FERMENTATION OF DEHYDRATED FRUIT JUICES BY *LACTOBACILLUS* SPP. WITH PREBIOTIC SUPPLEMENTATION AS NON-DAIRY FUNCTIONAL BEVERAGES

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by

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LIST OF SYMBOLS

%	Percentage
±	Standard Deviation
°C	Degree Celcius
μL	Microliter
eV	Electron Volt
g	Gram
h	Hour
kcal	Kilocalories
L	Liter
M	Molarity
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
N	Normality
nm	Nanometer
rpm	Revolutions Per Minute
U	Unit
v/v	Volume per Volume
w/v	Weight per Volume

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
ATP	Adenosine Triphosphate
CFU	Colony Forming Unit
CH_2Cl_2	Dichloromethane
$CuSO_4$	Copper Sulphate
DP	Degree of Polymerization
DPPH	2,2 Diphenyl-1- picrylhydrazyl radical scavenging assay
Ed	Edition
EDC	Euclidean Distance to Centriods
FOS	Fructo-oligosaccharides
FSQD	Food Safety and Quality Division
GC-MS	Gas-Chromatography-Mass Spectometry
GI	Gastrointestinal
GOS	Galacto-oligosaccharides
GRAS	Generally Recognized as Safe
H_2O	Water
HCl	Hydrochloric Acid
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
IPA	Isopropyl Alcohol
K_2SO_4	Potassium Sulphate
LAB	Lactic Acid Bacteria
Na_2CO_3	Sodium Carbonate

NaOH	Sodium Hydroxide
ND	Not Detected
NPRA	National Pharmaceutical Regulatory Agency
OD	Optical Density
PI	Prebiotic Index
PNPB	P-nitrophenylbutyrate
PNPG	P-nitrophenyl glucopyranoside
SCFA	Short-Chain Fatty Acid
Spp	Species
vs	Versus
WHO	World Health Organization
XOS	Xylo-oligosaccharides

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PENAPAIAN JUS BUAH-BUAHAN TERNYAHHIDRAT OLEH *LACTOBACILLUS* SPP. DENGAN SUPLEMEN PREBIOTIK SEBAGAI MINUMAN BERFUNGSI BUKAN TENUSU

ABSTRAK

Jus buah, yang diperoleh daripada isi buah yang boleh dimakan dan tidak mengalami proses fermentasi, boleh diperkaya melalui proses fermentasi menggunakan bakteria asid laktik (GRAS) untuk meningkatkan manfaat kesihatan. Kajian ini bertujuan untuk menilai pengeluaran asid laktik, profil pertumbuhan, dan daya tahan pelbagai spesies *Lactobacillus* dalam fermentasi jus buah serta mengkaji kesan penambahan prebiotik terhadap pembangunan minuman berfungsi. Lima spesies *Lactobacillus*, iaitu *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum, L. acidophilus, L. rhamnosus,* dan *Limosilactobacillus reuteri*, digunakan untuk menapai lima jenis jus buah, iaitu jus mangga, nanas, buah naga, kranberi dan beri campuran. Dalam kalangan spesies tersebut, *L. plantarum* menunjukkan pertumbuhan sel dan daya tahan yang lebih baik dengan penghasilan asid laktik yang standing dengan spesies *Lactobacillus* yang lain, dan kemudiannya dipilih untuk kajian lanjut. Kesan inulin dan galakto-oligosakarida (GOS) terhadap fermentasi turut dinilai selama 72 jam. Penambahan inulin secara signifikan meningkatkan pertumbuhan *L. plantarum*, terutamanya pada 12 jam dalam jus mangga (1.32 ± 0.014) dan 24 jam dalam jus nanas (0.68 ± 0.003). Inulin juga meningkatkan pengeluaran asid laktik, mencapai 10.22 ± 0.004 g/L dalam jus mangga berbanding 8.66 ± 0.007 g/L dalam sampel kawalan. Indeks prebiotik (PI) bagi jus yang ditambah inulin menunjukkan nilai melebihi satu, manakala hanya jus nanas yang ditambah GOS menunjukkan PI melebihi satu. Sifat fungsian jus yang ditapai kemudiannya dianalisis melalui ujian *in vitro* untuk menilai

aktiviti antioksidan, anti-diabetik, dan anti-obesitiPenambahan inulin didapati secara ketara meningkatkan aktiviti antioksidan dalam jus kranberi (43.33 ± 0.03%) dan jus beri campuran (27.05 ± 0.08%). Ujian *in vitro* anti-diabetik menunjukkan bahawa jus mangga yang ditapai dengan inulin mempunyai penyekatan α-amilase tertinggi (95.20 ± 0.00%), manakala jus kranberi dan jus beri campuran mencapai 100% penyekatan α-glukosidase, melebihi jus yang tidak ditapai. Selain itu, ujian *in vitro* anti-obesiti menunjukkan peningkatan aktiviti penyekatan lipase dalam jus yang ditapai dengan inulin, dengan jus kranberi yang ditapai mencatatkan penyekatan tertinggi (56.65 ± 0.02%), sementara tiada aktiviti dikesan dalam jus nanas dan buah naga. Analisis peta haba memberikan gambaran mengenai perubahan metabolik yang berlaku akibat fermentasi dan penambahan prebiotik. Secara keseluruhannya, kajian ini menunjukkan bahawa fermentasi jus buah menggunakan *L. plantarum* serta penambahan prebiotik, terutamanya inulin, bukan sahaja meningkatkan pertumbuhan bakteria dan penghasilan asid laktik, malah memperkayakan sifat fungsian minuman yang ditapai. Penemuan ini menekankan potensi fermentasi jus buah yang diperkaya dengan prebiotik, membuka peluang bagi pembangunan minuman berfungsi dengan implikasi yang meluas terhadap kesihatan usus dan kesejahteraan keseluruhan.

FERMENTATION OF DEHYDRATED FRUIT JUICES BY *LACTOBACILLUS* SPP. WITH PREBIOTIC SUPPLEMENTATION AS NON-DAIRY FUNCTIONAL BEVERAGES

ABSTRACT

Fruit juice, derived from edible fruit flesh and unfermented, can be enhanced by fermenting with lactic acid bacteria (GRAS) to improve health benefits. This study evaluated lactic acid production, growth profile, and cell viability of different *Lactobacillus* spp. in fruit juice fermentation and examined the effects of prebiotics on functional beverage development. In this study, five *Lactobacillus* spp., namely *Lacticaseibacillus paracasei, Lactiplantibacillus plantarum, L. acidophilus, L. rhamnosus*, and *LimosiLactobacillus reuteri* were utilized to ferment five different fruit juices; mango, pineapple, dragon fruit, cranberry and mixed berry juices. Among them, *L. plantarum* demonstrated superior growth and viability with comparable lactic acid production and was selected for further study. The impact of inulin and galacto-oligosaccharides (GOS) on fermentation was also assessed over 72 hours. Inulin significantly enhanced *L. plantarum* growth, especially at 12 hours in mango juice (1.32 ± 0.014) and 24 hours in pineapple juice (0.68 ± 0.003). It also increased lactic acid production, reaching 10.22 ± 0.004 g/L in mango juice compared to 8.66 ± 0.007 g/L in the control. The prebiotic index (PI) for inulin-supplemented juices consistently exceeded one, while only GOS-supplemented pineapple juice achieved a PI above one. Functional properties were further evaluated through *in vitro* antioxidant, anti-diabetic, and anti-obesity assays. Inulin supplementation significantly enhanced antioxidant activity in cranberry (43.33 ± 0.03%) and mixed berry (27.05 ± 0.08%) juices. *In vitro* anti-diabetic assays revealed that fermented mango juice with inulin exhibited the highest α-amylase inhibition (95.20 ± 0.00%), while cranberry and mixed berry juices achieved 100% α-glucosidase inhibition, surpassing non-fermented juices. Similarly, *in vitro* anti-obesity assays showed increased lipase inhibition in inulin-supplemented juices, with fermented cranberry juice exhibiting the highest inhibition (56.65 ± 0.02%), though no activity was detected in pineapple and dragon fruit juices. Notably, a comprehensive heat-map analysis provided insights into the metabolic alterations induced by fermentation and prebiotic supplementation. Overall, this study demonstrates that the fermentation of fruit juices with *L. plantarum* and the addition of prebiotics, particularly inulin, not only enhance bacterial growth and lactic acid production but also enrich the functional properties of the fermented beverages. These finding highlights the potential of prebiotic-enhanced fermentation in fruit juice, paving the way for the development of functional beverages with significant implications for gut health and overall wellness.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Fruits are valuable for their rich content of nutrients, including dietary fiber, vitamins, and polyphenols, making them a healthy food choice with minimal fat and calorie levels (Yuan et al., 2024). Fruit juice can be made either directly from the fruits or by reconstituting the fruit concentrate. Fruit juices are mainly known for their antioxidant, vitamin content, and minerals, and can vary widely in flavor and nutritional content depending on the fruit used and any processing methods applied (Naseem et al., 2023).

Fermentation is the process of converting sugar into an organic acid that occurs naturally in many foods. Lactic acid bacteria (LAB) such as *Lactobacillus, Streptococcus,* and *Leuconostoc* are predominant in fermented foods, which often serve as carriers for probiotic bacteria, although other bacteria, yeasts, and fungi also contribute to the fermentation process (Rezac et al., 2018). Fermented fruits are created through the complex interplay between microorganisms and the fruit's naturally abundant glucose and fructose, enabling convenient storage and transportation while gaining increasing attention in recent advancements (Yuan et al., 2024).

Probiotics, which are beneficial live microorganisms, are increasingly recommended for their health benefits and, while traditionally found in dairy products, are now commonly incorporated into nutrient-rich fruit and vegetable juices, offering a lactose-free, cholesterol-free alternative for delivering probiotics effectively (Rahmah et al., 2023). Fruit and vegetable juices have emerged as promising mediums for delivering or cultivating these beneficial microorganisms (Naseem et al., 2023).

Fermentation of juice introduce a spectrum of bioactive compound, position them as a novel functional food. The synergy between fruit juice and probiotic will enhance nutritional profile and offer potential health benefits. Probiotics are commonly found in dairy-based beverages because dairy products like milk, yogurt, and cheese effectively deliver beneficial bacteria such as *Lactobacillus* species by buffering stomach acid, thereby enhancing the likelihood that these bacteria will thrive in the intestines (Kaur et al., 2022). Nevertheless, certain individuals, such as vegetarians, lactose intolerant individuals, and those with dairy allergies, must abstain from dairy consumption (Nguyen et al., 2019). Consequently, alternative carriers for probiotics are sought to enable these individuals to access the health benefits associated with beneficial bacteria.

There are numerous strains of probiotics used in food but the most commonly used strains are *Lactobacillus* and *Bifidobacterium* (White & Hekmat, 2018). They are associated with antimicrobial properties against pathogens, antioxidant, reduce the chance of colon cancer, immune modulation, cholesterol reduction, diabetes management, and alleviation of conditions like diarrhea and lactose intolerance (Petrariu et al., 2024). *Lactobacillus* is a member of lactic acid bacteria. Lactic acid bacteria (LAB) are a group of microorganisms that produce lactic acid as their primary metabolite. Sugars are utilized by these bacteria via either homo- or heterofermentative pathways. LAB must possess certain phenotypic traits in order to grow efficiently in fruit juices, enhancing their safety as well as their nutritional and sensory value (Garcia et al., 2020).

Prebiotics are compounds that act as the fuel for probiotics that encourage the growth and activity of the beneficial microorganism. Prebiotics have been shown to increase probiotic viability, improve gastrointestinal health, and increase calcium absorption (White & Hekmat, 2018). They can be found in many fruits and vegetables, especially those which is rich in complex carbohydrates such as fiber and starch. Dietary prebiotics are normally non-digestible fiber compounds thus, they will go down to the lower digestive tract where they will act as food that helps the useful microorganism to grow and do their activity.

Prebiotics are fermented by beneficial bacteria in the intestine, producing short-chain fatty acids that are released into the bloodstream and impact other organs in addition to the gastrointestinal tract (Davani-Davari et al., 2019). According to the preceding classifications, plant-based carbohydrates, including oligosaccharides like fructans (fructo-oligosaccharides and inulins) and galactans (galacto-oligosaccharides), as well as other prebiotic fibers such as pectin, resistant starch, xylooligosaccharides, and beta-glucans are vital for fostering the growth and activity of beneficial gut bacteria (Anburaj, 2020).

There has been a significant increase in the demand for non-dairy probiotic products as a substitute for dairy probiotic foods. Further research is needed to understand how individual probiotic strains survive and remain viable during fermentation processes, as well as to clarify the impact of prebiotics on probiotic survival and the stability of fermented juices. This study will reveal the viability and biochemical activities of individual probiotics during the fermentation of fruit juice and the potential functional activities of probiotics with supplementation of prebiotics.

1.2 Problem Statement

In recent years, the demand for the consumption of probiotic beverages has increased. Dairy products are considered as the primary example of probiotic foods.

However, these products display some drawbacks, affecting some consumers' attitudes. There are increasing of people who cannot tolerate dairy products but want to enjoy the benefits of the probiotic that mostly can be found in dairy and its by-product. Such people have health problems that make them cannot consume any product with milk in it (lactose intolerance, milk allergy and high cholesterol or fat content). Therefore, a probiotic carrier that is appropriate for these individuals is required. Beverages made from fruits using regulated lactic acid fermentation are newly introduced functional drinks that meet the needs of consumers as replacements for dairy products. Therefore, the development of probiotic drinks using fruits and vegetables is essential to cater to the growing demand for dairy-free and plant-based beverage options.

There have been studies conducted on the addition of probiotics into fruit juices as an alternative. Adding probiotic without fermentation provides life microorganisms but lacks of bioactive, sensory and preservation advantages. For consumers seeking a health promoting beverages with functional benefit, fermented juice is likely to be far more attractive due to the richness of nutrients, flavours, bioactive compounds develop uniquely through fermentation. Fermentation of fruit juices by probiotics introduces a spectrum of bioactive compounds, positioning them as a novel category of functional foods (Putnik et al., 2020). This synergy between fruit juices and probiotics not only enhances their nutritional profiles but also offers potential health benefits (Žuntar et al., 2020). An important aspect to take into account when developing probiotic foods is how well non-dairy product protect to maintain high probiotic survival during gastrointestinal digestion, processing and storage. However, due to the pH of fruit juices that is low, high dissolved oxygen content, and deficiency in free amino acids and peptides, sustaining probiotic cultures' viability is difficult (da Costa et al., 2017).

The survival of probiotic cultures in fruit juices is affected by probiotics strain used as well as the juice parameters. Some probiotic might not be able to survive or has low growth in certain fruit juices. The limited exploration of fermentation performance differences, especially among various *Lactobacillus* strains in fruit juices underscores the need to identify the most effective strains, which could enhance the development of tailored functional beverages. As food regulation, the probiotic cell count should be at least 10^6 CFU/mL to be claimed effective. For Malaysia, one of the conditions in the Food Safety and Quality Division (FSQD) regulation state that the probiotic shall remain viable and the viable probiotic count shall not be less than 10^6 CFU/mL during the shelf life of such food. Additionally, more research is required to confirm the positive effects of the beverage on human health.

Ingredients that are both probiotic and prebiotic serve as an ideal target for the creation of novel functional foods (Green et al,, 2020). The inclusion of prebiotics in these beverages has been researched, and fruit juices have been proposed as the ideal substrate for the creation of non-dairy probiotic beverages. However, there is a restricted data accessible regarding the inclusion of prebiotics in specific fruit drinks, especially fermented fruit juice, to establish non-dairy functional drinks, despite numerous studies on the fermentation of fruit juices. Most studies focus on dairy-based fermentation, leaving a significant gap in understanding how prebiotics perform in fruit juice matrices. The effectiveness of functional beverages in enhancing the health of the digestive system should be investigated as well as the stability of the functional beverages. This suggests a requirement for additional research on the ideal pairing of probiotics and prebiotics in assorted fruit juices.

Lactobacillus spp. that is fermented in the fruit juices may survive in a condition that has high acidity and low pH to be able to be used as the starter culture. Consumption of prebiotics may assist in the growth and activity of the *Lactobacillus spp.* in fruit juices. Despite the fact that the concept of functional foods is not new, food companies and research organizations continue to develop foods that may have these properties; however, current studies have not yet fully tested for its functional effects in humans to confirm their alleged potential as anti-obesity or anti-diabetic (Granato et al., 2017). The studies on synbiotic effects on the addition of prebiotics into fermented fruit juices, especially on the impact towards human's health also is not fully discovered and still in research. This research aims to address this gap by exploring the synergistic effects prebiotics in fruit juice fermentation, providing new insights into the development of novel functional beverages with enhanced health benefits. Consequently, this study demonstrated that fruit juices containing probiotics could serve as a viable substitute for dairy-based probiotic products and with the addition of prebiotics, a new functional beverage with multiple benefits for human health may be formed.

1.3 Research Objectives

The objectives of this study that need to be achieved at the end of the research are:

1. To investigate the effects of fermenting various fruit juices using different *Lactobacillus* spp., focusing on growth profiles, cell viability, pH changes and lactic acid production.

2. To determine the effects of the addition of prebiotics (inulin and galacto-oligosaccharides) in the fermented juices on the survivability of selected *Lactobacillus* spp.

3. To evaluate the potent antioxidant, anti-obesity, and anti-diabetic activities *in vitro* of the fermented fruit juices supplemented with selected prebiotics.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Fermentation

Fermentation is a process where living cells generate energy by breaking down glucose and other simple sugars without requiring oxygen (anaerobic reaction). It is the conversion of sugar (carbohydrate) into an alcohol or inorganic acid. Fermentation products include yogurt, kefir, wine, vinegar, cheese, soy sauce and more. Fermentation involves microbial or enzymatic activity on food components, leading to beneficial biochemical changes that significantly alter the food (Sharma et al., 2020). In a broader sense, fermentation refers to the deliberate use of microorganisms like bacteria, yeast, and fungi to produce goods that are beneficial to humans (Paulová et al., 2013).

There are three types of fermentation which are lactic acid fermentation, alcohol fermentation and acetic acid fermentation. According to Ezemba & Ezemba (2022), lactic acid fermentation is an anaerobic process where yeast and bacteria transform starches or sugars into lactic acid without applying heat, alcohol fermentation involves yeast breaking down pyruvate into alcohol and carbon dioxide, a method used to make wine and beer, and acetic acid fermentation involves fermenting starches and sugars from grains and fruits to create vinegar and sour-flavored condiments. A fermentation system comprises three key elements: microorganisms such as bacteria, molds, and yeasts; the substrate that supplies nutrients and energy; and the environmental conditions (Septembre-Malaterre et al., 2018)

Humans have used the fermentation process for centuries as a preservative and to improve food quality. Fermented foods provide benefits such as enhanced antioxidants, peptide production, organoleptic and probiotic properties, antimicrobial activity, and reduced levels of toxins and anti-nutrients compared to unfermented foods (Sharma et al., 2020).

2.1.1 Lactic acid fermentation

Through lactic acid fermentation, carbohydrate molecules such as sugar are converted into lactic acid with the help of lactic acid bacteria (LAB). Some examples of LAB are *Lactobacillus* spp., *Pediococcus* spp., *Streptococcus* spp., and *Leuconostoc* spp..

During lactic acid fermentation, LAB will convert pyruvate into lactic acid and regenerate NAD+, enabling glycolysis to continue producing ATP under anaerobic conditions during lactic acid fermentation (Malakar et al., 2019). LAB synthesizes vitamins and minerals, produces health-benefiting biologically active peptides through enzymes like peptidase and proteinase, and removes certain non-nutrients during fermentation (Şanlier et al., 2019).

2.1.1(a) Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) are a non-pathogenic type of gram-positive, in the form of cocci or rods that do not form spores, normally anaerobic or facultative aerobic bacteria that use carbohydrates and metabolically produce lactic acid as a key product during fermentation (Mokoena, 2017). Lactic acid bacteria (LAB) differ in morphology, pH and salt tolerance, temperature preferences, habitats, and pathogenic potential, with safety traits often varying by strain rather than species (Pessione, 2012). Pessione (2012) further reported that emerging three billion years ago, LAB are welladapted to anaerobic and aerobic conditions, possessing proteins for respiration and enzymes for fermentative pathways. They have a strong tolerance to low pH. LAB can outcompete other bacteria in natural fermentation due to their ability to tolerate increased acidity from organic acids, making them crucial in the final stages of food fermentation when other microbes are inhibited by low pH levels (Khandelwal et al., 2016). Additionally, LAB can generate or release bioactive substances like peptides, vitamins, amino acids, and phenolics while also enhancing the antioxidant activity of the matrix (Ruiz Rodríguez et al., 2021)

According to Axelsson, (2004), the genera considered the principal of LAB included *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus and Weissella*. Lactic acid bacteria are classified into various genera based on their morphology, glucose fermentation pathways, growth temperature ranges, lactic acid configuration, tolerance to high salt concentrations, and ability to thrive in acidic or alkaline conditions (Axelsson, 2004). A phylogenetic tree illustrating the evolutionary relationships among LAB species is presented in Figure 1, providing insight into their genetic diversity and classification.

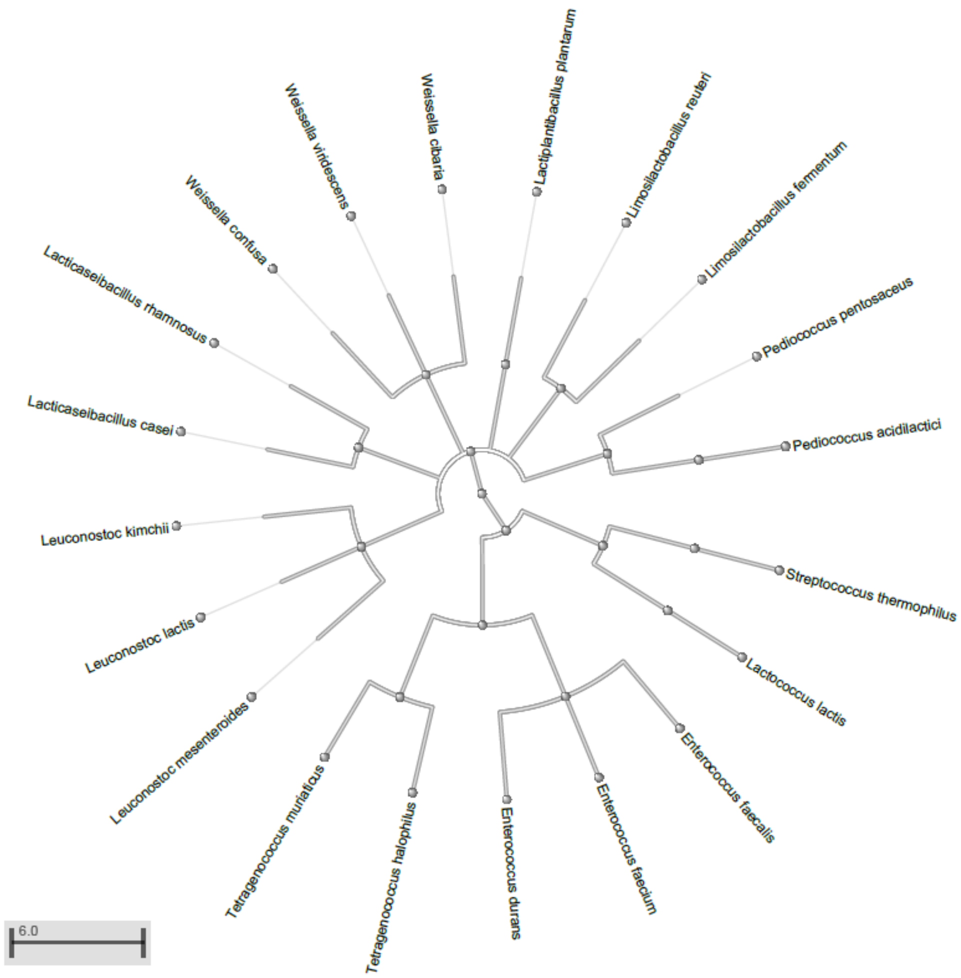

Figure 2.1 Phylogenetic tree of lactic acid bacteria (LAB) and related genera, highlighting their evolutionary relationships.

LAB produces an acidic environment that suppresses the growth of potentially harmful microorganisms in the early stages of food fermentation (Min et al., 2023). To produce energy through the fermentation of carbohydrates, LAB which are typically non-motile, and cell division happens in one plane use endogenous carbon sources rather than oxygen as the final electron acceptor where peroxidases shield them from oxygen byproducts like hydrogen peroxide, making them aerotolerant, except for *pediococci* (Mokoena, 2017). LAB is being employed in the food industry including the beverage industry as antioxidants, food preservatives and fermentation agents and they play a crucial role in both food-related and non-food applications. The growth and acidification of LAB in fruits are influenced by factors such as the level of fermentable carbohydrates, the highly acidic conditions, anti-nutritional elements, inhibitory substances like tannins and phenolics, and non-digestible nutrients including fiber, inulin, and fructo-oligosaccharides (Ruiz Rodríguez et al., 2021)

Lactic acid bacteria used as fermentation strains should possess key metabolic traits, including the ability to produce acid and aroma, hydrolyze proteins, generate viscous exopolysaccharides, and suppress the growth of other bacteria (Wang et al., 2021). Reported by Mantzourani et al., (2019), the production of lactic acid in the pomegranate juice fermented by *L. plantarum* ATCC 14917 at 24 hours is 1.26 ± 0.07 g/L. According to Abedi & Hashemi (2020), the production and efficiency of lactic acid are impacted by factors such as pH levels (3.5 to 9.6), temperature (5 to 45°C), the availability of nutrients like amino acids, peptides, nucleotides, and vitamins, and the specific strain of LAB used. The notable range of lactic acid to legally permitted for food applications is based on its proven antimicrobial action against pathogens and the resulting shelf-life extension (Ameen, 2020). There is no universally established maximum consumption level for lactic acid produced by LAB in fermented foods like fruit juice. The amount considered safe typically depends on the individual's tolerance and the specific context of consumption. Lactic acid serves as an alternative energy source, a signaling molecule that regulates gene expression and protein synthesis, and a pH balance regulator in the body (Kumari, et al., 2024). But too much of it could be uncomfortable for the digestive system, particularly for those who are sensitive to lactic acid or have gastrointestinal issues already. However, the body employs mechanisms to neutralize and clear excess lactic acid, maintaining the pH balance (Kumari, et al., 2024).

When selecting candidate LAB for fermenting fruit juice, factors such as the ability to grow and acidify the juice, enhance safety by eliminating pathogens, and avoid biogenic amine production must be considered (Garcia et al., 2020). *Lactobacillus* spp. is a commonly used genus in food fermentation due to its high acid tolerance, efficiency, and productivity, and its strains are commercially valuable because they can be engineered for targeted lactic acid production (Abedi & Hashemi, 2020). They can grow at pH levels as low as 3.5 to 3.8 and are generally more resistant to acidic conditions than other LAB, have an ecological competitive advantage, allowing them to thrive and dominate the final stage of many lactic acid fermentations when the pH drops too low for other LAB (Ameen, 2020). Effective LAB typically possesses properties such as the ability to adhere to human epithelial cells, lack of hemolytic and cytotoxic activity, acid and bile tolerance, and antagonistic activity against potential pathogenic bacteria, making them good probiotics (antimicrobial) (Ameen, 2020). Table 2.1 shows several products of lactic acid fermentation.

Table 2.1 Several products of lactic acid fermentation

Products	Commonly used microorganisms in the fermentation process	Biological Activity	References
Yogurt	*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus plantarum*	Plain yogurt displays antioxidant properties while fermentation of yogurt can generate small peptides and various amino acids with antioxidant effect. Consuming yogurt can strengthen the immune system, reduce the risk of cancer, help with weight management, and improve the absorption of vitamins and minerals.	Jovanović et al., (2020); Rashwan et al., (2023); Yang et al., (2023)
Kefir	*Lactobacillus kefiranofaciens*, *Lactiplantibacillus plantarum* (basonym *Lactobacillus plantarum*), *Lacticaseibacillus paracasei* (basonym *Lactobacillus paracasei*), *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Kluyveromyces marxianus* ssp. *Marxianus*, *Saccharomyces cerevisiae*, *Candida kefyr*, and *S. unisporus*,	Antibiotics, lactic acid and bactericides that are produced by microorganisms in kefir grains prevent the growth of pathogenic and degrading microorganisms in kefir milk. Kefir-isolated bacteria and yeast exhibit antimicrobial activity against spoilage fungi and enteropathogenic bacteria both in vivo and in vitro.	Azizi et al., (2021); González-Orozco et al., (2022); Prado et al., (2015);
Kombucha	*Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, *Komagataeibacter*, *Lactobacillus*, *Lactiplantibacillus*, *Lacticaseibacillus*, *Brettanomyces*, *Candida*, *Saccharomyces*, *Zygosaccharomyces*	High content of bioactive compounds, strong antioxidants and have detoxifying and antimicrobial effects. Kombucha fermentation combines alcohol, lactic and acetic acid processes.	Antolak et al., (2021); Içen et al., (2023); Jakubczyk et al., (2022); Villarreal-Soto et al., (2018)

Table 2.1 Continued

Products	**Commonly used microorganisms in the fermentation process**	**Biological Activity**	**References**
Fermented fruit and vegetables	*Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus lactis* subsp. *lactis, Lactobacillus buchneri, Lactobacillus paracasei, Lactobacillus pentosus, Lactobacillus helveticus, Lactobacillus acidophilus*	Provide nutritional and health benefits including controlling the body's microecological balance and gastrointestinal flora, regulating immunity, reducing the risk of cancer, cardiovascular disease, age-related macular degeneration, and coronary condition, promoting biological antagonism interaction, preventing free radical formation and lowering blood pressure.	Ajibola et al., (2023); Sajjad, et al., (2020); Saud et al., (2024); Swain et al., (2014)
Sour beer	*Lactobacillus brevis, L. buchneri* CD034, *Kluyveromyces lactis, Lachancea thermotolerans*	Advantages from biological acidification include smoother hop bitterness, increased zinc bioavailability and flavor stability, leading to notable variations in the sensory attributes of sour beers.	Ciosek et al., (2020); Dysvik et al., (2019); Piraine et al., (2021)
Cheese	*Lactobacillus rhamnosus, S. thermophilus, L. helveticus, Lacticaseibacillus casei, Lacticaseibacillus paracasei, Lacticaseibacillus rhamnosus,*	Starter LAB initiates curd formation by acidifying milk and curd, altering casein hydration and affecting the cheese's texture and structure, while their metabolites, which acidify the environment, inhibit undesirable microorganisms and remain in the food to prevent harmful bacteria from proliferating. Flavor compounds in cheese depend on the enzymes of each fermenting strain and their metabolic interactions, which enhance flavor quality and variety.	Bettera et al., (2023); Coelho et al., (2022); Li et al., (2020); Neviani et al., (2024); Zheng et al., (2021)

2.1.2 Advantage of fermentation

Fermentation enhances the nutritional value of foods by synthesizing vitamins, proteins, and essential amino acids, improving the digestibility of protein and fiber, increasing the bioavailability of micronutrients, and breaking down anti-nutritional factors (Sharma et al., (2020); Magala et al., 2015) Additionally, fermentation enhances food safety by reducing harmful substances, such as aflatoxins, and producing antimicrobial agents like lactic acid, bacteriocins, and ethanol, which help inhibit or eliminate food-borne pathogens (Magala et al., 2015). Food fermentation processes are advantageous because they; i) maintain and enhance food safety, primarily due to the formation of organic acids, ethanol, and antimicrobial compounds; ii) enhance nutritional value; and iii) maintain organoleptic quality (Ruiz Rodríguez et al., 2021)

As reported by Şanlier et al., (2019), fermentation increases the product shelf life, especially highly perishable food, improves the food's organoleptic qualities, protein and carbohydrate digestion, and vitamin and mineral bioavailability. It is also described by Şanlier et al., (2019) that fermented food has several health benefits including reducing the risk of hypertension, diabetes, obesity, high cholesterol, thrombosis and diarrhea. Similarly, Melini et al., (2019) discussed that fermented fruit juices show improved nutritional properties and health benefits through changes in vitamin content due to chemical alterations in the raw ingredients, and their probiotic activity may also enhance the gut microbiome, potentially influencing brain and central nervous system functions.

2.2 Fermentation of fruit juices

Fermented fruit juice is a functional fermented food with numerous health benefits where there is clinical evidence that suggests that fruit constituents are beneficial to human health and help to prevent degenerative processes caused by oxidative stress (Shet et al., 2017). Pinto et al., (2022), Shet (2017) and Slavin & Lloyd (2012) state that fruits are recommended for their dietary fiber, high antioxidant phytonutrients (like phenolics, carotenoids, and vitamins), and their rich concentrations of vitamins (especially C and A) and minerals (potassium, magnesium and iron). However, they are low in selenium and sodium (Pinto et al., 2022). There have been several studies on the fermentation of fruit juices, as shown in the Table 2.2. Starter strains must ensure good yields, viability for production, and application, with survival and colonisation in the host being essential for probiotic classification (Garcia et al., 2020). Table 2.2 shows the fermentation conditions and improvement of the nutritional properties of fruit juices by lactic acid bacteria.

Table 2.2 Fermentation conditions and improvement of the nutritional properties of fruit juices by lactic acid bacteria

Fruit juices	Bacteria used	Process condition	Observation	References
Blueberry	*Lactobacillus plantarum* J26	• 25 mL of juices were used. • The juice was supplemented with 0.75g (3 g/100 ml, w/v) of skim milk powder and 1.5g (6 g/100 ml, w/v) of glucose. • Inoculation of the strain with 1 mL (washed twice with 0.01 mol/L phosphate buffered saline (PBS, pH7.4)) • Incubated at 37°C for 24 h.	The phenolic content increased by 43.42% after fermentation and the anthocyanins content increased by 15.38%. The increased in the inhibition of α-glucosidase and α-amylase after fermentation process took place. The ability to scavenge DPPH, superoxide anion, and hydroxyl radicals improved significantly.	Zhang et al., (2021)
Loquat	*L. plantarum*, *L. acidophilus*	• The loquat flesh was blended with distilled water at a 1:2 (w/w) ratio, along with 0.08% (w/w) ascorbic acid, using a Y91A	Fermentation of loquat juice with *L. plantarum* and *L. acidophilus* significantly enhanced its antioxidant activity, phenolic content, and flavonoid content, while enriching bioactive metabolites such as phenolic	Meng et al., (2022)

Table 2.2 Continued

		blender (Joyoung, Hangzhou, China). Juice samples (90 mL each) were then pasteurized at 85 °C for 20 minutes. • The strain was inoculated into the bottles at a final concentration of 1 × 10^7 CFU/mL under sterile conditions • Incubated at 36°C for 48 h.	acids, flavonoids, and organic acids. Fermentation also reduced undesirable compounds and promoted the biosynthesis of health-beneficial metabolites.	
Apple (Red Fuji apple)	*L. acidophilus* BNCC 185342, *L. casei* ATCC 393, *L. plantarum* BNCC 337796	• 0.15% ascorbic acid was added during crushing. The juice's soluble solid content and pH were adjusted to 13% and 6.5 using glucose and NaOH. • The juice was sterilized at 110°C for 10 minutes before fermentation.	The LAB strains demonstrated robust growth in apple juice, with viable cell counts increasing from 7.5 to 8.3 log CFU/mL, lactic acid production rising to 4.2 g/L, and a pH reduction from 5.5 to approximately 3.8. Fermentation significantly enhanced the antioxidant and antibacterial properties of the juice, attributed to the metabolism of phenolic and organic acids. The total amino acid content of the fermented	Yang et al., (2022)

Table 2.2 Continued

		• Inoculation of 8 mL strains into 400 mL juice (0.02%), with initial viable bacterial count in apple juice was about 7.5 log CFU/mL. • Incubated at 37°C for 72 h.	juice significantly increased after 30 days of storage at 4°C. The stored fermented juice retained its antibacterial and antioxidant activities *in vitro*.	
Papaya	*L. plantarum*, *L acidophilus*	• 45% papaya puree, 45% distilled water, 5% edible glucose, and 5% skim milk powder – heat sterilized at 90°C for 10 min • Inoculation of strains – 5% • Incubated at 37°C for 48 h.	Fermented papaya juice with *L. plantarum* demonstrated changes in the antioxidant activity, with increased DPPH (77.39% to 86.25%), stable ABTS (inhibition >80%), and improved CUPRAC (1.26 to 1.57 mM trolox). The lactic acid concentration of papaya juice (266 ± 3 mg/100mL) increase after fermentation process with *L. plantarum* (571 ± 32 mg/100mL) and *L. acidophilus* (543 ± 68 mg/100mL).	Chen et al., (2018)
Orange, tangerine, grapefruit	*Lactobacillus acidophilus, Lactobacillus casei, Streptococcus thermophilus, Bifidobacterium animalis*	• 2.5 mL of 0.5 Mc Farland standard lactic acid bacteria suspensions	Free radical scavenging activity and total phenolic substance values increased whereas pH, aw, dry matter,	Akarca & Baytal (2023)

Table 2.2 Continued

	spp. lactis, Lactobacillus delbrueckii spp. bulgaricus, Lactobacillus delbrueckii spp. lactis, Lactobacillus kefir and lactiplantibacillus plantarum	• Incubated at 30°C for 48 h.	viscosity, brix and L* values decreased.	
Dragon fruit juice	*Lactobacillus plantarum FBS05*	• The inoculum (3% v/v) was prepared by cultivating the strain at 37 °C for 48 h, centrifuging the culture, washing it with filtered water, and adjusting it to a concentration of 10^6 cfu/mL. • Fermentation process: 37°C for 48 h.	Increased in the antibacterial activity of dragon fruit juice threefold and slightly enhanced its antioxidant activity. A mix of 10% fermented juice with 90% fresh juice had the highest acceptability and maintained low microbial load with a stable shelf life of three months at 8 °C.	Muhialdin et al., (2020)
Watermelon	*Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus brevis and Pediococcus pentosaceus*	• Inoculation of bacteria with 1% (v/v) • 30°C for 24 h (*L. plantarum, L. brevis and P. pentosaceus)* • 37°C for 24 h (*L. rhamnosus, L. casei)*	The concentrations of alcohols, ketones, monoterpenes, furans increased but aldehydes and alkanes decreased.	Mandha et al., (2021)

Table 2.2 Continued

Fermented star fruit juice	*Lactobacillus helveticus* L10, *Lactobacillus paracaei* L26, *Lactobacillus rhamnosus* HN001	• The star fruit juice with initial °Brix of 7.09 and pH of 3.58, which was adjusted to 5.9 using 1 mol/L NaOH. It was then aseptically filter-sterilized using 0.65-µm and 0.45-µm polyethersulfone membranes. • Inoculation of bacteria with 10% (v/v) • Incubation at 37°C for 48 h	The fermentation process led to a significant increase in antioxidant activity. This enhancement is attributed to the metabolic activities of the probiotic strains, which may produce bioactive compounds with antioxidant properties during fermentation. The fermented star fruit juices exhibited notable antibacterial activity, which is likely due to the production of organic acids and other antimicrobial substances by the probiotic lactobacilli during fermentation.	Lu et al, (2018)

Recently, there has been a rise in the production of fermented drinks from various fruits like oranges, mangos, pineapple, apples and raspberries, with probiotics often added to enhance their nutritional value due to increasing consumer demand (Žuntar et al, 2020; Pinto et al., 2022).

Fruit juices contain trace amounts of vitamins, minerals and nitrogenous compounds, along with water, carbohydrates and organic acids, and the juice's flavor comes from organic acids, sugars and phenolic; on the other hand, yeast growth and fermentation are often dependent on minerals, nitrogenous compound and vitamins (Shet et al., 2017). According to Shet et al., (2017), compared to fruit juice, fermented fruit juice is composed similarly, but it contains a wider variety of minor ingredients and much less sugar.

Commercial starter cultures are standardized inoculants employed in fermented food production to ensure quality and control, streamline the fermentation process, and minimize errors, with the selection of these cultures significantly impacting the quality and flavor of fermented fruit and vegetable juices due to each produce distinct dominant flora influenced by its particular physical and nutritional conditions (Saud et al., 2024).

2.3 Probiotic used in the beverage industry

Probiotics are tiny organisms that produce valuable impacts on human health when it is being consumed in sufficient quantities. According to Food Act 1983, Regulation 26A, probiotic cultures means live microorganisms which when administered in adequate numbers confer health benefits on the host. It promotes health to the gut flora, enhances and maintains the immune system of humans. Consuming probiotics has been shown to have an adverse effect on certain common diseases, including obesity, type 2 diabetes, autism, osteoporosis, irritable bowel syndrome, infections of the digestive tract, lactose intolerance, allergies, urogenital tract infections, cystic fibrosis, various cancers and some immunological disorders, which cause the progression of the disease to be delayed (Bodke & Jogdand, 2022; Ranjha et al., 2021). The action of probiotics against microorganisms is related to their capacity to alter the immune response of the host, combat pathogenic microbes, or compete for adhesion sites with them (Bodke & Jogdand, 2022).

Aseptic dosing technologies currently available allow for the direct inoculation of probiotics into fruit or vegetable juice where microencapsulation, vacuum impregnation, and prebiotics are used to keep probiotics alive for the duration of products (Ionela Istrati et al., 2019). There are several species and genera of bacteria, yeast and molds in probiotics including *Lactobacillus, Bifidobacterium, Streptococcus, Aspergillus niger*, *Leuconostoc, Bacillus, Pediococcus, Saccharomyces cerevisiae*, and *Enterococcus*, and among these, the most commonly used probiotic belong to lactic acid bacteria which are primarily represented by *Bifidobacterium* and *Lactobacillus* (Al-Yami et al, 2022). LAB produce lactic acid as the result of their metabolic activities.

Lactobacillus spp. often chosen as probiotics to make dairy products like cheese and yogurt because they exhibit essential properties such as high acid and bile tolerance, resistance to low pH and gastric juice, including harsh intestinal conditions, the ability to adhere to an intestinal surface and colonize the gastrointestinal system, act as an antibacterial, antagonistic, and inhibiting defence against potentially pathogenic species, antibiotic resistance, removing cholesterol and exopolysaccharide production (Fijan, 2014; Haghshenas et al., 2023; Somashekaraiah et al., 2019). They