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CHARACTERISTICS OF LACTOBACILLUS HELVETICUS FERMENTATION OF CAMEL AND BOVINE MILKS

Kobika Chelladhurai

MASTER THESIS NO. 2024: 80

College of Agriculture and Veterinary Medicine

Department of Food Science

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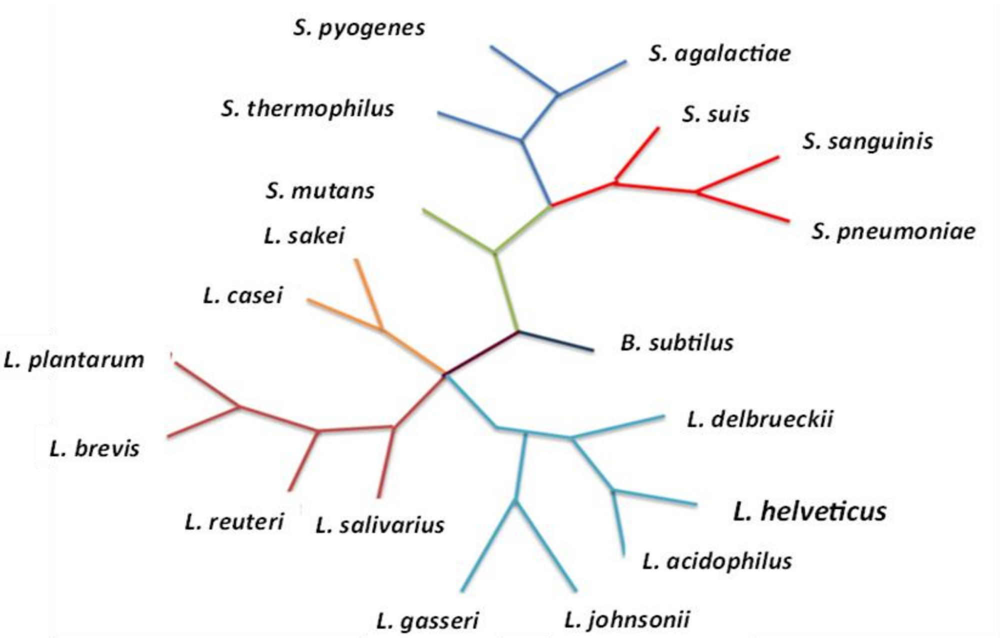

June 2024

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This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Food Science

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Cover: Phylogenetic super tree of 11 selected *Lactobacillus* bacteria

(Photo: By Kobika Chelladhurai)

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Declaration of Original Work

I, Kobika Chelladhurai, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Characteristics of Lactobacillus Helveticus Fermentation of Camel and Bovine Milks*", hereby, solemnly declare that this is the original research work done by me under the supervision of Prof. Afaf Kamal-Eldin, in the College of Agriculture and Veterinary Medicine at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma, or similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged by appropriate academic conventions. I further declare that there is no potential conflict of interest concerning the research, data collection, authorship, presentation, and/or publication of this thesis.

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Abstract

In this study, the fermentation of low-fat camel milk (CM) and bovine milk (BM) with *Lactobacillus helveticus* was compared to fermentation with *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* at 28°C, 35°C, and 42°C. The fermentation activities were followed by measuring bacterial counts, titratable acidity, pH, and changes in lactose, organic acids, and proteolysis for 96 hours. Although bacterial growth was comparable in CM and BM, the fermented milks showed differences in acidification and proteolysis. Negative correlations between TA (%) and pH were consistent in both CM and BM suggesting similar buffering capacities. In both milks, *L. helveticus* behavior was more comparable to *S. thermophilus* than *L. delbrueckii* subsp. *bulgaricus*. These findings provide valuable insights into the dynamics of *L. helveticus* offering possibilities for alternative fermentation processes in dairy product production.

Keywords: Milk, Fermentation, Lactic acid bacteria, *L. helveticus*, Acidification, Proteolysis.

Title and Abstract (in Arabic)

خصائص تخمير حليب الإبل والبقر بولسطة باكتيريا *Lactobacillus helveticus*

الملخص

في هذه الدراسة تم مقارنة عملية تخمر حليب الإبل والبقر قليل الدسم بواسطة باكتيريا *Lactobacillus helveticus*، *L. delbrueckii* subsp. *bulgaricus* و *S. thermophilus* عند درجات حرارة 28، 35، و42 درجة مئوية. تمت متابعة أنشطة التخمير عن طريق قياس أعداد الباكتيريا والحموضة القابلة للممعايرة والأس الهايدروجينى والتغيرات في اللاكتوز والأحماض العضوية والتحلل البروتيني لمدة 96 ساعة. على الرغم من أن النمو البكتيري كان متشابهًا في الحليبين إلا أنهما أظهرا اختلافات في درجة الحموضة وتحلل البروتينات. وقد كانت الارتباطات السلبية بين درجة الحموضة (٪) والأس الهايدروجينى متشابهة في كل من الحليبين. وفي كلا الحليبين، كان سلوك *L. helveticus* أقرب الى سلوك *S. thermophilus* منه الى سلوك *L. delbrueckii subsp. bulgaricus*. توفر هذه النتائج رؤى قيمة حول ديناميكيات *L. helveticus* فى تخمير الحليب وقد توفر إمكانيات لعمليات تخمير بديلة في إنتاج منتجات الألبان.

مفاهيم البحث الرئيسية: الحليب، التخمير، باكتيريا حمض اللاكتيك، L. helveticus، التحمض، تحلل البروتينات.

Author Profile

Kobika Chelladhurai is a graduate research student at the United Arab Emirates University, UAE. She received her bachelor's special degree in Food Science and Technology from the Sabaragamuwa University in Sri Lanka. She has published the literature review of this thesis under the title "*Lactobacillus helveticus*: Health effects, current applications, and future trends in dairy fermentation" in Trends in Food Science and Technology. She presented a poster at the UAE Microbiome Conference (2023) and oral presentations at the 8^{th} International Conference on Climate Change (2024) and UAE GSRC (2024). Kobika lives with her family in Al Ain city, UAE.

Acknowledgments

I extend my heartfelt gratitude to my esteemed committee for their invaluable guidance, unwavering support, and insightful assistance throughout the entire process of crafting this thesis. I am particularly indebted to my dedicated advisor, Prof. Afaf Kamal-Eldin, whose expertise and encouragement have been instrumental in shaping the trajectory of my research.

I am grateful to UAE University for its financial support, which enabled me to pursue and complete this research.

Finally, I would like to acknowledge the understanding and encouragement provided by my family during this challenging journey. Their unwavering support has been a source of strength and motivation.

This thesis is the result of the collaborative efforts and support from many individuals, and I am sincerely thankful for every contribution.

Dedication

To my beloved family, Dhruvit, and Prof. Afaf Kamal-Eldin

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List of Abbreviations

ACE	Angiotensin Converting Enzyme
ANOVA	Analysis of Variance
BM	Bovine Milk
CM	Camel Milk
DH	Degree of Hydrolysis
EPS	Extracellular Polysaccharides
HPLC	High-Performance Liquid Chromatography
OPA	o-Phthalaldehyde Assay
PCA	Principle Component Analysis
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
TA	Titratable Acidity
TA%	Titratable Acidity Percentage

Chapter 1: Introduction

1.1 Overview

Yogurt is a fermented milk product enjoyed worldwide for its delightful taste. (Aryana & Olson, 2017; Mehra et al., 2022). It holds a prominent place in various culinary traditions across the globe, serving as a staple in many diets (Gaur et al., 2018). Yogurt's versatility is evident in the diverse dishes and beverages it uses across different cultures (McKinley, 2005). Beyond its appealing taste and creamy texture, yogurt is recognized for its exceptional nutritional profile. It is a rich source of protein, calcium, B vitamins, and probiotics (Aryana & Olson, 2017; Fazilah et al., 2018). The increasing awareness of yogurt's potential health benefits has positioned it as a functional food, valued for its contribution to gut health, weight management, and the alleviation of lactose intolerance symptoms (Abdi-Moghadam et al., 2023).

Milk can ferment due to various types of lactic acid bacterial strains as noted earlier (Fazilah et al., 2018) and these types contribute in a great way towards the sensory characteristic of fermented milk products as well as supporting immunological function in the intestines in addition to digestive health (Sharma et al., 2020; Yerlikaya et al., 2020). Even though *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the most common bacteria used in making yogurt, there are other important strains as well (McKinley, 2005; Yerlikaya et al., 2020). For instance, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *lactis*, *Bifidobacterium bifidum*, and *Lactobacillus casei* are among the additional bacteria frequently employed (Talwalkar & Kailasapathy, 2004). Notably, *L. helveticus* stands out due to its significant proteolytic activities, contributing to improved flavor and texture in yogurt (Fan et al., 2019). This bacterium is gaining attention for its potential to generate low molecular weight peptides during fermentation, adding an intriguing dimension to the diverse array of bacteria used in yogurt production. The main effects of *L. helveticus* on dairy products quality are summarized in Figure (1.1).

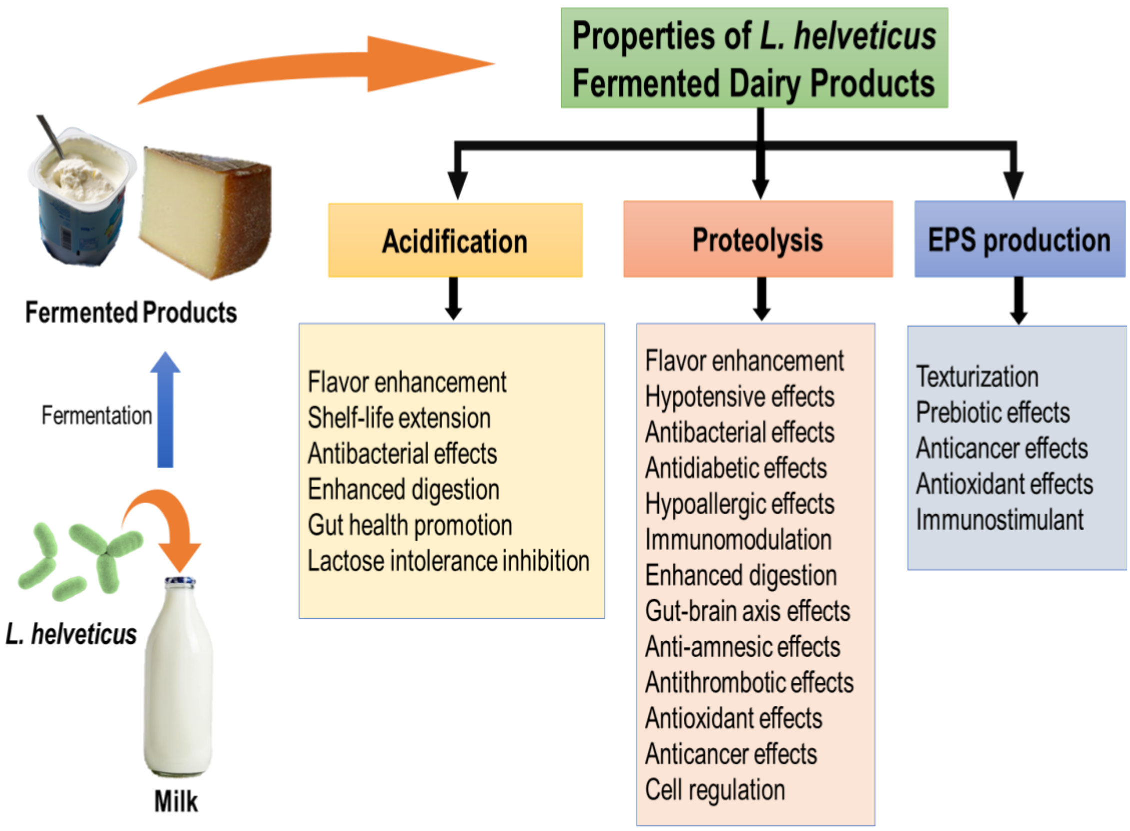

Figure 1.1: Benefits of adding *L. helveticus* to milk (Fan et al., 2019; Ge et al., 2022; Zago et al., 2012)

The lactic acid bacteria (LAB) have close phylogenetic links (Figure 1.2) and similar metabolic pathways. *L. helveticus* is closely related to *L. acidophilus* (98.4% sequence identity). Both are phylogenetically related to some gut bacteria and can survive in the gut and dairy environments (O'Sullivan et al., 2009). *L. helveticus* and *Lactobacillus acidophilus* are also closely related to *L. delbrueckii subsp. bulgaricus* (Callanan et al., 2008). The existence of niche varieties among closely related species shows that they have undergone significant genetic change during their evolution (O'Sullivan et al., 2009). The types and concentrations of available sugars and the capacity of the bacteria to deal with stress conditions are crucial environmental determinants for the bacterial niche population (Slattery et al., 2010). *L. helveticus* is acid-tolerant, generally non-spore-forming, and is linked by physiological and metabolic properties. Due to its ability to resist diverse environmental challenges such as high temperatures, low pH, osmotic pressure, and oxygen, *L. helveticus* may adapt to industrial fermentation environments more quickly than most probiotic lactobacilli and bifidobacterial, which allows its introduction into novel probiotic formulae (Taverniti & Guglielmetti, 2012). Biotypes isolated from the same niche have been shown to differ greatly and many of the bacteria's technological characteristics are strain specific (Griffiths & Tellez, 2013; Schuster et al., 2020).

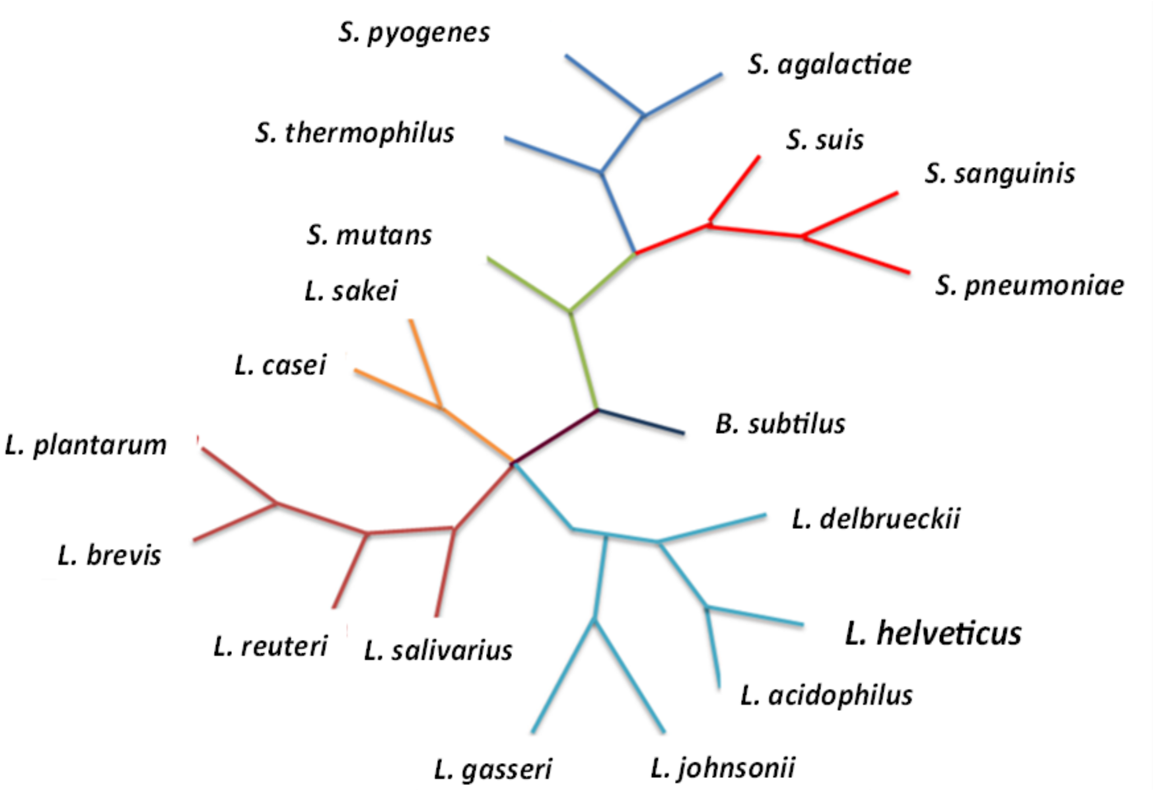

Figure 1.2: Phylogenetic super tree of 11 selected *Lactobacillus* bacteria (adapted from Callanan et al., 2008)

Different strains of LAB prefer specific temperatures for optimum growth. For example, thermophilic species like *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* grow best at higher temperatures approximately 40-45 degrees Celsius that are commonly used in making yogurt and other heat-treated dairy products (Radke-Mitchell & Sandine, 1986; Slattery et al., 2010). While the optimal range for growth is higher in temperature for such strains, their growth rate becomes slower when the temperature decreases.

1.2 Camel Milk Fermentation

Camel milk (CM) holds significant importance in societies where camels are prevalent, often serving as a staple food and symbolizing hospitality and well-being

(Agyei et al., 2020). Traditional practices often admire the assets of CM, attributing it to various medicinal properties and healing benefits (Cheikh Ismail et al., 2022). Over the past few years, studies have started to support the idea that camel milk can provide health benefits for conditions like diabetes, autoimmune diseases, and stomach problems (Abd El-Aziz et al., 2022; Seifu, 2023). Moreover, CM production is also known for its sustainability, especially in dry lands where camels can survive even in tough conditions (Pak et al., 2019). Their ability to tolerate water scarcity and harsh climates makes camels valuable assets to communities dependent on livestock for nutrition and livelihoods (Asiimwe et al., 2020). As awareness of CM's nutritional benefits continues to grow, efforts to promote its consumption and expand its availability are underway, offering promise for improved health outcomes and economic opportunities in regions where camels roam.

CM holds significance in fermentation practices due to its probiotic potential and cultural importance (Ipsen, 2017). CM is used as a substrate to make different fermented products like cheese, and fermented milk (Al-Zoreky & Al-Otaibi, 2015). The fermentation process not only prolongs the shelf-life of CM but also improves its digestibility and nutritional content. Beyond its practicality, raw and fermented CM products carry cultural significance in regions where camels are prevalent, forming part of traditional diets and offering unique flavors cherished by communities (Seifu, 2023). Fermentation improves the absorption of nutrients supports gut health and strengthens the immune system, highlighting the numerous health advantages of including fermented CM products in the diet (Ipsen, 2017; Seifu, 2023). CM and bovine milk (BM) yogurts offer distinct characteristics in terms of texture, flavor, and nutritional content. CM yogurt tends to be thinner in consistency with a smoother texture and may possess a slightly tangy flavor profile with hints of sweetness, reflecting the unique composition of CM (Sobti et al., 2019). In contrast, BM yogurt is known for its thicker and creamier texture, accompanied by a milder, slightly tart taste characteristic of yogurt made from cow's milk. The reason CM doesn't form firm, thick gels is primarily due to the absence of β-lactoglobulin and lower levels of κ-casein within its composition (Seifu, 2023; Sobti et al., 2019). Thus, CM fermentation represents a link of nutritional, cultural, and health-related values, enriching both dietary practices and scientific exploration in the scope of food science.

1.3 Statement of the Problem

In the context of dairy fermentation, particularly in the production of fermented milk products, there is a need to find alternative lactic acid bacteria that is able to offer sensorial as well as nutritional benefits and widen the range of available dairy products. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are the most common bacteria used in dairy fermentation. *L. helveticus* is a distinct bacteria characterized by high proteolytic activity and demands exploration. Previous studies have extensively investigated the fermentation capabilities of *L. helveticus* in fermented bovine milk products, emphasizing its role in enhancing flavor profiles and bioactive compound production (Begunova et al., 2020; Gandhi & Shah, 2014; Hassan et al., 2020; Li et al., 2019; Lukic et al., 2014; Tak et al., 2018; Zhou et al., 2019). Since *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are known to behave differently in camel than in bovine milk (Sobti et al., 2019, 2024), there is a notable gap in understanding the fermentation behavior of *L. helveticus* in the context of camel milk fermentation.

1.4 Research Aims

1. To evaluate the growth kinetics of three lactic acid bacteria strains *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* during the fermentation of CM and BM at three temperatures (28°C, 35°C, and 42°C).
2. To compare the effect of fermentation by the different bacteria on changes in titratable acidity (TA), lactose, organic acids, pH, and proteolysis during fermentation.

Chapter 2: *Lactobacillus helveticus*: Health Effects, Current Applications, and Future Trends in Dairy Fermentation

Redrafted from

Chelladhurai, K., Ayyash, M., Turner, M. S., Kamal-Eldin, A., 2023. *Lactobacillus helveticus*: Health effects, current applications, and future trends in dairy fermentation. Trends in Food Science and Technology 136, 159–168. https://doi.org/10.1016/J.TIFS.2023.04.013

2.1 Abstract

Background: Lactobacillus helveticus is a homofermentative, thermophilic starter bacterium commonly used in dairy processing to produce cheese and fermented milk. It is known for enhancing flavor and texture and improving the final products' health benefits. L. helveticus has a number of characteristic features that differentiate it from commonly used starter cultures, including the ability to metabolize galactose, high acidification rate, and strong proteolytic activity.

Scope and Approach: This article aims to review the important features of L. helveticus relevant to the dairy industry, i.e., technological advantages, health effects, current applications, and future trends in dairy fermentation. It provides an overview of the health benefits associated with the consumption of L. helveticus-fermented dairy products, which are enriched in bioactive components. The review covered some important strains, bioactive peptides, and their health advantages.

Key Findings and Conclusions: L. helveticus is characterized by strong proteolytic activity that leads to the production of technologically- and physiologically active peptides that contribute to products' flavor and health benefits. The consumption of L. helveticus-fermented dairy products was shown to contribute bioactive peptides with prebiotic, antimicrobial, antioxidant, antihypertensive, anticarcinogenic, gut-wellness, psychobiotic, and immunostimulatory effects. The fusion of L. helveticus-fermented milk products with clean-label trends, such as natural, healthy, and free from additives or preservatives, has the potential to cater to consumer demands and foster expansion in the dairy market. L.

helveticus is, thus, a potential bacterium for use in future starter cultures designed for the production of functional dairy products.

Keywords: *Lactobacillus helveticus*, Milk, Fermentation, Bioactive compounds, Health benefits.

2.2 Introduction

Lactobacillus helveticus is a Gram-positive rod-shaped lactic acid bacterium (Shi et al., 2018) commonly isolated from milk and fermented dairy products (Moser et al., 2018). It is an industrial homofermentative, thermophilic, non-spore-forming starter bacterium (optimum growth temperature, 42ºC) with a generally recognized safe status (Hassan et al., 2020; Kittibunchakul et al., 2019). Notably, *L. helveticus* prevents bitterness and provides nutty flavors in its fermented products (Garbowska et al., 2021; Oberg et al., 2022). Increasing evidence indicates that *L. helveticus* strains exhibit probiotic effects and health-improving qualities, which mark their industrial usefulness (Fontana et al., 2019; Skrzypczak et al., 2020; Toropov et al., 2020).

Owing to the recent rise in the interest of consumers in leading healthy lives, there is an excellent market opportunity for *L. helveticus*–fermented milk products (Skrzypczak et al., 2020). Food industries are currently limiting preservatives and artificial additives while investigating other ways to increase the natural flavors, quality, and safety of dairy and dairy-based products. Previous studies have reported that optimized application of *L. helveticus* strains may produce various functionally improved dairy products, thus showcasing its impact on product quality and public health (Bahrudin et al., 2020; Skrzypczak et al., 2021). However, it is important to note that the different features exhibited by this bacterium, including aroma formation in cheeses, are strain-dependent (Skrzypczak et al., 2020).

L. helveticus is used instead of *L. delbrueckii ssp. bulgaricus* to produce certain cheeses, such as aged Swiss and Italian hard cheeses, as well as fermented beverages (Oberg et al., 2022) with the main aim to improve their flavors. Owing to the high functional, probiotic, and proteolytic properties of *L. helveticus* and its ability to produce bioactive peptides with therapeutical potential (see below), it is worthwhile to evaluate the advantages of *L. helveticus*–fermented products in the dairy industry. Recently, research interest in the potential of different bacterial strains in food fermentation has increased. Different strains of *L. helveticus* are known to have numerous health benefits, including improved gut health, immune function, and mental health as recently reviewed (Verma et al. 2023).

The present review article aimed to focus on the ability of *L. helveticus* to influence the flavor and texture of fermented dairy products (mainly cheese and yoghurt) as well as its potential to contribute bioactive peptides with prebiotic, antimicrobial, antioxidant, antihypertensive, anticarcinogenic, gut-wellness, psychobiotic, and immunostimulatory effects. Furthermore, this review article also discussed the mechanisms involved in the technological and health-promoting effects of *L. helveticus*.

2.3 Taxonomy and Biodiversity

Lactic acid bacteria (LAB) include several dairy-related bacteria, such as lactococci, streptococci, and lactobacilli (Oberg et al., 2022). LAB exhibit close phylogenetic links and similar metabolic pathways among themselves. Recently, the diverse genus *Lactobacillus*, with ~261 distinct species, has been reclassified into 25 different genera (Zheng et al., 2020). Alongside the revised genus *Lactobacillus*, the new genera include *Lacticaseibacillus, Lactiplantibacillus, Latilactobacillus, Lentilactobacillus, Levilactobacillus, Ligilactobacillus, Limosilactobacillus, Loigolactobacillus*, *Paucilactobacillus,* C*ompanilactobacillus*, *Amylolactobacillus*, and *Lapidilactobacillus.* As members of the amended *Lactobacillus* genus, *L. helveticus* and *L. acidophilus* were identified as closely related (>98 sequence identity across the entire genome); both being phylogenetically associated with certain gut bacteria and can survive in gut and dairy environments (Fontana et al., 2019). Additionally, the genome of *L. helveticus* has been closely linked to *L. delbrueckii* subsp. *bulgaricus* (Zheng et al., 2020).

The types and concentrations of available sugars constitute crucial environmental determinants for bacterial niche populations and their capacity to deal with stress conditions, such as low pH, oxygen, osmotic pressure, and high temperatures (Skrzypczak et al., 2020; Zhong et al., 2021). *L. helveticus* can potentially adjust to dairy and dairy-based fermentation environments faster than numerous other probiotic bifidobacteria and lactobacilli, thus allowing its inclusion in novel probiotic formulas that can withstand diverse environmental challenges (Zago et al., 2021). The adaptation processes of different bacterial strains to changes in their natural habitat circumstances may engender phenotypic and genotypic differences among strains. Even biotypes isolated from the same niche differ considerably, with numerous technological characteristics of the bacteria being strain-specific (Schuster et al., 2020).

The geographic origin of *L. helveticus* plays a significant role in its evolution because the environmental variables, such as temperature, pH, and nutrient availability, affect the growth and survival of different strains (Skrzypczak et al., 2020; Zhong et al., 2021). *L. helveticus* strains inhabit various environments abundant in carbohydrates; with dairy products representing a primary habitat (Schuster et al., 2020). When strains of *L. helveticus* colonize variable ecological niches, they leverage metabolic and temperature adaptations suitable for those habitats (Schuster et al., 2020), e.g. *L. helveticus* 27058 is phenotypically well-suited to the milk environment (Kido et al., 2021). With distinct biotechnological profiles, *L. helveticus* strains may have variable effects on the quality of dairy products (Somerville et al., 2019), e.g. they may play critical roles in the production of compounds with specific flavors and debittering effects in aged cheddar cheeses (Garbowska et al., 2021). Table (1) summarizes the different functional properties and some of the functional properties associated with specific *L. helveticus* strains isolated from dairy and dairy-related products. Genome comparison indicated *L. helveticus* DPC4571 genes as prospective dairy-specific genes, whereas *L. acidophilus* NCFM genes were identified as potential gut-specific genes. The newly sequenced *L. helveticus* strains DSM 20075 and UC1267 demonstrated adaptability to the gut environment (Fontana et al., 2019).

Table 2.1: Characteristic features of selected *L. helveticus* strains isolated from bovine milk and milk products

Strain	**Properties**	**Reference**
B734	Used as adjunct culture in the manufacture of fermented milk products to improve their functional qualities, namely antihypertensive, ACE inhibitory, antithrombotic, antioxidative, antibacterial, immunomodulating, mineral binding, and opioid agonist activities	Skrzypczak et al., 2021
CAUH18	Possesses bioactive properties, specific production of novel exopolysaccharide (EPS), and prevention of intestinal cell aggregation, and pathogen colonization of the gastrointestinal tract	Yang et al., 2016
CD6	Maintain normal liver histology and reduce hyperlipidemia and weight gain caused by a high-fat diet.	Patil et al., 2021
CICC6024	Generates a variety of biopeptides with antihypertensive, ACE inhibitory, DPP IV inhibitory, immunomodulatory, antibacterial, and antioxidative activities	Fan et al., 2019
CPN 4	Produce the hypotensive peptides with ACE-inhibitory activity *in fermented bovine milk*	Baptista et al., 2020
D75, D76,	Have proteolytic and bacteriocin production activities	Toropov et al., 2020

Table 2.1: Characteristic features of selected *L. helveticus* strains isolated from bovine milk and milk products (Continued)

Strain	**Properties**	**Reference**
DSMZ 20075, T80	Possess antioxidant capabilities	Skrzypczak et al., 2018
481, 34.9, EMCC 1654	Possess antibacterial effect	Angelescu et al., 2022; Abdelhamid et al., 2018
H9	This probiotic strain, identified in traditionally fermented yak milk Kurut in China, has good growth during milk fermentation, the highest proteolytic activity out of 121 *L. helveticus* isolates tested and showed favorable antihypertensive effects in rats	Zhang et al., 2014
KLDS 1.8701	Effective in inhibiting oxidative stress and the growth of *Penicillium spp.* and EPS production	Li et al., 2019
MRTL91	Have antibacterial and antiadhesive effects against a variety of pathogenic and nonpathogenic microorganisms and are suitable alternatives to conventional food antibiotics	Sharma & Saharan, 2016
SBT2171	Increases β- defensin expression, improves periodontal disease caused by *Porphyromonas gingivalis*, alleviates collagen-induced arthritis, and attenuates experimental autoimmune encephalomyelitis	Kobatake et al., 2019

Table 2.1: Characteristic features of selected *L. helveticus* strains isolated from bovine milk and milk products (Continued)

Strain	**Properties**	**Reference**
SIM12, SIS16, 1734, and Lh43	Immunomodulatory properties	Zago et al., 2021
SNA12	Regulation of gut microbiota and production of EPS	Wang et al., 2022
T80, T105, B734	Generate variety of biopeptides with antihypertensive, ACE inhibitory, and antithrombotic activities by hydrolyzing α-lactalbumin and caseino-glycomacropeptide	Skrzypczak et al., 2021

2.4 Lactose Catabolism

Having a good tolerance to acid and temperature, *L. helveticus* is a powerful homofermentative milk acidifier that metabolizes lactose to lactic acid as the principal metabolic product (Begunova et al., 2020; Zago et al., 2021). The high acidification of dairy products by *L. helveticus* strains enhances their flavor, texture, and aroma (Akabanda et al., 2014). *L. helveticus* strain MTCC 5463 was reported to thrive in acidic conditions and exhibit optimum growth at pH 5.5 (Kittibunchakul et al., 2019) and markedly decrease the pH at the end of fermentation (Hati et al., 2017). *L. helveticus* BGRA43 was found to survive in simulated gastric and intestinal environments (Lukic et al., 2013). The abilities of *L. helveticus* strains to stand the high temperatures required for cheese cooking (i.e., 55ºC–65ºC) and high salt concentrations (Fontana et al., 2019; Zago et al., 2021) make them good candidates for cheese production.

During bacterial fermentation, milk lactose enters the bacterial cells *via* membrane transport and undergoes intracellular hydrolysis by galactosidase to produce the monosaccharides glucose and galactose (Kittibunchakul et al., 2019). Like other LAB, *L. helveticus* converts glucose to lactic acid *via* glyceraldehyde-3-phosphate, 1,3-diphosphoglycerate, and pyruvate (glycolysis pathway). In addition, *L. helveticus* strains are also able to metabolize galactose and utilize it as a carbon source (Fontana et al., 2019; Kido et al., 2021; Schuster et al., 2020) while other LAB (such as *L. delbrueckii* subsp. *bulgaricus*, *L. lactis*, and *L. acidophilus*) cannot accumulate free galactose in the external media (Oberg et al., 2022). Different behaviors were observed for *L. helveticus* and *L. lactis* in the production of Koumiss (fermented from mare milk), wherein *L. helveticus* used both galactose and glucose as energy sources whereas *L. lactis* was only able to use glucose (Tang et al., 2020; Oberg et al., 2022). In the presence of *L. helveticus*, lactose is depleted after a few days of cheese ripening, which allows avoidance of the growth of undesired secondary bacteria that might negatively affect cheese quality (Akabanda et al., 2014). The ability of *L. helveticus* to produce large amounts of lactic acid from glucose and galactose alongside its high acid tolerance and ability to express complex proteolytic enzymes are important features in cheese production. These enzymes, including endopeptidases, proteinases, and exopeptidases, are critical for reducing ripening time, expediting flavor development, and alleviating bitterness (Zhang et al., 2022).

Owing to its ability to metabolize both glucose and galactose, *L. helveticus* V3 produced more acid from the same amount of lactose than other bacteria that only metabolize glucose, e.g. *S. thermophilus* MD2, *L. acidophilus* (NCDC 15 and NCDC 298), and *L. rhamnosus* (NS6 and NS4) (Hati et al., 2018). Galactose fermentation using single-cell *L. helveticus* strains reduced the brown color of cooked cheese (Ah & Govind, 2017) as a result of reduced Maillard reactions (Xiang et al., 2021). A galactose-positive and lactose-negative adjunct culture, including *L. helveticus* 7995, reduced undesirable gas production, and crack and slit formation, and improved the quality and shelf-life of cheddar cheese (Green et al., 2021). This is due to the production of higher levels of lactic acid from galactose metabolism leading to a lowering of the pH and inhibition of the growth of bacteria, such as clostridia, responsible for gas and crack formation in cheese.

2.5 Proteolytic Activity

L. helveticus is regarded as one of the most efficient LAB in cheese and fermented milk proteolysis in milk environments and thereby considerably contributing to flavor development in these products (Begunova et al., 2020; Schuster et al., 2020). *L. helveticus* harbors cell-envelope–associated proteases and intracellular peptidases, which are released into the cheese matrix during bacterial autolysis (Moser et al., 2018). In terms of protease genes, *L. helveticus* strains exhibit high diversity, including the cell-envelope proteinases PrtH1, PrtH2, PrtH3, and PrtH4 (Zhong et al., 2021).

L. helveticus strains isolated from traditional Mongolian dairy products were found to exhibit high levels of proteolytic activity. In fact, *L. helveticus* is considered an efficient LAB in the proteolysis of α_{S1}-, k-, and β-caseins, and α-lactalbumin and the production of various bioactive peptides, which are functional in fermented milk and cheese (Table 2.2). Peptides extracted from fermented milk were found to be closely related to the strain's growth and acidification capacity in milk (Raveschot et al. 2020). As a result, the selection of specific strains for different applications, such as high acidification and growth rate in milk, may be necessary. Using integrated transcriptomics and proteomics analyses, studies of protein metabolism by *L. helveticus* CICC22171 provided solid foundations for the efficiency and cost-effectiveness of using proteins as nitrogen sources for *L. helveticus* strains (Xu et al., 2021).

Table 2.2: Reported functional peptides produced by *L. helveticus* in bovine milk

Protein	Peptide	Bioactivities	Reference
Bovine β-casein	NIPPLTQTPVVVPPFLQPE	Improves cognitive function	Ohsawa et al., 2017
	DKIHPF	ACE-Inhibitor/Antioxidant	Begunova et al., 2020
	VVPPFLQPE	ACE- Inhibitor	
	NIPPLTQTPV	ACE- Inhibitor	
	TQTPVVVPPFLQPE	Antioxidant	
	DVENLHLPLPLLQSWM	ACE- Inhibitor	
	LHLPLPLLQSW	ACE- Inhibitor	
	SLSQSKVLPVPQK	Antioxidant	
	KVLPVPQ	ACE- Inhibitor	
	LLYQEPVLGPVRGPFPIIV	ACE- Inhibitor /Antioxidant	
	QEPVLGPVRGPFPIIV	ACE- Inhibitor /Antioxidant	
	GPVRGPFPIIV	ACE- Inhibitor	
	KEMPFPK	Iron-chelating activity	Yang et al., 2021
	VLPVPQK	Antioxidant	
	DKIHPF	ACE- Inhibitor	
	EMPFPK	Bradykinin	

Table 2.2: Reported functional peptides produced by *L. helveticus* in bovine milk (Continued)

Protein	**Peptide**	**Bioactivities**	**Reference**
Bovine β-casein	YQEPVLGPVRGPFPIIV	Immunomodulating/ Antibacterial	Yang et al., 2021
	DELQDKIHPF	Immunomodulating	Fan et al., 2019
	HLPLPL	Antiamnesic	
	IPPLTQTPV	DPP IV inhibitor	
	LSQSKVLPVPQ	ACE- Inhibitor	
	MPIQAF	Antibacterial	
	NIPPLTQTPV	ACE- Inhibitor	
	PKYPVEPFTE	15-lipoxygenase Inhibitor	
	PLPLL	ACE- Inhibitor	
	QEPVLGPVRGPFP	Immunomodulating/ACE -Inhibitor/antioxidant	
	QPLPPTVMFP	Inhibition of apoptosis and other cellular damage	
	RELEELNVPGEIVE	ACE- Inhibitor	
	SKVLPVPQKAVPYPQ	Antioxidant	
	SQSKVLPVPQ	ACE- Inhibitor	

Table 2.2: Reported functional peptides produced by *L. helveticus* in bovine milk (Continued)

Protein	Peptide	Bioactivities	Reference
Bovine β-casein	VPPFL	Antihypertensive	Fan et al., 2019
	VPYPQRDMPIQ	Leptin-associated disorders inhibitor	
	VPYPQRDMPIQA	ACE- Inhibitor	
	YPFPGPIPN	ACE- Inhibitor	
	YPVEPFTE	ACE- Inhibitor	
	YQEPVLGP	Antioxidant	
	YQEPVLGPVR	ACE- Inhibitor	
	YQEPVLGPVRGPFP	Immunomodulation	
Bovine αS1-casein	FVAPFPEVF	ACE and DPP IV inhibitor	Baptista et al., 2020
	RPKHPIKHQ	ACE- Inhibitor	
	RPKHPIKHQ	ACE- Inhibitor	Begunova et al., 2020
	FVAPFPEVFGKE	ACE- Inhibitor /Antioxidant	
	VAPFPEVFGKE	ACE- Inhibitor	
	LYQGPIVLNPWDQVK	ACE- Inhibitor	
	EDVPSER	Iron-chelation	Yang et al., 2021
	SDIPNPIGSENSEK	Antibacterial	
	SDIPNPIGSENSEK	Antibacterial	

Table 2.2: Reported functional peptides produced by *L. helveticus* in bovine milk (Continued)

Protein	Peptide	Bioactivities	Reference
Bovine κ-casein	KYIPIQYVL	Antioxidant	Begunova et al., 2020
	AVRSPAQIL	Antibacterial	Fan et al., 2019
	YGLNYYQQKPVA	Improvement of human growth	
Bovine α-Lactalbumin	RPKHPIKHQ	ACE- Inhibitor	Skrzypczak et al., 2021
	LLYQEPVLGPVRGPFPIIV	Immunomodulation	
Bovine Caseino-glycomacropeptide	LLYQEPVLGPVRGPFPIIV	Immunomodulation	Skrzypczak et al., 2021
	RELEELNVPGEIVESLSSSEESITR	Mineral binding	
	MAIPPKKNQDK	Antithrombotic	
Camel β-casein	LSLSQFKVLPVPQ	ACE- Inhibitor	Alhaj et al., 2018
	SLSQFKVLPVPQ	ACE- Inhibitor	
	SQFKVLPVPQ	ACE- Inhibitor	
	TDLENLHLPLPL	ACE- Inhibitor	
	DLENLHLPLPL	ACE- Inhibitor	
	LENLHLPLPL	ACE- Inhibitor	

2.6 *L. helveticus* Application in Fermented Milk Products

2.6.1 Cheese

Different stains of *L. helveticus* were used in the production of cheese and fermented dairy products with improved functionality and health benefits (Table 3). The reported effects include the potential to improve the flavor, texture, and shelf-life of the produced dairy products as well as their antioxidant, antimicrobial, angiotensin-converting enzyme (ACE) inhibitory, immune-modulatory, and lactose tolerance effects. These advantages are mainly explained by the high proteolytic activity of this bacterium and its ability to produce functionally- and nutritionally active peptides.

L. helveticus is often employed for producing cheeses such as Swiss-style and Italian hard cheeses (Moser et al., 2018). *L. helveticus* is used as an adjunct culture in cheese processing and improves the flavor and bioactive potential of cheeses while reducing their salt content (Baptista et al., 2020). Similar to *L. delbrueckii* subsp. *bulgaricus*, *L. helveticus* is not typically used individually in the production of Swiss-type cheese and is often used in mixed starter cultures in the production of Emmental and Gruyère cheeses (Moser et al., 2018). *L. helveticus* is a suitable alternative to *L. delbrueckii* subsp. *bulgaricus* for making Swiss and Dutch-style cheeses due to its ability to ferment galactose. Additionally, *L. helveticus* produces both d- and l-lactate, while *L. delbrueckii* subsp. *bulgaricus* only produces d-lactate. This can be advantageous in Swiss cheese production as the l-lactate is preferred by propionibacteria, which are responsible for the production of carbon dioxide gas and the characteristic holes in Swiss cheese. The propionibacteria prefer to use l-lactate as a substrate for their metabolism, and the presence of this form of lactate produced by *L. helveticus* can increase their growth rate and overall activity, leading to a more desirable cheese product (Oberg et al., 2022).

Table 2.3: Overview of *L. helveticus* strains used in the production of functionally improved bovine cheese and fermented dairy product types

Type of cheeses and Yoghurts	**Strains**	**Reported Effects**	**Reference**
Hard cheeses	Lh209, Lh138, and LH-B02	Enhance proteolysis and accelerate flavor development	Cuffia et al., 2018 Sıçramaz et al., 2022
Swiss-type cheeses	T104, and T105	Accelerate flavor development, decrease bitterness, and exhibit the greatest amount of free amino acids and free radical scavenging capacity	Skrzypczak et al., 2020
Dutch-type cheese	LH-B01	Enhances proteolysis and ACE inhibitory activity. Increased proteolysis impacts products' texture, flavor, and health effects	Garbowska et al., 2021
Grana Padano cheese	MIMLh5	Enhances immunomodulatory properties	Stuknyte et al., 2014
Italian hard cheese	Lh43, SIM12, SIS16, and 1734	Alleviate lactose intolerance due to high β-galactosidase activity, immunomodulatory properties, and ability to produce folate	Zago et al., 2021
Prato cheese	LH-B02	Enhances ACE inhibitory and antimicrobial activity	Baptista et al., 2020; Baptista & Gigante, 2022
Raclette-type cheese	FAM1172, and FAM22157	Enhance proteolysis and produce diverse peptides	Egger et al., 2021

Table 2.3: Overview of *L. helveticus* strains used in the production of functionally improved bovine cheese and fermented dairy product types (Continued)

Type of cheeses and Yoghurts	**Strains**	**Reported Effects**	**Reference**
Cheddar cheese	LH-1.0612	Enhances antioxidant activity	Yang et al., 2021
Traditional Tibetan kurut	H9	Improves texture, viscosity, and water-holding capacity	Zhou et al., 2019
Sayram ketteki yoghurt	MB2-1	Improves texture, viscosity, and water-holding capacity and produces the potential prebiotic EPS	Ge et al., 2022
Functional yogurt powder	CNRZ32	Improves shelf-life	El-Sayed et al., 2020
Traditional Pakistani yoghurt	LBh5, and LBh4	Produce bacteriocin with antimicrobial activities	Hassan et al., 2020
High protein yogurt	LH-B02	Increases protein content and ACE inhibitory activity	Giacometti et al., 2020
Chortan (Heated strained yoghurt)	PTCC 1332	Enhances proteolysis	Hashemi & Gholamhosseinpour, 2020
Yogurt with flavourzyme	881315	Improves ACE inhibitory activity	Shi et al., 2017

The volatilome of Reggianito cheese produced by *L. helveticus* B02 and 209 was found to comprise 32 volatile substances, including ketones, esters, aldehydes, alcohols, acids, and other substances with aromatic rings compounds (Cuffia et al., 2018). The key metabolic activities of *L. helveticus* responsible for generating flavor include lactose metabolism as well as proteolysis and subsequent amino acid catabolism (Skrzypczak et al., 2020). The proteolytic enzymes of *L. helveticus* can expedite flavor improvement in cheese (Garbowska et al., 2021). *L. helveticus* NK1 strain exhibited more proteolytic activity than other LABs that are commonly used in the dairy industry (Begunova et al., 2020). Thus, *L. helveticus* strains can be used as an adjunct to expedite protein degradation, enhance flavor development, and eliminate bitterness during cheese ripening (Egger et al., 2021; Oberg et al., 2022). *L. helveticus* played a secondary role in S. *thermophilus* in acid production and flavor enhancement in Swiss cheese through increased proteolysis during the later stages of ripening (Oberg et al., 2022). The bacterial enzymes break down proteins and peptides, releasing amino acids that can then be broken down further into various flavor compounds (Garbowska et al., 2021), e.g. *L. helveticus* T105 was shown to produce free amino acids, which are important precursors of cheese aroma and flavor (Skrzypczak et al., 2020). During this process, nitrogen is also released from amino acids as non-protein nitrogen and is used by the microorganisms to produce additional flavor compounds. The volatile profiles of different *L. helveticus* strains may vary due to differences in enzymatic systems and they may produce variable cheese flavors upon mixing (Cuffia et al., 2018). During heat-induced ripening of Swiss-type cheeses, *L. helveticus* did not induce excessive casein hydrolysis but expedited the breakdown of pre-existing peptides and the release of nitrogen from amino acids, thereby increasing the intensity of the cheese flavor (Egger et al., 2021; Garbowska et al., 2021).

2.6.2 Fermented Milk

L. helveticus strains CNRZ32 (El-Sayed et al., 2020), H9 (Zhou et al., 2019), LBh5 and LBh4 (Hassan et al., 2020), LH-B02 (Giacometti et al., 2020), MB2-1(Ge et al., 2022), PTCC 1332 (Hashemi & Gholamhosseinpour, 2020), and 881315 (Shi et al., 2017) have been used in yogurt production (Table 3). *L. helveticus* CNRZ32 survived for up to three months in spray-dried powder at 120 °C and was able to create fresh yogurt powder with an adequate bacterial count of $\geq$6.0 log colony-forming unit/g at the end of this period without external or additional microorganisms (El-Sayed et al., 2020). *L. helveticus* NK1 exhibited the fastest growth rate and the highest maximum viable cell count, proteolytic activity, and acidification ability compared with *L. rhamnosus* F and *Limosilactobacillus reuteri* LR1 during bovine milk fermentation (Begunova et al., 2020).

When *L. helveticus* H9 was used as an additional starter bacterium in yogurt fermentation, it shortened the fermentation time and increased the concentrations of volatile components, particularly those of benzaldehyde and acetoin, compared with a control fermented using commercial starter bacterium (Zhou et al., 2019). In addition, two antihypertensive tripeptides, isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) were detected in *L. helveticus* H9–fermented bovine milk and remained stable during cold storage (Zhou et al., 2019). Moreover, *L. helveticus* 881315–fermented yogurt was reported to be rich in ACE inhibitory peptides (Shi et al., 2017). *L. helveticus*–fermented camel milk exhibited high levels of autoaggregation, coaggregation, and adhesion alongside several health benefits; these traits were associated with the ability of the isolate to attach to intestinal epithelial cells and fight pathogens (Mahmoudi et al., 2019). Thus, *L. helveticus* KMCH1 isolated from camel milk possesses all the required probiotic qualities for application in the production of functional dairy products.

2.7 Health Benefits and Potential Probiotic Mechanisms of *L. helveticus*

Recent developments in functional foods have been accompanied by a general reorientation toward natural and sustainable ways of improving health (Skrzypczak et al., 2020). Some of the health benefits associated with the ingestion of *L. helveticus*-fermented products are summarized in Table 4. Several mechanisms, including acidification and production of bioactive compounds and exopolysaccharides, are involved in the probiotic effects of *L. helveticus*. To date, 57 different types of *L. helveticus* strains have been identified, and probiotic abilities have been confirmed in 51 *L. helveticus* genomes (Fontana et al., 2019), including H9 (Zhou et al., 2019), MB2-1 (Ge et al., 2022a), KLDS1.8701 (Li et al., 2019), D75 and D76 (Toropov et al., 2020), B734 and T105 (Skrzypczak et al., 2021), R0052 (Carlman et al., 2022), and SNA12 (Wang et al., 2022).

Table 2.4: Reported health effects of *L. helveticus* strains as probiotic bacteria

Effects of fermentation	**Strains**	**Probiotic Effects**	**References**
Acidification	BLh	Antibacterial effects	Lukic et al., 2013
	ASCC 511	Improved digestion and gut health	Ho et al., 2020
	Lh43, SIM12, SIS16, and 1734	Improved lactose tolerance	Zago et al., 2021
Production of bioactive peptides	LH-B02	Hypotensive effects	Giacometti et al., 2020
	LZ-R-5	Immuno-stimulating effects	You et al., 2020
	ASCC 511	Improved digestion	Ho et al., 2020
	CM4	Improved cognitive function	Sivamaruthi et al., 2018
	ASCC 511	Anti-inflammatory effects	Ho et al., 2020
	T80, T105, and B734	Antithrombotic effects	Skrzypczak et al., 2021
	1.0612	Antioxidant potential	Yang et al., 2021
	1.0612	Iron chelating activity	Yang et al., 2021
	1.0612	ACE inhibition	Yang et al., 2021
	CICC6024	Dipeptidyl-peptidase 4 (DPP-IV) inhibition	Fan et al., 2019
	CICC6024 and 34.9	Antimicrobial effects (E.g., Bacteriocins)	Fan et al., 2019; Angelescu et al., 2022

Table 2.4: Reported health effects of *L. helveticus* strains as probiotic bacteria (Continued)

Effects of fermentation	**Strains**	**Probiotic Effects**	**References**
	T80, T105, and B734	Opioid peptides	Skrzypczak et al., 2021
	CICC6024	Anti-amnesic effects	Ohsawa et al., 2017
	CICC6024	Anticancer effects	Fan et al., 2019
	CICC6024	Cell regulation potential	Fan et al., 2019
Production of Extracellular polysaccharides (EPS)	MB2-1	Antitumor effects	Ge et al., 2022
	MB2-1	Antioxidant potential	Xiao et al., 2020
	LZ-R-5	Immunostimulatory effect	You et al., 2020
	SNA12	Prebiotic effect	Wang et al., 2022

According to FAO/WHO, probiotics can facilitate the activity and growth of beneficial bacteria in hosts, and thus, they not only survive but also be viable and active in the gut environment (Zendeboodi et al., 2020). During fermentation, probiotics produce one primary and various secondary metabolites from milk proteins, such as exopolysaccharides and peptides that are linked to sensory and nutritional effects (Raj et al., 2021). A probiotic supplement, containing a combination of *L. helveticus* Rosell®-52, *Bifidobacterium bifidum* Rosell®-71, and *B. infantis* Rosell®-33 together with fructooligosaccharides, has been marketed and sold since 2002 in more than 28 countries worldwide (Tremblay et al., 2021). Additionally, Polish *L. helveticus* strains have been used to produce dairy products and functional foods (Skrzypczak et al., 2018).

2.7.1 L. helveticus as a *Psychobiotic*

Psychobiotics are probiotics that may potentiate mental health through interactions leading to improvement of the performance of the gut-brain axis in disorders such as depression and anxiety (Dahiya & Nigam, 2022). The concept of the gut-brain axis has gained attention in recent years due to increased evidence suggesting that gut microbiota play an important role in influencing brain function and behavior (Verma et al., 2023). Psychobiotic gut microbiota may influence anxiety and other mental health conditions *via* the production of neurotransmitters and/or hormones that can interact with the central nervous system (Misra & Mohanty, 2019). *L. helveticus* was reported to have beneficial effects on individuals with anxiety, depression, and stress-related cognitive dysfunction (Verma et al., 2023). According to reports, consuming a combination of *L. helveticus* R0052 and *Bifidobacterium longum* R0175 (Probio'Stick) orally for one month may improve symptoms of anger, depression, and anxiety while also reducing the level of the stress hormone cortisol (Misra & Mohanty, 2019).

There is some evidence suggesting that drinking *L. helveticus* fermented milk may lead to cognitive improvement in humans. Ingestion of *L. helveticus* CM4–fermented milk for 8 weeks was found to improve the attention and memory of healthy middle-aged adults (Ohsawa et al., 2017). Similarly, *L. helveticus* CM4 fermented milk supplementation for 8 weeks was reported to improve the cognitive functions of Japanese middle-aged healthy adults (Sivamaruthi et al., 2018). Studies have shown that certain strains of *L. helveticus* produce bioactive peptides that have the potential to affect brain function and improve cognitive performance, e.g. lactononadecapeptide (NIPPLTQTPVVVPPFLQPE) (Ohsawa et al., 2017). There are also findings supporting the use of probiotics, such as *L. helveticus* R0052, as a non-pharmaceutical treatment for stress-related disorders (Carlman et al., 2022). Thus, probiotic intervention, including *L. helveticus* R0052, may be able to subtly alter brain activity and functional connectivity in areas known to regulate emotion and stress responses. However, more research is needed to provide stronger evidence and to enable a better understanding of the mechanisms responsible for any putative cognitive benefits of *L. helveticus* fermented products.

2.7.2 Gut Well-ness

As a probiotic, *L. helveticus* was reported to improve intestinal barrier integrity by reducing inflammatory responses (Ho et al., 2020). Regular administration of probiotics containing *L. helveticus* for a week relieved constipation-related symptoms and decreased fecal pH (Bahrudin et al., 2020). Moreover, *L. helveticus* milk fermentation reduced the antigenic/allergenic potential of milk proteins and lactose. In fact, *L. helveticus* BGRA43 was reported to reduce the extent of cow milk allergy by hydrolyzing the β-lactoglobulin allergen (Lukic et al., 2013). This suggests the potential of *L. helveticus* in reducing the allergenicity of these proteins, but more research is needed to confirm that this bacterium has the potential in reducing the allergenicity of dairy products *in vivo*.

Lactose intolerance occurs when lactose is not properly hydrolyzed due to the lack of β-galactosidase activity in the gut microbiome (Misselwitz et al., 2019). *L. helveticus* strains, including 1734, SIM12, SIS16, and Lh43, were found to exhibit extremely high β-galactosidase activities (Zago et al., 2021). Thus, *L. helveticus* could potentially alleviate lactose intolerance by providing a source of β-galactosidase to break down lactose in the gut.

When combined with *Lacticaseibacillus rhamnosus* R0011, *L. helveticus* R0052 had a positive effect on gut microbiome restoration in patients with alcoholic hepatitis (AH) (Gupta et al., 2022). The administration of *L. helveticus* R0052 was associated with several positive outcomes. Firstly, there was a decrease in the levels of γ-glutamyltranspeptidase and alanine aminotransferase, indicating improved liver function and reduced stress or damage. Additionally, the proportion of Fusobacteria and Proteobacteria decreased, which is beneficial for gut health. These findings imply that *L. helveticus* R0052 may improve AH by modulating the gut-liver axis (Gupta et al., 2022).

2.7.3 Bioactive Peptides

The high proteolytic activity of *L. helveticus* results in the generation of peptides with high antioxidative, ACE-inhibitory, antidiabetic, anticancer, and antiamnesic activities alongside other bioactive peptides, which are beneficial for treating leptin-related disorders and contribute to lipoxygenase inhibition (Fan et al., 2019; Yang et al., 2021). *L. helveticus* was shown to have lipoxygenase inhibition activity that is linked to its anti-inflammatory effects and improved regulation of various cellular processes (Verma et al., 2023). The lipoxygenase enzymes participate in the oxidation of polyunsaturated fatty acids resulting in the formation of various metabolites that contribute to inflammation and other health problems. *L. helveticus* inhibits lipoxygenase activity *via* the production of bitter cell-regulating peptides, particularly those associated with leptin. However, the exact mechanism for the conversion of linoleic to these aldehydes still needs to be investigated.

To generate industrially useful fermented milk, the isolation and creation of a new breed of *L. helveticus* strain with enhanced peptide-releasing ability in fermented milk is critical (Zhong et al., 2021). In addition to peptides, *L. helveticus* produces oligopeptides, which are hydrolyzed by gastrointestinal enzymes to produce bioactive peptides (Fan et al., 2019). Compared with other LABs, *L. helveticus* exhibits the highest extracellular proteinase activity and can generate antihypertensive peptides in fermented milk. Furthermore, in *L. helveticus*–fermented sour milk, an antihypertensive impact linked to the ACE-inhibitory peptides IPP and VPP was observed (Zhou et al., 2019). Through the production of antihypertensive peptides, *L. helveticus* can regulate high blood pressure (Zhou et al., 2019), even in individuals with hypertension (Baptista et al., 2020; Li et al., 2019). Long-term storage of *L. helveticus*–fermented kefir samples resulted in high ACE-inhibitory activity with a decrease in pH from 4.6 to 4.3 (Şanli et al., 2018). Using *L. helveticus* LH-B02 as an adjunct culture to produce Prato cheese affected the abundance of a peptide with bioactive potential favoring ACE-inhibitory action throughout cheese ripening (Baptista et al., 2020). *L. helveticus* KLDS.31 and *Lacticaseibacillus casei* KLDS 105 produced novel ACE-inhibitory peptides (i.e., KPAGDF, KAALSGM, KKAAMAM, and LDHVPGGAR) in fermented bovine milk (Li et al., 2019). Milk containing *L. helveticus* and *S. thermophilus* were superior to those containing *L. acidophilus* and *S. thermophilus* in ACE-inhibitory activity and antibacterial effects (Alhaj et al., 2018).

Fermented milk proteins are rich in natural dietary antioxidants due to the hydrolysis of proteins to bioactive peptides with increased radical scavenging power. Due to its high proteolytic activity, *L. helveticus* 1.0612 was found to increase the antioxidant activity during cheddar cheese ripening through the production of antioxidant peptides including SSEESITR, SSSEESITR, EDVPSER, KEMPFPK, VLPVPQK, ITVDDKHY, DKIHPF, VNELSKDIGSE, EMPFPK (Bradykinin), SDIPNPIGSENSEK, EIVPNSAEERL, FPKYPVEP, EMPFPKYP, YIPIQYVLSR, and YLGYLEQLLR (Yang et al., 2021). The hydrolysates of whole milk powders by *L. helveticus* T80, T10, and B734 were found to enhance Fe^{2+}-chelation and antioxidant activities (Skrzypczak et al., 2019).

In addition to its antioxidant-potentiating effect, It was suggested that *L. helveticus* LH-B02 could potentially inhibit dipeptidyl-peptidase 4 (DPP-IV) and stimulate the release of glucagon-like peptide-1 (GLP-1) (Baptista et al., 2020). Both these two effects are linked to regulating glucose levels in the body, and peptides that exhibit these effects could have potential therapeutic benefits in the management of type 2 diabetes. GLP-1 is a hormone that plays a role in regulating blood sugar levels and stimulating insulin secretion. By inhibiting DPP-IV, *L. helveticus* LH-B02 can increase the levels of GLP-1 in the body. This increase in GLP-1 can then led to improved blood sugar control, which is beneficial for people with type 2 diabetes. For example, *L. helveticus* CICC6024 was reported to produce DPP-IV-inhibitory peptides that were able to decrease plasma in cretin levels and counteract the inhibition of insulin synthesis and improve glucose tolerance suggesting benefits for patients with type 2 diabetes mellitus (Fan et al., 2019).

2.7.4 Antimicrobial Activity

L. helveticus can substantially alter the composition of gut microbiota (Ho et al., 2020). *L. helveticus* competes with other food bacteria *via* different mechanisms, including (i) antimicrobial peptide production *via* proteolysis; (ii) bacteriocin production; (iii) high ability to produce lactic acid *via* galactose metabolism; (iv) antibiofilm activity; and (v) anti bacteriophage activity. True probiotic cells secrete antimicrobial compounds such as bacteriocins, H_2O_2, and organic acids (butyric, lactic, and acetic acids) (Zendeboodi et al., 2020). Generally, bacteriocins are small peptides (<60 amino acids) that kill or limit the growth of a narrow range of bacterial strains from comparable or closely related species, such as Gram-negative and Gram-positive pathogens including *Staphylococcus aureus*, *Escherichia coli*, methicillin-resistant *S. aureus*, *Acinetobacter baumannii*, and *Salmonella paratyphi* (Angelescu et al., 2022). Particularly, a bacteriocin produced by *L. helveticus* exhibited higher efficacy against foodborne pathogens than a similar bacteriocin produced by *Lactiplantibacillus plantarum* (Hassan et al., 2020). *L. helveticus* 481 produced helveticin J, a bacteriocin comprising 249 amino acids that retain its activity throughout a broad pH range (2.0–10.0) (Angelescu et al., 2022). *L. helveticus* also produced helveticin 34.9, which inhibited the growth of other bacteria (such as *L. sakei*, *L. helveticus*, *L. delbrueckii*, *Enterococcus faecium*, *B. subtilis*, *S. aureus*, and *B. cereus*) and demonstrated considerable antagonistic effect against *Halobacillus hunanensis* (Angelescu et al., 2022). These bacteriocins are interesting for the food industry as they can be used as food bio-preservatives to combat spoilage and pathogenic microorganisms.

Dairy products fermented with *L. helveticus* may become infected with phages that negatively affect product quality and cause problems in the dairy industry (Zago et al., 2017). However, different strategies may be employed to mitigate the risk of phage infection in *L. helveticus* fermentation. For example, natural selection has led to the discovery of phage-resistant *L. helveticus* strains, including the bacteriophage-insensitive mutant LhM. Resistant variants exhibit multiple phage defense mechanisms that provide enhanced fitness and stability to the dairy environment (Zago et al., 2017). In fact, the *L. helveticus* strain EMCC 1654 exhibited the most potent antibiofilm activity against an *E. coli* WW1 isolate (Abdelhamid et al., 2018).

2.7.5 Anti-tumor Activity

The administration of *L. helveticus* R389–fermented milk induced a delay in breast tumor growth in mice owing to increased interleukin (IL)-10 and decreased IL-6 levels in the mammary glands, serum, and tumor-infiltrating immune cells (Chadha et al., 2021).

2.7.6 Immunity Enhancement

Milk fermented with *L. helveticus* WHH2580 enhanced immunity in mice (Yanjun et al., (2022) and milk fermented with *L. helveticus* LZ-R-5 exhibited an apparent substantial immunostimulatory effect (You et al., 2020). The probiotic and immunomodulatory activities of *L. helveticus* NS8 suggest its application for generating commercially viable functional foods or as therapeutic adjuvants for inflammatory illnesses (Rong et al., 2015). Furthermore, *L. helveticus* reportedly exhibits S-layer protein-associated immune-protective effects in intestinal epithelial cells (Ho et al., 2020).

2.7.7 Extracellular Polysaccharides (EPS) Production

The ability of *L. helveticus* to form EPS as postbiotics (i.e., probiotic-produced metabolites) can help improve the quality of dairy products and their nutritional content (Cundell & Tripepi, 2021; Ge et al., 2022). Owing to their potential applicability as viscosifiers, emulsifying, and texturizerizing agents in the fermented dairy industry, EPS exhibit distinctive physical and rheological properties, and EPS-producing lactobacilli are used to improve the texture, rheology, and mouthfeel of products such as yogurt and cheese (Ge et al., 2022). EPS (EPS-2) produced by *L. helveticus* MB2-1 can be a source of natural antioxidants with medicinal and nutritional food applications (Xiao et al., 2020). Moreover, this strain can produce *in-situ* EPS, which is identified as a novel heteropolysaccharide comprising fucose tightly linked to proteins that can effectively fill the three-dimensional network model typical of casein clusters (Ge et al., 2022a). The EPS-1 produced by *L. helveticus* KLDS1.8701 supplementation may reduce hepatic oxidative stress by influencing gut microbiota composition, and thus, can reduce oxidative damage (Li et al., 2019). R-5-EPS, isolated from *L. helveticus* LZ-R-5–fermented milk, was reported to exhibit an apparent substantial immunostimulatory effect and should be investigated as a possible immunomodulatory agent (You et al., 2020). Finally, SNA12-

EPS, extracted from *L. helveticus* SNA12–fermented milk, can regulate gut microbiota and be used as a new prebiotic in functional foods (Wang et al., 2022).

2.8 Perspectives for Future Trends

Recently, consumers have been focusing on foods and food formulations that will enable them to lead healthy lives. Thus, efficient industry practices to improve the health changes in the daily diets of consumers are critical. Such initiatives should alleviate the concerns and improve the knowledge of modern consumers regarding their wellness and healthy options (Skrzypczak et al., 2018). Fermented dairy products are considered "functional foods" because they exhibit several advantages over regular foods. According to recent studies, *L. helveticus*–fermented dairy products may reduce the risk of heart diseases and improve digestion and immunity (Şanli et al., 2018; Misselwitz et al., 2019; Begunova et al., 2020; Skrzypczak et al., 2020), and reduce the risk of type 2 diabetes (Fan et al., 2019). Moreover, the anti-inflammatory, antioxidant, anticancer, antihypertensive, and other properties of bioactive peptides produced by *L. helveticus* are being researched (Fan et al., 2019; Baptista et al., 2020a). In addition, bacteriocins produced by *L. helveticus* can be used as bio-preservatives to prevent food spoilage and mitigate pathogenic microorganisms in the food industry (Angelescu et al., 2022). In addition, *L. helveticus*–produced EPS exhibits unique rheological and physical properties and can be used as natural viscosifiers, emulsifying agents, and texturizers in the food industry instead of artificial chemical agents as well as a natural antioxidant and immunomodulatory agents (Ge et al., 2022; You et al., 2020).

The implementation of newly emerging multi-omics approaches in the dairy industry is necessary in order to gain a comprehensive understanding of the role and function of each strain of *L. helveticus* present in milk. This will serve to enhance our knowledge and increase the number of practical applications in this field (Zhou et al., 2019; Raveschot et al., 2020; Xu et al., 2021). Despite the positive consumer perception of probiotic dairy products, there remains substantial misinformation, especially regarding label information (Avila et al., 2020). Thus, clear labeling information can improve the chance of people buying *L. helveticus*–fermented functional dairy products.

2.9 Conclusions

Lactobacillus helveticus is a useful thermophilic bacterium, especially used in the production of cheese and fermented milk. This article reviews several studies that have been conducted in the last few years regarding the utilization of *L. helveticus* in milk fermentation. While additional research is warranted to fully comprehend the effects of *L. helveticus*–fermented foods on our health, a daily dose of digestive aids can be beneficial for the body. Furthermore, *L. helveticus can act as a bio preservative, enhancing the quality of dairy and dairy-based products without additional harmful effects.* Since some *L. helveticus* mutants exhibit phage resistance, more studies are required to recognize the defense systems in these mutants, which will help to develop successful starter cultures. The versatility of *L. helveticus* and its ability to quickly adapt to dairy and dairy-based industrial fermentation environments allow it to be included in novel probiotic formulas under different environmental challenges. Further research into the biotechnological profiles and functional properties of *L. helveticus* strains could lead the development of dairy products with distinct flavors and functional properties.

Different strains of *L. helveticus* can be developed to produce fermented dairy products with specific nutritional properties. Since it is efficient in the conversion of lactose into lactic acid, it can be utilized in the production of fermented liquid and powdered products for lactose-intolerant individuals. Thus, further investigations are warranted regarding the clinical potential of different *L. helveticus* strains in the production of lactose- and galactose-free milk products and the discovery of novel bioactive peptides in fermented milk products. Since *L. helveticus*–fermented products may have several benefits, it is important to expand the scope of its strains in different industrial applications aiming to satisfy consumer expectations regarding "probiotic" dairy products.

Declarations of Interest

The authors declare that they have no conflicts of interest to disclose.

Data Availability

The study described in the article did not utilize any data.

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“This research work is based in full on the previously published article listed below. I have permission from my co-authors/publishers to use the works listed below in my thesis/dissertation. [Chelladhurai, K., Ayyash, M., Turner, M. S., Kamal-Eldin, A., 2023. *Lactobacillus helveticus*: Health effects, current applications, and future trends in dairy fermentation. Trends in Food Science and Technology 136, 159–168. https://doi.org/10.1016/J.TIFS.2023.04.013]”.

Chapter 3: Differences in the Growth, Acidification, and Proteolytic Activities of *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* in Camel and Bovine Milk Fermentation

Redrafted from

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3.1 Abstract

Low-fat camel milk (CM) and bovine milk (BM) were fermented using *Lactobacillus helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* individually at 28°C, 35°C, and 42°C for 96 h and then measured and compared the bacterial counts, titratable acidity (TA), pH, changes in lactose and organic acids, and proteolytic activity. The above-mentioned parameters increased slightly with increasing temperature for each species. Although bacterial growth was similar in CM and BM, acidification and proteolysis differed following fermentation. Both CM and BM showed negative correlations between TA (%) and pH, suggesting similar buffering capacities. In both types of milk, the behavior of *L. helveticus* was more comparable to that of *S. thermophilus* than to *L. delbrueckii* subsp. *bulgaricus*, particularly in terms of proteolysis and acidification. Our findings provide valuable insights into the complex dynamics of bacterial interactions with different milk substrates, which can be applied to optimize fermentation processes in the dairy industry.

Keywords: Milk, Fermentation, Lactic acid bacteria, *Lactobacillus helveticus*, Acidification, Proteolysis.

3.2 Introduction

Camel milk (CM), either fresh or in a fermented form, plays a vital role in the diets of people residing in desert regions (Pak et al., 2019). Historically, the consumption of fermented milk products has been essential, particularly because fresh milk cannot be preserved under challenging environmental conditions. Compared with bovine milk (BM), CM has a unique chemical composition, which impacts the biological, functional, and sensory characteristics of its associated dairy products (El-Hatmi et al., 2023). CM is unique in its protein and lipid composition, immunoglobulins, and mineral balance; moreover, it has higher levels of peptides and free amino acids than BM (Abd El-Aziz et al., 2022; Seifu, 2023).

Yogurt, an important dairy product, is renowned for its rich taste and texture and serves as a versatile and nutritious ingredient in various diets worldwide (Mehra et al., 2022). The transformation of milk into yogurt and yogurt-like products, which have been treasured for centuries, involves fermentation by lactic acid bacteria (LAB) (Abarquero et al., 2022; Wang et al., 2021). This intricate microbial-driven transformation alters the composition and characteristics of milk, thereby significantly enhancing the quality of fermented products (Ayyash et al., 2018). The conversion of lactose into lactic acid increases total acidity and decreases pH, creating an unfavorable environment for the growth of harmful microorganisms (Coelho et al., 2022). One of the most significant uses of this fermentation process is the conversion of milk into a primary end product and a variety of secondary metabolites, such as exopolysaccharides and peptides (Raj et al., 2021; Wang et al., 2021).

LAB comprise a diverse group of microorganisms, such as *Lactobacillus* and *Streptococcus* species, most of them confer probiotic and postbiotic effects (Ge et al., 2022). Some LAB act as psychobiotics, which are probiotics that exert a positive psychiatric impact on humans, such as stress and anxiety control (Sarkar et al., 2016). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are commonly used for yogurt production. However, other bacteria, such as *Lactobacillus acidophilus* and *Bifidobacterium*, can also be used in fermented milk production. *Lactobacillus helveticus* is another bacterial species that is occasionally used in fermented milk production. *L.*

helveticus contributes to the fermentation process and enhances the texture and flavor of yogurt due to its high acidification and proteolytic activities (Chelladhurai et al., 2023). In addition, some strains of *L. helveticus* possess probiotic properties, offering potential health benefits when included in fermented milk formulations. In dairy products such as cheese and fermented milk, the proteolytic activity of LAB is crucial for liberating bioactive peptides (Begunova et al., 2020) that possess antioxidative, angiotensin-converting enzyme inhibitory, opioid, antidiabetic, antibacterial, anticancer, antiamnesic, and immunomodulatory properties (Alhaj et al., 2018; Fan et al., 2019). These bioactive peptides not only influence the nutritional properties and health benefits of milk products but also affect their sensory properties. The proteolytic system of LAB allows the utilization of milk protein as a nitrogen source, contributing to the production of various metabolites in fermented foods (Raveschot et al., 2020). The use of proteolytic probiotic LAB as a strategy for producing innovative functional foods rich in bioactive peptides to improve health in a sustainable manner is under consideration (Chelladhurai et al., 2023).

The bacterial species/strains differ in their ability to convert lactose into lactic acid and their tolerance for the generated acidity that creates unfavorable environment for the growth of other microorganisms (Coelho et al., 2022). The variability in milk fermentation by different bacteria leads to different effects on the metabolism of proteins and the generation of bioactive peptides (Raj et al., 2021; Begunova et al., 2020; Wang et al., 2021). A few studies have compared the fermentation behaviours of different LAB in camel and cow milks (Ayyash et al., 2018; Terzioğlu et al., 2023). Bacterial counts, titratable acidity, and pH were compared during the fermentation of camel and cow milks with *Lactococcus lactis* at 25°C for 24 hours (Attia et al., 2001) and with *Lactiplantibacillus plantarum*, *Lactiplantibacillus paraplantarum*, *Lentilactobacillus kefiri*, *Lactobacillus gasseri*, *Lacticaseibacillus aracasei*, *Leuconostoc lactis*, *Weissella cibaria*, and *Enterococcus faecium* at 37°C for 24 h (Soleymanzadeh et al., 2016). These and other studies have shown that bacteria growth in camel milk is much slower than in cow milk and attributed that to antimicrobial agents in camel milk (Hamed et al., 2024). Studies on cow milk fermentation have shown that *L. bulgaricus* has higher acidification ability than *S. thermophilus* (Rajagopal & Sandine, 1990) and that *L. helveticus* has higher proteolytic activity than both *L. bulgaricus* and *S. thermophilus* (Gandhi & Shah, 2014).

L. helveticus was found to have higher proteolytic activity during the fermentation of camel milk at 37°C due to its exceptional need for all amino acids (Alhaj (2017). These previous studies suggest complex relations exist between bacterial growth and acidification and proteolysis rates in milks. These interactions are variably affected by bacterial species and fermentation temperature (Rodríguez-Serrano et al., 2018). Thus, this study aimed to compare the growth of *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* at three temperatures (28°C, 35°C, and 42°C) as well as their effect on lactose consumption, acidification, and proteolysis in pasteurized CM and BM. Understanding the interplay between microorganisms and milk can provide insights into producing dairy products with improved shelf life, enhanced nutritional value, and distinct sensory qualities.

3.3 Material and Methods

3.3.1 Collection of Samples

Commercially pasteurized samples (HTST, 161°F/72°C for 15 sec) of low-fat camel milk and bovine milk were purchased from a local milk supplier in Al Ain, transported to the laboratory in an ice box, and stored for a maximum of 4 hours at 4°C until used. There was no detectable bacterial count during the storage.

3.3.2 Bacterial Cultivation

L. helveticus DSM 20075, *L. delbrueckii* subsp. *bulgaricus* DSMZ 20081, and *S. thermophilus* DSM 20617 were purchased from Leibniz-Institute DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). The bacterial vials, containing ~ 0.5 g bacteria, were diluted with 0.5 mL MRS broth (Man, Rogosa, and Sharpe broth, LAB M, Lancashire, UK) prepared by mixing with 50% glycerol (1:1, w/v) and stored at −80°C. Before use, each strain was thawed and propagated by inoculating 0.16 mL of the previous suspension into 3.33 mL of MRS broth, and anaerobic incubation at 37°C for 24 hours following the directions of the providing company. A portion of the MRS broth (100 µL, ~7.0 log10 CFU/mL) was further activated in 10 mL of sterilized skim camel or cow milk made from milk powder (12%, w/v) by incubation at 37°C for 24 hours. The volumes of bacteria used were based on a pilot study performed to determine bacterial counts during the propagations in MRS broth and milks.

3.3.3 Milk Fermentation

Both types of milk (6 L) were equilibrated to the specified fermentation temperatures (28°C, 35°C, or 42°C), followed by aseptic inoculation with the three commercial probiotic bacterial cultures individually to reach 10^7 CFU/mL and allowed to ferment for 96 h.

3.3.4 Bacterial Count

The fermented CM and BM samples were homogenized using a vortex mixer prior to quantification, as per Ayyash et al. (2018). In brief, serial 10-fold dilutions were prepared from 1 mL of each fermented sample using sterile 0.1% peptone water (LAB M, Lancashire, UK). Then, the pour-plate method with MRS agar was used to determine the total plate counts in duplicate. In addition, the bacterial populations were assessed by inoculating the samples on MRS agar plates in triplicate, followed by anaerobic incubation for 48 h at 37°C using an anaerobic jar system (Don Whitley Scientific Ltd., West Yorkshire, UK).

3.3.5 Titratable Acidity and pH

The TA of the fermented milk samples was determined according to the method described by Ayyash et al. (2018) where the sample titrated with 0.01 N NaOH and expressed as the percentage of lactic acid. The pH values were measured at room temperature using a calibrated Start-3100 digital pH meter (OHAUS Corporation, NJ, USA).

3.3.6 Lactose and Organic Acids Quantification by High-performance Liquid Chromatography (HPLC)

For lactose quantification (i.e. using HPLC), lactose was first extracted from fermented CM and BM using the method described by Troiano et al. (2023) with modifications. In brief, 1 g of each sample was precisely measured and placed into a 10-

mL volumetric flask. Then, each flask was filled up to the mark with 7% perchloric acid solution to precipitate the proteins, leaving the sugars in the solution. After vortexing for 10 min, the mixtures were adjusted to pH 7.0 using 0.1 N NaOH to prevent lactose hydrolysis, transferred to centrifuge tubes, and centrifuged at 4,032 × g for 20 min at 4°C. Then, 1 mL of supernatant was collected from each flask and passed through a 0.45-µm polytetrafluoroethylene (PTFE) filter into 1.5 mL vials. Next, 10 µL of each prepared sample was injected into an HPLC system for separation. HPLC was performed on a Bio-Rad Aminex HPX 87H cation-exchange resin column connected to a cation H+ guard column (Bio-Rad Laboratories, Hercules, CA, USA) with a 2% acetonitrile aqueous mobile phase under isocratic conditions. Detection was performed using a refractive index detector set at 55°C. Sample aliquots (70 µL) were injected and chromatographically separated at a flow rate of 0.8 mL min^{-1} and a column temperature of 65°C. The total run time was 20 min. Peak area calculations were used to construct standard curves for lactose. The standards were prepared using deionized water passed through 0.45-µm filters.

For organic acid (lactic, citric, acetic, malic, and formic acids) quantification, the organic acids were extracted from fermented BM and CM using a modification of the method of Costa et al. (2016). Briefly, aliquots (2 g) of each sample were vortexed for 2 min with 10 mL of 45 mmol/L H_2SO_4, stirred for 30 min at 240 rpm, vortexed for 1 min, and centrifuged at 6,000 × *g* for 20 min at 4°C. Then, the supernatant was filtered through a 0.45 mm PTFE membrane and stored at 4°C until analysis. The HPLC was performed using a Bio-Rad Aminex HPX 87H column and a DAD UV detector with a 3 mmol/L H_2SO_4 aqueous mobile phase under isocratic conditions and detection at 210 nm. Aliquots of 20 µL were injected and separated at a flow rate of 0.5 mL min^{-1} and a column temperature of 60°C. The total run time was 30 min. Chromatograms of the standards (Figure 3.1) were constructed for each acid across a diverse concentration range (Table 3.1), using peak area calculations and comparing samples with standards prepared in filtered deionized water.

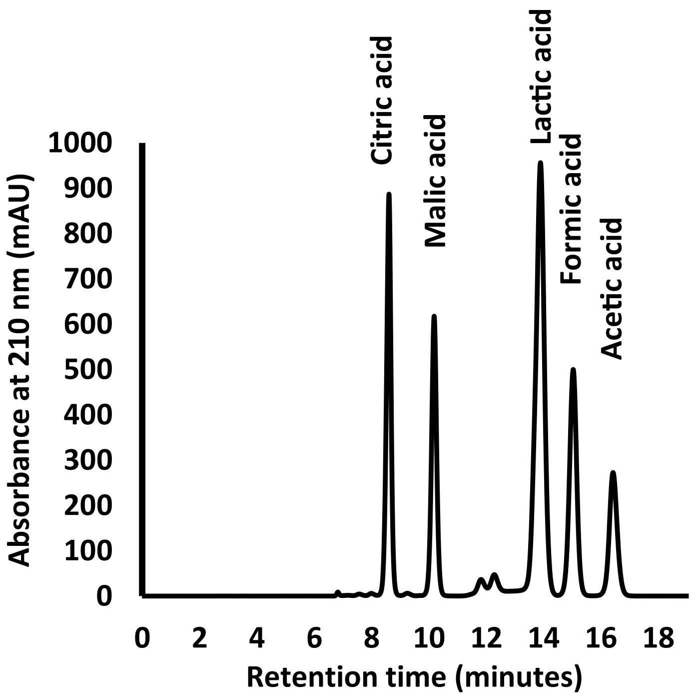

Figure 3.1: Typical chromatograms from HPLC of organic acid standards

Table 3.1: Formation of standard curves for lactose and organic acid quantification

Standard	Concentration range (mg/ml)	Regression equation	R^2 value
Lactose	0-152.42	y = 1.5762x	0.9965
Citric acid	0-397.25	y = 40.338x	0.9991
Malic acid	0-284.67	y = 30.342x	0.9891
Lactic acid	0-235.15	y = 23.386x	0.9999
Formic acid	0-364.56	y = 36.591x	0.9998
Acetic acid	0-220.46	y = 22.041x	0.9998

3.3.7 Separation of the Whey Fractions

The pH of samples of each fermented milk specimen was adjusted to 4.6 using either 1.0 M HCl or 1.0 M NaOH, followed by centrifugation at 9,000 × *g* for 15 min at 4°C. Then, the resultant supernatant was passed through a 0.45-μm syringe filter to obtain the water-soluble extract. The filtered solutions were stored at −20°C before o-Phthalaldehyde assay.

3.3.8 o-Phthalaldehyde Assay (OPA)

The o-phthalaldehyde (OPA) assay was used to determine the free amino groups in fermented milk samples by assessing the degree of protein hydrolysis as per the method of Soleymanzadeh et al. (2016). The OPA reagent was prepared by combining 0.503 g sodium tetraborate, 0.5 g sodium dodecyl sulfate, 40 mg OPA (dissolved in 1 mL methanol), and 100 µL β-mercaptoethanol in 45 mL of deionized water. The whey fraction of the fermented milk samples was diluted to a final volume of 50 mL, vortexed for 1 min, and separated by centrifugation at 10,000 × *g* for 5 min. Then, 100 μL of the whey supernatant extract was mixed with 1000 μL of OPA reagent, incubated in the dark for 2 min, and the absorbance at 340 nm was measured using a Jenway UV/VIS spectrophotometer (Jenway 6300, Cole-Parmer, Staffordshire, UK) exactly 2 min later. Proteolysis (%) was calculated as follows: (absorbance of sample − absorbance of blank) / absorbance of sample × 100, Milli-Q water used as a reference blank in a duplicated procedure.

3.3.9 Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted as per the procedure reported by Mbye et al. (2023). The samples (0.2 g) were prepared by vortexing for 1 min with 4.17 mL of 8 M urea solution, followed by incubation at 37°C for 2 h. After centrifugation at 9,150 × *g* for 35 min at 4°C, the resultant supernatant was mixed with 30 μL of a fresh solution of 50 mM dithiothreitol in 4× Laemmli buffer. Following vortexing and heating at 90°C for 5 min, 6 μL of each sample was loaded onto polyacrylamide gels containing 12% resolving gel buffer and 4% stacking gel buffer. After electrophoresis, the gel was scanned and analyzed using a ChemiDoc MP

Imaging System (Bio-Rad, USA), and band intensities were estimated from the obtained electropherograms.

3.3.10 Statistical Analysis

All analytical measurements and experiments for fermented milk preparation were performed in triplicate, and sample values were averaged to obtain the mean values and standard deviation (SD). Statistical differences were evaluated using a one-way analysis of variance with the Tukey range test for multiple comparisons in Minitab v21.1.0 (Minitab, LLC, Pennsylvania, USA) statistical software. Between-group mean comparisons were performed using the least significant difference test, with $p \leq 0.05$ considered statistically significant. Pearson correlation in Minitab was utilized for correlation analysis. To estimate the correlation structure of the variables, principal component analysis was used for both variables and observations.

3.4 Results and Discussions

3.4.1 Bacterial Growth and Survival in CM Compared to BM

The LAB (i.e. *L. helveticus*, *L. delbrueckii subsp. bulgaricus*, and *S. thermophilus*) counts in CM and BM following incubation at 28°C, 35°C, and 42°C are shown in Figure 3.2. The results revealed that the bacterial growth increased with an increasing temperature during the first 24 h and behaved differently thereafter (remained stable, increased or declined). This trend can be explained by the cumulative effects of nutrient depletion, increased acidity, and potentially adverse environmental conditions (Soleymanzadeh et al., 2016). At each temperature, there was no difference in bacterial counts of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* between CM and BM, in disagreement with previous literature (Attia et al., 2001). However, once acclimatized, these bacteria exhibited similar growth rates in both types of milk. *L. helveticus* behaved differently from the other two bacteria, showing a much slower growth rate in CM than in BM, particularly as the temperature increased ($p < 0.05$). Abu-Tarboush (1996) reported that although the growth of yogurt starter cultures was generally higher in BM than in CM, proteolysis was higher in CM than in BM, suggesting that specific metabolic activities, such as proteolysis, might be more pronounced despite the slower bacterial growth in CM. This indicates that the choice of starter cultures influences the microbial dynamics in CM, and certain combinations may promote increased acid production.

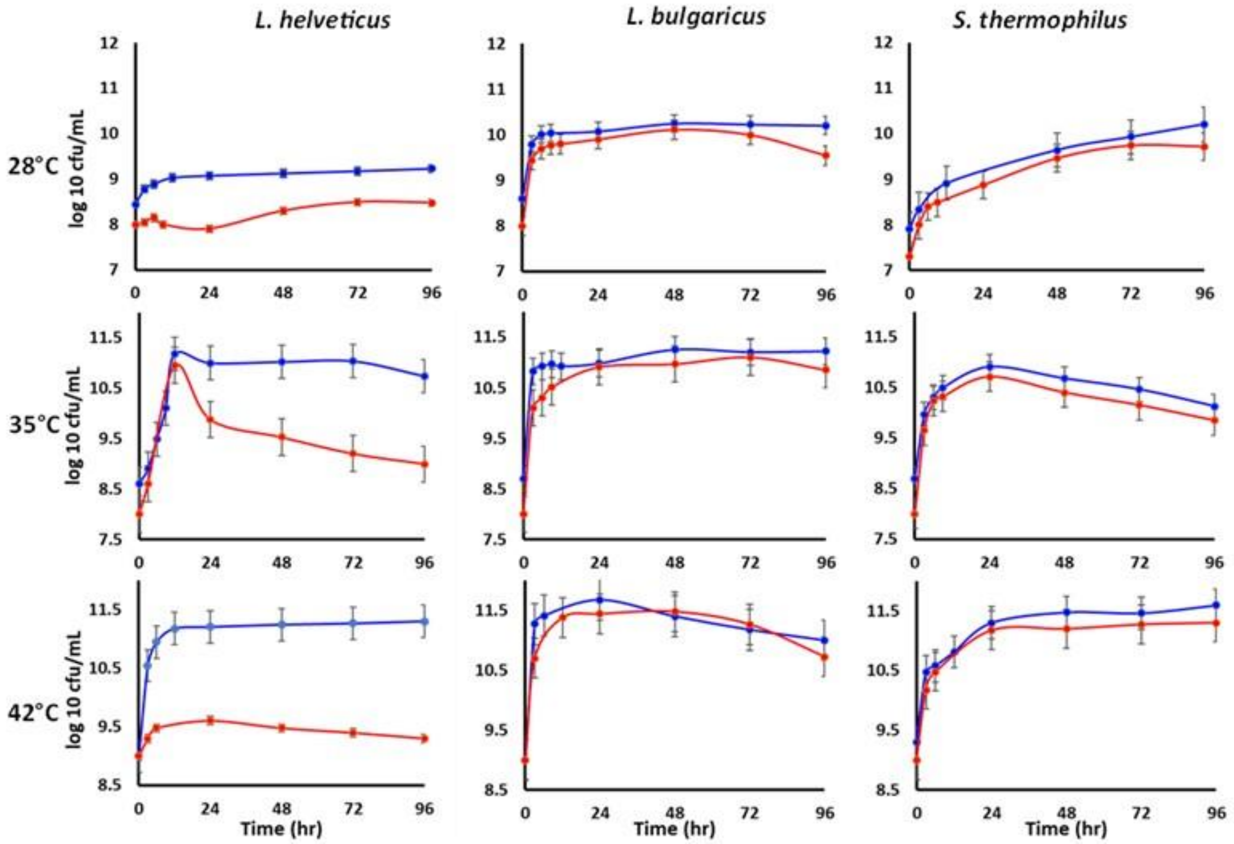

Figure 3.2: Growth of *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*), and *S. thermophilus* in camel milk (red color) and bovine milk (blue color) at 28°C, 35°C, and 42°C. Values mean ± SD (n = 3)

3.4.2 Acidification Capability

The changes in TA and pH during CM and BM fermentation by the three bacteria at the three temperatures are represented in Figure 3.3 The TA (%) increased in both milks upon fermentation and became more noticeable as the temperature increased. The rate of acidification was highest for *L. helveticus*, especially in BM. Thus, the interplay of temperature and milk type is crucial in determining TA, highlighting the importance of these factors in fermentation outcomes. *L. helveticus* showed the highest TA (%) and lowest pH in BM, which is supported by reports of its acidity tolerance and proficiency in metabolizing galactose (Tango & Ghaly 1999, Chelladhurai et al., 2023). Although all strains lowered the pH in both CM and BM, the acidification rate was significantly lower in CM compared with BM ($p < 0.05$), in agreement with Berhe et al. (2018). This variation is attributed to differences in proteolysis rather than the presence of inhibitory compounds in CM or nutrient depletion. Regarding the correlation of TA (%) and pH, there were no differences observed between CM and BM (Figure 3.4), suggesting similar buffering capacities, which disagrees with Attia et al. 2001).

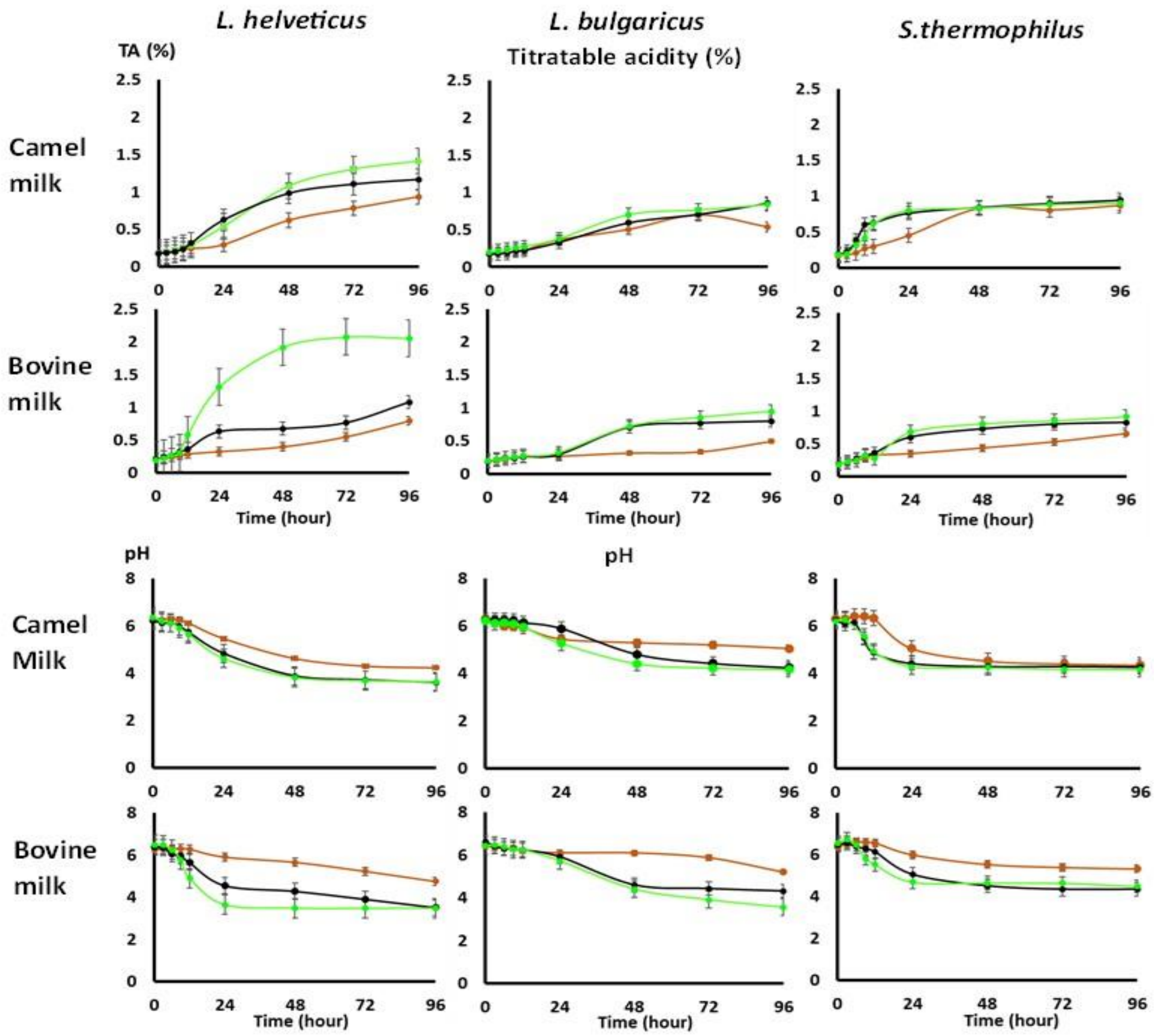

Figure 3.3: Titratable acidity (%) and pH changes during the fermentation of camel milk and bovine milk by *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus,* and *S. thermophilus* at 28°C (brown color line), 35°C (black color line), and 42°C (green color line). Values mean ± SD (n = 3)

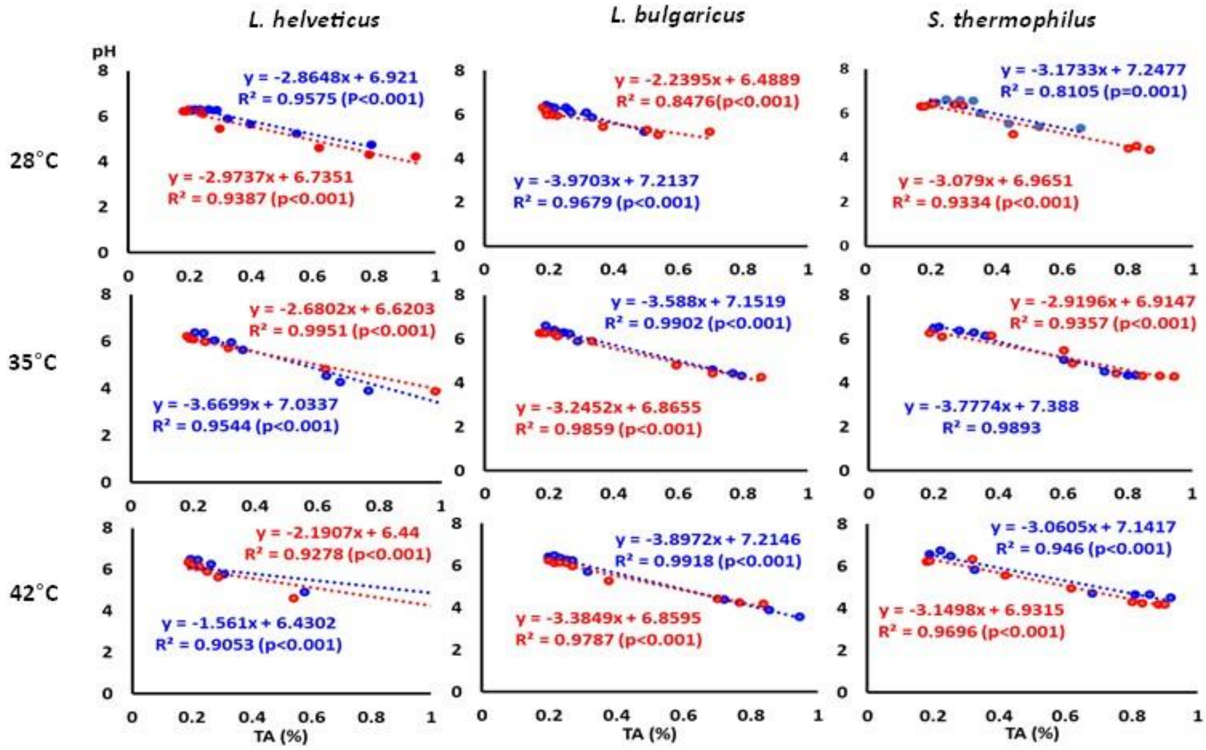

Figure 3.4: Correlation between pH and TA (%) during the fermentation of camel milk (red color line) and bovine milk (blue color line) by *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and S. *thermophilus*

3.4.3 Changes in Lactose and Organic Acids During Fermentation as Determined by HPLC

Organic acids result from the metabolic conversion of fermentable carbohydrates following glycolysis via the pentose phosphate pathway (Khedid et al., 2009, Jafari et al., 2021, Wang et al., 2021). Figure 3.5 shows the concentrations of lactose, as well as lactic, citric, formic, malic, and acetic acid in fermented CM and BM. The increase in TA (%) is mainly attributed to increased lactic acid concentration (Al-Zoreky & Al-Otaibi, 2015). Lactic acid production was higher in *L. helveticus*, suggesting enhanced lactose utilization (Tango & Ghaly, 1999), and the optimal temperature for lactic acid production by *L. helveticus* was identified as 42°C (Kulozik & Wilde, 1999). Citric acid concentrations decreased ($p < 0.05$) after 96 h, consistent with previous studies (Nassar et al., 2023). Acetic, malic, and formic acid concentrations peaked after 96 h of fermentation ($p < 0.05$). Acetic acid is an intermediary in citrate metabolism, representing an additional outcome of heterofermentative lactose metabolism (Wang et al., 2021). Organic acids contribute to flavor, aroma, and product preservation (Nassar et al., 2023). Organic acid production was higher in BM than in CM, suggesting that the metabolic activity of LAB was more pronounced in BM fermentation.

3.4.4 Proteolysis in CM and BM Fermented Milk

The OPA assay measures primary amines through fluorescent derivatives, used to assess the degree of proteolysis (%) in the fermented milks (Figure 3.6). CM exhibited a higher degree of proteolysis than BM ($p < 0.05$), but the increase in OPA over time was higher in BM, possibly due to differences in CM protein composition and structure that make them easier to hydrolyze than BM proteins. This phenomenon can be attributed to the higher proportion of β-casein in CM, higher proline content in CM caseins, and higher levels of micelle hydration and mineral composition, which render it more susceptible to proteolytic degradation during fermentation (Moslehishad et al., 2013, Ipsen, 2017). An increased rate of proteolysis has been reported to limit the growth rates of commercial starter cultures in pure CM (Berhe et al. 2018).

In agreement with the literature, *L. helveticus* showed higher proteolytic activity than the other two bacteria (Guron et al., 2023; Zhou et al., 2019; Chelladhurai et al., 2023). The impact of temperature on the OPA values was minimal, which may be because peptide utilization took precedence over protein hydrolysis by LAB proteases (Gandhi & Shah., 2014). The slower growth rate and increased lactic acid production of *L. helveticus* in milk cultures may also play a role in its higher degree of protein hydrolysis (Torino et al., 2001). The protein band intensity in non-fermented CM and BM was higher than that in fermented samples, particularly α-casein and β-casein (Figure 3.7). Supporting the OPA results, fermented CM exhibited several as-yet unidentified bands in the 10 to <75 kDa range, indicating considerable proteolysis. The electrophoresis results provide unequivocal evidence of *L. helveticus* displaying the highest proteolytic activity.

Figure 3.8 presents the principal component analysis results of the CM and BM samples fermented with three different bacterial species over 96 h. The loading plot revealed distinct correlation patterns between fermentation parameters. Principal component 1 (PC1), explaining 58.7% of the variance, showed strong negative correlations between pH and lactose concentration on one hand, and TA, lactic acid concentration, and OPA on the other hand. Principal component 2 (PC2), explaining 16.9% of the variance, revealed negative correlations between OPA values and bacterial counts, lactose, and lactic acid (%). The score plots showed that fermentation with *L. delbrueckii* subsp. *bulgaricus* led to lower OPA values compared to that with *L. helveticus* and *S. thermophilus* and that CM fermentation resulted in higher OPA values than BM fermentation.

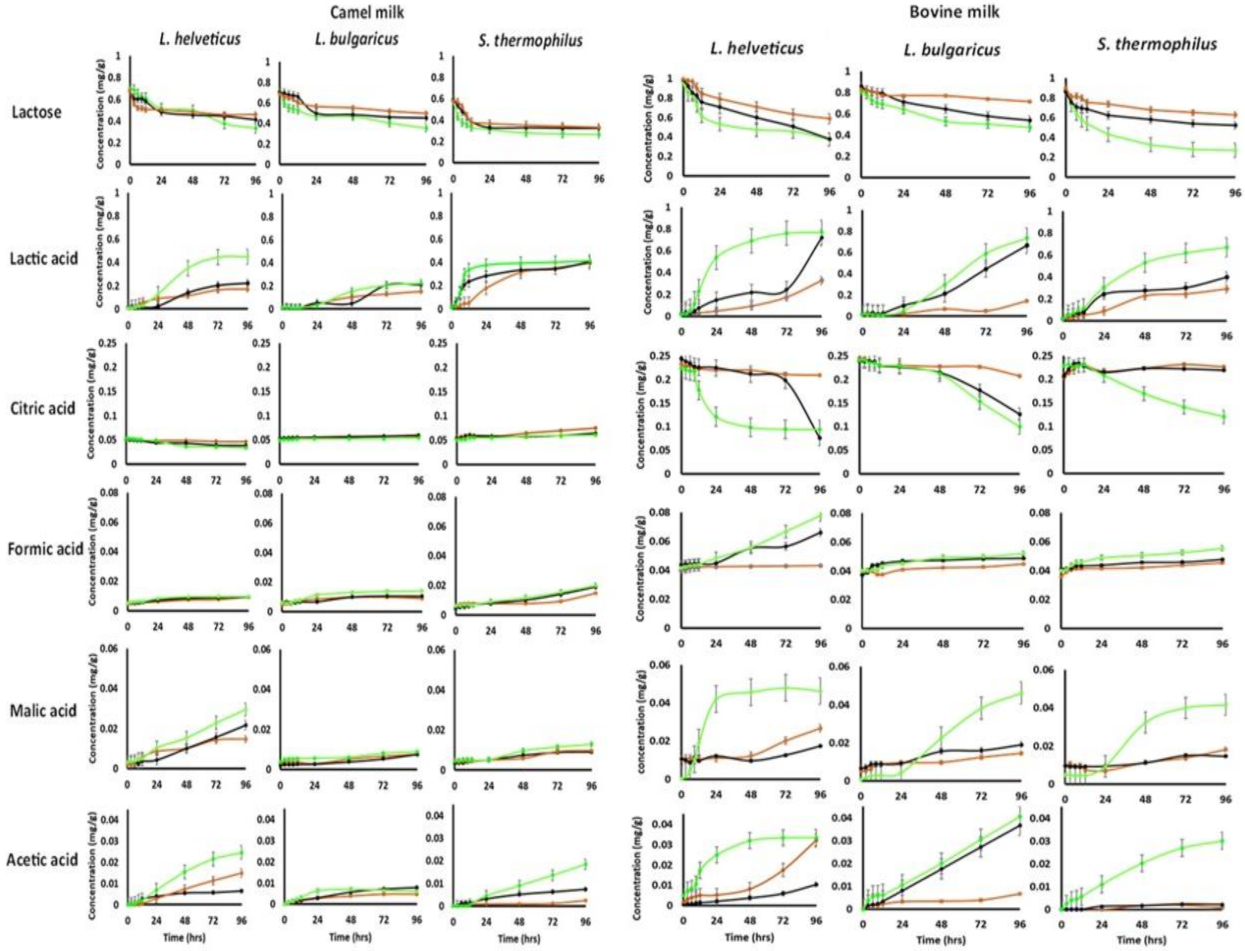

Figure 3.5: Effect of temperatures 28°C (brown color), 35°C (black color), and 42°C (green color) and fermentation time on the concentrations of lactose, lactic acid, citric acid, formic acid, malic acid, and acetic acid (mg/g) in fermented camel milk and bovine milk samples. Values mean ± SD (n = 3)

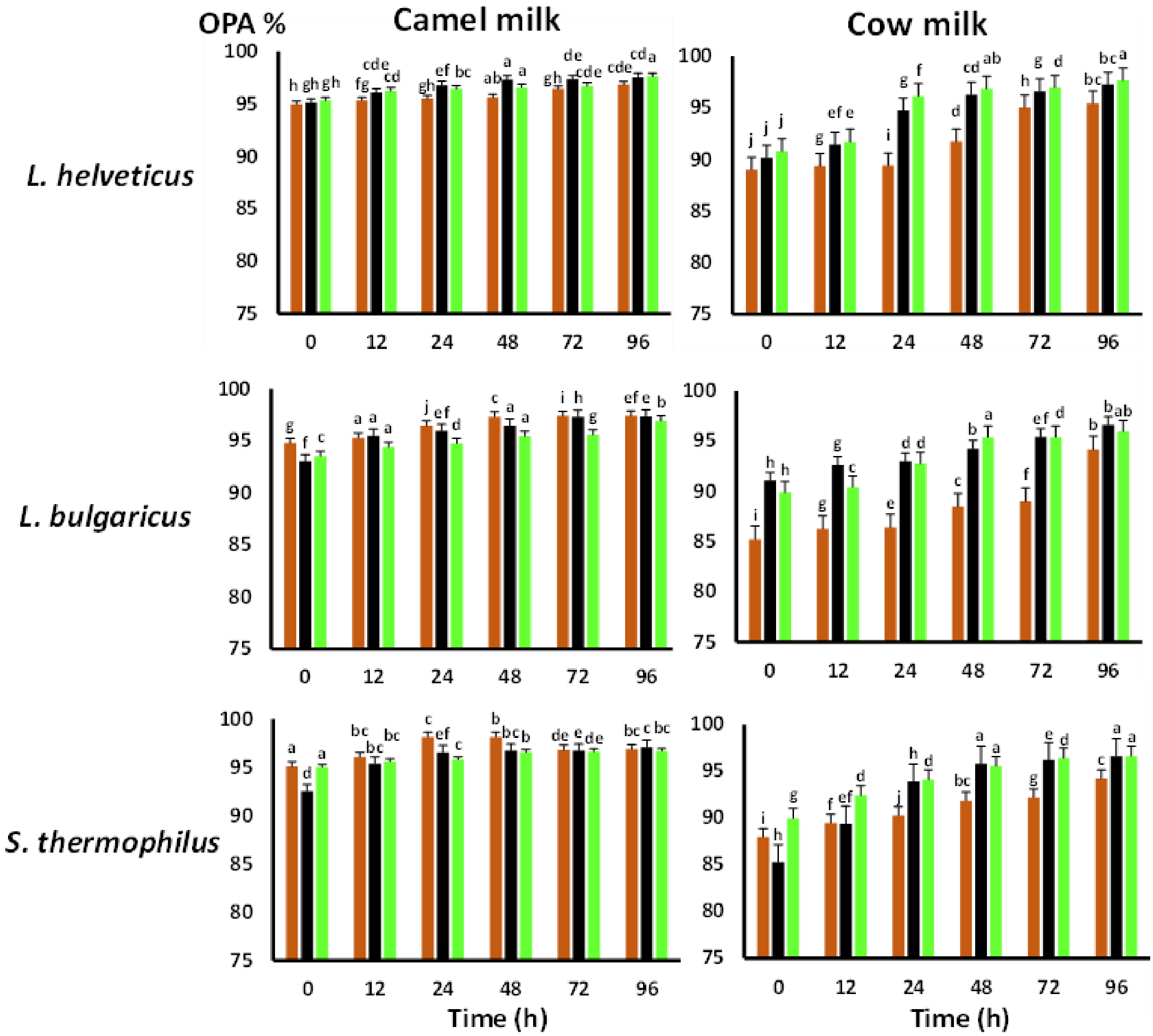

Figure 3.6: Degree of hydrolysis (OPA, %) in camel milk and bovine milk fermented with *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* at 28°C (brown color bar), 35°C (black color bar), and 42°C (green color bar). Values mean ± SD (n = 3)

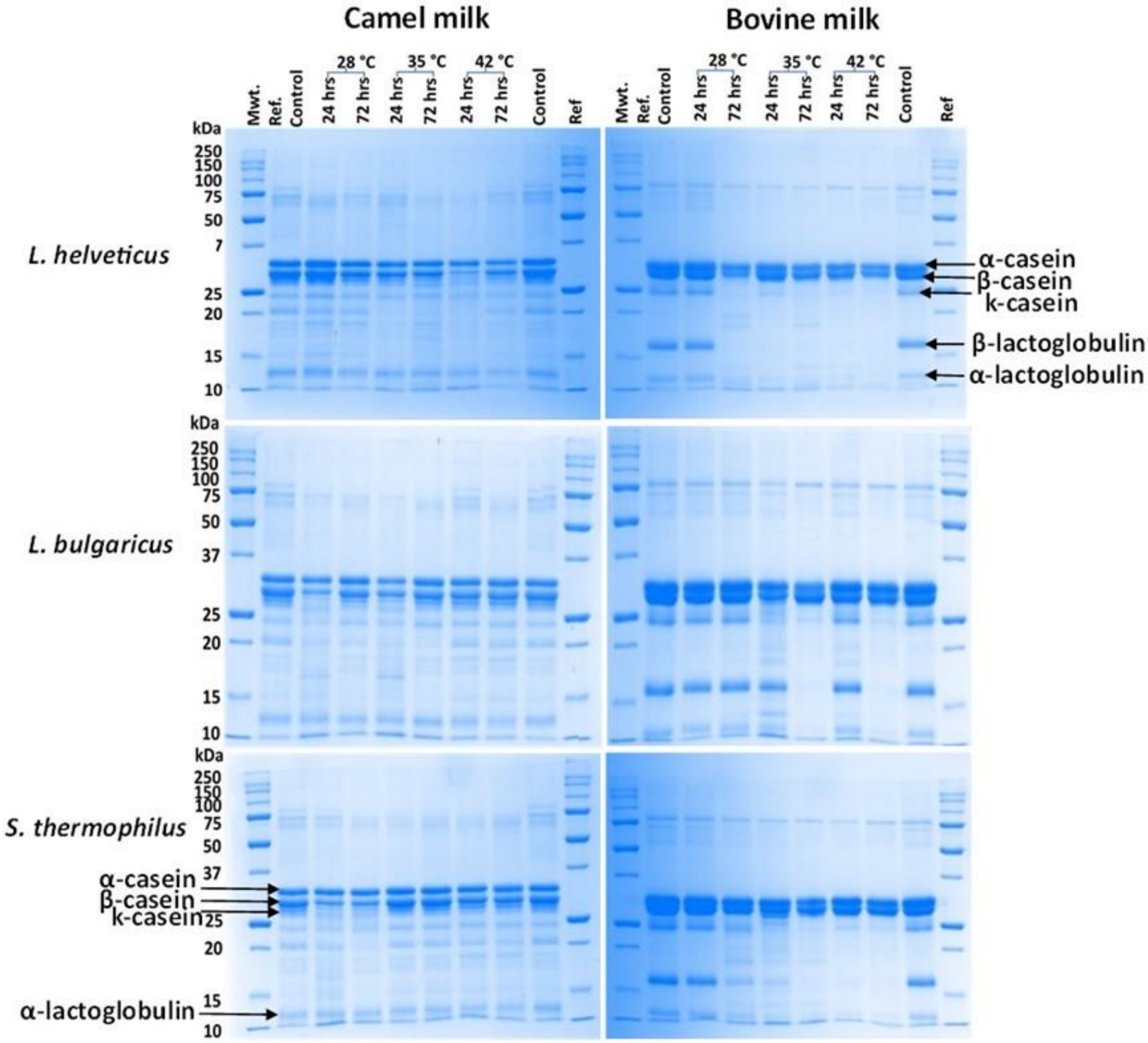

Figure 3.7: SDS-PAGE electropherograms of camel and bovine milks fermented with *L.helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* at 28°C, 35°C, and 42°C

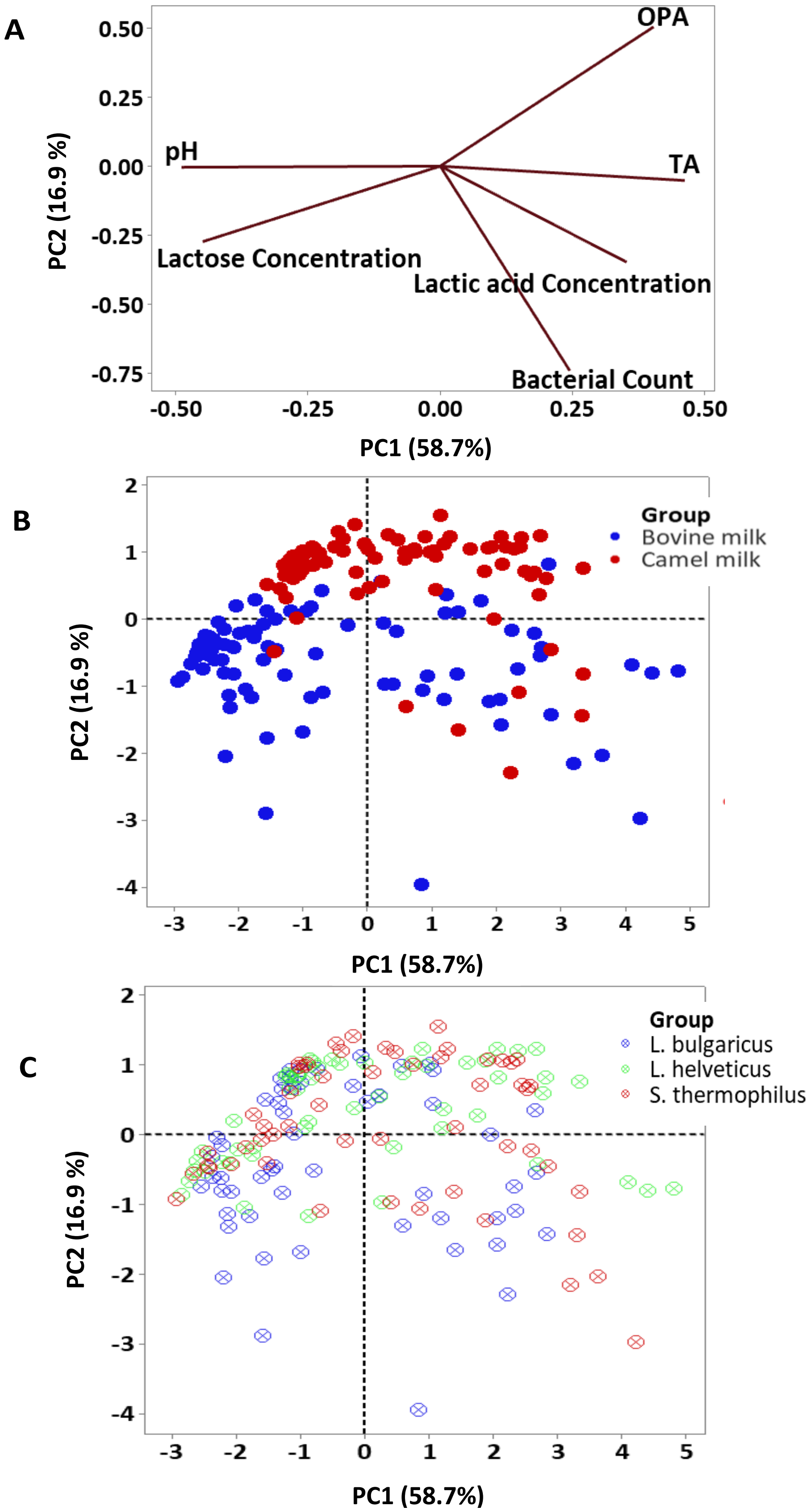

Figure 3.8: Principal components analysis (PCA): (A) loading plot, (B) score plot labelled according to the bacteria used, and (C) score plot labelled according to the milk type

Credit authorship contribution statement

Kobika Chelladhurai Data curation, Formal analysis, Writing- Original draft preparation. Santhoshani Warakaulle and Sifatun Nesa Ali Data curation. Mutamed Ayyash and Mark Turner Conceptualization, Methodology, Writing - Review & Editing. Afaf Kamal-Eldin Conceptualization, Methodology, Validation, Investigation, Resources, Supervision, Funding acquisition, Writing - Review & Editing.

Declaration of interest

None.

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Data statement

Data will be made available on request.

Chapter 4: Conclusions

The literature review, presented in Chapter 2, covers the current knowledge about *Lactobacillus helveticus* and its use in dairy fermentation. Chapter 3 presents an experimental study comparing the fermentation of camel milk (CM) and bovine milk (BM) with *L. helveticus* as compared to *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* at three different temperatures (28°C, 35°C, and 42°C) for a total of 96 hours. Fermentation behaviour was assessed through bacterial growth, changes in titratable acidity, lactose, organic acids, pH, and proteolysis.

The bacterial growth was comparable in CM and BM, but the fermented products showed differences in acidification and proteolysis. Negative correlations between TA (%) and pH were consistent in both CM and BM suggesting similar buffering capacities. *L. helveticus* was characterized by increased proteolysis and high acidification rates. In both milks, *L. helveticus* behavior was more comparable to *S. thermophilus* than *L. delbrueckii* subsp. *bulgaricus*. These findings provide valuable insights into the dynamics of *L. helveticus* offering possibilities for alternative fermentation processes in dairy product production.

The results presented in this thesis suggest fermentation of camel and bovine milk with *L. helveticus* might lead to the production of health promoting dairy products. Further studies in this direction is warranted.

References

Abarquero, D., Renes, E., Fresno, J. M., & Tornadijo, M. E. (2022). Study of exopolysaccharides from lactic acid bacteria and their industrial applications: a review. *International Journal of Food Science and Technology*, *57*(1), 16–26. https://doi.org/10.1111/IJFS.15227

Abd El-Aziz, M., Kassem, J. M., Assem, F. M., & Abbas, H. M. (2022). Physicochemical properties and health benefits of camel milk and its applications in dairy products: A review. *Egyptian Journal of Chemistry*, *65*(5), 101–118. https://doi.org/10.21608/EJCHEM.2021.92589.4383

Abdelhamid, A. G., Esaam, A., & Hazaa, M. M. (2018). Cell-free preparations of probiotics exerted antibacterial and antibiofilm activities against multidrug-resistant *E. coli*. *Saudi Pharmaceutical Journal*, *26*(5), 603–607. https://doi.org/10.1016/J.JSPS.2018.03.004

Abdi-Moghadam, Z., Darroudi, M., Mahmoudzadeh, M., Mohtashami, M., Jamal, A. M., Shamloo, E., & Rezaei, Z. (2023). Functional yogurt enriched and probiotic: A focus on human health. *Clinical Nutrition ESPEN*, *57*, 575–586. https://doi.org/10.1016/J.CLNESP.2023.08.005

Abu-Tarboush, H. M. (1996). Comparison of associative growth and proteolytic activity of yogurt starters in whole milk from amels and cows. *Journal of Dairy Science*, *79*(3), 366–371. https://doi.org/10.3168/JDS.S0022-0302(96)76373-7

Agyei, D., Owusu-Kwarteng, J., Akabanda, F., & Akomea-Frempong, S. (2020). Indigenous African fermented dairy products: Processing technology, microbiology and health benefits. *Critical Reviews in Food Science and Nutrition*, *60*(6), 991–1006. https://doi.org/10.1080/10408398.2018.1555133

Ah, J., & Govind, T. (2017). Functional properties of Mozzarella cheese for its end-use application. *Journal of Food Science and Technology*, *54*(12), 3766–3778. https://doi.org/10.1007/s13197-017-2886-z

Akabanda, F., Owusu-Kwarteng, J., Tano-Debrah, K., Parkouda, C., & Jespersen, L. (2014). The Use of Lactic Acid Bacteria Starter Culture in the Production of Nunu, a Spontaneously Fermented Milk Product in Ghana. *International Journal of Food Science*. https://doi.org/10.1155/2014/721067

Alhaj, O. A. (2016). Identification of potential ACE-inhibitory peptides from dromedary fermented camel milk. *CyTA – Journal of Food*, *15*(2), 191–195. https://doi.org/10.1080/19476337.2016.1236353

Alhaj, O. A. (2017). Identification of potential ACE-inhibitory peptides from dromedary fermented camel milk. *CyTA-Journal of Food*, *15*(2), 191–195. https://doi.org/10.1080/19476337.2016.1236353

Alhaj, O. A., Metwalli, A. A., Ismail, E. A., Ali, H. S., Al-Khalifa, A. S., & Kanekanian, A. D. (2018). Angiotensin-converting enzyme-inhibitory activity and antimicrobial effect of fermented camel milk (*Camelus dromedarius*). *International Journal of Dairy Technology*, *71*(1), 27–35. https://doi.org/10.1111/1471-0307.12383

Al-Zoreky, N. S., & Al-Otaibi, M. M. (2015). Suitability of camel milk for making yogurt. *Food Science and Biotechnology*, *24*(2), 601–606. https://doi.org/10.1007/s10068-015-0078-z

Angelescu, I. R., Grosu-Tudor, S. S., Cojoc, L. R., Maria, G. M., Chirițoiu, G. N., Munteanu, C. V. A., & Zamfir, M. (2022). Isolation, characterization, and mode of action of a class III bacteriocin produced by *Lactobacillus helveticus* 34.9. *World Journal of Microbiology and Biotechnology*, *38*(12), 1–13. https://doi.org/10.1007/ S11274-022-03408-Z

Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. *Journal of Dairy Science*, *100*, 9987–10013. https://doi.org/10.3168/jds.2017-12981

Asiimwe, R., Ainembabazi, J. H., Egeru, A., Isoto, R., Aleper, D. K., Namaalwa, J., & Diiro, G. M. (2020). The role of camel production on household resilience to droughts in pastoral and agro-pastoral households in Uganda. *Pastoralism*, *10*(1), 1–12. https://doi.org/10.1186/S13570-020-0160-X/TABLES/6

Attia, H., Kherouatou, N., & Dhouib, A. (2001). Dromedary milk lactic acid fermentation: microbiological and rheological characteristics. *Journal of Industrial Microbiology & Biotechnology*, 26, 263-270. doi: 10.1038/sj.jim.7000111

Avila, B. P., da Rosa, P. P., Fernandes, T. A., Chesini, R. G., Sedrez, P. A., de Oliveira, A. P. T., Mota, G. N., Gularte, M. A., & Roll, V. F. B. (2020). Analysis of the perception and behavior of consumers regarding probiotic dairy products. *International Dairy Journal*, *106*, 104703. https://doi.org/10.1016/J.IDAIRYJ.2020.104703

Ayyash, M., Al-Dhaheri, A. S., Al Mahadin, S., Kizhakkayil, J., & Abushelaibi, A. (2018). In vitro investigation of anticancer, antihypertensive, antidiabetic, and antioxidant activities of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. *Journal of Dairy Science*, *101*(2), 900–911. https://doi.org/10.3168/jds.2017-13400

Bahrudin, M. F., Abdul Rani, R., Tamil, A. M., Mokhtar, N. M., & Raja Ali, R. A. (2020). Effectiveness of sterilized symbiotic drink containing *Lactobacillus helveticus* comparable to probiotic alone in patients with constipation-predominant irritable bowel syndrome. *Digestive Diseases and Sciences*, *65*(2), 541–549. https://doi.org/10.1007/S10620-019-05695-3/FIGURES/5

Baptista, D. P., & Gigante, M. L. (2022). Prato cheese with the addition of *Lactobacillus helveticus*: Effect of culture preparation on peptide profile. *Applied Food Research*, *2*(1), 100107. https://doi.org/10.1016/J.AFRES.2022.100107

Baptista, D. P., Negrão, F., Eberlin, M. N., & Gigante, M. L. (2020). Peptide profile and angiotensin-converting enzyme inhibitory activity of Prato cheese with salt reduction and *Lactobacillus helveticus* as an adjunct culture. *Food Research International*, *133*, 109190. https://doi.org/10.1016/J.FOODRES.2020.109190

Baptista, D. P., Salgaço, M. K., Sivieri, K., & Gigante, M. L. (2020). Use of static and dynamic in vitro models to simulate Prato cheese gastrointestinal digestion: Effect of *Lactobacillus helveticus* LH-B02 addition on peptides bioaccessibility. *Lebensmittel Wissenschaft und -Technologie*, 134, Article 110229. https://doi.org/10.1016/J. LWT.2020.110229

Begunova, A. V., Savinova, O. S., Glazunova, O. A., Moiseenko, K. V., Rozhkova, I. V., & Fedorova, T. V. (2020). Development of antioxidant and antihypertensive properties during growth of *Lactobacillus helveticus*, *Lactobacillus rhamnosus* and *Lactobacillus reuteri* on ow’s Milk: Fermentation and peptidomics study. *Foods*, *10*(1), 17. https://doi.org/10.3390/FOODS10010017

Berhe, T., Ipsen, R., Seifu, E., Kurtu, M. Y., Eshetu, M., & Hansen, E. B. (2018). Comparison of the acidification activities of commercial starter cultures in camel and bovine milk. *LWT*, *89*, 123–127. https://doi.org/10.1016/J.LWT.2017.10.041

Callanan, M., Kaleta, P., O'Callaghan, J., O'Sullivan, O., Jordan, K., McAuliffe, O., Sangrador-Vegas, A., Slattery, L., Fitzgerald, G. F., Beresford, T., & Ross, R. P. (2008). Genome sequence of *Lactobacillus helveticus*, an organism distinguished by selective gene loss and insertion sequence element expansion. *Journal of Bacteriology*, *190*(2), 727–727. https://doi.org/10.1128/JB.01295-07

Carlman, H. M. T. E., Rode, J., Konig, ¨ J., Repsilber, D., Hutchinson, A. N., Thunberg, P., Persson, J., Kiselev, A., Pruessner, J. C., & Brummer, R. J. (2022). Probiotic mixture containing *Lactobacillus helveticus*, *Bifidobacterium longum* and *Lactiplantibacillus plantarum* affects brain responses to an arithmetic stress task in healthy subjects: A randomised clinical trial and proof-of-concept study. *Nutrients*, *14*(7), 1329. https:// doi.org/10.3390/NU14071329/S1

Chadha, J., Nandi, D., Atri, Y., & Nag, A. (2021). Significance of human microbiome in breast cancer: Tale of an invisible and an invincible. *Seminars in Cancer Biology*, *70*, 112–127. https://doi.org/10.1016/j.semcancer.2020.07.010

Cheikh Ismail, L., Osaili, T. M., Mohamad, M. N., Zakaria, H., Ali, A., Tarek, A., Ashfaq, A., Al Abdouli, M. A., Saleh, S. T., Daour, R. Al, Alrajaby, R., Stojanovska, L., & Al Dhaheri, A. S. (2022). Camel milk consumption patterns and perceptions in the UAE: a cross-sectional study. *Journal of Nutritional Science*, *11*, e59. https://doi.org/10.1017/JNS.2022.55

Chelladhurai, K., Ayyash, M., Turner, M. S., & Kamal-Eldin, A. (2023). *Lactobacillus helveticus*: Health effects, current applications, and future trends in dairy fermentation. *Trends in Food Science and Technology*, *136*, 159–168. https://doi.org/10.1016/J.TIFS.2023.04.013

Chen, Y. F., Zhao, W. J., Wu, R. N., Sun, Z. H., Zhang, W. Y., Wang, J. C., Bilige, M., & Zhang, H. P. (2014). Proteome analysis of *Lactobacillus helveticus* H9 during growth in skim milk. *Journal of Dairy Science*, *97*(12), 7413–7425. https://doi.org/10.3168/JDS.2014-8520

Chen, Y., Liu, W., Xue, J., Yang, J., Chen, X., Shao, Y., Kwok, L. yu, Bilige, M., Mang, L., & Zhang, H. (2014). Angiotensin-converting enzyme inhibitory activity of *Lactobacillus helveticus* strains from traditional fermented dairy foods and antihypertensive effect of fermented milk of strain H9. *Journal of Dairy Science*, *97*(11), 6680–6692. https://doi.org/10.3168/JDS.2014-7962

Chung, Y. C., Jin, H. M., Cui, Y., Kim, D. S., Jung, J. M., Park, J. il, Jung, E. S., Choi, E. K., & Chae, S. W. (2014). Fermented milk of *Lactobacillus helveticus* IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults. *Journal of Functional Foods*, *10*, 465–474. https://doi.org/10.1016/J.JFF.2014.07.007

Coelho, M. C., Malcata, F. X., & Silva, C. C. G. (2022). Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. *Foods*, *11*(15), 2276. https://doi.org/10.3390/FOODS11152276

Costa, M. P. Da, Frasao, B. D. S., Lima, B. R. C. D. C., Rodrigues, B. L., & Junior, C. A. C. (2016). Simultaneous analysis of carbohydrates and organic acids by HPLC-DAD-RI for monitoring goat's milk yogurts fermentation. *Talanta*, *152*, 162–170. https://doi.org/10.1016/J.TALANTA.2016.01.061

Cotter, P. D., Ross, R. P., & Hill, C. (2012). Bacteriocins — a viable alternative to antibiotics?. *Nature Reviews Microbiology 2012 11:2*, *11*(2), 95–105. https://doi.org/10.1038/nrmicro2937

Cuffia, F., Bergamini, C. v., Wolf, I. v., Hynes, E. R., & Perotti, M. C. (2018). Characterization of volatile compounds produced by *Lactobacillus helveticus* strains in a hard cheese model. *Food Science and Technology International*, *24*(1), 67–77. https://doi.org/10.1177/1082013217728628/ASSET/IMAGES/LARGE/10.1177_1082013217728628-FIG2.JPEG

Cundell, D. R., & Tripepi, M. (2021). The role of probiotics and prebiotics in the composition of the gut microbiota and their influence on inflammatory bowel disease, obesity, and diabetes. Microbiome-Host Interactions, 113–128. https://doi. org/10.1201/9781003037521-12

Dahiya, D., & Nigam, P. S. (2022). Clinical potential of microbial Strains, Used in fermentation for probiotic food, beverages, and in synbiotic supplements, as psychobiotics for cognitive treatment through gut-brain signaling. *Microorganisms 2022*, *Vol. 10*, *Page 1687*, *10*(9), 1687. https://doi.org/10.3390/MICROORGANISMS10091687

Egger, L., Ménard, O., Abbühl, L., Duerr, D., Stoffers, H., Berthoud, H., Meola, M., Badertscher, R., Blaser, C., Dupont, D., & Portmann, R. (2021). Higher microbial diversity in raw than in pasteurized milk Raclette-type cheese enhances peptide and metabolite diversity after in vitro digestion. *Food Chemistry*, *340*, 128154. https://doi.org/10.1016/J.FOODCHEM.2020.128154

El-Hatmi, H., Oussaief, O., Hammadi, I., Dbara, M., Hammadi, M., Khorchani, T., & Jrad, Z. (2023). Relation between color and chemical composition of dromedary camel colostrum. *Animals*, *13*(3), 442. https://doi.org/10.3390/ANI13030442

El-Sayed, H. S., Salama, H. H., & Edris, A. E. (2020). Survival of *Lactobacillus helveticus* CNRZ32 in spray-dried functional yogurt powder during processing and storage. *Journal of the Saudi Society of Agricultural Sciences*, *19*(7), 461–467. https://doi.org/10.1016/J.JSSAS.2020.08.003

Fan, M., Guo, T., Li, W., Chen, J., Li, F., Wang, C., Shi, Y., Li, D. X., & Zhang, S. (2019). Isolation and identification of novel casein-derived bioactive peptides and potential functions in fermented casein with *Lactobacillus helveticus*. *Food Science and Human Wellness*, *8*(2), 156–176. https://doi.org/10.1016/J.FSHW.2019.03.010

Fazilah, N. F., Ariff, A. B., Khayat, M. E., Rios-Solis, L., & Halim, M. (2018). Influence of probiotics, prebiotics, synbiotics, and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods*, *48*, 387–399. https://doi.org/10.1016/J.JFF.2018.07.039

Fontana, A., Falasconi, I., Molinari, P., Treu, L., Basile, A., Vezzi, A., Campanaro, S., & Morelli, L. (2019). Genomic comparison of *Lactobacillus helveticus* strains highlights probiotic potential. *Frontiers in Microbiology*, *10*(JUN), 1380. https://doi.org/10.3389/FMICB.2019.01380/BIBTEX

Gandhi, A., & Shah, N. P. (2014). Cell growth and proteolytic activity of *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, and *Streptococcus thermophilus* in milk as affected by supplementation with peptide fractions. *International Journal of Food Sciences and Nutrition*, *65*(8), 937–941. https://doi.org/10.3109/09637486.2014.945154

Garbowska, M., Berthold-Pluta, A., Stasiak-Rozanska, L., & Pluta, A. (2021). The impact of the adjunct heat-treated starter culture and *Lactobacillus helveticus* LH-b01 on the proteolysis and ace inhibitory activity in dutch-type cheese model during ripening. *Animals*, *11*(9). https://doi.org/10.3390/ani11092699

Gaur, S., Waller, A. W., & Andrade, J. E. (2018). Effect of multiple micronutrient fortification on physico-chemical and sensory properties of Chhash (traditional Indian yogurt-based drink). *Foods 2019*, *8*(1), 5. https://doi.org/10.3390/FOODS8010005

Ge, Z., Bao, X., Feng, X., Yin, X., Chen, X., & Dong, M. (2022). In situ exopolysaccharides from *Lactobacillus helveticus* MB2-1 in Sayram ketteki yoghurt: Set yoghurts properties and interactions with sodium caseinate. *International Journal of Biological Macromolecules*, *216*, 871–881. https://doi.org/10.1016/J.IJBIOMAC.2022.07.147

Ge, Z., Bao, X., Li, Z., Chen, X., Li, W., Rui, X., Wu, J., Zhang, Q., & Dong, M. (2022b). In situ exopolysaccharides produced by *Lactobacillus helveticus* MB2-1 and its effect on gel properties of Sayram ketteki yoghurt. *International Journal of Biological Macromolecules*, *208*, 314–323. https://doi.org/10.1016/J.IJBIOMAC.2022.03.027

Giacometti Cavalheiro, F., Parra Baptista, D., Domingues Galli, B., Negrão, F., Nogueira Eberlin, M., & Lúcia Gigante, M. (2020). High protein yogurt with the addition of *Lactobacillus helveticus*: Peptide profile and angiotensin-converting enzyme ACE-inhibitory activity. *Food Chemistry*, *333*, 127482. https://doi.org/10.1016/J.FOODCHEM.2020.127482

Giraffa, G. (2014). *Lactobacillus helveticus*: Importance in food and health. *Frontiers in Microbiology*, *5*(JULY), 338. https://doi.org/10.3389/FMICB.2014.00338/BIBTEX

Green, I. R., Oberg, C. J., Broadbent, J. R., Thunell, R. K., & McMahon, D. J. (2021). Galactose-positive adjunct cultures prevent gas formation by *Paucilactobacillus wasatchensis* WDC04 in a model gas production test. *Journal of Dairy Science*, *104*(10), 10540–10549. https://doi.org/10.3168/JDS.2021-20428

Griffiths, M. W., & Tellez, A. M. (2013). *Lactobacillus helveticus*: The proteolytic system. *Frontiers in Microbiology*, *4*(MAR), 30. https://doi.org/10.3389/fmicb.2013.00030

Grosu-Tudor, S.S., Maria, G.-M., & Zamr, M. (2021). Isolation, characterization, and applicability of Helveticin 34.9, a class iii bacteriocin produced by *Lactobacillus helveticus* 34.9. https://doi.org/10.21203/RS.3.RS-808205/V1

Gupta, H., Kim, S. H., Kim, S. K., Han, S. H., Kwon, H. C., & Suk, K. T. (2022). Beneficial shifts in gut microbiota by *Lacticaseibacillus rhamnosus* R0011 and *Lactobacillus helveticus* R0052 in alcoholic hepatitis. Microorganisms, *10*(7), 1474. https://doi.org/ 10.3390/MICROORGANISMS10071474/S1

Guron, G. K., Qi, P. X., McAnulty, M. J., Renye Jr, J. A., Miller, A. L., Oest, A. M., Wickham, E. D., & Harron, A. (2023). Differential behavior of *Lactobacillus helveticus* B1929 and ATCC 15009 on the hydrolysis and angiotensin-I-converting enzyme inhibition activity of fermented ultra-high-temperature milk and nonfat dried milk powder. *Journal of Dairy Science*, *106*, 4502–4515. https://doi.org/10.3168/jds.2022-22842

Hamed, N. S., Mbye, M., Ayyash, M., Ulusoy, B. H., & Kamal-Eldin, A. (2024). Camel milk: Antimicrobial agents, fermented products, and shelf life. *Foods 2024, 13*(3), 381. https://doi.org/10.3390/FOODS13030381

Hashemi, S. M. B., & Gholamhosseinpour, A. (2020). Fermentation of Chortan (heated strained yoghurt) by *Lactobacillus helveticus*: sonication treatment and biological activities. *International Journal of Food Science & Technology*, *55*(2), 908–915. https://doi.org/10.1111/IJFS.14365

Hassan, M. U., Nayab, H., Rehman, T. U., Williamson, M. P., Haq, K. U., Shafi, N., Shafique, F., & de Guía Córdoba, M. (2020). Characterization of bacteriocins produced by *Lactobacillus spp*. isolated from the traditional Pakistani yoghurt and their antimicrobial activity against common foodborne pathogens. *BioMed Research International*, *2020*. https://doi.org/10.1155/2020/8281623

Hassan, M. U., Nayab, H., Shafique, F., Williamson, M. P., Almansouri, T. S., Asim, N., Shafi, N., Attacha, S., Khalid, M., Ali, N., & Akbar, N. (2020). Probiotic properties of *Lactobacillus helveticus* and *Lactobacillus plantarum* isolated from traditional Pakistani yoghurt. https://doi.org/10.1155/2020/8889198

Hati, S., Patel, N., & Mandal, S. (2018). Comparative growth behaviour and biofunctionality of lactic acid bacteria during fermentation of soy milk and bovine milk. *Probiotics and Antimicrobial Proteins*, *10*(2), 277–283. https://doi.org/10.1007/s12602-017-9279-5

Hati, S., Patel, N., Sakure, A., & Mandal, S. (2017). Influence of whey protein concentrate on the production of antibacterial peptides derived from fermented milk by lactic acid bacteria. *International Journal of Peptide Research and Therapeutics 2017 24:1*, *24*(1), 87–98. https://doi.org/10.1007/S10989-017-9596-2

Ho, S. W., El-Nezami, H., & Shah, N. P. (2020). The protective effects of enriched citrulline fermented milk with *Lactobacillus helveticus* on the intestinal epithelium integrity against Escherichia coli infection. *Scientific Reports 2020 10:1*, *10*(1), 1–15. https://doi.org/10.1038/s41598-020-57478-w

Huang, R., Wang, K., & Hu, J. (2016). Effect of probiotics on depression: A systematic review and Meta-analysis of randomized controlled Trials. *Nutrients 2016*, *8*(8), 483. https://doi.org/10.3390/NU8080483

Ipsen, R. (2017). Opportunities for producing dairy products from camel milk: A comparison with bovine milk. *East African Journal of Sciences*, *11*(2), 93–98. https://www.ajol.info/index.php/eajsci/article/view/183085

Jafari, M., Shariatifar, N., Khaniki, G. J., Sayadi, M., & Abdollahi, A. (2021). Molecular Characterization of isolated lactic acid bacteria from different traditional dairy products of tribes in the Fars province, Iran. *Journal of Microbiology, Biotechnology, and Food Sciences*, *11*(2), 1–6. https://doi.org/10.15414/jmbfs.3621

Khedid, K., Faid, M., Mokhtari, A., Soulaymani, A., & Zinedine, A. (2009). Characterization of lactic acid bacteria isolated from the one-humped camel milk produced in Morocco. *Microbiological Research*, *164*, 81–91. https://doi.org/10.1016/j.micres.2006.10.008

Kido, Y., Maeno, S., Tanno, H., Kichise, Y., Shiwa, Y., & Endo, A. (2021). Niche-specific adaptation of *Lactobacillus helveticus* strains isolated from malt whisky and dairy fermentations. *Microbial Genomics*, *7*(4), 000560. https://doi.org/10.1099/MGEN.0.000560/CITE/REFWORKS

Kittibunchakul, S., Pham, M. L., Tran, A. M., & Nguyen, T. H. (2019). β-Galactosidase from *Lactobacillus helveticus* DSM 20075: Biochemical characterization and recombinant expression for applications in the dairy industry. *International Journal of Molecular Sciences*, *20*(4), 947. https://doi.org/10.3390/IJMS20040947

Kobatake, E., Kobayashi, R., Kabuki, T., & Kurita-Ochiai, T. (2019). *Lactobacillus helveticus* SBT2171 upregulates the expression of β-defensin and ameliorates periodontal disease caused by *Porphyromonas gingivalis*. *Microbiology and Immunology*, *63*(8), 293–302. https://doi.org/10.1111/1348-0421.12719

Kulozik, U., & Wilde, J. (1999). Rapid lactic acid production at high cell concentrations in whey ultrafiltrate by *Lactobacillus helveticus*. *Enzyme and Microbial Technology*, *24*(5–6), 297–302. https://doi.org/10.1016/S0141-0229(98)00122-7

Leung, K., & Thuret, S. (2015). Gut Microbiota: A Modulator of brain plasticity and cognitive function in ageing. *Healthcare*, *3*(4), 898–916. https://doi.org/10.3390/HEALTHCARE3040898

Li, B., Du, P., Smith, E. E., Wang, S., Jiao, Y., Guo, L., Huo, G., & Liu, F. (2019). In vitro and in vivo evaluation of an exopolysaccharide produced by *Lactobacillus helveticus* KLDS1.8701 for the alleviative effect on oxidative stress. *Food & Function*, *10*(3), 1707–1717. https://doi.org/10.1039/C8FO01920G

Li, J., Zhao, J., Wang, X., Qayum, A., Hussain, M. A., Liang, G., Hou, J., Jiang, Z., & Li, A. (2019). Novel angiotensin-converting enzyme-inhibitory peptides from fermented bovine milk started by *Lactobacillus helveticus* KLDS.31 and *Lactobacillus casei* KLDS.105: Purification, identification, and interaction mechanisms. *Frontiers in Microbiology*, *10*, 2643. https://doi.org/10.3389/FMICB.2019.02643/BIBTEX

Liang, S., Wang, T., Hu, X., Luo, J., Li, W., Wu, X., Duan, Y., & Jin, F. (2015). Administration of *Lactobacillus helveticus* NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neuroscience*, *310*, 561–577. https://doi.org/10.1016/J.NEUROSCIENCE.2015.09.033

Lukic, J., Terzic-Vidojevic, A., & Lozo, J. (2013). Use of *Lactobacillus helveticus* BGRA43 for manufacturing fermented milk products. *Food Technology and Biotechnology*, *51*(2), 257–265. https://www.researchgate.net/publication/257932261

Lukjancenko, O., Ussery, D. W., & Wassenaar, T. M. (2012). Comparative genomics of *Bifidobacterium*, *Lactobacillus*, and related probiotic genera. Microbial Ecology, 63(3), 651–673. https://doi.org/10.1007/s00248-011-9948-y

Mahmoudi, M., Khomeiri, M., Saeidi, M., Kashaninejad, M., & Davoodi, H. (2019). Study of potential probiotic properties of lactic acid bacteria isolated from raw and traditional fermented camel milk. *J. Agr. Sci. Tech*, *21*(5), 1161–1172.

Misra, S., & Mohanty, D. (2019). Psychobiotics: A new approach for treating mental illness? Critical Reviews in Food Science and Nutrition, *59*(8), 1230–1236. https://doi. org/10.1080/10408398.2017.1399860

Mbye, M., Ayyash, M., Mohamed, H., Abu-Jdayil, B., Kamleh, R., & Kamal-Eldin, A. (2023). Effects of ultrafiltration followed by heat or high-pressure treatment on camel and bovine milk cheeses. *NFS Journal*, *31*, 123–132. https://doi.org/10.1016/j.nfs.2023.04.004

McKinley, M. C. (2005). The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*, *58*(1), 1–12. https://doi.org/10.1111/J.1471-0307.2005.00180.X

Mehra, R., Kumar, H., Rafiq, S., Kumar, N., Buttar, H. S., Leicht, K., Okpala, C. O. R., & Korzeniowska, M. (2022). Enhancing yogurt products' ingredients: preservation strategies, processing conditions, analytical detection methods, and therapeutic delivery—an overview. *PeerJ*, *10*, e14177. https://doi.org/10.7717/PEERJ.14177/FIG-4

Misselwitz, B., Butter, M., Verbeke, K., & Fox, M. R. (2019). Update on lactose malabsorption and intolerance: pathogenesis, diagnosis, and clinical management. *Gut*, *68*(11), 2080–2091. https://doi.org/10.1136/GUTJNL-2019-318404

Moser, A., Schafroth, K., Meile, L., Egger, L., Badertscher, R., & Irmler, S. (2018). Population dynamics of *Lactobacillus helveticus* in Swiss Gruyère-type cheese manufactured with natural whey cultures. *Frontiers in Microbiology*, *9*(APR), 637. https://doi.org/10.3389/FMICB.2018.00637/BIBTEX

Moslehishad, M., Ehsani, M. R., Salami, M., Mirdamadi, S., Ezzatpanah, H., Naslaji, A. N., & Moosavi-Movahedi, A. A. (2013). The comparative assessment of ACE-inhibitory and antioxidant activities of peptide fractions obtained from fermented camel and bovine milk by *Lactobacillus rhamnosus* PTCC 1637. *International Dairy Journal*, *29*(2), 82–87. https://doi.org/10.1016/J.IDAIRYJ.2012.10.015

Mühlhansová, A., Zhexenbay, N., Kozybayev, A., Horáčková, Solichová, K., & Plocková, M. (2016). Quantification of *Lactobacillus helveticus* in a mixture of lactic acid bacteria using qPCR in cheese. *Acta Alimentaria*, *45*(4), 493–499. https://doi.org/10.1556/066.2016.45.4.5

Nassar, K. S., Attia, I., Korma, S. A., Ibrahim, S. A., Esatbeyoglu, T., & Ragab, E. S. (2023). Novel, functional fermented dairy product: preparation and evaluation of dried Kishk-like products from fenugreek seeds with cow's milk, camel's milk, and goat's milk. *Fermentation*, *9*(10), 919. https://doi.org/10.3390/FERMENTATION9100919

O'Sullivan, D. J., McSweeney, P. L. H., Cotter, P. D., Giblin, L., & Sheehan, J. J. (2016). Compromised *Lactobacillus helveticus* starter activity in the presence of facultative heterofermentative *Lactobacillus casei* DPC6987 results in atypical eye formation in Swiss-type cheese. *Journal of Dairy Science*, *99*(4), 2625–2640. https://doi.org/10.3168/JDS.2015-10503

Oberg, T. S., McMahon, D. J., Culumber, M. D., McAuliffe, O., & Oberg, C. J. (2022). Invited review: Review of taxonomic changes in dairy-related *Lactobacilli*. In *Journal of Dairy Science*, *105*(4), 2750–2770. https://doi.org/10.3168/jds.2021-21138

Ohsawa, K., Nakamura, F., Uchida, N., Mizuno, S., & Yokogoshi, H. (2017). *Lactobacillus helveticus*-fermented milk containing lactononadecapeptide (NIPPLTQTPVVVPPFLQPE) improves cognitive function in healthy middle-aged adults: a randomized, double-blind, placebo-controlled trial, *69*(3), 369–376. https://doi.org/10.1080/09637486.2017.1365824

Pak, V. V., Khojimatov, O. K., Abdiniyazova, G. J., & Magay, E. B. (2019). Composition of camel milk and evaluation of food supply for camels in Uzbekistan. *Journal of Ethnic Foods*, *6*(1), 1–8. https://doi.org/10.1186/s42779-019-0031-5

Patil, M. P., Ahire, J. J., Patil, U. K., Bhushan, B., & Chaudhari, B. L. (2021). Effect of *Lactobacillus helveticus* CD6 on serum lipid profile and indicators of liver function in high-fat diet fed Swiss albino mice. *3 Biotech 2021 11:6*, *11*(6), 1–8. https://doi.org/10.1007/S13205-021-02832-6

Radke-Mitchell, L. C., & Sandine, W. E. (1986). Influence of temperature on associative growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Journal of Dairy Science*, *69*(10), 2558–2568. https://doi.org/10.3168/JDS.S0022-0302(86)80701-9

Raj, T., Chandrasekhar, K., Kumar, A. N., & Kim, S. H. (2021). Recent biotechnological trends in lactic acid bacterial fermentation for food processing industries. *Systems Microbiology and Biomanufacturing*, *2*(1), 14–40. https://doi.org/10.1007/S43393-021-00044-W

Rajagopal, S. N., & Sandine, W. E. (1990). Associative Growth and Proteolysis of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in Skim Milk. *Journal of Dairy Science*, *73*(4), 894–899. https://doi.org/10.3168/JDS.S0022-0302(90)78745-0

Raveschot, C., Cudennec, B., Deracinois, B., Frémont, M., Vaeremans, M., Dugersuren, J., Demberel, S., Drider, D., Dhulster, P., Coutte, F., & Flahaut, C. (2020). Proteolytic activity of Lactobacillus strains isolated from Mongolian traditional dairy products: A multiparametric analysis. *Food Chemistry*, *304*, 125415. https://doi.org/10.1016/J.FOODCHEM.2019.125415

Richoux, R., Aubert, L., Roset, G., & Kerjean, J. R. (2009). Impact of the proteolysis due to lactobacilli on the stretchability of Swiss-type cheese. *Dairy Science & Technology*, *89*(1), 31–41. https://doi.org/10.1051/DST:2008030

Rode, J., Edebol Carlman, H. M. T., Konig, ¨ J., Repsilber, D., Hutchinson, A. N., Thunberg, P., Andersson, P., Persson, J., Kiselev, A., Lathrop Stern, L., Salomon, B., Mohammed, A. A., Labus, J. S., & Brummer, R. J. (2022). Probiotic mixture containing *Lactobacillus helveticus*, *Bifidobacterium longum* and *Lactiplantibacillus plantarum* affects brain responses toward an emotional task in healthy subjects: A randomized clinical trial. *Frontiers in Nutrition*, *9*, 865. https://doi.org/10.3389/ fnut.2022.827182

Rodríguez-Serrano, G. M., García-Garibay, J. M., Cruz-Guerrero, A. E., Gómez-Ruiz, L. D. C., Ayala-Niño, A., Castañeda-Ovando, A., & González-Olivares, L. G. (2018). Proteolytic System of *Streptococcus thermophilus*. *Journal of Microbiology and Biotechnology*, *28*(10), 1581–1588. https://doi.org/10.4014/JMB.1807.07017

Rong, J., Zheng, H., Liu, M., Hu, X., Wang, T., Zhang, X., Jin, F., & Wang, L. (2015). Probiotic and anti-inflammatory attributes of an isolate *Lactobacillus helveticus* NS8 from Mongolian fermented koumiss Microbe-host interactions and microbial pathogenicity. *BMC Microbiology*, *15*(1), 1–11. https://doi.org/10.1186/S12866-015-0525-2/FIGURES/5

Şanli, T., Akal, H. C., Yetişemiyen, A., & Hayaloglu, A. A. (2018). Influence of adjunct cultures on angiotensin-converting enzyme (ACE)-inhibitory activity, organic acid content and peptide profile of kefir. *International Journal of Dairy Technology*, *71*(1), 131–139. https://doi.org/10.1111/1471-0307.12346

Sarkar, A., Lehto, S. M., Harty, S., Dinan, T. G., Cryan, J. F., & Burnet, P. W. J. (2016). Psychobiotics and the Manipulation of Bacteria–Gut–Brain Signals. *Trends in Neurosciences*, *39*(11), 763. https://doi.org/10.1016/J.TINS.2016.09.002

Schuster, J. A., Vogel, R. F., & Ehrmann, M. A. (2020). Biodiversity of *Lactobacillus helveticus* isolates from dairy and cereal fermentations reveals habitat-adapted biotypes. *FEMS Microbiology Letters*, *367*(8), 58. https://doi.org/10.1093/FEMSLE/FNAA058

Seifu, E. (2023). Camel milk products: innovations, limitations, and opportunities. *Food Production, Processing, and Nutrition*, *5*(1). https://doi.org/10.1186/s43014-023-00130-7

Sharma, D., & Saharan, B. S. (2016). Functional characterization of biomedical potential of biosurfactant produced by *Lactobacillus helveticus*. *Biotechnology Reports*, *11*, 27–35. https://doi.org/10.1016/J.BTRE.2016.05.001

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. In *Fermentation*, *6*(4). https://doi.org/10.3390/fermentation6040106

Shi, M., Ahtesh, F., Mathai, M., McAinch, A. J., & Su, X. Q. (2017). Effects of fermentation conditions on the potential anti-hypertensive peptides released from yogurt fermented by *Lactobacillus helveticus* and Flavourzyme®. *International Journal of Food Science & Technology*, *52*(1), 137–145. https://doi.org/10.1111/IJFS.13253

Shi, Y., Cui, X., Gu, S., Yan, X., Li, R., Xia, S., Chen, H., & Ge, J. (2018). Antioxidative and probiotic activities of lactic acid bacteria isolated from traditional artisanal milk cheese from northeast China. *Probiotics and Antimicrobial Proteins 2018*, *11*(4), 1086–1099. https://doi.org/10.1007/S12602-018-9452-5

Sıçramaz, H., Güven, O. T., Can, A., Ayar, A., & Gül, Y. (2022). Impact of different starter cultures and *Lactobacillus helveticus* on volatile components, chemical and sensory properties of pasta filata cheese. *Current Research in Food Science*, *5*, 1009–1016. https://doi.org/10.1016/J.CRFS.2022.05.017

Sivamaruthi, B. S., Kesika, P., & Chaiyasut, C. (2018). Impact of fermented foods on human cognitive function—a review of outcome of clinical trials. Scientia Pharmaceutica, 86(2), 22. https://doi.org/10.3390/SCIPHARM86020022

Skrzypczak, K. W., Gustaw, W. Z., Jabłońska-Ryś, E. D., Michalak-Majewska, M., Sławińska, A., Radzki, W. P., Gustaw, K. M., & Waśko, A. D. (2018). Antioxidative properties of milk protein preparations fermented by Polish strains of *Lactobacillus helveticus*. *Acta Scientiarum Polonorum Technologia Alimentaria*, *16*(2), 199–207. https://doi.org/10.17306/J.AFS.2017.0479

Skrzypczak, K., Fornal, E., Domagała, D., Gustaw, W., Jabłońska-Ryś, E., Sławińska, A., Radzki, W., Kononiuk, A., & Waśko, A. (2021). Use of α-Lactalbumin and Caseinoglycomacropeptide as biopeptide precursors and as functional additives in ilk beverages fermented by *Lactobacillus helveticus*. *International Journal of Food Science*, *2021*. https://doi.org/10.1155/2021/8822161

Skrzypczak, K., Gustaw, W., & Waśko, A. (2015). Health-promoting properties exhibited by *Lactobacillus helveticus* strains. *Acta Biochimica Polonica*, *62*(4), 713–720. https://doi.org/10.18388/ABP.2015_1116

Skrzypczak, K., Gustaw, W., Fornal, E., Kononiuk, A., Michalak-Majewska, M., Radzki, W., & Waśko, A. (2020). Functional and technological potential of whey protein isolate in production of milk beverages fermented by new strains of *Lactobacillus helveticus*. *Applied Sciences 2020*, *10*(20), 7089. https://doi.org/10.3390/APP10207089

Skrzypczak, K., Gustaw, W., Kononiuk, A., Sołowiej, B., & Waśko, A. (2019). Estimation of the antioxidant properties of milk protein preparations hydrolyzed by *Lactobacillus helveticus* T80, T105, and B734. *Czech Journal of Food Sciences*, *37*(4), 260–267. https://doi.org/10.17221/477/2017-CJFS

Skrzypczak, K., Gustaw, W., Wasko, A., & Banach, T. (2020). Changes in Biochemical Properties during Ripening Process of Swiss-Type Cheeses Produced with Different *Lactobacillus helveticus* Strains. *Journal of Agricultural Science and Technology*, *22*(3), 693–707. http://jast.modares.ac.ir/article-23-18419-en.html

Slattery, L., O'Callaghan, J., Fitzgerald, G. F., Beresford, T., & Ross, R. P. (2010). Invited review: *Lactobacillus helveticus*—A thermophilic dairy starter related to gut bacteria. *Journal of Dairy Science*, *93*(10), 4435–4454. https://doi.org/10.3168/JDS.2010-3327

Sobti, B., Ayyash, M., Mbye, M., Ranasinghe, M., Nazir, A., Kamleh, R., Abu-Jdayil, B., & Kamal-Eldin, A. (2024). Effects of ultrafiltration followed by heat or high-pressure treatment on camel and bovine yogurts. *NFS Journal*, *35*, 100181. https://doi.org/10.1016/J.NFS.2024.100181

Sobti, B., Teneiji, H. A. A., & Kamal-Eldin, A. (2019). Effect of added bovine casein and whey protein on the quality of camel and bovine milk yoghurts. *Emirates Journal of Food and Agriculture*, *31*(10), 804–811. https://doi.org/10.9755/ejfa.2019.v31.i10.2022

Soleymanzadeh, N., Mirdamadi, S., & Kianirad, M. (2016). Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). *Dairy Science and Technology*, *96*(4), 443–457. https://doi.org/10.1007/s13594-016-0278-1

Somerville, V., Lutz, S., Schmid, M., Frei, D., Moser, A., Irmler, S., Frey, J. E., & Ahrens, C. H. (2019). Long-read based de novo assembly of low-complexity metagenome samples results in finished genomes and reveals insights into strain diversity and an active phage system. *BMC Microbiology*, *19*(1), 143. https://doi.org/10.1186/s12866-019-1500-0

Stuknyte, M., Brockmann, E. C., Huovinen, T., Guglielmetti, S., Mora, D., Taverniti, V., Arioli, S., de Noni, I., & Lamminmäki, U. (2014). *Lactobacillus helveticus* MIMLh5-Specific antibodies for detection of S-layer protein in Grana padano protected-designation-of-origin cheese. *Applied and Environmental Microbiology*, *80*(2), 694–703. https://doi.org/10.1128/AEM.03057-13/SUPPL_FILE/ZAM999105057SO1.PDF

Tak, L., Bais, B., Singh, R., & Singh, S. (2018). Study of the Anti-oxidant potential of Buffalo Milk using *Lactobacillus helveticus* and *Lactobacillus fermentum*. *Intl. J. Food. Ferment. Technol*, *8*(1), 69–74. https://doi.org/10.30954/2277-9396.01.2018.8

Talwalkar, A., & Kailasapathy, K. (2004). Comparison of selective and differential media for the accurate enumeration of strains of *Lactobacillus acidophilus, Bifidobacterium* spp. and *Lactobacillus casei* complex from commercial yoghurts. *International Dairy Journal*, *14*(2), 143–149. https://doi.org/10.1016/S0958-6946(03)00172-9

Tang, H., Ma, H., Hou, Q., Li, W., Xu, H., Liu, W., Sun, Z., Haobisi, H., & Menghe, B. (2020). Profiling of koumiss microbiota and organic acids and their effects on koumiss taste. *BMC Microbiology*, *20*(1), 1–11. https://doi.org/10.1186/S12866-020-01773-Z/FIGURES/8

Tango, M. S. A., & Ghaly, A. E. (1999). Effect of temperature on lactic acid production from cheese whey using *Lactobacillus helveticus* under batch conditions. *Biomass and Bioenergy*, *16*(1), 61–78. https://doi.org/10.1016/S0961-9534(98)00062-2

Taverniti, V., & Guglielmetti, S. (2012). Health-promoting properties of *Lactobacillus helveticus*. *Frontiers in Microbiology*, *3*(NOV), 392. https://doi.org/10.3389/FMICB.2012.00392/BIBTEX

Terzioğlu, M. E., Bakırcı, İ., Oz, E., Brennan, C. S., Huppertz, T., Amarowicz, R., Khan, M. R., Elobeid, T., Aadil, R. M., & Oz, F. (2023). Comparison of camel, buffalo, cow, goat, and sheep yoghurts in terms of various physicochemical, biochemical, textural and rheological properties. *International Dairy Journal*, *146*, 105749. https://doi.org/10.1016/J.IDAIRYJ.2023.105749

Torino, M. I., Taranto, M. P., & Font De Valdez, G. (2001). Mixed-acid fermentation and polysaccharide production by *Lactobacillus helveticus* in milk cultures. *Biotechnology Letters*, *23*(21), 1799–1802. https://doi.org/10.1023/A:1012417403787

Toropov, V., Demyanova, E., Shalaeva, O., Sitkin, S., & Vakhitov, T. (2020). Whole-Genome Sequencing of *Lactobacillus helveticus* D75 and D76 Confirms Safety and Probiotic Potential. *Microorganisms 2020*, *8*(3), 329. https://doi.org/10.3390/MICROORGANISMS8030329

Tremblay, A., Xu, X., Colee, J., & Tompkins, T. A. (2021). Efficacy of a multi-strain probiotic formulation in pediatric populations: A comprehensive review of clinical studies. *Nutrients*, *13*(6), 1908. https://doi.org/10.3390/NU13061908/S1

Troiano, R., Denaro, F., & Reuter, W. M. (2023). The analysis of lactose in milk and cheese products by HPLC with RI detection. Application note, Perkin Elmer, Shelton, CT, USA. https://resources.perkinelmer.com/lab-solutions/resources/docs/app-the-analysis-of-lactose-in-milk-and-cheese-products-by-hplc-note-012755-01.pdf

Verma, P., Dutt, R., & Anwar, S. (2023). Effect of probiotic Lactobacillus helveticus on various health disorders. *Current Traditional Medicine*, *9*(4), 105–113. https://doi.org/10.2174/2215083808666220623161236

Wakai, T., Yamamoto, N., Wakai, T., & Yamamoto, N. (2012). Antihypertensive peptides are specific to *Lactobacillus helveticus* fermented milk. *Biotechnology - Molecular Studies and Novel Applications for Improved Quality of Human Life*. https://doi.org/10.5772/28695

Wang, X., Xu, M., Xu, D., Ma, K., Zhang, C., Wang, G., Dong, M., & Li, W. (2022). Structural and prebiotic activity analysis of the polysaccharide produced by *Lactobacillus helveticus* SNA12. *Carbohydrate Polymers*, *296*. https://doi.org/10.1016/J.CARBPOL.2022.119971

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Frontiers in Bioengineering and Biotechnology*, *9*, 612285. https://doi.org/10.3389/fbioe.2021.612285

Wu, Q., Cheung, C. K. W., & Shah, N. P. (2015). Towards galactose accumulation in dairy foods fermented by conventional starter cultures: Challenges and strategies. *Trends in Food Science & Technology*, *41*(1), 24–36. https://doi.org/10.1016/J.TIFS.2014.08.010

Xiang, J., Liu, F., Wang, B., Chen, L., Liu, W., & Tan, S. (2021). A literature review on Maillard reaction based on milk proteins and carbohydrates in food and pharmaceutical products: Advantages, disadvantages, and avoidance strategies. In *Foods*, 10(9). MDPI AG. https://doi.org/10.3390/foods10091998

Xiao, L., Han, S., Zhou, J., Xu, Q., Dong, M., Fan, X., Rui, X., Chen, X., Zhang, Q., & Li, W. (2020). Preparation, characterization, and antioxidant activities of derivatives of exopolysaccharide from *Lactobacillus helveticus* MB2-1. *International Journal of Biological Macromolecules*, *145*, 1008–1017. https://doi.org/10.1016/J.IJBIOMAC.2019.09.192

Xu, M., Hu, S., Wang, Y., Wang, T., Dziugan, P., Zhang, B., & Zhao, H. (2021). Integrated transcriptome and proteome analyses reveal protein metabolism in *Lactobacillus helveticus* CICC22171. *Frontiers in Microbiology*, *12*, 1287. https://doi.org/10.3389/FMICB.2021.635685

Yang, W., Hao, X., Zhang, X., Zhang, G., Li, X., Liu, L., Sun, Y., & Pan, Y. (2021). Identification of antioxidant peptides from cheddar cheese made with *Lactobacillus helveticus*. *LWT*, *141*, 110866. https://doi.org/10.1016/J.LWT.2021.110866

Yang, Y., An, H., Zhai, Z., Wang, G., Li, J., & Hao, Y. (2016). Complete genome sequence of *Lactobacillus helveticus* CAUH18, a potential probiotic strain originated from koumiss. *Journal of Biotechnology*, *224*, 18–19. https://doi.org/10.1016/J.JBIOTEC.2016.03.004

Yanjun, L., Su, C., Lie, C., Cailing, C., Xueliang, R., Zhiyao, Z., Luwei, W., Hongjuan, G., Jian, W., Guanchen, L., & Xingqian, Y. (2022). Immunomodulatory effects of Lactobacillus helveticus WHH2580 fermented milk on an immunosuppressed murine model, *Journal of Functional Foods*, *99*,1756-4646. https://doi.org/10.1016/j.jff.2022.105353

Yerlikaya, O., Saygili, D., & Akpinar, A. (2020). Evaluation of antimicrobial activity and antibiotic susceptibility profiles of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains isolated from commercial yoghurt starter cultures. *Food Science and Technology*, *41*(2), 418–425. https://doi.org/10.1590/FST.03920

You, X., Li, Z., Ma, K., Zhang, C., Chen, X., Wang, G., Yang, L., Dong, M., Rui, X., Zhang, Q., & Li, W. (2020). Structural characterization and immunomodulatory activity of an exopolysaccharide produced by *Lactobacillus helveticus* LZ-R-5. *Carbohydrate Polymers*, *235*, 115977. https://doi.org/10.1016/J.CARBPOL.2020.115977

Zago, M., Massimiliano, L., Bonvini, B., Penna, G., Giraffa, G., & Rescigno, M. (2021). Functional characterization and immunomodulatory properties of *Lactobacillus helveticus* strains isolated from Italian hard cheeses. *PLOS ONE*, *16*(1), e0245903. https://doi.org/10.1371/JOURNAL.PONE.0245903

Zago, M., Orrù Luigi, Rossetti, L., Lamontanara, A., Fornasari, M. E., Bonvini, B., Meucci, A., Carminati, D., Cattivelli, L., & Giraffa, G. (2017). Survey on the phage resistance mechanisms displayed by a dairy *Lactobacillus helveticus* strain. *Food Microbiology, 66*, 110–116. https://doi.org/10.1016/j.fm.2017.04.014

Zago, M., Scaltriti, E., Fornasari, M. E., Rivetti, C., Grolli, S., Giraffa, G., Ramoni, R., & Carminati, D. (2012). Epifluorescence and atomic force microscopy: Two innovative applications for studying phage–host interactions in *Lactobacillus helveticus*. *Journal of Microbiological Methods*, *88*(1), 41–46. https://doi.org/10.1016/J.MIMET.2011.10.006

Zendeboodi, F., Khorshidian, N., Mortazavian, A. M., & da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Current Opinion in Food Science*, *32*, 103–123. https://doi.org/10.1016/J.COFS.2020.03.009

Zhang, H., Xu, M., Hu, S., Zhao, H., & Zhang, B. (2022). The enzyme gene expression of protein utilization and metabolism by *Lactobacillus helveticus* CICC 22171. *Microorganisms*, *10*(9), 1724. https://doi.org/10.3390/MICROORGANISMS10091724/S1

Zhang, W. Y., Chen, Y. F., Zhao, W. J., Kwok, L. Y., & Zhang, H. P. (2014). Gene expression of proteolytic system of *Lactobacillus helveticus* H9 during milk fermentation. *Annals of Microbiology 2014 65:2*, *65*(2), 1171–1175. https://doi.org/10.1007/S13213-014-0902-3

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., O'toole, P. W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G. E., Gänzle, M. G., & Lebeer, S. (2020). A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus beijerinck* 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, *70*(4), 2782–2858. https://doi.org/10.1099/IJSEM.0.004107/CITE/REFWORKS

Zhong, Z., Hu, R., Zhao, J., Liu, W., Kwok, L. Y., Sun, Z., Zhang, H., & Chen, Y. (2021). Acetate kinase and peptidases are associated with the proteolytic activity of *Lactobacillus helveticus* isolated from fermented food. *Food Microbiology, 94*, 103651. https://doi.org/10.1016/J.FM.2020.103651

Zhou, T., Huo, R., Kwok, L. Y., Li, C., Ma, Y., Mi, Z., & Chen, Y. (2019). Effects of applying *Lactobacillus helveticus* H9 as adjunct starter culture in yogurt fermentation and storage. *Journal of Dairy Science, 102*(1), 223–235. https://doi.org/10.3168/JDS.2018-14602

List of Publications

Chelladhurai, K., Ayyash, M., Turner, M. S., & Kamal-Eldin, A. (2023). *Lactobacillus helveticus*: Health effects, current applications, and future trends in dairy fermentation. *Trends in Food Science and Technology*, *136*, 159–168. https://doi.org/10.1016/J.TIFS.2023.04.013

Chelladhurai, K., Ayyash, M., & Kamal-Eldin, A. (2024). Nutraceutical properties of milk caseins. In Casein (pp. 289–298). Academic Press. https://doi.org/10.1016/B978-0-443-15836-0.00003-2

Chelladhurai, K., Santhoshani, W., Ali, S.N., Ayyash, M., Turner, M. S., & Kamal-Eldin, A. (2024). Differences in the growth, acidification, and proteolytic activities of *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* in camel and cow milk fermentation. *International Dairy Journal*, Article 106075. https://doi.org/10.1016/j.idairyj.2024.106075

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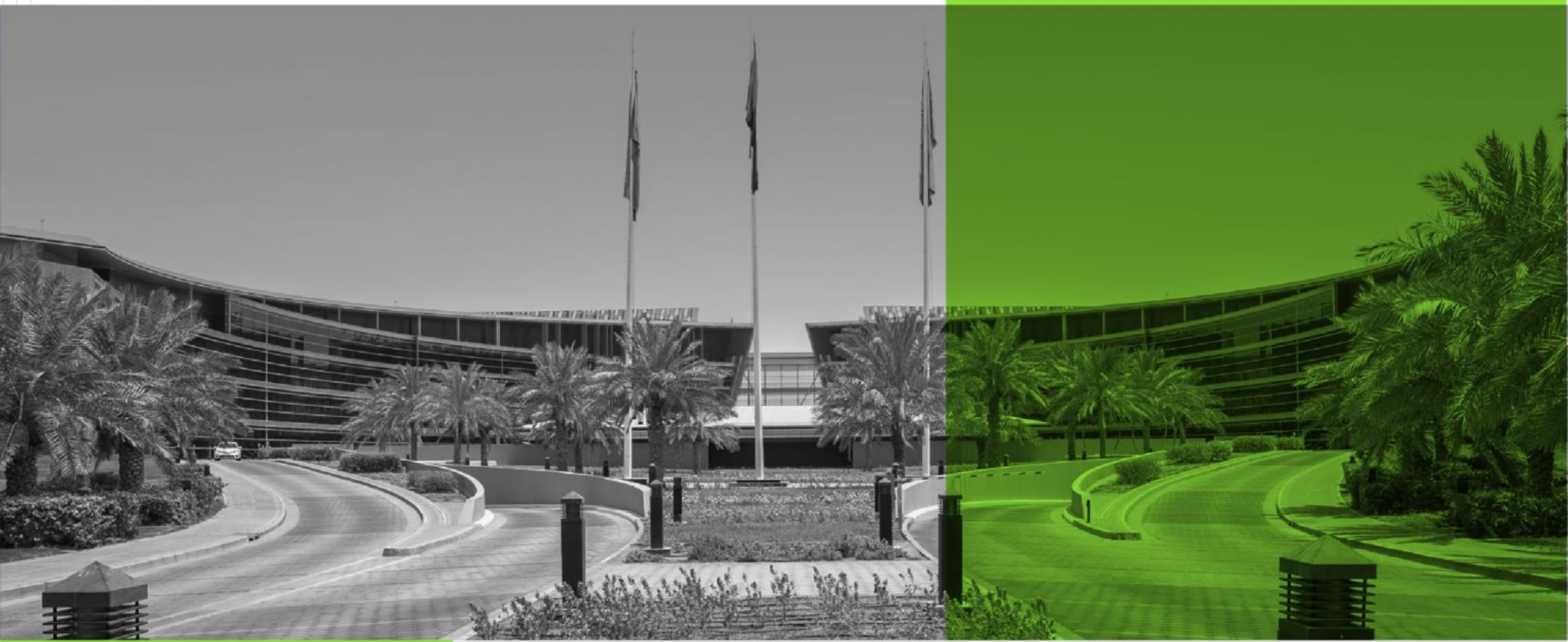

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The increasing consumer interest in fermented dairy products highlights the need for extensive research on different types of milk and various strains of lactic acid bacteria. In this study, the fermentation of low-fat camel milk and bovine milk with *Lactobacillus helveticus* was compared to fermentation with *L. delbrueckii* subsp. *bulgaricus*, and *S. thermophilus* at 28°C, 35°C, and 42°C.

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