UNIVERSITÀ DEGLI STUDI DI PADOVA
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EMERGING FERMENTED FOODS AND BEVERAGES: TREND OR HEALTH MATTER?

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“Let Food Be Thy Medicine and Medicine Be Thy Food”

Hippocrates

ABSTRACT: Fermentation, a traditional food preservation method, has gained immense popularity in recent years, being considered attractive among consumers seeking both culinary enjoyment and wellness. This thesis explores the production technologies, primary microorganisms involved, and the growing market for some emerging fermented products, with a focus on kombucha, kefir, kimchi and fermented soy products such as miso, natto, and tempeh.

In addition, a literature review examines the health benefits of these foods demonstrated by in vitro and in vivo studies in animals and humans. The aim is to determine whether the recent popularity of fermented products is merely a trend or whether they possess scientifically supported health properties.

1. INTRODUCTION: FERMENTATION AND FERMENTED FOODS

1.1 What is fermentation and its origin

The fermentation process was discovered long ago and has played crucial roles in food preservation, nutrient enhancement, and the production of alcoholic beverages. The earliest archaeological evidence of fermentation includes an alcoholic drink made from fruit, rice, and honey, found in pottery jars dating from 7000 to 6600 BC in the village of Jiahu in China, and evidence of winemaking dating from 6000 BC in Georgia. The spread of winemaking was significantly influenced by the Roman colonization of the Mediterranean, which facilitated its expansion into other regions, including Asia. These discoveries highlight that fermented beverages such as vinegar and wine are among the oldest fermented foods consumed by humans. However, the scientific understanding of fermentation began with the identification of microorganisms in 1665 by Antonie van Leeuwenhoek and Robert Hook. It further advanced when the French microbiologist Louis Pasteur discovered in the nineteenth century that live microorganisms were responsible for the souring of alcohol during fermentation.

The distinctive flavours and aromas resulting from fermentation have made these foods integral to human diets throughout history. Figure 1.1 presents a timeline tracing the history of fermentation [Nasir et al., 2022; Ray and Joshi, 2014].

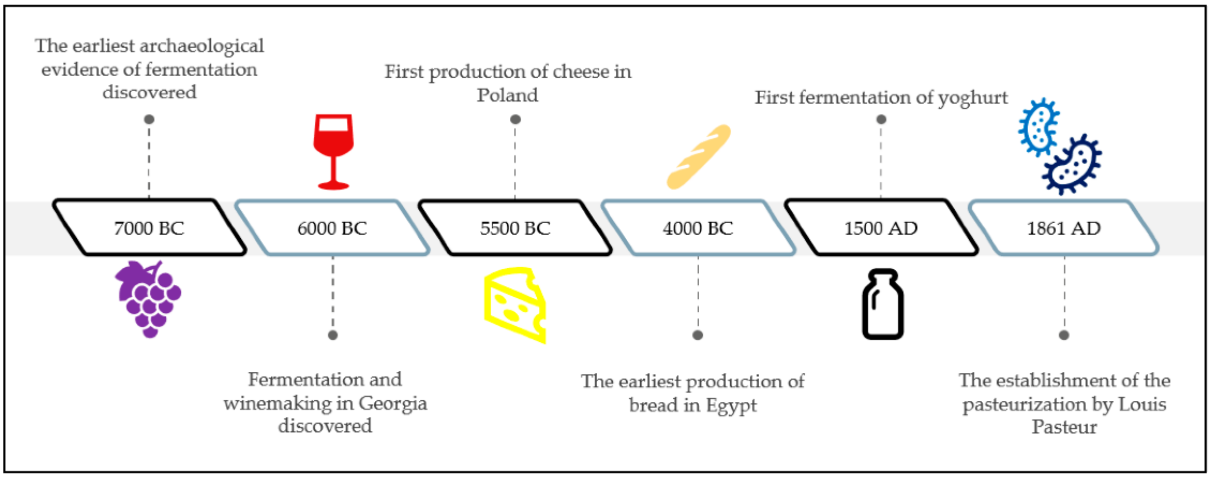


Figure 1.1-Timeline of fermentation history [Nasir et al., 2022]

Fermentation is recognized as one of the earliest biotechnological processes, historically utilized to produce food products with extended shelf-life and desirable sensory properties. Today, fermented foods are an integral part of our basic diet.

Biochemically, fermentation involves the anaerobic conversion of carbohydrates like glucose and starch into other molecules, such as alcohol and acids, facilitated by enzymatic action. It is a metabolic pathway through which energy is extracted from organic compounds without the involvement of an external oxidizing agent [Mannaa et al., 2021; Ray and Joshi, 2014].

However, there is also a broader meaning of fermentation, which considers it from a technological point of view as all transformations achieved by the action of microorganisms. In this thesis, the terms “fermented products” and “fermentation” will be understood in this broader sense, and thus used for foods and beverages produced by microorganisms, either through anaerobic fermentation or with the use of oxygen.

The process of fermentation relies on the enzymatic and catalytic actions of microorganisms such as bacteria, yeasts, and moulds. These microorganisms chemically transform complex organic compounds in substrates into simpler, bioactive, functional, and nutritious compounds. Fermented foods and beverages can be classified in various ways, primarily based on the substrate category used. Common substrates in commercial fermentation include milk, cereals, legumes, vegetables, fruits, meat, fish, and herbs. The diverse combinations of different food substrates and fermentation microbiota result in a wide array of fermented products worldwide, as summarized in Figure 1.2.

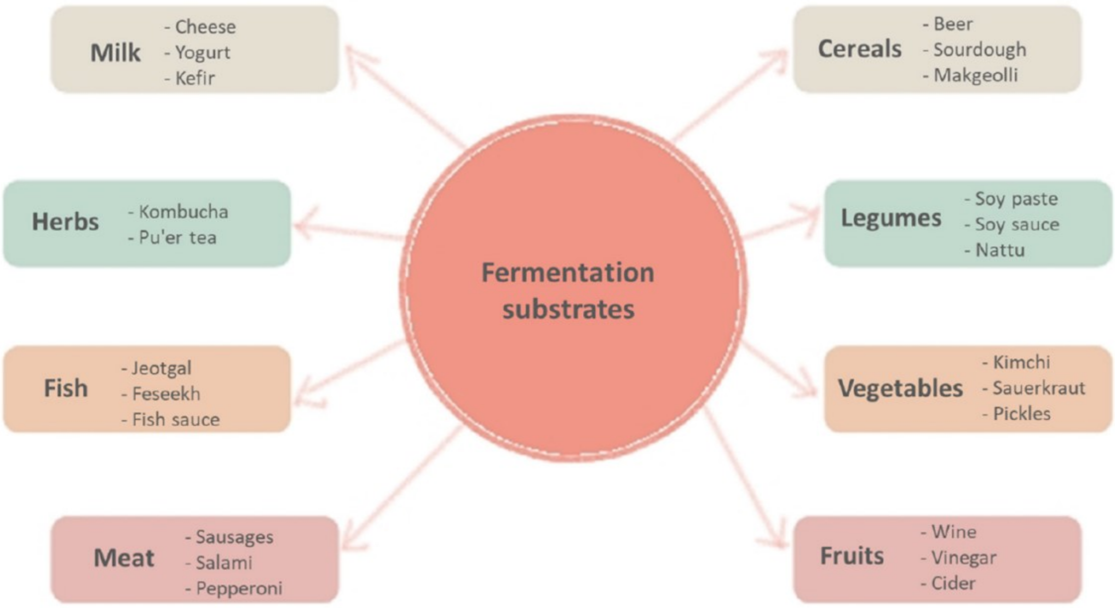


Figure 1.2-Common fermentation substrates and produced fermented foods and beverages [Mannaa et al., 2021]

Fermented foods and beverages are the products of different fermentation pathways - alcoholic, lactic, acetic or alkaline – mediated by the microbial cultures involved. Traditionally, these products were produced through spontaneous fermentation, relying on the naturally occurring microbiota on the food substrates. This method, dependent on autochthonous microbes, remains prevalent in domestic and small-scale settings. In spontaneous fermentation, conditions are adjusted to favour the growth of desirable microbes that impart unique sensory properties to the product while inhibiting spoilage-associated microbes. Often, a small amount of a previously successful fermented batch is used as an inoculum to start the fermentation of new ingredients, a process known as backslopping. This practice enhances the competitive ability of fermentation microbes and ensures the success of the process by allowing the desirable microbes to dominate the environment.

With advancements in microbiological techniques, specific starter cultures have been isolated, characterized, and preserved from fermented foods. These cultures are now used extensively, particularly in industrial-scale production, to ensure controlled fermentation and consistent quality. The use of defined starter cultures was first adopted in the production of beer, wine, vinegar, and bread, followed by dairy and meat products. Starter cultures accelerate the fermentation process and often consist of multiple strains and species that coexist [Mannaa et al., 2021; Ray and Joshi, 2014].

1.2 Microorganisms involved in fermentation

1.2.1 Bacteria

Lactic acid bacteria (LAB) such as *Lactobacillus, Pediococcus, Streptococcus,* and *Oenococcus* play a pivotal role in food fermentation, followed by *Acetobacter* species, which oxidize alcohol to acetic acid. A third group of bacteria significant in fermentation are the *Bacillus* species, which facilitate alkaline fermentation.

LAB are Gram-positive, non-respiring, non-spore forming bacteria that produce lactic acid as the major metabolic end product from carbohydrate fermentation. They are crucial in the production of various fermented foods, including bread, beer, dairy products, sausages, seafood, fruit, and vegetables. Due to their long history of safe use in food fermentation, they are considered “Generally Recognized As Safe” (GRAS) by the US FDA. Their antagonistic properties, which inhibit pathogenic bacteria and spoilage microorganisms, make them a cost-effective method for food preservation. LAB consist of 13 genera, with main genera including *Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Oenococcus,* and *Streptococcus*. Lactococci are particularly important for their role in rapid acidification, proteolysis, and flavour formation, with *Lactococcus lactis* being the dominant species in cheese production. *Lactobacillus plantarum* is essential in fermenting milk, vegetables, fruits, and meats, generating high levels of lactate and other metabolites like ethanol, acetate, and carbon dioxide during heterolactic fermentation under low oxygen and pH conditions. Additionally, species like *Lactobacillus curvatus* and *Lactobacillus sakei* are commonly found in fermented sausages. *Pediococcus* species, including *Pediococcus acidilactici* and *Pediococcus pentosaceus,* contribute to the fermentation of vegetables and sausages, enhancing food safety through antimicrobial peptide production, while *Pediococcus halophilus* is involved in soy-based fermentation. *Leuconostoc* species are utilized in dairy and vegetable fermentation, often in conjunction with lactococci.

Bacillus species consist of Gram-positive, aerobic, and facultative anaerobic endospore-forming rods found in diverse environments. These bacteria are particularly valuable in alkaline fermentation, causing the hydrolysis of protein to amino acids and peptides and releasing ammonia. Bacilli are also recognized for producing antimicrobial compounds effective against pathogens such as *Listeria monocytogenes, Bacillus cereus,* and *Staphylococcus aureus.* They are essential in the fermentation of various foods, including cereal, legume, and vegetable-based products. They play a crucial role in the fermentation of soybean foods; for example *Bacillus subtilis* is the primary microorganism in the fermentation of natto, chungkookjang, and sufu [Ray and Joshi, 2014; Liu and Han, 2014].

1.2.2 Yeasts

Yeasts are widely distributed in nature, found in orchards, vineyards, air, soil, and in the intestinal tracts of animals. In food fermentations, yeasts can have both beneficial and non-beneficial effects. While some, like *Pichia,* are considered spoilage organisms, others such as *Candida* are used for single-cell protein production. The most beneficial yeasts for food fermentations are from the *Saccharomyces* genus, particularly the species *Saccharomyces cerevisiae*, which is crucial for bread making and alcohol production in wine fermentations.

Yeasts are responsible for the production of many traditional fermented foods. They ferment sugars, generate secondary metabolites, and inhibit the growth of harmful moulds. Successful yeast fermentation requires strains that produce consistent gas levels, maintain reasonable fermentation rates across temperatures, degrade various sugars, survive unfavourable conditions, and have good storage properties.

Common yeasts in alcoholic fermentation include *Hanseniaspora (Kloeckera), Candida, Kluyveromyces, Pichia, Saccharomyces*, and *Torulaspora.*
In dairy fermentation and cheese production, common species include *Debaryomyces hansenii, Yarrowia lipolytica, Saccharomyces cerevisiae, Kluyveromyces marxianus*, and *Galactomyces geotrichum*, which improve cheese flavour and texture through protease and lipase production [Ray and Joshi, 2014; Liu and Han, 2014].

1.2.3 Moulds

Fungal fermentation is integral to the production of various foods, including beer, wine, bread, and cheese. The advantages of using food-grade fungi include the low cost of raw material, rapid cell growth, non-allergenic properties, and the absence of pathogenicity. Currently, diverse food substrates are employed for fungal fermentation to produce desirable foods.

Moulds are involved in the processes of many Asian fermented foods, often working alongside with yeasts. They serve also as starter cultures for some fermented meat products, enhancing the external appearance, aroma, and flavour by breaking down lipids and proteins. While, in the production of tempeh, moulds like *Rhizophus oligosporus* or *Rhizophus oryzae* ferment soybeans, binding them into a solid cake through mould mycelium and partially digesting them to enhance their nutritional value [Ray and Joshi, 2014; Liu and Han, 2014].

1.3 Main fermentation pathways

The fermentation process can be categorized into four primary types based on their main biochemical pathways: alcoholic, lactic, acetic, and alkaline fermentation, as summarized in the Table 1.1.

Alcoholic fermentation involves the conversion of sugars into alcohol and carbon dioxide. This type of fermentation is crucial in the production of beer, spirits, and wine, with yeasts being the predominant microorganisms responsible. In this process, ethanol and CO_2 produced by yeasts contribute to the characteristic texture and flavour of the final products.

Lactic acid fermentation is characterized by the conversion of sugars into lactic acid. This process is primarily carried out by LAB, which can be categorized into homofermentative (producing mainly lactic acid) and heterofermentative (producing lactic acid along with CO_2, acetic acid, and/or ethanol). This fermentation pathway enhances the flavour, texture, and preservation of foods by lowering pH and inhibiting spoilage microorganisms. Products of this fermentation include yogurt, sauerkraut, kimchi, milk kefir, sourdough bread, and fermented cereals.

Acetic acid fermentation involves the oxidation of alcohols and sugars into acetic acid by bacteria of the *Acetobacter* genus. It is fundamental in the production of vinegar, kombucha, water kefir, and cocoa. During this fermentation, organic acids like propionic and glucuronic acids are produced, contributing to the antimicrobial and antioxidant properties of the final products. The conversion of ethanol to acetic acid requires the presence of oxygen.

Alkaline fermentation is distinct in that it involves the hydrolysis of proteins into amino acids and peptides, resulting in the release of ammonia which raises the pH to 8-9. This high pH environment inhibits spoilage-associated microbes and contributes to the umami flavour of the products. Microorganisms such as *Bacillus* species and coagulase-negative *Staphylococcus* are primarily responsible for this fermentation. This type is commonly used in the preparation of traditional foods like Japanese natto and African fermented legumes and eggs.

Furthermore, certain fermented foods and beverages, such as kombucha, undergo multi-stage fermentation processes, involving different types of microbes at each stage (yeasts, LAB, acetic acid bacteria sequentially). This complexity highlights the intricate interplay of microorganisms in achieving the desired qualities in fermented products [Mannaa et al., 2021; Anal, 2019].

Type	**Biosynthetic Pathway**	**Responsible Microbes**	**Fermented Food**
Lactic	Sugars are converted into lactic acid	Lactic acid bacteria	Yoghurt and kimchi
Acetic	Several substrates are converted into acetic acid	*Acetobacter*	Vinegar and water kefir
Alcoholic	Sugars are converted to alcohols and CO_2	Yeast	Wine and beer
Alkali	Proteins are converted into amino acids, peptides, and ammonia	*Bacillus* and *Staphylococcus* spp.	Japanese nattu

Table 1.1- Classification of the major types of fermentation related to food production [Mannaa et al., 2021]

1.4 Technological properties of fermented products

1.4.1 Extended shelf-life and enhanced food safety

Biopreservation, a method of using microorganisms and their metabolites to preserve food, has gained considerable attention as consumers become increasingly aware of the health risks associated with chemical preservatives. Microorganisms used for natural preservation must be safe, produce non-toxic metabolites, maintain high activity during storage, and not adversely affect the sensory properties of the food.

LAB are particularly important in biopreservation due to their broad-spectrum activity against undesirable microflora. Most LAB are GRAS and are commonly used in the food industry. They also form part of the natural human gut microflora. During growth and fermentation, LAB produce various antimicrobial metabolites, including hydrogen peroxide, lactic acid, acetic acid, bacteriocins, antifungal compounds like phenyl lactate, propionate, hydroxyphenyl lactate, and low molecular weight substances such as diacetyl, fatty acids, reuterin, and reutericiclin. These metabolites destabilize pathogen cell membranes, inhibit cell wall enzyme synthesis, disrupt proton gradients, and induce reactive oxygen species formation, thereby enhancing food safety.

Moreover, LAB ability to lower pH through lactic acid production, creates an environment hostile to many pathogens, including *Salmonella spp*., which thrive at pH levels between 4.0 and 9.0.

Additionally, other organic acids produced, such as acetic acid and propionic acids, have antagonistic effects on bacteria, yeasts, and filamentous fungi. Bacteriocins like nisin also inhibit pathogenic microorganisms such as *Bacillus subtilis, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus aureus.* Studies have demonstrated that LAB can also inhibit biofilm formation by pathogens like *Salmonella enterica ssp. enterica, Bacillus cereus,* and *Escherichia coli* on various surfaces, which is crucial in preventing food spoilage and maintaining hygiene in production facilities. LAB also inhibit the growth of filamentous fungi of the genera *Aspergillus, Fusarium,* and *Penicillum* through metabolites that compromise fungal cell membrane integrity and amino acid absorption [Zapasnik et al., 2022].

1.4.2 Reduction of toxic or anti-nutritive factors

Fermentation plays a crucial role in reducing toxic and anti-nutritive factors in food, significantly enhancing food safety and nutritional quality.

Patulin, produced by fungi such as *Byssochlamis, Penicillium,* and *Aspergillus*, is a mycotoxin commonly found in contaminated fruits and responsible for damage to the intestinal barrier. While aflatoxins, produced by various *Aspergillus* and *Emericella* spp., are found in cereals, nuts, and dairy products and have been associated with serious health risks, including acute poisoning, immunosuppression, and cancer. During fermentation processes involving *Saccharomyces cerevisiae* and LAB, levels of mycotoxins such as patulin and aflatoxins can be reduced or even completely removed, resulting in safer food products.

Fermentation also effectively reduces FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) like raffinose, stachyose, and verbascose from soy, preventing flatulence and intestinal cramps in individuals with irritable bowel syndrome (IBS).

In addition to mycotoxins and FODMAPs, fermentation can eliminate various anti-nutritive factors. Trypsin inhibitors in legumes, which interfere with protein digestion, can be reduced by over 99% through fermentation, improving digestion and nutrient retention. Fermentation also degrades phytic acid and tannins in cereals and legumes, enhancing mineral bioavailability and preventing deficiencies associated with high-phytate diets.

Moreover, fermentation detoxifies vicine and convicine in faba beans, compounds that can cause favism in individuals with glucose-6-phosphate dehydrogenase deficiency. Fermentation with *Lactobacillus plantarum* can completely eliminate these compounds. Additionally, fermentation reduces cyanogenic glycosides in plants like cassava, preventing the release of toxic hydrogen cyanide and ensuring the safety of these foods [Ray and Joshi, 2014; Mukherjee et al., 2024].

1.4.3 Organoleptic properties

Fermentation also significantly enhances the organoleptic properties of food, improving texture, flavour, and aroma. Polysaccharides produced during fermentation by functional starter cultures of LAB, such as *Lactobacillus delbrueckii* subsp*. bulgaricus* and *Streptococcus thermophilus*, increase the viscosity and firmness of low-fat products and contribute to their mouthfeel.

LAB also produce various compounds that enhance the aroma and flavour of fermented foods. Their metabolic activities result in a tangy lactic acid taste and the production of aromatic compounds from amino acids. Control over LAB peptidase activities is crucial in cheese ripening, improving sensory qualities, as demonstrated by the overexpression of specific peptidases in *Lactobacillus lactic* subsp*. cremoris*, which enhances the flavour and texture of cheese [Ray and Joshi, 2014].

2. FERMENTED FOODS AND DRINKS: A NEW TREND IN THE MARKET

Fermented foods and beverages, such as kimchi, kombucha and kefir, are growing in popularity. These products are increasingly found on restaurant menus, in retail stores, and frequently discussed by food experts. Fermentation, a traditional method used to preserve food, is now celebrated for its ability to create complex flavours and enhance the dining experience. Noma, a renowned two-Michelin-starred restaurant in Copenhagen, exemplifies the importance of fermentation in modern cuisine, pillar of their restaurant.

Fermentation produces a wide range of products, including beer, wine, cheese, kimchi, and soy sauce. Despite their differences, these products share a common process where microbes, bacteria, moulds, and yeasts transform the molecules in food, creating new flavours. The best fermented foods retain some of their original character while transforming into something new and delicious.

These unique combinations of familiar and new flavours make fermented foods particularly appealing. In 2018 Renè Redzepi e David Zilber published the book "The Noma Guide to Fermentation", and in 2022, they launched the *Noma Projects* to share their fermentation knowledge and innovations globally, allowing people to experience Noma's flavours at home.

The interest in fermentation raises questions: why is this ancient technique capturing the passion and curiosity of so many people today? Is it solely due to the unique flavours, or is there more to this culinary revival? Exploring the world of fermented foods and drinks reveals an intricate relationship between flavour, tradition, and health that makes this trend so compelling.

2.1 Market analysis and consumer drivers

Between 2019 and 2023, the fermented food and beverage market grew at a compound annual growth rate (CAGR) of 7.5%, driven by rising health consciousness, high probiotic content demand, and the introduction of new products. In 2023, the Asia-Pacific region led with a 38.1% market share, where traditional fermented foods like kimchi, miso, natto, and tempeh hold cultural significance. Globally, the dairy fermented food segment dominated with a 46.2% share.

The increased availability of fermented foods in supermarkets, specialty stores, and online platforms has enhanced market accessibility, supporting growth. This accessibility, along with a diverse range of products, including sourdough bread, yogurt, kefir, kimchi, sauerkraut, kombucha, tempeh, and miso, further drives market expansion. The fermented foods and beverages market is projected to be valued at USD 575.6 billion in 2024 and is expected to grow at a CAGR of 8.1% from 2024 to 2034, reaching USD 1.25 trillion by 2034. This growth is driven by increasing consumer interest in health, wellness, and unique culinary experiences [Future Market Insights (1); Markwide Research Report].

Probiotic-rich foods, valued for their immunity and digestive benefits, are gaining attraction among health-conscious consumers. The trend toward clean labels and natural ingredients has increased the demand for fermented products with simple, recognizable ingredient lists. There is a rising demand for natural and organic fermented products free from artificial additives or preservatives. Preferences for natural, less processed foods align well with the perceived health benefits of fermented products, such as improved immunity, digestion, and gut health, resulting in significant opportunities for producers. Leading companies are investing in R&D to launch innovative, low-calories, and natural ingredient-based products that taste good to meet specific consumer needs. The increasing prevalence of health issues among the growing population is expected to drive the demand for fermented foods, along with innovations and new products launches by key market players [Mordor Intelligence (1)].

The popularity of fermented foods and drinks can be attributed to the growth of health consciousness, especially among young consumers, with campaigns to battle obesity and promote healthy living. According to Global Data's 2018 Q4 Survey, health and wellbeing impact the product choices of 67% of consumers aged 18-24 globally [Drinks Insight Network].

Moreover, environmental sustainability concerns are driving interest in fermented foods and beverages. Produced with minimal environmental impact through eco-friendly methods, this products appeal to consumers seeking sustainable and animal-free options, especially with the growing popularity of plant-based diets and veganism. This trend has led to the development of fermented products made from plant sources like soy, nuts and grains.

2.2 Emergent products: characteristics and market

The following sections will examine some of the emergent products within the fermented food and beverages market. Specifically, it will focus on kombucha and kefir among beverages, and kimchi, miso, natto and tempeh among foods. For each of these products, a detailed analysis will be provided, covering their technical characteristics, and exploring the market dynamics.

2.2.1 *Kombucha*

Kombucha is a fermented beverage of Asian origin, traditionally produced through the aerobic and static fermentation of sweetened black or green tea (derived from the leaves of *Camellia sinensis* plant) with the aid of a symbiotic culture of bacteria and yeasts (SCOBY), also known as the tea fungus or kombucha mother. During fermentation, osmophilic yeasts within the SCOBY convert the sugar into ethanol, while bacteria oxidize the alcohol to produce acetic acid. In addition to acetic acid, other organic acids such as gluconic, lactic, malic, citric, and tartaric acids are formed. These acids possess antibacterial properties, helping to prevent contamination by pathogenic bacteria.

The resulting beverage is slightly sweet and sour, with a trace of carbonation that enhances its acceptance among consumers. Kombucha can serve as a low-alcoholic substitute for sparkling wines or soft drinks due to its degree of carbonation, making it a healthier alternative. Recently, “hard kombuchas” with alcohol content ranging from 3% to 11% (v/v) have been developed, compared to the 0-1% (v/v) in traditional kombucha.

The production of kombucha involves preparing a tea base by brewing tea with 5 grams of tea leaves per litre of water and adding 50 grams of sugar per litre of tea, which serves as a substrate for the fermenting bacteria and yeasts. The sweetened tea is then cooled to room temperature (approximately 20°C) to prevent the inactivation of microorganisms. Once cooled, the SCOBY and 20% of already fermented kombucha are added, which contains high levels of indigenous yeast and bacteria and lowers the starting pH. Fermentation is conducted under aerobic conditions at ambient temperatures between 18-28°C for a period of 8 to 14 days, with the duration depending on factors such as microbial population composition, aeration, temperature, and the shape and size of the fermentation vessel.

Maintaining sanitized utensils and a clean workspace is crucial to controlling microbial growth and preventing contamination. Food safety methods include pasteurizing the product, adding preservatives such as 0.1% sodium benzoate and 0.1% potassium sorbate, and refrigerating the kombucha. Although pasteurization and cold storage can reduce the viability of probiotic microorganisms, recent studies suggest that non-viable microorganisms and their metabolic by-products (paraprobiotics and postbiotics) still offer health benefits to consumers.

It is essential to control pH levels during fermentation and preferably halt the process at a pH level of 4.2 to prevent the overproduction of acetic acid. Given the intense formation of organic acids, suitable fermentation containers should be made of glass or stainless steel to avoid corrosion and potential toxicity from other materials.

Kombucha fermentation is a natural process characterized by the method of backslopping, where specific microorganisms are selectively propagated under controlled conditions to ensure their dominance and effectiveness. The key to this process is the SCOBY, a cellulose-based, gelatinous film that forms on the surface of the tea. It houses a complex community of acetic acid bacteria (AAB) and yeasts, whose composition can vary depending on factors such as the origin, climate, geographic location, and the medium used for fermentation.

The primary bacteria in the SCOBY are acetic acid and gluconic acid producers, primarily from the genera *Acetobacter* and *Gluconobacter*. These bacteria play crucial roles in the fermentation process. *Acetobacter* species convert ethanol into acetic acid using the enzymes alcohol dehydrogenase and aldehyde dehydrogenase. This acetic acid is then further processed through the Krebs cycle to produce water and carbon dioxide. In contrast, *Gluconobacter* species oxidize glucose to produce gluconic acid, without further metabolism of acetate, leading to gluconate accumulation in the medium. Notably, *Komagataeibacter xylinus* is a significant bacterium within the SCOBY due to its ability to produce both acetic acid and cellulose, the latter being essential for SCOBY's structural integrity.

The yeast species present in kombucha, which include *Saccharomyces, Zygosaccharomyces, Dekkera/Brettanomyces,* and *Pichia*, are more abundant than AAB. These yeasts are responsible for converting sugars into ethanol and carbon dioxide, essential steps in the fermentation process.

Indeed, the kombucha fermentation process involves symbiotic interactions between these microorganisms. Sucrose, the common substrate, is hydrolysed into glucose and fructose by yeast invertase enzymes, increasing the concentrations of these simple sugars. Yeasts then ferment these sugars into ethanol, carbon dioxide, and glycerol. Concurrently, AAB utilize glucose to produce gluconic acid and ethanol to produce acetic acid, and some can also produce cellulose, contributing to the structure of the SCOBY. Occasionally, LAB are present and can ferment glucose via the pentose phosphate pathway, resulting in the production of lactic acid, acetic acid, and carbon dioxide (Figure 2.1).

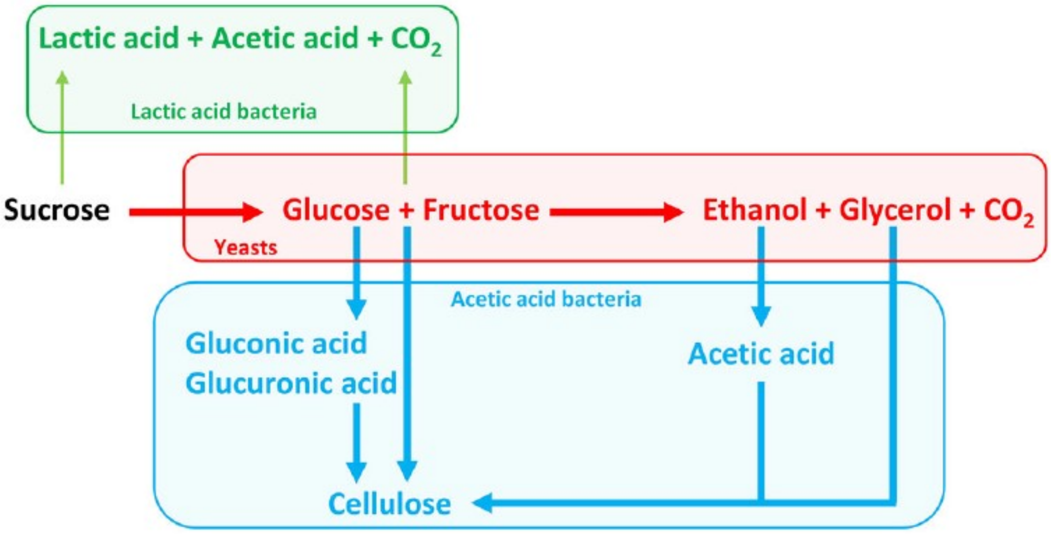


Figure 2.1- Main metabolic activities of yeasts, acetic acid bacteria, and lactic acid bacteria during kombucha fermentation [Laureys et al., 2020]

The final kombucha beverage is rich in microbial metabolites such as organic acids, vitamins, ethanol and minerals, and retains beneficial compounds from the tea, including antioxidant polyphenols like epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate [Coelho et al., 2020; Leal et al., 2018; Laureys et al., 2020].

Recently, kombucha, has gained significant popularity in Western cultures, particularly within the functional food movement due to its supposed health benefits. It has become one of the fastest-growing beverages in this category, with a +49% dollar growth from July 2017 to July 2018 [Laureys et al., 2020]. The kombucha market is estimated to be worth USD 2.97 billion in 2024 and is expected to reach USD 4.65 billion by 2029, growing at a CAGR of 9.48%. This growth is driven by rising health consciousness among consumers and a preference for healthy hydration options. The need to supplement daily nutrition to compensate for poor eating habits has also boosted kombucha sales. As consumers become more aware about health risks associated with soft drinks, many are shifting towards healthier alternatives like kombucha and other functional beverages (Figure 2.2).

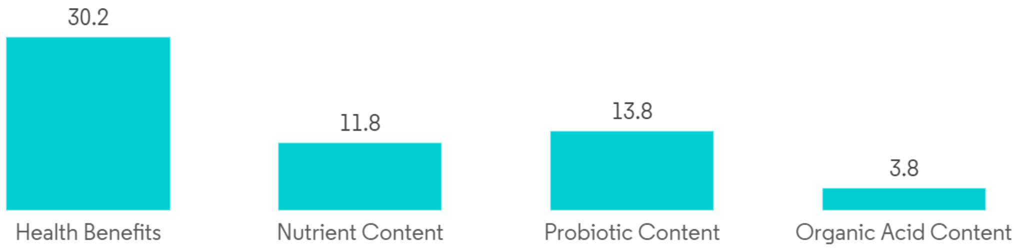


Figure 2.2- Kombucha consumption motivation, in percentage, data from United States, 2021 [Mordor Intelligence (3)]

In response to the increased demand for kombucha, manufacturers are introducing various flavour variants that are preferred over original kombucha. Traditional flavours such as ginger, grapefruit, and elderflower remain popular, while newer flavours like cherry blossom, winter mint, pumpkin spice, and spicy pineapple are attracting consumer interest.

North America is currently the largest market, while Asia-Pacific is the fastest-growing region in the kombucha market, with China being a leading market due to the perceived health benefits of packaged kombucha drinks. Rising urbanization and the easy availability of kombucha in local retail stores, especially in countries like Australia, China, and Japan, are also supporting market expansion (Figure 2.3)[Mordor Intelligence (3)].

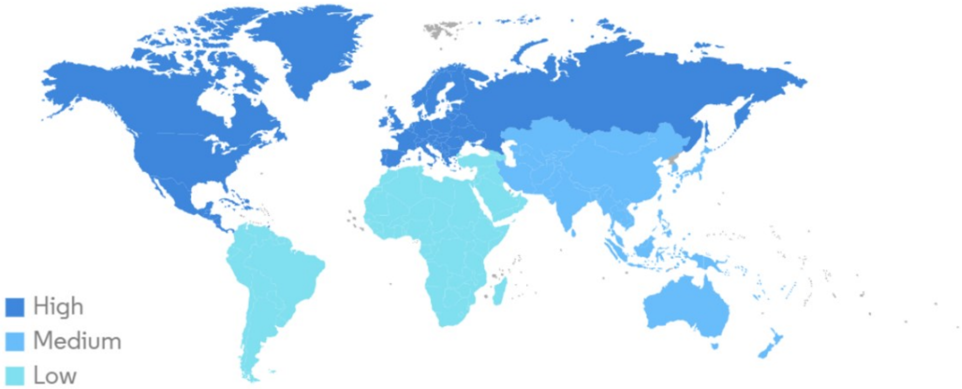


Figure 2.3- Kombucha Market Size by Geography, global data in 2021 [Mordor Intelligence (3)]

The kombucha market in Italy is gaining momentum. An article published in Gambero Rosso in January 2024 refers to this year as "the year of kombucha, the fermented tea that is conquering the world". Lorenzo Ruggeri writes, "Seattle […], Sydney […], we cultivated our passion for this diabolical drink while travelling […] Berlin […], Copenhagen or San Francisco […]. In Italy, it is much less known, but it is only a matter of time. Months. The movement is growing at a dizzying pace: in fine dining, in bars, in homebrewing […] Particularly appreciated by Generation Z and millennials, it is versatile, even at the table, and is produced by a network of young producers who speak the same language as their consumers. Industrial soft drinks producers cannot sleep soundly […] Kombucha is part of a new lifestyle with less alcohol, less caffeine, less sugar (between 2 and 5 grams of residual sugar per 100 ml) […] For now, the Italian network is fuelled by small producers who have recently started and are moving quickly. The common thread? They are young men and women who have travelled the world with a curious eye, only to replicate the recipe at home and venture into commercialization" [Ruggeri, 2024].

However, it is important to always check the ingredients listed on the label and the production methods used by the company. An industrialized kombucha that has been pasteurized and microfiltered is different from a living artisanal one. Kombucha drinkers must be informed consumers!

This rapid development within the Italian market mirrors global trends, indicating a promising future for kombucha as a mainstream beverage choice.

2.2.2 *Kefir*

Kefir is a fermented drink with a low alcohol content, characterized by its acidic and effervescent qualities resulting from the carbonation process during fermentation. Originating in the Balkans, Eastern Europe, and the Caucasus, its consumption has spread worldwide due to its reputed health benefits. Kefir can be categorized into dairy and non-dairy types, depending on the substrate used for fermentation. Both types are produced by inoculating the starter culture, kefir grains, into the substrates at variable ratios (ranging from 1 to 20% w/v) and allowing fermentation to occur for 18-24 hours at 20-25°C. The fermentation process initiates when the bacteria and yeasts in the kefir grains find suitable conditions, leading to an increase of 5-7% in grain biomass and the production of various metabolites. At the end of fermentation, the kefir grains may split into smaller grains and release viable cells into the substrate. These grains are then isolated from the kefir by sieving and can be reused for subsequent fermentations.

Kefir grains, the key starter in kefir fermentation, range in size from 1 to 4 cm and resemble small cauliflower florets in shape and colour, varying from white to light yellow. This gelatinous and slimy structure consists of a natural matrix of exopolysaccharides (EPS), particularly kefiran, and proteins that house a symbiotic community of LAB, yeasts, and AAB. The microbiota of kefir grains can vary based on their geographical origin, which is closely linked to climate conditions. Additionally, the microflora composition in kefir can differ depending on the substrate used in the fermentation process and the methods of culture maintenance, such as fermentation time, temperature, degree of agitation, and ratio of kefir grains to substrate. This microbial diversity is recognized as being responsible for the unique physicochemical properties and biological activities of each kefir, although certain major Lactobacillus species are consistently present due to their specific probiotic properties [Azizi et al., 2021].

Dairy kefir, a viscous and slightly effervescent fermented milk drink, originated as a method to prolong the shelf life of surplus milk. Characterized by an acidic taste and low alcohol content ranging from 0.08% to 2.0%, kefir is traditionally prepared using raw cow, camel, goat, sheep, or buffalo milk mixed with kefir grains. These grains introduce a unique yeast-like flavour to the drink, thanks to the symbiotic metabolic activities of various bacterial and yeast species, which include the proteolytic, glycolytic and lipolytic degradation of milk constituents. The fermentation process yields numerous metabolic products, including lactic and acetic acids, carbon dioxide, ethanol, acetaldehyde, acetoin, and other volatile compounds, alongside essential minerals, amino acids, vitamins, folic acid, bacteriocins, bioactive peptides and some nutraceutical components [Azizi et al., 2021].

Typically, kefir composition includes about 90% moisture, 3.0% protein, 0.2% lipid, 6.0% sugar, 0.7% ash, 1.0% lactic acid, 0.48% alcohol and 201.7-277.0 mL/L CO_2, varying with the quantity of kefir grains used. Its chemical composition is influenced by type of milk, the grains or cultures, additives, and the production technology. For instance, the levels of dry matter, fat, protein, carbohydrates, and ash content depend on the milk type, while the alcohol content is influenced by the kefir grains and fermentation pH. The starter culture used, significantly affects the viscosity and overall chemical profile of the final product [Farag et al., 2020].

Milk kefir grains contain a complex microbiota comprising LAB, AAB, and yeasts. Dominant LAB species include *Lactobacillus paracasei* ssp. *paracasei, L.acidophilus, L.delbrueckii* subsp. *bulgaricus, L.kefiranofaciens*, and *L.plantarum*, which collectively make up about 20% of the total LAB population. These bacteria hydrolyse lactose during fermentation, producing lactic acid, carbon dioxide, ethanol, acetic acid and various flavour compounds such as acetaldehyde, diacetyl, and acetoin. AAB, mainly from the genus *Acetobacter*, produce acetic acid, while yeasts species like *Kluyveromyces marxianus*, *Candida kefyr*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Torulaspora delbrueckii*, and *Pichia fermentans* convert lactose to ethanol and carbon dioxide (Figure 2.3) [Fan et al., 2022; Chong et al., 2023].

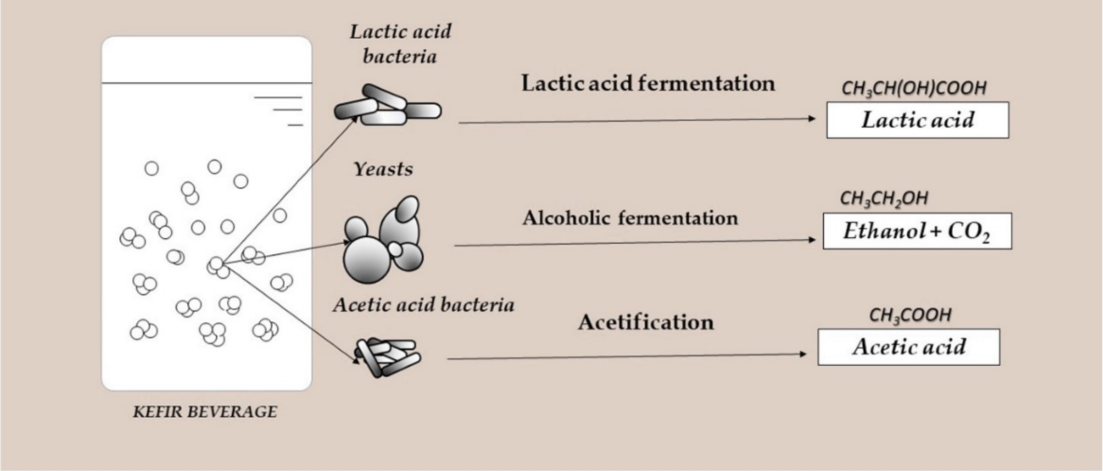


Figure 2.3- Kefir fermentation pathways and main microbial species involved: LAB, yeasts, and AAB [Ganatsios et al., 2021]

Each microbial species involved in the fermentation process plays a crucial role in the final quality of kefir. The overall flavour and aroma depend on the microbial interactions during fermentation, with LAB metabolizing milk lactose into glucose and galactose, which are further converted into lactic acid via the homofermentative pathway. This process results in the characteristic acidic taste. When AAB dominate, especially under aerobic conditions, the kefir exhibits a tarter flavour and slight biting odour due to acetic acid accumulation. The final quality of kefir is a result of both primary fermentation components, such as lactic acid, acetic acid, CO_2, and ethanol, and secondary metabolites like acetaldehyde, acetoin, diacetyl, and exopolysaccharides [Ganatsios et al., 2021].

The market for dairy kefir is experiencing significant growth. This beverage, with a long history of consumption in Central Asia and Eastern Europe, is now garnering immense interest globally due to its numerous proclaimed health benefits. This includes regions such as the United States, Germany, France, the United Kingdom, the Netherlands, Brazil, China, Japan, Turkey, Malaysia, Indonesia, Tibet, and both North and South America [Azizi et al., 2021].

The global kefir market, valued at USD 1.73 billion in 2022, is projected to grow at a rate of 6.4% annually over the next five years. This growth is driven by increasing demand for probiotic drinks due to rising consumer awareness of the importance of gut health for maintaining weight, energy levels, and overall physical health. A survey by the International Food Information Council in 2021 found that 51% of Americans consume probiotics for gut health, with significant numbers also consuming them for immune health, general wellness, and emotional health. The recent shift towards preventive health measures, coupled with a move away from carbonated drinks to healthier ready-to-drink functional beverages and probiotic drinks, is also a key factor in the market's expansion [Mordor Intelligence (2)]. The COVID-19 pandemic further influenced the market, with shifts in consumption patterns and an increased focus on immune health driving demand [Spizzirri et al., 2023].

Several commercial producers worldwide, such as Lifeway kefir (USA, UK, and Canada), Bionova (Italy), Evolve Kefir (USA), Wallaby Organic (Australia), and CocoKefir (USA), illustrate kefir expanding global popularity. Lifeway, one of the largest kefir companies, began with homemade kefir production in 1986 and now offers over 15 different types of kefir products. By 2017, Lifeway's annual revenues exceeded USD 120 million, with distribution extending throughout the United States, Mexico, the UK, Ireland, and parts of Central and South America and the Caribbean [Azizi et al., 2021].

Europe represents the largest market for kefir, where it is widely used to support digestive health, while North America is the fastest growing market. The competitive landscape includes both global and local players who focus on innovation, new product launches, and strategic market expansion. These companies invest heavily in research and development to create probiotic products that cater to diverse consumer needs and are increasingly targeting untapped markets [Mordor Intelligence (2)].

In recent years, the rise in lactose-intolerant, vegan, and vegetarian consumers have stimulated the development of healthy, fruit- or vegetable-based kefir formulas. Water kefir, a non-dairy alternative to milk kefir, is particularly suitable for these consumers. The fermentation process for water kefir is typically spontaneous, involving water kefir grains placed in a sucrose medium with or without dried fruits or fruit extracts. This fermentation occurs at temperatures between 21°C and 30°C for 4 to 8 days. Water kefir grains can be recovered and reused indefinitely by placing them in fresh sugar-water medium. Common carbon sources include table sugar or brown sugar, while nitrogen is provided by fresh or dried fruits, often figs and lemon slices. Additionally, other potential carbon and nitrogen sources for water kefir production include various fruit juices, vegetables, and molasses.

The primary microbial members of water kefir grains include LAB of the genus *Lactobacillus* (such as *Lactobacillus casei/paracasei*, *Lactobacillus hordei*, *Lactobacillus hilgardii*, and *Lactobacillus nagelii*), AAB of the genus *Acetobacter*, and yeasts like *Saccharomyces*. The dominant species can vary depending on the geographical origin of the grains and the fermentation substrate. Both the carbon source (typically sucrose) and nitrogen source (fresh or dried fruits) are crucial for the metabolism and growth of the water kefir grain microorganisms.

Water kefir presents a challenging environment due to its high sugar content (up to 100 g/L initially) and low nitrogen levels. Consequently, mutualistic cooperation within the microbial community is essential. Yeast species such as *Saccharomyces*, *Zygotorulaspora*, and *Dekkera* hydrolyse sucrose through intracellular or extracellular invertases, producing glucose and fructose. These monosaccharides are there taken up by the yeast cells via facilitated diffusion, utilized for metabolism, and converted into ethanol and carbon dioxide via the glycolytic pathway. This process also makes simple sugars available for the bacteria within the consortium. LAB can assimilate fructose, using it in glycolysis or converting it to mannitol, which imparts a sweet taste to the final beverage. Most LAB species found in water kefir, except for *L. hilgardii*, also produce lactic acid and exopolysaccharides from sucrose. AAB convert glucose into gluconic acid, and ethanol produced by the yeasts into acetic acid. Yeasts also supply LAB and other community members with peptides and amino acids (Figure 2.4).

The market for water kefir is growing as consumer lifestyle trends towards health and wellness continue to increase. Many consumers avoid dairy products due to lactose intolerance, allergies, or ethical reasons, leading to greater interest in non-dairy alternatives like water kefir. This beverage can be produced from a diverse range of substrates, and the ability to modulate fermentation processes and parameters allows for unique product and flavour development. A report from Mintel (2019) suggests that water kefirs are capitalizing on consumer preferences for multifunctional products and adventurous flavours. Water kefirs are viewed as a vegan version of dairy-fermented drinks and compete with water for hydration, juices for fruit content and nutrition, and ready-to-drink products for convenience. Additionally, they offer probiotic benefits and superior taste compared to kombucha. According to NewNutrition Business, while the probiotic market was once dominated by dairy, consumers are now seeking probiotics from a wider variety of foods, especially those with traditional and historical significance and water kefir is well-positioned to capitalize on this shift [Lynch et al., 2021].

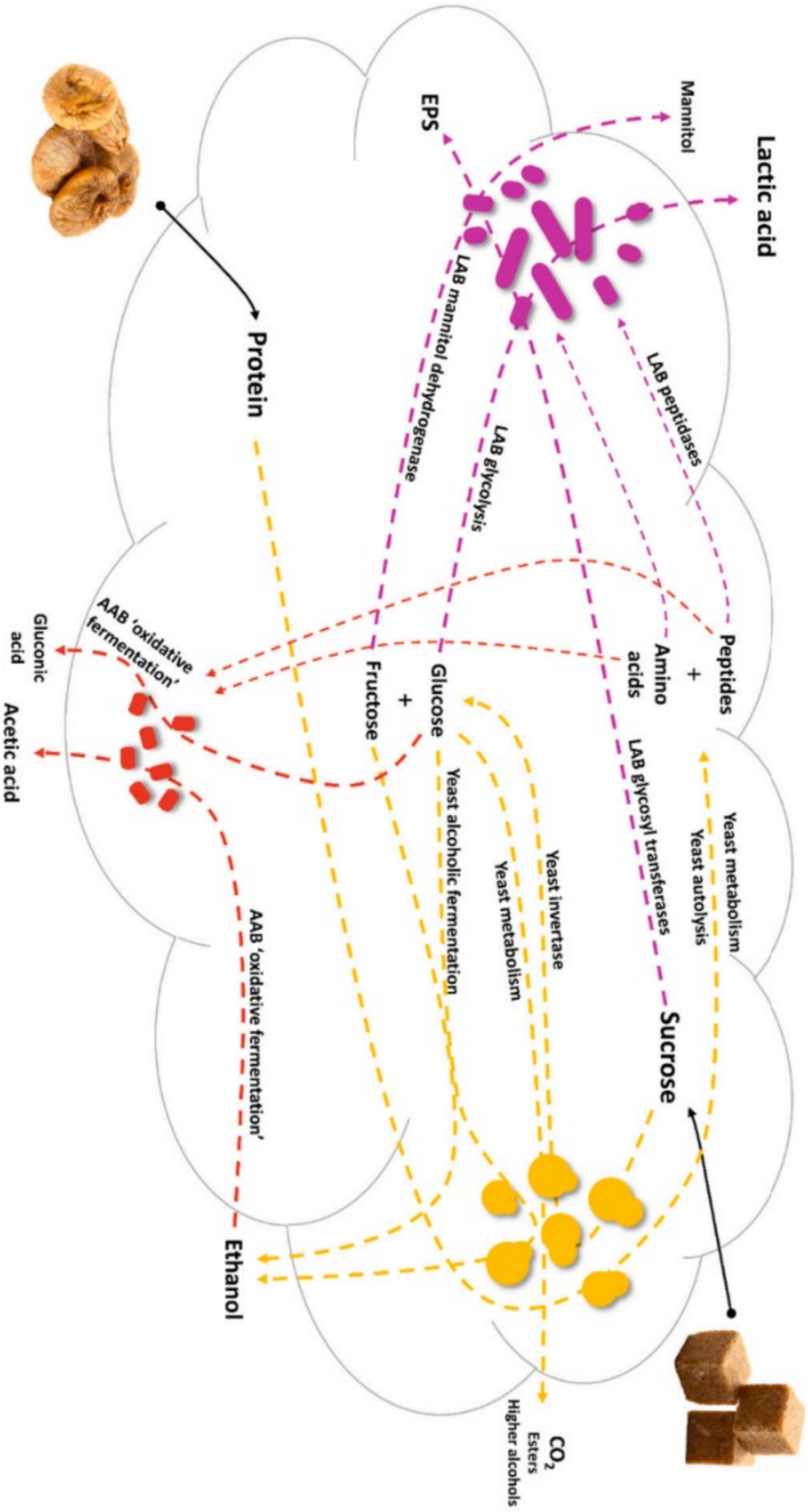


Figure 2.4- Primary metabolites and interaction between the water kefir microbiota. Yeast species (yellow) hydrolyse sucrose producing glucose and fructose, then metabolized and converted into ethanol and CO_2. Yeasts also transform proteins into peptides and amino acids used by LAB and AAB. LAB (pink) use sucrose to produce EPS, and convert glucose and fructose into lactic acid and mannitol respectively. Finally, AAB (red) convert glucose into gluconic acid, and ethanol produced by the yeasts into acetic acid [Lynch et al., 2021]

2.2.3 *Kimchi*

Kimchi is the quintessential traditional fermented food in Korea, widely appreciated across East Asian countries like Japan and China. This dish is primarily made by fermenting vegetables, such as cabbage, radish, and cucumber, with a variety of spices (including red and black pepper, cinnamon, garlic, ginger, onion, and mustard) and seasonings (like salt, salt-pickled seafood, corn syrup, sesame seed, and soy sauce). Depending on availability, geographical region, and desired taste, additional ingredients such as mushrooms, carrots, leeks, watercress, seafood (oysters and shrimp), cereals (barley and rice), fruits (apple and pear), and meats (pork and beef) may also be included.

The most popular type of kimchi in Korea is Baechu, which is primarily made with Chinese cabbage, constituting 74–90% of its ingredients. Preparation involves trimming the cabbage into small pieces, washing it thoroughly, and draining excess water before brining. Brining requires a small amount of table salt and a resting period of 2-3 hours. During this time, the remaining raw materials are washed, graded, cut, and mixed. Once brining is complete, the excess water is drained, and all ingredients are combined.

Kimchi fermentation is primarily driven by heterofermentative LAB, from the genera *Leuconostoc*, *Lactobacillus*, and *Weissella*. These bacteria produce organic acids, carbon dioxide, ethanol, vitamins, prebiotic factors, and various flavour compounds (mannitol and amino acids) under anaerobic conditions, contributing to kimchi's health benefits and distinctive taste. Minor populations of *Lactococcus* and *Pediococcus* are also present. Fermentation conditions vary based on consumption and storage needs: room temperature is suitable for short-term consumption, while long-term storage requires fermentation at low temperatures (5°C).

Spontaneous kimchi fermentation, without specific inoculants or starters, results in the growth of various LAB species from raw materials, which are considered to have potential health benefits or probiotic effects. These LAB, initially present in very low numbers in the vegetables and seasonings, quickly dominate due to the favourable anaerobic, low temperature, and saline (1.5-4.0% NaCl) conditions. They lower the pH of the kimchi by producing organic acids from carbohydrates during fermentation, causing significant changes in LAB communities at different fermentation stage. Early fermentation stages are dominated by *Leuconostoc* species like *Leu.mesenteroides* and *Leu.citreum*, which are less acid-tolerant and microaerophilic. As fermentation progresses, more acid-tolerant species such as *Lactobacillus* and *Weissella* (including *Lb.sakei*, *Lb.plantarum*, and *W.koreensis*) become dominant.

However, spontaneous fermentation results in variations in the taste and sensory qualities of kimchi, making it challenging to control the process for industrial production. With its increasing global popularity, kimchi market has expanded worldwide, necessitating consistent quality in industrial production. Starter cultures are frequently used to ensure uniform quality, along with standardized raw materials and fermentation conditions, to produce high-quality, standardized kimchi on industrial scale [Patra et al., 2016; Jung et al., 2014].

The global kimchi market was valued at USD 4.83 billion in 2023 and is expected to grow at a CAGR of 6.25% from 2024 to 2033 (Figure 2.5). This growth can be attributed to increasing awareness of the health benefits of kimchi, rich in vitamins, minerals, antioxidants and probiotics, the rise of veganism and plant-based diets, and the growing influence of social media and Korean food tourism [The Brainy Insights].

The surge in popularity can be linked to the growing interest in fusion cuisines and kimchi's ability to blend seamlessly with other culinary traditions due to its unique, distinct, savoury, and spicy flavour. Kimchi offers a delicious and nutritious option for those seeking unique and healthy dining experiences. Once a simple side dish, it has evolved into a dynamic component that enhances the flavours of many other foods. Its acidic, spicy, and fermented qualities can be added to salads, sandwiches, tacos, and even pizza, giving a new twist to familiar foods. This versatility has inspired culinary ingenuity, capturing the interest of food enthusiasts around the world and cementing kimchi's status as a leading innovator in the food industry. The rising popularity of Korean cuisine, facilitated by its visually appealing presentation on social media, as well as the cultural impact of K-pop and K-dramas, has further boosted its appeal. The globalization of food preferences, the proliferation of Korean restaurants abroad, and increased in culinary curiosity have expanded kimchi's reach [Polaris Market Research].

The Baechu kimchi variant leads in popularity as it is the most consumed type. Significant sales occur through supermarkets and hypermarkets, which are the leading distribution platforms. China and South Korea are the foremost global producers of kimchi, with high production and consumption rates contributing to positive market growth. North America is expected to witness significant growth in the coming years due to the rising trend of cross-cultural food, with the United States as the second-largest importer of kimchi globally, with an approximate value of US$ 9 million in 2018 [Fortune Business Insights].

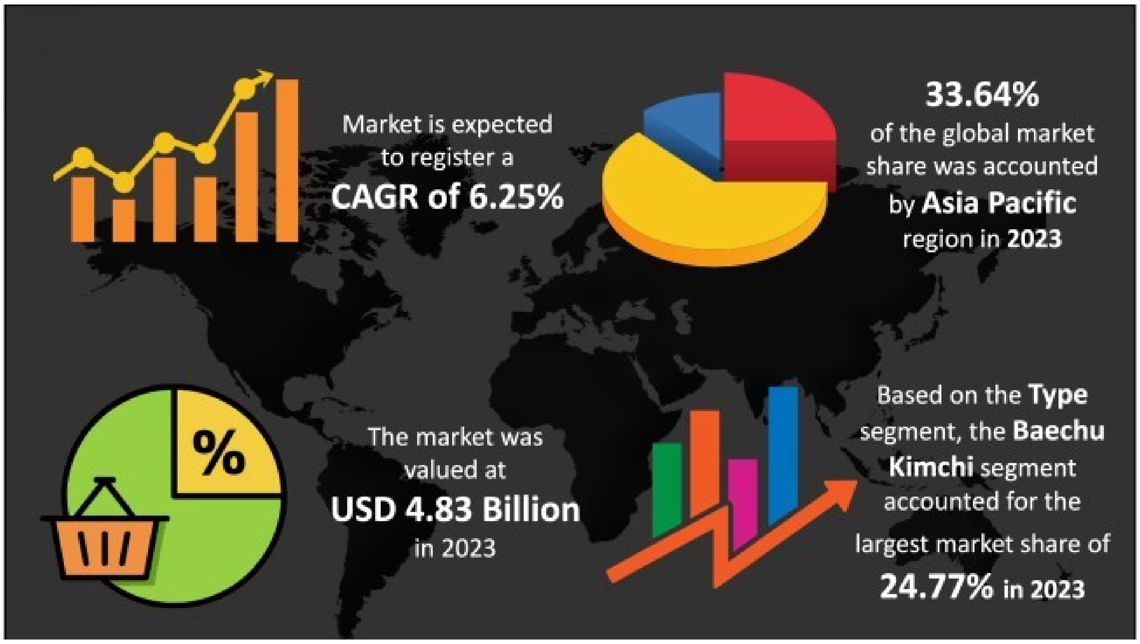


Figure 2.5- Kimchi Market [The Brainy Insights]

2.2.4 *Fermented soy products: Miso, Natto, and Tempeh*

Miso is a traditional Japanese fermented food known for its savoury flavour and aroma, often used as a key ingredient in miso soup [Allwood et al., 2021].

Recently, its unique taste has gained popularity in Western cuisine, where it is incorporated into marinades, dressings, dips, and even desserts [Saeed et al., 2022].

The production of miso involves a two-step fermentation process. Initially, a substrate such as rice, barley, or soybeans is inoculated with the mould *Aspergillus oryzae* and fermented at around 30°C for about 48 hours to create *koji*. The *koji* is then mixed with salt and a soybean mash, undergoing a second fermentation by bacteria and yeast at room temperature, which can last from 2 to 24 months (Figure 2.6). Depending on the substrate used for the *koji*, it can be categorized into varieties such as rice, barley or soybean *koji*. Then miso is further classified into white, red, or dark miso based on the amount of salt added and the fermentation duration. In Japan, rice miso, made from rice koji, soybeans, and salt, constitutes 80% of the miso produced [Allwood et al., 2021].

The substrates used in the first fermentation step, rich in starch, are broken down by enzymes from *Aspergillus*, yielding fermentable sugars for the subsequent fermentation by yeasts. *Aspergillus oryzae* thrives in low moisture environments and temperatures between 30°C and 43°C. Initially, heat is provided via incubation, and after 24 hrs, exothermic reactions generate heat, necessitating mixing to prevent overheating.

The addition of salt in the second fermentation step is crucial not only for flavour, but also to inhibit spoilage microorganisms by reducing water activity. Traditionally, miso relies on indigenous fermentation or the use of mature miso from previous batches, whereas commercial production often employs pure starter cultures like *Tetragenococcus halophilus* (LAB) and *Zygosaccharomyces rouxii* (yeast). These microorganisms can withstand miso's high salinity of miso, with *T. halophilus* producing acidic metabolites that lower the pH and promote the growth of *Z. rouxii*. The distinct umami flavour of miso arises from the activity of glutaminase, an enzyme produced during koji fermentation, which converts glutamine to glutamic acid. The LAB lower the pH from 5.8 to 4.9, favouring yeast growth, which produces ethanol and esters, further enhancing the flavour and aroma. The microbial community involved in miso fermentation is essential for its unique flavour, texture, and nutritional profile, though research on these microbial population and processes remains limited [Saeed et al., 2022; Allwood et al., 2021].

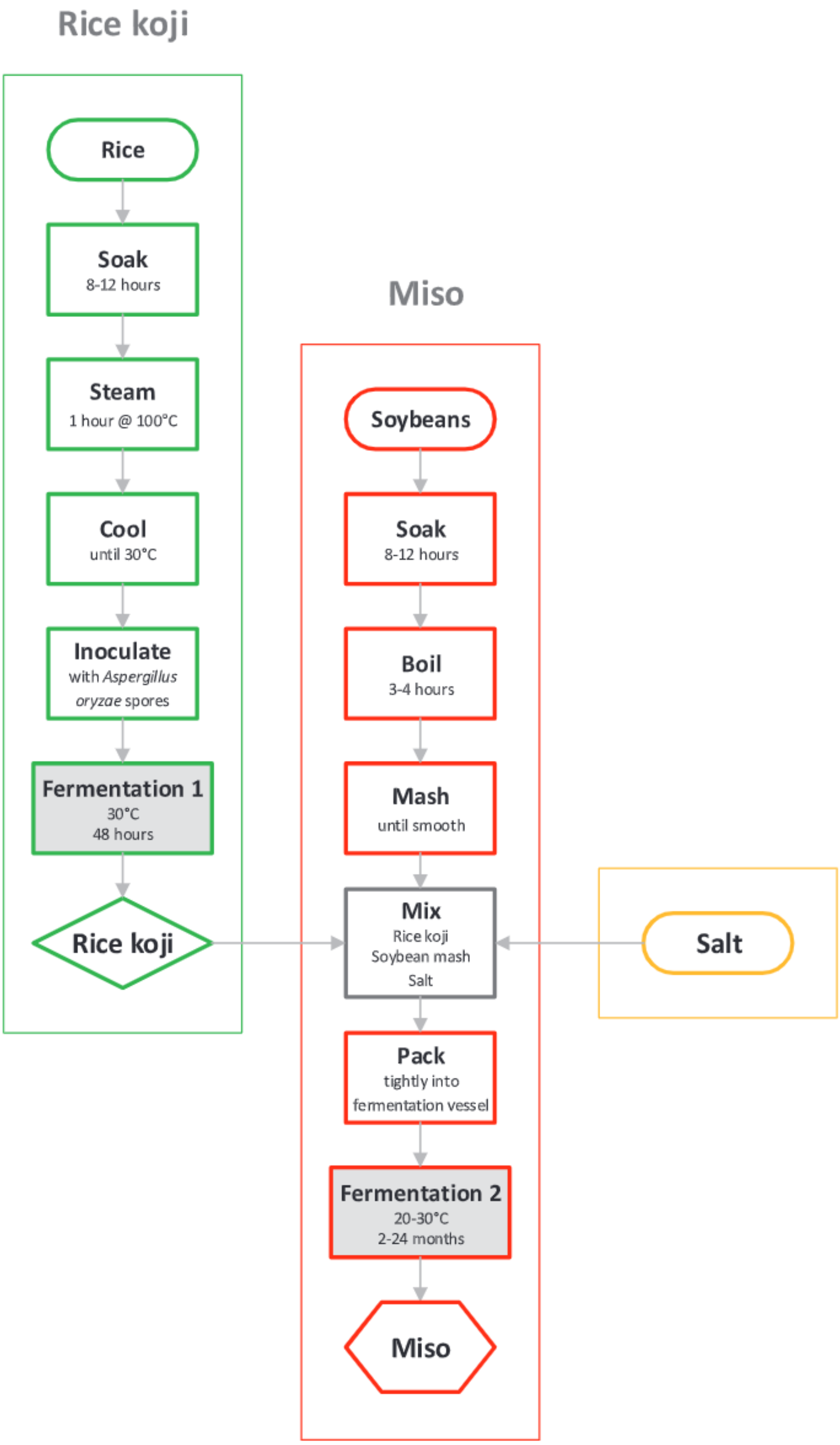


Figure 2.6- Miso production involves a two-step fermentation process. First, rice is inoculated with Aspergillus oryzae to create koji. Then, koji mixed with salt and a soybean mash, undergoes a second fermentation by bacteria and yeasts [Allwood et al., 2021]

Although traditional miso was once made in small batches in private homes, the production process has been largely commercialized since the 1960s. Japanese scientists developed methods to standardize miso production by using commercial strains of microorganisms, ensuring a more consistent product. Mechanized production methods and temperature control have reduced fermentation time, boosting production rates. The use of polyethylene bags and pasteurization techniques to extend shelf life has also contributed to the decline of traditionally made miso. Since the 1970s, soy foods like miso have gained popularity in Western countries, driven by growing interest in nutrition and health. Miso is prized for its umami flavour, created from the breakdown of protein into glutamic acid. Its complex taste makes it a popular ingredient in both savoury and sweet dishes among chefs and home cooks. The microorganisms involved in miso fermentation are believed to offer health benefits, further increasing its appeal in the West. Japanese miso exports have surged, with Japan exporting 16.000 tons worldwide in 2017, a significant increase from 2.800 tons in 1990 [Allwood et al., 2021; JETRO, 2020].

The international miso market is expected to grow at a CAGR of 4.7%, reaching an estimated value of USD 107.4 billion by 2032, up from USD 67.8 billion in 2022, according to a report by Future Market Insights. This growth is driven by rising consumer demand for nutrient-dense foods and increasing health consciousness. Miso's high protein, vitamin, and mineral content are key factors propelling the global market. North America leads the global miso market with a share of over 40% and is expected to maintain this dominance, driven by the growing popularity of Japanese cuisine. Other regions, such as Asia-Pacific and Europe, are also experiencing significant growth in miso demand. Europe, in particular, has become an attractive market for Japanese miso producers due to several factors: a growing population of Asian immigrants, increasing health consciousness among consumers, and a developed taste for Japanese cuisine. In fact, the growing popularity of Japanese dishes such as sushi has stimulated interest in authentic Japanese foods, including miso [Future Market Insights (2)].

Natto is a traditional Japanese fermented food made from soybeans, known for its sticky texture and potent aroma. In Japan, it is very popular in daily life and is considered to be a secret recipe for the longevity of the Japanese people [Wang et al., 2023].

The production process begins with selecting and cleaning soybeans to remove impurities. The beans are then soaked in water for about 18 hours to double their weight, ensuring they are evenly moistened. After soaking, the soybeans are steamed in a pressure cooker for an hour to an hour and a half, which preserves their nutrients better than boiling. Once steamed, the beans are sprayed with a pure culture of *Bacillus subtilis* natto, a heat-tolerant bacterium capable of withstanding temperatures between 70°C and 90°C. This bacterium ferments the soybeans in a controlled chamber at approximately 40°C with high humidity for 16 to 24 hours, during which it breaks down proteins, making them easier for the body to assimilate, creates the distinctive viscosity of natto, and generates healthy enzymes and vitamins, contributing to natto rich nutritional profile. The natto is then cooled to stop the fermentation and allowed to mature at 5°C, stabilizing its components and enhancing its umami flavour and characteristic stickiness [Natto Power].

The current outlook for the natto market is positive, with steady growth expected in the coming years. The natto market size is estimated to reach USD 340 million by 2030, growing at a CAGR of 6.4% during the forecast period from 2024 to 2030. Several key drivers are fuelling this growth. Increasing health consciousness and awareness about the benefits of fermented foods are leading consumers to seek out natural and nutritious options. Natto is recognized for its high nutritional content, including protein, fibres, vitamins and minerals (particularly vitamin K2), and its probiotic properties. Moreover, it contains the unique enzyme nattokinase, known for its potential cardiovascular benefits. The rise in environmental awareness is also boosting the popularity of plant-based protein sources like natto, which align with the growing trend of adopting vegan or vegetarian diets. Additionally, the increasing interest in ethnic foods and the influence of Japanese cuisine on global culinary trends are expanding natto's appeal. The global increase in soybean production is another fundamental driver, as soybeans are the primary ingredient in natto. This increase in cultivation makes soybeans more available and affordable, aiding the diversification and expansion of soy-based products into new markets.

Despite these positive trends, the natto market faces challenges. The strong smell and slimy texture of natto may deter some potential consumers, and limited awareness and availability in certain regions could hinder market growth. Overcoming these hurdles will require educational campaigns highlighting natto's nutritional benefits, cultural significance, and versatile uses. Product innovation, such as introducing flavoured or ready-to-eat natto variants, could attract new consumers and broaden the market appeal, helping to mitigate these challenges and support continued market growth [Anywhere Analytics; IndustryARC].

Tempeh, a traditional Indonesian soybean food, is unique among fermented soy products for not originating in China or Japan. It is produced through a series of processes including dehulling, soaking, boiling, and fermentation, making it a rich source of protein and suitable for various cooking methods such as boiling, frying, grilling, or steaming. Historically seen as a protein source for the lower classes, tempeh has now gained widespread acceptance and is readily available in supermarkets and upscale restaurants, particularly as a meat substitute for vegetarians due to its high protein, prebiotics, and an array of vitamins and minerals.

The production of tempeh involves a fermentation process using various strains of mycelium fungus, notably *Rhizopus* spp. (*R. oligosporus, R.oryzae, and R. stolonifera*). The process begins with washing and soaking soybeans overnight, which hydrates and softens them, making dehulling easier. After dehulling, the soybeans are boiled for 30 to 40 minutes to eliminate their raw taste and kill pathogens. The beans are then cooled to 25 to 38°C, inoculated with *Rhizopus* spp., and traditionally wrapped in banana leaves or perforated polyethylene bags to ferment at 27 to 32°C for 30 to 48 hours. This result in a dense, cottony, white mycelium cake that holds the soybeans together (Figure 2.7).

The wrapping material can influence the aroma of tempeh. Moreover, traditional tempeh uses soybeans, but various other substrates like peanuts, chickpeas, horsebeans, and wheat can also be used to produce tempeh, indicating the potential for ongoing innovation and improvement in tempeh production [Teoh et al., 2024; Ahnan-Winarno et al., 2021; Rizzo, 2024].

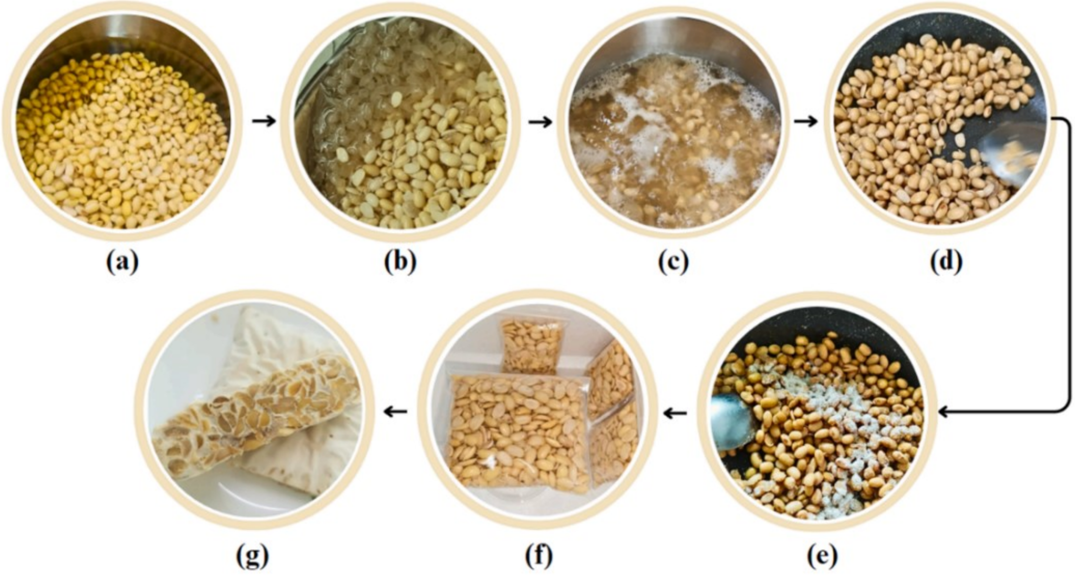


Figure 2.7- Tempeh-making process: (a) soaking, (b) dehulling, (c) boiling, (d) pan-drying, (e) inoculation with starter, (f) fermentation, (g) final product [Teoh et al., 2024]

The global tempeh market is experiencing significant growth, with its size in 2022 being 1753.8 million USD, expected to reach 4808.9 million USD by 2029, growing at a CAGR of 15.50% from 2023 to 2029 [International Research Report].

This growth is driven by the rising demand for plant-based foods due to increasing health awareness, environmental sustainability concerns, and ethical considerations regarding animal welfare. Tempeh, as one of the most commonly consumed plant-based meats, benefits from these trends [Mordor Intelligence (4)].

It is not only nutrient-dense but also offers environmental advantages over animal proteins. Tempeh production results in lower greenhouse gas emissions compared to pork, beef, chicken, fish, eggs, and milk. The most burdensome component on the environmental impact of tempeh appears to be land use and eutrophication derived from soy cultivation. This implies that, to optimize the environmental impact of tempeh production, it would be useful to use autochthonous legumes and grains that are adapted to a specific ecological niche.

The affordability of tempeh also makes it an attractive alternative, often costing less per kilogram than beef and sometimes even less than chicken, eggs, and milk [Rizzo, 2024].

The largest regional consumer of tempeh is China, followed by Indonesia and South Korea. Given this high demand, local soybean production in these regions is insufficient, leading to significant imports, particularly from the United States. In 2021, Indonesia imported more than 60% of its soybeans from the United States, valued at USD 1286.84 million. The market is highly competitive, with many domestic and multinational players. So, leading companies focus on product innovation and expanding their portfolios to cater to various consumer needs, particularly in the snacking category. Mergers, expansions, acquisitions, and partnerships are common strategies used by key players, aiming to enhance market presence and leverage brand value, distribution, and supply chains [Mordor Intelligence (4)].

3. HEALTH BENEFITS OF FERMENTED PRODUCTS

As evidenced by previous data on market analysis, there is a growing popular consensus that consuming fermented foods yields positive health benefits. This is primarily because traditional fermented products utilize unprocessed raw ingredients, contain few or no added preservatives, colours, or flavourings, and are produced using well-established techniques. Furthermore, fermentation process can lead to the generation and/or enrichment of compounds in the raw materials that are associated with beneficial effects on health, as supported by human trials or inferred from in vitro or animal studies.

The mechanisms through which fermented foods and beverages can promote human health include one or a combination of the following: the direct nutritional value of fermented foods (including the presence of compounds produced as a consequence of the fermentation process); the supply of nutrients that support the growth of indigenous gut microbes; and the ability of the microbes in fermented foods to survive gastric transit and to either become a component of the gut microbiome or to inhibit/compete with existing members of the gut microbiome [Leeuwendaal et al., 2022].

3.1 *Nutritional value of fermented foods*

Fermented products are known for their higher digestibility and improved nutrient bioavailability. This is primarily due to microbial and endogenous enzymatic actions within the food substrate during the fermentation process, which are responsible of the hydrolysis of proteins, polysaccharides, and fats. In the case of dairy products, fermentation leads to the destabilization of the casein micelle by bacteria present in milk, thereby enhancing milk protein digestibility. For cereals, which typically have low protein digestibility and suboptimal amino acid profile due to the presence of antinutritive factors, fermentation can significantly improve these aspects. It reduces inhibitors of digestive enzymes (such as trypsin and chymotrypsin), removes compounds that promote crosslinking (such as tannins), and produces microbial proteases that partially degrade and release proteins from the matrix. The benefits of enzymatic activities associated with fermented foods are particularly evident among individuals deficient in the enzyme lactase, which is required to break down lactose into glucose and galactose. These individuals can consume fermented dairy products such as yogurt, kefir, or ripened cheeses with reduced gastrointestinal symptoms related to lactose malabsorption. This is thanks to the action of fermenting microorganisms, such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, which metabolize lactose during fermentation, reducing its level in the final product. Additionally, these microorganisms deliver lactase enzyme that remain in the food matrix. Fermented foods can also be used to alter the composition and metabolism of the colonic microbiota, with the purpose of improving lactose digestion.

Fermentation not only enhances the digestibility and nutrient bioavailability of foods but also leads to the biosynthesis and consequent increase in concentrations of various vitamins and antioxidants, primarily due to the action of LAB. Fermented dairy and cereal products, for instance, have been reported to contain high levels of folate, riboflavin, cobalamin (B-group vitamins), and vitamin K, all of which play crucial roles in gut health. Vitamin K, in addition, has been associated with the suppression of colonic tumour development, improvement of intestinal integrity, and inhibition of gut pathogens. Moreover, fermentation increases the bioavailability of minerals such as zinc, calcium, iron and magnesium by breaking down complex structures formed when minerals bind to non-digestible matter.

Additionally, fermentation results in the production of exopolysaccharides (EPS), long sugar polymers, from simple sugars present in the raw food. These EPS have gained attention due to their potential health benefits. Since they are resistant to digestion in the upper gastrointestinal tract, they are fermented by intestinal microorganisms to produce short-chain fatty acids (SCFAs). EPS also play a role in immunomodulation by regulating cytokine production by immune cells, inhibiting pathogens or pathogen adhesion to the intestinal epithelium, suppressing inflammation, promoting intestinal barrier integrity, acting as antioxidants, and facilitating weight management. Examples of these EPS include acetan, xanthan and kefiran [Leeuwendaal et al., 2022; Mukherjee et al., 2024].

3.2 *Fermented foods and gut microbiome*

Humankind has co-evolved with microorganisms, particularly beneficial ones, and that microbial exposure has been crucial in training the immune system to respond appropriately to external challenges. Indeed, in the industrialized world, a lack of exposure to microorganisms is associated with the increased prevalence of allergic and autoimmune diseases. To address this issue, the consumption of fermented foods has been suggested as a safe way to increase microbial exposure in the modern era.

There are distinctions to be made among different types of fermented products. It is important to note that only live microorganisms have the potential to confer beneficial effects on gut health. Therefore, fermented products should not undergo pasteurization or filtration. Additionally, foods produced via spontaneous fermentation typically contain a higher number and diversity of microorganisms compared to those produced from starter cultures. Thus, it is desirable to preserve microbial communities of artisanal and traditional fermented food to retain their unique organoleptic profiles and potential health benefits, including positive modulation of the gut microbiota and interactions with the host immune system [Mukherjee et al., 2024].

A study conducted in 2021 investigated the consequences of consuming a diet rich in fermented foods on 18 healthy adults over a 17-week period. During the study, stool and blood samples were collected at various intervals, allowing researchers to assess changes in microbiota composition, function, and metabolic output and to generate a system-level view of the immune system.

The results showed significant decreases in inflammatory markers and increases in microbiota diversity from baseline (three-week pre-intervention time period) to the end of the intervention in participants consuming a fermented food-rich diet. Surprisingly, during the "choice" period (four weeks after the intervention period in which participants could maintain their diet to the desired extent), the alpha diversity in the microbiota of participants remained sustained. This suggests that the increase in microbiota diversity was not primarily due to consumed microbes but rather a result of gut ecosystem remodelling, including shifts or new acquisitions in the resident community (Figure 3.1).

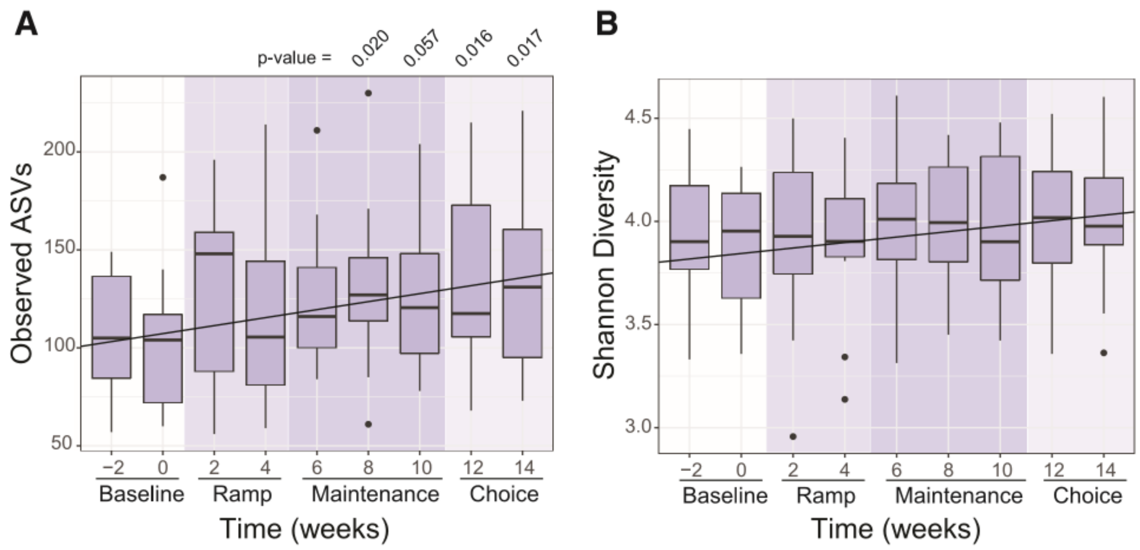


Figure 3.1- Alpha diversity is the diversity of microbial species within a given sample. In A) and B) it is described by observed Amplicon Sequence Variants (ASVs) and Shannon index respectively. They both increase over the course of the intervention, from the baseline to the choice period, suggesting that fermented food-rich diet increases microbiota diversity. The fact that this increase is sustained also during the choice period, when fermented food intake is higher than baseline but lower than at the end of maintenance, suggests that it is a result of a gut ecosystem remodelling [Wastyk et al., 2021]

Moreover, the consumption of a diet rich in fermented foods was found to lead to a decrease in markers of host inflammation, such as the cytokines IL-6, IL-10, IL-12 and other inflammatory factors, that have been linked to chronic, low-grade inflammation and observed in conditions such as rheumatoid arthritis, type-2 diabetes, and chronic stress (Figure 3.2) [Wastyk et al., 2021].

In another epidemiological study including 46,091 American adults, increased consumption of dietary microorganisms from a variety of foods, including fermented foods, was linked to modest positive health outcomes. These outcomes included improved markers for cardiovascular health and inflammation, as well as reduced BMI, plasma glucose levels and insulin levels [Hill et al., 2023].

This is possible because certain microorganisms present in fermented foods can survive gut transit and, when metabolically active in the gut, can become part of the gut microbiome or can be involved in cross-feeding and competition for nutrients with resident microorganisms. In the following paragraphs health benefits of the emergent fermented foods and beverages will be explored.

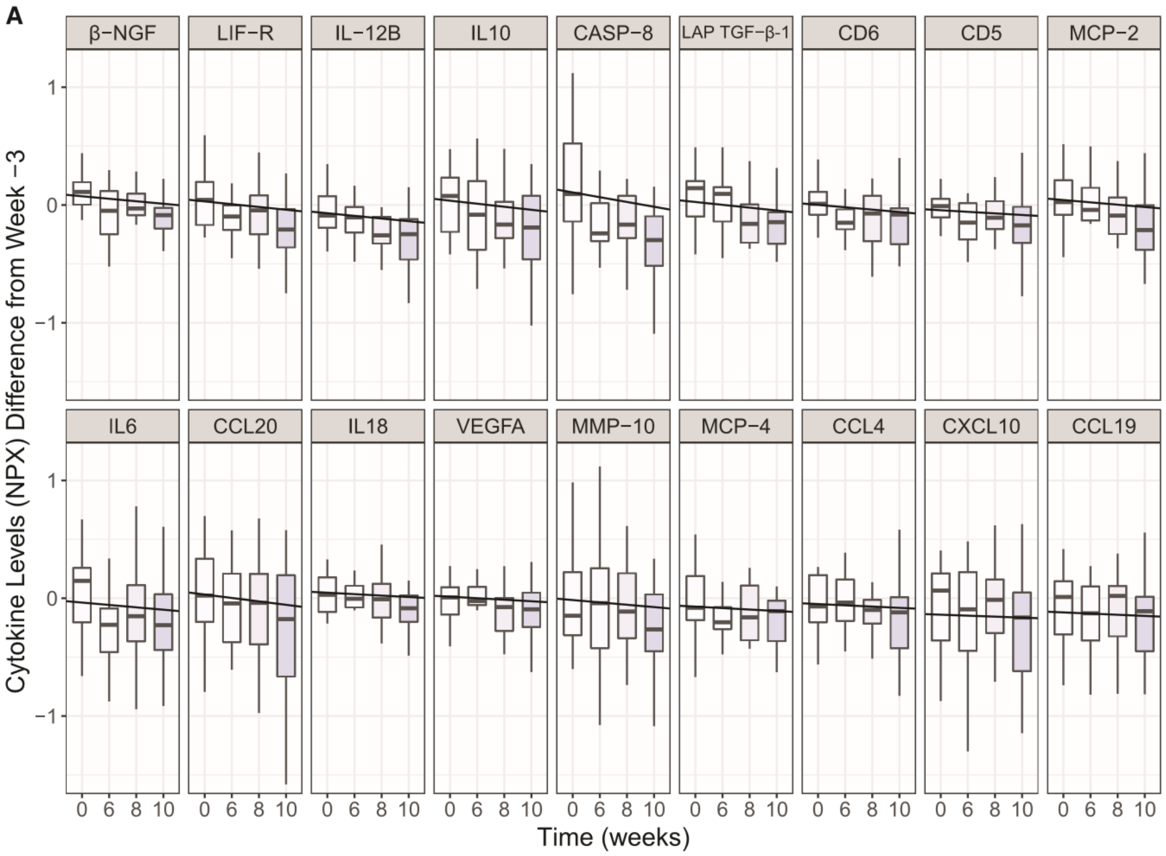


Figure 3.2- Circulating levels of 18 cytokines, chemokines, and other inflammatory serum proteins, have been found to decrease significantly over the fermented food intervention, from baseline to the end. In particular interleukin-6 (IL-6), commonly used metric of inflammation, interleukin-12β (IL-12β), and interleukin-10 (IL-10) [Wastyk et al., 2021]

3.3 *Kombucha*

The fermented kombucha beverage has been extensively studied for its wide array of health benefits, including antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, anticancer, antimutagenic and antidiabetic activities. These benefits are attributed to the numerous beneficial compounds present in kombucha, derived from both the raw material (green or black tea) and the microorganisms and materials used during fermentation, such as the SCOBY (Symbiotic Culture of Bacteria and Yeast). Among the most significant compounds found in kombucha there are organic acids like acetic acid and glucuronic acid, which exhibit antimicrobial activity and possess detoxifying properties. Additionally, D-saccharic acid-1,4-lactone (DSL) present in kombucha has been identified for its antioxidant and detoxifying effects. Kombucha also contains essential vitamins (B1, B2, B6, B12, C), minerals, and polyphenols.

However, the presence and quantity of these chemical components can vary depending on several factors, including the specific microorganisms present in the symbiotic culture used for fermentation, fermentation time and temperature, sucrose content and the type of tea used as the base ingredient [Leal et al., 2018].

The elderly population is increasing worldwide, accompanied by a rise in chronic diseases such as metabolic disorders, cardiovascular diseases, neurodegenerative diseases, musculoskeletal diseases, and cancer. These conditions are often linked to physiological function impairments and homeostatic imbalances, leading to oxidative stress.
Excessive accumulation of free radicals, including reactive nitrogen species (RNS) and reactive oxygen species (ROS), generated during the physiological modulation of different organs and cellular activities, can cause oxidative damages to proteins, lipids, DNA, cells and tissues resulting in cellular dysfunction and cell death. While the body has endogenous antioxidant defence mechanisms, also natural sources of exogenous antioxidants found in medicinal plants, vegetables, fruits, cereals, and spices can play a crucial role in combating oxidative stress.

Tea, known for its rich polyphenol content, especially catechins in green tea and theaflavins and thearubigin in black tea, imparts antioxidant properties to kombucha. Fermentation further enhances the antioxidant potential of the beverage due to the interdependent relations between the microbes and compounds within the beverage.
In kombucha, AAB and yeasts release enzymes that catalyse the conversion of tea polyphenols into smaller antioxidant molecules, such as phenolic acids, phenolics, and flavonoids. The antioxidant effects of these polyphenols can be further enhanced when they conjugate with gluconic acid (GlcUA), an organic acid produced by the SCOBY, thereby increasing the bioavailability and transport of polyphenols.
Adequate antioxidants in the body can prevent cardiovascular diseases (CVDs) by inhibiting the oxidation of low-density lipoprotein (LDL), regulating cholesterol metabolism, and promoting smooth muscle relaxation, thereby lowering blood pressure. The antioxidants present in kombucha protect endothelial cells by reducing oxidative stress, thus lowering the risks of atherosclerosis and heart attacks. Catechins in green tea, as well as theaflavins and thearubigins in black tea, act as potent antioxidants and help protect against the development of various diseases, including certain types of cancers,

cardiovascular diseases, and hypertension. Additionally, DSL (D-saccharic acid lactone), a main functional component of kombucha, contributes to its hypocholesterolaemic effect [Leal et al., 2018; Chong et al., 2023; Yang et al., 2009].

In animal models, traditional black tea kombucha has demonstrated its ability to inhibit ROS production and lipid peroxidation, while also enhancing the activities of antioxidant enzymes in alloxan-induced diabetic rats. The study by S. Bhattacharya documented an increase in free-radicals in ALX-induced diabetic rats, leading to excessive lipid peroxidation and impaired protein synthesis, ultimately resulting in tissue damage at the level of pancreas, liver, heart and kidney. Overall, both kombucha tea and black tea effectively mitigated oxidative stress-mediated tissue damage in ALX-induced diabetic rats. However, kombucha tea was found to be more effective than black tea (Figure 3.3), and in some cases, its efficacy was comparable to glibenclamide, a well-known antidiabetic drug that reduces oxidative stress in diabetic organ pathophysiology by increasing the body's antioxidant status.

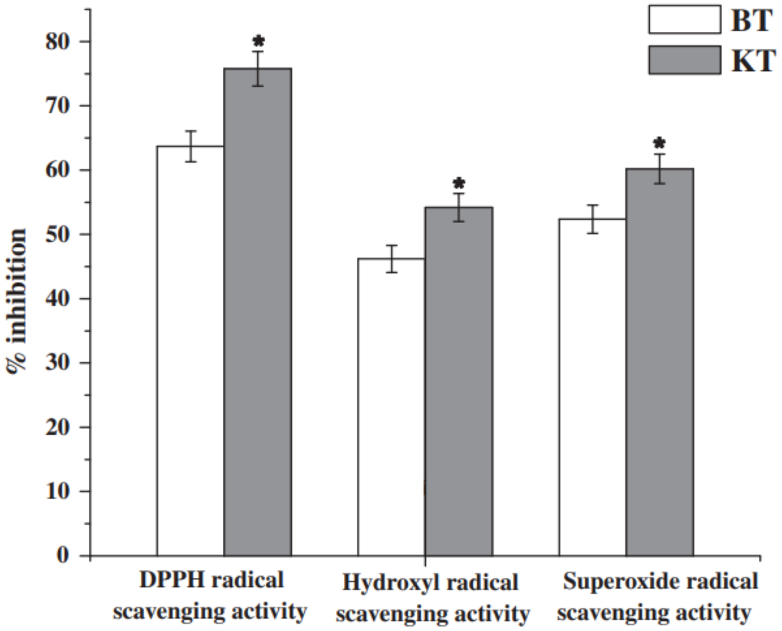


Figure 3.3- Free radical scavenging activities of kombucha tea (KT) and black tea (BT). DPPH = 2,2-Diphenyl-1-picrylhydrazyl. "" indicates significant differences between BT and KT [Bhattacharya et al., 2013].*

The superior efficacy of kombucha tea over black tea may be attributed to the formation of antioxidant compounds during the fermentation period, such as D-saccharic acid lactone (DSL), which possesses protective effects against oxidative/nitrative modifications of plasma proteins and blood platelets. When combined with phenolic compounds, DSL augments its antioxidative property. In addition, organic acids like gluconic acid and glucuronic acid present in kombucha tea act as detoxifying agents by facilitating the detoxification process through the conjugation with toxins, which are then solubilized and eliminated from the body [Bhattacharya et al., 2013].

Due to its antioxidant properties, daily consumption of kombucha is associated with protection against cancer. Kombucha tea contains polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins and DSL, all of which possess anticancer properties. Specifically, these components are responsible for antiproliferative activity [Deghrigue et al., 2013].
In a study conducted by Srihari et al. (2013) on a human prostate cancer cell line (PC-3), kombucha was found to effectively reduce cell viability, migration and activities of angiogenic-stimulating molecules such as HIF-1α, IL-8, VEGF, COX-2, MMP-2 and MMP-9 in a dose dependent manner for tumour cells, reducing the potential for metastasis [Srihari et al., 2013].

In addition to its antioxidative properties, polyphenols naturally present in kombucha tea may help ameliorate inflammatory reactions and support immune function by modulating inflammatory molecules and oxidative stress. Kombucha beverages have been found to prevent and attenuate the severity of allergies, infections, and various inflammatory disorders. Apart from their immunosuppressive effects during inflammation, the beneficial components in kombucha also modulate the immune system by boosting immunity. Vitamin C and B2, both strong antioxidants found in kombucha, play a crucial role in aiding and supporting the immune system.

Furthermore, kombucha exhibits significant antimicrobial effects. During the fermentation process, the microbiota in kombucha releases organic acids, especially acetic acid, which play a crucial role in the antimicrobial activity of the final product. Both black tea and green tea kombucha have been found to exhibit antimicrobial activity against a wide range of pathogens, including Gram-positive bacteria (e.g. *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus cereus*, *Enterococcus faecalis, Listeria* *monocytogenes*, and *Micrococcus luteus)* and Gram-negative bacteria (e.g. *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Shigella sonnei*, *Shigella dysenteriae*, *Salmonella enteritidis*, *Salmonella enterica*, *Salmonella typhimurium*, *Salmonella typhi*, *Aeromonas hydrophila*, *Yersinia enterolitica*, *Campylobacter jejuni, Klebsiella pneumoniae, Haemophilus influenzae*, *Helicobacter pylori*, and *Vibrio cholerae*), as well as antifungal properties against Candida yeasts (*C. albicans, C. krusei, C. glabrata, C. tropicalis*) and other pathogenic fungi (*Aspergillus flavus*, *Aspergillus niger*, and *Microsporum gypseum*) [Deghrigue et al., 2013].

The antimicrobial activity of acetic acid is attributed to its ability to lower the pH value through proton release, creating an environment unfavourable for pathogenic bacteria by altering cell membrane permeability, disturbing cell membrane function and enzymatic activity. Some studies have reported the inhibitory effect against pathogenic microorganisms of neutralized kombucha while it was not observed in most of the unfermented samples. These results suggested that apart from organic acids, other bioactive compounds in kombucha such as alkaloids, flavonoids, tannins, proteins, and bacteriocins generated during fermentation may be responsible for antimicrobial effects. Studies have shown that the antimicrobial capabilities of kombucha increase with fermentation time, due to the increase in the production of acetic acid, other organic acids, and other metabolites, as well as the low pH value or acidic property during the fermentation process. Moreover, some differences can be attributed to the raw material: it has been observed stronger antimicrobial capabilities of green tea kombucha compared to black tea kombucha. These differences were explained by the higher antibacterial catechins and acidity, plus the sole presence of antibacterial verbascoside in green tea. In summary, the antimicrobial properties of kombucha make it a potential candidate for reducing or preventing microbial infections within the body. Kombucha can be used also as natural preservative in food products, offering a safer alternative to synthetic preservatives that may pose health risks.

Kombucha tea has been also studied for its potential antidiabetic activity in several studies using diabetic animal models. Diabetes mellitus (DM) is one of the major epidemic health issues in the 21st century, characterized by hyperglycaemia (an increase in plasma glucose level) due to deficiency in insulin action or secretion. Hyperglycaemia consequently causes macrovascular complications including cardiovascular diseases (CVD) and microvascular complications. Although several groups of synthetic drugs have been developed for type 2 diabetes mellitus (T2DM), they have limitations and side effects. So, natural agents are now garnering more attention as alternatives for the management of DM.

Different studies have evaluated the effect of kombucha consumption on diabetic mice, showing a decrease in fasting blood glucose and food intake, with a significant recover in body weight (the yellow line in the Figure 3.4, KT) compared to diabetic mice not treated (the green line in the Figure 3.4, DC). These results indicate that kombucha can effectively improve the hyperglycaemic characteristics of diabetic mice, approaching the characteristics of healthy mice (the orange line in the Figure 3.4, control group NC) [Xu, 2022].

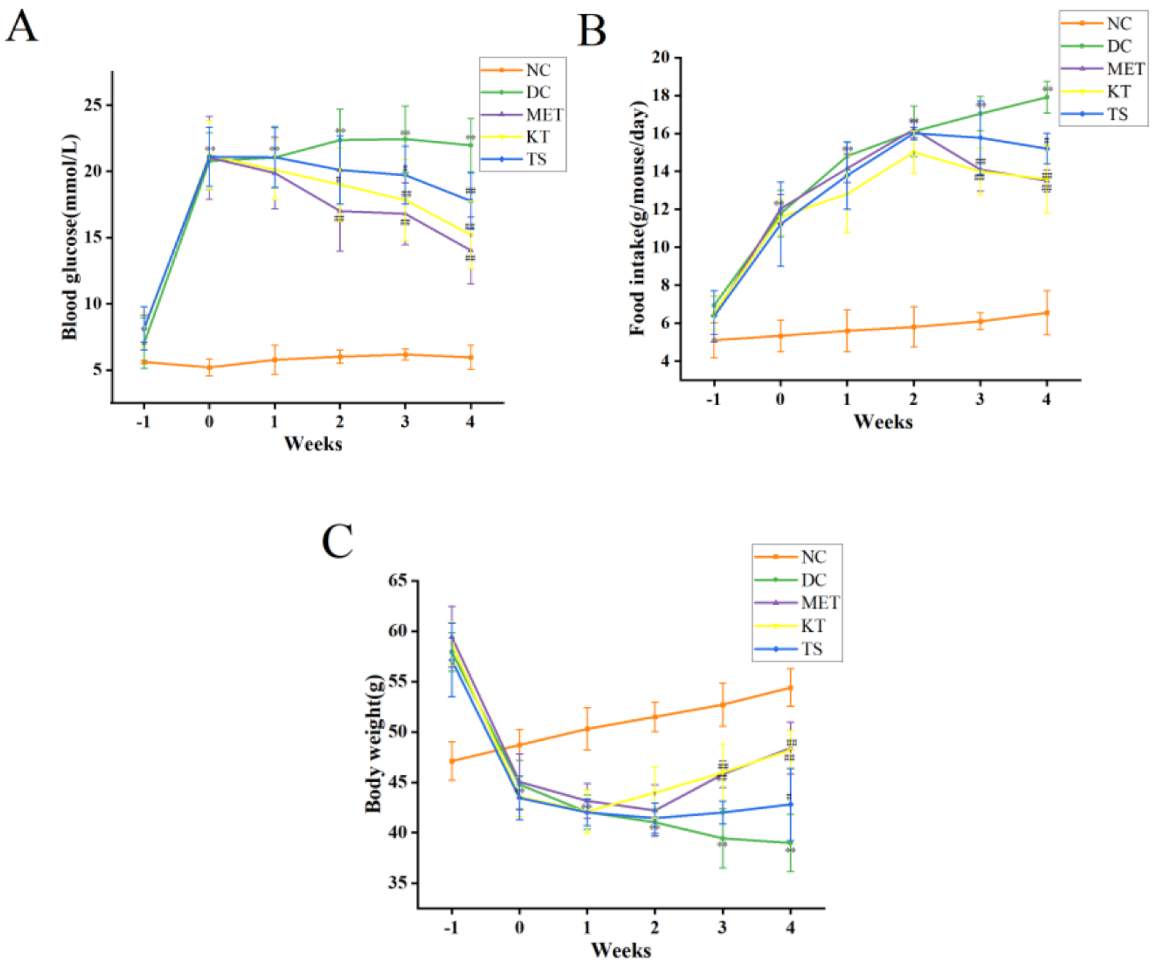


Figure 3.4- Effects of kombucha on fasting blood glucose (A), food intake (B) and body weight (C) in T2DM mice. Mice are feed with HFHSD and intraperitoneally injected with STZ (streptozotocin) to induce T2DM. NC = control group, mice following normal diet. DC = diabetic mice following high-fat high-sugar diet (HFHSD). MET = positive medicinal control group, mice following HFHSD and administered with metformin. KT = mice following HFHSD and administered with kombucha tea. TS = mice following HFHSD and administered with tea soup. [Xu et al., 2022]

This improvement is attributed to the polyphenols and organic acid active substances present in this beverage, that together with DSL, are able to alter enzyme activity in glucose regulatory pathways, such as glycolysis and gluconeogenesis. Plasma and pancreas α-amylase activities are reduced in the digestive tract of rats treated with kombucha, delaying starch hydrolysis, and reducing the spike in postprandial glucose levels. Furthermore, kombucha restored the activities of the enzymes crucial for glycolysis and gluconeogenesis, including glucose-6-phosphatase, fructose-1,6-bisphosphatase, and hexokinase, to a level close to normal in diabetic rats [Anantachoke et al., 2023].

Moreover, it has been observed that kombucha also protects tissues from injury in T2DM mice, particularly at the level of the liver and pancreatic islets, resulting in an improvement in the function of these tissues.

Recent years have seen numerous studies confirming the close relationship between gut microbiota and T2DM. Altering the gut microbiota could potentially alleviate the symptoms of this disease. Kombucha has been found to modulate the composition of gut microbiota in diabetic mice by increasing *Firmicutes*, which are usually considered anti-inflammatory due to their production of SCFA metabolites, and by reducing *Proteobacteria*, which often include various pathogens, and are diagnostic markers for gut ecological disorders and disease risks. This effect reduces the lipopolysaccharide (LPS) content in the blood, thereby alleviating inflammation and insulin resistance. Specifically, kombucha boosts the population of SCFAs-producing bacteria, which improve β-cell function in T2DM (Figure 3.5) [Xu, 2022].

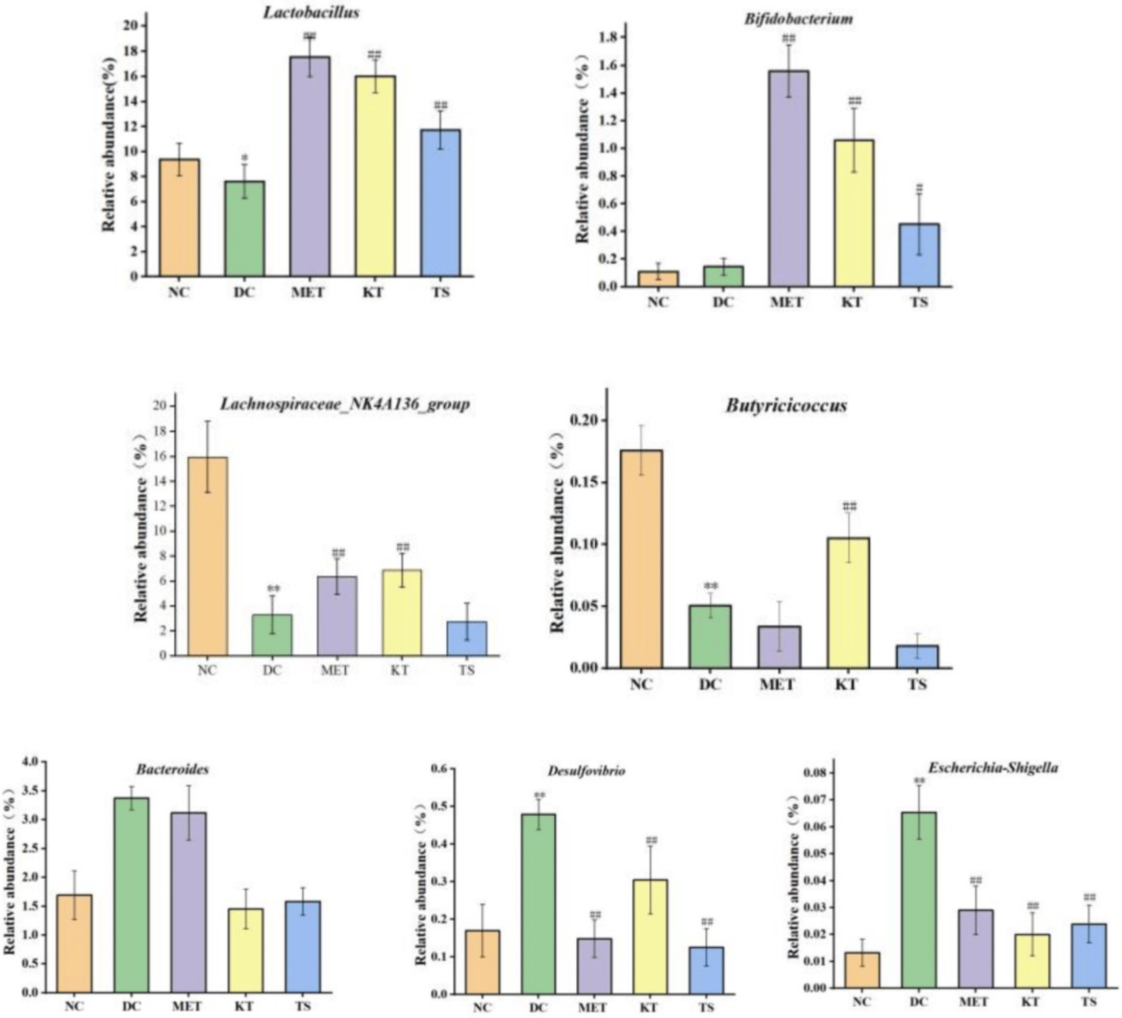


Figure 3.5- Relative abundance of SCFAs-producing bacteria (Lactobacillus, Bifidobacterium, Lachnospiraceae_NK4A136_group, and Butyricicoccus) and LPS-producing GRAM-negative proinflammatory bacteria (Bacteroides, Desulfovibrio, and Escherichia-Shigella).
NC = control group, mice following normal diet. DC = diabetic mice following high-fat high-sugar diet (HFHSD). MET = positive medicinal control group, mice following HFHSD and administered with metformin. KT = mice following HFHSD and administered with kombucha tea. TS = mice following HFHSD and administered with tea soup [Xu et al., 2022]

3.4 *Kefir*

Milk kefir, like other fermented dairy products, has been associated with a variety of health benefits, including cholesterol metabolism, angiotensin-converting enzyme (ACE) inhibition, antimicrobial activity, tumour suppression, and modulation of the immune system, which includes alleviating allergy and asthma symptoms.

Kefir is a highly nutritious dairy product, rich in minerals, sugars, carbohydrates, proteins, peptides, vitamins, and fats. The fermentation process further enhances its nutritional value by introducing secondary metabolites such as catechin, vanillin, ferulic acid, and salicylic acid. This fermented beverage is enriched in vitamins (B1, B2, B5 and C), macro-elements in high concentration (calcium, magnesium, potassium, and sodium for cell growth, maintenance, and energy), micro-elements in low concentration (iron, zinc and copper for cellular metabolism and blood production) as well as essential amino acids (serine, threonine, alanine, lysine, valine, isoleucine, methionine, phenylalanine, tryptophan) [Farag et al., 2020].

While commercial, industrial-scale production of kefir often involves the use of selected starter cultures of microbes isolated from kefir or kefir grains to ensure product consistency, traditional production method use kefir grains for fermentation. The studies examined in this review focus on traditionally produced kefir. Although industrially produced kefir may offer health benefits of its own, research examining these benefits and the difference between artisanal and industrial production methods remain controversial [Bourrie et al., 2016].

Milk kefir is rich in antioxidant molecules such as exopolysaccharides (including kefiran) and phenolic compounds and contain microorganisms with antioxidant properties. These bioactive compounds contribute to kefir’s antioxidant activity, protecting cells from oxidative stress and preventing cardiovascular diseases, which are mainly associated with hypertension and hypercholesterolemia. Consumption of kefir is associated with a reduction in blood pressure, a decrease in adipocyte fat, and a decrease in cholesterol level. Kefir peptides and EPS have been shown to reduce blood pressure by inhibiting the actions of angiotensin-converting enzyme (ACE), a key component of the renin-angiotensin system involved in regulating blood pressure [Vieira et al., 2021].

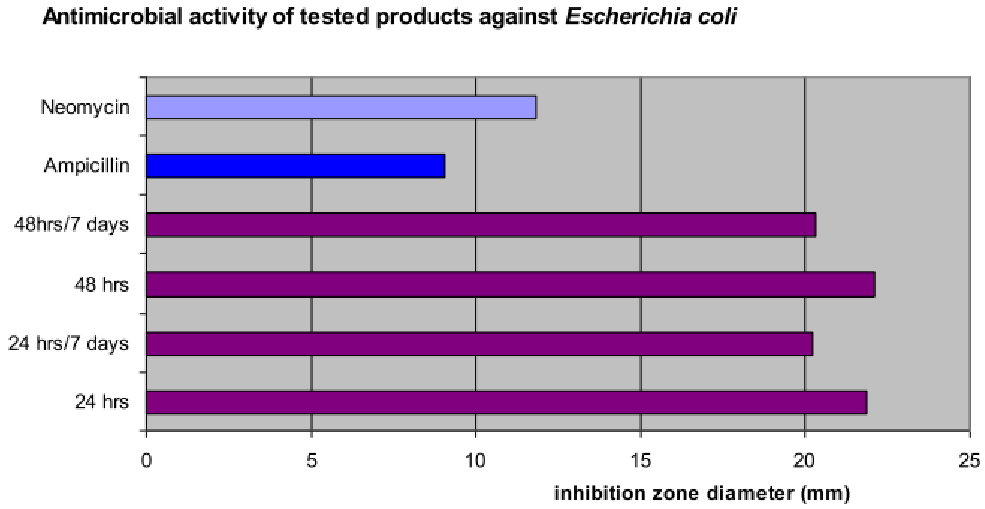
ACE causes hypertension by breaking down bradykinin, which encourages vasodilation, and catalysing the formation of the angiotensin II hormone, leading to vasoconstriction and rising blood pressure. Rats fed with kefiran produced by *Lactobacillus kefiranofaciens* had notably reduced ACE activities in the serum to 19.8 units/L and thoracic aorta to 19.9 milliunits/mg of protein as compared with the control at 21.7 units/L and 23.2 milliunits/mg of protein, respectively. Moreover, in the same study, spontaneously hypertensive and stroke prone rats fed with high fat diet and supplemented with kefiran showed a significant reduction in serum total cholesterol, LDL-cholesterol and triglycerides, and liver cholesterol and triglycerides [Maeda et al., 2004].

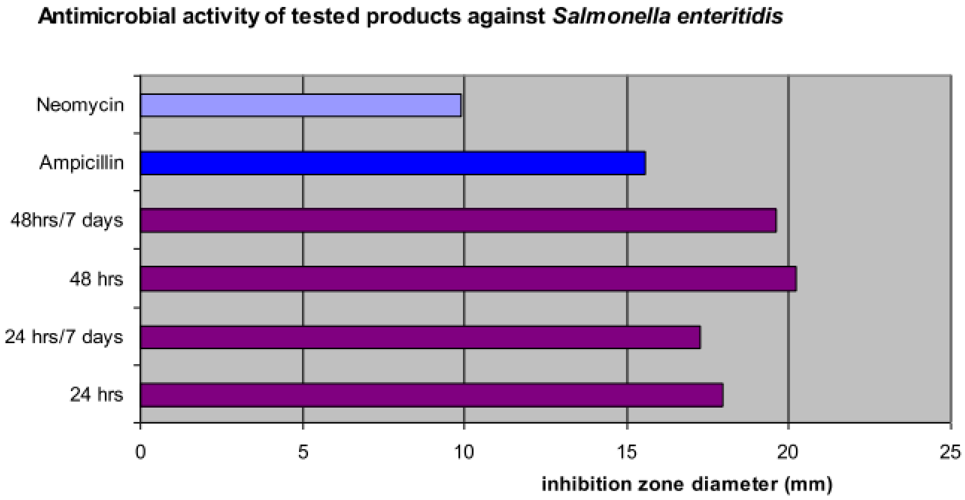
The cholesterol-lowering effect of kefir have been attributed also to the action of cholesterol-assimilating LAB present in kefir. These bacteria act through various mechanisms, including the prevention of cholesterol absorption, the inhibition of cholesterol-synthesizing HMG-CoA reductase, and the deconjugation of bile acids [Chong et al., 2023].

Milk kefir, considered a probiotic-containing food, exerts beneficial effects on the intestinal microbiome and, consequently, on the immune system together with the EPS kefiran which act as a postbiotic. Modulation of intestinal microbiota can occur through the introduction of new species or strains into the gastrointestinal tract, or by promoting the growth of beneficial microbes already present. Consumption of kefir in animal models has been associated with an increase in beneficial microbes such as *Lactobacillus* and *Bifidobacterium*, while simultaneously inhibiting the proliferation of pathogenic bacteria. This has the consequence of maintaining the integrity of the intestinal barrier, reducing exposure to the allergen, and sensitization to antigens. Indeed, the imbalance among resident bacterial population, known as intestinal dysbiosis, can potentiate food allergy through immune dysregulation. Therefore, through the modulation of microbiome, kefir can regulate the innate immune response, and may play a role in treating allergic symptoms and preventing the development of allergies. Studies show that the bacterial strains in kefir can modulate the Th1/Th2 equilibrium through the production of cytokines such as IL-12 and IFN-γ. In a study involving 18 health individuals who consumed kefir for 6 weeks, an increase in the Th1-type immune profile, a reduction in the Th2-type response and, consequently, a reduction in the allergic response were observed. These authors also reported that kefir promotes a reduction in the expression of the cytokine IL-8, which can lead to neutrophil chemotaxis, in addition to increasing the expression of IL-5 and the secretion of IgA in the gastric and intestinal mucosa. Moreover, kefir has demonstrated an antiasthmatic effect by inhibiting eosinophilia in lung tissue and mucosal hypersecretion by goblet cells, and by blocking the NF-kB signalling pathway to reduce the synthesis of pro-inflammatory cytokines in the lung tissue [Barros et al., 2021].

Similar results have been obtained when evaluating the anti-inflammatory action of kefir in healing situations or in the case of viral infections. Kefir has been shown to reduce the expression of proinflammatory cytokines such as IL-6, IL-1, TNF-α, and interferon-γ, suggesting its potential as a protective agent also against viral infections. Its antiviral mechanisms involve enhancing macrophage production, increasing phagocytosis, and boosting the production of various immune cells (CD4+, CD8+, IgG+ and IgA+ B cells, T cells, and neutrophils) as well as cytokines like interleukin IL-2, IL-12, and interferon gamma-γ. Furthermore, kefir derivatives such as polysaccharides, protein, and peptides, can suppress viral activity by disrupting viral adhesion. Activity of kefir has been reported against the Zica virus, HCV, hepatitis-B virus, influenza virus (H1N1), HSV, rhinoviruses and retroviruses [Hamida et al., 2021].

Kefir has also demonstrated numerous antibacterial and antifungal activities, attributed to EPS, but also to organic acids and bacteriocins produced by kefir microorganisms. In a study conducted by Chifiriuc et al., the antimicrobial activity of kefir was tested using the *in vitro* disk diffusion method. The intensity of the antimicrobial activity was interpreted by comparison with two antibiotics (ampicillin and neomycin). The antimicrobial activity was observed against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli, Enterococcus faecalis* and *Salmonella enteritidis*, and it was found to be superior to that of tested antibiotics (Figure 3.6A and 3.6B), suggesting that kefir exhibits a broad spectrum and strong antibacterial activity [Chifiriuc et al., 2011].





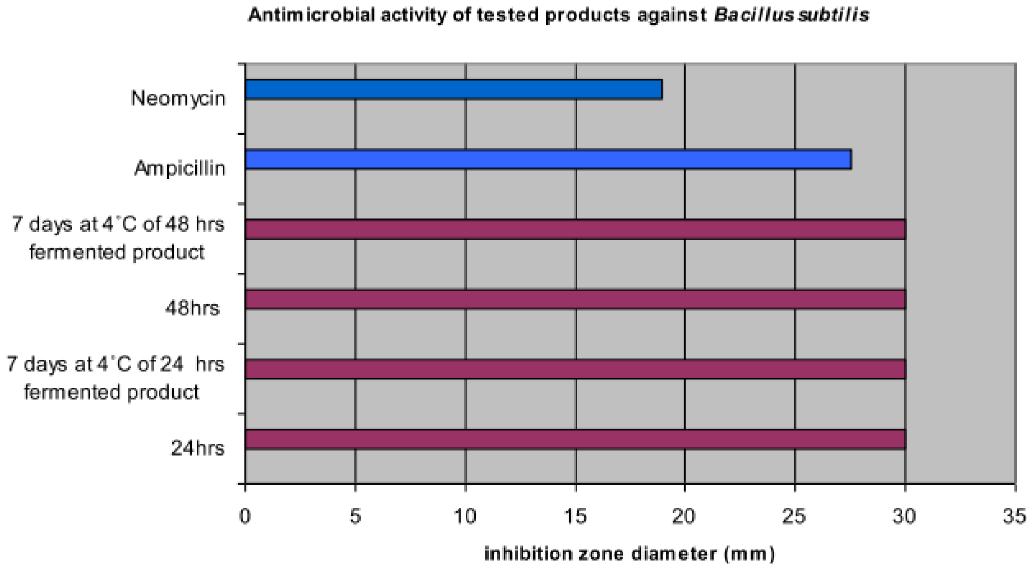
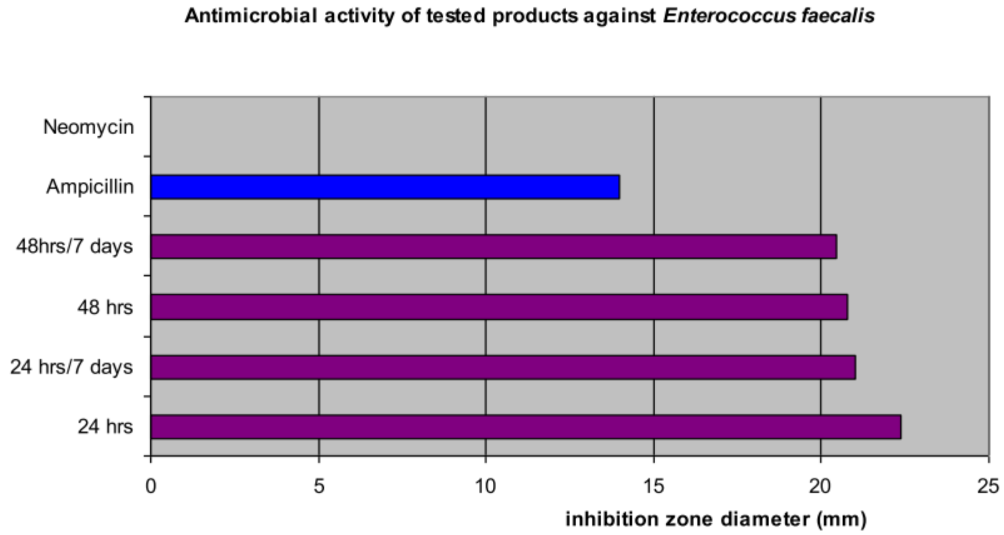


Figure 3.6A - Antimicrobial activity of 24h as well as 48h fermented kefir, fresh or after 7 days preservation at 4-8°C, tested using in vitro disk diffusion method and evaluating inhibition zone diameter. For E.coli, S.enteritidis, and B.subtilis the antimicrobial activity is superior to both control antibiotics (neomycin and ampicillin) [Chifiriuc et al., 2011]



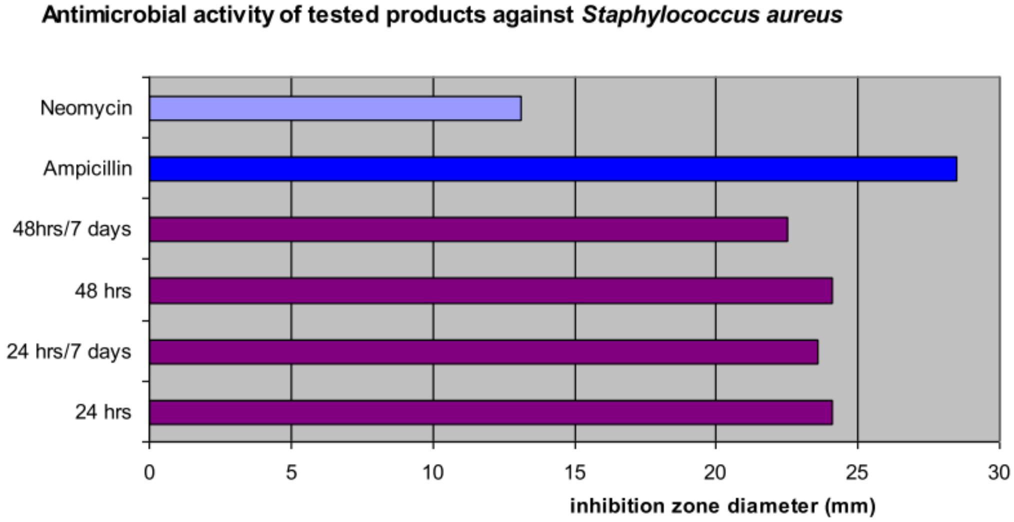


Figure 3.6B - Antimicrobial activity of 24h as well as 48h fermented kefir, fresh or after 7 days preservation at 4-8°C, tested using in vitro disk diffusion method and evaluating inhibition zone diameter. For E.faecalis and S.aureus the antimicrobial activity is superior to one antibiotic (neomycin or ampicillin) [Chifiriuc et al., 2011]

Finally, kefir can interact with several cellular pathways and regulate biological processes exhibiting anti-carcinogenic properties. It induces apoptosis and has an anti-proliferative effect in cancerous cells. Additionally, it is able of controlling oxidative stress, reducing DNA damage and mutagenicity. The role of kefir in anti-tumour process has been investigated revealing promising results for different cancers such as colorectal cancer, malignant T lymphocytes, breast cancer, and lung carcinoma.

In some studies, conducted by de LeBlanc et al. (2006), utilizing a murine breast cancer model it has been found that feeding kefir to mice before tumour challenge, led to a decrease in tumour size and increase in tumour apoptosis. Additionally, the levels of IgA+ cells and CD4+ T cells, serum IL-10 and IL-4 were elevated in these mice. These demonstrated an increase in immune cell populations and recruitment, suggesting a potential mechanism for the reduction in tumour size [Sharifi et al., 2017].

In addition to milk kefir, there is also water kefir. Water kefir grains differ significantly from milk kefir grains; they have a much lower protein content and microbial content, resulting in different nutritional properties compared to milk kefir [Gökırmaklı and Seydim, 2022]. Scientific research on the health benefits of water kefir has seen considerable growth in recent years. These positive health effects are attributed to a combination of live microorganisms present in the fermented beverage and bioactive components released in during the fermentation process. Among the beneficial health attributes of water kefir, its antioxidant activity (attributed to LAB, AAB, yeasts and their metabolites), antihyperglycemic, and antimicrobial activity are noteworthy [Pendòn et al., 2022].

3.5 *Kimchi*

Kimchi, a traditional Korean fermented vegetable dish, is rich in diverse nutrients and bioactive compounds that offer numerous health benefits. Research indicates its potential in combating various diseases such as cancer, oxidative stress, diabetes and obesity. These benefits are attributed to the diverse ingredients of kimchi, whose bioactivities are magnified during the fermentation process.

Fermented kimchi is particularly abundant in LAB, enhancing its probiotic properties. With only 18 kcal per 100g of product, kimchi contains high levels of vitamins (C, B complex, β-carotene), minerals (Na, Ca, K, Fe, P), dietary fibres and other phytochemicals. Incorporating kimchi, mainly prepared with cancer-preventing cruciferous vegetables, into the diet can booster immunity, combat aging, and prevent constipation.

Key components such as garlic, gingerol from ginger, capsaicin from red pepper powder, and other functional components in kimchi like benzyl isothiocyanate, indole compounds, thiocyanate, and β-sitosterol are associated with anticancer, antioxidative and anti-obesity effects. The fermentation process not only enhances the taste, but also augments the functional qualities of this food, making it a valuable addition to a healthy lifestyle.

For instance, studies have shown that kimchi can help maintain normal body weight in rats. Capsaicin, found in red pepper, can increase metabolism and energy expenditure. Rats fed a diet containing red pepper powder and high fat content showed significantly decreased body weight compared to rats fed only a high-fat diet. However, kimchi showed a better body weight-lowering effect than red pepper powder alone [Park et al., 2014].

The anti-obesity effect of kimchi has also been demonstrated in humans. In a study conducted by Kim et al. (2011), overweight and obese subjects who consumed 300 g/day of fresh or fermented kimchi for 4 weeks, experienced reductions in weight, BMI, and body fat resulting from the beneficial effects of kimchi ingredients (napa cabbage, hot red pepper, garlic, green leek, and ginger that contain dietary fibres, vitamins, calcium, capsaicin and niacin). The fermented kimchi group showed additional benefits on waist-hip ratio, systolic and diastolic blood pressure, blood glucose, cholesterol levels, fasting insulin levels, and leptin compared to the fresh kimchi group. These results indicate that the process of fermentation could affect the favourable effects of kimchi in the case of metabolic syndromes, in particular obesity, indirectly decreasing the risk of CVD too [Kim et al., 2011].

To understand the molecular mechanisms underlying the impact on metabolic parameters and the anti-obesity effect of kimchi, Han et al. (2015) conducted a study on 24 obese women. These women were assigned to either a fresh or fermented kimchi group (180 g/day) for eight weeks of intervention, after which blood gene expression and gut microbial populations were evaluated. The study revealed significant changes in gut microbiota and blood gene expression profiles in response to kimchi intervention.

Consumption of fermented kimchi resulted in changes in the gut microbiota composition, including a decrease in the *Firmicutes/Bacteroidetes* ratio, shown to be related to weight loss, and an increase in *Proteobacteria* and *Actinobacteria*, showing negative correlation with body fat percentage. Additionally, the relative abundance of *Bacteroides* and *Prevotella* increased, while that of *Blautia* decreased after the intake of fermented kimchi. These changes were associated with improvements in obesity-related clinical parameters, suggesting that specific strains of gut microbiota could be linked with obesity and metabolic disorders.

Furthermore, the impact of kimchi intake on the gene expression profile of blood was assessed using microarray analysis. It was observed that consumption of fermented kimchi led to changes in the expression of genes with a wide range of functionalities, including those related to metabolism, immunity, blood circulation, and digestion. One of the significantly up-regulated genes in the fermented kimchi group was ACSL1, which play a vital role in the metabolic pathway. ACSL1 showed a significant negative correlation with systolic and diastolic blood pressure, possibly due to its involvement in lipid metabolism prevention and promotion of fatty acid degradation. Other significantly up-regulated genes in the fermented kimchi group also showed a negative correlation with systolic and diastolic blood pressure. Among these genes, ANPEP is known to play a significant role in the regulation of pain, angiogenesis, inflammation, and apoptosis [Han et al., 2015].

So, to prevent or improve metabolic syndromes, consumption of dietary fibre and probiotic is recommended. Obesity is the most well-known metabolic disorder, and it is related with other diseases like CVD, T2D, dyslipidaemia, and hypertension that are becoming important health problems worldwide. All of them are related to dietary habits and human gut microbiota [Kim and Park, 2018].

Prediabetes can be addressed with kimchi consumption too. In a study by So-Yeon An et al. (2021), 21 prediabetic individuals who consumed fermented kimchi experienced reductions in body weight, BMI, waist circumference, insulin resistance, blood pressure, and improved glucose tolerance, insulin sensitivity and β-cell function [So-Yeon An et al., 2013].

Optimizing the health-promoting, probiotic and functional food properties of kimchi can be achieved by manipulating the kinds and amounts of ingredients, using appropriate probiotic starters, and employing specific kimchi preparation methods. Thus, optimally produced kimchi can be one of the best healthy foods [Park et al., 2014].

3.6 *Fermented soy products*

Soybean (*Glycine max*) is one of the most widely grown oilseeds crops globally and serves as a primary source of vegetable protein. Comprising approximately 40% protein (such as glycinin and conglycinin), 20% lipids, 35% carbohydrates, 5% minerals, and 10% moisture, soybeans also contain fatty acids, vitamins (vit. E), flavonoids, isoflavones, phenolic acids, and saponins. Fermented soy products are extensively produced and consumed in Asian countries, serving as a protein source in a diet with limited meat consumption.

Originally aimed at food preservation, soybean fermentation is now a subject of research interest as it increases the bioactive components responsible for health benefits and reduces anti-nutritional factors. Fermentation of soybeans can occur with bacteria only (e.g., Natto production), filamentous fungi only (e.g., miso and tempeh production), or with a combination of both microorganisms. Microorganisms possessing specific hydrolytic enzymes, including protease, amylase, and β-glucosidase, play a crucial role in increasing the functional properties of fermented soy products.

Soybean protein and isoflavones are the primary functional constituents of fermented soybean foods. During fermentation, isoflavones are modified by microbial β-glucosidase enzymes, resulting in increased amounts of isoflavone aglycones, such as genistein and daidzein, which exhibit higher bioactive potential. Aglycone forms also exhibit greater antioxidant activity, contributing to the reduction of chronic diseases associated with the consumption of fermented soybean products in Asian countries. Soy protein fraction contain inhibitory enzymes like proteinase and trypsin, making them less digestible. However, during fermentation, microbial proteolytic enzymes hydrolyse proteins into peptides and free amino acids, enhancing antioxidant activity and increasing the digestibility of soybean protein. In addition, the content of polyphenols, phenolic acids, saponins, sterols and flavonoids in fermented soy products confer to them potent antioxidant properties, protecting against oxidative damage.

Fermented soy products offer a range of bioactive properties beyond their antioxidant effects. These include anticancer, anti-obesity, antidiabetic, anti-inflammatory, neuroprotective, and anti-aging effect. The anticancer effect of fermented soy products, mainly attributed to soybean isoflavones like genistein, is well-documented. Genistein demonstrates a broad-spectrum anticancer effect against various cancers, including breast, prostate, oesophageal, pancreatic, gastric, colorectal, metastatic carcinoma, lymphoma, and neuroblastoma. Fermented soy products also exhibit anti-obesity and antidiabetic effects, with isoflavones (such as daidzein and genistein) and soy proteins and peptides promoting favourable alterations in lipid profiles (increase in HDL cholesterol and decrease in total cholesterol, LDL, and triglycerides) and insulin sensitivity, and reducing both hypertrophy and hyperplasia. Furthermore, fermented soy products exert anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and the activation of inflammatory signalling pathways due to isoflavones. These products also demonstrate neuroprotective effects, protecting against neuronal cell death and enhancing neuronal function. Fermented soy products show an increase in gamma-aminobutyric acid (GABA), which regulates the central nervous system (CNS).

Additionally, isoflavones aglycone genistein and daidzein found in fermented soybean food products are associated with anti-aging properties, including improvements in skin quality, bone strength, and cognitive function. The high concentration of spermidine found in foods like natto and tempeh, contributes to these anti-aging effects by reversing memory loss, improving the blood lipid profile, and reducing cardiovascular risks inducing autophagy in damaged cells. Fermentation of soybeans is also responsible for the generation of ACE inhibitory peptides, which help regulate blood pressure and reduce the risk of cardiovascular disease [Prado et al., 2022].

3.6.1 Miso

Miso, a fermented soybean product, boasts numerous health properties, confirmed by several studies. Its antioxidant activity is potent, with compounds like daidzein, genistein, and syringic acid exhibiting stronger antioxidant effects than α-tocopherol.

Miso also has notable gastrointestinal beneficial effects. Its fermentation process transforms proteins into peptides and incorporates highly active enzymes, enhancing the digestion and absorption of vital nutrients. The Nagahama Study, conducted on a general Japanese population of 9.364 subjects, demonstrated that daily intake of miso soup is associated with fewer epigastric symptoms, reflux and dyspepsia, independently of age, sex, BMI, smoking, drinking alcohol, and unfavourable dietary habits. This effect could be due to histidine, glutamate and aspartate, three amino acids that promote gastric emptying [Mano et al., 2018]. Moreover, isoflavones present in miso, such as genistein, have inhibitory effects against *Helicobacter pylori*, a bacterium involved in stomach inflammation and peptic ulcers.

The anticancer potential of miso is also significative. Compounds like genistein and glycitein have shown strong cytotoxic effects against HL-60 leukaemia cells and demonstrated efficacy in preventing mammary and colon cancer in rats. Human clinical trials have suggested that miso consumption may reduce the risk of hepatocellular carcinoma (HCC), possibly by lowering cell proliferation and directly affecting angiogenesis and tumorous cells [Sharp et al., 2005].

Miso protective properties extend to radiation injury, where consumption aids in the faster elimination of radioactive components from the body, as observed in rat studies. Though the exact mechanisms remain unknown, miso's radiation-protective properties are promising [Watanabe, 2013].

Furthermore, miso has anticholesterolemic and anti-aging effects. It contains plant sterols, linoleic acid, and vitamin E, contributing to its cardioprotective role. Substituting soy protein for animal protein or directly including soy protein in diets has been clinically and experimentally proven to lower blood cholesterol levels. For example, a study on healthy premenopausal women showed a significant decrease in total blood cholesterol after consuming 50 g of miso daily for nine months [Minamiyama and Okada, 2003]. Recent studies also suggest a potential inverse relationship between miso soup consumption and heart rate, particularly among middle-aged and elderly Japanese individuals, indicating broader cardiovascular benefits.

Contrary to concerns about its salt content, studies indicate that miso does not increase blood pressure. In Dahl salt-sensitive rats, long-term miso consumption attenuated blood pressure increases, hypertension, and kidney damage, possibly due to delayed sodium absorption in the gastrointestinal tract. This is also associated with decreases in cardiovascular damage [Yoshinaga et al., 2012; Saeed et al., 2022; Chan et al., 2021].

3.6.2 Natto

Natto contains many nutrients originating from soybeans and the metabolites of *Bacillus subtilis (natto)* cells, which are used to initiate fermentation and are considered potential probiotics. Studies have demonstrated that the ingestion of natto (50g/day) significantly affects the composition and metabolic activity of human faecal microflora, increasing *Bifidobacterium* spp. and lactobacilli, and can have an immunostimulant effect [Hosoi and Kiuchi, 2003].

Among the bioactive components generated during fermentation are proteases such as nattokinase (NK) and bacillopeptidase F (BPF), vitamin K2 (with natto containing 124 times more than soybeans prior to fermentation), dipicolinic acid (DPA), and γ-polyglutamic acid (γ-PGA), which gives natto its sticky consistency [Chan et al., 2021].

Nattokinase, found in the sticky component of natto, has strong fibrinolytic activity both *in vitro* and *in vivo*. Human trials have demonstrated NK ability to enhance fibrinolysis and antithrombosis after a single-dose administration, suggesting that NK improves blood circulation and provides protective effects against cardiovascular diseases. This has led to its sale as a dietary supplement in the US, Canada, and Europe [Kurosawa et al., 2015; Weng et al., 2017].

Furthermore, nattokinase has shown anti-hypertensive activity. In a study of subjects with pre-hypertension or stage 1 hypertension, NK supplementation reduced both systolic and diastolic blood pressure (Figure 3.7), possibly through a mechanism involving renin activity [Kim et al., 2008].

Vitamin K2 (menaquinone-7) plays crucial roles in blood coagulation and regulation of bone metabolism, stimulating bone formation and suppressing bone degeneration. Natto may help prevent osteoporosis and bone loss, as evidenced by studies on Japanese women. One study found that natto intake decreased bone mass loss at the femoral neck and possibly at the distal third of the radius in postmenopausal women [Ikeda et al., 2006]. Another long-term study involving 1417 postmenopausal Japanese women suggested that habitual natto intake is associated with a decreased risk of osteoporotic fractures. These benefits may be mediated not only through the preservation of bone mass but also through maintenance of bone microarchitecture, which includes the number, spacing, and thickness of trabecular bone [Kojima et al., 2020].

In summary, natto is a nutritious fermented soybean products with numerous health benefits, including enhanced gastrointestinal health, cardiovascular protection, blood pressure regulation, and bone health maintenance.

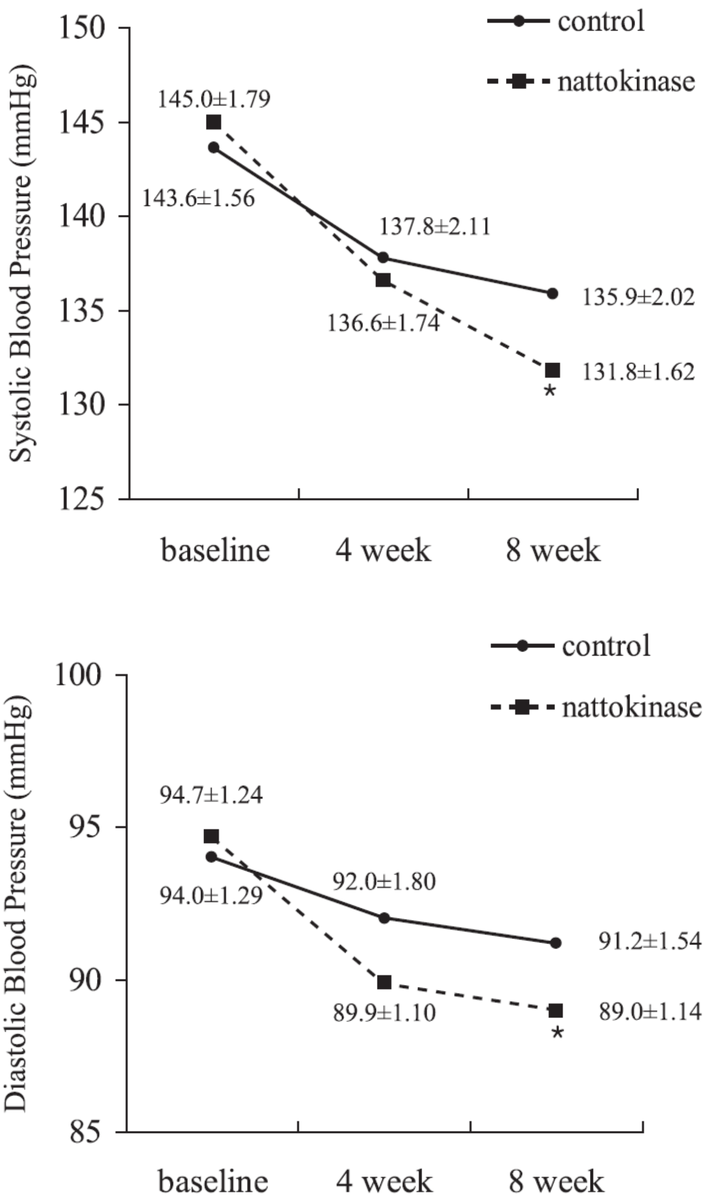
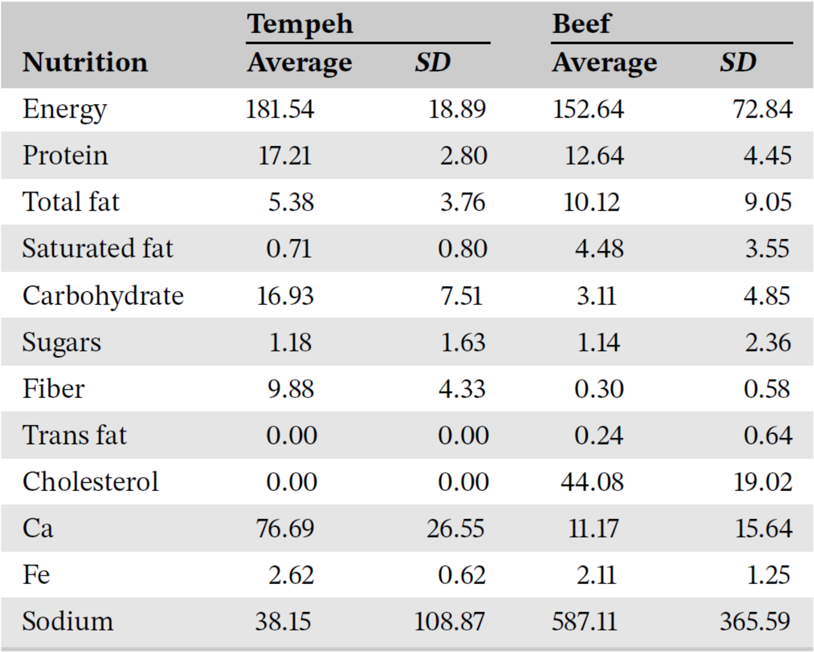


Figure 3.7- Effect of nattokinase supplementation on systolic and diastolic blood pressure. At the 8-week visit, the net changes in SBP and DBP were statistically significant (= $p < 0.05$ compared with the values of control group) [Kim et al., 2008]*

3.6.3 Tempeh

As meat consumption and production have been deemed unsustainable in terms of public health and the environment, tempeh emerges as a favourable food source due to its health benefits, affordability, and sustainability. Tempeh is known for its significant amounts of protein, vitamin B12, and bioactive compounds. The fermentation process not only decreases antinutrient and allergen contents, but also increases essential micronutrient content, making it suitable for vegetarians as meat replacer. Tempeh is high in protein, prebiotics, and a wide array of vitamins and minerals, making it a comprehensive meat substitute [Ahnan-Winarno et al., 2021; Teoh et al., 2024].

Commercial tempeh is rich in proteins and fibres, low in saturated fat and salt, free of sugar, cholesterol, and trans fatty acids, and typically contains substantial amounts of calcium, potassium, and healthy fatty acids. Nutritional analyses reveal that tempeh is a nutritious protein source, comparable to beef, and potentially more favourable regarding protein, total fat, saturated fat, fibre, cholesterol, calcium, iron, and sodium content (Table 3.1) [Ahnan-Winarno et al., 2021].



	Tempeh		**Beef**	
Nutrition	**Average**	***SD***	**Average**	***SD***
Energy	181.54	18.89	152.64	72.84
Protein	17.21	2.80	12.64	4.45
Total fat	5.38	3.76	10.12	9.05
Saturated fat	0.71	0.80	4.48	3.55
Carbohydrate	16.93	7.51	3.11	4.85
Sugars	1.18	1.63	1.14	2.36
Fiber	9.88	4.33	0.30	0.58
Trans fat	0.00	0.00	0.24	0.64
Cholesterol	0.00	0.00	44.08	19.02
Ca	76.69	26.55	11.17	15.64
Fe	2.62	0.62	2.11	1.25
Sodium	38.15	108.87	587.11	365.59

Table 3.1- Nutritional profiles of tempeh and beef. Tempeh has a higher protein and fibre content compared to beef, while having lower levels of total fat, saturated fat, and cholesterol. Additionally, tempeh is more favourable in terms of mineral content, with higher levels calcium and iron, and lower levels of sodium [Ahnan-Winarno et al., 22021]

Consumption of tempeh has been linked to various health benefits, including antidiabetic effects, cholesterol-lowering properties, improved cognitive function, antitumor and anticancer properties, anti-aging effects, improved gut health and reduced cardiovascular disease risk [Teoh et al., 2024]. These benefits are attributed to its isoflavone, protein, mineral, para- and probiotic contents [Ahnan-Winarno et al., 2021].

In terms of antidiabetic effects, tempeh's fermentation process elevates levels of phytoestrogens, bioactive soy peptides, and isoflavonoids, while reducing starch and fat content, which helps lower blood glucose levels, body weight, and total cholesterol levels in T2DM [Teoh et al., 2024].

Regarding gut health, a human study showed that supplementation of steamed tempeh (100g/person per day) for two weeks in healthy young adults enhanced IgA production, showing that tempeh could act as prebiotics and paraprobiotics agent for human body modulating human intestinal immune system. Moreover, tempeh supplementation is responsible for an increase in the population of *Akkermansia muciniphila* of the intestinal tracts. This bacterial strain regulates immune system, cell proliferation, adhesion and apoptosis, and mucosal gene expression contributing to weight loss and T2DM reductions (Figure 3.8) [Stephanie et al., 2017].

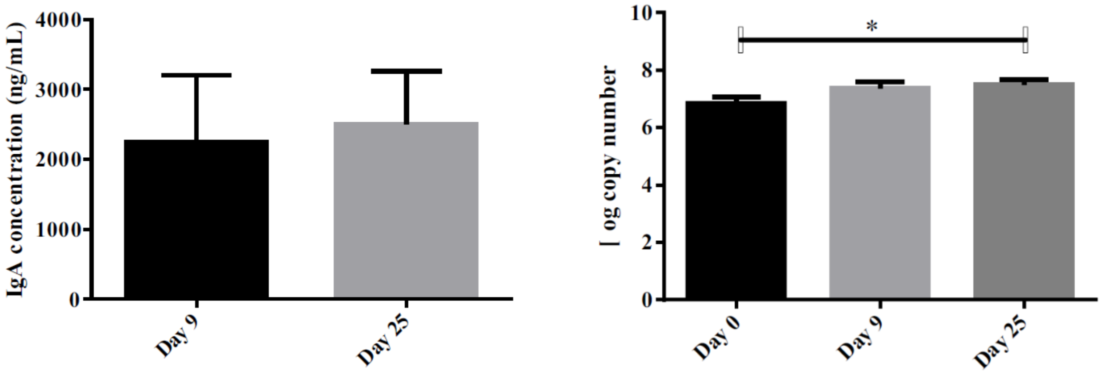


Figure 3.8- On the left, enhancement of immunoglobulin A (IgA) secretion after tempeh supplementation. IgA in the digestive tract act as a first defence barrier, protecting intestine from pathogenic bacteria and toxins.
On the right, enhancement of A.muciniphila population after tempeh supplementation.
Day 0 and day 9 = before tempeh supplementation, Day 25 = after tempeh supplementation.
[Stephanie et al., 2017]

Gut microbiota directly influences brain function, and alterations in gut microbiota can affect both peripheral and central nervous systems. Probiotics from fermented foods like tempeh have demonstrated anxiolytic and antidepressant effects in animal studies, and oral consumption of probiotics can modulate the gut-brain-axis (GBA), potentially benefiting cognitive function [Teoh et al., 2024]. Tempeh, enriched in vitamins B2, B12 and γ-aminobutyric acid (GABA) due to the fermentation process, can improve memory, brain functionality, and reduce dementia risk. An experimental study on elderly individuals with memory impairment found that tempeh probiotics (*L.fermentum*) improved the cognitive domains of memory, language and visuospatial function [Handajani et al., 2022].

Tempeh also exhibits anticancer properties, with isoflavones like genistein and daidzein inhibiting cancer cell proliferation, suppressing angiogenesis, and inducing apoptosis in cancer cells. These anticancer activities have been evaluated against breast cancer, cervical cancer, and ovarian cancer, although more clinical trials are needed [Teoh et al., 2024].

For cardiovascular health, tempeh's soy protein, isoflavones and phytosterols reduce serum total cholesterol and low-density lipoprotein (LDL) levels, improving heart health. Clinical studies have shown that tempeh supplementation can decrease LDL, triglycerides, total cholesterol levels, and systolic blood pressure in hyperlipidaemic and hypercholesterolemic individuals [Ahnan-Winarno et al., 2021; Zulaikha et al., 2023]. Tempeh protein extracts also exhibit anti-ACE activity, indicating their potential as functional foods for hypertension prevention and treatment, alongside GABA content associated with high blood pressure management [Teoh et al., 2024].

CONCLUSIONS

Fermentation is a practice deeply rooted in human history. As Katz (2018) writes, humans began fermenting food long before they learned to write, make pottery, or cultivate the soil [Katz, 2018]. However, this tradition, maintained for millennia, has largely been forgotten in modern times, overshadowed by industrial food production. Now, it is making a comeback as it meets the new needs of modern consumers and fits perfectly into the current perspective of human and social development. It intersects with several major food movements: it is natural, artisanal, sustainable, innovative, functional, global, tasty, and healthy [The Fermentation Association].

The contemporary consumer is very health-conscious and aware of the correlation between nutrition and well-being. Modern eating styles are increasingly oriented towards the consumption of high-quality raw materials that are sustainable, locally sourced, and produced in a natural or artisanal way. The famous aphorism “We are what we eat”, coined by Feuerbach in 1850, is more relevant than ever, emphasising the importance of nutrition as a driving force in human history. Today, this awareness leads us to understand the importance of food preparation and transformation processes and their effects on our diet and health [Gasbarrini, 2022].

Western diets, low in fibre and rich in industrialized and processed foods, are considered one of the leading causes of maladaptive gut microbiome changes along human evolution, likely contributing to the increasing incidence of chronic non-communicable diseases like cardiovascular disease, cancer, and diabetes. In contrast, fermentation makes food more nutritious. Fermented foods often contain probiotic bacteria that are beneficial to our bodies. By interacting with each other and with our microbiome, they can improve digestion, immune system, mental health and many other aspects of our overall well-being.

The term “ecoimmunonutrition” emphasises that an organism’s immune system depends on its gastrointestinal microecological equilibrium, and it is possible to cultivate and develop this microbial ecosystem inside us through our diet by consuming foods rich in beneficial bacteria and plant fibres, such as fermented products [Bengmark, 1998; Sima and Vetvicka, 2014].

As highlighted in various points of this thesis, the benefits of fermented foods are numerous. For example, kombucha, kefir, and soy-based fermented products are rich in antioxidants, which help fight free radicals and reduce cellular damage, and also possess anti-inflammatory and anti-carcinogenic properties.

Furthermore, due to the ability of these foods to modulate the gut microbiota, fermented products impact metabolic parameters and are beneficial in preventing many chronic non-communicable diseases. For instance, kefir, kimchi, and tempeh are effective in weight loss and obesity prevention. Products such as kefir, miso, natto, and tempeh, on the other hand, are important in the prevention of cardiovascular diseases, lowering cholesterol levels and blood pressure, and having antithrombotic functions. Many of these fermented foods, such as kombucha, kimchi, and tempeh, also have antidiabetic effects. Finally, natto has been shown to be important for bone health, particularly in menopausal women. While tempeh offers mental health benefits due to its anxiolytic, antidepressant, and cognitive-enhancing effects.

Therefore, the growing curiosity of the modern consumers towards fermented foods is mainly due to their health-promoting properties. The demand for foods containing probiotics is increasing. Many fermented foods, especially those discussed in this thesis, can be consumed raw, which is the most nutritious way to consume them. It is therefore important to read the labels of products such as kombucha, kefir, or kimchi if purchased industrially, to ensure that contain live cultures. Another need of the modern consumer concerns healthy, non-alcoholic beverage options that can also be consumed in social settings. Kombucha and kefir meet this need, providing alternatives to commonly used alcoholic beverages or soft drinks.

Awareness of environmental issues is fortunately becoming more and more effective, and consumers are starting to pay attention to this food-related aspect as well, looking for products that are sustainable. The growing market for products such as water kefir, kimchi, natto, and tempeh reflects this need. These plant-based foods offer interesting nutritional values due to the action of the microbes than inhabit them. Spontaneous fermentation then has an even broader ecological value. According to the “One Health” approach, the health of humans, the planet, and other organisms are closely interconnected and must be considered together for sustainable development.

Western culture, terrified of germs and obsessed with hygiene, has led to a change in biodiversity of human microbiota, which has been shown to be responsible for some emerging health problems. In reality, the microorganisms we host in our bodies are more numerous than our human cells and provide a wide range of essential services. They enable efficient digestion, synthesize essential nutrients, and are crucial to the immune system. Katz writes, “Bacteria are not germs, but rather the elements that germinate, and structure, all life on earth. In declaring war on them, we declare war on the living structure at the basis of the planet, on all forms of life we know, on ourselves”. Health and homeostasis therefore require coexistence with microorganisms, in a mutually beneficial and dependent relationship so complex that we cannot presently fully understand. Consuming a wide variety of “live” fermented foods promotes microbial diversity in our organism. And if biodiversity is recognised as crucial for the survival of ecosystems on a large scale, it is equally important at the microscopic level. Our bodies function efficiently when populated by numerous species of different microorganisms. Spontaneous fermentation allows this natural biodiversity to enter our bodies, helping us to adapt to changing conditions and reducing our vulnerability to diseases [Katz, 2018].

Finally, in a globalised world, the possibility of spreading the traditional fermented products of each culture makes it easy to explore and learn about geographically distant worlds also through food. Natto, miso and kimchi belong to the Asian gastronomic tradition, while tempeh originates from Indonesia and is also spreading to the West. These flavours, new to our palate, allow us to expand our culinary and world knowledge.

In “The Noma Guide to Fermentation”, Renè Redzepi and David Zilber, the chef and master fermenter of Noma, express their strong belief in fermentation. Renè Redzepi states, “I believe in fermentation wholeheartedly, not only as a way to unlock flavours, but also as a way of making food that feels good to eat […] I personally feel better eating a diet full of fermented products […] I dream about the restaurants of the future, where you go not just for an injection of new flavours and experiences, but for something that’s really positive for your mind and body” [Redzepi and Zilber, 2019].

To answer the question posed in this thesis title, I borrow the words of Amelia Nielson-Stowell, editor of the Fermentation Association, who states, “Fermentation is not a trend. It’s experiencing a resurgence, a renaissance. Fermentation never went away. It just became less of a common type of food craft. Humans have long consumed fermented foods for thousands and thousands of years, but now we have the scientific techniques to dive into fermented foods and analyse their nutritional properties, their microbial composition and better understand how they may improve a person’s health” [The Fermentation Association, 2023].

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