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Ghazal Hamsho

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Health Benefits of Fermented Milk Protein Concentrate

Insider consultant: Kálmán Botond Süli

PhD student

Dr. Erika Bujna

Associate professor

Insider consultant's Institute/department: Food Science and Technology / Bioengineering and Alcoholic Drink Technology

Created by: **Ghazal Hamsho**

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1. Introduction and Objectives

1. 1. Introduction

As recognition of the crucial role of diet in maintaining health becomes widespread, research is increasingly focusing on how dietary interventions can enhance well-being and prevent diseases like metabolic syndrome. This growing awareness has spurred interest in bioactive peptides (BAPs) within the emerging field of foodomics, which uses advanced technologies to explore the impact of food on health and consumer confidence and this interest in BAPs extends beyond mere academic curiosity. Where research in this area not only illuminates the health benefits of dietary proteins but also lays the groundwork for their practical applications in industries such as functional food production or pharmaceuticals (Hejel et al., 2021; Raveschot et al., 2018). However, challenges remain in BAP production and commercialization. These challenges include identifying and isolating new BAPs, understanding the mechanisms underlying their bioactivities, and developing efficient bioprocesses for large-scale peptide production. While *in silico* methodologies facilitate the discovery of new peptides, the industrial production of BAPs is hindered by the limited availability of large-scale technologies and the high cost of enzymes used for protein hydrolysis (Park & Nam, 2015).

Microbial fermentation already widely applied in the dairy industry, is a cost-effective method for bioactive peptide production, which is a biological mechanism, harnesses microorganisms to enrich foods by altering their nutritional composition and enhancing their quality. Lactic acid bacteria (LAB) stand out in fermentation practices owing to their capability to generate advantageous substances like lactic acid and antimicrobial peptides, known as bacteriocins. With a history of use in food preservation, LAB play a pivotal role in elevating the nutritional content, sensory attributes, and technological characteristics of fermented foods. Their popularity in fermentation is due to their recognized safety profile and their efficiency to achieve the desired fermentation outcomes (Rizwan et al., 2023).

1. 2. Objectives

This thesis aims to deepening the knowledge of the bioactive constituents within fermented milk protein concentrate (MPC) and their potential applications in therapy. It seeks to pioneer the development of innovative functional foods and dietary strategies geared towards enhancing human health and well-being. The research will focus on the pivotal role of lactic acid bacteria (LAB), especially lactobacilli, in orchestrating fermentation processes and elevating the nutritional profile of dairy products. Additionally, it aims to clarify the complex mechanisms by which LAB generate bioactive peptides during fermentation and their implications for human health.

The study places particular emphasis on health benefits of the different types of bioactive peptides found in dairy products, including the management of hypertension through ACE-inhibitory peptides, assessment of antioxidant activity through various detection methods, and investigate the antibacterial properties of fermented milk protein concentrate, with a focal point on their effectiveness against pathogenic microorganisms such as *Escherichia coli, Listeria, Enterococcus faecalis,* and *Enterococcus cloacae.* Substantially exploring the potential of the fermented milk protein concentrate (MPC) as a natural antioxidant, antihypertension and antimicrobial agent.
An important aspect of the investigation involves exploring how glucose levels and heat treatment influence the physiological impacts of bioactive peptides, and the impact of different concentrations of cherry juice on antioxidant activity. By clarifying these relationships, the study aims to determine the optimal conditions for maximizing the health benefits of fermented milk protein concentrate. Ultimately, the thesis seeks to contribute to the advancement of scientific knowledge in the field of functional foods and foster the development of evidence-based dietary interventions aimed at promoting human health and well-being.

2. Literature Review

2. 1. The Fermentation and Lactic Acid Bacteria

2. 1. 1. History and Significance of Lactic Acid Bacteria

Traditional classification of lactic acid bacteria laid by Jensen-Orla's (in 1919), dividing them into *Streptococcus, Pediococcus, Leuconostoc,* and *Lactobacillus* based on factors like cell arrangement and glucose fermentation. Schleifer and colleagues later proposed a separation of enterococci from hemolytic streptococci, forming the new genus *Lactococcus* in 1985.

In contrast modern classification methods rely on molecular techniques, focusing on nuclear and genetic DNA analysis for enhanced precision. Gel electrophoresis and PCR amplification techniques have revolutionized species identification, facilitating differentiation between closely related species (Al-Mariri & Sharabi, 2008).

Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming, but aerotolerant microorganisms that are generally characterized by the production of lactic acid as a key fermentation product. It representes a diverse group with immense industrial potential, particularly in dairy fermentation.

Lactobacillus, the largest genus within LAB, plays a pivotal role in numerous food production processes and is renowned for its probiotic properties. These microorganisms belonging to the Firmicutes phylum, Bacilli class, Lactobacillales order, and Lactobacillaceae family, holds Generally Recognized as Safe (GRAS) status, ensuring its safety for food applications (El-Sayed et al., 2021). *Lactobacillus acidophilus*, in particular, has obtained Qualified Presumption of Safety (QPS) status from the European Food Safety Authority (EFSA), granting it a high level of safety assurance (Meng et al., 2021).

2. 1. 2. Fermented Dairy Products

Fermentation, an age-old culinary practice rooted in civilizations across India, Iraq, and Egypt, harnesses the power of microbial growth and enzymatic conversion to transform food. This time-honoured process not only preserves but also enhances the quality of food, resulting in products with distinct sensory profiles and functional attributes. Microorganisms like bacteria, yeasts, and

fungi, along with their enzymes, orchestrate fermentation across a wide array of food groups, from milk and cereals to vegetables, fruits, meats, and legumes, and during fermentation, numerous key metabolites and microbial byproducts are generated, contributing to food safety, prolonging shelf life, and augmenting nutritional properties. These include lactic acid, acetic acid, alcohol, carbon dioxide, ammonia, and various fatty acids (Górska et al., 2007; Deveci et al., 2023).

Milk, recognized as a crucial reservoir of essential macro- and micronutrients vital for human health, boasts a diverse spectrum of components including protein, conjugated linoleic acid, calcium, riboflavin, and phosphorus. Milk proteins, which include both whey and casein, provide a wide range of health benefits. These benefits range from promoting feelings of fullness and regulating body weight to exhibiting hypotensive, antimicrobial, anti-inflammatory, anticancer, and antioxidant properties (Deveci et al., 2023; Hejel et al., 2021).

Fermented dairy products, including yogurt, cheese, and kefir, are crafted using lactic acid bacteria (LAB), imparting distinctive flavors and textures. Among them, strains of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* are commonly employed as starter cultures for yogurt production. These LAB strains also double as probiotics, conferring various health benefits when administered appropriately (Ayivi et al., 2022). Fermentation not only enhances these products quality but furthermore, microbial metabolic activities yield bioactive compounds such as exopolysaccharides, oligosaccharides, bioactive peptides, phenolic compounds, vitamins, short-chain fatty acids (SCFAs), conjugated linoleic acid (CLA), and γ-aminobutyric acid (GABA), which confer diverse health benefits. For instance, LAB and propionibacteria play pivotal roles in boosting vitamin B12 and folic acid levels in fermented milk products.

In addition, fermented milk fortified with *Lactobacillus acidophilus* LA-5 offers a myriad of health benefits. It promotes the growth of beneficial bacteria while suppressing harmful pathogens, lowers *Streptococcus* mutans levels in saliva, reduces risk factors associated with nonalcoholic fatty liver disease, and contributes to various other health aspects, including cholesterol management, gut microbiota balance, immune function enhancement, lactose digestion facilitation, and potentially anticancer properties (Meng et al., 2021).

Yogurt, a prime example of milk undergoing lactic acid fermentation, undergoes a transformation as lactose is converted into lactic acid by bacteria (Figure 1), creating an acidic environment that

initiates curd formation. As lactic acid concentrations peak, bacterial growth slows (Yassin, 2020; Deveci et al., 2023).

It is widely agreed that yogurt should maintain a minimum level of viable starter bacteria ($\geq 10^7$ cfu/g) throughout its shelf life, and this requirement has been incorporated into the food regulations of countries with advanced dairy industries (Varga, 2016).

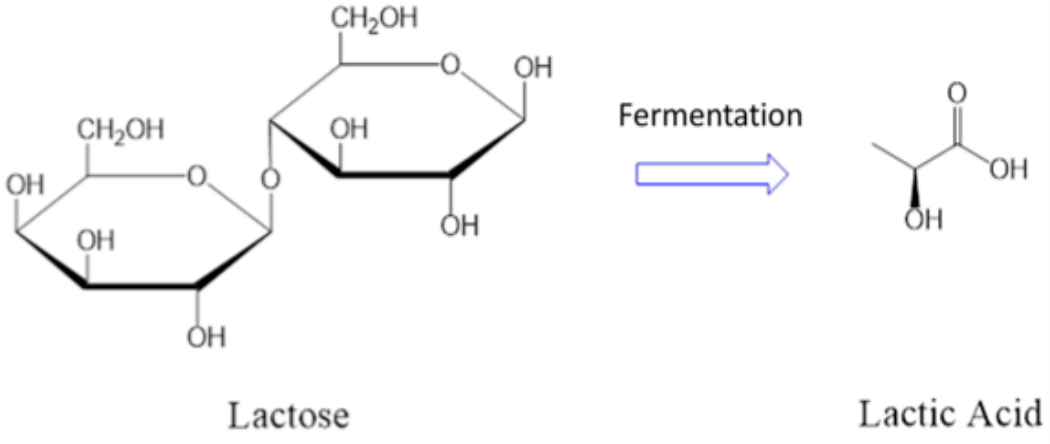


Figure 1. Breakdown of lactose due to fermentation (Marvin, 2017)

https://ncdnadayblog.org/2017/01/19/the-bacterial-life-inside-your-breakfast/

2. 1. 3. The Role of LAB in the Production of Bioactive Peptides

The interplay between genetic signals and environmental factors significantly impacts human health, with microorganisms, including endogenous symbiotic microbiota and food-derived bacteria and yeasts, playing pivotal roles in modulating physiological processes. Fermented foods, containing live organisms, offer a valuable source of bioactive molecules, influencing various aspects such as appetite, and mood. Nitrogen-bearing molecules, particularly amino acids and their derivatives, exhibit diverse effects on human physiology.

Lactobacilli and yeasts are capable of synthesizing amino acid derivatives such as selenocysteines and selenomethionines, which play crucial roles in counteracting oxidative stress and modulating immune function. Additionally, selenoproteins associated with these derivatives have been linked to protective effects against cancer development (Pessione & Cirrincione, 2016)

However, the production of biogenic amines, derived from bacterial decarboxylation of free amino acids, presents a notable health risk in both non-fermented and fermented foods. While starters in controlled fermentations are carefully selected to mitigate this risk, autochthonous or contaminant lactic acid bacteria can contribute to biogenic amine accumulation due to their metabolic pathways. LAB, in particular, are known for their robust production of biogenic amines (Mangiapane et al., 2014 a,b).

LAB also play a significant role in releasing bioactive peptides from food proteins, which have been shown to possess various health-promoting properties. These peptides, initially inactive when encrypted within proteins, become biologically active upon liberation through proteolytic processes (Hayes et al., 2007; Pessione, 2012).

LAB employ a sophisticated proteolytic system involving cell envelope proteinases (CEPs), peptide transporters, and intracellular peptidases to obtain amino acids from external protein sources. CEPs initiate the degradation of proteins, yielding peptides containing 4 to 30 amino acid residues (Chaudhary et al., 2021).

These peptides are then transported into the cells by peptide transporters, such as Opp, DtpT, and Dpp (Figure 2), facilitating their utilization as a nitrogen source for LAB growth and proliferation.

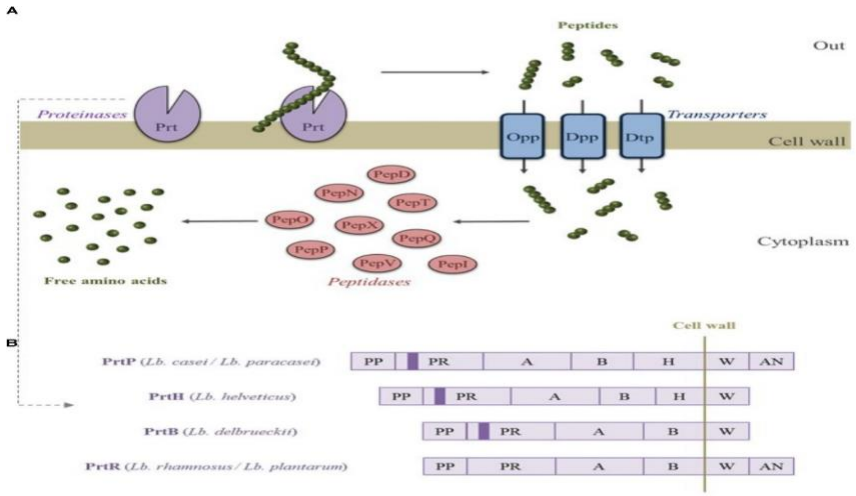


Figure 2. The proteolytic system of *Lactobacillus* species. (Sadat-Mekmene et al., 2011)

Subsequently, internal peptidases, including endopeptidases, aminopeptidases, tripeptidases, dipeptidases, and proline-specific peptidases, further degrade these peptides into individual amino acids.This intricate proteolytic machinery ensures that LAB efficiently break down proteins into constituent amino acids, meeting their nutritional requirements for growth and survival,

particularly in nitrogen-limited environments like milk (Savijoki et al., 2006; Sadat-Mekmene et al., 2011; Griffiths & Tellez, 2013).

The depicted proteolytic system of *Lactobacillus* species begins with the action of cell envelope proteinases (CEPs and Prt), which initiate the breakdown of proteins. Subsequently, peptides are transported into the cell. Within the cell, various peptidases convert peptides into free amino acids. Notably, CEPs from different *Lactobacillus* species exhibit diverse structures, featuring various domains. (Sadat-Mekmene et al., 2011).

2. 2. Bioactive Compounds in Milk

2. 2. 1. Overview of Milk Bioactive Compounds

Milk from various mammalian species contains a plethora of bioactive compounds (Figure 3), including proteins, lipids, and carbohydrates such as caseins, whey proteins, immunoglobulins, antibacterial peptides, antimicrobial proteins, and oligosaccharides (Park & Nam, 2015).

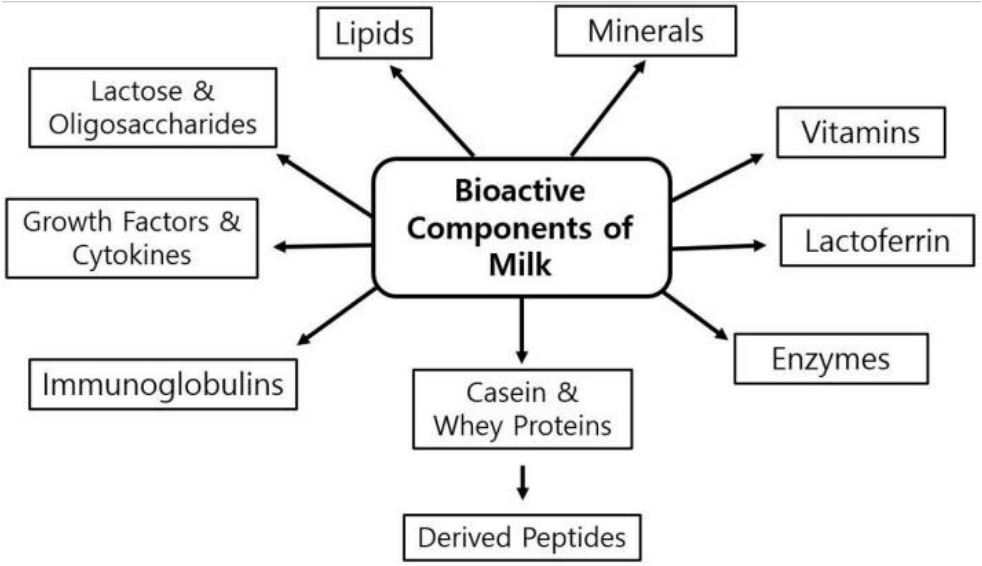


Figure 3. Major bioactive functional compounds of milk (Park, 2009; Park & Nam, 2015).

Cow's milk is a rich source of diverse proteins, particularly evident in colostrum, which contains approximately 514 types of proteins. These proteins are distributed between the cell fraction and

the liquid phase, with 352 found in the former and 162 in the latter. Colostrum is particularly rich in exclusive proteins, with 50 unique to it, while 13 are exclusive to milk, and 99 are present in both.

Beyond their nutritional role, many of these proteins exhibit bioactive properties, contributing to various physiological functions. Some proteins play regulatory roles in metabolic and immunological processes, while others contribute to maintaining the integrity and barrier function of the digestive system (Hejel et al., 2021).

Bioactive peptides (BAPs) are short chains of amino acids derived from proteins in food sources, exhibiting physiological effects beyond basic nutrition (Rizwan et al., 2023). Recent advancements in technology have facilitated the identification and understanding of these peptides, paving the way for the development of functional foods and nutraceuticals aimed at improving health and quality of life, where bioactive peptides (BPs) derived from various dietary proteins have been the subject for many of research (Rizwan et al., 2023; Smith et al., 2022).

2. 2. 2. Mechanisms of Bioactive Peptide Generation

Bioactive peptides crucial for functional nutrition can be obtained through fermentation, utilizing the proteolytic systems of microorganisms to hydrolyze parental proteins, peptides released through three primary mechanisms (Park & Nam, 2015; Korhonen & Pihlanto, 2007).

1-Hydrolysis by Digestive Enzymes: One-way bioactive peptides are generated through the process of hydrolysis by digestive enzymes. When dietary proteins are consumed, they undergo enzymatic breakdown in the gastrointestinal tract. Proteolytic enzymes, such as pepsin, trypsin, and chymotrypsin, cleave the peptide bonds within proteins, leading to the liberation of bioactive peptides. These peptides may exhibit various physiological activities, contributing to health benefits.

2-Hydrolysis by Proteolytic Microorganisms: Another mechanism involves the action of proteolytic microorganisms present in the digestive system. Certain microorganisms, such as bacteria in the gut, have proteolytic enzymes that can cleave protein molecules, releasing bioactive peptides. This microbial hydrolysis is an additional pathway for the generation of peptides with potential health-promoting properties.

3-Action of Proteolytic Enzymes from Microorganisms or Plants: Apart from endogenous digestive enzymes, bioactive peptides can also be produced through the action of proteolytic enzymes derived from external sources, such as microorganisms or plants. Some fermented foods, for instance, involve the use of specific microorganisms that produce proteolytic enzymes during the fermentation process. These enzymes can break down proteins in the food matrix, releasing bioactive peptides with functional properties.

Understanding these mechanisms of bioactive peptide release is crucial in the context of functional food development. Researchers and the food industry leverage this knowledge to identify and isolate bioactive peptides with desirable health effects. These peptides can then be incorporated into food products to enhance their nutritional and functional profiles, offering consumers options for improving health through dietary choices (Park & Nam, 2015; Korhonen & Pihlanto, 2007).

2. 2. 3. Identifying Regions for Bioactive Peptides in Milk Proteins

The protein fraction in milk of minor dairy species can be classified into three main groups: casein, whey protein, and non-protein nitrogenous compounds. Casein, which precipitates at pH 4.6, encompasses αs1-casein, αs2-casein, β-casein, and κ-casein. Whey protein includes noncasein soluble proteins, while non-protein nitrogen compounds in milk consist of free amino acids, peptides, creatine, urea, ammonia, uric acid, and orotic acid (Guha et al., 2021).

The ongoing exploration for newly discovered bioactive peptides has led to the identification of 202 additional peptide sequences matched to specific functions, expanding the unique entries in the Milk Bioactive Peptide Database (MBPDB) by 20%. These peptides demonstrate diverse functionalities, such as antioxidant, ACE-inhibitory, DPP-IV inhibitory, anti-inflammatory, and antimicrobial properties (Nielsen et al., 2023).

The majority of bioactive peptides originate from bovine β-casein, making a substantial contribution to the total entries in the MBPDB. Other sources include bovine αs1-casein, bovine β-lactoglobulin, bovine lactoferrin, human β-casein, bovine αs2-casein, bovine κ-casein, bovine α-lactalbumin, and human lactoferrin. With the addition of new data, the total number of unique bioactive peptide sequences annotated in the MBPDB has increased by 14%, totaling 691 sequences. These bioactive peptides from milk exhibit a wide range of potential sites of action

throughout the body, although there *in vivo* bioactivities are yet to be extensively examined, particularly in humans (Nielsen et al., 2023). Furthermore, bioactive peptides are derived from various sites within the parent sequences of milk proteins. The majority of identified bioactive peptides are derived from β-casein, particularly bovine β-casein, with additional contributions from other proteins like bovine αs1-casein, bovine αs2-casein, and bovine κ-casein. Specific regions within these proteins have been identified as major sources for bioactive peptides. For instance, amino acids 1–35, 80–109, and 142–199 in bovine αs1-casein contain significant regions for bioactive peptides. Bovine αs2-casein is notable for its antimicrobial peptides, while bovine κ-casein's AA 106–169 regions, known as caseinomacropeptide, exhibits multiple functions, including antimicrobial, ACE-inhibitory, and anti-inflammatory activity (Nielsen et al., 2023).

2. 2. 4. Diverse Health Benefits of Bioactive Peptides

This table presents an overview of the various categories of bioactive peptides found in milk, along with their corresponding peptides and highlights the multifaceted roles of bioactive peptides in milk (Table 1).

Table 1. Functional Diversity of Bioactive Peptides in Milk

own work based on (Rizwan et al., 2023; Park & Nam, 2015).

Bioactive Peptides Category	**Peptides**	**Function**
Antioxidative Peptides	αs-Casein	Inhibits lipid peroxidation, Possesses free radical-scavenging activity
Cellular Protection Peptides	Whey Protein	Yields dipeptides that promote glutathione synthesis for cellular protection
Antithrombotic Peptides	Caseinomacropeptide and Casoplatelin	Inhibit blood clot formation
Hypocholesterolemic Peptides	β-Lactoglobulin	Lowers serum cholesterol levels

Bioactive Peptides Category	**Peptides**	**Function**
Opioid Peptides	Casoxins and Casomorphins	Act as agonists or antagonists on opioid receptors
Antiappetizing Peptides	-	Suppress appetite to aid in weight management
Antimicrobial Peptides	Lactoferricins	Display antibacterial activity
Immunomodulatory Peptides	Caseins and Whey Proteins	Modulate immune responses
Cytomodulatory Peptides	-	Affect cell viability by inhibiting or stimulating cell growth
Mineral-Binding Peptides	Caseinophosphopeptides	Enhance mineral absorption
Growth Factors	Milk Growth Factor (MGF)	Influence cell proliferation
Unique Bioactive Peptides	α-Lactorphin and Lactoferricin	Possess specific health-promoting properties
Antihypertensive Peptides	-	Control arterial blood pressure, inhibit elevation of blood pressure by degrading bradykinin and enkephalins

2. 3. Angiotensin-Converting Enzyme –Inhibitory Effect in Milk

2. 3. 1. Hypertension and ACE Inhibitory Peptides

Hypertension is a chronic condition associated with severe diseases like arteriosclerosis, cardiovascular disease, myocardial infarction, stroke, and renal failure. ACE inhibitors (angiotensin-converting enzyme inhibitors) play a crucial role in treating hypertension and various cardiovascular conditions by promoting vasodilation, which widens blood vessels. These medications work by blocking the action of the angiotensin-converting enzyme, involved in converting angiotensin I to angiotensin II. ACE inhibitors are commonly prescribed for conditions

such as hypertension, heart failure, and kidney diseases associated with diabetes (Jumaaha et al., 2023; Úri et al., 2014).

The structural features of ACE-inhibitory peptides are not fully determined, but those with hydrophobic amino acids at their C termini are more likely to exhibit ACE-inhibitory actions. The Milk Bioactive Peptide Database (MBPDB) contains 355 ACE-inhibitory peptides and five antihypertensive peptides, mainly from β-casein, αs1-casein, and β-lactoglobulin (Úri et al., 2014). Various *in vitro* assays, such as spectrophotometric and fluorometric approaches, measure ACE-inhibitory activity. Many peptides derived from milk proteins have shown *in vitro* ACE-inhibitory activity. However, their digestive survival and bioavailability have not been fully examined in most cases (Nielsen et al., 2023).

several examples from Nielsen et al., (2023) Studies, such as the β-casein-derived peptide LLYQEPVLGPVR (leucine, leucine, tyrosine, glutamine, glutamic acid, proline, valine, leucine, glycine, proline, valine, arginine), which exhibited robust *in vitro* ACE inhibition and stability during simulated gastric digestion. Shorter peptide fragments generated after simulated intestinal digestion showed increased ACE-inhibitory activity. Enzymatic hydrolysis of yak milk β-casein yielded ACE-inhibitory peptides, with PFPGPIPN (proline , phenylalanine , proline, glycine, proline, isoleucine, proline, asparagine) demonstrating stabsility throughout *in vitro* gastrointestinal digestion. The ACE inhibitory peptide LPYPY (leucine, proline, tyrosine, proline, tyrosine.), derived from bovine κ-casein, underwent hydrolysis after *in vitro* pepsin digestion, resulting in a significant increase in ACE-inhibitory activity. Similarly, the ACE-inhibitory peptide YQKFPQYLQY (tyrosine, glutamine, lysine, phenylalanine, proline, glutamine, tyrosine, leucine, glutamine, tyrosine.), derived from bovine αs2-casein, was further digested to YQK upon incubation with pepsin and trypsin (Nielsen et al., 2023).

As shown in (Table 2) several ACE-inhibitory peptides were identified by *in vitro* enzymatic digestion of milk proteins or chemical synthesis of peptide analogs (Gobbetti et al., 2004). The ACE-inhibitors derived from milk proteins are attributed to different fragments of casein, named (table .2) casokinins, or whey proteins, named lactokinins (Park & Nam, 2015).

Table 2. Bioactive Compounds Derived from Milk Precursors and Their ACE-Inhibitory Activities Source (Park and Nam, 2015)

Bioactive compounds	**components (Milk precursors)**	**Bioactivities**
Casokinins	α-, β-caseins	ACE inhibitory (Increase blood flow to intestinal epithelium)
Lactokinins	α-La, β-La and Serum albumin	ACE inhibitory

An overview of ACE inhibitory peptides in camel, goat, and sheep milk, highlighting the source, identified peptides, and additional notes for each milk type (Table 3).

Table 3. ACE Inhibitory Peptides in Various Types of Milk

own work based on (Guha et al., 2021).

Milk Type	**Source of ACE Inhibitory Peptides**	**Identified Peptides**	**Notes**
Camel Milk	Proline-Rich Casein Fraction	MVPYPQR (Met-Val-Pro-Tyr-Pro-Gln)	Camel milk shows superior ACE inhibition and antioxidant activity. ACE inhibitory peptides are enhanced by pepsin digestion and fermentation with *Lactobacillus* species. Colostrum and fermented skim camel milk also exhibit significant ACE inhibitory properties.
Goat Milk	Casein and Whey Fractions	TGPIPN (Thr-Gly-Pro-Ile-Pro-Asn), SLPQ (Ser-Leu-Pro-Gln), SQPK (Ser-Gln-Pro-Lys)	Commercial proteases like Alcalase and Subtilisin are effective in producing ACE inhibitory peptides. Fermentation with *Lactobacillus* species yields potent ACE inhibitory hydrolysates. Casein and whey fractions show higher ACE inhibitory activities compared to undigested proteins.
Sheep Milk	Sheep Sodium Caseinate	ACE inhibitory peptides from αs2-casein (residues 182–185) and αs1-casein (residues 1–6), Hydrophilic peptides from β-Lactoglobulin	ACE inhibitory peptides are produced after hydrolysis with proteases like *Bacillus sp*. P7 protease and *Lactobacillus helveticus* PR4 proteinase. Trypsin hydrolysis of β-Lactoglobulin also yields ACE inhibitory peptides, with hydrophilic ones showing higher activity.

2. 3. 2. Comparing Two Different Method to Detecting ACE-I

An overview of different methods used for investigating the ACE-inhibitory peptides in fermented milk. These methods, aim to explore various aspects such as the impact of bacterial strains, ingredients, and analytical techniques on peptide detection (Table 4).

Table 4. ACE-Inhibitory Peptide Detecting Methods

own work based on (Panayotova et al., 2018; Rubak et al., 2020).

Method	**Aim**	**Analytical Technique**	**Key Features and Findings**
Method (1) milk fermented with selected lactic acid bacteria (Panayotova et al., 2018)	Investigate ACE-inhibiting peptides in yogurt	Ultra High-Pressure Liquid Chromatography with Tandem Mass Spectrometry (UPLC-MS2)	- Peptide Analysis: Evaluated VPP and IPP concentrations using UPLC-MS2. - Bacterial Strains: Used specific strains in yogurt production. - Comprehensive sensory, physicochemical analyses. - Enumeration of viable bacterial cells.
Method (2) milk fermented by indigenous lactic acid bacteria) (Rubak et al., 2020)	Explore ACE-I peptide production by indigenous LAB in fermented milk	Stepwise enzymatic process involving substrate preparation, incubation, enzyme addition, reaction termination, and product extraction.	- Detailed enzymatic process for ACE-I activity determination. - Broader range of LAB strains from indigenous sources.

2. 4. Antioxidant Activity

2. 4. 1. Overview of Antioxidant in Milk

Oxidation occurs when essential compounds lose hydrogen atoms or electrons, often due to factors like radiation or high-heat cooking, leading to oxidative stress. This imbalance between free radical production and the body's ability to neutralize them can result in cellular damage, linked to diseases such as cancer, neurodegenerative disorders, and circulatory issues (Saber, 2009; Hussein, 2020).

Antioxidants play a crucial role in neutralizing free radicals, the body's defense mechanism against oxidative stress includes both enzymatic and nonenzymatic components. Enzymatic antioxidants like catalase and superoxide dismutase act as barriers against reactive oxygen species, while nonenzymatic antioxidants obtained from food, particularly from natural sources like milk, play a crucial role in neutralizing free radicals (Stobiecka et al., 2022).

Milk and dairy products provide antioxidants in their protein, fat, and water fractions, such as β-lactoglobulin and lactoferrin in proteins, vitamins E, A, β-carotene in fats, and vitamins C and microelements in water (Khan et al., 2019). Milk proteins supply crucial amino acids, with casein and whey proteins being the two main types. Whey proteins, such as α-lactalbumin, β-lactoglobulin, lactoferrin, immunoglobulins, serum albumin, and glycomacropeptides, play various roles in the antioxidant defense of milk.

Caseins exhibit antioxidant activity attributed to their phosphate content. They act as scavengers for free radicals, inhibit lipoxygenase-catalyzed lipid autoxidation, and can bind non-heme iron, affecting lipoxygenase activity. Whey proteins, especially β-lactoglobulin, showcase high antioxidant potential due to their rich content of sulfur amino acids, particularly cysteine. Additionally, lactoferrin exhibits antioxidant activity by chelating iron and suppressing inflammatory responses. The β-casein fraction, known for its high proline content, also demonstrates significant antioxidant activity (Stobiecka et al., 2022).

Carotenoids, lipophilic molecules found in milk, function as scavengers of singlet oxygen and peroxyl radicals. β-carotene, a specific carotenoid, acts as a preventive antioxidant by quenching singlet oxygen and inhibiting photo-oxidation. The oxidative stability of milk is vital to prevent off-flavors and nutritional decline, depending on various factors such as fatty acids, metal ions, tocopherols, and carotenoids (Khan et al., 2019). The antioxidant capacity of milk is not only important for human health but also plays a crucial role in preserving the quality of milk itself. Oxidation negatively impacts the shelf life and nutritional value of milk. Natural antioxidants derived from sources like milk are considered valuable and contribute to strengthening the body's defense mechanisms without the potential risks associated with synthetic alternatives (Stobiecka et al., 2022).

2. 4. 2. Methods to Detecting the Antioxidant

Various methods are employed to evaluate the antioxidant activity of milk and dairy products, categorized into single electron transfer (SET) and hydrogen atom transfer HAT mechanisms. SET methods include FRAP, ABTS, and DPPH assays, where antioxidants reduce oxidants. (Stobiecka et al,2022).

The DPPH assay, devised by Blois in 1958, serves as a common method for gauging antioxidant activity by employing a stable free radical known as α, α-diphenyl-β-picrylhydrazyl (DPPH). This assay relies on measuring the scavenging capacity of antioxidants against DPPH, where the odd electron of the nitrogen atom in DPPH is reduced upon receiving a hydrogen atom from antioxidants, resulting in the formation of hydrazine. DPPH is a stable free radical due to the delocalization of the spare electron over the entire molecule, that preventing dimerization. Its deep violet color, attributed to delocalization, fades upon reaction with substances capable of donating hydrogen atoms. The primary reaction involves the reduction of DPPH radical by the donor molecule, leading to the formation of the reduced form and a free radical. This assay is rapid, cost-effective, and widely used for assessing the ability of compounds to scavenge free radicals or donate hydrogen. It finds application in quantifying antioxidants in various biological and food samples, offering flexibility in solvent choice. However, limitations include sensitivity to certain compounds, solvent types, and environmental factors like light and oxygen (Kedare & Singh, 2011; Baliyan et al., 2022). The absorbance drop at 517 nm is measured in organic solvents like methanol or ethanol to assess the ability of compounds to scavenge the DPPH radical. Methanol provides a suitable environment for the reaction to occur and allows for the accurate measurement of the antioxidant capacity of substances by observing the reduction in the DPPH radical's color intensity. (Kedare & Singh, 2011).

The ABTS assay, widely known as ABTS/TAC or ABTS/TEAC, is a highly utilized method for assessing antioxidant capacity across diverse fields like food science, agriculture, and nutrition. Originally used for detecting fecal occult blood and glucose determination, ABTS evolved into a key tool for peroxidase activity assessment and subsequent kinetic studies. It relies on spectrophotometric analysis, leveraging the oxidized ABTS radical cation (ABTS•+) to react with antioxidants, resulting in the reduction of ABTS radical and loss of its bluish-green color. ABTS•+ boasts favorable attributes for colorimetric assays, making it a versatile choice for evaluating

antioxidant potential. (Cano et al., 2023). The (Table 5) outlines various antioxidant assays used to evaluate the antioxidant capacity of compounds. The mechanisms, color changes, and assessment methods are summarized for two common assays: DPPH and ABTS.

Table 5. Antioxidant Assays and Assessment Methods

(Mishra et al., 2015; Cloetens et al., 2013; Stobiecka et al, 2022).

Assay	**Mechanism**	**Color change**	**Assessment**
DPPH	Reduction of the purple-colored stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical to yellow 2,2-diphenyl-1-picrylhydrazine.	Purple to Yellow	Visual or Spectrophotometric analysis
ABTS	Antioxidants it lead to the reduction of the cation radical ABTS^+. can causes discoloration of the blue-green solution.	Blue-Green to Colorless	Visual or Spectrophotometric analysis

2. 5. Antibacterial Bioactive Peptides in Milk and Fermented Milk

Milk-derived antimicrobial bioactive peptides (ABPs) represent a diverse group of biochemical compounds with a molecular weight below 10 KD, produced through various processes such as organic synthesis, enzymatic proteolysis, and molecular cloning. These peptides, typically ranging from 5 to 90 amino acids in length, exhibit antibacterial activity when separated from parental proteins and can exist in various forms. They are categorized into milk-derived, whey-derived, casein-derived, and lysozyme-derived groups, with their antibacterial activities influenced by biophysical properties such as charge, size, conformation, and hydrophobicity (Khan et al., 2018). The antibacterial activity of ABPs relies on their composition of cationic and hydrophobic amino acids, which impart a mild positive charge under physiological conditions. These peptides disrupt lipid membranes by altering their permeability and transport properties, particularly affecting both Gram-positive and Gram-negative bacteria. They may also interact with bacterial DNA and RNA, form hydrogen bonds with specific substances, and lead to membrane dissolution or binding to nucleic acids (Khan et al., 2018).

Fermentation of caseins by lactic acid bacteria leads to the production of small peptides with antimicrobial properties. These peptides, like isracidin, k-casecidin, and kappacin, are generated through proteolytic activity and exhibit both bactericidal and bacteriostatic effects. LAB strains

such as *Lactococcus lactis*, *Lactobacillus* species, and *Streptococcus thermophilus* are adept at producing these peptides from milk proteins. They act by disrupting bacterial membranes, interfering with proton gradients, and chelating membrane-bound iron. This natural preservative ability makes them valuable in food production and infection control (Pessione and Cirrincione, 2016).

Additionally, protein hydrolysis with enzymes like pepsin can yield antibacterial peptides from milk proteins, such as isracidin and casiocidine, which combat various pathogens.

While over 880 antibiotic peptides have been identified globally, research on peptides from milk by LAB has primarily emphasized their nutritional and health benefits over their antimicrobial activity. These peptides interact with bacterial cell membranes, disrupt transcription or protein synthesis, or form pores in the membrane. Cationic peptides with hydrophobic amino acids adopting an α-helical structure are particularly effective in inhibiting pathogenic bacteria growth and damaging cell membranes. Peptides derived from alpha S1 and beta-casein, often containing phosphate groups, may play a role in mineral element transportation (Muhialdin & Algboory, 2018).

Overall, milk-derived ABPs are of significant interest due to their biological versatility and potential applications in pharmaceuticals and health-promoting food supplements. They contribute to the recognition of milk as a functional food, offering health benefits including antibacterial effects and inflammation reduction (Khan et al., 2018).

3. Materials and methods

3. 1. Materials

Microorganisms

The applied microorganisms – lactic acid bacteria and pathogens – can be seen in Table 6.

Table 6. The applied lactic acid bacteria and pathogen strains

Names of microorganisms	
Lactic acid bacteria	Pathogens
Lactobacillus acidophilus LA-5	*Escherichia coli* O157:H7
Lactobacillus acidophilus 150	*Escherichia coli* 8739
Lactobacillus acidophilus N2	*Listeria monocytogenes* 4ab
Lactobacillus rhamnosus GG ATCC 53103	*Enterococcus faecalis*
	Enterobacter cloacae

Milk protein concentrate solution

Milk Protein Concentrate (MPC) at a 5% concentration with distilled water was prepared. The desired amount of MPC powder was weighed and mixed with distilled water. The mixture was stirred until the solution's uniformity was ensured by employing gentle agitation or continuous stirring until homogeneity was achieved. These mixtures were sterilized by autoclaving at 121°C and 15 min.

Milk protein concentrate solution with cherry juice

Milk Protein Concentrate solution at a 5% concentration with distilled water was prepared as described above. Once sterilization was completed, cherry juice was added to each MPC sample in different concentrations (0%, 10%, 20%, 30%), ensuring thorough mixing.

MRS media

The MRS formulation, developed in 1960 by de Man, Rogosa, and Sharpe, was aimed at providing a medium conducive to the growth of lactobacilli. This medium composition consists of various

components that cater to the specific nutritional needs of lactobacilli, promoting their growth while inhibiting the growth of competing microorganisms (Table 7).

Table 7. Composition of MRS Media (Tille, 2014)

Ingredients	**Gramm/ Liter**
Proteose peptone	10.0
Beef extract	10.0
Yeast extract	5.0
Polysorbate 80	1.0
Dextrose	20.0
Ammonium citrate	2
Sodium acetate	5
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2.0

The media was sterilized by autoclave at 121°C for 15 minutes.

Tryptic soy broth agar

Tryptic Soy Broth Agar was prepared by weighing out 9g of Tryptic Soy Broth and 3.9g of agar. These precise measurements were then combined in a flask, to which 300ml of distilled water was added. The components were meticulously mixed to ensure complete dissolution. Subsequently, the mixture sterilized by autoclave at 121°C for 15 minutes.

ABTS Radical Cation Solution (Şanlıdere Aloğlu & Öner, 2011)

3.60234 grams of ABTS was being weighed out using a precision balance, the ABTS was simultaneously dissolved in water to create a 7 mM stock solution in a volumetric flask, During the same timeframe, 0.6623 grams of potassium persulfate were weighed out using a precision balance and added to the ABTS solution in the volumetric flask. The solution was mixed thoroughly until all solids were dissolved, and the flask was covered with aluminum foil or dark paper to shield it from light and incubate the solution in the dark at room temperature

(approximately 20-25°C) for 12 to 16 hours to allow the formation of ABTS radical cation. After the incubation period, the ABTS radical cation solution is ready for use in experiments.

Sodium Borate Buffer (pH 8.3)

Sodium Borate Buffer was prepared by dissolving 1.24g of Boric acid (H_3BO_3) in 90ml of distilled water. Subsequently, 10ml of 1N Sodium hydroxide (NaOH) solution was added. The pH of the solution was then adjusted to 8.3 by slowly adding approximately 39.3ml of 1M Hydrochloric acid (HCl), with careful monitoring. Thorough mixing was ensured after each addition to maintain homogeneity.

Hippuryl-L-Histidyl-L-Leucine (HHL) substrate

A solution of 50 mM Hippuryl-L-Histidyl-L-Leucine (HHL) substrate was prepared in 0.1 M sodium borate buffer with 0.3 M NaCl at pH 8.3. The substrate, sourced from SIGMA-ALDRICH (Catalog No. SLBW5368) with a purity >98% (HPLC), was stored at -20°C. 1.753g NaCl was added to 100 ml buffer to achieve a concentration of 0.3 M.

Angiotensin-Converting Enzyme (ACE)

The Angiotensin-Converting Enzyme solution, sourced from SIGMA-ALDRICH, had an activity of 2 Units/mg protein and was stored at -20°C.

3. 2. Methods

3. 2. 1. Fermentation of MPC Solution

• First, microorganism strains were inoculated into the MRS media and allowed to grow at 37°C for 24 hours. 5% MPC solutions were prepared with varying concentrations of glucose (0%, 0.1%, 0.5%, 1%, 1.75%, 2.5%)

• Following sterilization, one sample was taken from each flask without inoculation as a control and the other samples were inoculated with the refreshed bacterial strains (at a 5% inoculum size). The fermentation process was monitored over 8 hours and 24 hours, with samples collected at these specific time points to observe changes in fermentation.

3. 2. 2. Determination of pH

• Mettler Toledo seven Multi-pH meter was calibrated using two buffer solutions with known pH values (4 and 9). The electrodes of the pH meter were sequentially immersed in each buffer solution, and the meter was adjusted accordingly to match the pH values of the buffers.

•After calibration, the electrodes of the pH meter were thoroughly washed with deionized water to remove any contaminants between measurements. The sample to be measured was placed in a suitable container, and the electrodes of the pH meter were immersed into the sample solution.

The pH meter displayed the pH value of the sample, which was then recorded for analysis.

3. 2. 3. Determination of Antioxidant Activity

DPPH Assay

• The DPPH solution was prepared by dissolving 0.00142g of DPPH in 100ml of methanol.

• Next, the sample was mixed with the prepared DPPH solution. Specifically, one hundred microliters of the sample were added to 3.9ml of the prepared DPPH solution. DPPH solution and methanol without the sample serve as blank. Three replicates were made for each sample, and the mixture was left in the dark for 30 minutes.

• Following the incubation period, the mixture was centrifuged at 10000 rpm for 10 minutes (Hettich centrifuge), the absorbance drop at 517 nm was measured by Helios Alpha spectrophotometer and the radical scavenging activity of extracts was calculated as follow:

Radical scavenging activity % = ((Blank Abs -Normal Sample Abs) ×100)/ Blank Abs

ABTS Assay

• The prepared ABTS radical cation was diluted in methanol until the absorbance at 734 nm reached 0.70 ± 0.02 after equilibration at 30°C.

• Each fermented milk sample was centrifuged at 10,000 rpm for 10 minutes (Hettich centrifuge). After centrifugation, volumes of 50, 40, 30, 20, and 10 microliters were taken from each sample and diluted with distilled water to obtain a final volume of 50 microliters. Subsequently, each

diluted sample was mixed with 2 ml of the prepared ABTS and methanol solution. Three replicates were prepared for each sample, and the mixtures were left at room temperature for 6 minutes.

• Triplicates were also prepared using the ABTS solution and methanol as a control under the same conditions as the samples.

• Following incubation, the absorbance drop at 734 nm was measured, and the radical scavenging activity of extracts was calculated as follow-

Radical scavenging activity % = ((Blank Abs - Sample Abs) ×100)/ Blank Abs.

• The obtained data was utilized to calculate the half-maximal inhibitory concentration (IC50), representing the sample concentration needed to inhibit 50% of the ABTS radical cation activity

IC50= (50-b)/a where a = concentration of the antioxidant.

b= y-intercept of the dose-response curve, represent the maximal inhibition achieved by the antioxidant.

3. 2. 4. Determination of Angiotensin-Converting Enzyme Inhibitor Activity

• fifty microliters of the substrate (50 mM Hippuryl-L-Histidyl-L-Leucine in 0.1 M sodium borate buffer containing 0.3 M NaCl at pH 8.3) was mixed with 50 µL of the sample and incubated at 37°C for 5 minutes.

• Following incubation, 50 µL of 0.1 U/mL ACE solution (Angiotensin-Converting Enzyme) was added and incubated at 37°C for another 5 minutes (initiate the reaction).

• The reaction was stopped by adding 250 µL of 1 M HCl, and the resulting hippuric acid (HA) was extracted with 1.5 mL of ethyl acetate.

• The mixture was centrifuged at 2000× g (6500rpm) for 5 minutes.

• Subsequently, 0.8 mL of the ethyl acetate layer was transferred to a clean tube and evaporated at 85°C for 60 minutes. After evaporation, 4 mL of distilled water was added to dissolve the HA in the tube.The sample was centrifuged at 6500rpm for 5 minutes.

• Finally, the optical density of the dissolved HA was measured at 228 nm using a UV spectrophotometer. And calculated the percentage inhibition of absorbance at 228 nm using the formula: the extent of inhibition as 100% = ([B-A]/B), where: A is the optical density in the presence of ACE and ACE-I component, and B is the optical density without ACE-I component

3. 2. 5. Determination of Antimicrobial activity

Cultivation on plates

•Firstly, 200 µl of the pathogens was spread evenly into sterile Petri dishes. The tryptic soy broth agar medium was poured into the Petri dishes as needed, thoroughly mixed and the medium was allowed to solidify completely before using it for the test.

•Afterward, six holes (wells) were created in each plate, and the fermented milk protein concentrate solution was added into the wells. Because of the diffusion, the Petri dishes was placed to 5ºC for 1 hour.

•Finally, the plates were incubated at 37°C for 24 hours to allow lactic acid bacteria to interact with the pathogens. After incubation the clear inhibition zones around the well can be detected.

4. Results and Evaluations

This thesis aims to deepening the knowledge of the bioactive constituents within fermented milk protein concentrate. The study places particular emphasis on health benefits of the different types of bioactive peptides found in dairy products, including the management of hypertension through ACE-inhibitory peptides, assessment of antioxidant activity through various detection methods, and investigate the antibacterial properties of fermented milk protein concentrate, with focusing on the pivotal role of lactic acid bacteria (LAB), especially lactobacilli to maximize it.

4. 1. Results of PH Measurement of Fermented Milk Protein Concentrate with Glucose

5% MPC solution completed with glucose in different concentration were fermented by four lactic acid bacteria: *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* 150, *Lactobacillus acidophilus* N2, *Lactobacillus rhamnosus* GG ATCC 53103.

During the fermentation, the acidity through pH measurement was monitored to know the outcome of fermentation processes. Comparing the pH values before and after fermentation shows the acidity changes over time and with different fermentation agents and concentrations. The initial pH values ranged from pH 7.14 to 6.56 across MPC samples with varying glucose concentrations. This indicates that higher concentrations of glucose generally led to lower initial pH values.

4. 1. 1. Evaluation of Fermentation with *Lactobacillus acidophilus* LA-5

The results of changes of pH during MPC fermentation with *Lactobacillus acidophilus* LA-5 are summarized on the Figure 4.

After 8 hours of fermentation: The pH decreased significantly in all samples compared to their initial values. For MPC without glucose the pH decreased to 5.89, and still decreased with higher sugar concentrations until 5.47with 2.5% glucose. This demonstrates that *Lactobacillus acidophilus* LA-5 effectively initiated fermentation across all glucose concentrations.

After 24 hours of fermentation: without glucose and with 2.5% glucose the pH decreased to 5.36 and 5.24, respectively. It shows further reduction compared to 8-hour fermentation, indicative of continued fermentation activity. The results demonstrate a clear correlation between glucose concentration, fermentation kinetics, and pH evolution during *Lactobacillus acidophilus* LA-5 fermentation of MPC.

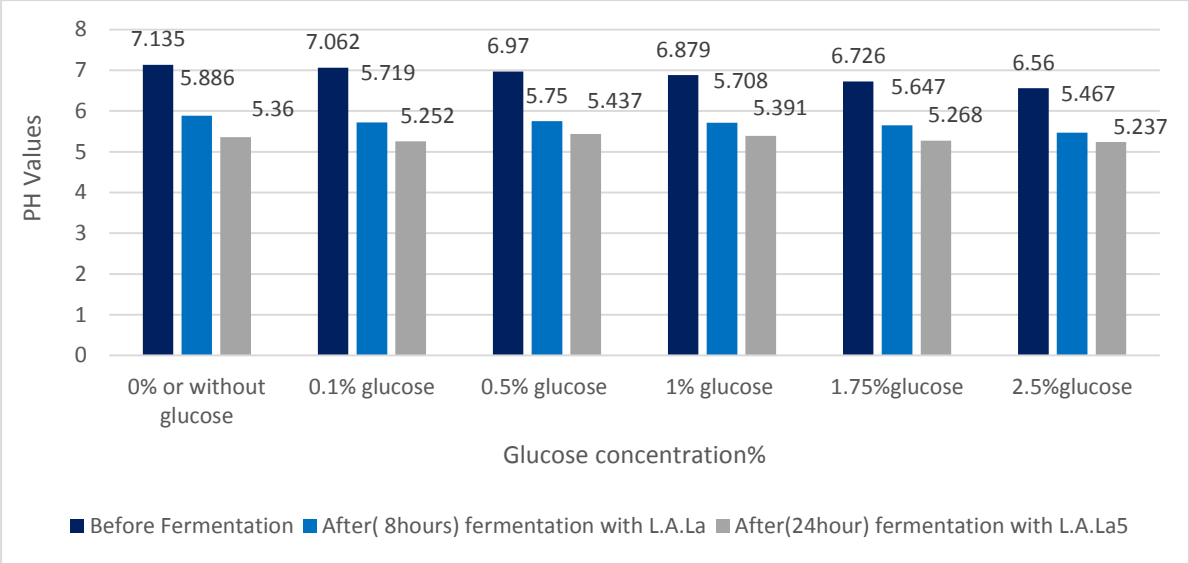


Figure 4. PH changing of milk protein concentrate with glucose during fermentation by *Lactobacillus acidophilus* LA-5

4. 1. 2. Evaluation of Fermentation with *Lactobacillus acidophilus* 150

The results of changes of pH during MPC fermentation with *Lactobacillus acidophilus* 150 are summarized on the Figure 5.

After 8 hours of fermentation: The pH decreased significantly in all samples compared to their initial values. For MPC without glucose, the pH dropped to 6.16, and still decreased with higher sugar concentrations. and decreased further to 5.52 with 2.5% glucose, this demonstrates that *Lactobacillus acidophilus* 150 effectively initiated the fermentation across all glucose concentrations.

After 24 hours of fermentation: Further reduction in pH was observed compared to the 8-hour fermentation period. The pH values decreased to 5.57 for MPC without glucose and to 4.34 for MPC with 2.5% glucose. The difference in glucose concentration had a significant effect on pH

after 24 hours. The results suggest a clear relationship between glucose concentration, fermentation kinetics, and pH evolution during *Lactobacillus acidophilus* 150 fermentation of MPC.

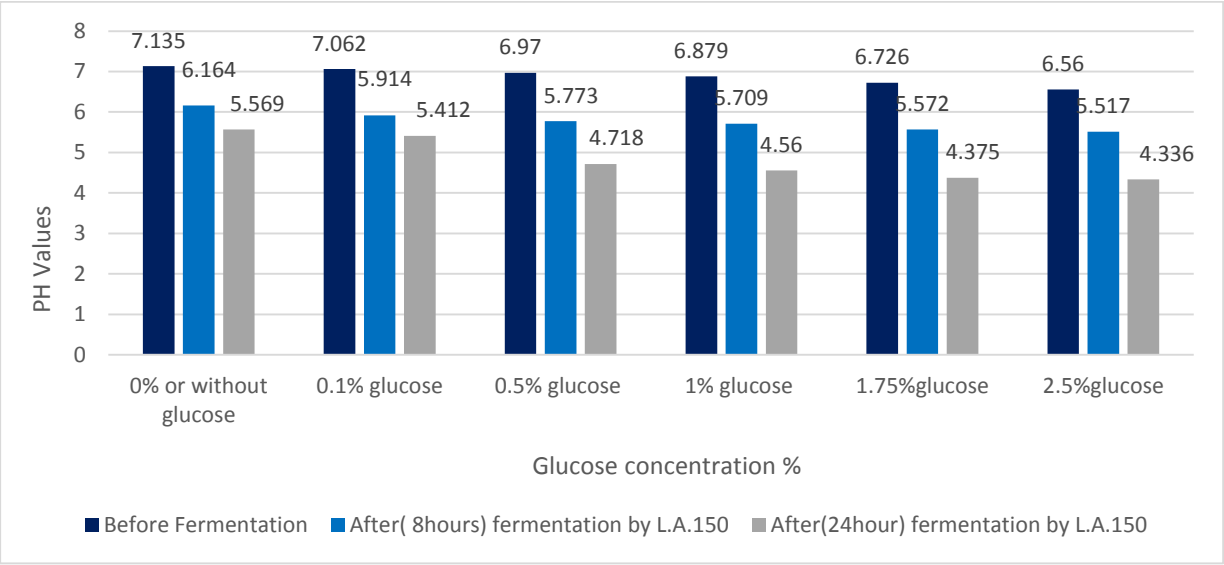


Figure 5. PH changing of milk protein concentrate with glucose before during fermentation by *Lactobacillus acidophilus* 150

4. 1. 3. Evaluation of Fermentation with *Lactobacillus acidophilus* N2

The results of changes of pH during MPC fermentation with *Lactobacillus acidophilus* N2 are summarized on the Figure 6.

After 8 hours of fermentation: very significant decreases in pH were observed across all samples containing 0.5-2.5% of glucose compared to their initial values. For MPC without glucose, the pH dropped to 6.3, while with 2.5% glucose, it decreased further to 4.28. This indicates effective fermentation activity initiated by *Lactobacillus acidophilus* N2 across all glucose concentrations.

After 24 hours of fermentation: Further reductions in pH were noted compared to the 8-hour fermentation period specially with high glucose concentration. The pH values decreased to 6.27 for MPC without glucose and to 3.79 for MPC with 2.5% glucose.

The results demonstrate a clear correlation between glucose concentration, fermentation kinetics, and pH evolution during *Lactobacillus acidophilus* N2 fermentation of MPC.

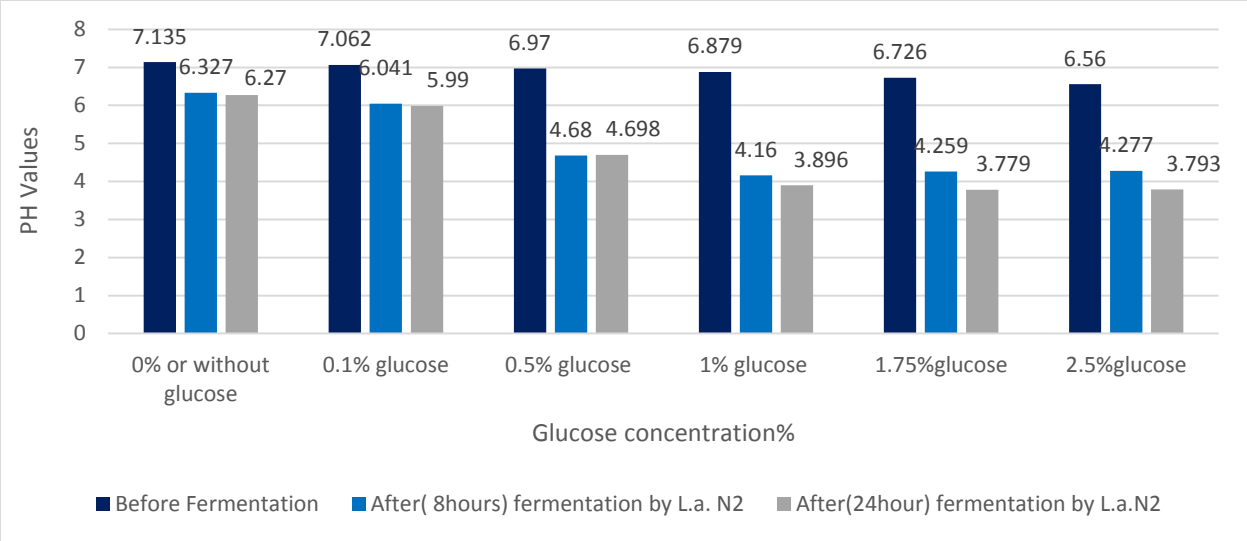


Figure 6. PH changing of milk protein concentrate with glucose before during fermentation by *Lactobacillus acidophilus* N2

4. 1. 4. Evaluation of Fermentation with Lactobacillus rhamnosus GG ATCC 53103

The results of changes of pH during MPC fermentation with *Lactobacillus rhamnosus* GG ATCC 53103 summarized on the Figure 7.

After 8 hours of fermentation: Significant decreases in pH were observed across all samples compared to their initial values. For MPC without glucose, the pH dropped to 6.31, and still decreased with higher glucose concentration, to 4.46 with 2.5% glucose. This indicates effective fermentation activity initiated by across all glucose concentrations.

After 24 hours of fermentation: Further reductions in pH were noted compared to the 8-hour fermentation period. The pH values decreased to 6.26 for MPC without glucose and to 3.66 for MPC with 2.5% glucose.

The results demonstrate a clear correlation between glucose concentration, fermentation kinetics, and pH evolution during *Lactobacillus rhamnosus* GG ATCC 53103 fermentation of MPC.

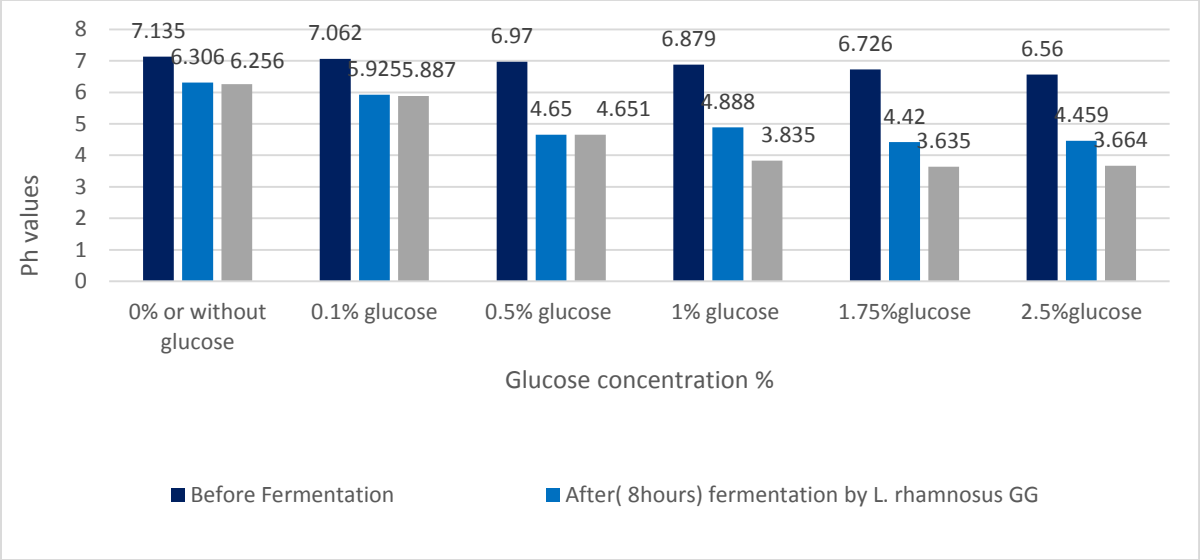


Figure 7. PH changing of milk protein concentrate with glucose before during fermentation by *Lactobacillus rhamnosus* GG ATCC 53103

4. 1. 5. Comparing the pH results during MPC fermentation with different lactic acid bacteria

The evaluation of fermentation with four different strains *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* 150, *Lactobacillus acidophilus* N2 and *Lactobacillus rhamnosus* GGATCC53103 reveals notable similarities and differences in their fermentation kinetics and pH evolution in milk protein concentrate (MPC) with varying glucose concentrations.

all strains demonstrated effective fermentation activity, as evidenced by significant decreases in pH after 8 hours of fermentation across all glucose concentrations.

However, differences emerged in the extent of pH reduction and the stability of fermentation activity over time. *Lactobacillus acidophilus* LA-5 and *Lactobacillus acidophilus* 150 exhibited similar patterns, with a gradual decrease in pH over 24 hours but with different value and a diminishing impact of glucose concentration on pH reduction. In contrast, *Lactobacillus acidophilus* N2 and *Lactobacillus rhamnosus* GGATCC53103 displayed more pronounced decreases in pH, particularly with higher glucose concentrations, suggesting potentially higher fermentation activity.

Overall, while all strains demonstrated the ability to ferment MPC and modulate pH, their specific fermentation kinetics and responses to glucose concentration vary. These differences highlight the importance of strain selection in optimizing fermentation processes for the production of functional dairy products.

4. 2. Result and Evaluations of Antioxidant by DPPH Assay

5% milk protein concentrate solution was supplemented with varying concentrations of glucose and subjected to fermentation by four distinct lactic acid bacteria strains: *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* 150, *Lactobacillus acidophilus* N2 and *Lactobacillus rhamnosus* GG ATCC53103. Following fermentation, the resulting fermented solutions was determined by DPPH method (Figure 8). Optical density measurements were then taken to quantify the antioxidant activity of each fermented solution.

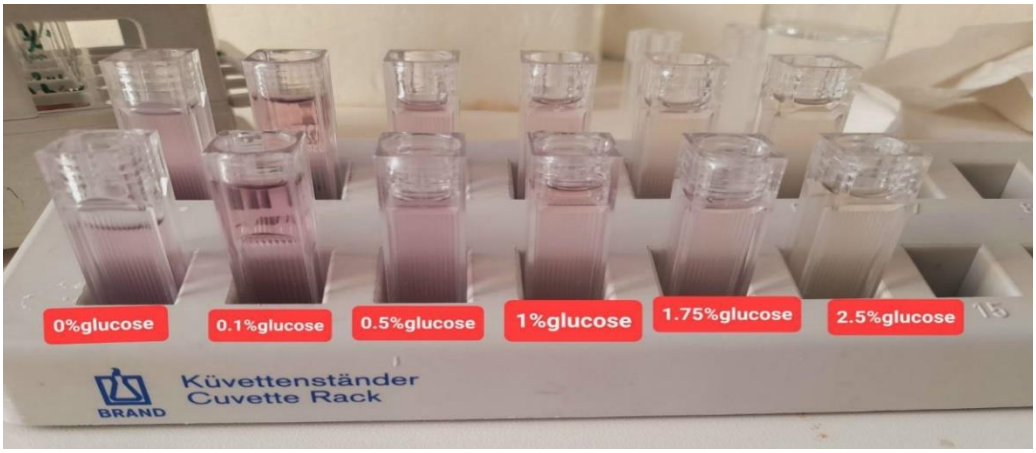


Figure 8. Color Change in Fermented Milk Protein Concentrate by *Lactobacillus acidophilus* LA-5 with DPPH Solution

The color change in fermented milk protein concentrate (MPC) with DPPH solution after 30 minutes is depicted. As the glucose concentration increases, the color of the solution transitions from purple to yellow.

4. 2. 1. Evaluations the result of antioxidant with *Lactobacillus acidophilus* LA-5

The results of changes of antioxidant activity during MPC fermentation with *Lactobacillus. acidophilus* LA5 summarized on the Figure 9.

Effect of glucose concentration: After 8 hours of fermentation, a clear trend of increasing antioxidant activity with higher glucose concentrations is observed. The highest antioxidant activity is recorded at 2.5% glucose concentration (91.83%), indicating that glucose supplementation enhances the production of antioxidant compounds during fermentation. Similarly, after 24 hours, the trend persists, with a continued increase in antioxidant activity observed with higher glucose concentrations. This suggests that glucose has a big effect for the synthesis of antioxidant compounds during fermentation, with higher concentrations leading to greater production of these bioactive compounds.

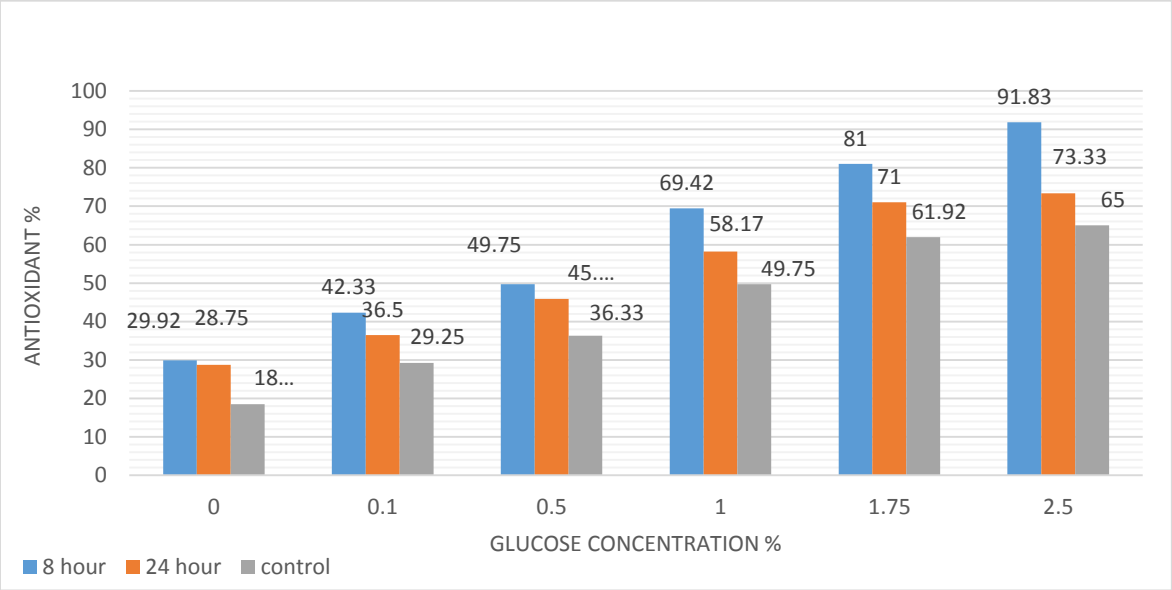


Figure 9. Antioxidant changing of milk protein concentrate with glucose during fermentation by *Lactobacillus acidophilus* LA-5

Effect of fermentation time: Comparing the antioxidant activity at 8 hours and 24 hours of fermentation, it is noted that the antioxidant activity after 8 hours (91.83%) is higher than that after 24 hours (73.33%). This observation indicates that the antioxidant potential reaches its peak at 8 hours of fermentation and gradually decreases over time. This could be attributed to the consumption of substrate (glucose) and the depletion of nutrients necessary for the synthesis of antioxidant compounds during prolonged fermentation.

Comparison with control: When compared to the control sample, the antioxidant activity of the fermented MPC solutions consistently surpasses that of the control. This highlights the significant role of fermentation in augmenting the antioxidant potential of dairy products.

4. 2. 2. Evaluation of Antioxidant Activity with *Lactobacillus acidophilus* 150

The results of changes of antioxidant activity during MPC fermentation with *Lactobacillus acidophilus* 150 summarized on the Figure 10.

Effect of glucose concentration: After 8 hours of fermentation, there is a noticeable increase in antioxidant activity with higher glucose concentrations. The highest activity is recorded at 2.5% glucose concentration, indicating that glucose supplementation significantly enhances the production of antioxidant compounds during fermentation. This trend persists after 24 hours of fermentation.

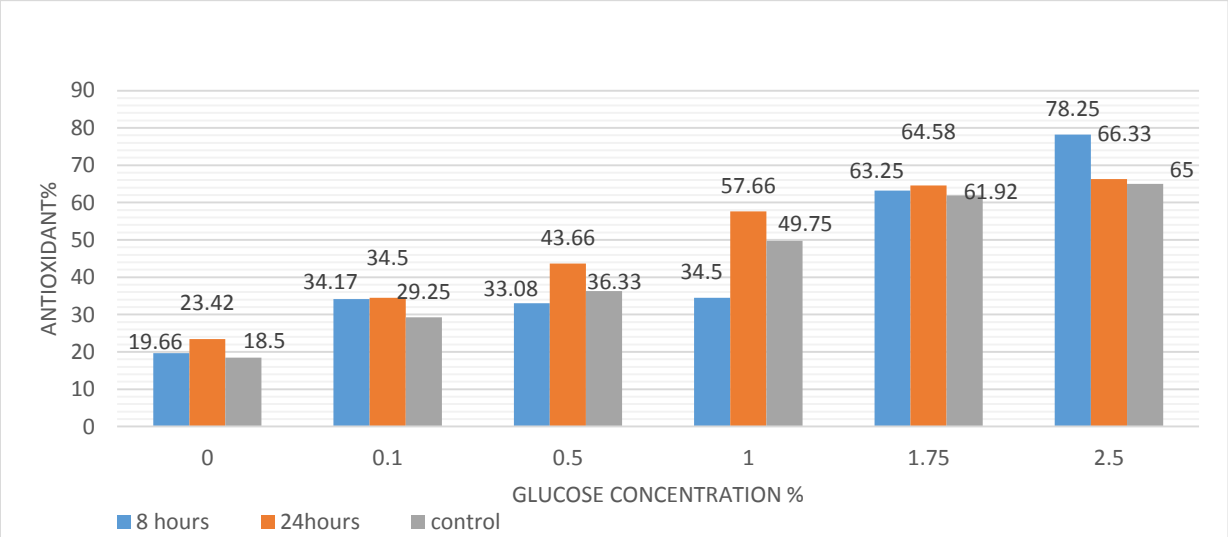


Figure 10, Antioxidant changing of milk protein concentrate with glucose during fermentation by Lactobacillus acidophilus 150

Effect of fermentation time: Comparing the antioxidant activity at 8 hours and 24 hours reveals an interesting trend. The antioxidant activity after 8 hours (78.25%) is higher than that after 24 hours (66.33%), indicating that the peak antioxidant potential is reached earlier in the fermentation process. This decline in antioxidant activity over time may be attributed to the depletion of

nutrients and substrates necessary for antioxidant compound synthesis during prolonged fermentation.

Comparison with Control: When compared to the control sample, the fermented MPC solutions consistently exhibit higher antioxidant activity. This underscores the significant contribution of fermentation to enhancing the antioxidant potential of dairy products. Fermentation with *Lactobacillus acidophilus* 150 leads to the production of bioactive compounds with antioxidant properties, surpassing the antioxidant activity of the control sample.

4. 2. 3. Evaluation of Antioxidant Activity with *Lactobacillus acidophilus* N2

The results of changes of antioxidant activity during MPC fermentation with *Lactobacillus acidophilus* N2 summarized on the Figure 11.

Effect of glucose concentration: After 8 hours of fermentation, there is a noticeable increase in antioxidant activity with higher glucose concentrations. The highest activity is recorded at 2.5% glucose concentration, suggesting that glucose supplementation significantly enhances the production of antioxidant compounds during fermentation. This persists after 24 hours of fermentation, emphasizing the crucial role of glucose in augmenting antioxidant potential during the fermentation process.

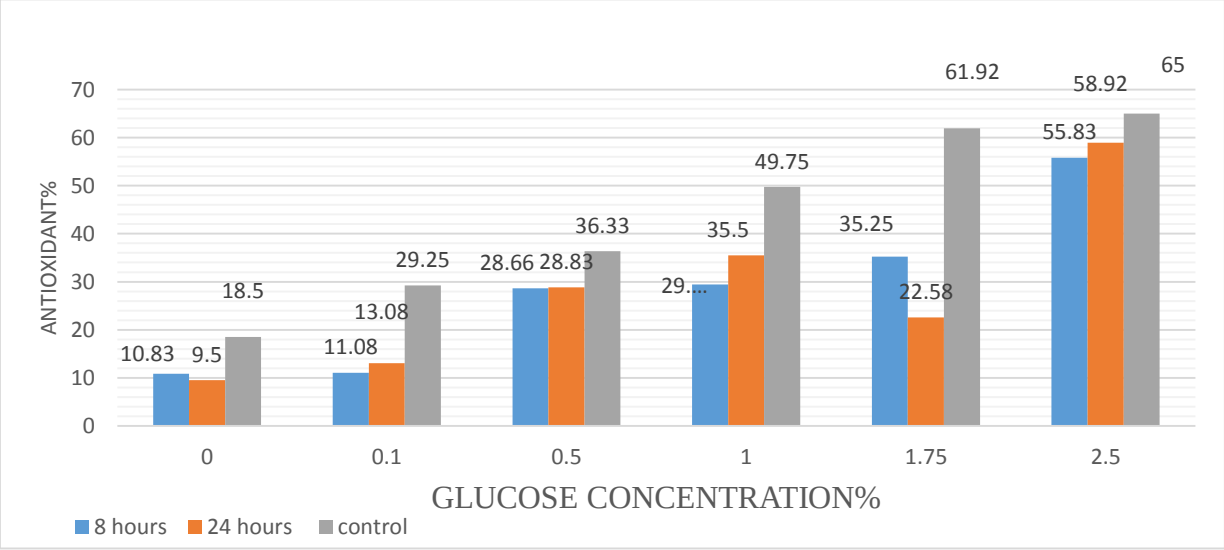


Figure 11. Antioxidant changing of milk protein concentrate with glucose during fermentation by *Lactobacillus acidophilus* N2

Effect of fermentation time: Comparing the antioxidant activity at 8 hours and 24 hours, there is a variation in the trend. The antioxidant activity after 8 hours (55.83%) is lower than after 24 hours (58.92%).

Comparison with control: the fermented MPC solutions exhibit lower antioxidant activity.

4. 2. 4. Evaluation of Antioxidant Activity with *Lactobacillus rhamnosus* GG ATCC 53103

The results of changes of antioxidant activity during MPC fermentation with *Lactobacillus rhamnosus* GGATCC 53103 summarized on the Figure 12.

Effect of glucose concentration: After 8 hours of fermentation, the antioxidant activity increases with higher glucose concentrations. The highest activity is observed at 1% glucose concentration. However, interestingly, after 24 hours of fermentation, the antioxidant activity peaks at 0.1% glucose concentration. This suggests that moderate glucose concentrations may be more effective in enhancing antioxidant activity during prolonged fermentation with *Lactobacillus rhamnosus* GGATCC 53103.

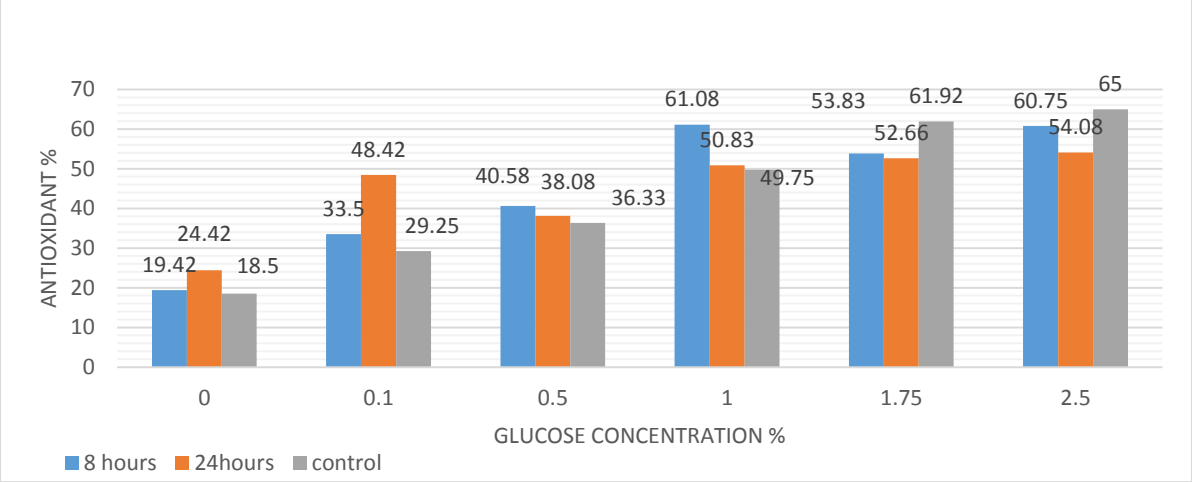


Figure 12. Antioxidant changing of milk protein concentrate with glucose during fermentation by *Lactobacillus rhamnosus* GG ATCC 53103

Effect of fermentation time: Comparing the antioxidant activity at 8 hours and 24 hours, it is noted that the antioxidant activity varies depending on the glucose concentration and fermentation time. While some variations are observed, there is not a consistent trend in the effect of fermentation time on antioxidant activity, but the highest result is at 1% glucose after 8 hours fermentation (61.08%).

Comparison with control: When comparing to the control sample, the fermented MPC solutions exhibit varying degrees of antioxidant activity. In some cases, the antioxidant activity surpasses that of the control, indicating the potential of *Lactobacillus rhamnosus* GGATCC 53103 fermentation to enhance antioxidant properties. However, there are instances where the antioxidant activity remains lower than the control.

4. 3. Result and Evaluations of Antioxidant by ABTS Assay

To study the antioxidant activity by ABTS method of the fermented milk protein concentrate, the sample of *Lactobacillus acidophilus* LA-5 was chosen, because it gave the best antioxidant activity up to 90% with DPPH assay. Absorbance measurements were then taken to quantify the antioxidant activity of each fermented solution. The results of antioxidant activity of fermented milk samples with varying glucose concentrations (0%, 0.1%, 0.5%, 1%, 1.75%, 2.5%) by *Lactobacillus acidophilus* LA-5 after incubation can be seen on the Figure 13.

The color change in different amount of fermented milk protein concentrate (MPC) with ABTS solution after 6 minutes is depicted. As the sample concentration increases, the color of the solution transitions from Blue to colorless.

Figure 13. Color Change in Fermented Milk Protein Concentrate by *Lactobacillus acidophilus* LA-5, with ABTS Solution

The obtained data was utilized to calculate the half-maximal inhibitory concentration (IC50), representing the sample concentration needed to inhibit 50% of the ABTS radical cation activity.

4. 3. 1. Evaluation of Antioxidant Activity at 0% Glucose (without Glucose)

A serial dilution from fermented milk protein concentrate without glucose was prepared to determine IC50 number. The results of changes of antioxidant activity during MPC fermentation without glucose summarized on the Figure 14. Lower dilutions (higher concentrations) generally lead to higher antioxidant activity.

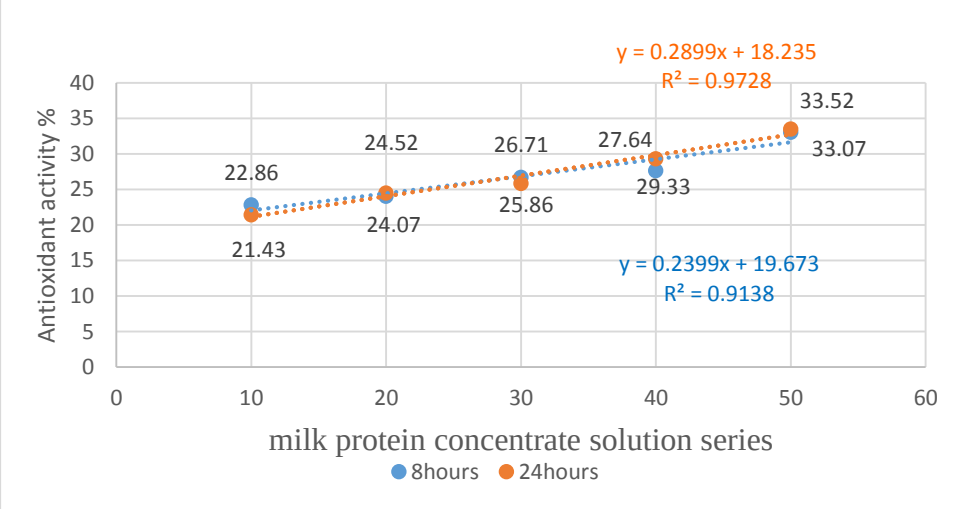


Figure 14. Antioxidant changing of milk protein concentrate without glucose during fermentation for different milk protein concentrate solution series

Effect of glucose and fermentation time: The highest antioxidant activity during 8 hours of fermentation is observed with fifty microliter milk, with an antioxidant activity of 33.07%.
The best antioxidant activity during 24 hours of fermentation is observed with fifty microliter milk, with an antioxidant activity of 33.52% (no big changing between 8 and 24 hours).

4. 3. 2. Evaluation of Antioxidant Activity at 0.1% Glucose

The results of changes of antioxidant activity during MPC fermentation at 0.1% glucose summarized on the Figure 15.

The highest antioxidant activity during 8 hours and 24 hours of fermentation is 32.43% and 35.19%, respectively. This indicates that the antioxidant continues to increase over time, even in small glucose concentration.

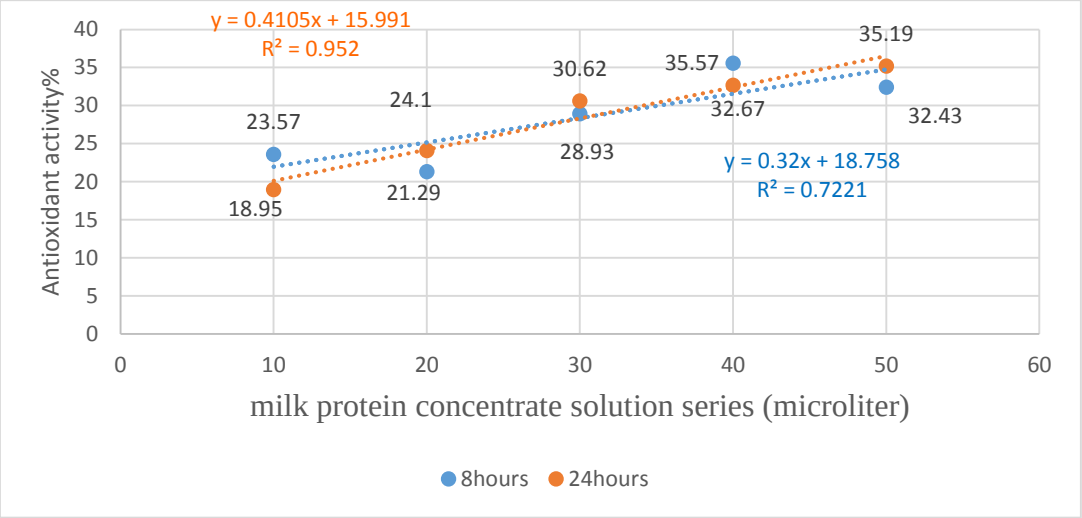


Figure 15. Antioxidant changing of milk protein concentrate with 0.1%glucose during fermentation for different milk protein concentrate solution series

4. 3. 3. Evaluation of Antioxidant Activity at 0.5% Glucose

The results of changes of antioxidant activity during MPC fermentation at 0.5% glucose summarized on the Figure 16.

Effect of glucose and fermentation time: The highest antioxidant activity during 8 hours of fermentation is 61.66%.

After 24 hours of fermentation, the antioxidant activity decreases to 31.76%.

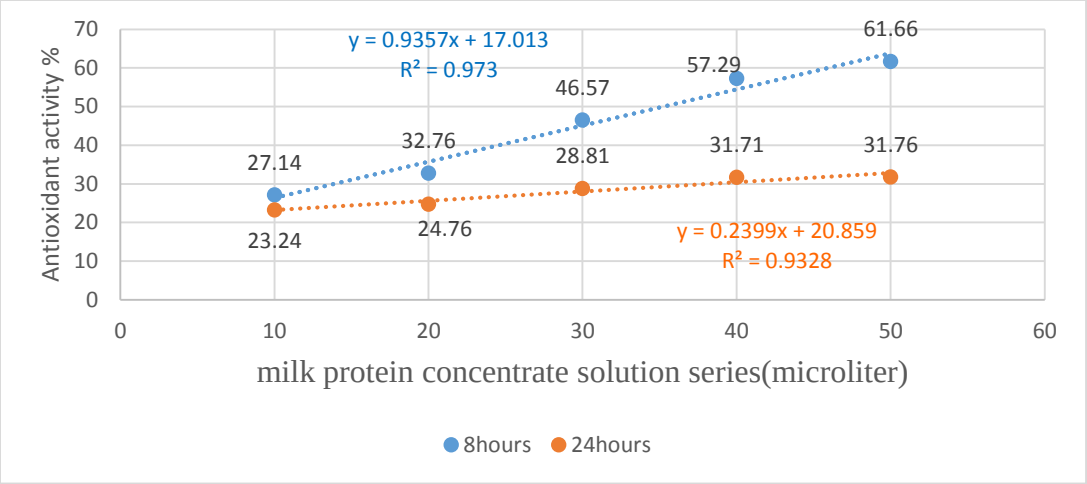


Figure 16. Antioxidant changing of milk protein concentrate with 0.5% glucose during fermentation for different milk protein concentrate solution series

4. 3. 4. Evaluation of Antioxidant Activity at 1% Glucose

The results of changes of antioxidant activity during MPC fermentation at 1% glucose summarized on the Figure 17.

Effect of glucose, and fermentation time: The highest antioxidant activity during 8 hours of fermentation is 75.38%.

The best antioxidant activity during 24 hours of fermentation is 49.38%.

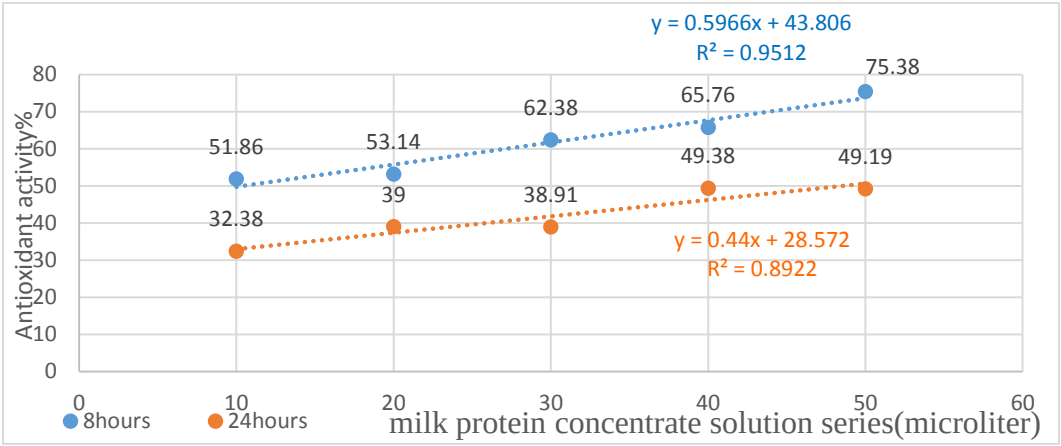


Figure 17. Antioxidant changing of milk protein concentrate with 1%glucose during fermentation for different milk protein concentrate solution series

4. 3. 5. Evaluation of Antioxidant Activity at 1.75% Glucose

The results of changes of antioxidant activity during MPC fermentation at 1.75% glucose summarized on the Figure 18.

Effect of glucose, and fermentation time: The highest antioxidant activity after 8 and 24 hours of fermentation is 90.9% and 91.62%, respectively

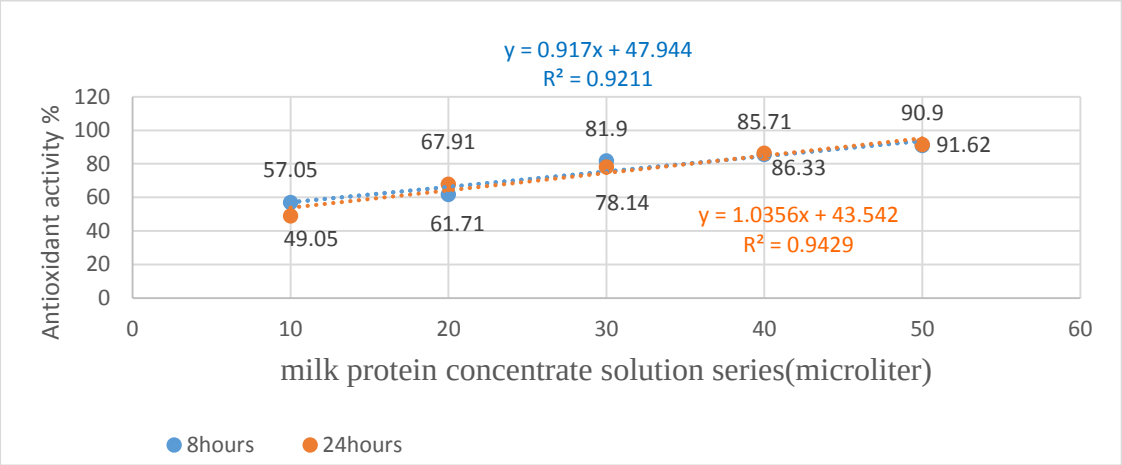


Figure 18. Antioxidant changing of milk protein concentrate with 1.75% glucose during fermentation for different milk protein concentrate solution series

4. 3. 6. Evaluation of Antioxidant Activity at 2.5% Glucose

The results of changes of antioxidant activity during MPC fermentation at 2.5% glucose summarized on the Figure 19.

Effect of glucose, and fermentation time: The highest antioxidant activity during 8 hours of fermentation is 92.81%.

The best antioxidant activity during 24 hours of fermentation 87.14%.

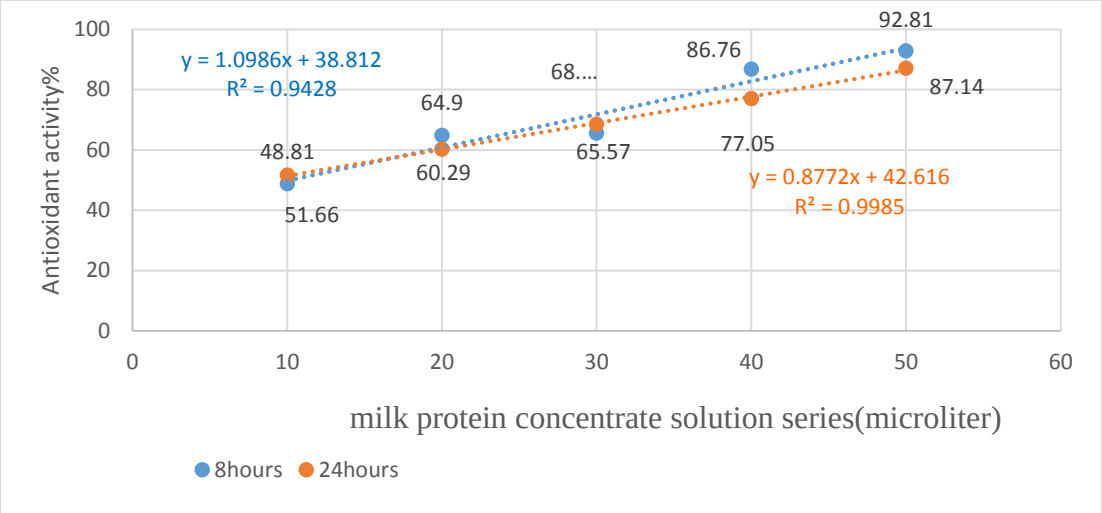


Figure 19. Antioxidant changing of milk protein concentrate with 2.5%glucose during fermentation for different milk protein concentrate

4. 3. 7. Comparing the Antioxidant with different Glucose Concentrations

Antioxidant activity is influenced by both glucose concentration and fermentation time.

The best antioxidant activity after 8 hours of fermentation is observed with 2.5% glucose concentration 92.81%.

After 24 hours of fermentation, the highest antioxidant activity is observed with 1.75% glucose concentration 91.62%.

The obtained data was utilized to calculate the half-maximal inhibitory concentration (IC50), (Table 8)

Table 8. IC50 values after 8 and 24 hours of fermentation with different glucose concentrations

samples	**IC50 after 8 hours (microliter)**	**IC50 after 24 hours(microliter)**
0%or without glucose	126.38	109.52
0.1% glucose	97.63	82.95
0.5% glucose	35.10	121.42
1% glucose	10.32	48.70
1.75% glucose	2.23	6.21
2.5% glucose	10.17	8.39

After 8 hours of fermentation, the lowest IC50 value (indicating the highest antioxidant activity) is observed with 1.75% glucose concentration, requiring only 2.23 microliters to achieve IC50. However, after 24 hours of fermentation, although 1.75% glucose concentration remains the best, the IC50 value increases to 6.21 microliters after 24 hours, suggesting a decrease in antioxidant activity compared to the 8-hour time point. The highest IC50 with the sample without glucose, It decreases with increasing glucose, except at 0.5% glucose after 24 hours.

4. 4. Result and Evaluations of ACE inhibitory Activity

To determine the ACE enzyme inhibitory effect of the fermented MPC samples, 5% milk protein concentrate (MPC) solution with 0% and 1% concentrations of glucose was applied. MPC without glucose to know the effect of glucose and 1% concentration because it has a significant effect previously. The chosen samples were fermented by *Lactobacillus acidophilus* LA-5. Following fermentation, the resulting fermented solutions were mixed with a substrate, enzyme and follow the description the ACE-I% was calculated.

Based on the results in can be said, that ACE-I activity was low in MPC without glucose 14.09% (after 8 hours fermentation) and 13.63 % (after 24 hours fermentation).

MPC supplemented with 1% glucose exhibited the highest ACEI activity, with values of 18.94% at 8 hours and 27.63% at 24 hours.

Glucose appears to enhance the ACEI activity of the fermented MPC solutions, evident at 1% glucose concentration comparing to 0% glucose.

Overall, these results indicate that the addition of glucose enhanced the ACE-I inhibitory effect, particularly after 24 hours of incubation (The fermentation time is important here). The observed increase in ACE-I activity percentage in the presence of glucose after 24 hours could be attributed to several factors. Glucose might have facilitated conditions favorable for ACE inhibition, such as providing additional energy sources for enzymatic processes or altering the microenvironment surrounding the ACE enzyme. Additionally, prolonged incubation time might have allowed for more efficient interaction between the ACE inhibitor and the enzyme, leading to enhanced inhibition. The results of changes of ACEI activity during MPC fermentation with *Lactobacillus acidophilus* LA-5 summarized on the Figure 20.

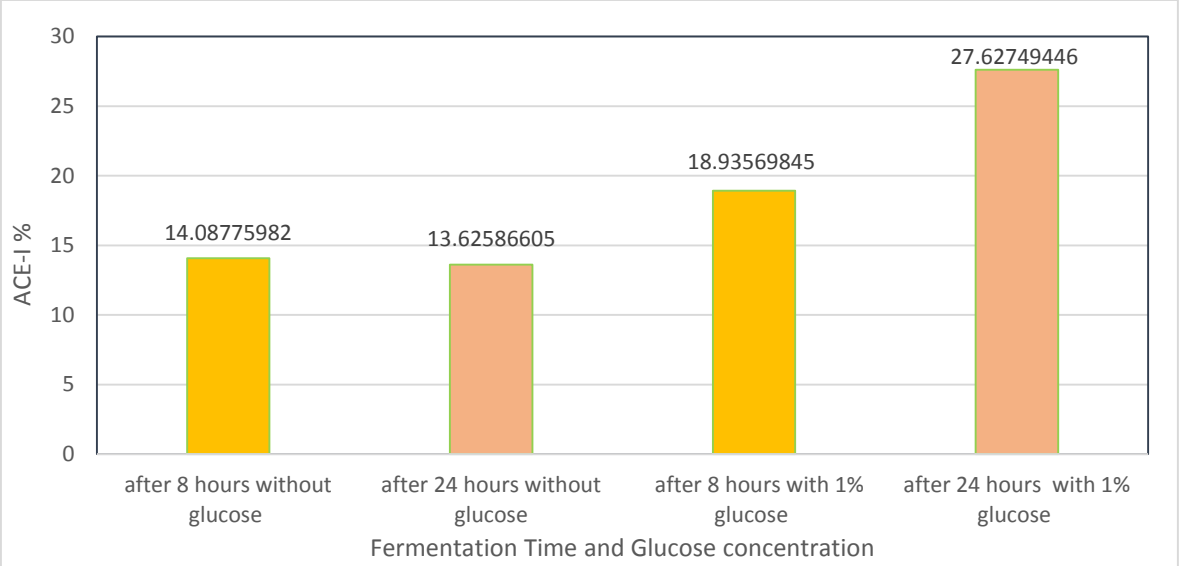


Figure 20. ACE-I activity changing of milk protein concentrate during fermentation by *lactobacillus acidophilus* LA-5

4. 5. Result and evaluations of Antimicrobial Activity

After incubating the plates at 37°C for fermented MPC sample over 8 and 24 hours, the antimicrobial activity of the fermented milk protein concentrate with varying concentrations of glucose was assessed against *Escherichia coli* O157:H7, *Escherichia coli* 8739, *Listeria*, *Enterococcus faecalis*, and *Enterococcus cloacae.* The presence or absence of inhibition zones around the wells indicated the effectiveness of the fermented solution against each pathogen (Figure 21).

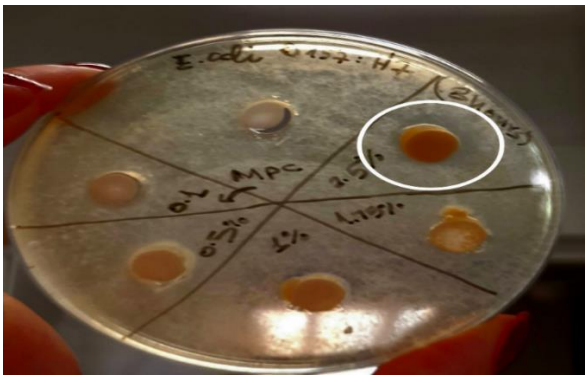
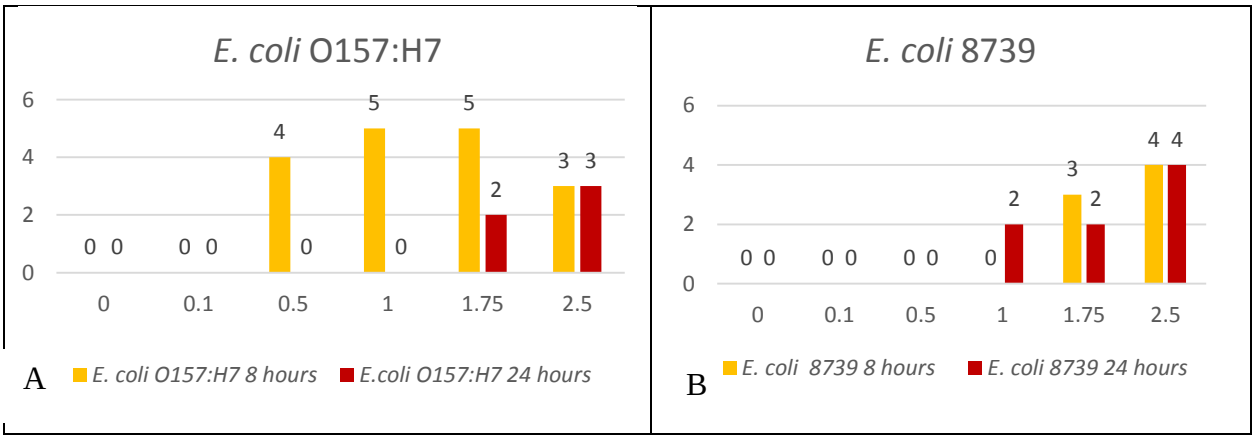


Figure 21. Antimicrobial activity of fermented milk protein concentrate with glucose after 8hours by *Lactobacillus acidophilus* LA-5 against *E. coli* O157:H7

The antimicrobial experiment conducted using fermented milk produced by *Lactobacillus acidophilus LA-5* with varying glucose concentrations showed notable inhibitory effects against the tested bacterial strains. Across different concentrations of glucose and time points, distinct patterns of antimicrobial activity emerged, as evidenced by the formation of inhibition zones.

At both the 8-hour and 24-hour time points, the fermented milk exhibited varying degrees of inhibition against the bacterial strains tested, including *E. coli* 8739, *E. coli* O157:H7, *E. faecalis*, *Listeria*, and *E. cloacae*. The inhibition zones ranged from 0 to 5mm, indicating differences in susceptibility among the bacterial species.

Notably, the antimicrobial activity appeared to be concentration-dependent, with higher concentrations of glucose in the fermented milk generally resulting in larger inhibition zones. This suggests that glucose concentration influences the production or effectiveness of antimicrobial compounds during fermentation by *Lactobacillus acidophilus* LA-5. The results of changes of antimicrobial activity during MPC fermentation with *Lactobacillus acidophilus* LA-5 summarized on the Figure 22.



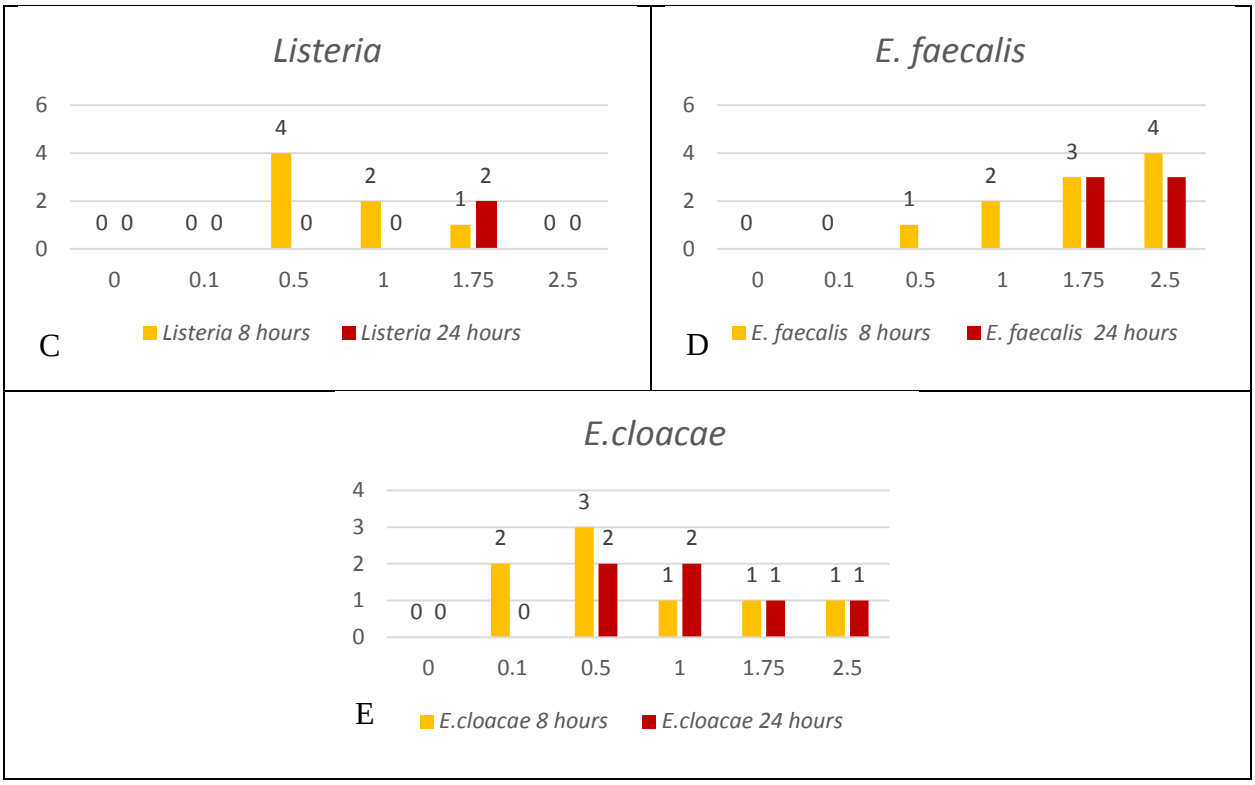


Figure 22. Antimicrobial activity of fermented milk protein concentrate with glucose after 8 and 24 hours by *Lactobacillus acidophilus* LA-5 against A: *E. coli* O157:H7, B: *E. coli* 8739, C: *Listeria*, D: *E. faecalis*, E: *E. cloacae.*

Among the tested bacterial strains, *E. coli* O157:H7 exhibited the highest susceptibility to the antimicrobial activity of the fermented milk, with inhibition zones reaching up to 5mm at 1.75% glucose concentrations after8 hours fermentation and time points. In contrast, other bacterial strains, such as *E. faecalis* and *Listeria*, showed moderate to low susceptibility, as indicated by smaller inhibition zones.

The observed variations in antimicrobial activity among the bacterial strains could be attributed to differences in their cell wall structure, metabolic pathways, and susceptibility to antimicrobial compounds produced during fermentation. Additionally, the duration of fermentation might have influenced the synthesis and accumulation of bioactive peptides or metabolites with antimicrobial properties.

Overall, the results suggest that fermented milk produced by *Lactobacillus acidophilus* LA-5 has the potential to serve as a natural antimicrobial agent against a range of pathogenic bacteria.

4. 6. Result and Evaluations of Fermented MPC with Juice

4. 6. 1 Results of Change in PH

5% MPC solution completed with cherry juice in different concentrations were fermented by *Lactobacillus acidophilus* LA-5 strain.

Cherry juice is known for its high antioxidant content, primarily due to compounds like anthocyanins, flavonoids, and vitamin C. These antioxidants can complement the antioxidant peptides derived from milk during fermentation, potentially enhancing the overall antioxidant capacity of the fortified product.

Monitoring acidity through pH measurement to know the outcome of fermentation processes Comparing the pH values before and after fermentation how acidity changes over time and with different fermentation agents and concentrations. The results of changes of pH during MPC fermentation with *Lactobacillus acidophilus* LA-5 summarized on the Figure 23.

Before fermentation, the initial pH values ranged from 7.27 without cherry juice to 5.34 with 30% cherry juice,

After 8 hours of fermentation, significant decreases in pH were observed across all samples. The pH dropped to values ranging from 5.69 without cherry juice to 4.09 with 30% cherry juice, indicating active fermentation by *Lactobacillus acidophilus* LA-5. Interestingly, higher concentrations of cherry juice resulted in more substantial reductions in pH, suggesting enhanced fermentation activity.

After 24 hours of fermentation, further clear decreases in pH were noted across all samples, indicative of continued fermentation activity. The final pH values ranged from 5.59 to 3.73, with higher concentrations of cherry juice leading to lower final pH values. Overall, the results demonstrate the effectiveness of cherry juice as a substrate for fermentation by *Lactobacillus acidophilus* LA-5. Higher concentrations of cherry juice resulted in more pronounced decreases in pH, suggesting increased fermentation activity.

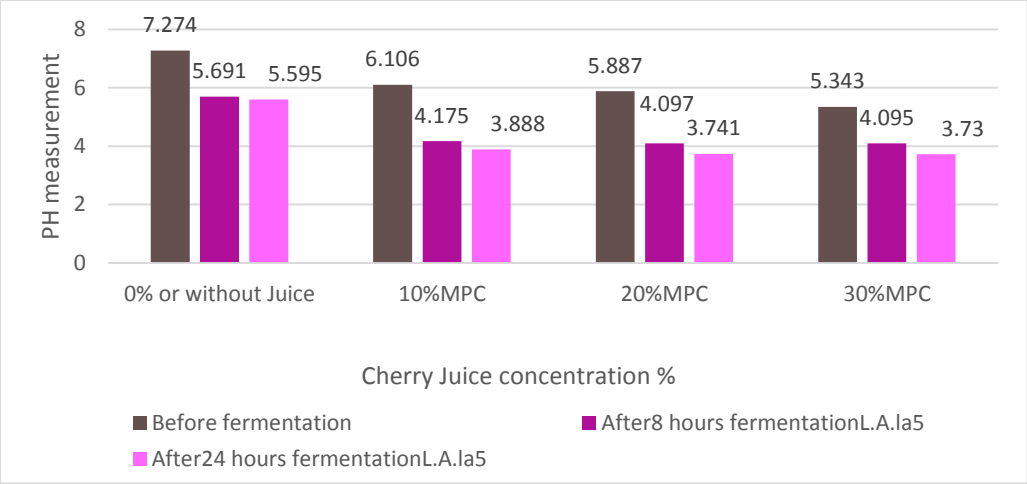


Figure 23. PH changing of milk protein concentrate with cherry juice during fermentation by *Lactobacillus acidophilus* LA-5

4. 6. 2. Result and Evaluations of Antioxidant of MPC with Juice

5% milk protein concentrate (MPC) solution was supplemented with varying concentrations of cherry juice and subjected to fermentation by *Lactobacillus acidophilus* LA-5. Following fermentation, the resulting fermented solutions were mixed with a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution to assess their antioxidant activity. Measurements of absorbance were then taken to quantify the antioxidant activity of each fermented solution

The results of changes of antioxidant activity during MPC fermentation with *L. acidophilus LA-5* summarized on the Figure 24.

Effect of cherry juice concentration: After 8 hours of fermentation, there is a noticeable increase in antioxidant activity with higher cherry juice concentrations. The highest antioxidant activity is observed at 30% cherry juice concentration, indicating a significant enhancement in the production of antioxidant compounds during fermentation.

This trend persists after 24 hours of fermentation, with antioxidant activity continuing to increase with higher cherry juice concentrations.

Higher cherry juice concentrations lead to greater production of bioactive compounds with antioxidant properties, highlighting the importance of cherry juice supplementation in enhancing antioxidant potential during fermentation.

Effect of fermentation time: Comparing the antioxidant activity at 8 hours and 24 hours, it is noted that the antioxidant activity consistently increases over time for all cherry juice concentrations. This indicates that fermentation progresses, leading to the accumulation of antioxidant compounds in the fermented MPC solutions. The highest antioxidant (88.25%) activity is generally observed after 24 hours of fermentation, suggesting that prolonged fermentation enhances the overall antioxidant potential of the fermented MPC solutions.

Comparison with control: When compared to the control sample, the fermented MPC solutions with cherry juice concentrations consistently exhibit significantly higher antioxidant activity only in case of 24 hours fermentation.

This highlights the substantial contribution of cherry juice supplementation in augmenting the antioxidant potential of dairy products during long fermentation.

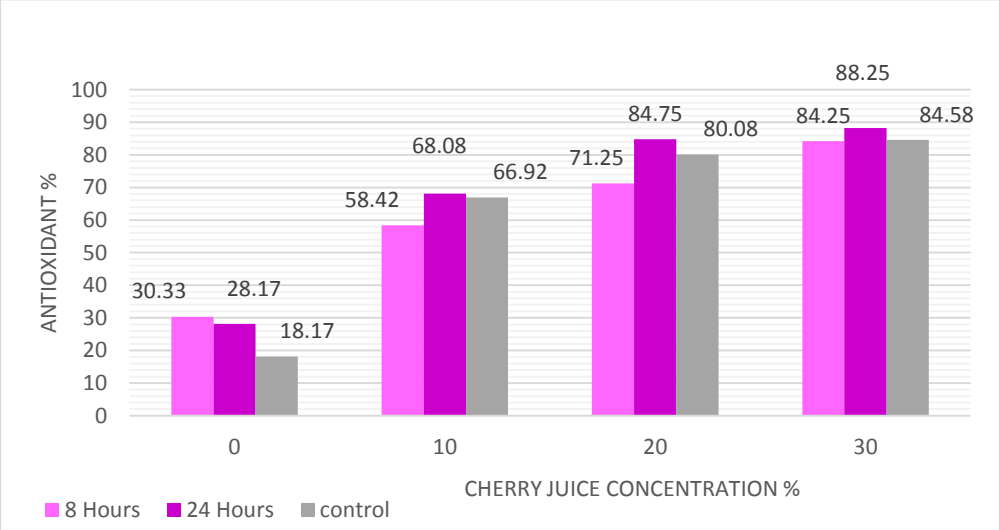


Figure 24. Antioxidant changing f milk protein concentrate with cherry juice during fermentation by *Lactobacillus acidophilus* LA-5

4. 7. Comparative Analysis with Literature

- Bioactive peptide activity become better after 24 hours specially in ACE-I activity: evidence: LAB also play a significant role in releasing bioactive peptides from food proteins, these peptides, initially inactive when encrypted within proteins, become biologically active upon liberation through proteolytic processes (Hayes et al., 2007; Pessione, 2012)

- Decreasing antioxidant and antimicrobial activity after 24 hours fermentation comparing to 8 hours fermentation in some cases. And this could be attributed to the consumption of substrate (glucose) and the depletion of nutrients necessary for the synthesis of antioxidant compounds during prolonged fermentation.

Evidence: proteolytic machinery ensures that LAB efficiently break down proteins into constituent amino acids, meeting their nutritional requirements for growth and survival, particularly in nitrogen-limited environments like milk (Savijoki et al., 2006; Sadat-Mekmene et al., 2011; Griffiths and Tellez).

- The importance of glucose on LAB and biologically active peptides:

Evidence: during fermentation, glucose can act as a substrate for microbial metabolism, affecting the growth and activity of lactic acid bacteria (LAB). Additionally, glucose availability can impact the proteolytic activity of LAB, potentially influencing the release of bioactive peptides from milk proteins (Tagliazucchi et al., 2019).

5. Conclusion and proposals

The results of the evaluation of antioxidant activity using the DPPH and ABTS methods, as well as the assessment of ACE-I activity and antimicrobial effects, provide valuable insights into the multifaceted health-promoting properties of fermented milk. Notably, the research elucidated the influence of glucose concentration and fermentation time on the antioxidant, ACE-I inhibitory, and antimicrobial activities of fermented milk protein concentrate.

The pH measurements serve as a reliable indicator of fermentation progress. This is crucial for monitoring the outcome of fermentation processes over time and with different fermentation agents and concentrations.

The assessment of antioxidant activity reveals significant variations across different strains of lactobacilli, glucose concentrations, and fermentation times. Notably, *Lactobacillus acidophilus* LA-5, *Lactobacillus acidophilus* 150, and *Lactobacillus rhamnosus* GGATCC 53103 demonstrate promising antioxidant properties, with enhancements observed at higher glucose concentrations. And because the *Lactobacillus acidophilus* LA-5 was the best, it focused on it with further experiment.

The evaluation of antioxidant activity using the ABTS method indicates that milk protein concentrate, glucose concentration, and fermentation time significantly influence the IC50 values., and the optimal conditions for achieving lower IC50 values vary depending on the fermentation duration.

The evaluation of antioxidant activity in milk samples supplemented with cherry juice using the DPPH method highlights the beneficial effects of fermentation, particularly in combination with *Lactobacillus acidophilus* LA-5. Overall, these findings underscore the potential of incorporating cherry juice into fermented milk products, particularly for enhancing their antioxidant activity, thereby offering further health benefits to consumers.

The evaluation of ACE-I activity in fermented milk protein concentrate samples reveals intriguing insights into the potential cardiovascular health benefits associated with different formulations, it is evident that *Lactobacillus acidophilus* LA-5 alone had minimal impact on ACE inhibition. However, the addition of glucose, particularly at a concentration of 1%, significantly enhanced the ACE-I inhibitory effect, with notable increases observed after 24 hours of incubation. This suggests that glucose may play a pivotal role.

Similarly, the antimicrobial activity of fermented milk produced by *Lactobacillus acidophilus* LA-5 exhibits notable inhibitory effects against pathogenic bacterial strains specially with higher glucose concentrations, suggesting its potential as a natural antimicrobial agent.

This is confirmed by the reviewed literature, especially the importance of *Lactobilius acidophilus* on health and the importance of glucose on biologically active peptides is also according to (Tagliazucchi et al., 2019) that during fermentation, glucose can act as a substrate for microbial metabolism, affecting the growth and activity of lactic acid bacteria (LAB). Additionally, glucose availability can impact the proteolytic activity of LAB, potentially influencing the release of bioactive peptides from milk proteins.

Some future suggestions: Investigation how LAB produce bioactive peptides during fermentation to enhance yields.

Exploring novel LAB strains for fermenting milk protein concentrate to expand functional dairy products.

Exploring synergistic effects with other components for enhanced health benefits.

Investigating additional health benefits beyond known effects.

6. Summary

The investigation conducted in this thesis sheds light on the bioactive compounds in fermented milk protein concentrate (MPC), and their potential therapeutic applications. It conducted a series of experiments to analyze the bioactive properties of fermented milk protein concentrate (MPC). Initially, microorganism strains were cultured and inoculated into MPC solutions with varying glucose concentrations in all experiments except one experiment with cherry juice. Fermentation progress was monitored over 8 and 24 hours, alongside pH measurements to track acidity changes.

Through experimentation and analysis Antioxidant potential was assessed using DPPH and ABTS assays, measuring radical scavenging activity and determining IC50 values. ACE-I activity was evaluated, Antimicrobial activity was tested by culturing pathogens on agar plates and adding fermented MPC solutions.

several key findings have emerged, which contribute significantly to the understanding of functional foods and dietary strategies aimed at enhancing human health and well-being.

Firstly, the study elucidates the pivotal role of lactic acid bacteria (LAB), particularly lactobacilli, in orchestrating fermentation processes and enhancing the nutritional profile of dairy products. LAB play a crucial role in generating bioactive peptides during fermentation, which have been shown to offer a myriad of health benefits.

The investigation into antioxidant activity using both DPPH and ABTS assays reveals significant variations across different strains of lactobacilli, glucose concentrations, and fermentation times. Notably, *Lactobacillus acidophilus* LA-5 emerges as a promising strain, demonstrating enhanced antioxidant properties, particularly at higher glucose concentrations. These findings underscore the potential of fermented milk products, enriched with specific LAB strains, to serve as natural sources of antioxidants, thereby promoting health and well-being.

Moreover, the assessment of ACE-inhibitory activity highlights the potential cardiovascular health benefits associated with fermented milk protein concentrate. While *Lactobacillus acidophilus* LA-5 had minimal impact on ACE inhibition, the addition of glucose significantly enhanced ACE-I inhibitory effects. This suggests a synergistic interaction between glucose and LAB fermentation, potentially offering therapeutic benefits for cardiovascular health.

Furthermore, the evaluation of antimicrobial activity underscores the efficacy of fermented milk protein concentrate in inhibiting the growth of pathogenic microorganisms, such as *Escherichia coli, Listeria, Enterococcus faecalis* and *Enterobacter cloacae.* The findings suggest that fermentation, in combination with specific LAB strains, enhances the antimicrobial properties of dairy products, thereby contributing to food safety and public health.

In the end, the study provides insights for the development of evidence-based dietary interventions aimed at enhancing human health and well-being. underscores the importance of strain selection, glucose concentration and fermentation time in optimizing fermentation processes for the production of functional dairy products. in addition to understanding the fermentation kinetics and functional properties of different *Lactobacillus* strains.

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8. List of Tables and Figures

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DECLARATION

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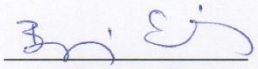

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