirable [55]. Interestingly, other yeasts can be used these fermentations to get a faster and more stable process, and to improve the growth of LAB during the first month after brining (e.g., by production of growth factors such as vitamins). Thus, *L. thermotolerans* has been described as a yeast with high production of lactic acid [76], even with values above 10 g/L (w/v) in culture media. However, *L. thermotolerans* was identified only once in fermentations of Manzanilla table olives by Spanish style [41]. Moreover, different *L. thermotolerans* strains have showed great resistance to table olives parameters and kept their high lactic acid production in fermentation conditions [77]. This acidification can help deeply to the development of the initial steps of table olive fermentation and by implementating the initial growth of LAB cultures [77]. Besides, this pH reduction can offer natural protection against pathogens [78, 79].

3.2 Functional characterization

The utilization of yeasts as starter cultures in the fermentation of table olives represents a promising biotechnological approach for the delivery of beneficial microorganisms to the human gastrointestinal system. Traditionally, gut microbiota has been regarded as the principal source of probiotic strains; however, increasing evidence supports the probiotic potential of microorganisms isolated from fermented foods [44, 80]. Among these, table olives have gained recognition as an effective matrix for probiotic administration [81], thereby stimulating scientific interest in the functional characteristics of associated yeast populations.

A critical prerequisite for the probiotic functionality of any microorganism is its capacity to endure the hostile environment of the gastrointestinal tract, specifically the acidic and enzymatic conditions encountered during gastric and pancreatic digestion. *In vitro* simulations of these conditions revealed that *S. boulardii*, used as the probiotic control, exhibited the highest survival rate under gastric stress (95.9%). Nonetheless, comparable levels of resistance were also observed in different strains belonging to *S. cerevisiae*, *C. diddensiae*, *C. boidinii*, and *W. anomalus* [29]. Although survival rates generally declined under simulated pancreatic conditions, several strains demonstrated superior resilience compared to *S. boulardii*. These results align with prior research highlighting the tolerance of olive-associated yeasts to gastrointestinal challenges [82, 83]. Conversely, reference [52] reported reduced acid resistance in *S. cerevisiae* strains isolated from Portuguese olives.

Other studies focused on the probiotic and technological attributes of native yeasts from various olive cultivars, including Galega and Cordovil [52], Negrinha de Freixo [84], and Kalamata [29], propose *P. membranifaciens* as a particularly promising multifunctional starter candidate. This species exhibits a range of desirable properties for olive fermentation, including vitamin biosynthesis, antimicrobial and mycocins production. These functional traits not only enhance product safety and stability

but also positively influence the sensory and nutritional profiles of table olives. The synergistic combination of biocontrol potential and enzymatic activity positions *P. membranifaciens* as a strong candidate for the development of controlled and probiotic-driven fermentation processes [52].

In addition to acid and bile tolerance, the cholesterol-lowering ability of various yeast strains was evaluated in different culture media. The most significant cholesterol removal was observed in a minimal culture medium supplemented with cholesterol and glucose, with reduction percentages ranging from 20.4% for *C. boidinii* to 26.3% for *Z. mrakii* [29]. By contrast, strains of *C. boidinii* and *S. cerevisiae* consistently demonstrated high cholesterol assimilation across all tested conditions, outperforming the probiotic control. These findings represent the first documented instance of cholesterol metabolism by yeasts derived from table olives and agree with earlier studies involving yeasts isolated from cheese and intestinal sources [85, 86].

Another critical aspect of yeast functionality in olive fermentation is their role in biofilm formation on the fruit surface. Ref. [11] assessed the biofilm-forming capabilities of selected yeast strains on inert plastic substrates, both in monoculture and coculture with *Lp. pentosus*. Many strains, including those of *Z. mrakii*, *S. boulardii*, *N. molendinolei*, *C. diddensiae*, *C. boidinii*, and *S. cerevisiae*, exhibited significantly enhanced biofilm formation when co-cultured with *Lp. pentosus*. Conversely, specific strains of *W. anomalus*, *C. diddensiae*, and *C. boidinii* demonstrated greater biofilm formation in monoculture, with one strain of *C. boidinii* showing particularly strong performance. These observations are consistent with findings by reference [87], who reported increased biofilm development in *C. boidinii* and *W. anomalus* in the presence of *Lp. pentosus*, likely due to synergistic interactions. However, these effects also depend on the compatibility between the bacteria and yeast strains used. Thus, several *L. thermotolerans* strains have displayed a better performance to form biofilms with *Lp. plantarum* (**Figure 4**), and faster than those biofilms formed by *W. anomalus* (as control) and the same lactic acid bacteria strain [77]. To investigate whether direct cell-to-cell contact was necessary for biofilm stimulation, *C. boidinii* and *Lp. pentosus* were cultured in separate chambers divided by a membrane. Scanning electron microscopy revealed that *C. boidinii* retained biofilm-forming ability even in the absence of direct contact, suggesting that diffusible metabolites produced by *Lp. pentosus* were sufficient to trigger biofilm formation. This hypothesis is further supported by reduced biofilm production observed in monoculture, underscoring the

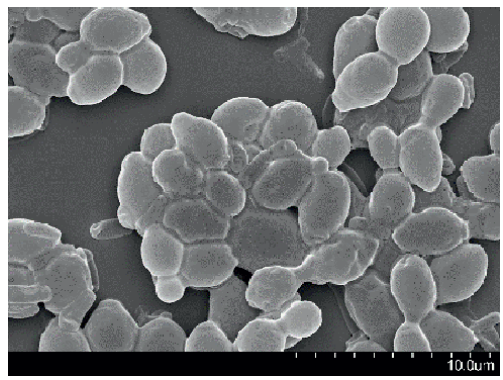

Figure 4.
SEM picture showing the formation of a biofilm by yeasts and BALs coculture. Picture were obtained on the 7th day of fermentation. Source: Author's original work.

importance of lactic acid bacteria (LAB)-derived compounds. These findings corroborate earlier studies indicating that biofilm formation may be induced by bacterial metabolites [87, 88].

Collectively, these findings highlight the multifunctional potential of table olive-associated yeasts, particularly in terms of gastrointestinal resilience, cholesterol assimilation, and biofilm formation, supporting their prospective application as novel probiotic candidates.

4. Linking yeast activity to volatile profile development

Soluble sugars, including glucose and fructose, serve as fundamental substrates for microbial fermentation, thereby facilitating the synthesis of both primary and secondary metabolites that critically contribute to the organoleptic attributes and characteristic aroma of the final product. Understanding the intricate interplay between microbial communities and volatile compounds is therefore essential for optimizing table olive fermentation processes.

In Ref. [28], yeasts emerged as principal contributors to volatile compounds formation. In particular, *P. membranifaciens* displayed strong positive correlations with a range of esters such as ethyl propanoate and ethyl octanoate, as well as ketones like heptan-2-one. Similarly, *C. boidinii* showed significant associations with esters including ethyl 2-phenylacetate and ethyl 2-hydroxybenzoate. Other yeasts, including *Z. mrakii* and *Z. hellenicus*, also correlated with multiple aroma-active volatiles. These findings align with earlier reports emphasizing the pivotal influence of yeast metabolism on the volatile profile of fermented olives [26, 59]. Moreover, a core mycobiota comprising *W. anomalus*, *C. nyonsensis*, *Z. mrakii*, *C. boidinii*, and *P. membranifaciens* was consistently identified across fermentation stages, thereby confirming their central role in the production of aroma compounds [28, 44, 68]. Specifically, in the mid-to-late phases of fermentation, the increased prevalence of *Z. mrakii*, *P. membranifaciens*, and *C. boidinii* coincided with elevated levels of fruity esters and higher alcohols such as 2-phenylethanol which impart floral and fruity sensory notes [88, 89].

During the fermentative process of different olive cultivars (Cellina di Nardò, Leccino, Konservolia, Kalamata, Manzanilla) the volatile profiling was carried out [25]. Thus, a broad spectrum of aroma compounds, predominantly aldehydes, esters, and alcohols were detected. More closely, during fermentations some trends can be revealed, such as a decline of aldehyde levels, while esters (e.g., ethyl acetate, isoamyl acetate, and ethyl octanoate) and higher alcohols were increased significantly. These trends reflect the enzymatic esterification and alcohol-yielding activities of yeasts [62, 90–92]. Further statistical analysis displayed that early fermentation stages are marked by herbaceous aldehydes, mid-stages by higher alcohols and terpenes, and late stages by ester-rich profiles indicative of active yeast metabolism [93, 94]. Reference [37] also highlights the role of yeasts as a central role in shaping the volatile profile of table olives during fermentation, particularly in the later stages. These authors revealed the predominant of hydrocarbons, followed by alcohols and aldehydes in olives, while in brine samples the main compounds were hydrocarbons, alcohols, and esters [25]. They also detected that compounds such as 1-heptanol, 3-methylbenzaldehyde, and several esters were strongly associated with *Pichia* spp., *C. minutum*, and *Y. diddensiae*, highlighting their contribution to fruity and floral aromas. In contrast, early colonizing species like *A. pullulans* and *W. anomalus* showed weaker or negative correlations with key volatiles. In Gordal olives, *K. lactis*

notably increased the concentration of alcohols, terpenes, esters, and phenols, yielding a more complex volatile profile than uninoculated controls or previous fermentation seasons [36]. In Manzanilla olives, both *K. lactis* and *C. adriatica* enhanced key aroma compounds, including phenylethyl alcohol and furan-derived esters [36]. These results confirm that specific yeast taxa are responsible for the synthesis of aroma-active compounds, supporting their biotechnological potential in enhancing sensory quality through controlled fermentation.

Taken together, these results underscore the dominant and multifaceted role of yeast species in generating volatile compounds during table olive fermentation. Specifically, the production of isoamyl alcohols, phenylethanol, and ester derivatives greatly enriches aroma complexity. Furthermore, differences in volatile patterns across olive cultivars likely reflect distinctive consortia of yeast species and fermentation kinetics, offering promising targets for the design of tailored starter cultures to enhance sensory quality and consistency in industrial fermentations.

5. Conclusions

In this chapter, several examples of biotechnological applications of table olive yeasts were presented and discussed. It is evident that these microorganisms can play an important role during this fermentation process and lead to the production of a variety of final products with different organoleptic profiles due to the generation of diverse organic acids and volatile compounds. In addition, the great diversity of yeast species can be used for improving functionality, a faster acidification and the lactic acid bacteria growth. Further research will definitely suggest more such applications in the near future. Although table olive yeasts have always been in the background of attention compared to other microbial groups, the information provided allows us to think of yeasts as a precious biotech tool for industry and a significant player in the maintenance of the microbial safety conditions.

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Conflict of interest

The author declares no conflict of interest.

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Chapter 5

Pulque: More than an Alcoholic Beverage – History, Native Microbiota, Fermentation Process and Its Potential Health Benefits

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Abstract

Pulque is a traditional Mexican fermented drink made from the sap of the maguey (*Agave spp.*) plant. Besides its reputation as a handcrafted beverage, pulque holds deep cultural, historical, and nutritional significance. It has been used for centuries by indigenous civilizations like the Aztecs. Initially, it was reserved for religious ceremonies and exclusively for nobility, priests, and warriors. Its production depends on the spontaneous fermentation of agave sap, known as *aguamiel*, by native microorganisms. These are mainly lactic acid bacteria and yeasts, crucial for the drink's distinctive flavor, probiotic potential, and thick texture. The latter is mainly due to the exopolysaccharides produced by *Leuconostoc mesenteroides*. The fermentation process is relatively quick. It typically lasts between 24 and 48 hours. Occurring in open containers that allow natural microbial inoculation, pulque has a complex and diverse microbiome, including beneficial strains such as *Lactobacillus*, *Leuconostoc*, and *Saccharomyces cerevisiae*. These microorganisms aid in fermentation and may also offer health benefits, such as improved gut health, enhanced immune function, and antioxidant activity. This positions pulque alongside other functional fermented foods. Currently, it is experiencing a cultural resurgence, especially in urban areas, driven by the increased interest in artisanal and traditional products. However, its short shelf life and lack of standardized production methods remain significant obstacles to large-scale commercialization. Despite these challenges, *pulque*'s unique microbiological profile, along with its historical and nutritional significance, makes it a fascinating subject for further research. It is also a promising candidate in the field of functional nutrition.

Keywords: pulque, agave, spontaneous fermentation, functional fermented beverage, traditional Mexican fermentation

1. Introduction

Pulque is an ancient Mexican fermented beverage obtained from the *aguamiel* (Agave sap, also known as "*aguamiel*") of maguey plants such as *Agave salmiana* and

Agave mapisaga [1–3]. Its consumption dates back to pre-Hispanic times, where it held central ritual and social value in various Mesoamerican civilizations [4, 5]. Although historically associated with the cultural and traditional contexts, scientific interest in *pulque* has grown in recent years due to the complexity of its fermentation process and its potential functional benefits [6, 7].

Unlike other controlled fermentations, *pulque* production is a spontaneous and unstandardized process that involves complex microbial communities composed of lactic acid bacteria (LAB), yeasts, acetic acid bacteria, and exopolysaccharide-producing LAB [1, 2, 8, 9]. These microorganisms convert *aguamiel* into a final product known as *pulque*, which has specific organoleptic and functional properties, as well as the potential uses in the food and biotechnology industries [1, 6].

However, the spontaneous nature of pulque fermentation introduces considerable variability in microbial composition across regions, seasons, and production batches. This variability is largely determined by the local microbial environment, which is influenced by factors such as temperature, altitude, relative humidity, soil pH, and overall environmental conditions. In some tinacales (traditional fermentation facilities), a form of pre-inoculum from previous batches may be present, yet even under these circumstances the bacterial diversity and community dynamics remain highly dependent on the environmental factors mentioned above.

Unlike other traditional fermented beverages such as kefir or kombucha, which rely on defined starter cultures like kefir grains or the Symbiotic Culture of Bacteria and Yeast (SCOBY) to maintain relatively stable microbial consortia despite environmental variations, pulque fermentation typically begins without any external inoculum and depends entirely on the native microbiota present in the aguamiel and the surrounding environment. These ecological factors shape the initial inoculum, microbial succession, and metabolic activity throughout the fermentation process, resulting in a complex interaction between microbiota and environmental conditions that makes standardization at both microbiological and technological levels particularly challenging. This intrinsic variability, reflecting local ecosystems, seasonality, artisanal practices, and the variable presence of pre-inocula in some tinacales, not only distinguishes pulque from other traditional fermented beverages but also positions it as a valuable model for studying non-standardized microbial ecosystems.

This ecological dependency underscores its cultural and biological uniqueness and positions it as a valuable model for studying non-standardized fermentation ecosystems. *Pulque*'s microbial communities have been studied using the traditional methods and, more recently, molecular techniques such as next-generation sequencing, which have allowed us to identify a wide variety of bacterial and fungal genera, as well as their main metabolic pathways [6, 9, 10]. These pathways include lactic acid fermentation (homolactic and heterolactic), alcoholic fermentation, and the production of exopolysaccharides such as dextrans, which contribute to the characteristic viscosity of the final product [1, 2, 8]. Besides its ethnobiological significance, *pulque* has been suggested as a potential source of functional microorganisms. Strains with probiotic properties, resistance to gastrointestinal conditions, the production of antimicrobial compounds, and ability to adhere to the intestinal epithelium have been identified [3, 11, 12].

These characteristics not only underline their functional relevance in fermented beverages but also point to their potential as candidates for industrial applications [11, 12]. Native strains isolated from pulque could be exploited in the formulation of functional foods, probiotics, and biotechnological products aimed at improving gut health, modulating immunity, or serving as sources of novel biomolecules.

Highlighting these possibilities broadens the significance of pulque beyond its cultural and nutritional context, situating it within the expanding field of microbial biotechnology and health-oriented innovations.

These properties position them as a relevant model for studying traditional fermentations within the modern functional contexts. The aim of this chapter is to compile the available scientific evidence on the *pulque* fermentation process, with emphasis on: (i) the characterization of the microbial communities involved, (ii) the main metabolic pathways driving its transformation, (iii) the biotechnological potential of native strains isolated during the process, and (iv) the health benefits associated with *pulque* consumption.

2. History of *pulque*

Pulque, traditionally known as *neutle*, is considered the oldest alcoholic beverage in Mexico. It is made from the sap collected from certain species of the Agave plant and has played a significant role in the rituals, social customs, and cultural life of the Central Mexican Plateau.

2.1 Mythical origins and archaeological evidence

Various Mesoamerican traditions explain the origin of *pulque* through mythology. One of the most common stories credits the goddess Mayáhuel, a symbol of fertility, with discovering *aguamiel* (fresh maguey sap, prior to fermentation), while the god Patecatl is believed to have discovered its fermentation, thus creating the so-called "wine of the earth." Another version suggests that the noble Papántzin was the first to offer *aguamiel* to the god Tecpancaltzin during the Toltec period (900–1042 CE) [13].

Pre-Hispanic iconography highlights the religious significance of *pulque.* Its use was originally restricted to priests and elders and was limited to ceremonial occasions. Widespread consumption, including by children and young people, was only permitted during the Pillahuana festival. Outside of this ritual context, illegal *pulque* consumption was considered a serious crime, punishable by death [13]. From an archaeological standpoint, tools related to *aguamiel* extraction, like maguey scrapers, have been found, with evidence dating back to the fifth century BCE in Huapalcalco, Hidalgo, and between 1 and 500 CE in Teotihuacan, confirming its ancient use [4, 5]. Direct archaeological evidence of fermentation vessels or chemical residues linked to pulque remains scarce. As a result, most conclusions about its pre-Hispanic production are based on the identification of extraction tools, such as maguey scrapers, rather than on preserved containers or residues [4]. This limitation highlights the importance of combining archaeological findings with ethnographic and historical sources to reconstruct the early development of pulque.

2.2 *Pulque* during the colonial period and the porfiriato

During the colonial period (1521–1821), *pulque* gained significant commercial value, becoming a major source of income for "*haciendas pulqueras*" (large estates focused on maguey farming and *pulque* production) [6]. Despite this, between 1629 and 1786, colonial authorities banned it over health and order concerns. The ban was eventually lifted due to its economic importance [14]. During the Mexican War of Independence (1810–1821), production was disrupted but recovered by the late nineteenth century. Under the Porfirio Díaz regime (1877–1911), *pulque* was widely

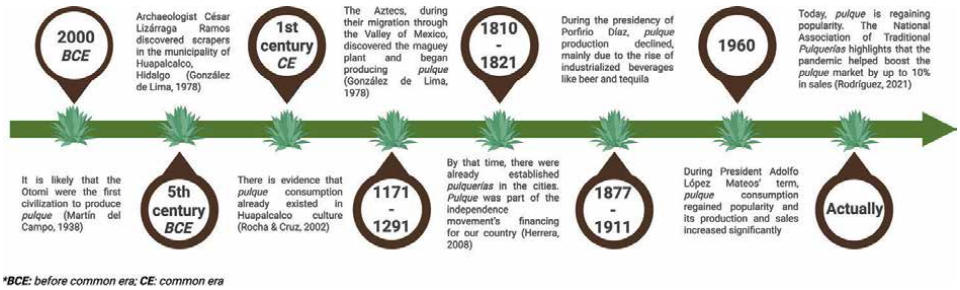

Figure 1.
Pulque *timeline.*

seen as a national symbol and a marker of cultural identity among different social groups [15]. However, after 1911, its consumption started to decline as beverages like tequila and beer became more popular [16].

2.3 The twentieth century and present outlook

Throughout the twentieth century, a lack of scientific and industrial investment hindered the progress of *pulque.* To try to reverse this trend, President Adolfo López Mateos established the Patronato del Maguey in 1960, aiming to improve maguey cultivation and promote commercialization [15]. Despite these efforts, the decline continued for several decades. In recent years, especially after the COVID-19 pandemic, *pulque* has regained popularity, particularly among younger consumers, according to the National Association of Traditional *Pulquerías* (ANTP). The *pulque* market grew by 10% during this period [17]. Although only about 25 traditional *pulquerías* remain nationwide, more than 100 new establishments have opened in Mexico City, many offering modern and innovative ideas. This renewed interest has driven innovations like *pulques curados* flavored with fruits or seeds that mellow their naturally tart flavor and thick texture, making the drink more attractive to new audiences.

Beyond local consumption, this revival has also been linked to cultural heritage marketing and tourism. Pulque is increasingly promoted not only as a beverage but also as part of Mexico's intangible cultural heritage, featured in gastronomic routes, festivals, and urban tourism initiatives. This cultural positioning aligns pulque with a global trend of renewed interest in artisanal and traditional fermented beverages, enhancing its visibility and contributing to its contemporary resurgence [17].

Figure 1 shows a timeline illustrating the historical development of *pulque*, from its mythological origins to its archaeological evidence.

3. *Pulque* production process

Figure 2 illustrates the *pulque* production process, beginning with growing and maturing the maguey plant. When the plant is ready, the sap, called *aguamiel*, is collected and taken to the *tinacal*, where it is prepared and fermented. After fermentation ends, the final product is packaged and stored, ready for distribution and consumption. **Figure 2** outlines each step in *pulque* production, from maguey cultivation and *aguamiel* extraction to fermentation, storage, and distribution.

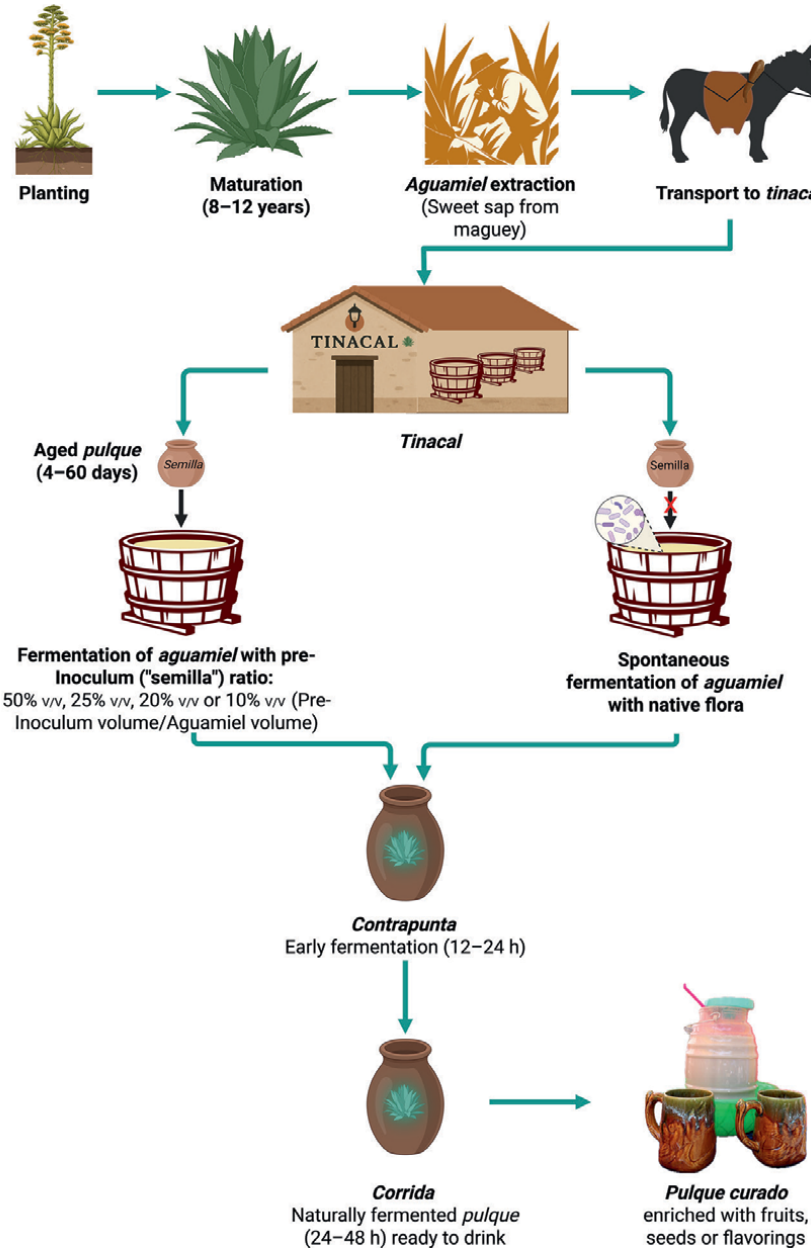

Figure 2.
Pulque *production process.*

3.1 Maguey cultivation

Understanding maguey cultivation is essential to fully appreciating the production process and the unique qualities of pulque. Historical data show that in the first half of the nineteenth century, about 250,000 hectares were planted with an estimated 206 million maguey plants for pulque production [18]. In stark contrast, the state of Hidalgo now reports only 6152 hectares, equivalent to 194,579 tons of

maguey or approximately 194.6 million liters of aguamiel. This reflects a drastic reduction in cultivated area and productivity compared to historical levels. Despite this decline, Hidalgo remains the top pulque-producing state in Mexico, accounting for 78.14% of the country's output [19, 20]. The agave species used in pulque production belong to the family Agavaceae (**Figure 3**). The most used species include *Agave salmiana*, *Agave americana*, *Agave ferox*, *Agave mapisaga*, and *Agave atrovirens*.

The maguey plant has long been, and continues to be, a symbol for the people of the region known as "Los Llanos de Apan," which covers parts of the Mexican states of Hidalgo, Tlaxcala, Puebla, and the State of Mexico. Additionally, states like Querétaro, Mexico City, and Michoacán are also known for their *pulque* production, highlighting the agricultural and cultural importance of maguey in various parts of the country (**Figure 4**).

Maguey plants generally require 8–12 years to reach full maturity, although this period can vary with the environmental and agricultural conditions. Climate is a key determinant: warmer temperatures tend to accelerate growth, whereas colder weather slows it. Rainfall amount and frequency also influence the development adequate irrigation fosters vigorous growth, while prolonged drought delays maturation. Ambient humidity affects nutrient uptake and metabolic activity, while soil

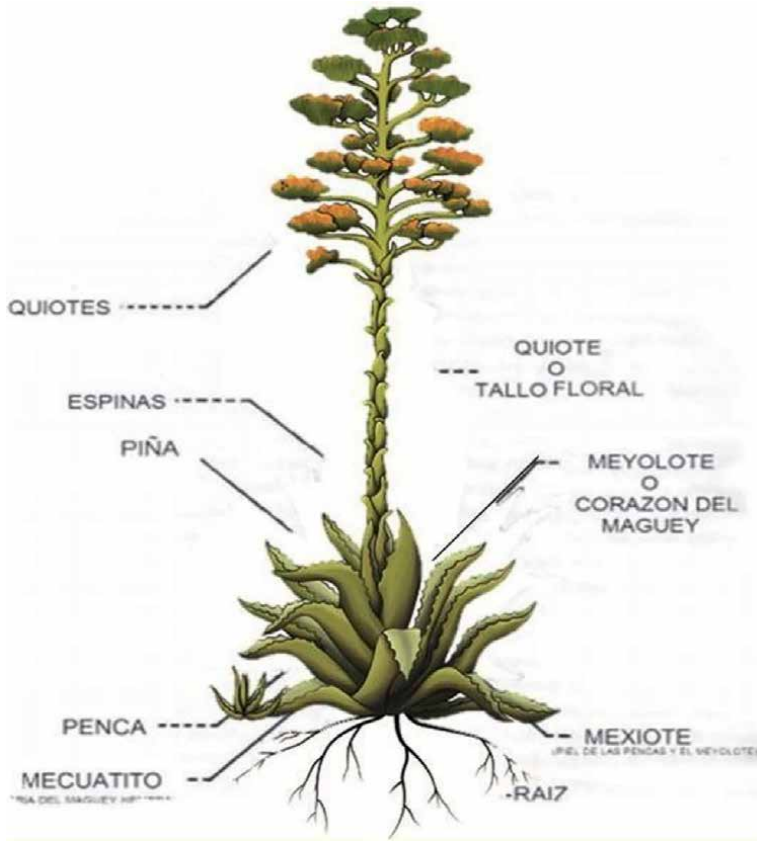

Figure 3.
Parts of the maguey *plant are used for* pulque *production [21].*

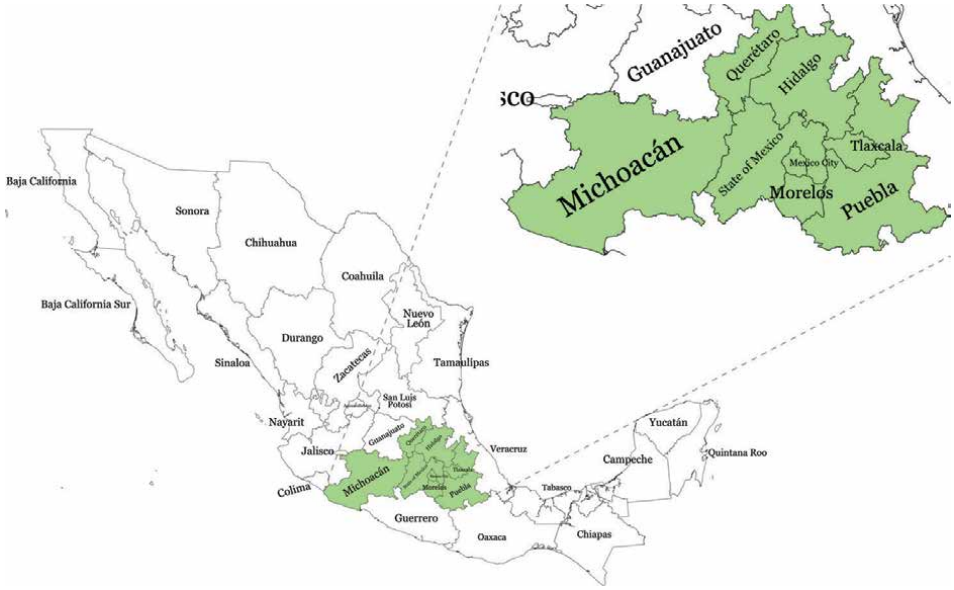

Figure 4.
Pulque-*producing states in the Mexican Republic.*

type, altitude, sun exposure, and local cultivation practices further shape the growth cycle. Consequently, maguey maturation is highly dependent on its environment and management practices. Once mature (**Figure 5**), the plant's sap (*aguamiel*) is carefully extracted. This sweet, nutrient-rich liquid is the essential raw material for pulque production.

The extraction of *aguamiel* is carried out by skilled workers known as *tlachiqueros*. This process starts with a technique called *capado*, where the central leaves

Figure 5.
Maguey pulquero *(*pulque *agave plant). Photograph taken in the municipality of Altzayanca, Tlaxcala.*

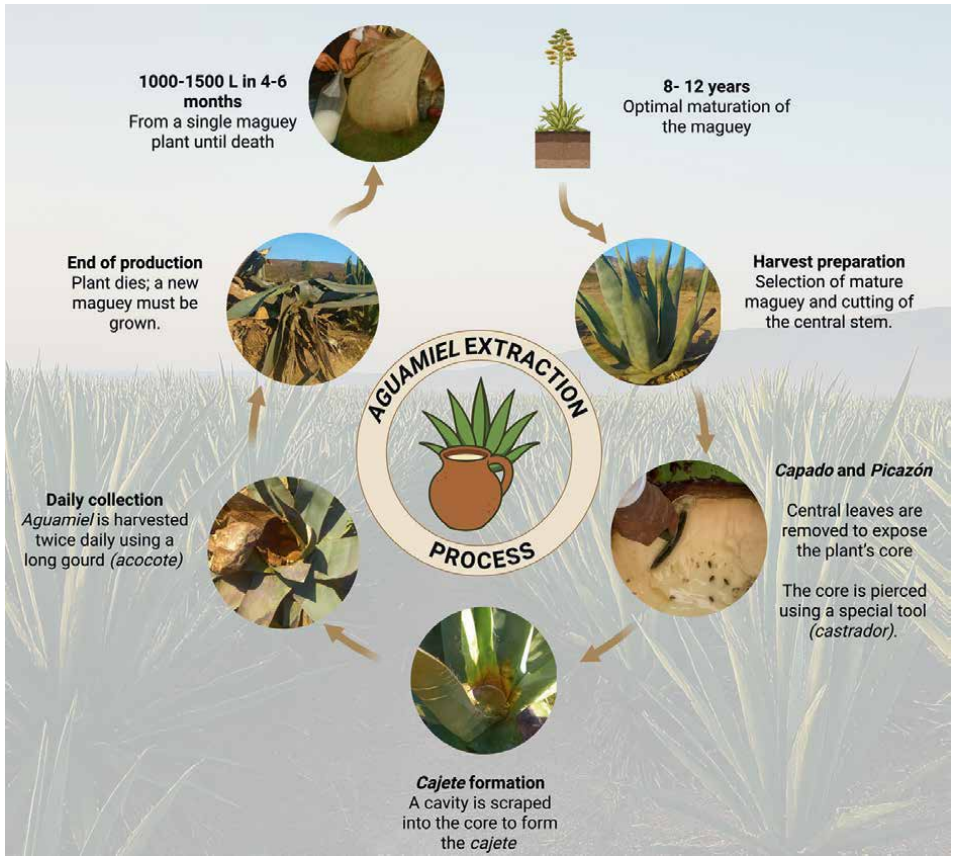

Figure 6.
Aguamiel *extraction process.*

(*pencas*) of the maguey are cut to expose the plant's core. Then, an initial scraping called *picazón* is performed, where the center of the piña is pierced with a specialized tool called a *castrador*. After approximately two weeks, a second scraping is done using the same tool to form a concave cavity called the *cajete*, which serves as the reservoir for *aguamiel*. The process concludes when the maguey ceases vegetative growth and redirects all its metabolic activity toward *aguamiel* production, marking the final stage of its life cycle [22]. **Figure 6** shows the *aguamiel* extraction process, beginning once the maguey plant reaches maturity, and highlights the steps involved in scraping the core and collecting the *aguamiel*.

The accumulated *aguamiel* is extracted using a traditional tool known as the *acocote*, which is shown in **Figure 7**.

The maguey is scraped twice daily until it dries completely, producing between 8 and 12 liters of *aguamiel* each day. The total amount depends on both the skill of the *tlachiquero* and the plant's capacity to produce. Each maguey can yield between 1000 and 1500 liters of *aguamiel* over a continuous period of 4–6 months, after which the plant finishes its productive cycle [23]. The *aguamiel* is collected in a traditional container known as a *cuero*, shown in **Figure 8.** Nowadays, many *tlachiqueros* have replaced this vessel with 25-liter plastic bottles, which are more readily available and easier to handle.

Figure 7.
Acocote.

Figure 8.
Traditional cuero *used for transporting* pulque.

4. Fermentation, native microbiota of aguamiel and metabolic pathways

Beyond its cultural and historical relevance, pulque's production process hosts a complex microbial ecology, where bacteria, yeasts, and other microorganisms interact to transform *aguamiel* into the final beverage.

4.1 Fermentation

The collected *aguamiel* (unfermented maguey sap) is transported to the *tinacal* to initiate the fermentation process, which can occur in two different ways:

Fermentation with inoculum ("semilla"): This method begins with the addition of previously fermented *pulque*, known as *semilla*, aged between 4 and 60 days. The ratio of *aguamiel* to *semilla* may vary (1:1, 1:4, 1:5, or 1:10), depending on the practices of each *tinacal* [24].

Spontaneous fermentation: In this process, *aguamiel* is left to ferment naturally for 24 hours, relying on the native microbiota present in the sap.

The fermentation process of *pulque* involves four main stages:

- *Aguamiel*: the sweet sap extracted from maguey.
- *Semilla*: aged *pulque* (4–60 days), used as a starter culture.
- *Contrapunta*: the initial fermentation stage, which involves either spontaneous fermentation or the mixture of *aguamiel* and *semilla*. *Pulque* can be obtained after 12–24 hours.
- *Corrida*: *pulque* that has fermented for 24–48 hours. This represents the final stage, after which the beverage is ready for consumption and sale.

4.2 Native microbiota of *aguamiel*

Bacterial diversity was shown to vary across the fermentation stages, reaching its peak during the intermediate *contrapunta* phase and being the lowest at the initial *aguamiel* stage characterizes *pulque* fermentation. Rocha et al. [25] identified 2855 bacterial operational taxonomic units (OTUs) and 1494 yeast and fungal species. Bacterial diversity was shown to change throughout the fermentation stages, peaking during the intermediate *contrapunta* phase and being lowest at the initial *aguamiel* stage [25].

Several studies have demonstrated that the time of year significantly affects the composition and abundance of microorganisms in *aguamiel*. Villarreal et al. reported that summer had the highest microbial diversity and prevalence of the following genera and species: *Leuconostoc sp.* (*L. sp.*), *Leuconostoc gelidum* (*L. gelidum*), *Lactococcus lactis* (*L. lactis)*, *Pediococcus sp.* (*P. sp.*), *Trichococcus sp.* (*T. sp*), *Kazachstania zonata* (*K. zonata*), and *Kluyveromyces marxianus* (*K. marxianus*). In contrast, autumn, winter, and spring were linked to the lower microbial counts.

Rocha and Cruz also reported the presence of *Candida, Pichia, Cryptococcus, and Clavispora* in *aguamiel* [4]. Similarly, Chacón et al. identified the most common genera as *Acinetobacter, Leuconostoc, Zymomonas*, and *Lactobacillus* [26]. The dominant species included *Lactococcus plantarum* (*L. plantarum*), *Zymomonas mobilis* (*Z. mobilis*), *Acinetobacter nectaris* (*A. nectaris*), *L. gelidum, Leuconostoc citreum* (*L. citreum*), *Leuconostoc piscium* (*L. piscium*), *Acinetobacter boissieri* (*A. boissieri*), and *L. lactis*. It is important to note that these last authors did not specify the season during which their *aguamiel* samples were collected.

These findings show that while some genera such as *Leuconostoc*, *Lactobacillus*, and *Zymomonas* are consistently reported, the overall microbial profiles differ between studies. This partial overlap reflects the non-standardized nature of pulque

fermentation, where the microbial environment, seasonality, and artisanal practices shape unique microbial communities in each context. Additionally, they created a timeline of the main research studies on the microbial community in *pulque*, from the twentieth century to the present. **Figure 9** displays the microorganisms identified in *aguamiel*, *contrapunta*, and *pulque*.

Figure 10 presents a chronological record of microorganisms isolated from *aguamiel* and *pulque* between 1870 and 1991. As shown, *pulque* exhibits considerable microbial diversity responsible for the fermentation of *aguamiel* into alcohol, lactic acid, and dextrans (the latter contributing to the beverage's characteristic viscosity). The compounds formed during fermentation define the sensory profiles and physicochemical properties of the final product.

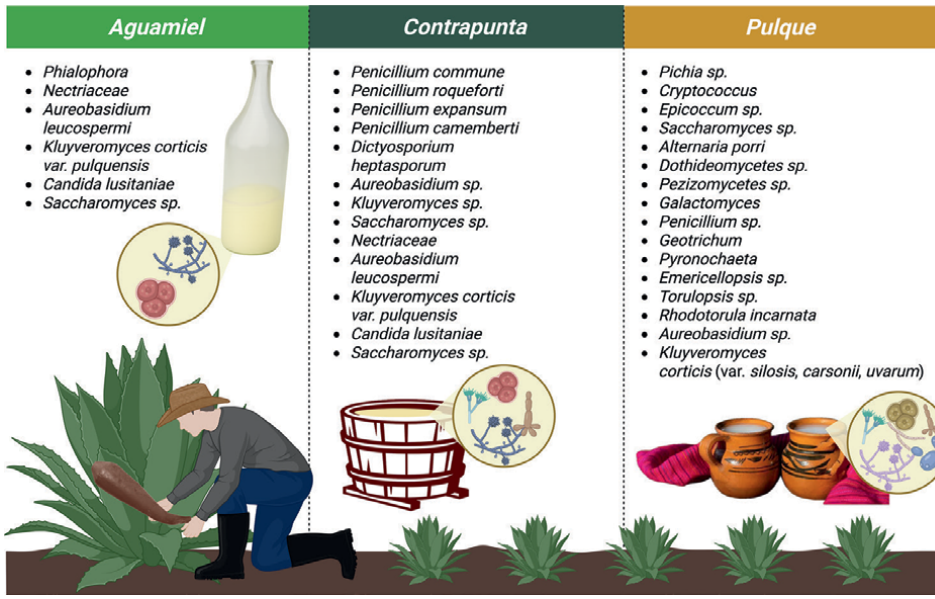

Figure 9.
Microorganism associated with each stage of pulque *fermentation.*

Figure 10.
Microorganisms identified over time in pulque.

Pulque has significant microbial diversity, recognized from the late nineteenth century to today. This microbiota is responsible for converting *aguamiel* into ethanol, lactic acid, and exopolysaccharides (dextrans), which are linked to the beverage's characteristic viscosity. The compounds produced during fermentation directly influence the sensory profile and physicochemical properties of the final product [4].

Fructose is the main sugar in *aguamiel*, while glucose, sucrose, and fructooligosaccharides are present in smaller amounts [27]. During fermentation, native microorganisms such as yeasts, lactic acid bacteria, ethanologenic bacteria, and exopolysaccharide-producing bacteria utilize these sugars as their main carbon source [24].

Unlike traditional solid-state fermentations, *pulque* is fermented in a liquid and viscous medium, which prevents the formation of microenvironments or gradients that often allow enterobacteria to persist even under acidic conditions [28]. This feature reduces the survival of pathogenic bacteria, helping to maintain the safety and microbiological quality of the final product.

4.3 Methabolic pathways

Three main types of fermentation occur during *pulque* production:

- Alcoholic fermentation
- Lactic acid fermentation
- Exopolysaccharide production (dextrans and gums)

This complexity defines pulque as a diverse microbial system with potential for bioprospecting genes of industrial relevance. Among this microbial diversity, genes involved in sugar transport, hydrolytic enzymes, lactic acid and ethanol production, and extracellular polysaccharides (EPS) can be found, with applications in the food, pharmaceutical, and biotechnology industries [1].

4.3.1 Alcoholic fermentation

One of the pioneers in *pulque* research, Dr. Sánchez Marroquín, identified *Saccharomyces cerevisiae* (*S. cerevisiae*), also called *S. carbajali* as the most important yeast in the process because of its ability to convert *aguamiel* sugars into ethanol under the anaerobic conditions [10].

Besides *S. cerevisiae*, the bacterium *Z. mobilis* also plays an important role in *pulque*'s alcoholic fermentation. This species uses an alternative metabolic pathway, the Entner–Doudoroff pathway (**Figure 11**), where glucose-6-phosphate is oxidized to 6-phosphogluconic acid, producing NADPH. This intermediate is then converted into pyruvate and glyceraldehyde-3-phosphate (G3P), producing NADH and ATP. Although this pathway generates less ATP than glycolysis, it allows for rapid ethanol production and is also found in some heterofermentative lactic acid bacteria [29].

Under the anaerobic conditions, *Z. mobilis* converts approximately 45% of glucose into ethanol and carbon dioxide, while producing small amounts of acetic acid, acetoin, and exopolysaccharides that contribute to *pulque*'s characteristic viscosity [30]. This pathway involves key enzymes such as 2-keto-3-deoxy-6-phosphogluconate aldolase.

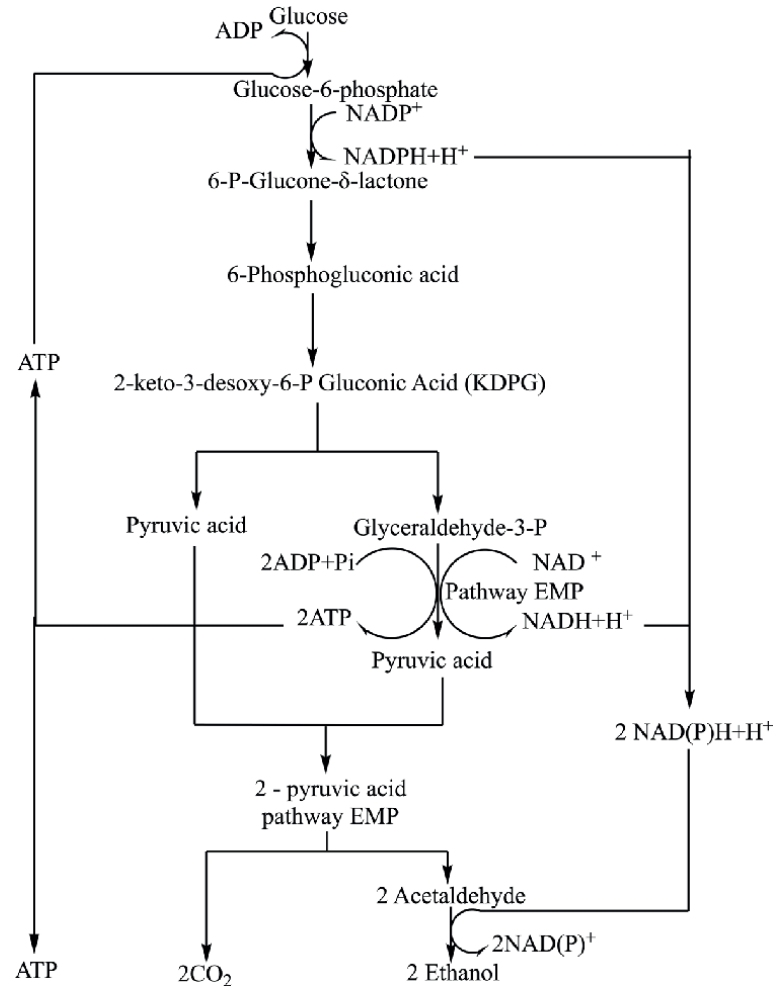

Figure 11.
Entner–Doudoroff pathway used by Zymomonas mobilis.

In contrast, *S. cerevisiae* utilizes the Embden–Meyerhof pathway (classic glycolysis; **Figure 12**). This process starts with the phosphorylation of glucose into glucose-6-phosphate by hexokinase and then isomerizes to fructose-6-phosphate. It is further phosphorylated to fructose-1,6-bisphosphate, which is split into DHAP and G3P. These intermediates continue through glycolysis to produce pyruvate while generating ATP and NADH. Under the anaerobic conditions, pyruvate is decarboxylated to acetaldehyde by pyruvate decarboxylase and then reduced to ethanol by alcohol dehydrogenase using the previously produced NADH. Comparison between *S. cerevisiae* and *Z. mobilis*:

S. cerevisiae is known for its metabolic efficiency, ethanol tolerance, and adaptability to various fermentation conditions.

Z. mobilis stands out for its high specific substrate consumption rate, rapid ethanol production, lower biomass formation, and independence from oxygen.

However, a major limitation of *Z. mobilis* is its ability to ferment only glucose, fructose, and sucrose [31].

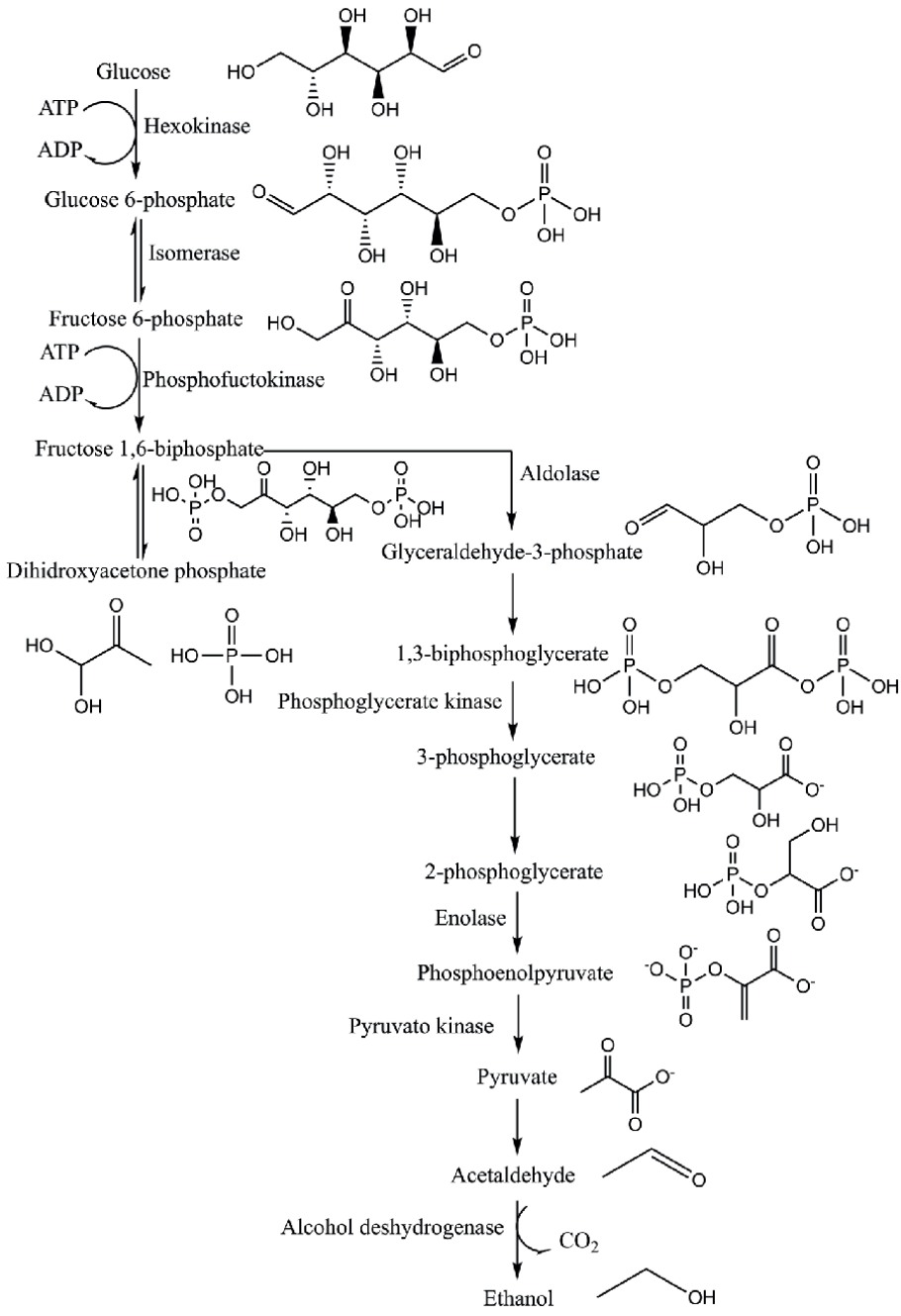

Figure 12.
Embden–Meyerhof pathway used by Saccharomyces cerevisiae.

4.3.2 Lactic fermentation in Pulque

Lactic acid bacteria (LAB) are Gram-positive cocci or bacilli, usually non-motile, whose main metabolic product is lactic acid. These bacteria are physiologically divided into two main groups:

Homofermentative LAB, which convert a single molecule of glucose entirely into lactate.

Heterofermentative LAB, which, besides lactate, produce volatile compounds such as ethanol, carbon dioxide, and acetic acid [32].

Both types of fermentation take place during the *pulque* fermentation process. Homofermentative species of the genus *Lactobacillus* are mainly responsible for producing lactic acid, helping to acidify the beverage and maintain microbial stability. Meanwhile, genera such as *Leuconostoc* and certain heterofermentative *Lactobacillus* species generate secondary compounds that affect the flavor, acidity, and overall stability of the final product.

4.3.3 Homolactic fermentation

Homofermentative bacteria use the Embden–Meyerhof–Parnas pathway (commonly known as glycolysis) to convert glucose into lactic acid. In this process, glucose acts as both an electron donor and the final electron acceptor, enabling the regeneration of NAD^+, which is essential for maintaining anaerobic metabolism. This pathway is shown in **Figure 13**.

This process is divided into three main phases:

1. Activation phase: Glucose is phosphorylated by ATP through the action of hexokinase to produce glucose-6-phosphate, which is then isomerized into fructose-6-phosphate. A second phosphorylation by phosphofructokinase creates fructose-1,6-bisphosphate. This molecule is split by aldolase into two triose sugars: glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). DHAP is converted into G3P by triose phosphate isomerase, resulting in two G3P molecules per glucose.

2. Oxidation and energy generation phase: G3P is oxidized to 1,3-bisphosphoglycerate, generating NADH. This high-energy intermediate donates a phosphate group to ADP, forming ATP. Overall, the process produces four ATP molecules (a net gain of two) and NADH.

3. Cofactor regeneration and lactate production: In the absence of oxygen, the NADH formed must be oxidized back to NAD^+. This occurs when pyruvate (produced at the end of glycolysis) is reduced to lactate by the enzyme lactate dehydrogenase, thereby sustaining anaerobic metabolism.

4.3.4 Heterolactic fermentation

In contrast, heterofermentative bacteria such as *Leuconostoc* spp. The phosphoketolase pathway is an alternative metabolic route in which glucose is converted into multiple end products: lactic acid, ethanol, and acetic acid. This type of fermentation contributes to the acidic and slightly alcoholic profile of *pulque*, as well as its aromatic complexity. This pathway is illustrated in **Figure 14.**

The pathway starts with the conversion of glucose into glucose-6-phosphate, then its oxidation to 6-phosphogluconate, releasing CO_2. This compound is then transformed into ribulose-5-phosphate, which is further converted into xylulose-5-phosphate, a key intermediate that leads to a metabolic split.

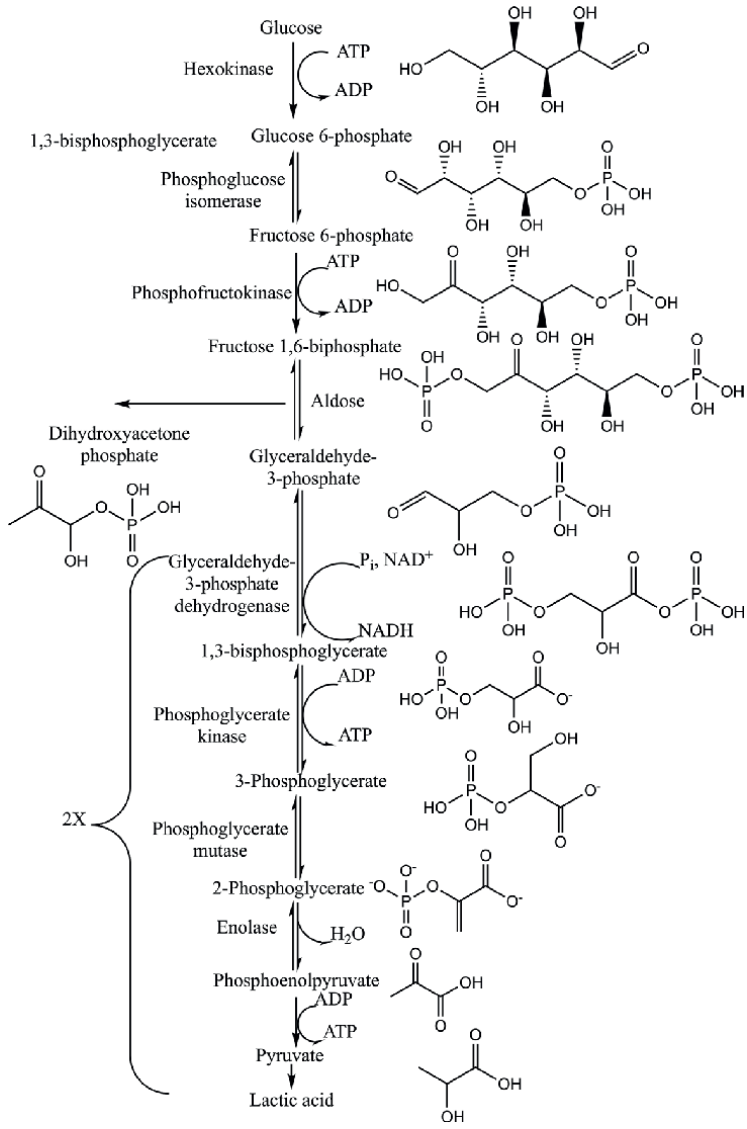

Figure 13.
Homolactic fermentation.

- First branch: Glyceraldehyde-3-phosphate is converted into pyruvate and subsequently into lactic acid *via* the enzyme lactate dehydrogenase. This product is mainly responsible for the acidic pH of *pulque*.

- Second branch: Acetyl-phosphate is converted into acetyl-CoA, which serves as a precursor for acetic acid or, alternatively, is reduced to ethanol depending on the availability of cofactors and environmental conditions.

This balanced metabolic network enables a versatile fermentation process that contributes not only to the preservation of *pulque* but also to its organoleptic complexity, combining acidity, residual sweetness, and light alcoholic notes.

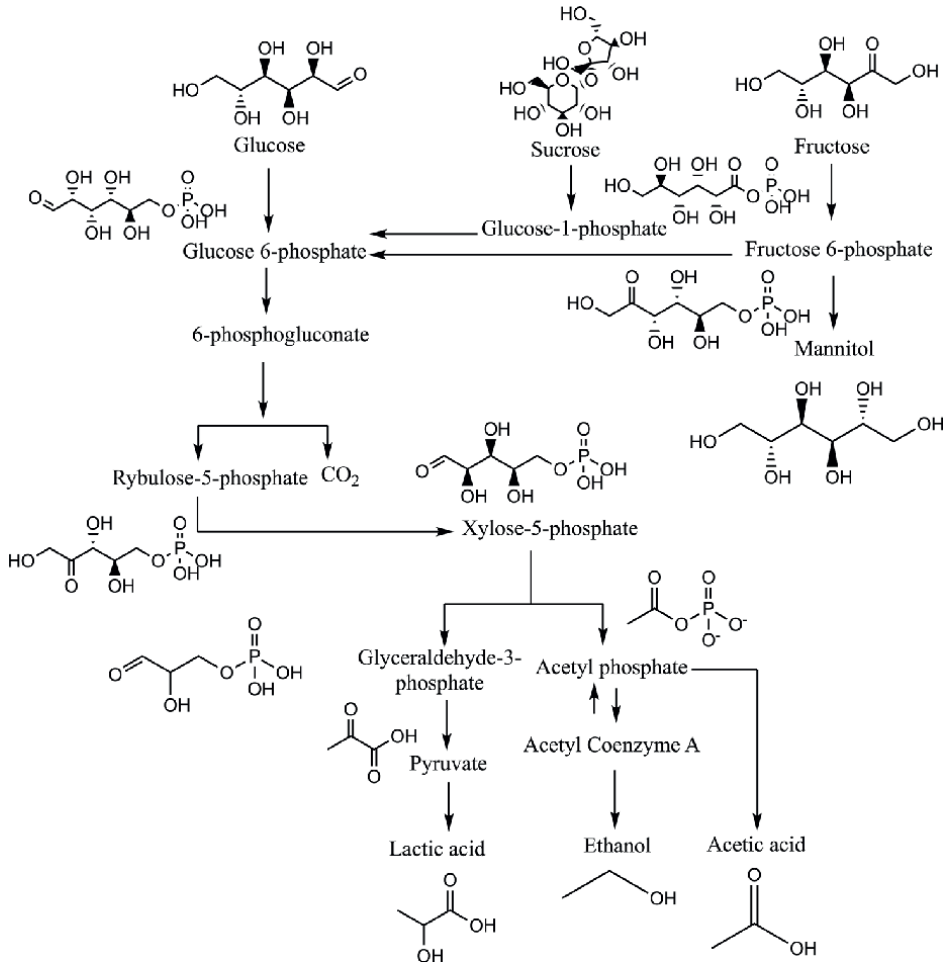

Figure 14.
Heterolactic fermentation.

4.3.5 Exopolysaccharide production (viscosity formation in Pulque)

In addition to alcoholic and lactic fermentations, *pulque* exhibits a third key fermentative feature: the development of a viscous or gelatinous texture, a trait that significantly influences consumer acceptance of the final product [28].

This viscosity results from the production of exopolysaccharides (EPS), particularly dextrans (**Figure 15**) and β-glucans, synthesized by lactic acid bacteria of the genus *Leuconostoc*, especially *L. mesenteroides*. This species has been identified as the main contributor to the characteristic consistency of pulque. Other *Leuconostoc* species also capable of dextran production, though in smaller amounts, include *L. kimchi*, *L. citreum*, *L. gasicomitatum*, and *L. pseudomesenteroides* [28].

These heterofermentative, Gram-positive bacteria, typically occurring in pairs or chains, metabolize sucrose through the action of the enzyme dextransucrase, which catalyzes dextran synthesis from the glucose released. This enzyme follows a double-displacement mechanism consisting of two phases:

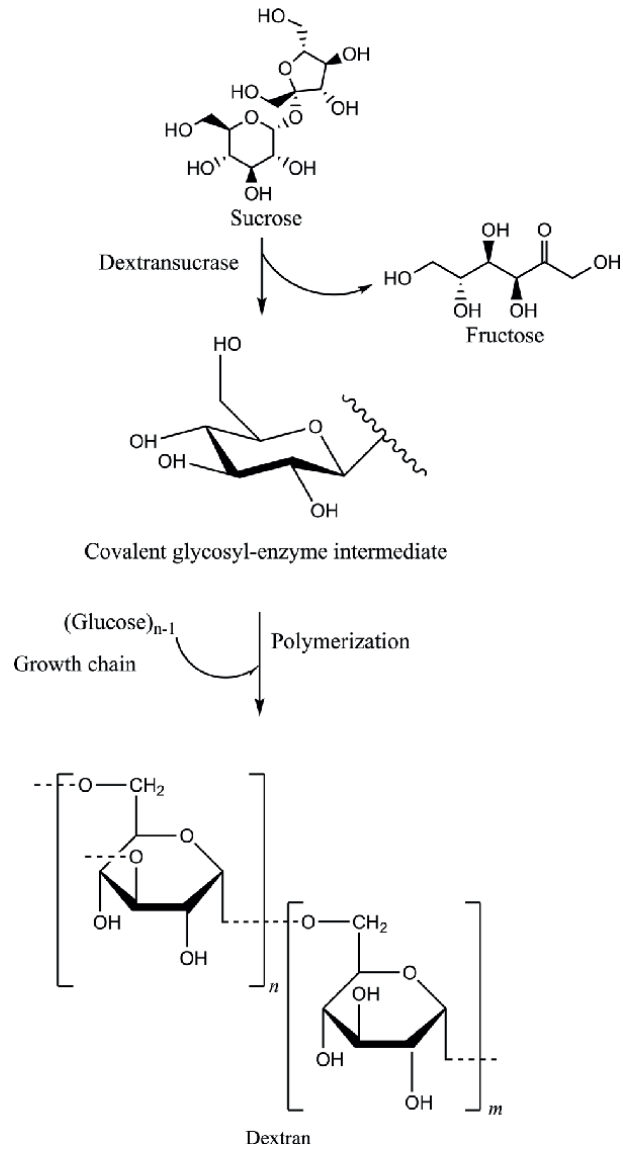

Figure 15.
Dextran synthesis catalyzed by the dextransucrase of L. mesenteroides *[32].*

- Cleavage phase: The α-(1 → 2) glycosidic bond in sucrose is broken, releasing fructose and forming a covalent intermediate known as a glucosyl-enzyme.

- Transfer phase: The glucose is transferred to the non-reducing end of a growing glucan chain, forming a new α-glycosidic bond and progressively elongating the dextran polymer [33].

The dextrans produced by *L. mesenteroides* are predominantly linear polymers composed of α-(1 → 6) glycosidic bonds, with occasional branching through α-(1 → 2), α-(1 → 3), and α-(1 → 4) linkages [34, 35]. These polysaccharides not only

contribute to the distinctive viscous texture of *pulque* but also serve several important functional roles:

- They increase the acidity of the medium.
- They generate carbon dioxide, promoting the anaerobic conditions.
- They facilitate the growth of lactic acid bacteria (LAB), such as *Lactobacillus* spp., by suppressing *Leuconostoc* populations once a critical acid threshold is reached, thereby reducing the proliferation of undesirable or pathogenic bacteria [15].

Historically, the isolation of *L. mesenteroides* from *pulque* is attributed to Dr. Paul Lindner, a pioneer in the microbiology of traditional fermented beverages [10]. More recently, research groups at the Institute of Biotechnology at UNAM have studied this native strain to assess its biotechnological potential and functional properties.

At this stage, the role of microbial communities has been described mainly from a metabolic perspective, emphasizing their contribution to acidity, stability, and sensory properties of pulque. Beyond these functions, several strains have also been associated with the potential functional and probiotic effects, which will be addressed in detail in Section 6.

5. Chemical composition of *aguamiel* and *pulque*

The fermentation of *aguamiel* leads to significant changes in its physicochemical and nutritional composition. *Aguamiel*, the sap of the maguey plant, contains high water content (86.96%), fermentable sugars (fructose, glucose, sucrose), proteins (3.41 g/L), as well as essential vitamins and minerals (see **Table 1**). Its initial pH is neutral (6.29), and it displays a high °Brix value (11.10), reflecting its sweetness and energetic richness.

During fermentation, these physicochemical characteristics undergo significant changes. *Pulque* shows increased moisture content (98.3%), reduced soluble solids (4.3°Brix), higher titratable acidity (up to 1.25%), and moderate ethanol production (3.5–4.2%). These shifts result from the active metabolism of microorganisms such as *Z. mobilis* and *S. cerevisiae*, which efficiently consume the simple sugars present in *aguamiel* (see **Table 2**).

Regarding the protein and amino acid profile, fermentation reduces the total protein content but preserves, and in some cases increases, the concentration of certain essential amino acids. For instance, leucine increases significantly in *pulque* (24.75 vs.

Component	*Aguamiel*	*Pulque*
Moisture (%)	86.96	98.3
Proteins (g/L)	3.41	3.5
Ethanol (% alcohol/vol)	0	3.5–4.2
pH	6.29	3.5–4.0
°Brix	11.10 ± 0.10	4.3

Table 1.
Comparison of the basic chemical composition of aguamiel *and* pulque *[36–38].*

Sugar	*Aguamiel* (%)	*Pulque* (%)	Commentary
Fructose	5.22	Significant reduction	Utilized by *Zymomonas* and *S. cerevisiae*
Glucose	3.48	Significant reduction	Utilized by *Zymomonas* and *S. cerevisiae*
Sucrose	4.69	Almost absent	Early hydrolysis

Table 2.
Fermentable sugar content in aguamiel *and its transformation during* pulque *fermentation [38].*

Amino acid	*Aguamiel* (mg/100 g)	*Pulque* (mg/100 mL)	Variation
Leucine	4.67	24.75	↑ Significant → Significant increase
Lisine	5.17	5.11	~ Equal → Approximately unchanged
Methionine	2.18	3.25	↑ Moderate → Moderate increase

Table 3.
Essential amino acid content in aguamiel *and* pulque *[39].*

4.67 mg/100 g in *aguamiel*), while lysine remains relatively stable (~5 mg/100 g), as shown in **Table 3**.

These data highlight the nutraceutical value of *pulque* as a fermented product with the functional properties derived from both its microbiota and its profile of bioactive compounds.

6. Probiotics and prebiotics in *pulque*: Functional and microbiological implications

The World Health Organization (WHO) defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [40, 41]. In this context, *pulque* emerges as a highly relevant fermented beverage, not only due to its nutritional contribution but also because of its complex microbiota, composed of lactic acid bacteria, yeasts, and fungi.

Researchers at UNAM identified *L. mesenteroides* P45 in *pulque*, a strain capable of producing antimicrobial compounds with the beneficial gastrointestinal effects [12].

Recent studies have identified various strains with probiotic potential in *pulque*, including *L. mesenteroides* subsp. *mesenteroides* SD23, *Lactobacillus sanfranciscensis* (*L. sanfranciscensis*), *Lactobacillus casei* (*L. casei*), and representatives of the genus *Pediococcus* [42].

These strains have demonstrated the beneficial effects, including improved metabolism in obese murine models, cholesterol reduction, cytokine modulation, and antimicrobial activity against pathogens such as *Salmonella enterica* (*S. enterica*), *Helicobacter pylori*, and *Staphylococcus aureus* (*S. aureus*). **Table 4** from the study by Cerero et al. [42] summarizes the specific applications of these strains, reinforcing the functional relevance of *pulque* as a potential source of probiotics with implications for metabolic and immune health.

6.1 Prebiotics in *pulque*: Functional ingredients from native substrates

Prebiotics are defined as fermentable ingredients that selectively modify the gut microbiota, promoting the growth of beneficial bacteria [2]. In the case of *aguamiel*

Isolated strains	Applications
L. mesenteroides subsp. *mesenteroides* SD23	Improves metabolic dysfunction associated with obesity. Reduces body weight, glucose, cholesterol, and leptin levels.
Strains of the genera *Lactobacillus* and *Pediococcus*	Significant antibacterial activity against *Helicobacter pylori* ATCC 43504, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213. Potential preventive effect against multidrug-resistant bacterial infections.
L. mesenteroides P45	Antagonistic effect against strain L1334 of *Salmonella enterica* serovar Typhimurium resistant to streptomycin (murine model).
Lactobacillus sanfranciscensis LBH1068	Anti-inflammatory properties, weight loss, decreased intestinal permeability, and modulation of cytokines (murine model).
Lactobacillus casei J57	Bile salt hydrolase activity. Potential cholesterol reduction.

Table 4.
Pulque-*derived strains with probiotic potential and their applications [42].*

and *pulque*, their prebiotic activity is attributed to their content of fructooligosaccharides (FOS), compounds composed of 3–10 fructose units linked to glucose *via* β-2,1 or β-2,6 glycosidic bonds. When these chains exceed 10 units, they are referred to as fructans, and can be classified as inulin, levan, or agavin, depending on the type of linkage [19].

In addition, *pulque* contains exopolysaccharides (EPS), biopolymers synthesized by microorganisms during fermentation. These EPS are composed of glucose, galactose, rhamnose, and other sugars, and exhibit the prebiotic properties due to their resistance to gastrointestinal digestion and their ability to generate short-chain fatty acids (SCFAs) through microbial metabolism [43]. Some *pulque*-derived bacteria involved in EPS production include *Bacillus subtilis* (levan), *Lactobacillus plantarum* (xyloses, galactose, and other sugars), *Lactobacillus casei* (glucose and rhamnose), and *Leuconostoc citreum* (dextran) [19].

Together, these components position pulque as a unique matrix with both probiotic and prebiotic potential. While native microbial strains contribute to the probiotic activity by modulating gut microbiota and producing bioactive metabolites, the oligosaccharides and exopolysaccharides present in aguamiel and generated during fermentation provide the fermentable substrates that selectively stimulate the beneficial bacteria. This symbiotic relationship highlights the dual functional role of pulque, making it a promising candidate for the development of next-generation functional foods.

Furthermore, Curado (pulques produced by adding fruits, herbs, or other ingredients after fermentation) provides a strategy to diversify and enhance the sensory profile of the beverage. Beyond delivering vitamins and antioxidants, the inclusion of fiber-rich fruits such as apples, pears, bananas, and prickly pears, together with polyphenol-rich herbs and other bioactive substrates, enhances the nutritional and functional properties of pulque by supplying fermentable components in the same way as the oligosaccharides, exopolysaccharides, and other prebiotics fiber generated or added during fermentation that selectively stimulate beneficial gut microbiota.

This strategy not only augments the health-promoting potential of curados through synergistic probiotic-prebiotic interactions but also preserves their cultural and culinary relevance, positioning them as promising candidates for functional food innovation within traditional fermentation frameworks. The fermentable fibers, polyphenols, and bioactive molecules provided by these ingredients interact with the native microbiota of pulque, collectively supporting gut health and metabolic regulation.

7. Conclusions and future perspectives

Pulque is a complex traditional fermentative system in which diverse microbial communities and key metabolic pathways interact. This ancestral beverage not only holds deep cultural and ethnobiological value but also harbors microorganisms with the recognized functional and biotechnological potential. This chapter integrates recent studies demonstrating the involvement of lactic acid bacteria, yeasts, and ethanologenic bacteria in lactic, alcoholic, and exopolysaccharide-producing fermentations (processes that shape the texture, stability, and functionality of the final product).

Several native strains with the validated probiotic properties have been identified, including *L. mesenteroides*, *Lactobacillus casei*, and *Pediococcus* spp., which exhibit gastrointestinal tolerance, antimicrobial activity, and immunomodulatory effects. Moreover, the natural presence of fructooligosaccharides (FOS) and exopolysaccharides (EPS) in aguamiel and pulque reinforces their classification as foods with prebiotic properties, derived from both the raw substrate and microbial metabolism.

Despite its potential, pulque remains underexplored in terms of strain standardization, genomic analysis, and optimization of controlled fermentations. Addressing these research gaps will facilitate its industrial use in developing functional foods, probiotic supplements, and biomaterials. Strengthening collaboration among science, industry, and traditional producer communities will be essential to preserving the practices that have sustained this ancient beverage.

Ultimately, *pulque* can be envisioned as more than a traditional fermented drink: it represents a model system where ethnobiology and modern biotechnology converge. Its study illustrates how ancestral knowledge, artisanal practices, and microbial ecology intertwine to shape a complex product that is both culturally symbolic and scientifically relevant. Positioning *pulque* in this way not only strengthens its identity as part of Mexico's intangible cultural heritage but also highlights its potential to inspire contemporary innovations in functional foods, probiotics, biomaterials, and microbial applications. By bridging cultural authenticity with the technological advancement, pulque exemplifies how traditional fermentations can inform future biotechnological strategies and global discussions on sustainable, innovative, and culturally rooted food systems.

Conflict of interest

The authors declare that they have no conflict of interest.

Notes/thanks/other declarations

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Section 3

Industrial and Nutritional Applications

Chapter 6

Fermented Rice Beverages in the Food Industry: Advances in Processing, Nutrition, and Commercialization

Sivashankari Manickam

Abstract

The consumption of fermented rice-based drinks has been a common practice in several cultures for many years, because of their interesting tastes, strengthened nourishing value, and possible health benefits. Interest in healthier and probiotic-enriched drinks has led the food industry to develop ways to scale up the production and commercial selling of fermented rice beverages. As a result of these processes, rice wine, sake, amazake, handia, tapai, and other rice-based probiotic drinks become easier to digest, get improved nutrients, and contain beneficial bacteria. This part of the chapter looks at the microbiology, chemistry, and engineering parts of making rice beverages. It considers the function of microbial teams, the impact of various enzymes, and how parameters in the fermentation process can be improved to increase production in industries. The focus of this part is on controlled fermentation, bioreactors, help from enzymes during processing, and new ways of packaging food. Also, the opportunities in the industry for commercial products, the issues caused by regulations, and consumers' trends are discussed. At the end of the chapter, possible future projects and ways to increase the production, features, and marketing of fermented rice beverages are discussed.

Keywords: rice fermentation, fermented beverages, microbial diversity, functional drinks, probiotic strains, enzymatic hydrolysis, alcoholic fermentation

1. Introduction

For countless generations, humans have valued fermented beverages for their tastes, ability to be stored, and the health benefits they may provide. Many people looking for healthy drinks have contributed to the rise of the global market for fermented beverages. The increase is linked to people realizing the value of probiotics and bioactive compounds in fermented foods. Since rice is a key staple for half the world, it is often used as a substrate for several fermentation processes. Since it contains plenty of carbohydrates, it is ideal for fermentation by microorganisms and helps create various fermented drinks. For a very long time, people in Japan and Korea have been drinking sake and makgeolli, which are traditional types of rice drinks. There has

recently been a rise in people enjoying these drinks, along with the creation of new rice drinks that best fit today's lifestyle. By using microbes from nature and letting things happen naturally, typical methods of fermentation create unique characteristics in the products made. Yet, adopting such approaches can produce differences in both quality and safety. The purpose of industrial-scale fermentation is to guarantee the same process and quality by controlling things. Here, the use of certain starter cultures, appropriate fermentation conditions, and advanced methods are applied.

The chapter explores how rice becomes fermented beverages and what impacts microbiological, biochemical, and engineering principles have. It discusses both old and modern traditions, the health and nutritional benefits, and the possibilities and difficulties in launching such drinks in the market.

2. Traditional and industrial fermented rice beverages

2.1 Traditional rice-based beverages and their microbial profiles

2.1.1 Alcoholic beverages

Fermented rice has been used in many parts of Asia, like India, to make different kinds of local alcoholic drinks that people enjoy and remember for their taste and traditions [1]. Among the best known in the world is Sake from Japan, which is made with a careful two-step fermentation process [2]. In this method, people put the *Aspergillus oryzae* mold on sticky cooked rice to break down starches into simple sugars the yeast and bacteria can eat [3]. These sugars then turn into ethanol when yeast called *Saccharomyces cerevisiae* do their job [4]. Critical processing steps like how much rice is polished (seimaibuai), thee minerals in the water, and keeping the temperature steady, are adjusted to help improve the sake's quality and get the smooth and nice aroma that you find in good sake [5].

Just like Zaklyvka and Niyama, the Korean beverage Makgeolli uses nuruk to culturally enliven it with wild yeasts, molds, and lactic acid bacteria [6]. Makgeolli is famous for having a low alcohol level 96–9% and for containing live microbes that may help promote health [6]. Thanks to recent technology, fermentation in Makgeolli is now standardized using specific strains of bacteria to keep the drink traditional and safe for health [7].

Heritage rice-based fermented alcoholic beverages have always been a traditional part of food traditions for various Indian ethnic groups in the north-eastern states and tribal regions [8]. For example, there is Apong in both Assam and Arunachal Pradesh, Xai in Assam, and Handia in several Indian states like Jharkhand, Odisha, and Chhattisgarh. The beverages are brewed using starter cultures such as ranu tablets, made from rice flour plus majorly yeasts, molds, and lactic acid bacteria [9–11]. Though fermentation in wine occurs naturally, it shares a lot in common with the saccharification and alcohol fermentation steps that occur when producing sake and makgeolli. Efforts have been put in place in recent times to scientifically ensure that indigenous rice beers are produced safely, keeping for longer, and are liked by buyers, so that the age-old methods are not forgotten [12].

2.1.2 Non-alcoholic beverages

People are paying more attention to these types of drinks for their wellness and health properties. Amazake is made in Japan by fermenting rice with *Aspergillus*

oryzae, which hydrolyzes the starch in the rice into sugar [13, 14]. The amount of alcohol in amazake varies depending on the duration and method used during fermentation. Since amazake contains plenty of B-vitamins and essential proteins, plus oligosaccharides and antioxidants, it is recommended for the digestive system, better skin health, and as a quick source of energy, making it suitable for different groups such as children and the elderly [15, 16].

Low-alcohol and non-alcoholic fermented beverages made from rice are common in India, and especially fermented beverages made from rice are common in India, especially among some tribal communities [17]. Pachwai is made in Jharkhand and Odisha by soaking or cooking rice that is then left to ferment in small amounts for a naturally sweet and mildly sour drink [18]. Studies have shown that consuming these drinks increases Lactic acid bacteria (LAB) strains in your microbiome and improves your digestion since they are a good source of probiotics [19]. Modern food science is starting to create probiotic drinks from rice using the process of lactic acid fermentation. To grow *Lactobacillus plantarum* and *Lactobacillus fermentum*, steamed or gelatinized rice is used as a food source [20]. They are developed to satisfy the increasing interest in non-dairy, plant-based probiotic drinks, mainly from people who cannot tolerate lactose [21–23]. Fermentation of rice has helped micronutrients such as zinc, iron, and B-complex vitamins to be absorbed better, so the resulting beverages have become an attractive and promising area for the Indian food industry [24, 25].

2.2 Industrial fermentation and standardization

2.2.1 Scaling up traditional fermentation for mass production

Glasses of fermented rice beverages are made today on an industrial scale, making it important to use careful strategies to ensure quality, safety, microbe management, and economics [26]. Traditional methods of fermenting foods are unpredictable mainly because the quality of raw materials changes and the environment is not controlled [27]. Ensuring uniform rice by choosing the same starch and moisture type and controlling fermentation temperature, oxygen levels, and moisture makes the mass production process possible [9, 28, 29]. Bioreactors and automated tanks with real-time sensors are used to control the conditions during industrial fermentation [30–32]. Moreover, using hazard analysis and critical control points (HACCP) allows for efficient management of food safety during the entire production process. More interest in Handia, Apong, and Pachwai drinks in India has encouraged researchers to explore developing larger-scale factories for the drinks. Attention has been given to improving the processing of substrates (including a liquefying process for the rice starches), managing the quality of the starter, and choosing ways to keep products fresh and flavorful while they remain on the shelves [7, 33]. Studies in research reactors have found that using the same procedures boosts the similarity of batches, an essential aspect for successful commerce [34, 35].

2.2.2 Use of microbial starter cultures in industrial processing

Using particular microbial starters is one important development in the industrialization of rice-based drinks [9]. Unlike past techniques that depend on natural, assorted microbes, industries today use well-defined yeasts (e.g., *Saccharomyces cerevisiae*), lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus brevis*), and filamentous fungi (such as *Aspergillus oryzae*) [36–39].

By choosing starter cultures, it is possible to ensure accurate, reliable fermentation, lower the chances of contamination, always get a consistent taste and boost probiotic and antioxidant activity [7, 40, 41]. In industrial manufacturing of sake, choosing specialized yeast strains such as Kyokai No. 7 has helped achieve sake with both high ethanol and fine aromas [42, 43]. Indian researchers have worked on finding local microbes in traditional beverages and on developing starter cultures perfect for their environment. LAB strains identified in Handia are currently being used to create starter cultures that aim to increase safety, improve taste and lengthen shelf life of the finished drink [10, 44]. Thanks to genomic and proteomic advances, researchers can now choose starter strains that are tough, have a strong capacity to ferment, and create desirable metabolic products [45].

By creating set microbial starters, industry was able to process rice-based fermented drinks [9]. While naturally occurring microbes were often used in traditional fermentation, now industrial fermentation uses carefully picked single-strain microbes. When starter cultures are present, fermentation is steady and level, the fermentation is less likely to be contaminated, sensory qualities are more the same, and probiotics become more effective. Employed in industrial sake making is a yeast strain, Kyokai No. 7 that has seen the production of more ethanol and better taste [42, 43]. Experts in India are analyzing traditional rice beverages to find special microbes that can become Indian-specific starters. In fact, adding cultures produced from Handia LAB helps improve the taste, keeps the drink safe to drink, and increases its freshness [10, 44]. Thanks to recent improvements in genomic and proteomic techniques, it is simpler to identify strong starter strains that work efficiently, can resist stresses, and create ideal metabolites [45].

2.2.3 Process standardization to ensure consistency and quality

Standardizing the production process is vital to reach the desired quality for every batch and comply with manufacturers' regulations and consumer expectations [46]. Key elements that are monitored during standardization are called:

- The temperature for fermentation should lie between 25 and 30°C for yeasts and 30–37°C for LAB.
- Control the pH level so that the acidity is between pH 4.0 and 5.0 to prevent infections.
- Period of fermentation, which varies with each drink from 24 to 96 hours.
- Colony-forming units per mL (or grams) in microbial inoculum.

To achieve precise fermentation, current practices in food engineering involve using fermentation modeling software, computer apps, and automated control systems [47–49]. Scientists use HPLC and GC-MS techniques to continuously track metabolites such as ethanol, organic acids, and flavor oils [50–52]. With the help of SOPs developed by CSIR-CFTRI and similar organizations, entrepreneurs and industries in India find it simpler to produce traditional, rice-based beverages while meeting the required food safety standards set by the FSSAI. Standardizing products encourages the government to award geographical indication (GI) tags, which improve their appeal to consumers [53, 54].

3. Microbial diversity in rice beverage fermentation

3.1 Key microorganisms in fermented rice beverages

3.1.1 Yeasts

It is thanks to yeasts that the alcoholic fermentation of rice-based beverages occurs, as they convert fermentable sugars into ethanol and carbon dioxide [55–57]. The yeast species mostly used is *Saccharomyces cerevisiae*, which is capable of fermenting sugars, withstanding ethanol, and creating flavors people find desirable [58, 59]. After *S. cerevisiae, S. bayanus, Pichia anomala, Pichia kudriavzevii, and Candida tropicalis* have each been spotted in rice fermenations, each species adding different flavor notes with their esters, higher alcohols, and organic acids [60, 61]. Rice drinks like Apong (Assam), Handia (Jharkhand, Odisha), and Zutho (Nagaland) in India contain many different types of indigenous yeasts. Work done in North-Eastern India suggests that various yeasts live together in these traditional starters, contributing to shaping the flavors and properties of these beverages [62].

3.1.2 Lactic acid bacteria

Lactic acid bacteria (LAB) make up the other significant microbial group involved in fermenting rice beverages. Typically, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Pediococcus pentosaceus* are found in rice fermentations [63]. LABs create lactic acid, lowering the pH and preventing the growth of harmful microorganisms in the product [64–66]. Also, they produce various volatile compounds that affect the drink's taste and aroma, and can offer benefits like antioxidants and probiotics [40, 67–69]. Researchers found that LAB from traditional starters of fermented rice drinks have the ability to deal with acidity, bile, release antimicrobials, and adhere to cells found in the intestine [41]. During co-fermentation, the interaction of yeasts and LAB plays a key role in making the food taste, feel, and be nutritious [70].

3.1.3 Molds

Aspergillus oryzae, the primary type of mold used in the fermentation of beverages, is critical in the saccharification of rice. *A. oryzae*, a filamentous fungus belonging to the phylum Ascomycota, also generates various hydrolytic enzymes (mainly amylases (α-amylase, glucoamylase) as well as proteases), which are important in converting the starch and proteins that are contained in rice into fermentable sugars and peptides [71, 72]. This enzymatic process leads to fermentation of traditional beverages of Japan such as sake and amazake. Similarly, in Indian rice based drinks such as handia, apong and ruhi, the starter cultures use molds such as *Rhizopus oryzae* and *Mucor indicus* these belong to the phylum Mucoromycota perform an early hydrolysis of rice starch and proteins. The *Rhizopus oryzae* can be characterized by the cottony-white growth and the black-spored sporangia, secretes both proteases and amylases, and saccharification occurs prior to the yeast activity. Another rapidly growing mold with round sporangia, and white mycelium, is *Mucor indicus*, which provides comparable contribution by its own set of amylases, proteases and lipases [17, 73]. These fungi assist to breakdown complex macromolecules to simpler forms which yeast can subsequently ferment into alcohol. Other studies verify the fact that most of the native Indian starter cultures already have a blend of *Aspergillus, Rhizopus* and *Mucor*. They are crucial to the manufacture of traditional and commercial rice-based

beverages owing to their enzymes in a mix that effectively breaks down starches and proteins to alcoholic fermentation [74].

3.2 Role of microbial enzymes in sugar conversion, alcohol production, and flavor development

Fermentation of rice into tasty and useful beverages mainly depends on the action of the enzymes produced by the fermenting microorganisms [75, 76]. Key enzymatic actions include:

- Yeast relies on amylases (α-amylase and glucoamylase enzymes) to breakdown the starch present in the lima bean into glucose and maltose, which they use for ethanol production.
- Breaking proteins into peptides and free amino acids, proteases make the food both more nutritious and tasty because of the umami. Breaking down amino acids also gives rise to aroma compounds called aldehydes and ketones.
- Lipases cause the breakdown of triglycerides to glycerol and three fatty acids. Adding fatty acids allows the beverage to undergo ester formation and become more aromatic.

A significant amount of reports indicates that starters from Jharkhand and Manipur are highly active and allow for better sugar release during fermentation [11, 77]. The cooperation of these enzymes is needed for increasing the taste, amount of alcohol, and texture of rice-based beverages.

3.3 Industrial application of probiotics in fermented rice beverages

The rise in popularity of functional foods has led to fortifying fermented rice beverages with scientifically pickled probiotic strains. Using *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium bifidum* as probiotics can boost the positive effects of these drinks, such as aiding gut bacteria, improving immunity, and boosting antioxidant properties [78–80]. Now, the industry aims to combine rice substrates with probiotic strains during fermentation or adds microencapsulated probiotics after fermentation to protect the cells [81]. In India, efforts are being made to bring probiotic drinks made from rice to markets that require lactose-free and gluten-free beverages. Probiotic LAB were successfully added to Handia and Apong by research done at ICAR institutes and CFTRI without changing the sensory features [17].

3.4 Impact of fermentation conditions on microbial activity and metabolite formation

A variety of physical and chemical conditions in the fermentation process can strongly influence the activity and formation of metabolites by microbes [82]. Critical factors include:

- Temperature: High temperatures can speed up yeast growth but can slow the growth of LAB; ideal temperatures vary by strain (usually between 28 and 32°C for yeast, and 30 and 37°C for LAB)

- pH: A pH value of 4.5 to 5.5 creates an environment where LAB thrive and other bacteria that may spoil the food cannot grow.

- Oxygen levels: Molds thrive in saccharification under aerobic conditions, but yeasts require mainly anaerobic conditions to do their fermentation

- Nutrient availability: If a sufficient amount of nitrogen, vitamins, and minerals is provided, microbes are better able to work and create metabolites.

It has been found through research that fine-tuning the process improves ethanol yield and provides the added benefits of producing different organic acids, vitamins (such as B-complex), and antioxidant peptides [83]. By optimizing the fermentation, Indian researchers made the beverages more appealing since they contain more GABA and polyphenols [84–86].

4. Biochemical and nutritional transformations in fermented rice beverages

The fermentation of rice results in many changes in its chemistry that make it healthier, have good tolerability, and taste better [87, 88]. The changes in food are due to the metabolic actions of various microorganisms, such as lactic acid bacteria (LAB), yeasts, and molds. Interactions between the bacteria and rice cause greater absorption of important vitamins, nutrients, and the production of healthy substances helpful for people [89].

4.1 Macronutrient modifications

4.1.1 Carbohydrates

Microbial enzymes in fermentation, such as amylases, help breakdown starch into maltose and glucose, which make the beverage from rice simpler to digest and more palatable [90, 91]. Furthermore, fermenting foods can create resistant starch fractions that act much like prebiotics and support specifically the growth of helpful gut microbes [92, 93].

4.1.2 Proteins

Enzymes from fermenting microorganisms split the proteins in rice into small peptides and free amino acids. This improves how our body digests and absorbs proteins, and it also results in the creation of bioactive peptides that help control blood pressure, protect from oxidative stress, and play an important role in the immune system [94].

4.1.3 Lipids

Although rice contains very little lipid, the fermentation process can lead to changes in lipids [95, 96]. Specific types of yeast and LAB produce essential fatty acids and create new volatile compounds that improve the odor and taste of the beverage [97].

4.2 Micronutrient enhancements

4.2.1 B-vitamins

The process of fermentation can boost the amount of B2, B3, and B3, and B5 vitamins in rice beverages [98, 99]. The improvement is mostly due to how LAB and certain yeast strains are involved in the fermenting process [100].

4.2.2 Minerals

Through degrading phytic acid and similar antinutritional agents, microbial fermentation allows for better access and use of iron, zinc, and selenium [101]. The improvements to the fermented rice beverage help it become a good choice as a functional food that addresses deficiencies of micronutrients.

4.3 Bioactive compounds

4.3.1 Gamma-aminobutyric acid (GABA)

As a result of fermentation involving lactic acid bacteria and certain yeast strains, GABA, a non-protein amino acid, is made. This acid helps the nervous system by reducing stress, helping you fall asleep, and stabilizing your blood pressure [86, 102].

4.3.2 Antioxidants

As a result of fermentation, the fermented drink has more antioxidants like phenolic acids and flavonoids. Because they target free radicals, these bioactive molecules decrease oxidative stress and lower the risk of long-term diseases as a result [103, 104].

4.3.3 Organic acids

The formation of lactic acid and acetic during fermentation helps the drink develop a unique sour taste and, moreover, protects if from bacteria that spoil or contaminate the drink [40].

5. Innovations in industrial processing of fermented rice beverages

Since fermented rice drinks are becoming more common in the market, companies must use innovative technology to maintain product safety, ensure it stays the same each time, work well in large quantities, and last for a good length of time. Innovations in food engineering, together with knowledge from food chemistry and microbiology, have allowed traditional food production to become standardized in industry.

5.1 Starter culture development

Using specially made and fine-tuned starter cultures has revolutionized the industrial process for making fermented rice beverages. By using natural fermentation, some variability and inconsistency often occur in the product, as it is different with every

batch [29, 105]. Starter cultures make it possible to control fermentation, leading to more consistent and similar fermented products. Starter cultures are usually made from certain strains of microorganisms, like LAB, *Saccharomyces*, or *Aspergillus*, depending on their ability to ferment, make bioactive substances, and give the desired flavor [106]. Experts are continuously working to find and isolate strains that have set qualities like:

5.1.1 High fermentative capacity

Yeasts that are able to change carbohydrates into alcohol and acids, improving the rates of fermentation and how the beverage tastes [107, 108].

5.1.2 Probiotic potential

There are many starters available, but *Lactobacillus and Bifidobacterium* made from "Lactic Acid Bacteria," can support healthy bacteria in the gut [109]. Bacteria that have been shown to thrive in the gut are being used more often in making fermented rice beverages for better health reasons [56].

5.1.3 Flavor-enhancing abilities

There are yeast and LAB strains that make flavor compounds called esters, organic acids, and phenolic compounds during the fermentation process [110, 111]. Such additives help make rice-based beverages taste and smell better, helping them sell in different markets [110]. Having starter cultures allows for larger and more consistent production of good-quality fermented rice and is vital to the fermentation process in large bioreactors.

5.2 Bioreactor fermentation

Despite working efficiently on a small scale, these traditional methods are set back by changes in each batch of fermentation, excess time spent, and a lack of ways to control the environment. By contrast, using bioreactor technology in industrial production allows fermented rice beverages to be made with strict control over parameters such as temperature, pH, supply of oxygen, and the amount of stirring [112, 113].

Bioreactor-based fermentation enables:

5.2.1 Higher microbial productivity

Fermentative microbes increase their biomass and metabolite production when kept in optimal fermentation conditions [114].

5.2.2 Reduced batch-to-batch variability

By having specific environmental rules, the quality of products remains unchanged throughout their production [115].

5.2.3 Automation and scalability

The use of bioreactors allows for ongoing monitoring and control, enabling businesses to produce on a large scale [112].

Continuous fermentation systems with *Saccharomyces cerevisiae* cells fixed in gel have been successfully used to produce good sake with steady flavor and an increased amount of alcohol [116, 117].

5.3 Novel processing technologies

The use of non-thermal and soft processing has made fermented rice beverages safer, longer-lasting on the shelf, and higher in quality while keeping their special flavors and active substances [118, 119].

5.3.1 High-pressure processing (HPP)

The high-pressure process exposes the beverage to up to 600 MPa, which kills spoilage microorganisms and pathogens while still keeping its nutrients and organoleptic properties [120]. As a result of HPP processing, fermented rice beverages keep their natural color, taste, and functions, making them more attractive for high-end markets [121].

5.3.2 Membrane filtration

In the industry, rice beverages are now clarified more often with membrane methods, separating unwanted particles and yeast while also preserving the beneficial ingredients [122]. Such a process improves the appearance, steadiness, and length of the product's life [28, 123].

5.3.3 Spray drying and freeze drying

Amazake and similar drinks are processed by spray drying or freeze drying into powder so they can be sold more conveniently. Spray drying is effective for mass production, but it may reduce or destroy some heat-sensitive nutrients [124–126]. While it costs more, freeze-drying preserves the active compounds, odor, and nutrients of food [126–128]. Fermented rice products have evolved into instant powders and nutrient supplements [56, 128–130].

5.4 Process optimization

Developing the best fermentation process helps enhance both the performance and taste of fermented rice beverages. Advanced statistical and modeling methods are now commonly used to improve the process.

5.5 Response surface methodology

Response surface methodology (RSM) is a statistical technique. Many investigations use RSM to measure and enhance GABA content, antioxidant activity, alcohol levels, and the characteristics of different wine qualities [131].

RSM has been proven to be successful by several studies.

- The end result is a boost in production of GABA and polyphenols
- Fermentation conditions were adjusted to result in a better flavor, texture, and aroma

- The fermentation process was cut down, producing healthy and reliable results.

Combining optimization methodologies and advanced bioprocessing technologies is a key approach to producing high-quality, health-promoting fermented rice beverages in industry [7].

6. Food engineering considerations in industrial rice beverage production

6.1 Optimization of fermentation parameters

Rice beverages rely on proper fermentation, so controlling its parameters is fundamental for getting the right flavor and texture [9, 28, 132]. Studies conducted in recent years have revealed that these factors are very important:

6.1.1 Temperature

Temperature settings determine the growth rate of bacteria in fermentation and affect what kinds of substances are produced, including alcohol and organic acids. According to studies, by setting the temperature from 20 to 30°C, it is possible to optimize fermentation and ensure that the process is not stressed by too much heat [133].

6.1.2 pH

How much the pH changes can influence the growth of bacteria and the time it takes to ferment? Usually, keeping the pH at 4–6 is necessary for ensuring microbial stability and correct flavor formation [134]. Having a pH outside the accepted values can cause an increase in harmful microbes and change the way the final product tastes and smells [9, 135].

6.1.3 Aeration

Proper supply of oxygen is needed by the aerobic microorganisms, which strongly affects the flavor and alcohol content [9, 132]. In the production of rice drinks, aeration stimulates both the growth of microbes and the formation of compounds that influence rice drink flavor [77].

Fermentation parameters are typically adjusted to perfect the final product's taste, make it more consistent, and improve its quality and yield of beneficial substances [136, 137].

6.2 Equipment design

Improving the fermentation equipment can enhance scaling efficiency and quality in rice beverage production [31, 138]. It is important for equipment to have several key aspects in its design to be effective in industry.

6.2.1 Sterile conditions

One of the main reasons for using sterile conditions is to stop unwanted microorganisms from getting in [139]. In order to control microbes, industrial fermentation tanks need to have effective sanitation units and also use aseptic inoculation methods [9, 82].

6.2.2 Continuous monitoring

Modern bioreactors use sensors to check temperature, pH, dissolved oxygen, and pressure very frequently [112, 140]. They make sure that the conditions for the fermentation process do not fluctuate [141].

6.2.3 Mass transfer efficiency

It is essential for the system to transfer mass effectively so nutrients, oxygen, and the resultant products are spread evenly [142]. Stirred tank reactors (STR) are widely used in industrial rice beverage production due to their ability to effectively mix the solution, giving all the microbial cells access to necessary nutrients and avoiding settling [143].

The use of automation systems allows for real-time updates to settlings, leading to improvements in the whole batch and consistency of the process [47, 141, 144]. With the right control algorithms, the process and environment during fermentation are properly managed, leading to more output and consistent results [144, 145].

6.3 Packaging innovations

The quality, safety, and shelf life or fermented rice beverages are protected by using appropriate packaging [40, 146, 147]. Many new innovations have been introduced in packaging to fit the needs of each product:

6.3.1 Aseptic packaging

Under aseptic packaging, both the product and its wrapper are made sterile to prevent contamination. Because rice beverages need to remain live and unspoiled, aseptic packaging plays a key role in keeping them fresh over time and without refrigeration [28, 148]. Thanks to this technology, drinkable rice can be stored at room temperature for an extended period without losing its nutrient value and taste [149, 150].

6.3.2 Modified atmosphere packaging

MAP packaging is an innovative way to keep fermented rice beverages fresh for longer periods of time [119]. Changing the amount of oxygen and carbon dioxide inside the packaging is one way MAP can limit the growth of spoilage microbes and deterioration caused by oxidation [151]. Since this method protects the useful bacteria in probiotic drinks, it can ensure their continued presence even after storage [152, 153].

It is important that aseptic packaging and MAP are used to preserve the probiotics and other features of fermented rice beverages during their shelf life. Such developments are essential for expanding the commercial value of fermented rice beverages on a global scale.

7. Health benefits and functional properties

People are drinking more fermented rice beverages because they are tasty and have many healthy and beneficial effects. This category of beverages, featuring bioactive compounds, probiotics, and prebiotics, has been indicated to be beneficial to the body in many ways [57, 154–156]. In the last decade, research has supported the idea

that these beverages are beneficial for digestion, the immune system, heart health, metabolism, and anti-aging.

7.1 Digestive health

Fermented rice beverages contain beneficial bacteria called lactic acid bacteria, helping maintain good health of the gut. Having enough of these microbes is beneficial for health, mainly by influencing the bacteria in the gut. Science suggests that probiotics boost the diversity of microbes in the gut, enhance how the body absorbs nutrients, and make it easier to digest both carbohydrates and fibers [157, 158]. Also, the fermented rice beverages in this diet could bring benefits to gut health as prebiotics that help good bacteria grow [159, 160]. For people with irritable bowel syndrome (IBS), fermented rice drinks improve stomach function and ease both bloating and constipation [57, 161, 162].

7.2 Immunomodulatory effects

Adding probiotics, particularly lactic acid bacteria, to fermented rice drinks can improve our immune responses. Evidence shows that these microorganisms help stimulate the creation of cytokines that regulate various immune reactions and limit inflammation [163–166]. LAB support proper functioning of the intestinal barrier that is vital for limiting infection risk and stopping inflammation [167, 168].

Studies have found that rice drinks that include LAB may help relieve chronic low-grade inflammation linked to metabolic syndrome, cardiovascular diseases, and certain cancers [169–171].

7.3 Cardiovascular health

Scientists have discovered that drinking beverages made from GABA-enriched rice is beneficial for cardiovascular health. Gamma-aminobutyric acid (GABA) is an important substance made during fermentation that has been linked to promoting good health, especially in blood pressure regulation [51, 88, 172, 173]. Researchers believe that drinking GABA-rich beverages regularly may help to reduce blood pressure by relaxing blood vessels and improving how they function [88, 174, 175].

GABA-rich fermented rice beverages also have been found to enhance lipid profiles by reducing total cholesterol, LDL cholesterol, and triglyceride levels and increasing good HDL cholesterol [174]. Thanks to these effects, these drinks play a role in lowering the risks of hypertension and atherosclerosis.

7.4 Anti-diabetic and anti-obesity effects

When you consume fermented rice beverages, they have a low GI, so they allow for slower and more steady rises in blood glucose [176]. People with diabetes or at risk of metabolic disorders can greatly benefit from using this feature. Better glucose regulation happens when insulin sensitivity increases due to changes in carbohydrates from fermentation [177].

Studies also reveal that fermented rice beverages help fight obesity [178, 179]. Regular use of these drinks may lessen body fat and waist size, both due to their effects on fat metabolism and their appetite-reducing benefits [56, 180–182].

Fermented rice is a functional food that may be helpful in dealing with type 2 diabetes and avoiding obesity.

7.5 Antioxidant and anti-aging effects

Rice wine and sake are known to contain plenty of phenolic compounds and antioxidants that counteract damage from harmful free radicals [56]. Thanks to their antioxidant activity, these substances help prevent oxidative stress, which lead to many dangerous health effects, especially as we grow older [183].

Researchers have shown that fermentation leads to a higher level of phenolics and better absorption of antioxidants in rice drinks [184]. Besides supporting health, these compounds are believed to have anti-aging effects like improving skin's flexibility and reducing signs of aging [132, 185, 186].

Studies have shown that these drinks also provide anti-inflammatory benefits, helping to cut down the risk of age-related diseases such as Alzheimer's disease and arthritis [169, 187, 188].

8. Commercialization and market challenges

Turning fermented rice beverages into a commercial product requires making the product consistent, meeting regulations, ensuring products do not go bad quickly, and winning customer approval. Entering the markets of other countries, where competition is high, requires these points to be taken into account.

8.1 Microbial contamination and spoilage

Microbial contamination is still a major issue in the industry of fermented rice beverages, even with the use of starter cultures. Even though lactic acid bacteria (LAB) and *Saccharomyces cerevisiae* are commonly used to manage fermentation and stabilize the product, contamination by spoilage bacteria, molds, or yeast can happen [38, 153, 189]. The buildup of microbes in foods can cause quality problems, like bad odors and flavors, make things spoil and be dangerous for health, leading to a lesser chance that consumers will buy them.

After fermentation, the microbial contamination risk tends to be the highest during storage and handling when not all precautions are taken. These pathogens have been found in fermented foods and beverages, primarily because of poor health and sanitation practices during fermenting [9, 190–192]. They can weaken the positive properties of the beverage by lowering probiotics, GABA, and antioxidant levels [40, 129, 193].

It is essential to have strict hygiene and control systems for safety during the whole production process. Manufacturers should rely on GMP, HACCP, and SSOPs to minimize chances of microbial contamination [194]. According to the research of some experts, using natural preservatives such as essential oils and plant extracts, along with MAP, can improve the microbial safety and shelf life of fermented rice beverages, as well as maintain their health benefits [195–197].

To check for pathogenic bacteria and spoilage organisms, PCR, 16S rRNA sequencing, and other similar methods are often used throughout fermentation and storage of fermented foods [49, 198–201].

8.2 Standardization and regulatory compliance

A main issue in introducing fermented rice beverages to the market is the variety of ways they are fermented, which can result in inconsistency in their quality. Depending on the strain and fermentation process, and the materials used, the flavor, texture, and nutritional value of a product may change, possibly affecting its approval by customers and regulating bodies [202]. Since different fermentation cultures may produce varying results, it is essential to define the best culture and strains (*lactic acid bacteria* and *Saccharomyces cerevisiae*) to get optimal results [48]. Making these procedures standard reduces inconsistencies, making sure the product is both nutritious and pleasing to everyone [203].

To ensure safety and quality, using certificates such as HACCP and ISO is important for every step of producing fermented rice beverages [204]. They are crucial for being trusted in the food industry, given the tough regulations worldwide [205]. Food safety guidelines help avoid contamination and preserve both the beneficial effects and safety probiotics and bioactive substances [206, 207].

8.3 Shelf life and stability

A major challenge in selling fermented rice beverages is ensuring that live probiotics and key nutrients stay stable while being transported and kept. The effectiveness of probiotics declines as they are subjected to both time and changes in temperature, light, and oxygen [208]. In a similar way, the breakdown of GABA and antioxidants can impact the taste and usefulness of the beverage [85].

To fix these problems, companies are investigating innovative ways to package and preserve food. By reducing the amount of oxygen around fermented beverages, processes such as MAP and vacuum sealing can stop or slow spoilage [157]. High-speed freezing and spray drying methods are currently being explored to help preserve the bioactive content and probiotics in powders of fermented rice drinks [209]. These technologies play an important role in storing the product to maintain both its smell and function, which is important for a pleasing user experience.

8.4 Consumer acceptance and market education

Fermented rice beverages can only sell well if there is broad acceptance among consumers. The potential benefits of such beverages include helping the gut, boosting the immune system, and protecting the heart, but many consumers might not know much about their nutritional assets or what they mean culturally. This is why it is vital to have education campaigns to encourage consumers to see fermented rice beverages in a better light [210].

Efforts to educate should stress these drinks' role in enhancing digestion, building resistance to disease, improving metabolic issues, and their probiotic and antioxidant properties. Reasons behind the popularity of fermented rice beverages in Asian countries, where they are an important part of daily life, will help increase appreciation in international markets [1]. Rice-based drinks are also a sustainable option for those who look for plant-based options and want to help the environment.

You can build more trust with consumers by involving health influencers, nutritionists, or dieticians in your campaigns. When the labels on drinks are clear and honest, shoppers can choose better products for their health [211].

9. Future prospects and research opportunities

Fermented rice beverages will grow and improve through new ideas and sustainable practices, especially by increasing their benefits to health and the environment. With various new trends and areas of research, there is great potential to improve rice beverage production, quality of nutrition, and profits.

9.1 Development of novel functional beverages

The popularity of functional foods keeps rising, and fermented rice beverages are set to play an important role in it. The idea of adding functional ingredients is gaining momentum. Many studies are now focusing on combining herbal medicine, peptides from plants, and synbiotics in beverages to enhance their health effects.

The addition of Moringa, Tulsi, and Ashwagandha might increase the antioxidant and anti-inflammatory qualities of fermented rice beverages, according to research [212]. In addition to what fermented rice already offers, these herbs may help improve gut health and immunity further.

Bioactive peptides that emerge during fermentation are receiving more focus due to their benefits in antioxidant capacity, antihypertension, and immune-related tasks [213]. Adding bioactive peptides can make these beverages more useful for the body.

A combination of probiotics and prebiotics called synbiotics can be very beneficial for a healthy gut and improved digestion.

The arrival of these innovations means that fermented rice beverages may cater to people wanting solutions for their health and wellness, including immune support and digestion.

9.2 Precision fermentation

With synthetic biology advances and precision fermentation, the possibilities for customization in fermented rice beverages are clear. With this strategy, flavors can be modified, health benefits can be aimed at specific people, and special functional features can be created.

Through synthetic biology, it is possible to engineer certain bacteria with the goal of boosting their output of things like GABA or anti-oxidants. As a result, production may become more time-saving and output more reliable. In precision fermentation, the environment for fermentation is carefully managed to boost the number and quality of useful ingredients.

Altering the microbes using in production enables greater flavor variety in fermented rice drinks, satisfying many people looking for different tastes [112]. It may be possible to produce drinks that give personalized benefits for gut health or brain function.

9.3 Integration with sustainable practices

The industry of fermented rice beverages can support and adapt to the shift toward sustainability in food production. Making use of by-products (such as rice bran) and less-energy intensive fermentation can be key approaches toward a better environment and a stronger economy.

In addition to rice, the by-products of its processing contain essential vitamins, fibers, and minerals. Adding them to fermented rice drinks can increase their

nutritional value and prevent any waste. Using rice bran both meets nutritional needs and follows the principles of the circular economy.

Food companies are turning to low-energy fermentation processes to reduce the amount of CO_2 released. By using technology that operates at low temperatures or harnesses renewable sources like Solar power or biomass, the sustainability of making fermented rice beverages can be increased without changing their taste [214]. With these practices in place, the industry can provide what customers and regulators need and still maintain sustainability.

9.4 Exploration of underutilized rice varieties

The exploration and utilization of *underutilized rice varieties* could help diversify the flavor and nutritional profiles of fermented rice beverages. In particular, *pigmented rice*, *aromatic rice*, and *indigenous rice varieties* offer unique properties that could differentiate products in the market.

9.4.1 Pigmented rice varieties

Pigmented rice varieties, such as *black rice* and *red rice*, are rich in *anthocyanins* and other *polyphenols*, which are known for their *antioxidant* and *anti-inflammatory* properties. The inclusion of these varieties could enhance the *nutritional value* and *health-promoting effects* of fermented rice beverages [108].

9.4.2 Aromatic rice varieties

Aromatic rice varieties, such as *Basmati* and *Jasmine*, are prized for their distinctive flavor profiles. These could be used to create *premium fermented rice beverages* with a unique sensory experience, appealing to consumers seeking new and exotic flavors.

9.4.3 Indigenous rice varieties

Indigenous rice varieties, which are often adapted to specific geographical areas and climates, offer additional *genetic diversity* and *nutritional value* that could contribute to the development of regionally unique and nutritionally enriched fermented rice beverages.

10. Conclusion

Fermented rice beverages, deeply rooted in *traditional practices*, are experiencing a resurgence in the global food and beverage industry. Their unique *sensory qualities*, combined with a growing body of evidence supporting their *health-promoting properties*, make them appealing to modern consumers seeking *functional foods* with *nutritional* and *therapeutic benefits*.

Advances in *food microbiology*, *food chemistry*, and *food engineering* have facilitated the *standardization*, *scaling up*, and *diversification* of these products, making them accessible to a wider consumer base. As the market for functional beverages continues to grow, continued research into microbial dynamics, *biochemical transformations*, and *innovative processing technologies* will enhance the *commercial viability* of fermented rice beverages.

By *integrating traditional wisdom* with *cutting-edge science*, the potential exists to transform *fermented rice beverages* from regional specialties into *globally recognized health foods*, meeting both *cultural appreciation* and *consumer demand* for *health-enhancing*, *nutritionally rich* beverages.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Fruit Vinegar Production

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Abstract

This book chapter describes the fermentation processes involved in the production of fruit vinegar, in which the sugar in the fruit is converted into acetic acid in a two-stage fermentation process (alcoholic fermentation and acetic acid fermentation) by the action of yeasts and bacteria (*Saccharomyces cerevisiae* and *Acetobacter* and *Gluconobacter* species). A systematic overview of the microorganisms used and the biochemical changes during the process is given and the importance of optimising fermentation is emphasised. In addition, the sensory properties of common types of fruit vinegar (apple cider vinegar, grape vinegar, berry vinegar, pineapple vinegar and various fruit vinegars) and their widespread use in food technology and catering are described.

Keywords: fruit vinegar, fermentation processes, yeast, acetic acid bacteria, sensory evaluation

1. Introduction

Vinegar is a liquid condiment produced by the fermentation of various starchy, sugary or alcoholic substances [1, 2] and is used worldwide as a condiment and food preservative [2–4]. The earliest known use of vinegar dates back more than 10,000 years [5], while one of the oldest historical records of grain-based vinegar comes from China, described in the book Ceremony Notes (ca. 800 BC), which states that vinegar played an important ceremonial role during the Zhou dynasty (1000 BC–256 BC) [6]. In ancient times, vinegar production was long considered a chemical process until the Dutch scientist Boerhaave clarified in 1732 that the 'vinegar mother' (gel-like mass of cellulose and acetic acid bacteria (AAB)) was a living organism [4]. Later, in 1864, Louis Pasteur proved that the transformation of wine into vinegar is based on the development of a film of *Mycoderma aceti* on the surface of the wine [4].

Depending on the type of raw materials used, vinegars can be categorised into grain vinegars, fruit vinegars and alcohol-based vinegars. As a rule, East Asian countries produce grain-based vinegars, while Western countries mainly use fruit as the main substrate [7]. Vinegar production is an example of microbial biotransformation and takes place in a two-stage fermentation process, alcoholic and acetic acid fermentation [8]. The vinegar market is constantly evolving, and new products are being developed that have diverse and improved sensory properties as well as improved functional characteristics [9]. In Europe, vinegar is primarily produced by

underwater fermentation, usually using fruit as the main raw material [10], while in Asia, especially in China and Japan, traditional solid-state fermentation (SSF) methods are still the most commonly used [11].

The increasing popularity of fruit vinegar can be attributed to its documented health benefits and wide culinary applicability. Fruit vinegars are rich in organic acids – primarily acetic, citric, and malic acids – as well as phenolic compounds, which exhibit significant anti-inflammatory, antioxidant and probiotic properties. These bioactive constituents contribute to improved digestive health and attenuation of inflammatory responses [12, 13]. Furthermore, a growing body of evidence indicates that fruit vinegar may aid in the management of metabolic disorders such as obesity, diabetes and cardiovascular diseases. This is primarily achieved through its positive influence on metabolic pathways and glycaemic control [14]. Given its favourable organoleptic properties and ease of incorporation into daily dietary routines, fruit vinegar is emerging as a viable alternative to conventional functional foods and dietary supplements [15].

2. Raw materials and production methods

The raw materials used for vinegar production vary considerably depending on the geographical location and cultural heritage of the individual regions. In general, fruit-based substrates, especially grapes, are predominantly used in Europe, while grain-based substrates are rarely used. In this context, vinegar production in Europe primarily involves fermentation processes in a liquid state using fruit or other sugary sources.

Among the less commonly known fruit vinegars, mulberry vinegar (*Morus alba*) stands out due to its notable popularity in China – a country with a long-standing tradition of mulberry cultivation, primarily for the sericulture industry (*Bombyx mori*). Mulberry is a fruit species widely recognised for its nutritional and pharmacological properties; however, its biotechnological potential remains largely underexploited due to its high moisture content, which contributes to a short shelf life and increased perishability of the fruit [16].

In recent years, research has intensified towards the development of mulberry vinegar. By fermenting a mixture of crushed mulberries, sugar and mixed cultures of bacteria and yeasts, this perishable raw material can be effectively transformed into a microbiologically stable product [17]. Due to its high acidity, mulberry vinegar is resistant to spoilage and represents a sustainable approach to valorising this functional fruit resource [18].

Pineapple vinegar is particularly prominent in tropical regions of Africa, where favourable climatic conditions support the abundant cultivation of pineapple. Several studies have confirmed the functional health-promoting properties of pineapple vinegar. It has been demonstrated to inhibit the proliferation and metastasis of breast cancer cells, induce apoptosis, downregulate the expression of genes associated with tumour invasion and exert immunomodulatory effects [19].

Furthermore, in a study conducted on high-fat diet-fed mice, pineapple vinegar was shown to significantly reduce body weight and adiposity, increase the expression of genes involved in energy metabolism and induce favourable changes in gut microbiota composition, including an increase in beneficial *Bifidobacterium* species. Enhanced antioxidant activity was also observed [20].

The best-known European vinegars include sherry vinegar, balsamic vinegar from Modena, malt vinegar and apple cider vinegar [21]. In contrast, vinegar in Asian countries, especially in China and Japan, is traditionally produced by SSF [11]. In the

course of its long historical development, China has developed a unique system of solid-state fermentation, which shows considerable regional differences in vinegar production technologies [10].

The raw material used for vinegar production plays a crucial role in determining the final characteristics of the product [2, 21]. Although grapes are most commonly used due to the well-established winemaking tradition in many Western European countries, various other fruit are also utilised for vinegar production [2]. This is understandable given their high content of bioactive and health-promoting compounds such as amino acids, organic acids, phenolic compounds, vitamins and minerals [22]. **Figure 1** shows the most studied fruit species for vinegar production, based on their occurrence in the scientific literature published between 1990 and 2020. As can be seen, there has been an exponential growth in recent years, which would demonstrate the growing interest of the scientific community in this type of product.

Apple cider vinegar is the most commonly produced fruit vinegar. Sherry vinegar is made from sherry wines in the Jerez–Xérès–Sherry, Manzanilla de Sanlúcar and Vinagre de Jerez regions in south-west Spain using traditional vinegar-making methods [23]. Its unmistakable aroma and flavour are the result of the 'soleras y criaderas' system, which involves slow acetification and ageing in American oak barrels. The final product is a blend of vinegars of different ages to achieve a balanced flavour profile [24].

When it comes to vinegar produced by liquid fermentation, two primary fermentation methods are usually used: the surface fermentation method and the submerged fermentation method (immersion). The surface culture or surface fermentation method is characterised by an abundant growth of acetic acid bacteria on the surface of the medium, at the interface between liquid and gas, where the highest oxygen concentration is present due to the aerobic metabolism of the bacteria. This is a static method, as the presence of the bacteria is physically limited to the surface layer [2]. Examples of surface fermentation methods include traditional spontaneous fermentation, which can extend over several months. This category also includes the well-known Orléans method, in which fermentation takes place under partially controlled

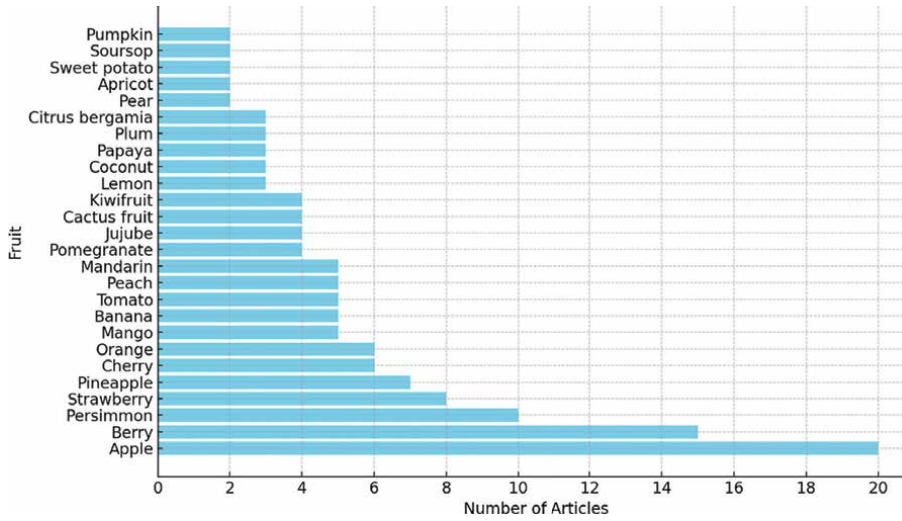

Figure 1.
Number of articles in the scientific literature per fruit used for the production of fruit vinegar published between 1990 and 2020 (modified from Ref. [2]).

conditions and the production process is continuous. The acetic acid concentrations achieved by surface culture fermentation are generally low.

Surface fermentation methods are traditionally considered suitable for producing high-quality vinegar. Originally, acetification was carried out using simple static methods in which the alcoholic media stood still and had only a minimal contact surface for oxygen diffusion. However, these approaches evolved into techniques where alcoholic media are poured over various support materials harbouring bacterial cultures. This innovation increased the oxidation surface area and significantly accelerated the acetification process. A variety of materials are used to increase the oxidation surface area, with wood crisps being the most commonly used carriers for AAB due to their ability to enhance oxygen exposure.

This principle underlies both the Schützenbach method and the Frings generator system. Submerged fermentations, on the other hand, are characterised by improved fermentation conditions, including aeration, stirring and temperature control [25]. As generator-based systems are relatively slow and costly, submerged fermenters have become established in industrial vinegar production [25]. These systems are based on bacterial cultures that float freely in the fermentation liquid [2]. Continuous aeration is ensured, and the bacteria are not offered a solid carrier [26].

The fermentation period in submerged fermentations is usually much shorter than in surface cultures. Cejudo-Bastante et al. [27] compared the rate of surface and submerged fermentations using the same alcoholic substrate and found that surface fermentation took 6 weeks, while submerged culture was completed in only 22 hours, highlighting the significant difference in the rate of acetification between the two methods. The submerged fermentation methods or acetators used for these processes are usually automated, ensure high oxygen transfer and yield larger quantities of vinegar than surface fermentation methods.

Grain vinegars are traditional acidic condiments used mainly in China, where their quality depends on the type of raw materials, the fermentation methods and the cooking conditions used [7]. Notable examples are the Japanese vinegar Kurosu and the Chinese vinegar Zhenjiang, both made from rice [28]. In the production of rice vinegar, the rice is soaked to promote starch hydrolysis. It is then heated, cooled and inoculated with yeast to produce ethanol. Acetic acid fermentation is then carried out and the resulting product is matured [29]. Among other raw materials, vinegar produced through the fermentation of sugarcane juice is known for its mild flavour and is commonly used in food preparation in the Philippines [5]. Vinegar production by traditional SSF is mainly used in Asian countries, especially in China. SSF is widely used in the production of vinegar, Chinese brandy, soy sauce, furu and other traditional foods [30]. SSF refers to the growth of microorganisms on a moist solid substrate in the absence of free-flowing water [31]. In general, the SSF process for Chinese vinegar involves four stages: Koji preparation preparation (a starter culture obtained by inoculating cereals, most commonly rice, with moulds from the *Aspergillus* genus.), saccharification and alcoholic fermentation, acetic acid formation and maturation [32]. Traditional SSF processes are constantly being optimised. Vinegar produced by dry gelatinisation has a more pronounced umami flavour (fifth basic taste, has a rich, deep, savoury flavour), higher lactic acid concentrations and a significantly higher content of 2-methylbutanal, a compound associated with fruity and chocolate-like flavours. These results indicate that the dry gelatinisation process outperforms traditional methods in several important sensory and chemical properties [10]. The new process shows a 39.1% improvement in alcohol conversion efficiency and achieves a 14% higher vinegar yield compared to the traditional method [10].

3. Fermentation

During alcoholic fermentation, the yeast *S. cerevisiae* converts the fermentable sugar contained in various raw materials into ethanol. AAB are then added and the ethanol is oxidised to acetic acid [33]. Fermentation is a key process in the production of fruit vinegars, in which numerous volatile compounds, polyphenols and organic acids are converted by chemical and microbial processes [2]. The specific fermentation processes that take place can vary greatly depending on the production methods used [34]. Vinegar is produced using either fast or slow fermentation processes. In the slow or natural process, the barrels containing the fruit wines are left open at room temperature so that the fruit juices can ferment into alcohol over several months, followed by oxidation to acetic acid. In traditional production, slow methods are generally used in which fermentation progresses gradually over weeks or even months. Prolonged fermentation facilitates the accumulation of a non-toxic slime layer of AAB and soluble cellulose, commonly referred to as the 'mother of vinegar' [35].

The preparation and processing of the juice for vinegar production using various methods as well as the different conditions and procedures during alcoholic and acetic fermentation significantly influence the final properties of the resulting vinegar [2]. In alcoholic fermentation, the choice between spontaneous and inoculated processes in conjunction with the specific microorganisms present plays a decisive role. In acetic acid fermentation, the type of acetic acid system used – whether on the surface or under water – is one of the most decisive factors influencing the final physicochemical properties of the vinegar [2]. In addition, the use of commercial starters to initiate acetic acid fermentation, the use of thermotolerant bacterial strains and the use of innovative technologies such as high hydrostatic pressure, ultrasound, microwaves or pulsed electric fields can enhance the production of high-quality vinegar [2].

3.1 Alcoholic fermentation

In the initial phase, the sugars glucose and fructose contained in the fruit juice undergo alcoholic fermentation under anaerobic conditions, which is supported by yeasts and leads to the formation of ethanol and CO_2. These are usually yeasts of the genus *Saccharomyces*, which belong to the *Saccharomycetaceae* family, whereby fermentation is usually carried out with selected strains of *S. cerevisiae*. The choice of yeast strain can have a significant impact on the flavour profile of the fermented wine, as certain strains can influence the fermentation rate, ethanol content, residual sugar, tannins, esters, methanol and volatile acids [36].

The use of *Saccharomyces bayanus* for the alcoholic fermentation of cornelian cherry juice (*Cornus mas* L.) has been shown to significantly increase the content of bioactive compounds and antioxidant activity [37]. The duration of alcoholic fermentation depends on the sugar content of the fruit juice, expressed in degrees Brix (i.e., indicates the proportion of soluble dry matter in a solution, primarily sugars), which in turn influences the potential alcohol content of the resulting fruit wine [38]. In addition to the choice of yeast, several factors affect the fermentation time, including the type of fruit, its physical condition, microbial load and fermentation temperature [2]. Alcoholic fermentation can be carried out either spontaneously or with the help of starter cultures, which also has an influence on the fermentation time and the characteristics of the final product [39].

In addition to ethanol, alcoholic fermentation produces a variety of by-products, including glycerol. Glycerol is the second most frequently utilised alcohol by acetic

acid bacteria after ethanol, but its excessive concentration can reduce the ethanol yield in wine production. Glycerol contributes significantly to the sensory quality of wine by adding sweetness and body. It also serves as a carbon source for Acetobacter species and protects them under unfavourable conditions such as high pH [40]. This promotes the survival and activity of AAB in glycerol-rich media. The ratio of ethanol to glycerol is a crucial parameter for vinegar quality, with a high ratio being desirable for optimal acetification performance.

3.2 Acetic fermentation

AAB, especially *Acetobacter* species, are Gramme-negative, ellipsoidal to rod-shaped microorganisms that metabolise aerobically, with oxygen serving as the terminal electron acceptor [41]. However, some strains can survive under low-oxygen conditions by utilising quinones as alternative electron acceptors [42]. Elevated ethanol concentrations during the initial stages of fermentation and high pH values in later stages indicate that AAB are the predominant organisms responsible for the bioconversion of ethanol to acetic acid [2].

Acetobacter aceti is the most widespread species in vinegar production, which is optimally active at 28°C and sufficient aeration. Its metabolic activity is inhibited below 20°C and above 33°C [35]. Nevertheless, studies have shown that certain Acetobacter strains can also work effectively at higher temperatures [43].

The use of selected strains and commercial starter cultures allows for better process control and a consistent sensory and chemical composition of the final product [2]. Further research on strain diversity and its effects on the properties of fruit vinegar could support the development of novel vinegar products with differentiated sensory profiles, thus increasing commercial diversity and functional value [2].

4. Microbial ecology of acetic fermentation

The production and quality of vinegar are closely linked to a diverse consortium of microorganisms present in the raw material, each of which fulfils a specific role in the fermentation processes [44]. During alcoholic fermentation, yeasts, especially *S. cerevisiae*, convert the sugars in the raw material into ethanol [44, 45]. In addition, yeasts such as generas *Hanseniaspora*, *Metchnikowia*, *Pichia* and *Candida* produce secondary metabolites that can either enhance or impair the flavour and overall quality of the vinegar [46]. *Saccharomyces cerevisiae* is the predominant yeast in spontaneous fermentations due to its high tolerance to sulphur dioxide, together with properties such as ethanol, heat and osmotic stress resistance, the Crabtree effect and a low oxygen demand [47].

The interactions between yeasts and AAB are essential for the fermentation process and the quality of port wine vinegar. These interactions are both symbiotic and competitive, with community dynamics influenced by factors such as osmotic pressure, temperature, acetic acid concentration and nutrient availability [48].

AAB represent a taxonomically diverse group characterised by different ecological preferences and versatile metabolic capacities [49]. Taxonomically (**Figure 2**), AAB belong to the family *Acetobacteraceae*, the order *Rhodospirillales*, the class *Alphaproteobacteria* and are categorised into two main groups based on ecological and phylogenetic studies: the acetic acid group and the acidophilic group [50].

The former includes genera such as *Acetobacter*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter* and *Komagataeibacter*, which share general physiological

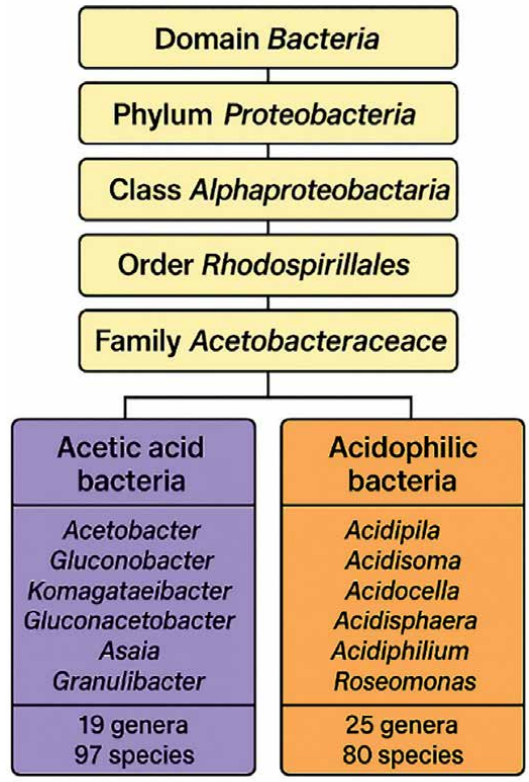

Figure 2.
Taxonomic origin and classification of acetic acid bacteria (modified from Ref. [50]).

characteristics, while the latter consists of more heterogeneous acidophilic and neutrophilic genera, including *Acidiphilum* and *Roseomonas* [51].

Members of the *Acetobacteraceae* are obligate aerobes, and their metabolic processes are highly dependent on the availability of oxygen, which fundamentally shapes their adaptive strategies in an aerobic environment [49]. Morphologically, they are non-spore-forming, ellipsoidal to rod-shaped cells that occur singly, in pairs or in short chains [49]. Their metabolism is strictly aerobic and relies on molecular oxygen (O_2) as the terminal electron acceptor, and the cells are Gram-negative or Gram-variable coloured [51]. Nevertheless, some strains of *Acetobacter* and *Gluconobacter* can tolerate low concentrations of dissolved oxygen [52].

AAB are widely distributed in nature and inhabit sugar- and ethanol-rich environments such as fruit juices, wine, cider, beer and vinegar, where they exhibit strong oxidative metabolism [53]. In an industrial context, Acetobacter and *Komagataeibacter* play a key role in the oxidation of ethanol to acetic acid, while *Gluconobacter* plays a crucial role in the oxidation of glucose to gluconic acid [53]. These genera – *Acetobacter*, *Gluconobacter*, *Gluconacetobacter* and *Komagataeibacter* – dominate the fermentation processes in food and vinegar production. Recent studies have reported the presence of more than 47 genera and 207 species within this microbial community [51].

Species of the genus *Acetobacter* preferentially oxidise ethanol over glucose, whereas *Gluconobacter* species oxidise glucose rather than ethanol [54]. Their optimal growth conditions are typically between 25 and 30°C and a pH of 5.0–6.5, although

many AAB can also grow at lower pH values, such as 3.0–4.0 [55]. The complexity of the microbial ecosystem in vinegar fermentation is further increased by the possible presence of lactic acid bacteria (LAB) and other microorganisms, especially in unfortified wine vinegars, which can influence both the fermentation dynamics and the properties of the final product [56].

The quality spectrum of vinegar is characterised by numerous factors, including the composition of the raw materials, the technological processes and the complexity of maturation, all of which are significantly influenced by the activity of AAB [57]. Recent genomic analyses have also shown that members of the *Acetobacteraceae* family can serve as a reservoir for undiscovered specialised bacterial metabolites, highlighting their potential biotechnological importance beyond traditional vinegar production [50].

5. Aromatic profile and sensory evaluation of vinegar

Acetic fermentation involves the sequential activity of yeasts and AAB that transform substrates such as wine or fruit juices into products with rich aroma and flavour profiles [8]. During alcoholic fermentation, yeasts produce ethanol and primary aromatic compounds, including esters and higher alcohols [58, 59], while AAB subsequently oxidise ethanol to acetic acid and produce additional volatile and non-volatile metabolites [60, 61]. These are gluconic acid, ketones, aldehydes and esters, all of which contribute to the complexity of the aromatic profile [62, 63].

The microbial interactions can be either synergistic or competitive, and factors such as temperature, oxygen availability and nutrient content have a major influence on the fermentation outcome [61, 64]. Submerged fermentations require careful control to avoid reductive environments that hinder ethanol oxidation [65].

In contrast, traditional methods of surface fermentation, such as the Orléans process, lead to a richer sensory profile due to prolonged fermentation and maturation in wooden barrels, an effect that is particularly noticeable in port and sherry vinegars [62, 66]. These processes promote the development of compounds such as diacetyl, isoamyl acetate and sotolone [67, 68], while the presence of specific *S. cerevisiae*, *Z. rouxii* and LAB strains further increases organoleptic complexity [8, 59].

Fruit vinegars, which can contain up to 160 volatile organic compounds, are aromatically complex products whose quality and consumer acceptance are highly dependent on the control of microbial dynamics and fermentation parameters [69, 70].

6. Conclusion

The production of fruit vinegar is a complex biotechnological process in which a two-stage fermentation, alcoholic and acetic, is carried out by yeasts and AAB. The composition of the raw material, in particular the sugar and bioactive content of the fruit, significantly influences both the microbial activity and the chemical properties of the end product. The choice of fermentation method – surface or underwater fermentation – together with the use of specific microbial strains, plays a crucial role in shaping the aromatic complexity, sensory profile and overall quality of the vinegar. Traditional methods, especially maturation in wooden barrels, tend to deliver

more nuanced sensory characteristics compared to industrial immersion methods. Optimising the microbial ecology and fermentation parameters is therefore crucial to improve both the functional and sensory value of fruit vinegars and meet consumer demand for high-quality, authentic products.

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Chapter 8

Reduction of Acrylamide in Plant-Based Foods through Traditional and Innovative Fermentation Techniques

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Abstract

Acrylamide (AA), a heat-induced compound classified as a probable carcinogen, is commonly formed in plant-based baked goods and chips through the Maillard reaction. As the demand for healthier and more sustainable food production increases, innovative and natural solutions for acrylamide reduction have gained significant attention. This chapter explores the role of fermentation in reducing acrylamide levels, the impact of different starter cultures and fermentation parameters on acrylamide precursors, and the sensory quality improvements associated with this process. Furthermore, sensory analysis results are examined to assess consumer acceptance, providing valuable insights for industrial applications. By highlighting fermentation as a sustainable technology that enhances food safety and sensory attributes, this chapter aims to contribute to the scientific literature and support the development of safer, high-quality food products.

Keywords: fermentation, acrylamide reduction, plant-based foods, starter cultures, sensory analysis

1. Introduction

Plant-based food acrylamide reduction through fermentation research shows great promise because it simultaneously solves health-related problems and quality needs. Through fermentation processes, the formation of precursors that create acrylamide is lowered by affecting the transformation of both asparagine and reducing sugars. Through this process acrylamide reduction occurs while taste and texture of the food improves to meet consumer preferences.

Acrylamide reduction through fermentation occurs because asparaginase enzymes in microbial cultures transform the acrylamide precursor asparagine into safer breakdown compounds [1]. Earthen bacteria strains used in fermentation process influence chemical changes, which lead to different degrees of acrylamide decrease. The effectiveness of reducing acrylamide depends on the specific strains of bacteria, which researchers identify through studies [2].

Plant-based foods experience better acceptability due to fermentation because it enhances both their flavor and texture profile. People tend to pick fermented goods in sensory evaluations because these foods possess superior taste elements [3]. Fermented foods gain acceptance as reported by studies, so products with lowered acrylamide become more market desirable to consumers [4]. Fermentation emerges as a proper acrylamide reduction method, but traditional cooking creates considerable risks because heat and extended cooking durations increase acrylamide formation probabilities. A proper synergy between fermentation methods and defensive cooking procedures should be used to ensure both food quality and safety.

2. Fermentation and acrylamide

2.1 Acrylamide risk in plant-based foods

Acrylamide is a substance formed during the cooking of starchy foods such as potatoes, bread, or cereals at high temperatures, by frying, baking, or roasting. Because it appears in so many common foods that are cooked in this way, it has become an increasing concern for food safety and public health. Products such as biscuits, crackers, chips, coffee, and cereals, among others, may develop acrylamide as the result of high temperature cooking through baking, roasting, or frying or through processes that involve high temperatures of food, such as steam cooking, extrusion cooking, deep fat frying, or roasting. Raw food does not contain acrylamide, and the formation of acrylamide during thermal processing of carbohydrate-rich products is a direct consequence of thermal processing [5]. The majority of acrylamide is formed through the Maillard reaction, which involves a reaction between reducing sugars and the amino acid asparagine above 120°C [6, 7].

The process is accentuated at high temperatures and long heating times [8, 9]. Also, acrylamide is formed to the greatest extent when Maillard reaction occurs in dry heat cooking environments, as such heating increases in formation of acrylamide [6, 8]. From a compositional point of view, potatoes and other starchy foods containing high levels of free asparagine and reducing sugars are likely to be processed into more acrylamide [5, 7]. Research demonstrates that knowledge of raw material composition and processing conditions is essential for developing effective mitigation strategies. Acrylamide exists primarily in the following dietary sources:

1. The high starch content of potatoes in French fries and chips, along with their typical deep-frying preparation methods, make them a primary source of acrylamide [10].

2. The consumption of roasted coffee beans maintains elevated acrylamide levels [7].

3. Baked goods such as bread, biscuits, and breakfast cereals that undergo prolonged high-heat exposure [11, 12].

4. Toasted grains along with other wheat-based baked products appear frequently in daily food consumption habits [10].

The International Agency for Research on Cancer views acrylamide as a Group 2A probable human carcinogen since it likely causes cancer in humans [6, 11]. Studies

have found that mitigation strategies depend on knowledge about raw material composition as well as processing conditions. The major dietary sources of acrylamide are listed as being primarily:

- It is neurotoxic, and both cognitive and motor system impairments have been observed [11, 13];
- Nevertheless, there is a widely accepted background of carcinogenicity [14];
- Reproductive and developmental toxicity, especially under high exposure [15, 16];
- Its results consisted of hepatic and cardiovascular damage induced by the mechanisms of oxidative stress [15, 17].

Regulatory bodies assess the risk posed by acrylamide exposure through marginal exposure (MOE) assessments, which suggest that consumer risk exposure may have increased in recent years [7, 18]. Food regulatory organizations from different nations employ assessment results and standardized national criteria to determine their maximum allowed acrylamide levels. Research into acrylamide in plant-based products such as potatoes and cereals and coffee has received continuous regulatory attention from the EU, leading to benchmarks for these substances. European nations take the front position in implementing specific rules to lower the consumption of acrylamide while boosting food security monitoring systems.

According to Commission Regulation (EU) 2017/2158, food manufacturers operating in the European Union must follow mandated benchmark levels and reduction strategies for acrylamide substances in foods [10, 18]. The Codex Alimentarius Commission creates standardized testing standards and risk management procedures for public health through an international scope [9]. Food producers must always follow regulation-compliant mitigation procedures as outlined in official verifications while avoiding any changes to the approved methods [11, 12].

2.2 Relevance of fermentation strategies

The growing concern over acrylamide formation has motivated researchers to implement fermentation strategies as innovative food processing methods for acrylamide reduction. The production of fermented foods offers a natural and sustainable approach to enhancing the health profile of plant-based products, meeting clean-label requirements for both consumers and industry. The resistance of acrylamide to thermal processes is reduced due to chemical changes in food matrices induced by traditional or modern fermentation techniques involving lactic acid bacteria and yeast. The reduction of acrylamide during fermentation occurs through multiple operational pathways:

- Microbial metabolism plays a substantial role in depleting asparagine, the key amino acid precursor in acrylamide formation.
- Lowering the pH inhibits the Maillard reaction and, consequently, acrylamide synthesis.
- The profile of reducing sugars in food products can be modified to minimize the availability of Maillard reaction substrates [8, 11].

Tests show LAB fermentation reduces acrylamide levels in bread and vegetable-based substances, and yeast fermentation proves effective for coffee bean processing [19]. Noxferm technologies deliver a double result since they increase safety standards and create better flavor perceptions alongside longer shelf-life durations and nutritional value benefits.

The rise in customer interest for plant-based minimally processed items enables manufacturers to adopt fermentation methods that help fulfill regulatory needs and maintain product quality through EU Regulation 2017/2158. Product sustainability improves through fermentation because the technique reduces the dependence on chemical additives and significant formulation adjustments. Food safety benefits from fermentation because this process works as both a risk reduction measure and an enhancement technique, which integrates nutritional improvement with sensory quality development.

3. Fermentation techniques for acrylamide reduction

3.1 Traditional LAB fermentation

The fermentation process requires lactic acid bacteria (LAB) from grains and dairy products as well as vegetables to function. Production of lactic acid creates conditions that are hostile to microbes, leading to longer product shelf life together with superior product quality. Several studies now show that fermentation improves product safety along with nutritional quality [20].

LAB are gram-positive, non-sporing, non-respiring cocci or rod-shaped bacteria that are important microorganisms for food production, agriculture, and clinical applications. They ferment carbohydrates to produce lactic acid [21]. The four main genera that scientists agree on are *Streptococcus, Leuconostoc, Pediococcus, and Lactobacillus. Weissella, Enterococcus*, and A*erococcus* are new genera added in recent reviews. These harmless bacteria produce organic acids and other metabolites through the hydrolysis of food sugars. LAB has a GRAS (Generally Recognized as Safe) status, enhanced by its widespread use and occurrence. There is no clinical evidence of adverse effects for these fermenting bacteria except for enterococci, as per the EFSA's "Panel on Biological Hazards" [22].

It has been shown that LAB, which are commonly used in conventional fermentation processes, reduce acrylamide formation through the degradation of asparagine and other reducing sugars. Research conducted on potato slices using LAB-fermented solutions showed a significant reduction in acrylamide formation when the potato slices were fried [23, 24]. LAB strains in sourdough fermentation, together with prolonged fermentation times, help decrease acrylamide formation in bread-making processes [25, 26].

The reduction of acrylamide in food production depends heavily on the fermentation processes conducted by lactic acid bacteria (LAB). Environmental conditions developed by microorganisms through enzymatic activity lead to reduced pH levels that help decrease acrylamide formation from asparagine precursors. The food industry relies on LAB as an essential approach to reduce acrylamide content within diverse food products [8].

3.2 Sourdough fermentation and LAB-yeast synergy

During sourdough fermentation yeast and LAB together create a synergistic reduction of acrylamide in bread alongside baked goods. The incorporation of LAB into

sourdough fermentation means lower acrylamide content as well as delivers better texture and taste to the product. Through this strategy, researchers succeeded in lowering acrylamide formation and created higher nutritional value within the bread product [19].

The blending of yeast with lactic acid bacteria (LAB) through sourdough fermentation produces successful acrylamide content reduction in food items. By employing this method, the chemical acrylamide formation decreases, and the products achieve a better texture together with tastier sensory properties. The fermentation process that combined sourdough with yeast resulted in substantial acrylamide reduction in whole-wheat bread compared to yeast fermentation alone [27].

Yeast and LAB fermented whole-wheat bread contained acrylamide levels between 6.9 and 20 μg/kg, whereas yeast-only bread contained 47.6 μg/kg [27]. Specific sourdough recipes reduced acrylamide formation by 79.6% in rye crispbread [28]. *Lactobacillus paracasei* generated the smallest amount of acrylamide (131.06 μg/kg) through its impact on dough acidity and glucose content [29].

Through its nutritional advantages and premium bread quality, the technology offers exciting commercial prospects especially when it integrates frozen products with substitutions of other ingredients [30]. Sourdough represents one of the oldest technologies through which people produce cereal-based foods. The traditional sourdough method allows fermentation by wild yeast and Lactobacillus bacteria found in raw materials instead of contemporary yeast (*Saccharomyces cerevisiae*) commercial methods [31].

The fermentation process used in sourdough bread production leads to productive acrylamide content reduction. Research shows that bread samples achieve reduced acrylamide content when fermentation proceeds for longer durations with increased sourdough concentrations up to 20% [25, 26].

3.3 Probiotic and functional microbial fermentation

Studies show that fermentation using Lactobacillus together with yeast produces promising results for lowering acrylamide concentrations. Adding probiotics to plant-based substrates such as soy and coffee beans promotes the breakdown of asparagine and other cooking precursors that lead to acrylamide formation [19]. The application of probiotic fermentation creates superior safety conditions while improving the nutritional benefits of final products.

The fermentation process with probiotics utilizes plant-based materials, such as fruits, vegetables, and cereals, to replace dairy substances in food production. Fermentation of soy drinks with sea buckthorn syrup and probiotic co-cultures of *Lactobacillus paracasei* and *Bifidobacterium animalis enhances* both bacterial viability and antioxidant activity [32]. Different microbial strains that are mixed during fermentation result in the generation of various functional attributes. A combination of *Saccharomyces cerevisiae* var. *boulardii with* kombucha microorganisms during fermentation results in probiotic mead, which shows better probiotic survival along with enhanced antioxidant features [33].

4. Mechanisms of acrylamide mitigation in fermentation

4.1 LAB mechanisms: pH, adsorption, precursors

Plant-based foods undergo acrylamide reduction during fermentation through various biochemical transformations. Acrylamide reduction occurs through enzymatic

asparagine degradation and specific microorganisms that enhance this process. The reduction of acrylamide occurs via the enzymatic degradation of asparagine, facilitated by specific microorganisms. Several mechanisms contribute to this reduction, which will be discussed in the following sections. Asparaginase breaks down asparagine into aspartic acid and ammonia through its enzymatic activity, thus blocking acrylamide formation when food undergoes high-temperature cooking [1, 34].

4.1.1 Reducing sugars

Fermentation by lactic acid bacteria (LAB) reduces acrylamide formation by controlling the amounts of reducing sugars and asparagine, both of which serve as key precursors in the Maillard reaction. The decrease in precursor amounts because of LAB strain enzymatic activities prevents thermal processing from producing detectable levels of acrylamide. LAB fermentation leads to decreased concentrations of glucose and fructose in fermented food products. Studies have shown that LAB treatment of fried potato products reduces the levels of essential reducing sugars that create conditions for acrylamide formation [24]. The impact of LAB strains on lowering asparagine concentration remains mild, thus only mildly reducing acrylamide formation potential.

The reduction in glucose and the simultaneous decrease of fructose along with asparagine affect Maillard reaction potential, thus lowering the final acrylamide content. LAB fermentation proves to be a powerful method for reducing acrylamide. The central role of reducing sugars in acrylamide formation can be modified through LAB fermentation, alongside alternative cooking techniques. Research suggests that adding Vitamin C and Vitamin B1 to food products reduces acrylamide concentrations. These vitamins interfere with the Maillard reaction; however, they do not affect LAB fermentation [35]. The cooking process determines how acrylamide is formed. Air frying results in reduced acrylamide content compared to traditional frying methods, as reported in [8], due to differences in heat transfer mechanisms and temperature profiles.

Acrylamide is formed through the Maillard reaction, which depends on reducing sugars that serve as key precursors in food processing. LAB fermentation has the potential to decrease acrylamide content by reducing both reducing sugars and asparagine in food substrates. The reduction of reducing sugars is the primary mechanism for minimizing acrylamide formation, while LAB fermentation has a lesser effect on amino acid modulation. Optimizing LAB fermentation techniques for different food products and processing conditions requires further research. A comprehensive framework for acrylamide reduction in foods can be developed by combining LAB fermentation with other mitigation strategies, including enzymatic treatments and modifications to cooking methods.

4.1.2 Asparagine depletion

The reduction of acrylamide depends on fermentation conditions, which include pH levels, temperature settings, and time duration. The effectiveness of acrylamide reduction depends on fermentation parameters within the pH range of 4.5–6.5 and temperatures between 32 and 42°C, which enhance microbial fermentation activities [36].

4.1.3 pH reduction and maillard inhibition

Acrylamide reduction is enhanced at lower pH values between 3.5 and 4.5 because acidic conditions inhibit the Maillard reaction [30, 36]. LAB grow best at

temperatures ranging from 30 to 37°C and effectively contribute to acrylamide reduction within this range [36, 37]. The process of acrylamide reduction through fermentation becomes more effective with longer fermentation periods extending between 12 and 24 hours [25].

LAB fermentation of Habanero peppers results in better flavor profiles because it adjusts pH levels and stimulates volatile compound synthesis. The pH value of Habanero peppers typically decreases from 4.78 to 4.47 when LAB fermentation occurs [38]. The production of organic acids becomes more favorable at lower pH values because these acids enhance both the preservation and flavor complexity of the peppers [39]. Food producers utilize LAB fermentation as a standard process because these microorganisms enhance product quality by changing pH values. The fermentation process generates lactic acid and various organic acids that decrease pH values while creating essential sensory features of fermented foods. LAB strains that speed up pH reduction in kimchi fermentation cause fast sugar consumption and acid generation. The optimal sensory preference for kimchi occurs when acetic acid and lactic acid coexist during fermentation because pH-based acid equilibrium controls flavor development [39, 40].

Volatile and non-volatile compound generation during LAB fermentation is directly influenced by changes in pH. LAB fermentation of cereal products produces ethanol, acetic acid, and carbon dioxide, which contribute to the texture and appearance of the final product. The reduction in pH leads to increased compound formation, enhancing the sensory qualities of the final product [41].

4.2 Chemical changes in fermented plant-based foods

Through lactic acid fermentation, the production of probiotic-rich foods becomes possible using microbes such as Lactobacillus and Bifidobacterium, among others. The preservation process enables these bacteria to convert sugars into lactic acid, which establishes a suitable environment for probiotic growth. Antioxidant components and bioactive properties significantly increase after fermenting probiotic purees containing different strains, including *Lactobacillus fermentum* and *Lactobacillus plantarum*. Complex laboratory studies have shown that specific microbial populations flourish under precisely engineered lactic acid fermentation conditions, developing nutritionally enhanced products that can greatly support public health when consumed daily with over 10 billion CFU per serving [42].

4.2.1 Role of amino acids

The development of acrylamide during LAB fermentation depends significantly on amino acids since these compounds interact through the Maillard reaction primarily with sugars. Several factors influence the concentration of acrylamide present in final products because different amino acids participate in the reaction. Asparagine functions as the leading substance that leads to acrylamide synthesis. Throughout fermentation, the treatment of asparagine with reducing sugars results in oxoaldehyde and oxoacid formation, which significantly enhances acrylamide production [43].

Acrylamide production during Maillard reactions occurs when valine, alanine, phenylalanine, and leucine amino acids participate in the process to generate materials that function as precursors for acrylamide synthesis [44]. The amino acid essential to the Maillard reaction to produce acrylamide is asparagine, which particularly reacts with reducing sugars to produce acrylamide. Cys, Lys, and Glu are involved in

Maillard reactions (flavor, etc.) and other biochemical changes during fermentation, but they are not the biggest contributors to acrylamide formation in amino acids [45].

Most other amino acids are generally less directly involved in acrylamide formation than asparagine. Various amino acids can participate in the Maillard reaction, but only asparagine is indispensable for the formation of acrylamide because its side-chain amide group is important for the unique reaction mechanism related with acrylamide formation [45]. As such, all other amino acids, even if engaged in Maillard reactions with effects on flavor and the formation of bioactive compounds, are not acting in the same manner as in product formation as during acrylamide formation during LAB fermentation or cooking.

However, some other studies suggest an indirect or modulating role of other amino acids in acrylamide formation. For instance, glutamine (Gln) could increase the amount of acrylamide production by the release of ammonia in a higher-temperature gradient, which potentially elevates the supply of the acrylamide precursor indirectly [36]. In thermal degradation, serine (Ser) can create substances that can be regarded as acrylamide precursors [46]. It is reported that certain amino acids such as alanine (Ala) and phenylalanine (Phe) can also affect the development of Maillard reactions, producing a variety of aroma compounds [47]. On the other hand, proline (Pro) and glycine (Gly) have been found to suppress the formation of acrylamide through the binding with reactive intermediates to minimize the exposure levels of acrylamide [47, 48].

Laboratory studies have confirmed that cysteine and lysine amino acids successfully decrease the amount of acrylamide formation during heating. The amino acids participate in competitive interactions with asparagine or modify the mechanisms of chemical changes that occur [49].

The ability of proline and glycine to reduce acrylamide formation increases notably when their amounts increase [44]. The formation of acrylamide becomes less challenging when glucose and similar reducing sugars are present during processing. Heat processing between amino acids and reducing sugars triggers chemical reactions that produce higher amounts of acrylamide [50]. Amino acids affect acrylamide formation by complex reaction processes that depend on the types of amino acids and fermentation parameters such as pH value and temperature and reducing sugar concentrations.

During fermentation the prevention of acrylamide formation occurs through non-toxic compound generation from acrylamide by cysteine and lysine chemicals. The reduction of acrylamide occurs at higher pH levels and temperatures because the reactivity of these amino acids intensifies under said conditions. Mechanism of action:

- Both cysteine and lysine contain reactive functional groups that initiate acrylamide reactions to produce stable non-toxic compounds. Acrylamide reacts with amino acids through first-order kinetics because of their direct structural relationship.

- Cysteine: The elimination rate of acrylamide through cysteine reaches up to 94.4% when applied. Acrylamide reactivity increases when pH levels are elevated, which leads to better acrylamide reduction during processing [51].

- Lysine: The reactivity of lysine remains high when its molar ratio with other substances increases. The compound demonstrates effectiveness for acrylamide

reduction and works best when combined with glucose during heat-based processing [50].

- Interaction with glucose: The addition of cysteine and lysine to glucose solution results in improved acrylamide elimination performance. This case illustrates the beneficial impact these amino acids have on blocking acrylamide generation throughout various food production methods.

- Impact of other amino acids: Cysteine and lysine decrease acrylamide levels, yet glutamine and possibly other amino acids may increase acrylamide formation. The opposite reaction patterns between these amino acids demonstrate the requirement for selecting appropriate ingredients when food processing to prevent acrylamide formation [49].

During LAB fermentation, amino acids either raise or decrease acrylamide levels through their chemical interactions. These amino acids show effectiveness in combination with pH conditions and temperature levels as well as reducing sugars, but controlling acrylamide formation proves challenging.

In summary, although asparagine is the dominant and essential precursor for acrylamide formation, the distribution and the reaction conditions also contribute to the level of acrylamide generation. This emphasizes the necessity to consider the complicated interactions between amino acids and processing parameters in strategies to reduce acrylamide.

4.2.2 Food matrix and fermentation conditions

Acrylamide production experiences significant chemical modifications in plant-based foods throughout fermentation. A decrease in acrylamide production in heated foods occurs because fermentation reduces the starting materials used for acrylamide formation. Antifungal food processing modifies both nutritional elements and acrylamide risk levels in plant-based food. The fermentation process and added substances in various plant-based meat products influence acrylamide content [52].

Besides microbial activity, other components in the food matrix, including dietary fiber content, protein-carbohydrate interactions, and water activity, play important roles concerning acrylamide formation. Higher matrix density could impair heat transfer and reduce the extent of Maillard reaction, leading to lower acrylamide content [53, 54].

The physical structure of the food (e.g., purée vs. solid slices) also influences acrylamide formation dynamics, as a denser matrix may restrict the diffusion of reactants like free asparagine and reducing sugars during heat treatment [55, 56]. Furthermore, matrix viscosity and moisture-binding ingredients such as hydrocolloids and dietary fiber could influence acrylamide formation by modifying water activity (a_1), an important factor in Maillard reactions [57, 58].

Enzymatic treatments (such as asparaginase) are more effective in matrices with moderate pH and low buffering capacity, conditions that allow better enzyme access and activity [56]. Plant matrices with high levels of polyphenols may also serve as scavengers of free radicals generated during processing at high temperatures, and thus, contribute to acrylamide chemistry [54].

Fermentation has been observed to lower the total sugar content of food products, which directly translates to lower acrylamide formation. The Bacillus strains used

in potato slice fermentation led to a 96.1% decrease in acrylamide content before deep-frying [23]. Long-term bread fermentation using sourdough resulted in reduced acrylamide formation, and specifically, using 20% sourdough starter maintained better gluten quality compared to higher concentrations [25]. The effectiveness of acrylamide reduction through fermentation depends on the specific fermentation methods used because different techniques produce dissimilar results. The results of acrylamide formation and food safety vary based on fermentation conditions, together with the selected microorganisms.

In addition, the matrix structure of plant foods, for example, the fat content and porosity, may favor or impair acrylamide formation during thermal treatment. For instance, in the high-fat matrix, the mobility of acrylamide precursors could increase, enhancing their reactivity [59]. On the contrary, high-fiber or viscous mediums may impede precursor migration and water evaporation, leading to decreases in the potential of acrylamide risks [56, 57].

Fermentation products are also modulated by the food matrix. Organic acids (e.g., lactic acid) generated during fermentation may bind or decompose acrylamide precursors, but the degree of their effects depends on the buffering capacity of the matrix and the mutualistic relationship with acidic molecule retention in the matrix [60]. In the case of meat analogs with soy, pea or a mycoprotein base, the incorporation of precursors in the protein network reduced acrylamide formation in the presence of reducing sugar [61]. Also, in cereal-based matrices, the effect of fermentation on gluten and arabinoxylans characteristics, affecting moisture holding/trapping and thermal conduction, which indirectly have an influence on acrylamide levels, has been observed as well [54].

5. Enzymatic approaches: The case of asparaginase

5.1 Enzyme mechanism and specificity

Asparaginase enzyme functions chips by transforming L-asparagine into its end products, aspartic acid and ammonia. Thermal processes halt the conversion process, preventing asparagine from participating in the Maillard reaction and subsequently reducing acrylamide formation. The enzyme demonstrates reactivity toward asparagine while it does not affect glutamine or other amino acids, thus helping maintain food matrix nutritional value and sensory quality [62, 63]. Research into food enzymatic treatment techniques has discovered L-asparaginase as an effective method to decrease acrylamide levels in starchy foods. L-asparaginase transforms L-asparagine (the acrylamide precursor) into L-aspartic acid, thus halting the acrylamide production process that occurs during high-temperature extended cooking of processed foods [5, 10]. Enzyme sources and applications:

- Multiple microbial strains of L-asparaginase, such as *Streptomyces koyangensis* SK4, *Fusarium culmorum*, and *Bacillus spp.*, have demonstrated acrylamide reduction in starchy food products [64, 65].

- When L-asparaginase from *Streptomyces koyangensis* SK4 was tested under optimal conditions, it reduced acrylamide content in potato chips by about 81%. In vitro enzyme activity reached its peak when potato chips were treated with sodium chloride treatment before undergoing air frying at 160°C. The authors

noted that enzyme concentration and other processing conditions need to be optimized for maximal reduction [65].

- The acrylamide levels in potato chips and sweet bread decreased by 94 and 86%, respectively, when using L-asparaginase derived from *Fusarium culmorum* (ASP-87). The authors stated that the enzyme also inhibited acrylamide levels in fried and baked starchy food products and could be used for commercial production [66].

- The tested enzyme from *Bacillus spp.* L-asparaginase demonstrated complete acrylamide reduction in potato slices after a 30-minute treatment. *Bacillus spp.* L-asparaginase I achieved better acrylamide reduction after purification and increased concentration during testing on potato slices [67].

- The results from L-asparaginase enzyme testing demonstrated that various enzyme concentration levels achieved better acrylamide reduction with enhanced potato chip sensory quality than the control group [68].

Asparaginase is selected as a prime example because it is the most selective toward L-asparagine, the main precursor of acrylamide in heat-treated food. Such specificity makes it possible to achieve a large decrease in acrylamide formation with no harm to the content in the remaining amino acids and the nutritional and sensorial properties of the food. The successful reduction of acrylamide by asparaginase has been confirmed in several strains of microorganisms and food products and serves as an excellent example of the use of enzymatic application relative to food safety improvement. Studies by Mottram et al. [46] and Zyzak et al. [69] proposed the Maillard reaction step that led to acrylamide formation in which asparagine was found to be the main precursor, and Amrein et al. [70] demonstrated that asparaginase use could be a practical means to decrease acrylamide in fried potato products. Therefore, asparaginase is a unique and prototype enzyme illustrating how enzymatic methods can be used to mitigate food contaminants [46, 69, 70].

5.2 Applications in fried, baked, and roasted products

Research indicates that the use of asparaginase throughout plant-based food production and fermentation activities effectively lowers acrylamide formations.

- *Potato-based products*: Conditions optimized for frying allowed the application of asparaginase before potato processing to decrease acrylamide formation by 81% [65].

- *Cereal-based baked goods*: Asparaginase treatment reduced the acrylamide content in baked cookies as well as breads and biscuits by 97 percent. Enzymatic pre-treatment produced an 89% reduction of acrylamide in fried pizza crusts, yet wood-fired pizza cuts displayed only a 50% acrylamide reduction [71, 72].

- *Coffee*: The enzyme treatment of green coffee beans produces an 80.7 and 75.8% acrylamide reduction in light roasts and dark roasts, respectively [62].

- *Fried dough products*: Model system research demonstrated that using asparaginase for dough treatment yielded about 90% reduction in acrylamide formation [73].

- The enzymatic system produced by *Aspergillus fumigatus* through Acrylamide amidohydrolase breaks down acrylamide and other substances in food products until reaching 95% degradation. This enzyme is used in food safety and quality management as it serves as an effective biocatalyst. After all, it functions as a vital instrument to convert acrylamide into harmless decomposition products through hydrolysis. Acrylamide amidohydrolase uses enzymatic action to break down acrylamide into acrylic acid, which reduces its hazardous features. Acrylamide processing occurs best at pH 7.5 and 40 degrees Celsius but retains stable for 13.37 hours when operating at 50 degrees Celsius [74].

- Acrylamide breakdown occurs through the enzyme in different foods such as bread alongside potato chips and cookies and meat products [74]. Through this method, the process creates safe food items, which preserve expected quality.

5.3 Combined processing strategies (HPP, blanching, coatings)

The food industry achieves effective acrylamide reduction in its products when different reduction methods are used in combination, ensuring both consumer safety and regulatory compliance. The fermentation process with certain probiotic strains, including *L. paracasei* and *B. breve*, results in substantial acrylamide reduction through their ability to break down acrylamide and its precursors [36, 75]. The optimization of fermentation parameters at their optimal levels requires adjustments of temperature, pH, and inoculum ratio. The optimization of cascara tea fermentation through Response Surface Methodology (RSM) resulted in enhanced antioxidant and microbial content according to previous studies [76].

The enzymatic approach, alongside asparaginase application in food production, provides an effective method to reduce acrylamide levels. Multiple enzymatic solutions used together could improve the effectiveness of acrylamide management systems. Research on acrylamide amidohydrolase demonstrates encouraging outcomes; however, scientists still need to verify how much acrylamide remains in food products and complete safety evaluations of enzymatic food processing methods.

Most producers obtain asparaginase from microbial fermentation processes. Various bacterial and fungal species such as *Bacillus sp.*, *Zymomonas mobilis*, and *Penicillium crustosum* have been optimized for large-scale enzyme production [77]. The development of enzyme engineering together with continuous fermentation techniques produced thermostable and pH-stable variants, which can be used for food processing applications [78, 79]. Acrylamide mitigation strategies become more effective when asparaginase works alongside additional food processing approaches.

- *Blanching*: Potato slices receive improved enzyme penetration after blanching, which results in acrylamide reductions reaching 90% [80].

- *High-Pressure Processing (HPP)*: The combination of HPP with enzymatic treatment enables better asparaginase penetration, thus resulting in acrylamide reduction levels between 26 and 47% [81].

- *Hydrocolloid coatings*: Zein-pectin coatings combined with asparaginase application result in more than 70% acrylamide reduction during the frying process of potato slices [82].

Enzymatic efficacy depends on multiple parameters that influence the outcome of asparaginase treatment for acrylamide mitigation.

- *Enzyme source and stability*: Variants of the enzyme that originate from *Mycobacterium gordonae* and *Pseudomonas sp.* demonstrate improved stability at extreme temperatures and pH values, leading to wider application opportunities across various food substrates [78, 79].

- *Enzyme dose and contact time*: Higher concentrations and longer exposure improve effectiveness. A treatment of potato slices with 84 U/mL purified asparaginase solution for 30 minutes eliminated detectable acrylamide levels [67].

- *Process conditions*: The maximum activity of the enzyme along with its ability to access the substrate requires specific conditions of temperature and pH and moisture content in the process.

Asparaginase presents a specific strategy for acrylamide minimization in plant food products. Implementing fermentation and processing workflows with pre-treatment and physical enhancement techniques creates opportunities for generating safer high-quality food products. Further industrial feasibility of the process is supported by ongoing research into enzyme stabilization and delivery systems despite present barriers including high costs and process changes.

6. Sensory, functional, and nutritional considerations

6.1 Volatile and non-volatile flavor compounds

Fermentation results in the creation of essential volatile compounds such as 1-hexanol and linalool that determine the aroma and flavor. Sensory evaluations demonstrate that different fermentation durations combined with LAB strains can produce unique taste and aroma profiles, which consumers find more appealing [25]. The research showed that 340 volatile compounds exist in fermented peppers, where specific compounds had substantial odor activity values, which improved sensory perception [39]. LAB fermentation of cereal products produces volatiles and non-volatiles, which affect sensory characteristics of fermented products. The compounds improve both nutritional value and safety, together with flavor and aroma properties, leading to better acceptance by consumers.

LAB fermentation results in the production of vital volatile compounds, which include acids along with alcohols and aldehydes that determine flavor characteristics. Acetic acid stands out for its sour taste, but other volatiles introduce sweet and malty flavors [83]. The research found 63 volatile compounds in fermented barley beverages that demonstrate fermentation's ability to generate diverse flavors. Non-volatile compounds, which include organic acids and amino acids, change during fermentation, leading to better nutritional value and improved product functions. Indole-3-lactic acid demonstrated significant growth in fermented barley beverages [84]. Non-volatile compounds such as Maillard-derived products determine the distinctive taste characteristics of sourdough bread, so they play an essential role in developing flavors [85]. The long-term health benefits and flavor stability of fermented cereal products depend heavily on non-volatile compounds, even though volatile compounds receive

more attention for their direct sensory effects, thus requiring a balanced approach for product development.

The combination of pH variations with volatile compound synthesis affects the flavor characteristics of fermented foods. The wild strain of *Lactobacillus plantarum* at 48 hours during Habanero pepper fermentation reached its highest levels of 3,3-dimethyl-1-hexanol and trans-2-hexene-1-al, which produced the most preferred odor. The final product's aroma and flavor receive their characteristics through pH changes during fermentation [86]. LAB fermentation of cereal products produces volatiles and non-volatiles, which affect the sensory characteristics of fermented products. The compounds improve both nutritional value and safety, together with flavor and aroma properties, leading to better acceptance by consumers. LAB-induced pH modifications of volatile compounds produce balanced flavor characteristics [87].

6.2 Managing trade-offs between taste and acrylamide reduction

The process of balancing acrylamide reduction with sensory qualities of plant-based foods during fermentation proves difficult to achieve. Plant-based products gain texture and flavor through fermentation, but this process could affect acrylamide concentrations and sensory quality. The fibrous structure of plant-based meat analogs becomes better during fermentation, yet prolonged fermentation causes unpleasant sliminess and decreased firmness. The enhancement of flavors through fermentation can sometimes produce unfavorable off-flavors that reduce sensory acceptance unless fermentation processes remain strictly controlled [88].

The combination of salt concentration optimization with pH value control during kohlrabi fermentation leads to enhanced sensory results. The combination of 3.5% NaCl solution at pH 4.2 provides optimal fermentation conditions, which produce acceptable quality along with superior sensory scores after 2 days of fermentation. The maintenance of sensory properties depends heavily on pH control because high temperatures tend to result in reduced product quality and decreased hardness [89]. The promising results of LAB and yeast combined for acrylamide reduction and bread quality improvement depend on the fermentation conditions and specific strains used. The inconsistent results between different bread batches might stem from the fermentation process.

The implementation of asparaginase as a method to decrease acrylamide content needs to be balanced with the preservation of product taste qualities [90]. The precise management of fermentation parameters enables the control of acrylamide formation alongside the preservation of sensory characteristics but demands exact monitoring to prevent product deterioration. Combining fermentation with proper management of processing parameters provides a solution to boost plant-based foods while reducing acrylamide risks. Recent studies also highlight the role of various compounds—such as cations, acids, and antioxidants—in further mitigating acrylamide formation in plant-based foods, especially cereals and potatoes [87, 91]. The complex relationships between sensory qualities and acrylamide formation require additional research to develop effective optimization methods.

6.3 LAB fermentation in plant-based alternatives

LAB fermentation of citrus vinegar by *Saccharomyces cerevisiae* together with *Lactobacillus plantarum* results in elevated levels of formic acid, lactic acid, and total

organic acids. The modification of pH along with organic acid composition generates improved antioxidant capacity and better sensory characteristics that include sweetness and umami taste along with flowery and fruity fragrance notes [92]. Dry-fermented sausages can maintain their probiotic *Lactobacillus plantarum* L125 viable cell counts throughout the storage duration. The fermentation process, which decreases pH levels, results in better sensory qualities of taste and texture without negatively affecting product quality [93].

Mixed fermentation of *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* in traditional Chinese paocai leads to significant nitrite content reduction. The production of organic acids, especially lactic acid, functions as a key factor for nitrite destruction while simultaneously improving flavor characteristics. The fermentation process generates enhanced taste and aroma qualities through increased production of alcohols, esters, and acids [94].

Paocai fermentation through the application of homo-fermentative and hetero-fermentative LAB strains produces samples with different physicochemical features and flavor compositions. The fermentation process of LAB produces different amounts of lactic acid among homo-fermentative strains, but acetic acid production occurs in hetero-fermentative strains, which leads to divergent sensory outcomes. The metabolic processes between paocai samples prepared using homo-fermentative LAB and hetero-fermentative LAB show different patterns as demonstrated by Principal Component Analysis [95].

7. Conclusions

Plant-based foods benefit from lactic acid bacteria (LAB) fermentation as this technique effectively enhances food quality attributes as well as nutritional content. During LAB fermentation, multiple volatile and non-volatile substances are formed, such as alcohols acids, amino acids, and esters, which enhance sensorial properties and create appealing textures, thus gaining consumer popularity. Laboratory research found that fermentation time length together with selected specific strains and pH control determine the end sensory characteristics of fermented bread and cereal beverages as well as fermented vegetables.

LAB fermentation provides multiple effective methods to decrease acrylamide content in products using strategies that include modifying pH levels and removing asparagine and combining fermentation with yeast or using specific enzyme technologies. Strategies to optimize performance benefits alongside sensory quality maintenance or enhancement present themselves as complex optimization objectives. The production of unwanted textures alongside off-flavors occurs through fermentation processes that extend beyond normal ranges or when acids accumulate irregularly during the process.

Biotechnology experts leverage homo- and hetero-fermentative LAB strains as part of fermentation processes to manage safety aspects together with antioxidant potential and product taste in plant-based alternatives. The correct implementation of fermentation alongside strict parameter management enables balance between lowered acrylamide formation and sensory attributes that consumers appreciate.

Plant-based system production using LAB fermentation requires expertise in biological transformations together with instances of strain behavior and customer sensory demands. Additional interdisciplinary research needs to be conducted both to optimize processes and determine large-scale food industry solutions.

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Conflict of interest

The authors declare no conflict of interest.

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Fermentation has long been a cornerstone of food science, linking ancient traditions with modern biotechnology. *Fermentation in the Food Industry* offers a comprehensive overview of how microbial processes continue to shape the quality, safety, and sustainability of food worldwide. This volume presents recent advances in microbial fermentation, including lactic acid bacteria, yeasts, and mixed microbial cultures, and examines their roles in enhancing nutritional value, improving preservation, and developing innovative food products. Emphasizing both fundamental science and industrial application, the book highlights progress in fermentation process design, microbial ecology, metabolic pathways, and bioengineering strategies. Readers will discover how fermentation contributes to cleaner production, reduced food waste, and the creation of functional and health-promoting foods. Combining insights from microbiology, food technology, and biotechnology, this book serves as a bridge between traditional practices and cutting-edge research. It is an essential resource for researchers, graduate students, and professionals in food science, microbiology, biotechnology, and related industries who seek to understand and apply fermentation technology for sustainable food development and future innovation.

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