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SELECTION AND COMBINATION OF PROTECTIVE, POTENTIAL PROBIOTIC LACTOBACILLI AND FOOD INDUSTRY BY-PRODUCTS IN EDIBLE COATINGS FOR CHEESE SAFETY AND QUALITY ENHANCEMENT

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APSAUGINIŲ IR PROBIOTINIŲ SAVYBIŲ LAKTOBACILŲ IR ANTRINIŲ MAISTO PRAMONĖS ŽALIAVŲ ATRANKA BEI JŲ PRITAIKYMAS VALGOMOSIOSE DANGOSE SŪRIO SAUGAI IR KOKYBEI DIDINTI

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CONTENTS

ABBREVIATIONS

AI – atherogenicity index
ANOVA – analysis of variance
ASV – amplicon sequence variant
ATCC – American Type Culture Collection
a_w – water activity
BHI – brain heart infusion
BSE – back-scattered electron
C – control
CFU – colony-forming units
D_{SEC} – digestibility based on size exclusion chromatography
DNA – deoxyribonucleic acid
DM – dry matter
E – exponential phase
EAB – elongation-at-break
EDS – energy-dispersive X-ray spectroscopy
EFSA – European Food Safety Authority
EPS – exopolysaccharides
FA – fatty acids
FAO/WHO – Food and Agriculture Organization/World Health Organization
GRAS – generally recognized as safe
h/H – hypocholesterolemic/hypercholesterolemic ratio
LAB – lactic acid bacteria
LAWPC – liquid acid whey protein concentrate
LAWP – liquid acid whey permeate
LCFA – long-chain fatty acids
L. p – *Lacticaseibacillus paracasei* A11
LQI – lipid quality indices
MCFA – medium-chain fatty acids
MIC – minimum inhibitory concentration
MRS – de Man, Rogosa and Sharpe medium
MUFA – monounsaturated fatty acids
n – flow behavior index
NSLAB – non-starter lactic acid bacteria
OD – optical density

omega-3 – omega-3 polyunsaturated fatty acids
omega-6 – omega-6 polyunsaturated fatty acids
PCA – principal component analysis
PCR – polymerase chain reaction
PERMANOVA – permutational multivariate analysis of variance
PUFA – polyunsaturated fatty acids
P – probiotic cheese
QIIME2 – Quantitative Insights into Microbial Ecology 2
RNA – ribonucleic acid
rRNA – ribosomal ribonucleic acid
R – regular cheese
SCFA – short-chain fatty acids
SDA – Sabouraud dextrose agar
SEC – size exclusion chromatography
SE – secondary electron
SEM – scanning electron microscopy
SFA – saturated fatty acids
SLAB – starter lactic acid bacteria
TBC – total bacteria count
TCD (ΔE) – total color difference
TI – thrombogenicity index
TS – tensile strength
UFA – unsaturated fatty acids
UHT – ultra-high temperature
UK – United Kingdom
U.S. – United States
WVP – water vapor permeability

INTRODUCTION

In recent years, natural strategies for improving food safety and quality have received increasing attention [1]. In the context of food safety, clean-label preservation strategies involve naturally derived or minimally processed substances that provide microbial control without the use of synthetic chemical additives, addressing consumer demand for safer food products [2,3]. Among these, naturally derived microorganisms, particularly lactic acid bacteria (LAB), have gained attention in the food industry [4].

LAB are essential in fermented foods, including dairy products, where they contribute to preservation, flavor development, texture enhancement, and overall product stability [5]. In cheese production, LAB play a key role not only during fermentation and ripening, but also in maintaining product safety by inhibiting undesirable microorganisms [6]. This effect is further supported by the ability of some LAB species to exhibit antibacterial activity through a combination of competitive growth and the production of antibacterial compounds, such as organic acids and bacteriocins [7].

Cheese, as one of the most widely consumed dairy products, is a complex matrix in which microbiological stability is essential for both quality and safety [8,9]. It has been estimated that up to 20% of dairy products are lost or wasted in Europe [10]. During storage, cheeses are particularly susceptible to contamination by bacteria, mold, and yeast [11]. The growth of these microorganisms causes a range of cheese defects, such as visual mycelial growth, pigment formation, off-odors, and off-flavors, leading to economic losses and potentially posing a risk to consumer health due to the production of toxins or other harmful metabolites [12,13].

To address these challenges, edible coatings and films have received attention, as they are made from thin layers of food-grade biopolymers that can be directly applied to food surfaces, thereby supporting product safety [14]. Their role is to extend shelf life by acting as barriers to oxidation, gas exchange, and moisture loss while enabling the incorporation of functional components [15,16]. In dairy products, edible coatings have been explored as carriers for antioxidants, antimicrobials, flavor compounds, and probiotics, allowing targeted control of microbial growth at the surface where contamination primarily occurs [17,18].

When edible coatings are used as carriers for probiotics, they are also relevant for the development of functional foods [14]. In cheese production, probiotic adjunct cultures have gained increasing importance due to their contribution to nutritional value and potential health benefits, promoting the development of cheeses with probiotic properties [19]. However, while

probiotics are commonly incorporated directly into the cheese matrix, less information is available on the behavior of additional protective potential probiotic microorganisms applied through edible coatings.

Another challenge related to LAB-incorporated edible coatings is the viability of probiotic LAB, as they are exposed to environmental stresses when deposited on the surface of cheese, including oxygen, reduced moisture, and temperature fluctuations [20,21], which may reduce viability during digestion. Various technological solutions have been investigated to improve the survival of probiotics, including the incorporation of prebiotics, such as oligosaccharides and polyphenols [22], as well as the application of more complex processing methods, including encapsulation [23].

Despite the growing body of research on LAB and edible coatings, most studies focus on edible coatings that include supplementary protective ingredients, such as essential oils, enzymes (lysozyme and bioactive peptides), polysaccharides (chitosan), and microbial source-based metabolites (bacteriocins and organic acids) [24,25]. As a result, limited information is available on the effectiveness of edible coatings in which LAB function as the main protective and probiotic component, especially in cheese. Furthermore, the relationship between coating formulation, microbial behavior, interactions between adjunct and coating-derived microorganisms, and product quality during storage and digestion is still not well understood.

Aim of the Study:

To develop and evaluate the effects of an edible coating with incorporated protective, potential probiotic lactobacilli on the safety and quality of Edam-type cheese.

Objectives:

1. To isolate lactobacilli with protective and probiotic properties from fermented cow's milk using an *in vitro* digestion model, and to identify, characterize, and evaluate their safety and probiotic properties.
2. To cultivate selected protective, potential probiotic lactobacilli in various dairy industry by-products and determine the most suitable medium for their cultivation based on biomass yield.
3. To develop a protective edible coating from food industry by-products and cultivated lactobacilli, and to evaluate its physicochemical properties.
4. To apply the protective edible coating to regular and probiotic Edam-type cheeses and assess their safety and quality during ripening and storage.

5. To investigate the effect of simulated gastrointestinal digestion on the microbiota composition and protein digestibility of coated regular and probiotic Edam-type cheeses during ripening.

Scientific novelty

The study is the first to apply the harmonized INFOGEST *in vitro* digestion method before the initial stage of lactobacilli isolation from fermented cow's milk. This approach enabled early selection of strains with probiotic potential, reducing the number of isolates subjected to further characterization.

The study demonstrates the dual utilization of dairy industry by-products, liquid acid whey protein concentrate (LAWPC) and liquid acid whey permeate (LAWP), for both lactobacilli cultivation and as structural components of edible coatings, contributing to sustainable food processing.

Unlike most previous research, the developed edible coatings rely on probiotic lactobacilli as the main antimicrobial and functional component, without the need for additional protective additives.

The ability of *L. paracasei* to remain viable in cheese and after *in vitro* digestion without encapsulation or added protective components represents a novel finding and simplifies industrial application.

Furthermore, a comprehensive assessment was carried out, linking coating formulation, microbial activity, cheese quality, and digestion results, providing insight into the performance of probiotic coatings from cheese ripening and storage to digestion.

Practical significance

This study provides relevant scientific and practical insights for both researchers and dairy industry producers by advancing knowledge on the use of LAB as the primary bioactive component in edible coatings. It expands current understanding of the interactions between probiotic microorganisms, coating matrices, and cheese microbiota during storage and simulated gastrointestinal digestion.

From a technological perspective, the results demonstrate that edible coatings incorporating protective, potential probiotic lactobacilli represent a practical and scalable strategy to enhance cheese safety and quality without the use of synthetic additives. The findings confirm the feasibility of replacing conventional preservatives with LAB-based bioprotective systems, while maintaining or improving product characteristics throughout ripening and storage. In particular, the selected *L. paracasei* A11 strain can be successfully incorporated into coatings without additional antimicrobial ingredients, simplifying formulation and facilitating industrial application.

The study also highlights the potential of dairy industry by-products, such as LAWPC and LAWP, as alternative raw materials for both probiotic cultivation and edible coating production. This approach supports waste reduction and valorization of low-value resources, contributing to circular economy principles while reducing production costs.

Importantly, the application of LAB-based edible coatings offer a natural solution aligned with clean-label trends, supporting the development of functional dairy products that meet the expectations of modern, health-conscious consumers. By effectively controlling spoilage microorganisms, extending shelf life, and preserving sensory quality, such systems can help reduce food losses and improve product sustainability.

Finally, the survival of probiotic bacteria during simulated digestion indicates that these coatings can serve as a delivery system for beneficial microorganisms, adding extra health value to commonly consumed foods.

1. LITERATURE REVIEW

1.1. Functional role of LAB

LAB have historically played a key role in the production of fermented foods and beverages, acting as natural agents for preservation, flavor development, and nutritional improvement [4]. They are Gram-positive, non-spore-forming microorganisms, typically occurring as rods or cocci, lacking catalase activity, classified as facultative or obligate anaerobes, and are well adapted to survive in low-pH environments [26]. LAB includes a diverse range of genera, including *Lactococcus, Leuconostoc, Pediococcus, Streptococcus*, and others, including *Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Oenococcus, Tetragenococcus, Vagococcus*, *Weissella,* and taxa formerly classified within the genus *Lactobacillus* [27]. The genus *Lactobacillus* was extensively reclassified based on whole-genome analysis, resulting in the former genus being divided into 25 genera, including the revised genus *Lactobacillus* and several newly proposed genera such as *Lacticaseibacillus*, *Lactiplantibacillus*, *Companilactobacillus,* and *Levilactobacillus,* commonly found in carbohydrate-rich environments [28,29].

LAB play a crucial role in the fermentation process by metabolizing substrates to produce by-products with applications in the pharmaceutical, chemical, and food industries [30–32]. Their significance is mostly associated with their safe metabolic activity in foods, utilizing available sugar to produce organic acids and other metabolites, including peptides, lactic acid, exopolysaccharides (EPS), bacteriocins, and enzymes such as amylases, proteases, and lipases [33–35]. Several of these metabolites contribute to antimicrobial activity through disruption of microbial and fungal cell membranes, thereby inhibiting spoilage and pathogenic microorganisms [30]. This natural preservation potential positions LAB as a sustainable alternative to synthetic preservatives in various food systems. Numerous studies have investigated the application of protective LAB in cheese, demonstrating their efficacy in inhibiting pathogenic bacteria. For instance, *Levilactobacillus brevis*, *Lactiplantibacillus plantarum*, and *Enterococcus faecalis* reduced *Listeria monocytogenes* counts by up to 4 $\log_{10}$ CFU/g in semi-hard cheese [36]. Similarly, *Lacticaseibacillus rhamnosus* inhibited *Staphylococcus aureus* and *L. monocytogenes* in semi-hard goat cheese [37], while *Lacticaseibacillus paracasei*, isolated from fermented milk, suppressed *Bacillus cereus* and *L. monocytogenes* in model Cheddar-like cheese [38].

Additionally, LAB facilitates the breakdown of proteins and fats, generating flavor precursors like free amino acids and fatty acids (FA), which further improve the sensory profile of fermented products (such as kimchi, sauerkraut,

plant-based milks, vinegar, fermented dough, and fermented dairy products [39–41]. The latter consists of the largest fermented food group, including yogurt, cheese, kefir, and fermented milk [42]. The fermentation of milk with LAB not only enhances product stability and preserves its nutritional value but also contributes to desirable sensory characteristics, making it a widely adopted method in dairy processing [43]. These functional qualities make LAB essential in modern food production, combining technological benefits with enhanced safety and quality.

1.2. Selection and characterization of LAB for food use

In recent years, there has been an increase in screening of novel LAB strains with targeted technological and functional attributes for use in the food industry [44–46]. This screening usually involves a multistep, time-consuming process [47,48], as no single assay can fully predict the performance of a strain in food or under gastrointestinal conditions. Since LAB isolation yields a large number of isolated strains, only a small fraction remains as potential probiotics after successive selection steps [49]. These are strains identified based on preliminary *in vitro* traits; however, such evidence alone is insufficient to confer probiotic status, which requires confirmation of efficacy *in vivo*, particularly in human studies, as emphasized by Food and Agriculture Organization/World Health Organization (FAO/WHO) guidelines [50]. For instance, four potential probiotic strains were selected from approximately one hundred isolates from traditional Korean fermented foods [51], whereas a similar number was identified from ninety-three isolates obtained from raw and fermented milk of local animals in Saudi Arabia [52]. Therefore, there is a clear need for strategies that allow early narrowing of the isolated bacterial pool. *In vitro* digestion models are usually applied to assess the viability of LAB under simulated gastrointestinal conditions, most often at later stages of strain characterization [53–56]. In this context, applying *in vitro* digestion directly to fermented foods containing diverse LAB populations may enable early identification of potential probiotic strains before detailed safety, technological, and probiotic characterization.

1.2.1. Safety assessment

Lactobacilli are one of the most well-known species of probiotic microorganisms, widely recognized for their long history of consumption and their status as “generally regarded as safe (GRAS)” [57,58]. However, recent safety concerns have emphasized the need for comprehensive evaluation. The FAO/WHO and European Food Safety Authority (EFSA) have provided guidelines that emphasize that probiotic strains should be evaluated for

potential adverse effects, including antibiotic resistance patterns, hemolytic activity, toxigenicity, and pathogenicity [57,59,60].

Intrinsic resistance, in contrast to acquired, has a low potential for horizontal gene transfer and is therefore not generally considered a safety concern for non-pathogenic bacteria [61,62]. Most *Lactobacillus* species exhibit intrinsic resistance to aminoglycosides (e.g., gentamicin, kanamycin, neomycin, and streptomycin), as well as vancomycin, ciprofloxacin, and trimethoprim. In contrast, they are generally susceptible to β-lactam antibiotics such as penicillin and ampicillin, and to protein synthesis inhibitors including tetracycline, erythromycin, chloramphenicol, and linezolid [63,64]. However, resistance to chloramphenicol [65–67], clindamycin [64], and tetracycline [63,68,69] has been reported in lactobacilli isolated from various sources, emphasizing the importance of assessing antibiotic resistance in newly isolated LAB for use in food.

Hemolytic activity is an important feature of many pathogenic bacteria, as it allows them to access heme iron *in vivo* [70,71]. Because of that, bacteria with hemolytic activity can damage intestinal epithelial cells, disrupt the integrity of the mucosal barrier, and promote the invasion of pathogens and other harmful compounds [72]. Although lactobacilli are generally recognized as commensal microorganisms with beneficial effects on the host [73,74], assessment of hemolytic activity remains a key step in evaluating the safety of LAB strains, especially for probiotic applications [75]. Previous studies have reported that LAB isolated from Korean fermented seafood [76], artisanal Colombian double-cream cheese [77], traditional Indian fermented foods [46], kimchi [67], goat milk [78] and camel milk [79] did not exhibit hemolytic activity. However, contrasting findings have also been reported. For example, β-hemolysis was observed in seven out of twenty LAB isolates isolated from traditional North Sumatran foods [80], in two out of sixteen isolates isolated from traditional Daqu [81], and among isolates isolated from traditional Egyptian dairy products, where fourteen showed β-hemolysis and nine showed α-hemolysis out of a total of 157 isolates [82].

1.2.2. Technological characterization

LAB can be used as a bioprotective culture to increase safety, extend shelf life, improve texture, and contribute to better sensory profile of the final product [7,83]. Therefore, technological characterization is an important step in selecting LAB for incorporation into edible coatings, as it determines their contribution to product quality.

The main technological function of LAB is to convert carbohydrates into lactic acid, rapidly acidifying the food matrix [84]. In addition, LAB contribute

to flavor and texture development through proteolysis and lipolysis, which degrade proteins and fats, resulting in the release of peptides, amino acids, and FA [85,86]. Furthermore, some LAB produce EPS, which improves rheology, texture, viscosity, and colloidal stability of fermented foods [87–89].

Antimicrobial activity is another key technological trait. During fermentation, LAB produce organic acids such as formic acid, lactic acid, and acetic acid, which decrease the pH and thus inhibit the growth of foodborne pathogens [90,91]. Also, LAB produce antimicrobial compounds, including bacteriocins, which are characterized by high thermal stability and resistance to both acidic and alkaline conditions, allowing them to remain active in a wide range of environmental conditions [92]. An essential attribute of bacteriocins is the antimicrobial activity against Gram-positive bacteria by targeting the bacterial cell envelope [93,94]. Reported studies have demonstrated that bacteriocin nisin A produced by *Lactococcus lactis* inhibited *L. monocytogenes* [95], while novel bacteriocin (LSB1) produced by *Lactiplantibacillus* disrupted the cell integrity of *Streptococcus mutans* [96]. Similarly, a newly identified bacteriocin (BMP11) produced by *Companilactobacillus crustorum* has shown inhibitory activity against *L. monocytogenes* [97] thus contributing to food preservation and safety.

1.2.3. Probiotic characteristics

The probiotic properties of LAB are assessed by their ability to survive, persist, and exert beneficial effects in the gastrointestinal tract [98,99], with minimum viable cell counts of approximately 6 $\log_{10}$ CFU/g generally required to ensure efficacy according to FAO/WHO guidelines [100]. In particular, LAB exert beneficial effects through various mechanisms, including strengthening the intestinal barrier, inhibiting pathogens, maintaining the balance of intestinal microbiota, producing antimicrobial compounds, and modulating the immune system [101,102]. These effects have been demonstrated in several studies. For example, probiotic supplementation with *Lactobacillus acidophilus*, *L. plantarum*, and *L. rhamnosus* has been shown to promote colonization of the intestinal tract and increase short-chain fatty acid (SCFA) production through interactions with the gut microbiota [103]. Furthermore, *L. plantarum* and *L. acidophilus* strains have been associated with modulation of microbiota composition and immune function, supporting their role in maintaining host health [104,105].

A suitable and efficient probiotic must fulfill several functional criteria, such as resistance to low pH and bile salts [106], indicating the ability to survive during passage through the stomach and small intestine [107]. Different studies have shown that selected strains, particularly *L. acidophilus*,

Lactobacillus delbrueckii, *L. brevis*, and *Latilactobacillus sakei* can maintain viability after exposure to simulated gastric and pancreatic fluids [108,109].

Additionally, adhesion to intestinal epithelial cells is another important probiotic trait [110], as it promotes colonization capacity and prolongs residence time in the gut [111,112]. This ability is related with cell surface properties such as autoaggregation and hydrophobicity [113]. For instance, mixtures of LAB strains have been shown to exhibit enhanced adhesion to Caco-2 and HT-29 intestinal epithelial cells, indicating a synergistic effect [114]. Moreover, a LAB strain, *Lactobacillus* sp. has been reported to reduce the adhesion of *Salmonella* through antagonistic and competitive mechanisms, supporting its probiotic potential [115].

Certain LAB strains produce proteolytic enzymes that can degrade milk proteins, thereby reducing their allergenicity [116]. In addition, LAB can improve lactose digestion by producing β-galactosidase, which hydrolyzes lactose [117]. Therefore, individuals with lactose intolerance may tolerate LAB-fermented products better, and in some cases, they may have a lower allergenic potential [118]. These properties emphasize the importance of selecting LAB strains with strong probiotic potential for functional food applications.

1.3. Edible coatings and incorporation of LAB

Edible coatings are thin layers of edible materials applied to the surface of food products to improve their shelf life by reducing moisture transfer, gas exchange, and microbial contamination [119,120]. They have been studied as an alternative to conventional plastic packaging and as carriers of additives like antimicrobials and antioxidants to improve both functional and physicochemical properties [121].

Edible coatings and films are based on polysaccharides, proteins, lipids or composite [122]. Polysaccharide-based coatings, such as those derived from starch, chitosan, cellulose, alginate, pectin, and their blends, are widely used due to their good film-forming ability [123,124]. Such coatings generally provide limited resistance to water vapor and low extensibility [125]. Lipid-based edible coatings, including waxes, beeswax, parafilm, carnauba, and oils, are known for their ability to maintain flexibility, hydrophobicity, and cohesion, serving as an excellent barrier against moisture [126]. However, they exhibit poor homogeneity and mechanical strength and are often brittle and opaque [127]. For the preparation of functional edible coatings and films, the most utilized proteins are casein, gelatin, wheat gluten, soy protein, and zein [128]. These protein-based edible coatings can be incorporated into foods to impart nutritional value, but the hydrophilic nature of proteins results in poor

water barrier properties [129]. Composite coatings combining two or more classes of materials are often developed to overcome the limitations of single-component coatings. In general, combining materials with different properties (e.g., hydrophilic and hydrophobic) allows the integration of molecules with different attributes, resulting in coatings with improved functional properties [130].

The circular economy approach, promoted by the European Union, encourages the use of by-products from the food industry as sources of biopolymers to form edible coatings and reduce waste [121]. Fruit and vegetable by-products, including peels, seeds, pomace, and leaves, are rich sources of biopolymers such as cellulose, starch, pectin, and plant proteins suitable for forming edible coatings [131]. In addition to plant-based by-products, the dairy industry produces large quantities of by-products such as whey and acid whey. Acid whey, produced in large quantities during the acid coagulation process of cheese production, has traditionally been considered a low-value by-product. However, recent research has shown that acid whey is a rich source of nutrients, including carbohydrates, proteins, isoflavones, and micronutrients [132], and can be used as a coating material or as a component in edible coating formulations. Studies have shown that whey proteins are a promising material for the production of edible coatings due to their good tensile strength, oxygen barrier properties, moderate elongation, and ability to reduce moisture loss, thereby preserving the sensory properties of food products [133]. When combined with plasticizers or other bioactive additives (e.g., essential oils, antimicrobial agents, and LAB), low-cost whey protein-based coatings can exhibit improved functional properties [134].

The functionality of edible coatings can be improved by the incorporation of antimicrobial compounds, including plant extracts, essential oils, bacteriocins, low molecular weight peptides, enzymes, organic acids, and microbial cultures [135]. In particular, LAB through the production of organic acids, ethanol, FA, hydrogen peroxide, diacetyl, antifungal compounds, bacteriocins (enterocin, pediocin, nisin, and lacticin) can act as biopreservatives and stop the growth of undesired microorganisms [24]. Several authors reported inhibitory effects of edible coatings with incorporated LAB against molds in strawberries [136], *Listeria innocua* in blueberries [137], *Staphylococcus* spp., *Pseudomonas* spp., *Enterobacteriaceae* and fungi in sliced ham [138], and *L. monocytogenes* in smoked salmon and fresh cheese [139,140]. Collectively, the incorporation of LAB into edible coatings represents a promising natural preservation strategy for enhancing food safety.

In addition to their antimicrobial role, the incorporation of LAB can influence coating performance. While some studies reported that LAB do not affect the physicochemical and mechanical properties of films or

coatings [141,142], others have observed significant changes [143–145]. The composition of the edible coating matrix influences probiotic survival, and conversely, the viability of incorporated probiotics can influence the properties of the edible coating. Probiotic viability can be further enhanced by incorporating prebiotics into coatings and films, which may also enhance or plasticize their structure [146]. For example, the incorporation of *L. rhamnosus* into mucilage-based edible films derived from quince, flax, and basil improved moisture retention, thermal stability, elasticity, and probiotic viability [147]. Similarly, the addition of *Heyndrickxia coagulans* (formerly *Bacillus coagulans*) to pregelatinized starch-based films increased tensile strength and enhanced probiotic viability [148]. These findings indicate that interactions between LAB and coating components are formulation-dependent features and should be considered when developing edible coating for LAB incorporation to ensure functional performance and microbial viability.

1.3.1. Application of edible coatings on various food products

The effectiveness of edible coatings depends largely on the application method, as it influences coating uniformity, thickness, adhesion, and barrier properties [149]. Various application techniques have been described in the literature, such as dipping, spraying, vacuum impregnation, brushing, foaming, layer-by-layer deposition, fluidized bed coating, panning and dripping [150]. The selection of an application method depends on the properties of the food product, the coating formulation, and the intended functionality.

Among these techniques, dipping is one of the most commonly used methods for applying edible coatings. It involves immersion of the food product in the coating solution for a defined period, followed by draining and drying [151]. This method ensures complete surface coverage and is therefore effective for a wide range of food products. Its limitations include longer drying time and may result in thicker coating due to excess solution [152,153]. Edible coating solution can also be sprayed to the surface of food products using a spray nozzle [151]. This application technique enables the development of a thin film with uniform thickness and reduces drying time, as the sprayed solution evaporates directly from the surface [154]. The disadvantages of this application technique involves the risk of uneven coverage and requires equipment [150]. Research, in which the aim was to compare different coating application techniques on Mozzarella cheese, results showed that spraying and electrostatic spraying led to thinner film thickness whereas film structure were more homogeneous using dipping method [155]. Considering that dipping application techniques do not need any equipment and can be feasible in laboratory and industrial scale by modifying edible

coating solution viscosity to achieve lower thickness to obtain thinner films, it is considered a simple and cost-effective coating method.

1.4. Effects of edible coatings on dairy products

The microbiological quality is a critical factor determining the safety of dairy products [156], particularly cheeses with extended shelf life. Cheese surfaces are highly susceptible to microbial contamination due to favorable acidity conditions, high water activity (a_w), and moisture content [157]. During prolonged storage, uncontrolled growth of spoilage and pathogenic microorganisms on rennet cheeses can lead to quality deterioration, economic losses, and potential safety concerns [158]. Therefore, effective control of product quality and safety is important in cheese production.

Edible coatings have been investigated as a method to improve the stability of dairy products by forming a protective layer on the surface of the product [159,160]. In this context, when incorporating antimicrobial components such as LAB, coatings may influence microbial populations in two directions: reducing the undesirable microorganisms including spoilage bacteria, yeasts, and molds, while simultaneously serving a carriers for beneficial microorganisms such as incorporated LAB [24,161]. For instance, whey-based antimicrobial coatings were reported to inhibit yeast and mold growth, while maintaining the viability of incorporated *L. paracasei* and *L. rhamnosus* until the end of the cheese ripening period [25].

Beyond microbiological effects, edible coatings can also influence physicochemical and sensory attributes of coated dairy products. Researchers have reported that immobilized probiotics in coated cheese enhanced biochemical activity during ripening, leading to higher content of medium- and long-chain free FA, acetaldehyde, and diacetyl than the control cheese [162]. Similarly, edible coatings containing *B. animalis* subsp. *lactis* effectively reduced weight loss and maintained the softness and color characteristics of the sliced processed cheese during storage [163]. Overall, it can be stated that edible coatings with incorporated LAB can influence cheese quality and safety due to both physicochemical and microbiological effects.

1.4.1. Control of spoilage and pathogenic microorganisms

Within the diverse microbial community, molds are ubiquitous contaminants that can colonize cheese surfaces at any stage of production and contribute to spoilage [164]. Mold populations in traditional cheeses are highly diverse and vary greatly between cheese types; the predominant genera are *Penicillium* and *Mucor*, while *Cladosporium*, *Aspergillus* and *Fusarium* are found less frequently [165]. Most of these molds can grow at low temperatures, low a_w,

low pH, and low oxygen conditions [166]. Fungal species in dairy products can cause spoilage, characterized by visible surface growth, off-flavors, color and texture changes [167]. Furthermore, molds, such as *Penicillium* and *Aspergillus* genera may produce mycotoxins including ochratoxin A, cyclopiazonic acid and sterigmatocystin [164].

In addition to spoilage fungi, pathogenic bacteria represent a critical safety concern in cheese production [168]. Foodborne pathogens commonly associated with cheese include *L. monocytogenes*, characterized by psychrotrophic nature, *S. aureus*, known for enterotoxin production and osmotolerance, Shiga toxin-producing *Escherichia coli*, associated with intestinal complications, and *Salmonella* spp., which can cause gastroenteritis [169]. Of these, *L. monocytogenes* is particularly important due to its ability to persist and grow under refrigerated storage in cheeses [170]. Although pathogenic bacteria are generally less prevalent than spoilage microorganisms [18,167], their presence poses a risk to public health and requires effective control during cheese ripening, storage, and distribution.

In response to these microbiological challenges, edible coatings have been studied as surface treatments to reduce the growth and persistence of spoilage fungi and pathogenic bacteria, while reducing the need for chemical additives without affecting safety [25]. Bioprotective LAB cultures have been studied and showed their inhibitory activity in cheese against pathogens like *B. cereus* [38], *L. monocytogenes* [38,171], *S. aureus* [172,173], *E. coli* [174], *Brucella abortus* [175], as well as fungal species such as *Penicillium commune, Mucor racemosus, Rhodotorula mucilaginosa* [176], *Penicillium chrysogenum*, and *Aspergillus parasiticus* [172]. Although LAB have been widely studied in cheese and shown to enhance microbiological stability, relatively few studies have focused on edible coatings where LAB are used as the only antimicrobial agents against spoilage fungi and pathogenic bacteria. Available research indicates that LAB-incorporated edible coatings can inhibit specific microorganisms, including *Penicillium nordicum* [177], *Candida albicans* [178], *L. monocytogenes* [140] in cheese, supporting the relevance of LAB-based edible coatings for cheese safety.

1.4.2. Survival and persistence of probiotic LAB

The survival of probiotic LAB in cheese is essential for their technological and functional effectiveness [179]. Cheese is considered a suitable carrier for probiotic LAB due to its relatively high buffering capacity, dense protein and fat matrix, compact structure, and nutrient-rich composition that can protect bacterial cells during manufacturing and storage [180,181]. Nevertheless, several studies have shown that the viability of probiotics in food products,

including cheese, depends on multiple factors, including strain-specific characteristics, cheese composition (pH, organic acid content, fat and moisture content), storage conditions, and the method of probiotic incorporation [182–184].

Various technological approaches, including microencapsulation, have been investigated to improve the viability of probiotics in food [185]. The main encapsulation processes for probiotics are freeze-drying, spray-drying, ionic gelation, complex coacervation, electrospraying, and electrospinning [186]. Encapsulation has been shown to increase the viability of encapsulated probiotics through *in vitro* digestion [187]. However, the microencapsulation phase of probiotics adds additional cost to food processing [188]. Therefore, alternative approaches that are more cost-effective and technologically efficient are of interest.

Edible coatings represent an alternative for delivering probiotic LAB to the cheese surface. When LAB are incorporated into edible coatings, they are applied to the surface of the product, where microbial interaction and contamination primarily occur [189]. Specific cheese microbes are adapted to a range of abiotic stresses, including fluctuations in pH, salinity, temperature, and moisture, as well as biotic stresses such as microbial competition and invasion resistance, both at the individual and community levels [190]. In contrast, when LAB are deposited on the surface of cheese, they are exposed to environmental stresses, including oxygen, reduced moisture, and temperature fluctuations, which may reduce viability over time [20]. As a result, the use of proper carriers, such as an edible coating, is necessary to support LAB survival.

Survival in the gastrointestinal tract represents an additional challenge for probiotic LAB [100]. Losses in bacterial viability commonly occur during digestion due to gastric acidity and the presence of bile salts [191]. Previous studies have shown that probiotic survival in edible coatings on cheese surfaces and during simulated digestion was improved when edible coatings were supplemented with prebiotics [192,193]. The use of prebiotics in the edible coatings can improve the survival of probiotic bacteria by providing nutritional support and protection against environmental stress [146]. Nevertheless, this strategy relies on additional functional ingredients, and evidence on the survival of LAB in coatings without additional protective ingredients remains limited.

2. MATERIALS AND METHODS

2.1. Investigation venue and study design

The experiments were conducted between 2022 and 2026 at the Lithuanian University of Health Sciences Veterinary Academy Department of Food Safety and Quality, University of Minho, Centre of Biological Engineering, Latvia University of Life Sciences and Technologies, Faculty of Food Technology, Food Institute, BioCC OÜ Biocompetence Centre (Estonia), Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima).

The overall structure of the study is presented in Fig. 2.1.1.

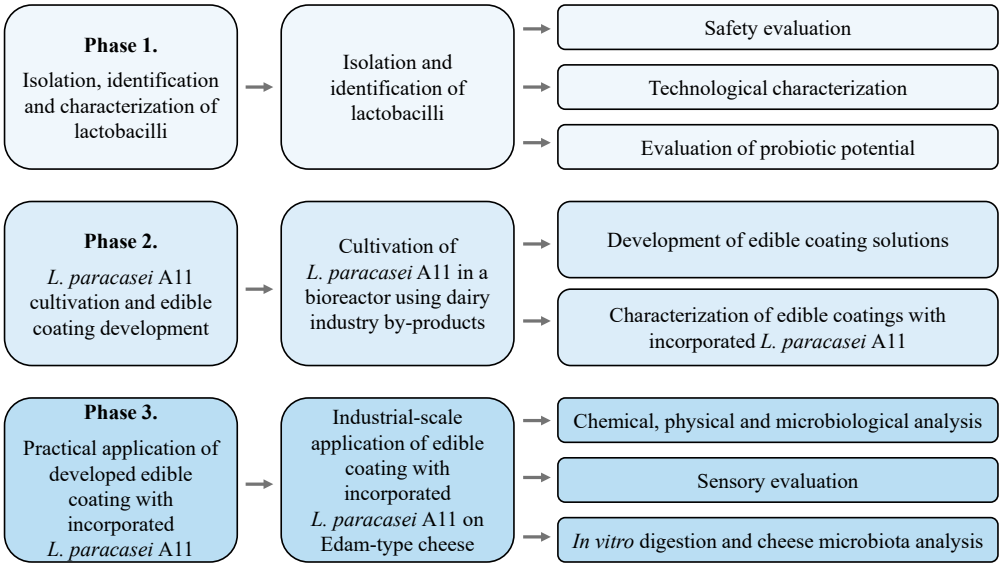


Fig. 2.1.1. *The design of the study*

2.2. Materials

Fresh organic cow's milk samples were collected from local organic farm and fermented using traditional methods for the isolation of LAB.

The microorganisms used in the antibacterial activity study were *Listeria monocytogenes* ATCC 35152, *Staphylococcus aureus* ATCC 9144, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* NCTC 6750, *Bacillus cereus* ATCC 11778, *Salmonella* Typhimurium ATCC 13311, *Pseudomonas fluorescens* ATCC 13525, *Brochothrix thermosphacta* ATCC 11509, which

were obtained from the laboratory collection of the Food Safety and Quality Department at the Lithuanian University of Health Sciences.

The microorganisms used in the antifungal activity assessment were yeasts *Debaryomyces hansenii* (BTL, M-31/Deb. 4 (T)), *Yarrowia lipolytica* (BTL, M-48/C.6.1), *Candida glabrata* (BTL, 8B), *Candida albicans* (BTL, M-7/C.17) and moulds *Mucor racemosus* (BTL, G-134/1064), *Cladosporium herbarum* (BTL, G-86/KŠ-2/7), *Penicillium commune* ML 21-3, *Aspergillus versicolor* (CBS 113090), which were obtained from the Nature Research Centre, Institute of Botany, Vilnius, Lithuania.

LAWPC and LAWP were obtained from the Lithuanian dairy company Pieno žvaigždės.

The film-forming solutions were prepared using glycerol (99% purity) as a plasticizer, Tween 80 as a surfactant, and sunflower (*Helianthus annuus*) seed oil as the lipid component, all supplied by Sigma-Aldrich (Darmstadt, Germany). Sugar beet pectin (54% degree of esterification), used as a thickening and emulsifying agent, was obtained from Herbstreith & Fox (Neuenbürg, Germany).

Fresh cheese samples were collected from the Estonian cheese and butter producer E-Piim.

2.3. Methods

2.3.1. Isolation and identification of LAB

Isolation of LAB. Fresh organic cow's milk samples were fermented at 37 °C for 96 hours and subsequently digested using the harmonized INFOGEST *in vitro* digestion method, which is suitable for food [194]. This method was selected as a potential approach for isolating lactobacilli with potential probiotic properties for incorporation into protective edible coatings. The model mimics the oral, gastric, and small intestinal digestion using simulated digestive fluids and enzymes, with standardized parameters. All enzymes and bile were purchased from Sigma-Aldrich, St. Louis, USA. Samples were subjected to the oral phase, and the digestion was performed in duplicates per sample. An aliquot of the final small intestinal phase was plated onto de Man, Rogosa and Sharpe (MRS) agar (Biolife, Milan, Italy), followed by incubation at 37 °C anaerobically. After incubation, representative colonies from each sample were selected and purified by several transfers on MRS agar. Pure colonies were selected and subjected to Gram staining test. Gram-positive LAB colonies were stored at –80 °C in MRS broth (Biolife, Milano, Italy) in the presence of 30% glycerol until further analysis. Prior to further

experiments, the strains were revitalized on MRS agar by growing for 48 h at 37 °C anaerobically.

Identification of lactobacilli strains. DNA was extracted using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) according to the manufacturer's instructions for Gram-positive bacteria. The polymerase chain reaction (PCR) assays were applied to identify *Lactobacillus* species. The primers used for species identification are summarized in Table 2.3.1.1. The PCR primers and conditions followed those previously described by Torriani et al. (2001) [195], Ventura et al. (2003) [196] and Gaspar et al. (2019) [197].

PCR products were separated on a 2% agarose gel using a 100 bp DNA ladder (Thermo Fisher Scientific, Vilnius, Lithuania) as the molecular weight marker and visualized by ethidium bromide staining (Sigma-Aldrich).

Table 2.3.1.1. *Primers used in this study for Lactobacillus species identification*

Primer	Sequence (5'→3')	Size (bp)	Reference
paraF	GTCACAGGCATTACGAAAAC	107	Torriani et al. (2001) [195]
pREV	TCGGGATTACCAAACATCAC		
pentF	CAGTGGCGCGGTTGATATC	218	
pREV	TCGGGATTACCAAACATCAC		
planF	CCGTTTATGCGGAACACCTA	318	
pREV	TCGGGATTACCAAACATCAC		
RHA	GCGTCAGGTTGGTGTTG	520	Ventura et al. (2003) [196]
CPR R	CAANTGGATNGAACCTGGCTTT		
PAR F	GACGGTTAAGATTGGTGAC	240	
CPR R	CAANTGGATNGAACCTGGCTTT		
LfermF	TAGGTGGTGGTGGTCACAGT	814	Gaspar et al. (2019) [197]
LfermR	GCCCGGTGGTTAACCTTCAA		
LacidF	CGTGATAGGGCCATTTGTGC	646	
LacidR	ACAAGCGTAAAACTGCGCTA		
LreutF	CCATGCAGTCACAACAACCT	386	
LreutR	GTCCCCGGTACATGTGTGAA		
LcaseF	TGGCATTGTCGGCTTAAATGG	268	
LcaseR	TGATGTTAAATGCTCAACCCGC		
LgassF	CAACGGAACCCCGCGACTATGC	219	
LgassR	GGCAGCATCTAAAACGCCAAGAC		
LrhamF	ATTTAACCGCAAGTGGCAGC	124	
LrhamR	AAATTGTGTGAACCGGCGTA		

2.3.2. Methods of evaluating safety characteristics of lactobacilli strains

Antibiotic susceptibility. Following the manufacturer's instructions, Minimum Inhibitory Concentrations (MIC) Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) were used to measure antibiotic susceptibility on Mueller-Hinton agar (Oxoid, Basingstoke, UK). The experiment was repeated using Mueller-Hinton agar supplemented with 10% MRS agar (Biolife, Milan, Italy) because certain strains did not grow on Mueller-Hinton agar. The antibiotics tested included chloramphenicol, clindamycin, streptomycin, gentamicin, tetracycline, erythromycin, ampicillin, vancomycin, and kanamycin. The MIC reading scale was used to calculate the MIC, which was stated in μg/mL. Isolates were designated as resistant and susceptible following the breakpoints given by EFSA [198].

Hemolytic activity. Hemolytic activity of the tested strains was assessed on blood agar plates that contained 5% sheep blood (E & O Laboratories Ltd., Bonnybridge, UK). The measurements were performed after 48 h of incubation at 37 °C and recorded as β-hemolysis, α-hemolysis and γ-hemolysis represented as clear zones, green zones, or no hemolysis around the colonies [199].

Enzymatic activity. The enzymatic activity of each selected strain was evaluated using the API ZYM kit (bioMérieux, Marcy-l'Étoile, France). The assay was performed according to Kondrotiene et al. (2020) [200], and the results were interpreted following the manufacturer's guidelines. Enzymatic activities were semi-quantitatively assessed based on the intensity of color development, rated on a scale from 0 to 5. A score of 0 indicated no activity, scores of 1 and 2 reflected weak activity (5 to <20 nmol of substrate hydrolyzed), while scores from 3 to 5 represented strong enzymatic activity (>20 nmol of substrate hydrolyzed).

2.3.3. Methods of evaluating technological aspects of *L. paracasei* A11

Extracellular proteolytic activity. Skim milk agar was used to evaluate the extracellular proteolytic activity of the strain. A 2 μL aliquot of revitalized culture was spotted onto skim milk agar containing 20% sterile skim milk and 80% water agar. After incubation at 30 °C for 4 days, the appearance of clear zones around the colonies indicated proteolytic activity.

Caseinolytic and lipolytic activities. Caseinolytic and lipolytic activities were assessed following the methods of Pisano et al. (2015) [201] with minor modifications. Caseinolytic activity was evaluated on PCA (Liofilchem, Roseto degli Abruzzi, Italy) supplemented with 10% sterile reconstituted skim milk (Oxoid, Basingstoke, UK); after 18 h of incubation at 30 °C under

anaerobic conditions, clear zones around the colonies indicated caseinolytic activity. Lipolytic activity was tested on MRS agar (Biolife, Milan, Italy) containing 0.01% calcium chloride (Sigma-Aldrich, Darmstadt, Germany) and 0.1% Tween 80 (Liofilchem, Roseto degli Abruzzi, Italy), where lipolytic colonies produced cloudy halos.

Acidifying activity. To assess acidification, UHT low-fat milk (1.5%) was inoculated with 1% revitalized cultures, and pH values were recorded after 6 and 24 hours. Non-inoculated milk served as the control.

Carbohydrate metabolism. Carbohydrate fermentation profile of *L. paracasei* A11 was evaluated using the API 50 CH test (BioMerieux, Marcy-l'Etoile, France) and following manufacturer's instructions. During incubation, carbohydrates were fermented to organic acids, resulting in a decrease in pH. This change was detected by a color shift of the indicator. The results were interpreted as follows: strong fermentation (yellow), moderate fermentation (green), weak fermentation (dark green), and no fermentation (blue).

Antibacterial and antifungal activity of *L. paracasei* A11 strain. The agar spot test was used to evaluate the antibacterial and antifungal activity of *Lacticaseibacillus paracasei* A11 strain. The revitalized strain (3 μL) was spotted onto the surface of MRS agar (Biolife, Milan, Italy) and incubated anaerobically at 37 °C for 48 hours. After incubation, the plates were overlaid with 7 mL of soft agar (0.7%) containing 100 μL of an indicator food spoilage microorganisms or pathogenic bacteria suspension and further incubated for 24 hours aerobically at temperature optimal for the indicator strain. For antifungal assessment, plates were overlaid with 5 mL SDA (Oxoid, Basingstoke, UK) containing 10^5 mold spores or yeast cells per mL, followed by incubation for 24 hours aerobically under appropriate temperature for fungal growth. The list of microorganisms used is provided in Table 2.3.3.1. The diameter of the clear inhibition zone surrounding the tested strain colonies was used to measure the antibacterial and antifungal activity.

Table 2.3.3.1. *Food spoilage microorganisms, pathogenic bacteria, molds, and yeasts used in the study*

Strains	Growth media	Incubation temperature (°C)
Debaryomyces hansenii (BTL, M-31/Deb. 4 (T))	SDA	25
Yarrowia lipolytica (BTL, M-48/C.6.1)	SDA	25
Candida glabrata (BTL, 8B)	SDA	25
Candida albicans (BTL, M-7/C.17)	SDA	25
Mucor racemosus (BTL, G-134/1064)	SDA	25
Cladosporium herbarum (BTL, G-86/KŠ-2/7)	SDA	25
Penicillium commune ML 21-3	SDA	25
Aspergillus versicolor (CBS 113090)	SDA	25
Listeria monocytogenes ATCC 7644	BHI	37
Staphylococcus aureus ATCC 25923	BHI	37
Escherichia coli ATCC 25922	BHI	37
Pseudomonas aeruginosa ATCC 27853	BHI	37
Bacillus cereus ATCC 11778	BHI	30
Salmonella Typhimurium ATCC 14028	BHI	37
Pseudomonas fluorescens ATCC 13525	BHI	30
Brochothrix thermosphacta ATCC 11509	BHI	25

ATCC – American Type Culture Collection; SDA – Sabouraud Dextrose Agar; BHI – Brain Heart Infusion medium.

2.3.4. Assessment of *L. paracasei* A11 probiotic potential

Acid and bile salt resistance. For the acid tolerance assay, *L. paracasei* A11 were incubated in MRS broth (Oxoid, Basingstoke, UK) at 37 °C for 18 hours. Subsequently, 1 mL of the culture was inoculated into 9 mL of PBS (Biolife, Milan, Italy) (pH 2.5), adjusted with 5 M HCl, and incubated at 37 °C. Viable cell counts were determined at 0 and 3 hours of incubation by plating on MRS agar (Biolife, Milan, Italy). All assays were performed in triplicate. For the bile salt tolerance assay, *L. paracasei* A11, which were grown in MRS broth at 37 °C for 18 hours, were transferred (1 mL) into 9 mL of MRS broth supplemented with 0.3%, 0.5%, and 1% (w/v) bile salts and incubated at 37 °C. Viable counts were assessed at 0 and 24 hours on MRS agar plates. Each experiment was conducted in triplicate [202].

2.3.5. Cultivation of *L. paracasei* A11 strain

L. paracasei A11 was cultivated in LAWPC, LAWP, and MRS broth (Oxoid, Basingstoke, UK) as control. The strain was grown in flasks and in real-time bioreactor (RTS-1C, Biosan, Riga, Latvia) to evaluate biomass

yield and growth kinetics, with cell growth monitored in real time following the manufacturer's instructions. The strain was initially cultured on MRS agar at 37 °C for 48 hours under anaerobic conditions. LAWPC and LAWP media were pasteurized at 93–95 °C for 10 minutes. After cooling to 37 °C, 29 mL of each medium and 1 mL of an *L. paracasei* A11 suspension (adjusted to 1 McFarland unit) were placed in 30 mL TubeSpin vessels. The cultures were incubated in a bioreactor at 37 °C for 96 hours under aerobic conditions. Optical density at 600 nm was recorded every 10 minutes at 110 rpm, and the growth rate was calculated automatically. After the cultivation in flasks, the number of viable bacteria was determined by plating on MRS agar and incubating anaerobically at 37 °C for 0, 24, and 48 hours.

The pH of each sample was measured directly with a pH meter (Sartorius Professional meter for pH Measurement, Germany).

Analysis of sugar content in dairy by-products following cultivation of *L. paracasei* A11. The sugar content in the fermentates was analyzed in triplicate using liquid chromatography for specific sugars according to ISO 22184:2021 [203], and an enzymatic method to determine lactic acid and lactate levels according to ISO 8069:2005 [204].

2.3.6. The preparation of edible coatings

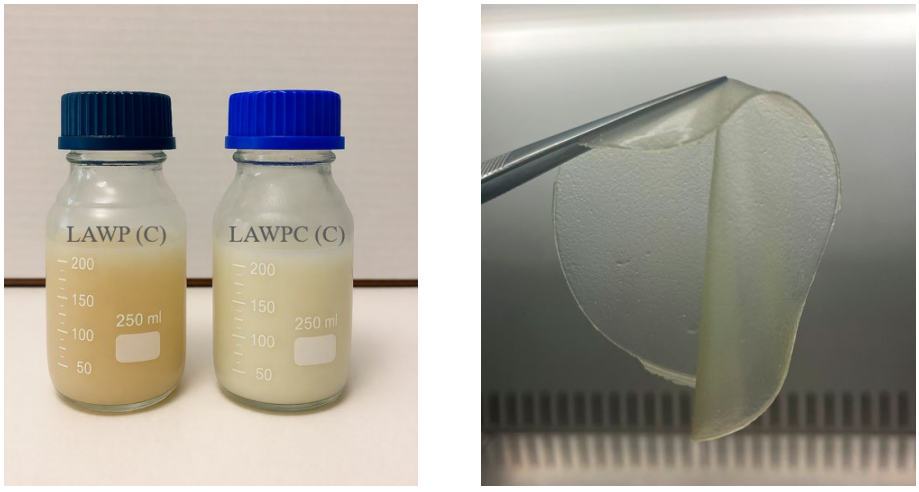
The development of edible coatings solutions. Four different coating solutions are presented in Table 2.3.6.1. The dry components (sugar beet pectin, Tween 80, sunflower oil, and glycerol) were dissolved in 50% of LAWPC or LAWP required in the recipe to prepare the stock coating solution. The mixture was agitated for 3 min at 10,000 rpm, thermally processed for 10 min at 93 to 95 °C in a water bath and then cooled to 35 °C. The strain *L. paracasei* A11 was cultivated in the portion of pasteurized LAWPC. The LAWPC cultivate containing 8.3 $\log_{10}$ CFU/g were added to the stock solution and thoroughly mixed to incorporate the strain into the experimental film-forming solutions at a final concentration of approximately 7 $\log_{10}$ CFU/g which were measured using a photometer (DEN-600, Biosan, Riga, Latvia). The stock solution was supplemented with half of the required LAWPC amount, excluding the strains to produce control film-forming solutions.

Table 2.3.6.1. *The composition of four different coating solutions*

Coating formulations	Pectin (% w/w)	Sunflower oil (% v/v)	Glycerol (% v/v)	Tween 80 (% v/v)	LAWP (% w/w of total solution)	LAWPC (% w/w of total solution)
LAWPC (C)	2	2	5	0.2	–	90.8
LAWPC + L. p		2			–	90.8
LAWP (C)		1			50	41.8
LAWP + L. p		1			50	41.8

LAWPC – liquid acid whey protein concentrate; LAWP – liquid acid whey permeate; C – control; L. p – *Lacticaseibacillus paracasei* A11.

The preparation of edible films. Films were prepared by pouring 28 mL of the film-forming solution into 150-mm Petri dishes and drying them at 37 °C and 50% ± 5% relative humidity for 12 hours. The dried films were then stored for 21 days under refrigeration (4 ± 1 °C) to evaluate the survival of *L. paracasei* A11. The appearance of the coating solutions and the obtained dried films is presented in Fig. 2.3.6.1.



(a) (b)

Fig. 2.3.6.1. *Edible coating solutions (a) and dried edible films (b)*

2.3.7. Characterization of edible coating solutions and their films

The rheological measurements were conducted according to Vieira et al. (2021) [205] method. The rheological properties of the film-forming solutions were measured using an AR1500ex rheometer (TA Instruments, USA) equipped with a stainless-steel cone-plate geometry (6.0 cm diameter,

2° angle, 67 μm truncation). All measurements were performed at 25 °C. Flow curves were obtained across a range of shear stresses using an up-down-up step program, covering a shear rate from 0 to 300 s^{-1}. The experimental data were fitted to the Newtonian (Eq. (1)) and power-law (Eq. (2)) models to characterize the rheological behavior of the film-forming solutions.

$$\sigma = \eta \times \gamma \tag{1}$$

$$\sigma = k \times \gamma^{n} \tag{2}$$

where σ is the shear stress (Pa), η is the viscosity (Pa.s), k is the consistency index (Pa.s^n), γ˙ is the shear rate (s^{-1}) and n is the flow index.

The color of the film samples was evaluated using a Minolta CR-400 colorimeter (Konica Minolta, Japan) following the CIE 1976 L*, a*, b* color system. In this method, the L* value represents lightness (ranging from 0 for black to 100 for white), a* indicates the red-yellow axis, and b* the blue-green axis. A white standard plate (L* = 97.6, a* = 0.01, b* = 1.60) was used as the background during measurements. Color measurements were performed in triplicate per film sample.

Film opacity was assessed using the Hunter Lab method. Opacity measurements were performed in triplicate for each film and calculated according to Equation (3):

$$Opacity(\%) = \left(Y_b / Y_w\right) \times 100 \tag{3}$$

where Y_b and Y_w were the opacity of the samples on black and white standard plates, respectively [206].

A handheld digital micrometer (Mitutoyo, Japan) with a sensitivity of 0.001 mm was employed to measure the films' thickness. For each sample, ten thickness measurements were made at several randomly chosen locations. The mean results were then used to calculate other film features, such as mechanical and barrier properties.

Mechanical attributes: tensile strength (TS) and elongation-at-break (EAB). An Instron Universal Testing Machine (Model 4500, Instron Corporation, USA) was used to measure TS and EAB in accordance with ASTM Standard Method D 882-91 guidelines. The initial grip separation was set at 30 mm, and the crosshead speed was set at 5 mm/min. TS was calculated and expressed in MPa by dividing the maximum load (N) by the initial cross-sectional area (m2) of the sample. By dividing the length of the extended specimen by its initial length (30 mm), EAB was calculated as a percentage. Five duplicates of the TS and EAB tests were conducted for all types of film.

The films were assessed for moisture content and water solubility in accordance with Ortiz de Elguea-Culebras et al. (2019) [207]. Gravimetric analysis based on weight differences was used to determine the moisture content. Film circles with a diameter of 2 cm were weighed first, dried for 24 hours at 105 °C, and then weighed once again to determine the moisture content. Equation (4) was used to determine the moisture content of each film in triplicate, as shown below:

$$Moisture\ content\ (\%) = \left(\left(M_0 - M \right) / M_0 \right) \times 100 \quad (4)$$

where M_0 and M were the film sample mass before and after drying, respectively. To determine the water solubility, the dried film samples were placed in beakers with 50 mL of distilled water and agitated for 24 hours at 150 rpm. The insolubilized films were then weighed, dried for 24 hours at 105 °C, and their solubility was assessed in triplicate using equation (5):

$$Solubility\ (\%) = \left(\left(S_0 - S \right) / S_0 \right) \times 100 \quad (5)$$

where S_0 was the film's initial dry mass and S represented the film insoluble dry mass.

The ability of water to permeate the film was measured using gravimetric analysis in accordance with the Standard Test Methods for Water Vapor Transmission of Materials (ASTM E96/E96M-16, 2016). To increase air uniformity, desiccated film circles (45 mm in diameter) were placed inside a silica gel-filled desiccator with a fan after being sealed on top of cups filled with distilled water. To track weight loss, samples were weighed every two hours for ten hours. Water vapor permeability (WVP) (g s^{-1} m^{-1} Pa^{-1}) was measured in triplicate for each film sample and calculated using equation (6):

$$WVP = \left(WVTR \times L \right) / \Delta P \quad (6)$$

The linear regression of the weight loss (g m^{-2} s^{-1}) yielded the following values: $WVTR$ is the water vapor transmission rate, L is the film thickness (m), and ΔP is the water vapor partial pressure difference across the two sides of the film (2,337 Pa). It was determined that the area of the permeation film was 5.2×10^{-3} m^2.

The morphology of the prepared film samples was examined using scanning electron microscopy (SEM) with a "Mira3" Scanning Electron Microscope (Tescan Orsay Holding, a. s., Brno-Kohoutovice, Czech Republic). The films were manually cut into small pieces measuring 0.4 cm × 0.4 cm using a surgical blade and mounted onto a 51 mm silicon wafer (MicrotoNano, Haarlem, Netherlands). Silicon was chosen as the substrate due to the organic

composition of the samples, predominantly containing carbon, hydrogen, oxygen, and nitrogen (CHON), which helps improve the signal-to-noise ratio and minimizes interference from other elements. Observations were performed in high vacuum mode using a combination of Back-Scattered Electron (BSE) and Secondary Electron (SE) detectors. Film samples were analyzed at magnifications up to 1.0 kx for accurate dimensional and elemental analysis, with the electron beam operated at an acceleration voltage of 5 kV.

Elemental composition of the film samples was determined using Energy-Dispersive X-Ray Spectroscopy (EDS) with an energy-dispersive X-ray spectrometer equipped with an INCA x-act LN2-free Analytical Silicon Drift Detector with PentaFET® Precision (Oxford Instruments Inc., Bognor Regis, UK). Measurements were taken at a 15 mm working distance to collect energy spectra. Elemental mapping was performed on selected film areas (0.7 × 0.7 mm) to assess the spatial distribution of elements.

The viable counts of LAB in the coating solutions and films were assessed in triplicate using selective media. Measurements were taken on days 0 and 1 for the coating solutions, and on days 0, 1, 3, 7, 14, and 21 for the films during storage. Total mesophilic LAB were enumerated following the ISO 15214:1998 [208].

2.3.8. Industrial-scale application of edible coatings on Edam-type cheese

Surface application of edible coatings on Edam-type cheese was carried out based on our previous research, with some modifications [209]. Two types of unripened Edam-type cheese blocks (regular Edam-type cheese as the control, and probiotic cheese containing *Lactiplantibacillus plantarum* Tensia (DSM 21380) as an adjunct culture) were cut into uniform pieces weighing 100 ± 5 g in a sterile, controlled airflow box. Six sets of cheese samples were prepared in triplicate: regular cheese control (no coating), regular cheese with plain LAWPC coating, regular cheese with LAWPC coating containing *L. paracasei* A11, probiotic cheese control (no coating), probiotic cheese with plain LAWPC coating, and probiotic cheese with LAWPC coating containing *L. paracasei* A11. The pre-cut cheese pieces were immersed in the coating solution, allowed to dry for one hour, and then vacuum-packed in bags. The appearance of coated cheese samples during the drying stage is shown in Fig. 2.3.8.1. The bags were sealed using a vacuum sealer (Status d.o.o., Metlika, Slovenia). The samples were stored in a ripening chamber at 12 °C at 80% relative humidity (φ) for 45 days, followed by refrigeration at 4 °C for an additional 90 days. Because different milk batches were used, regular and probiotic cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them.

Fig. 2.3.8.1. *Edam-type cheese samples during drying after coating application*

The pH was determined at room temperature using a calibrated benchtop Mettler Toledo JENWAY3510 pH meter (Barloworld Scientific Ltd., Essex, UK). A 10 g cheese sample was homogenized with 30 mL of distilled water (Lawson Scientific, Ningbo, China) and analyzed immediately.

A_w was determined using a LabSwift-aw water activity meter (Novasina AG, Lachen, Switzerland). The cheese was ground and promptly placed onto a measurement plate for analysis.

Dry matter (DM) content determination in samples was performed according to prescribed method ISO 5534:2004 [210].

The color of cheese samples was measured using a using a Minolta CR-400 colorimeter (Konica Minolta CR-400, Osaka, Japan) and expressed in CIE L*a*b* color coordinates, as well as total color difference (TCD, ΔE). L* represents lightness (0 = black, 100 = white), a* represents redness–greenness, and b* represents yellowness–blueness. Measurements were taken in triplicate, and ΔE was calculated using the following equation: [211]:

$$\Delta E^* = \sqrt{\left(L^* - L_0\right)^2 + \left(a^* - a_0\right)^2 + \left(b^* - b_0\right)^2} \tag{7}$$

where L_0, a_0 and b_0 are the initial lightness, redness and yellowness, respectively; L^*, a^* and b^* are the lightness, redness and yellowness at time t, respectively.

Microbiological analysis was conducted in triplicate to determine the viable counts of microorganisms typically present in this type of cheese. Samples were analyzed on days 1, 45, and 135 of storage using selective media for each microorganism. The microbial counts were measured both on the surface ($\log_{10}$ CFU/g) and inside ($\log_{10}$ CFU/g) the cheese sample. The surface area of the samples was measured; total area was calculated and swabbed. Enumeration followed standardized methods: total bacteria count (TBC) according to ISO 4833-1:2013 [212], total mesophilic LAB count according to ISO 15214:1998 [208], enterobacteria count according to ISO 21528-2:2017 [213], and yeast and mold count according to ISO 6611:2004 [214]. Samples from the surface and from the cheese matrix were collected according to ISO 18593:2018 [215].

To evaluate the concentrations of FA in cheese samples, one gram of cheese was mixed in 2 mL of type III water and homogenized. The sample was kept at room temperature for 1 hour during occasional shaking, centrifuged for 6 min at 4000 × g at room temperature. To 1 mL of supernatant 100 µL of 0.6 M oxalic acid solution was added to the supernatant, shaken and centrifuged. FA were determined by gas chromatograph Agilent 6890A, capillary column CP-Wax 52 CB (30 m x 0.25 mm, 0.25 µm), flame ionization detector at 280 °C. The oven temperature program was 175 °C 1 min, increased by 10 °C/min to 115 °C, and held for 3 min, increased by 20 °C/min to 190 °C, and held for 5 min. Each FA was expressed in g/100 g of total FA content.

FA were classified according to chain saturation and number of double bonds into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), long-chain fatty acids (LCFA), medium-chain fatty acids (MCFA), short-chain fatty acids (SCFA), omega-6 polyunsaturated fatty acids (omega-6), omega-3 polyunsaturated fatty acids (omega-3), and trans FA. Lipid quality indices (LQI) related to atherogenicity, thrombogenicity, hypocholesterolemic and hypercholesterolemic ratio (h/H), and desirable FA content were calculated as described by [216,217] using provided equations:

$$Index\ of\ atherogenicity\left(AI\right)=\frac{\left(C12:0+\left(4\,xC14:0\right)+C16:0\right)}{\left(PUFA+MUFA\right)} \tag{8}$$

$$Index\ of\ thrombogenicity\left(TI\right)=$$

$$=\frac{\left(C12:0+C16:0+C18:0\right)}{\left(0.5\,xMUFA\right)+\left(0.5\,xn6PUFA\right)+\left(3\,xn3PUFA\right)+\left(n3PUFA/n6PUFA\right)} \tag{9}$$

$$h/H = \frac{(C18:1 + PUFA)}{(C14:0 + C16:0)} \quad (10)$$

$$Desirable\ fatty\ acids\ (DFA) = UFA + C18:0 \quad (11)$$

Following cheese ripening (day 45), sensory analysis was carried out. Seven trained panel members (of both sexes, aged 20 to 50) participated in the evaluation, which was conducted in a sensory room. The panelists were chosen and instructed in accordance with ISO 8586:2023 [218]. Samples were served at room temperature and coded with three-digit randomized numbers prior to evaluation. The panel members were randomly given identical plastic plates containing six 2 × 2 cm blocks of cheese. With a maximum score of 100 points, the sensory evaluation scorecard, which was based on Bodyfelt et al. (1988) [219], gave 50 points for flavor, 40 points for body and texture, and 10 points for appearance.

2.3.9. *In vitro* digestion of cheese samples

To evaluate the survival of the cheese-associated bacteria (total LAB, *L. paracasei* A11 and *L. plantarum* Tensia) during digestion, the same INFOGEST *in vitro* digestion method, as previously described, was employed in this experiment. The cheeses were grinded before subjected to the oral phase, and the digestion was performed in duplicates per cheese type, including blank control without cheese. An aliquot of the final small intestinal phase was plated onto MRS agar (pH 5.7) and MRS agar with mannitol instead of glucose (pH 5.7), followed by incubation at 30 °C anaerobically. MRS with mannitol was used to support growth of *L. paracasei* instead of the dominating lactococci, however, *L. paracasei* was still difficult to exactly enumerate on these agar plates due to other LAB. Verification of *L. paracasei* positive colonies was obtained with a Bruker MALDI-TOF MS. As a comparison, enumeration of the LAB in the cheese before digestion was determined in a similar manner but was prior to agar plating homogenized in buffered peptone water.

Protein digestibility of the cheeses was evaluated after cheese ripening (day 45) and completion of the intestinal phase, after which the enzymes were heat-inactivated in boiling water for 10 minutes, followed by separation into supernatant and pellet with centrifugation at 4000 rpm for 10 minutes. Protein concentration of the cheeses and after digestion (supernatant and pellet) was determined by the Dumas method, involving combustion of the samples with a Vario EL cube (Elementar), and described by Rieder et al. (2021) [220]. The supernatant from the intestinal phase was analyzed by size exclusion chromatography (SEC) to determine peptide size distribution as previously

described Wubshet et al. (2017) [221]. Calculation of protein digestibility was done as described by Rieder et al. (2021) [220], which was estimated based on the relative proportion of absorbable (soluble) small peptides from the total protein. In detail, the equation (8) includes both function of protein solubility (soluble/total protein) and extent of protein break (small peptides as SEC area 9.4 to 15 min/all peptides as SEC area 5 to 15 min):

$$\%Digestibility_{SEC}(D_{SEC})(\%) = \\ = \left(\frac{Soluble\ protein}{Total\ protein} \times \frac{SEC\ area\ 9.4\ \text{to}\ 15\ \text{min}}{SEC\ area\ 5\ \text{to}\ 15\ \text{min}} \right) \times 100 \quad (12)$$

Cheese microbiota was analyzed in cheese samples before digestion to assess the effects of time (D1–D45), coating, and *L. paracasei* A11 on both regular and probiotic cheese microbiota, and to determine the presence of *L. paracasei* and *L. plantarum*. Bacterial DNA was extracted from pellets obtained from the cheese homogenized in buffered peptone water using DNeasy Power Soil 96 Pro Kit (Qiagen), following the manufacturer's protocol. The microbiota was analyzed by 16S rRNA amplicon sequencing (2 × 150 bp) targeting the V4 region, as described by Caporaso et al. (2012) [222]. The sequencing was done on a MiSeq (Illumina) using pooled PCR samples, which were based on triplicate PCRs per DNA sample using sample-specific barcoded forward primers. PhiX Control v3 was included and accounted for 10% of the reads.

Data processing of the sequencing reads was performed using the open-source bioinformatics pipeline Quantitative Insight into Microbial Ecology 2 (QIIME2) version 2023.5 [223]. Briefly, the sequences were demultiplexed and paired ends joined, quality filtered and denoised using dada2, and taxonomy was determined using the Greengenes2 2022.10 [224] database, where each unique taxonomic feature represents an amplicon sequence variant (ASV). Beta diversity was performed for overall microbial community comparison, where PCoA from weighted unifrac was presented. Fasta sequences per unique taxonomic feature were extracted from QIIME2, as well as taxonomic summary tables. Overall, taxonomic summary table at genus level was used to present taxonomic data. Fasta sequences and corresponding ASV's likely represent *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum* were identified, with the feature ID's and fasta sequence. Searched against the NCBI blast16S rRNA ribosomal database (high similarity sequence) showed 100% identical sequences to *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*.

2.3.10. Statistical analysis

The SPSS statistical software was used for all data processing and analysis (SPSS 24, SPSS Inc., Armonk, NY, USA). Descriptive statistics were obtained using *Explore*. Depending on the experimental design, one-way or two-way analysis of variance (ANOVA) was applied to evaluate the effects of treatment factors and storage time, followed by Tukey's post hoc test. Paired *t*-tests were used for dependent measurements, and Pearson correlation analysis was performed where appropriate. The results were considered statistically significant at $p < 0.05$.

Microbiome sequencing data were analyzed in Python (version 3.12) using the libraries *pandas*, *NumPy*, *scikit-bio*, *scikit-learn*, and *matplotlib*. Differences in overall bacterial community structure were evaluated using permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity. Pairwise PERMANOVA comparisons were performed to assess differences between treatment groups within sampling days. Differences in the relative abundance of individual genera were analyzed using the Kruskal–Wallis test.

3. RESULTS

3.1. Isolation and identification of *Lactobacillus* strains

Local fresh organic cow's milk was fermented and subsequently digested *in vitro* using the INFOGEST protocol to isolate acid-tolerant, antimicrobial, potential probiotic *Lactobacillus* strains that could be used in edible acid whey-based coatings. As shown in Table 3.1.1, 20 samples were processed, from which 22 colonies were grown and subjected to further analysis. Of these, 12 colonies (55%) exhibited characteristics typical of *Lactobacillus*, and 4 colonies (18%) were confirmed as *Lactobacillus* spp. (3 – *Lacticaseibacillus paracasei*, 1 – *Lactiplantibacillus plantarum*) by molecular identification.

Table 3.1.1. *Isolation of Lactobacillus strains from in vitro digested fermented cow milk*

Sample name	Number of samples	Number of colonies grown	Number of colonies with *Lactobacillus* phenotype (%)	Number of colonies identified as *Lactobacillus* spp. (%)
In vitro digested fermented cow milk	20	22	12 (55)	4 (18)

3.2. Safety evaluation of isolated *Lactobacillus* strains

All isolated lactobacilli were assessed for hemolytic activity (Table 3.2.1), and each showed γ-hemolysis, meaning no breakdown of red blood cells. This is the pattern expected from safe, food-grade strains, since bacteria with α- or β-hemolytic activity are considered undesirable and unsuitable for use in foods [225,226].

Antibiotic resistance screening is essential for LAB isolates, as these bacteria may transfer resistance genes to pathogenic species. To manage this risk, EFSA (2012) established antibiotic cut-off values for various LAB, indicating the highest antibiotic concentration at which a strain should remain susceptible [198]. If a strain is able to grow above this threshold, it is classified as resistant. In the present study, all tested strains were sensitive to ampicillin, erythromycin, clindamycin, streptomycin, gentamicin, kanamycin, and tetracycline. In contrast, all strains demonstrated intrinsic resistance to vancomycin (>256 μg/mL). Additionally, two of the four isolates exceeded the cut-off values for chloramphenicol. Only *L. paracasei* A11 and *L. plantarum* A154-d1 remained within the acceptable range and were therefore selected for further investigation.

Table 3.2.1. *Hemolysis and antibiotic susceptibility (MIC, µg/mL) of Lactobacillus strains*

	Isolate			
	***L. paracasei* A161-1**	***L. paracasei* A11**	***L. paracasei* A173-2**	***L. plantarum* A154-d1**
Hemolysis	γ	γ	γ	γ
Ampicillin	1 (4)	0,75 (4)	0,75 (4)	0,125 (2)
Erythromycin	0,19 (1)	0,19 (1)	0,25 (1)	0,25 (1)
Clindamycin	0,50 (1)	0,64 (1)	0,75 (1)	0,75 (2)
Chloramphenicol	6 (4)	4 (4)	8 (4)	6 (8)
Streptomycin	24 (64)	12 (64)	24 (64)	8 (n.r.)
Gentamycin	4 (32)	6 (32)	6 (32)	3 (16)
Kanamycin	64 (64)	24 (64)	48 (64)	12 (64)
Vancomycin	>256 (n.r.)	>256 (n.r.)	>256 (n.r.)	>256 (n.r.)
Tetracycline	0,5 (4)	0,5 (4)	0,75 (4)	4 (32)

n. r. not required. Values above the breakpoint, that are indicated in parentheses, provided by EFSA.

Bacterial strains in the large intestine, including some lactobacilli, that show high β-glucuronidase or β-glucosidase activity may have negative effect in the colon [227,228]. To test the enzymatic activity of lactobacilli isolates API ZYM kit was used, and the results are presented in Table 3.2.2. Neither of the two tested lactobacilli exhibited enzymatic activity of alkaline phosphatase, lipase, trypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. In contrast, both isolates showed strong enzymatic reactions of leucine arylamidase and valine arylamidase. *L. paracasei* A11 also displayed a strong activity of α-glucosidase, whereas *L. plantarum* A154-d1 showed a strong β-glucosidase activity therefore, it was not used in further studies.

Table 3.2.2. *Enzymatic activity of selected strains*

Enzyme	**Lactobacilli isolates**	
	***L. paracasei* A11**	***L. plantarum* A154-d1**
Alkaline phosphatase	0	0
Esterase (C4)	2	0
Esterase lipase (C8)	1	0
Lipase (C14)	0	0
Leucine arylamidase	5	4
Valine arylamidase	5	3
Cystine arylamidase	1	0
Trypsin	0	0
α-chymotrypsin	1	0

Table 3.2.2 cont.

Enzyme	Lactobacilli isolates	
	***L. paracasei* A11**	***L. plantarum* A154-d1**
Acid phosphatase	2	0
Naphthol-AS-BI phosphohydrolase	3	2
α-galactosidase	0	0
β-galactosidase	0	3
β-glucuronidase	0	0
α-glucosidase	4	0
β-glucosidase	0	4
N-Acetyl-β-glucosaminidase	0	4
α-mannosidase	0	0
α-fucosidase	0	0

reaction grade 0 on the API-ZYM test scale was interpreted to correspond to a negative reaction, grades 1 and 2 corresponded to a weak reaction, grades 3, 4 and 5 corresponded to a strong reaction.

3.3. Technological aspects of *L. paracasei* A11

The primary technological properties of *L. paracasei* A11 were examined to determine the potential of the isolated strains for application in the dairy industry (Table 3.3.1). *L. paracasei* A11 showed positive caseinolytic activity, indicating its ability to hydrolyze milk proteins (casein) into smaller peptides and amino acids [229], while no extracellular proteolytic or lipolytic activity was detected. The strain exhibited moderate acidifying activity after 6 h and moderate-to-high acidifying activity after 24 h, decreasing the pH by 1.48 ± 0.01 and 2.46 ± 0.01 units, respectively.

Table 3.3.1. *Technological characteristics of L. paracasei A11*

Strain	Extracellular proteolytic activity	Caseinolytic activity	Lipolytic activity	Acidifying activity	
				pH (6 h)	pH (24 h)
***L. paracasei* A11**	-	+	-	1.48 ± 0.01	2.46 ± 0.01

values presented are means of three replicates ± SD.

Another important technological aspect, the carbohydrate fermentation profile of *L. paracasei* A11 revealed a selective ability to utilize a range of carbohydrates (Table 3.3.2). The strain showed positive fermentation of several monosaccharides, including D-ribose, galactose, glucose, fructose, mannose, and mannitol. Among disaccharides, positive reactions were observed for cellobiose, maltose, lactose, and trehalose. In addition, *L. paracasei* A11 was able to ferment the trisaccharide melezitose. Positive reactions were also detected for the glycosides esculin and salicin.

Table 3.3.2. *Carbohydrate fermentation profile of L. paracasei A11*

Carbohydrate		*L. paracasei* A11	Carbohydrate		*L. paracasei* A11
0	Control	-	25	Esculin	+
1	Glycerol	-	26	Salicin	+
2	Erythritol	-	27	Cellobiose	+
3	D-Arabinose	-	28	Maltose	+
4	L-Arabinose	-	29	Lactose	+
5	D-Ribose	+	30	Melibiose	-
6	D-Xylose	-	31	Sucrose	+/-
7	L-Xylose	-	32	Trehalose	+
8	Adonitol	-	33	Inulin	-
9	ß-Methyl-D-Xyloside	-	34	Melezitose	+
10	Galactose	+	35	Raffinose	-
11	Glucose	+	36	Starch	-
12	Fructose	+	37	Glycogen	-
13	Mannose	+	38	Xylitol	-
14	Sorbose	-	39	Gentibiose	+/-
15	Rhamnose	-	40	Turanose	-
16	*Dulcitol*	-	41	D-Lyxose	-
17	Inositol	-	42	D-Tagatose	+
18	Mannitol	+	43	D-Fucose	-
19	Sorbitol	-	44	L- Fucose	-
20	α-Methyl-D-Mannoside	-	45	D-Arabitol	-
21	α-Methyl-D-Glucoside	-	46	L- Arabitol	-
22	N-Acetyl-Glucosamine	+	47	Gluconate	+/-
23	Amygdalin	-	48	2-Keto-Gluconate	-
24	Arbutin	+/-	49	5-Keto-Gluconate	-

Results evaluated using the API 50 CH test as '–' = negative; '+' = positive; '+/-' = weak.

In contrast, weak fermentation responses were recorded for sucrose, arbutin, gentiobiose, and gluconate. No fermentation was observed for glycerol, erythritol, D- and L-arabinose, D- and L-xylose, adonitol, β-methyl-D-xyloside, sorbose, rhamnose, dulcitol, inositol, sorbitol, α-methyl-D-mannoside, amygdalin, melibiose, inulin, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, and keto-gluconates.

Foodborne pathogens and spoilage fungi create major challenges for the food industry in keeping products safe. As a result, there is increasing interest in using microbial cultures with bioprotective properties as a natural way to improve food safety [230,231]. Therefore, in this study, antifungal and antibacterial activity of *L. paracasei* A11 was tested, and the results are

presented in Table 3.3.3. The strongest inhibition was observed against the yeast *D. hansenni* (25 mm) and pathogenic bacteria *L. monocytogenes* (15 mm). Moderate (7 – 11 mm) inhibition zones were noted for *P. fluorescens* (11 mm), *E. coli* (10 mm), *B. thermosphacta* (9 mm), *B. cereus* (9 mm), *P. aeruginosa* (9 mm), and *A. versicolor* (7 mm). Lower inhibitory effects (2 – 5 mm) were noted for several fungal species such as *C. herbarum* (3 mm), *Y. lipolytica* (2 mm), *C. glabrata* (2 mm), *P. commune* (2 mm), and for bacteria including *S.* Typhimurium (3 mm) and *S. aureus* (2 mm). No inhibition was observed against *C. albicans* and *M. racemosus*, indicating that *L. paracasei* A11 had no antifungal effect on these microorganisms.

Table 3.3.3. *Antifungal and antibacterial activity of L. paracasei A11*

Strains	Diameter of inhibition zone in mm
Debaryomyces hansenii (BTL, M-31/Deb. 4 (T))	25
Yarrowia lipolytica (BTL, M-48/C.6.1)	2
Candida glabrata (BTL, 8B)	2
Candida albicans (BTL, M-7/C.17)	0
Mucor racemosus (BTL, G-134/1064)	0
Cladosporium herbarum (BTL, G-86/KŠ-2/7)	3
Penicillium commune ML 21-3	2
Aspergillus versicolor (CBS 113090)	7
Listeria monocytogenes ATCC 35152	15
Staphylococcus aureus ATCC 9144	2
Escherichia coli ATCC 8739	10
Pseudomonas aeruginosa NCTC 6750	9
Bacillus cereus ATCC 11778	9
Salmonella Typhimurium ATCC 13311	3
Pseudomonas fluorescens ATCC 13525	11
Brochothrix thermosphacta ATCC 11509	9

ATCC – American Type Culture Collection; NCTC – National Collection of Type Cultures, a Culture Collection of Public Health England; MRS – De Man Rogosa Sharpe medium; BHI – Brain Heart Infusion medium.

3.4. *L. paracasei* A11 probiotic potential

The ability of *L. paracasei* A11 to survive in acidic conditions and in the presence of bile salts was assessed to determine its functional robustness. The strain's survival was measured after exposure to low pH (2.5) and varying concentrations of bile salts, simulating gastrointestinal conditions, with the results presented in Table 3.4.1. The initial viability of *L. paracasei* in acidic conditions was 8.58 $\log_{10}$ CFU/g, and after 3 hours of exposure, it maintained 100% survival, indicating strong acidic tolerance. The results of bile salt

resistance showed that in the control MRS broth, the bacterial count was 9.16 $\log_{10}$ CFU/g, and at the highest bile salt concentration (1%), the count decreased to 6.66 $\log_{10}$ CFU/g after 24 hours of incubation, indicating a 73% survival rate ($p < 0.05$). These results suggest that the strain is well-suited for gastrointestinal conditions.

Table 3.4.1. *L. paracasei A11 resistance to acid and bile salt*

Strain	**Resistance to acid (2.5 pH)**		**Resistance to bile salt**			
Media	**0 h**	**3 h**	**MRS broth**	**MRS broth + 0.3% bile**	**MRS broth + 0.5% bile**	**MRS broth + 1% bile**
***L. paracasei* A11 ($\log_{10}$ CFU/g)**	8.58 ± 0.03	8.61 ± 0.06	9.16 ± 0.12^{a}	7.40 ± 0.14^{b}	6.38 ± 0.02^{c}	6.66 ± 0.20^{c}

Values presented are means of three replicates ± standard deviation. Means in the same row with different lowercase letters indicate significant differences ($p < 0.05$) among treatments.

3.5. Cultivation of *L. paracasei* A11 in dairy by-products

During flask cultivation, statistically significant differences in pH and LAB counts were observed between time points and among growth media ($p < 0.05$), as shown by changes in *L. paracasei* A11 cultivation in MRS broth, LAWPC, and LAWP presented in Figure 3.5.1. In MRS broth, the pH (Fig. 3.5.1a), decreased from 6.25 ± 0.01 at 0 h to 4.50 ± 0.02 at 24 h and further to about 3.46 ± 0.01 after 48 h. In LAWPC, the pH showed a slight decrease from 4.70 ± 0.01 at 0 h to 4.65 ± 0.02 at 24 h and remained relatively stable up to 48 h. In LAWP, pH values remained nearly constant throughout the cultivation period, ranging from 4.36 ± 0.01 to 4.25 ± 0.02.

LAB counts (Fig. 3.5.1b) in MRS broth increased from 5.51 ± 0.02 $\log_{10}$ CFU/g at 0 h to 8.46 ± 0.02 $\log_{10}$ CFU/g at 24 h and reached 9.23± 0.03 $\log_{10}$ CFU/g after 48 h, representing the highest biomass yield among all evaluated time points. In LAWPC, LAB counts increased from 6.28 ± 0.02 $\log_{10}$ CFU/g at 0 h to 6.85 ± 0.01 $\log_{10}$ CFU/g at 24 h and to 8.30 ± 0.02 $\log_{10}$ CFU/g at 48 h. In LAWP, LAB counts decreased from 5.40 ± 0.03 $\log_{10}$ CFU/g at 0 h to 5.0 ± 0.04 $\log_{10}$ CFU/g at 24 h and increased to 6.3 ± 0.03 $\log_{10}$ CFU/g at the end of storage.

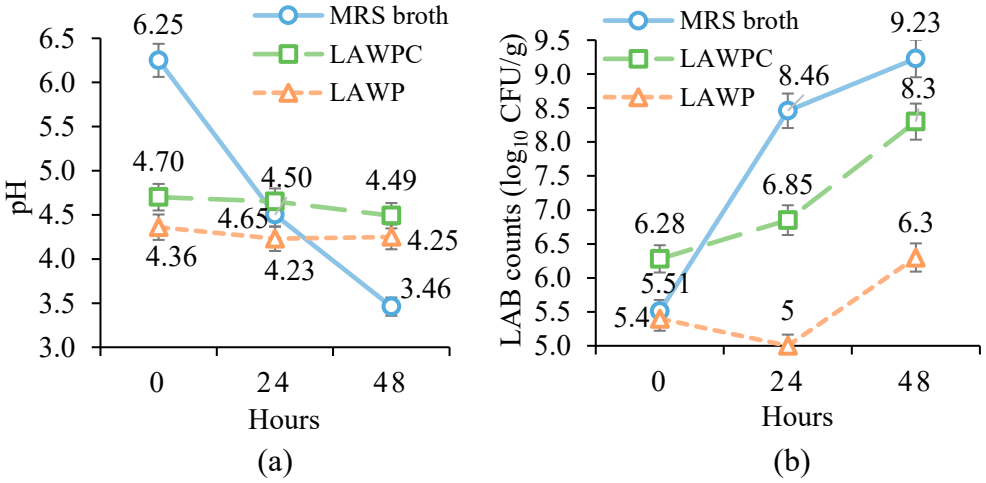


Fig. 3.5.1. *Changes in pH (a) and LAB counts (b) of L. paracasei A11 in MRS broth, liquid acid whey protein concentrate (LAWPC), and liquid acid whey permeate (LAWP)*

The consumption of sugars and the production of lactic acid and lactate by *L. paracasei* A11 were measured in MRS broth, LAWPC, and LAWP over 48 hours (Table 3.5.1). Statistical analysis showed significant differences in sugar utilization and metabolite production both among time points within each media and between media at the same time point ($p < 0.05$), indicating that substrate composition strongly influenced the metabolic activity of the strain [32].

In MRS broth, glucose was rapidly consumed, decreasing from 1.67 ± 0.03% to 0.15 ± 0.01%. In LAWPC, glucose decreased slightly from 0.66 ± 0.01% to 0.57 ± 0.02%, while in LAWP it dropped from 0.45 ± 0.01% to 0.02 ± 0.01%. Galactose and lactose were used only minimally. Galactose decreased slightly in LAWPC (1.05 ± 0.03% to 0.97 ± 0.02%) and LAWP (0.65 ± 0.01% to 0.61 ± 0.04%), while lactose showed a small reduction in LAWP (3.04 ± 0.07% to 2.84 ± 0.05%) and remained largely unchanged in LAWPC.

The production of D/L lactic acid and D/L lactate followed the pattern of sugar consumption. In MRS broth, lactic acid increased from 34.45 ± 0.33 to 1462.95 ± 18.45 mg/100 g, and lactate from 34.06 ± 0.32 to 1442.17 ± 20.25 mg/100 g. In LAWPC, lactic acid increased from 847.01 ± 1.01 to 1030.23 ± 7.07 mg/100 g, and lactate from 837.20 ± 1.06 to 1019.09 ± 5.01 mg/100 g. In LAWP, lactic acid increased from 120.0 ± 0.9 to 431.0 ± 0.08 mg/100 g, and lactate from 113.01 ± 1.0 to 416.01 ± 0.09 mg/100 g.

Table 3.5.1. *Kinetics of lactic acid, lactate, and sugar utilization by L. paracasei A11 in MRS broth, liquid acid whey protein concentrate (LAWPC), and liquid acid whey permeate (LAWP)*

Parameter	Hours	Growth media		
		MRS broth	LAWPC	LAWP
Glucose (%)	0	1.67 ± 0.03^{Aa}	0.66 ± 0.01^{Ab}	0.45 ± 0.01^{Ac}
	48	0.15 ± 0.01^{Bb}	0.57 ± 0.02^{Ba}	0.02 ± 0.01^{Bc}
Galactose (%)	0	-	1.05 ± 0.03^{Aa}	0.65 ± 0.01^{b}
	48	-	0.97 ± 0.02^{Ba}	0.61 ± 0.04^{b}
Lactose (%)	0	-	9.84 ± 0.04^{Aa}	3.04 ± 0.07^{Ab}
	48	-	9.76 ± 0.04^{Ba}	2.84 ± 0.05^{Bb}
D/L lactic acid (mg/100 g)	0	34.45 ± 0.33^{Bc}	847.01 ± 1.01^{Ba}	120.0 ± 0.9^{Bb}
	48	1462.95 ± 18.45^{Aa}	1030.23 ± 7.07^{Ab}	431.0 ± 0.08^{Ac}
D/L lactate (mg/100 g)	0	34.06 ± 0.32^{Bc}	837.20 ± 1.06^{Ba}	113.01 ± 1.0^{Bb}
	48	1442.17 ± 20.25^{Aa}	1019.09 ± 5.01^{Ab}	416.01 ± 0.09^{Ac}

Values are presented as means of three replicates ± standard deviation. "–" = not detected. Means within the same column followed by different uppercase (A–B) letters indicate significant differences ($p < 0.05$) among time points within the same treatment. Means within the same row followed by different lowercase (a–c) letters indicate significant differences ($p < 0.05$) among treatments at the same time point.

No growth of *L. paracasei* A11 was observed in LAWP in the bioreactor due to its low glucose content and limited availability of nitrogen sources, despite the presence of lactose. In contrast, LAWPC and MRS broth provided suitable conditions for growth as shown by optical density (OD) measurements in Figure 3.5.2. The effects of growth medium, cultivation time, and their interaction on OD, growth rate, and biomass yield were statistically significant ($p < 0.05$). In MRS broth, rapid increase in OD was observed, with doubling time of 2.14 h. Cultivation in LAWPC resulted in diauxic growth, with an initial exponential phase (E1) characterized by a doubling time of 3.5 h. During the second exponential phase (E2), the doubling time increased to 15 h. Overall, these results indicate that LAWPC provides a nutritionally adequate matrix for the growth and controlled acid production of *L. paracasei* A11, and therefore LAWPC was used as a fermentation substrate for the preparation of edible coatings in the further experiments.

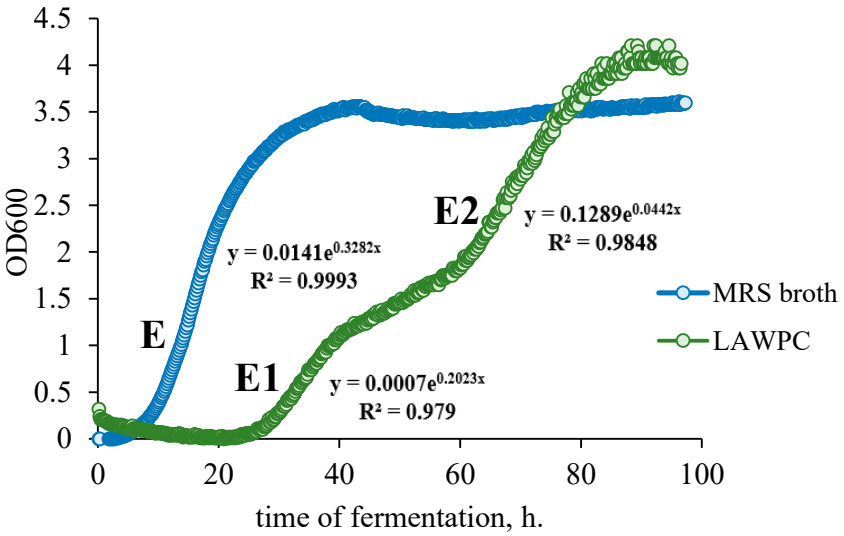


Fig. 3.5.2. *Growth kinetics of L. paracasei A11 in MRS broth and liquid acid whey protein concentrate (LAWPC). Growth phases: exponential phase (E), first exponential phase (E1) and second exponential phase (E2)*

"y" describes the linear relationship between x and y, while the "R²" value indicates how well the regression line fits the data.

3.6. Characterization of edible coating film-forming solutions and film properties

3.6.1. Properties of film-forming solutions and edible films

Rheological properties, particularly viscosity, are critical for food producers when selecting an appropriate method for applying film-forming solutions to product surfaces [155]. The rheological data (Fig. 3.6.1.1.) showed that all film-forming solutions exhibited non-Newtonian, time-independent shear behavior, with flow behavior index (n) lower than 1, indicating a decrease in apparent viscosity with increasing shear rate, while the shear stress–shear rate curves revealed significant differences in viscosity among the formulations ($p < 0.05$). In general, LAWP film-forming solutions exhibited lower shear stress values than LAWPC solutions, indicating lower viscosity (about 19.64 and 37.58 Pa, respectively; $p < 0.05$). Notably, the incorporation of *L. paracasei* A11 into the LAWPC film-forming solution resulted in a reduction of approximately 13.24 Pa ($p < 0.05$).

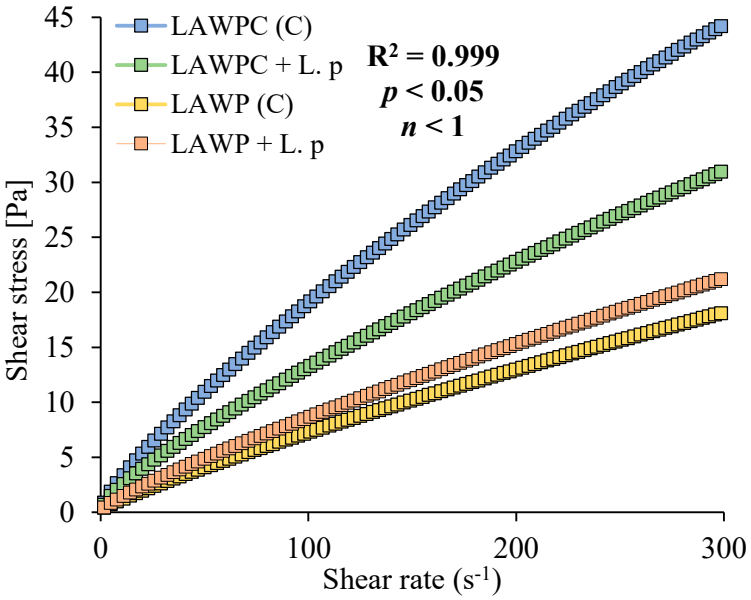


Fig. 3.6.1.1. *Flow curves of the different film-forming solutions*

LAWPC – liquid acid whey protein concentrate; LAWP – liquid acid whey permeate; C – control; L. p – *Lacticaseibacillus paracasei* A11. "R^2" value indicates how well the regression line fits the data, while "n" value indicates the flow behavior index.

Table 3.6.1.1 summarizes the physicomechanical and optical properties of edible films prepared from LAWPC and LAWP, with and without the incorporation of *L. paracasei* A11.

Significant differences among film formulations were observed for most physicomechanical attributes ($p < 0.05$). Film thickness ranged from 0.13 to 0.28 μm. LAWPC (C) film exhibited the highest thickness, while LAWP-based films showed significantly lower and more uniform thickness values. The tensile strength (TS) did not differ significantly among the formulations ($p > 0.05$), whereas elongation-at-break (EAB) values were significantly affected ($p < 0.05$). In LAWPC films, the addition of *L. paracasei* A11 significantly increased EAB compared to the control (LAWPC + L. p: 23.87 ± 4.84%; LAWPC (C): 9.36 ± 2.24%), indicating enhanced film extensibility. When comparing the two film bases, higher EAB values were observed in LAWP samples compared with LAWPC. Water solubility was relatively high across all films, with an overall mean of 71.79%. Moisture content was significantly higher in LAWP films (approx. 32.91%) compared to LAWPC (approx. 22.84%; $p < 0.05$), whereas water vapor permeability (WVP) showed no significant differences among the formulations ($p > 0.05$).

Table 3.6.1.1. *Physicomechanical and optical characterization of edible films*

Properties	Film samples			
	LAWPC (C)	**LAWPC + L. p**	**LAWP (C)**	**LAWP + L. p**
Thickness (µm)	0.28 ± 0.03^{b}	0.20 ± 0.08^{ab}	0.13 ± 0.01^{a}	0.13 ± 0.03^{a}
TS (MPa)	0.07 ± 0.02^{a}	0.27 ± 0.16^{a}	0.09 ± 0.01^{a}	0.05 ± 0.00^{a}
EAB (%)	9.36 ± 2.24^{c}	23.87 ± 4.84^{b}	31.31 ± 2.52^{a}	26.18 ± 4.93^{ab}
Water solubility (%)	66.56 ± 1.05^{a}	69.01 ± 2.81^{a}	75.25 ± 8.01^{a}	76.32 ± 1.11^{a}
Moisture (%)	23.37 ± 1.26^{a}	22.30 ± 0.92^{a}	32.63 ± 1.31^{b}	33.18 ± 0.59^{b}
WVP (g/(m.s.Pa))	$2.29 \times 10^{-6} \pm 6.20 \times 10^{-7a}$	$1.99 \times 10^{-6} \pm 8.69 \times 10^{-7a}$	$2.69 \times 10^{-6} \pm 1.14 \times 10^{-6a}$	$2.85 \times 10^{-6} \pm 1.24 \times 10^{-6a}$
Opacity (%)	21.59 ± 1.94^{a}	17.09 ± 1.33^{b}	12.23 ± 0.59^{c}	12.22 ± 0.87^{c}
L*	84.34 ± 1.98^{c}	88.23 ± 1.24^{b}	92.85 ± 0.27^{a}	92.82 ± 0.38^{a}
a*	-2.08 ± 0.09^{b}	-3.14 ± 0.45^{c}	-1.11 ± 0.18^{a}	-1.06 ± 0.14^{a}
b*	13.05 ± 0.63^{b}	17.48 ± 3.10^{a}	7.45 ± 0.71^{c}	7.20 ± 0.67^{c}

LAWPC – liquid acid whey protein concentrate; LAWP – liquid acid whey permeate; C – control; L. p – *Lacticaseibacillus paracasei* A11; TS – tensile strength; EAB – elongation-at-break; WVP - water vapor permeability. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) between samples. Results are mean ± SD.

The optical properties of the films, including opacity and color coordinates, were also assessed due to their direct impact on the appearance of coated products [232]. Notably, the optical properties varied significantly among formulations ($p < 0.05$). Opacity was highest in LAWPC (C) films and significantly decreased with the incorporation of *L. paracasei* A11. LAWP-based films showed lower opacity overall, indicating greater transparency. Color measurements revealed higher lightness (L*) values for LAWP films, while LAWPC films were darker. Film type (LAWPC vs LAWP) significantly influenced the a* and b* color parameters ($p < 0.05$), with LAWPC-based films showing lower a* values and higher b* values than AWP films.

3.6.2. Morphological characteristics of edible films

SEM analysis was performed to assess the microstructure of the film samples (Fig. 3.6.2.1). LAWPC-based films exhibited irregular, non-smooth surfaces with the presence of crystallized regions (indicated by white arrows). In contrast, LAWP-based films, particularly the LAWP (C) sample, showed surface cavities (indicated by dashed white circles), that were more pronounced than those observed in the film containing entrapped bacteria.

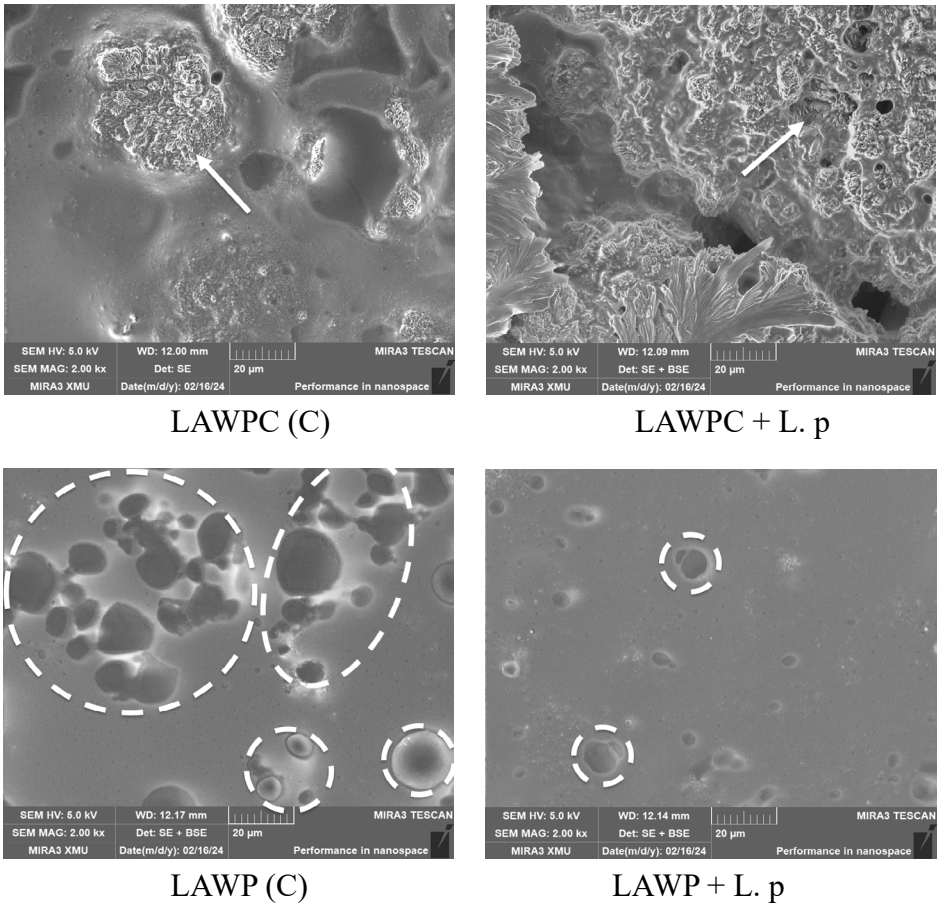
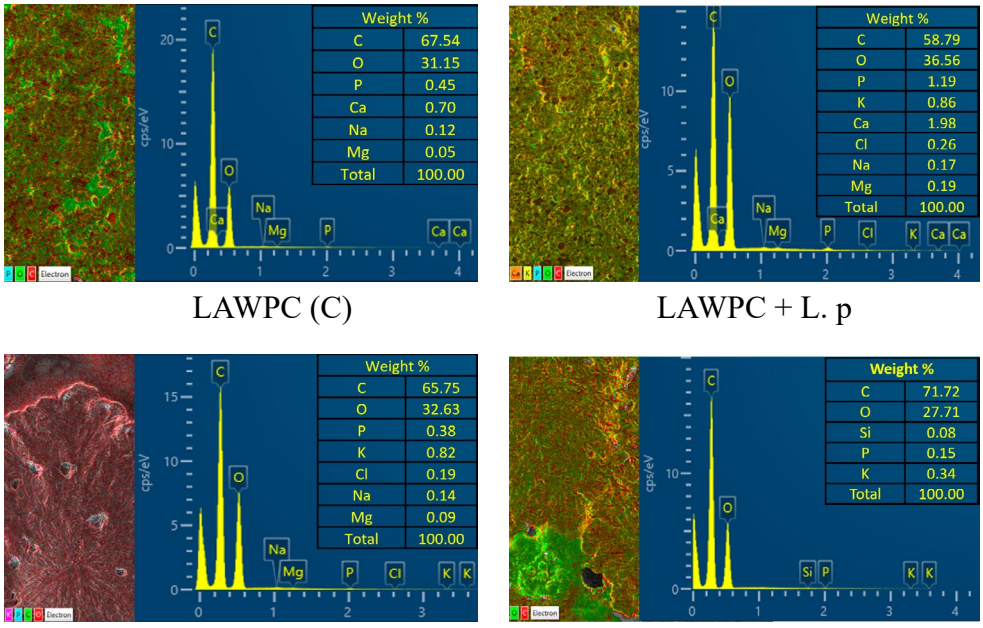


Fig. 3.6.2.1. *Scanning electron microscopy images of the surface of different edible films*

LAWPC – liquid acid whey protein concentrate; LAWP – liquid acid whey permeate; C – control; L. p – *Lacticaseibacillus paracasei* A11.

The element composition and distribution of the edible films, determined by Energy-Dispersive X-Ray Spectroscopy (EDS), are presented in Fig. 3.6.2.2. The EDS spectra obtained from two randomly selected regions of each film sample indicated that carbon (C) and oxygen (O) were the dominant elements, with their concentrations differing significantly among the films ($p < 0.05$). Smaller amounts of phosphorus (P), calcium (Ca), sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), and silicon (Si) were also detected, each representing less than 2% of the total weight.



LAWPC (C) LAWPC + L. p

LAWP (C) LAWP + L. p

Fig. 3.6.2.2. *EDS analysis and element X-ray mapping of the film samples*

LAWPC – liquid acid whey protein concentrate; LAWP – liquid acid whey permeate; C – control; L. p – *Lacticaseibacillus paracasei* A11.

3.6.3. Viability of *L. paracasei* A11 in edible films

Figure 3.6.3.1 shows the effect of the film drying process (a) and storage conditions (b) on the viability of *Lacticaseibacillus paracasei* A11 in edible films.

Prior to drying (Fig. 3.6.3.1a), LAB counts were significantly higher ($p < 0.05$) in LAWP + L. p films (7.60 $\log_{10}$ CFU/g) than in LAWPC + L. p films (7.16 $\log_{10}$ CFU/g). After drying, LAB counts increased significantly ($p < 0.05$) in LAWPC + L. p films to 7.67$_{10}$ log CFU/g, whereas a slight but significant decrease ($p < 0.05$) was observed in LAWP + L. p films (7.53 $\log_{10}$ CFU/g).

During storage at 4 °C for 21 days (Fig. 3.6.3.1b), LAB counts decreased significantly over time in both film formulations ($p < 0.05$), with a more pronounced reduction in LAWP + L. p films. In LAWPC + L. p films, LAB counts declined significantly ($p < 0.05$) from 7.67 $\log_{10}$ CFU/g on day 1 to 5.21 $\log_{10}$ CFU/g on day 21. In contrast, LAWP + L. p films showed a rapid and significant decrease ($p < 0.05$) from 7.53 $\log_{10}$ CFU/g to 4.76 $\log_{10}$ CFU/g by day 14. At all storage times, LAB viability was

significantly higher ($p < 0.05$) in LAWPC + L. p films than in LAWP + L. p films, therefore LAWPC coatings were used for further experiments.

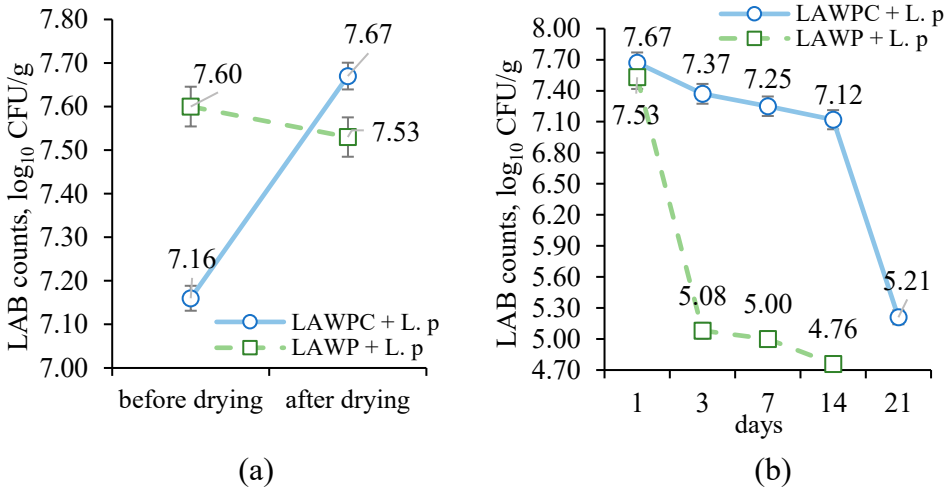


Fig. 3.6.3.1. *Effect of film drying process (a) and storage conditions (b) on the survival of L. paracasei A11*

LAWPC – liquid acid whey protein concentrate; LAWP – liquid acid whey permeate; L. p – *Lacticaseibacillus paracasei* A11.

3.7. Industrial-scale implementation of edible coatings with incorporated *L. paracasei* A11 for Edam-type cheese

3.7.1. Physicochemical and microbiological characterization of coated Edam-type cheese

The pH, water activity (a_w), dry matter (DM), and color difference (ΔE) of regular and probiotic Edam-type cheeses were determined on days 1 (before ripening), 45 (after ripening), and 135 (end of storage) (Table 3.7.1.1). Because different milk batches were used, regular (R) and probiotic (P) cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them.

Table 3.7.1.1. *Changes in pH, water activity (a_w), dry matter content (DM), and color (ΔE) of regular and probiotic Edam-type cheeses coated with edible coatings during ripening and storage*

Day	Sample	pH	a_w	DM (%)	ΔE
1	R (C)	5.18 ± 0.01^{C}	0.948 ± 0.001^{A}	54.23 ± 0.01^{A}	–
	R + LAWPC	5.18 ± 0.02^{B}	0.948 ± 0.002^{A}	54.23 ± 0.02^{A}	–
	R + LAWPC + L. p	5.18 ± 0.01^{B}	0.948 ± 0.001^{A}	54.23 ± 0.03^{A}	–
	P (C)	5.23 ± 0.02^{Z}	0.945 ± 0.002^{X}	56.77 ± 0.01^{X}	–
	P + LAWPC	5.22 ± 0.04^{X}	0.946 ± 0.002^{X}	56.76 ± 0.01^{Y}	–
	P + LAWPC + L. p	5.23 ± 0.03^{X}	0.944 ± 0.001^{X}	56.76 ± 0.01^{X}	–
45	R (C)	5.37 ± 0.02^{Ba}	0.946 ± 0.004^{Aa}	53.08 ± 0.02^{Ca}	3.77 ± 2.37
	R + LAWPC	5.04 ± 0.01^{Cc}	0.948 ± 0.004^{Aa}	52.93 ± 0.02^{Bb}	3.39 ± 2.32
	R + LAWPC + L. p	5.15 ± 0.02^{Cb}	0.943 ± 0.005^{Bb}	52.63 ± 0.03^{Cc}	3.97 ± 3.25
	P (C)	5.27 ± 0.03^{Yx}	0.943 ± 0.005^{Xx}	52.95 ± 0.02^{Zz}	9.18 ± 3.39
	P + LAWPC	5.07 ± 0.04^{Zy}	0.941 ± 0.004^{Yx}	57.25 ± 0.03^{Xx}	7.31 ± 3.67
	P + LAWPC + L. p	5.03 ± 0.02^{Yz}	0.927 ± 0.004^{Zy}	53.41 ± 0.02^{Zy}	5.36 ± 1.85
135	R (C)	5.51 ± 0.02^{Aa}	0.939 ± 0.002^{Bb}	53.45 ± 0.03^{Bb}	5.05 ± 0.57
	R + LAWPC	5.36 ± 0.02^{Ab}	0.941 ± 0.004^{Bab}	51.86 ± 0.01^{Cc}	3.67 ± 1.06
	R + LAWPC + L. p	5.29 ± 0.04^{Ac}	0.943 ± 0.004^{Ba}	53.72 ± 0.02^{Ba}	3.22 ± 1.42
	P (C)	5.33 ± 0.03^{Xx}	0.940 ± 0.003^{Yxy}	55.29 ± 0.04^{Yx}	5.61 ± 3.44
	P + LAWPC	5.15 ± 0.02^{Yz}	0.941 ± 0.002^{Yx}	53.13 ± 0.04^{Zz}	4.00 ± 2.17
	P + LAWPC + L. p	5.24 ± 0.01^{Xy}	0.939 ± 0.003^{Yy}	55.26 ± 0.03^{Yy}	2.07 ± 1.69
Cheese group	**Significance of factors and their interactions**				
Regular	Sample	$p < 0.01$	0.029	$p < 0.01$	ns
	Day	$p < 0.01$	$p < 0.01$	$p < 0.01$	ns
	Sample x Day	$p < 0.01$	$p < 0.01$	$p < 0.01$	ns
Probiotic	Sample	$p < 0.01$	$p < 0.01$	$p < 0.01$	ns
	Day	$p < 0.01$	$p < 0.01$	$p < 0.01$	0.025
	Sample x Day	$p < 0.01$	$p < 0.01$	$p < 0.01$	ns

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11. Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among R cheese samples, and different lowercase letters (x–z) indicate significant differences ($p < 0.05$) among P cheese samples. Different uppercase letters (A–C) indicate significant differences ($p < 0.05$) among R cheese storage times within the same treatment, while different uppercase letters (X–Z) indicate significant differences ($p < 0.05$) among P cheese storage times within the same treatment. Results are presented as mean of three replicates ± SD.

For regular cheeses, sample type and storage day significantly affected pH, a_w, and DM content, and a significant sample × day interaction was observed for

all three parameters ($p < 0.01$). Overall, pH increased significantly ($p < 0.05$) during storage, particularly by day 135, with the control sample exhibiting higher pH values than the LAWPC-coated samples. A_w decreased over storage time, especially at the end of storage, but coated samples maintained higher a_w values compared with the control. The control sample showed the highest DM content on day 45, whereas at the end of storage the R + LAWPC + L. p sample exhibited significantly higher DM content ($p < 0.05$). No significant effects were observed for ΔE, indicating that the coatings did not significantly influence color changes in regular cheese.

In probiotic cheeses, similar trends were observed. Sample type, storage day, and their interaction significantly affected pH, a_w, and DM ($p < 0.01$), demonstrating that the presence of LAWPC coatings, with or without *L. paracasei* A11, modified storage-related changes. Probiotic control cheeses exhibited a gradual and statistically significant increase in pH during storage ($p < 0.05$), whereas LAWPC-coated samples showed greater fluctuations, particularly on day 45. A_w decreased during storage, with significant differences ($p < 0.05$) observed among probiotic cheese samples, and the lowest a_w value was recorded for the P + LAWPC + L. p sample on day 45. DM content increased significantly ($p < 0.05$) in the P + LAWPC sample on day 45 compared with the other samples. In contrast, at the end of storage, the DM content of the P + LAWPC sample was significantly lower ($p < 0.05$) than the remaining samples. Unlike regular cheeses, storage day had a significant effect on ΔE in probiotic cheeses, suggesting that color changes progressed during storage, although the lack of a significant sample × day interaction indicates that coatings did not differentially influence color development over time.

To assess the impact of whey-pectin-based edible coatings with entrapped *L. paracasei* A11 strain on regular and probiotic Edam-type cheeses FA content and LQI were examined during days 1 (before ripening), 45 (after ripening), and 135 (end of storage) and the results are presented in Table 3.7.1.2.

On day 1, no significant differences ($p > 0.05$) were observed among regular cheese samples (Table 3.7.1.2a) for saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), long-chain fatty acids (LCFA), medium-chain fatty acids (MCFA), short-chain fatty acids (SCFA), omega-6 polyunsaturated fatty acids (omega-6), omega 3 polyunsaturated fatty acids (omega-3), omega-6/omega-3 ratio (n-6/n-3), trans FA, and all LQI including atherogenicity (AI), thrombogenicity (TI), and hypocholesterolemic/ hypercholesterolemic (h/H) indices.

Table 3.7.1.2. *Fatty acid (FA) groups and lipid quality indices (LQI) of regular (a) and probiotic (b) Edam-type cheeses coated with edible coatings during ripening and storage*

(a)	Day 1			Day 45			Day 135		
FA groups and LQI	**R (C)**	**R + LAWPC**	**R + LAWPC + L. p**	**R (C)**	**R + LAWPC**	**R + LAWPC + L. p**	**R (C)**	**R + LAWPC**	**R + LAWPC + L. p**
SFA	68.91^{A}	68.91^{A}	68.91^{A}	68.71^{B}	68.71^{B}	68.71^{B}	68.92^{Aa}	68.73^{Bb}	68.66^{Cc}
MUFA	26.89^{B}	26.89^{C}	26.89^{C}	27.11^{A}	27.13^{A}	27.12^{A}	26.81^{Bc}	27.03^{Bb}	27.09^{Ba}
PUFA	4.21^{B}	4.20^{B}	4.20^{B}	4.17^{C}	4.16^{C}	4.17^{C}	4.27^{Aa}	4.24^{Ab}	4.25^{Aab}
UFA	31.09^{B}	31.09^{B}	31.09^{C}	31.29^{A}	31.29^{A}	31.29^{B}	31.08^{Bc}	31.27^{Ab}	31.34^{Aa}
LCFA	70.18	70.22	70.23	70.15	70.17	70.25	70.10^{a}	69.84^{b}	69.92^{b}
MCFA	23.60^{A}	23.57^{B}	23.55	23.63^{A}	23.72^{A}	23.65	23.30^{Bc}	23.61^{Ba}	23.56^{b}
SCFA	6.22^{B}	6.22	6.22^{AB}	6.22^{B}	6.11	6.10^{B}	6.60^{Aa}	6.55^{ab}	6.52^{Ab}
Omega-6	2.05	2.06	2.05	2.05	2.06	2.07	2.07	2.06	2.06
Omega-3	0.87	0.87	0.88	0.87	0.87	0.88	0.87	0.87	0.86
n-6/n-3	2.37	2.36	2.37^{B}	2.37	2.36	2.35^{B}	2.37^{b}	2.38^{ab}	2.40^{Aa}
AI	2.52^{A}	2.52^{A}	2.52^{A}	2.50^{C}	2.51^{B}	2.51^{B}	2.51^{Ba}	2.50^{Cb}	2.49^{Cc}
TI	1.38	1.38	1.38	1.38	1.38	1.38	1.37	1.37	1.37
h/H	0.66	0.67	0.66	0.67	0.67	0.67	0.67	0.67	0.67
DFA	39.96^{B}	39.97	39.96^{B}	40.13^{A}	40.06	40.16^{A}	39.92^{Bc}	40.06^{b}	40.14^{Aa}
Trans	3.12^{B}	3.12^{C}	3.12^{B}	3.22^{Aa}	3.16^{Bb}	3.20^{Aab}	3.20^{Aab}	3.19^{Ab}	3.22^{Aa}
Mean of ±SD	0.02	0.02	0.03	0.03	0.06	0.04	0.03	0.01	0.01

Table 3.7.1.2 cont.

(b)	Day 1			Day 45			Day 135		
FA groups and LQI	**P (C)**	**P + LAWPC**	**P + LAWPC + L. p**	**P (C)**	**P + LAWPC**	**P + LAWPC + L. p**	**P (C)**	**P + LAWPC**	**P + LAWPC + L. p**
SFA	68.34^{X}	68.34	68.33	68.14^{Yy}	68.28^{x}	68.30^{x}	68.30^{X}	68.23	68.16
MUFA	27.54^{Y}	27.54	27.54	27.74^{Xx}	27.60^{y}	27.59^{y}	27.56^{Y}	27.60	27.64
PUFA	4.12	4.12	4.12	4.12	4.12	4.11	4.15	4.17	4.20
UFA	31.66^{Y}	31.66	31.67	31.86^{Xx}	31.72^{y}	31.70^{y}	31.70^{Y}	31.77	31.84
LCFA	72.23	72.16^{X}	72.44^{X}	72.31	72.19^{X}	72.32^{X}	71.58	71.70^{Y}	71.45^{Y}
MCFA	21.67^{x}	21.60^{xy}	21.56^{y}	21.65	21.65	21.46	21.58^{y}	21.58^{y}	21.78^{x}
SCFA	6.10	6.24^{XY}	6.00^{Y}	6.03	6.16^{Y}	6.22^{Y}	6.83	6.72^{X}	6.77^{X}
Omega-6	2.05	2.05	2.05	2.04y	2.06^{xy}	2.07^{x}	2.07	2.07	2.06
Omega-3	0.77^{Y}	0.77	0.77	0.83^{Xx}	0.81^{xy}	0.80^{y}	0.75^{Y}	0.76	0.78
n-6/n-3	2.67^{Y}	2.67	2.66	2.46^{Zz}	2.56^{y}	2.59^{x}	2.75^{X}	2.71	2.66
AI	2.40^{X}	2.40^{X}	2.40^{X}	2.40^{XYy}	2.41^{Xx}	2.41^{Xx}	2.38^{Y}	2.38^{Y}	2.37^{Y}
TI	1.48^{X}	1.48	1.48	1.44^{Yy}	1.45^{xy}	1.46^{x}	1.48^{X}	1.47	1.45
h/H	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
DFA	41.15^{Y}	41.14	41.13	41.35^{X}	41.22	41.23	41.06^{Y}	41.16	41.25
Trans	3.31^{Y}	3.31^{Y}	3.31^{Y}	3.34^{Y}	3.30^{Y}	3.31^{Y}	3.39^{X}	3.41^{X}	3.42^{X}
Mean of ±SD	0.06	0.04	0.01	0.09	0.03	0.07	0.03	0.08	0.09

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11. Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among R cheese samples, and different lowercase letters (x–z) indicate significant differences ($p < 0.05$) among P cheese samples. Different uppercase letters (A–C) indicate significant differences ($p < 0.05$) among R cheese storage times within the same treatment, while different uppercase letters (X–Z) indicate significant differences ($p < 0.05$) among P cheese storage times within the same treatment. Results are presented as mean of three replicates.

After 45 days of cheese ripening, significant differences among storage times ($p < 0.05$) were observed within the same treatment for SFA, MUFA, PUFA, UFA, MCFA, SCFA, n-6/n-3, AI, and trans FA. Among samples, differences were detected for trans FA with R (C) sample showing the highest value compared with R + LAWPC sample ($p < 0.05$).

At the end of storage (day 135), significant differences among samples were evident for most FA groups. The lowest SFA content was recorded in cheese coated with LAWPC + L. p (68.66 g/100 g total FA), while the highest was observed in the control (68.92 g/100 g total FA). Conversely, MUFA and UFA contents were highest in the LAWPC + L. p-coated sample compared with the other samples. PUFA content increased significantly ($p < 0.05$) in all samples by the end of storage, with the highest value ($p < 0.05$) observed in the R (C) sample. LCFA and SCFA contents were significantly reduced in coated samples compared with the control, whereas MCFA content was highest in the R + LAWPC sample ($p < 0.05$). The contents of omega-6 and omega-3 FA remained constant during storage; however, a significant increase ($p < 0.05$) in the n-6/n-3 ratio over storage time and among samples at day 135 was observed in the LAWPC + L. p sample. The AI decreased progressively during storage and reached its significantly lowest ($p < 0.05$) value in the LAWPC + L. p sample. DFA content increased significantly ($p < 0.05$) over storage and was highest in the LAWPC + L. p sample (40.14 g/100 g total FA) compared with the R + LAWPC and R (C) samples (40.06 g/100 g total FA and 39.92 g/100 g total FA, respectively). Trans FA content increased during storage and differed significantly among samples at day 135, with the highest value recorded in the LAWPC + L. p sample ($p < 0.05$).

On day 1, there were no significant differences ($p > 0.05$) among probiotic cheese samples (Table 3.7.1.2b) for LQI and FA groups, except MCFA content, which was significantly highest ($p < 0.05$) in the P (C) sample.

On day 45, SFA content was significantly higher ($p < 0.05$) in the coated samples, compared with the control. In contrast, MUFA and UFA content were significantly lower in the coated samples, while in the control the content increased during storage and was significantly highest ($p < 0.05$). PUFA, MCFA, and SCFA content did not change during cheese ripening and did not differ among samples ($p > 0.05$), while the content of LCFA in coated samples increased significantly ($p < 0.05$) after the ripening process compared with the R (C) sample. Omega-6 content was significantly higher in P + LAWPC + L. p sample (2.07 g/100 g total FA) compared to P (C) sample (2.04 g/100 g total FA), while omega-3 content was significantly lower in P + LAWPC + L. p sample (0.80 g/100 g total FA) compared to P (C) sample (0.83 g/100 g total FA) ($p < 0.05$). In the control sample, the n-6/n-3 ratio, AI, and TI indices were significantly lower ($p < 0.05$) compared to the coated samples.

The trans FA content and h/H and DFA indices did not differ significantly ($p > 0.05$) between samples.

On day 135, SFA, n-6/n-3 ratio, and TI significantly increased ($p < 0.05$) in the control sample, while MUFA, UFA, omega-3, and DFA decreased. In the coated samples, LCFA significantly decreased and SCFA increased ($p < 0.05$). In all samples, trans FA content significantly increased, while AI decreased ($p < 0.05$). The only significant difference between samples was observed for MCFA content, which was significantly lowest ($p < 0.05$) in the P + LAWPC + L. p sample. No significant difference ($p > 0.05$) was observed between storage days and between samples for PUFA, omega-6, and h/H.

The TBC, LAB, and *Lactiplantibacillus plantarum* counts were determined in the cheese matrix (sliced samples), while TBC, LAB, mold and yeast, and *Lacticaseibacillus paracasei* counts were determined on the cheese surface (swabs samples) of regular and probiotic Edam-type cheeses on days 1 (before ripening), 45 (after ripening), and 135 (end of storage) (Table 3.7.1.3). This dual strategy was used to assess the impact of the coatings and incorporated LAB on microbial distribution and growth within the cheese matrix and on the surface, where the conditions for microbial proliferation differ. Enterobacteria counts were also evaluated in these cheeses. However, no enterobacteria was detected throughout the storage period.

Table 3.7.1.3. *Microbiological profile of regular and probiotic Edam-type cheeses coated with edible coatings during ripening and storage*

Day	Sample	Cheese matrix			Cheese surface (swabs)			
		TBC $\log_{10}$ CFU/g	LAB $\log_{10}$ CFU/g	*L. plantarum* $\log_{10}$ CFU/g	TBC $\log_{10}$ CFU/g	LAB $\log_{10}$ CFU/g	Mold and yeast $\log_{10}$ CFU/g	*L. paracasei* $\log_{10}$ CFU/g
1	R (C)	7.62 ± 0.03^{Cc}	8.48 ± 0.01^{Ab}	n. d.	n. d.	n. d.	n. d.	n. d.
	R + LAWPC	8.45 ± 0.05^{Bb}	8.20 ± 0.09^{Ac}	n. d.	n. d.	n. d.	n. d.	n. d.
	R + LAWPC + L. p	8.53 ± 0.02^{Ba}	8.51 ± 0.05^{Aa}	n. d.	5.90 ± 0.03^{C}	5.81 ± 0.09^{C}	n. d.	5.65 ± 0.05^{C}
	P (C)	7.37 ± 0.22^{Zy}	8.38 ± 0.02^{Zx}	6.11 ± 0.02^{Y}	n. d.	n. d.	n. d.	n. d.
	P + LAWPC	8.33 ± 0.10^{Yx}	7.58 ± 0.19^{Yy}	6.17 ± 0.05^{Z}	n. d.	n. d.	n. d.	n. d.
	P + LAWPC + L. p	8.42 ± 0.11^{Yx}	8.20 ± 0.10^{Yx}	6.19 ± 0.03^{Y}	6.08 ± 0.01^{Z}	5.86 ± 0.05^{Z}	n. d.	5.60 ± 0.05^{Z}
45	R (C)	7.78 ± 0.04^{Bb}	8.11 ± 0.03^{Ba}	n. d.	8.79 ± 0.08^{a}	n. d.	n. d.	n. d.
	R + LAWPC	8.63 ± 0.11^{Aa}	7.94 ± 0.04^{Bb}	n. d.	7.54 ± 0.05^{c}	n. d.	n. d.	n. d.
	R + LAWPC + L. p	8.82 ± 0.02^{Aa}	7.80 ± 0.02^{Bc}	n. d.	8.18 ± 0.02^{Ab}	6.58 ± 0.12^{B}	n. d.	6.52 ± 0.03^{B}
	P (C)	7.80 ± 0.04^{Yy}	8.85 ± 0.04^{Xx}	6.80 ± 0.10^{Xy}	8.54 ± 0.05^{x}	n. d.	n. d.	n. d.
	P + LAWPC	8.80 ± 0.26^{Xx}	8.72 ± 0.03^{Xy}	7.09 ± 0.10^{Yx}	7.43 ± 0.02^{z}	n. d.	n. d.	n. d.
	P + LAWPC + L. p	8.90 ± 0.17^{Xx}	8.49 ± 0.09^{Xz}	6.30 ± 0.11^{Yz}	7.67 ± 0.01^{Xy}	6.76 ± 0.03^{X}	n. d.	6.27 ± 0.15^{Y}
135	R (C)	8.89 ± 0.01^{Aa}	7.65 ± 0.06^{Ca}	n. d.	7.23 ± 0.01^{b}	6.36 ± 0.10^{c}	2.30 ± 0.11	n. d.
	R + LAWPC	7.85 ± 0.05^{Cc}	7.18 ± 0.09^{Cc}	n. d.	7.28 ± 0.01^{a}	7.00 ± 0.05^{b}	2.97 ± 0.16	n. d.
	R + LAWPC + L. p	8.07 ± 0.07^{Cb}	7.41 ± 0.07^{Cb}	n. d.	7.28 ± 0.01^{Ba}	7.26 ± 0.04^{Aa}	n. d.	6.70 ± 0.01^{A}
	P (C)	8.63 ± 0.07^{Xx}	8.48 ± 0.05^{Yx}	6.90 ± 0.02^{Xz}	6.59 ± 0.04^{y}	6.56 ± 0.04^{y}	2.55 ± 0.12	n. d.
	P + LAWPC	8.08 ± 0.04^{Yy}	7.77 ± 0.01^{Yz}	7.30 ± 0.01^{Xx}	6.04 ± 0.07^{z}	5.85 ± 0.07^{z}	n. d.	n. d.
	P + LAWPC + L. p	7.99 ± 0.02^{Zz}	7.81 ± 0.02^{Zy}	7.08 ± 0.03^{Xy}	6.63 ± 0.09^{Yx}	6.66 ± 0.02^{Yx}	n. d.	6.57 ± 0.06^{X}

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11. Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among R cheese samples, and different lowercase letters (x–z) indicate significant differences ($p < 0.05$) among P cheese samples. Different uppercase letters (A–C) indicate significant differences ($p < 0.05$) among R cheese storage times within the same treatment, while different uppercase letters (X–Z) indicate significant differences ($p < 0.05$) among P cheese storage times within the same treatment. Results are presented as mean of three replicates ± SD.

On day 1, in regular cheeses matrix, the lowest TBC was observed in the control samples, while significantly higher ($p < 0.05$) values were recorded in samples coated with LAWPC and LAWPC + L. p coatings. A similar pattern was observed in probiotic cheese, where coated samples exhibited higher TBC compared to the control. LAB counts on same day were significantly higher ($p < 0.05$) in both cheeses covered with LAWPC + L. p coating. *L. plantarum* counts were detected only in probiotic cheese samples at approximately 6.15 $\log_{10}$ CFU/g.

After 45 days of cheese ripening, TBC counts significantly increased ($p < 0.05$) in all samples, ranging from 7.78 to 8.82 $\log_{10}$ CFU/g in regular cheese matrix and from 7.80 to 8.90 $\log_{10}$ CFU/g in probiotic cheeses matrix. In both cheese types, the lowest TBC counts were observed in control samples compared to their coated counterparts ($p < 0.05$). LAB counts significantly decreased ($p < 0.05$) in regular cheese samples, with the lowest counts in the control. Conversely, LAB counts significantly increased ($p < 0.05$) in probiotic cheese, although the control sample still exhibited the lowest LAB counts. In probiotic cheeses, *L. plantarum* counts increased significantly ($p < 0.05$) in the P (C) sample to 6.80 $\log_{10}$ CFU/g, while decreasing in coated samples. Nevertheless, among the samples, the significantly highest ($p < 0.05$) counts were observed in P + LAWPC (7.09 $\log_{10}$ CFU/g) sample.

By day 135 of storage, TBC counts significantly increased ($p < 0.05$) in the matrix of both regular and probiotic control cheeses compared with coated samples. LAB counts significantly decreased ($p < 0.05$) in both cheese groups, with the controls showing the highest counts. The counts of *L. plantarum* increased significantly ($p < 0.05$) in all probiotic cheese samples and differed among samples ($p < 0.05$), with the highest counts observed in the sample P + LAWPC, followed by P + LAWPC + L. p and P (C).

On day 1, TBC and LAB counts were not detected on the surface of the control and plain LAWPC-coated samples in either cheese type. In contrast, cheeses coated with LAWPC + L. p showed detectable surface TBC, with 5.90 $\log_{10}$ CFU/g on regular cheese and 5.86 $\log_{10}$ CFU/g on probiotic cheese. Corresponding LAB counts were 5.81 $\log_{10}$ CFU/g and 5.86 $\log_{10}$ CFU/g, respectively. On the cheese surface, *L. paracasei* counts were detected only in samples coated with LAWPC + L. p coating. In regular cheeses, counts reached 5.65 $\log_{10}$ CFU/g, while in probiotic cheeses the corresponding counts were 5.60 $\log_{10}$ CFU/g. Mold and yeast were not detected on any surface samples at this time.

On day 45, following ripening, surface TBC increased significantly ($p < 0.05$) in cheeses coated with LAWPC + L. p in both cheese types, and the samples differed significantly ($p < 0.05$), with highest TBC counts observed in the control samples for both cheese types. Surface LAB counts also

significantly increased ($p < 0.05$) but remained detectable only in LAWPC + L. p-coated regular and probiotic cheeses. The counts of *L. paracasei* increased significantly ($p < 0.05$) to 6.52 $\log_{10}$ CFU/g in regular cheese R + LAWPC + L. p sample and to 6.27 $\log_{10}$ CFU/g in the probiotic cheese P + LAWPC + L. p sample. Mold and yeast were still not detected on any surface samples.

By day 135, surface TBC differed significantly ($p < 0.05$) among samples within the cheese groups, with the lowest counts observed in control samples. Similarly, surface LAB counts significantly differed ($p < 0.05$) between samples, showing an increase on the surface of regular cheeses and a decrease on probiotic cheese surfaces coated with LAWPC + L. p ($p < 0.05$). Mold and yeast counts were detected on the surface of control samples for both cheese types (2.30 $\log_{10}$ CFU/g in regular cheese and 2.55 $\log_{10}$ CFU/g in probiotic cheese), as well as in the LAWPC-coated regular cheese (2.97 $\log_{10}$ CFU/g). Mold and yeast were not detected in regular and probiotic cheese surface samples coated with LAWPC + L. p coating. At the end of storage, *L. paracasei* counts increased significantly ($p < 0.05$) and were detected on the surface of cheeses coated with LAWPC + L. p, with counts of 6.70 $\log_{10}$ CFU/g on regular cheese and 6.57 $\log_{10}$ CFU/g on probiotic cheese. No *L. paracasei* was detected in control or LAWPC-coated samples throughout storage.

3.7.2. Sensory evaluation of coated Edam-type cheese

Sensory attributes of regular and probiotic Edam-type cheese samples were evaluated at the end of cheese ripening (day 45) and at the end of storage (day 135) to determine the impact of edible coatings on overall sensory quality (Table 3.7.2.1).

On day 45, among regular cheese samples, the R + LAWPC + L. p sample showed the highest scores for flavor and overall acceptability, with overall acceptability reaching 85.54 ± 7.14, which was significantly higher ($p < 0.05$) than that of the control sample (61.52 ± 4.32). A similar trend was observed for the probiotic cheese samples, where P + LAWPC + L. p sample resulted in significantly higher ($p < 0.05$) scores for flavor and overall acceptability compared with the remaining samples, as well as the highest appearance score compared with the control sample.

After 135 days of storage, the R + LAWPC + L. p sample received significantly higher ($p < 0.05$) scores for flavor, appearance, and overall acceptability compared with the R (C) sample. In addition, the R + LAWPC sample showed a significantly higher appearance score (8.06 ± 0.38) compared with the control (6.89 ± 0.50) and a significantly higher ($p < 0.05$) body and

texture score between storage days. However, a significant decrease ($p < 0.05$) in appearance was observed in regular cheese samples at the end of storage. In probiotic cheeses, the P + LAWPC + L. p sample also resulted in significantly higher ($p < 0.05$) scores for flavor and appearance compared with the P (C) sample, as well as significantly higher ($p < 0.05$) scores for body and texture and overall acceptability compared with the remaining samples. Similar to the regular cheese samples, a significant decrease ($p < 0.05$) in appearance was observed at the end of storage, except for P + LAWPC + L. p sample.

Table 3.7.2.1. *Sensory evaluation of regular and probiotic Edam-type cheeses coated with edible coatings at the end of ripening (day 45) and storage (day 135)*

Day	Sample	Flavor	Body and texture	Appearance	Overall acceptability
45	R (C)	29.17 ± 2.30^{b}	23.94 ± 4.08	8.41 ± 0.67^{A}	61.52 ± 4.32^{b}
	R + LAWPC	36.62 ± 3.87^{ab}	24.55 ± 2.12^{B}	9.34 ± 0.32^{A}	70.51 ± 6.18^{ab}
	R + LAWPC + L. p	43.31 ± 4.22^{a}	32.73 ± 4.01	9.42 ± 0.04^{A}	85.45 ± 7.14^{a}
	P (C)	34.34 ± 0.79^{y}	24.75 ± 3.64	8.11 ± 0.39^{Xy}	67.20 ± 4.46^{y}
	P + LAWPC	36.11 ± 1.94^{y}	24.65 ± 1.40	8.84 ± 0.44^{Xxy}	69.60 ± 2.77^{y}
	P + LAWPC + L. p	45.58 ± 0.58^{Xx}	31.72 ± 3.81	9.09 ± 0.30^{x}	86.39 ± 3.30^{x}
135	R (C)	32.83 ± 3.09^{c}	30.30 ± 2.37	6.89 ± 0.50^{Bb}	70.03 ± 5.90^{b}
	R + LAWPC	38.01 ± 0.95^{b}	31.62 ± 0.76^{A}	8.06 ± 0.38^{Ba}	77.68 ± 1.75^{ab}
	R + LAWPC + L. p	43.43 ± 0.44^{a}	33.94 ± 2.41	8.59 ± 0.29^{Ba}	85.96 ± 2.97^{a}
	P (C)	31.69 ± 2.09^{y}	28.38 ± 0.97^{y}	7.07 ± 0.32^{Yy}	67.15 ± 2.37^{y}
	P + LAWPC	36.36 ± 2.00^{xy}	26.87 ± 0.70^{y}	7.60 ± 0.44^{Yxy}	70.83 ± 2.25^{y}
	P + LAWPC + L. p	41.04 ± 2.09^{Yx}	33.84 ± 2.35^{x}	8.26 ± 0.55^{x}	83.13 ± 4.33^{x}

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11. Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among R cheese samples, and different lowercase letters (x–z) indicate significant differences ($p < 0.05$) among P cheese samples. Different uppercase letters (A–C) indicate significant differences ($p < 0.05$) among R cheese storage times within the same treatment, while different uppercase letters (X–Z) indicate significant differences ($p < 0.05$) among P cheese storage times within the same treatment. Results are presented as mean of three replicates ± SD.

3.8. Changes in coated Edam-type cheese properties during *in vitro* digestion

3.8.1. Microbiological analysis and Edam-type cheese microbiome

Considering the use of edible coatings containing *Lacticaseibacillus paracasei* A11 and *Lactiplantibacillus plantarum* Tensia as probiotic adjunct culture, the viable LAB, *L. paracasei*, and *L. plantarum* in regular and probiotic Edam-type cheeses before and after *in vitro* digestion on days 1 and 45 of ripening are presented in Table 3.8.1.1.

On day 1, LAB counts in regular cheese ranged from 8.47 to 8.69 $\log_{10}$ CFU/g, with significantly lower counts ($p < 0.05$) observed in control sample. *L. paracasei* was detected only in the R + LAWPC + L. p sample (5.99 $\log_{10}$ CFU/g), while *L. plantarum* was not detected in any regular cheese samples before and after digestion. Following *in vitro* digestion, LAB counts decreased significantly (paired t-test, $p < 0.05$) in all regular cheese samples, with reductions ranging from – 0.50 to – 0.96 $\log_{10}$ CFU/g. Among the samples, the R + LAWPC + L. p sample exhibited a significantly lower LAB count than the remaining regular cheese samples ($p < 0.05$). No significant difference after *in vitro* digestion was observed for *L. paracasei* with counts reaching 5.06 $\log_{10}$ CFU/g ($p > 0.05$).

In the probiotic cheese group on day 1, prior to digestion, LAB counts were significantly highest ($p < 0.05$) in the P + LAWPC sample (8.68 $\log_{10}$ CFU/g), whereas the lowest counts were observed in the P + LAWPC + L. p sample (8.47 $\log_{10}$ CFU/g). *L. plantarum* was detected in all probiotic cheese samples, with counts ranging from 6.14 to 6.28 $\log_{10}$ CFU/g, whereas *L. paracasei* was detected only in the P + LAWPC + L. p sample (6.29 $\log_{10}$ CFU/g). After *in vitro* digestion, a similar trend to that observed in regular cheeses was noted for LAB counts, with a significant reduction (paired t-test, $p < 0.05$) in all probiotic cheese samples ranging from –0.68 to –0.86 $\log_{10}$ CFU/g. Conversely, *L. plantarum* exhibited a significant increase following digestion in all probiotic cheeses (paired t-test, $p < 0.05$), ranging from 0.48 to 0.81 $\log_{10}$ CFU/g, with significantly higher count ($p < 0.05$) observed in the P + LAWPC + L. p sample compared with the other probiotic cheese samples. With counts of 6.46 $\log_{10}$ CFU/g, no significant effect of *in vitro* digestion was noted for *L. paracasei* ($p > 0.05$).

Table 3.8.1.1. *Microbiological changes in regular and probiotic Edam-type cheeses coated with edible coatings, before and after in vitro digestion at days 1 and 45 of ripening*

Day	Sample	Before *in vitro* digestion (log_{10} CFU/g)			After *in vitro* digestion (log_{10} CFU/g)		
		LAB	*L. paracasei*	*L. plantarum*	LAB	*L. paracasei*	*L. plantarum*
1	R (C)	8.47 ± 0.01^{Bb}	n. d.	n. d.	$7.97 \pm 0.08^{Aa*}$	n. d.	n. d.
	R + LAWPC	8.68 ± 0.08^{Ba}	n. d.	n. d.	$8.04 \pm 0.03^{Aa*}$	n. d.	n. d.
	R + LAWPC + L. p	8.69 ± 0.08^{Ba}	5.99 ± 0.98^{B}	n. d.	$7.73 \pm 0.11^{b*}$	5.06 ± 0.19^{B}	n. d.
	P (C)	8.58 ± 0.02^{Yy}	n. d.	6.14 ± 0.06^{Y}	$7.90 \pm 0.15^{X*}$	n. d.	$6.63 \pm 0.01^{Yz*}$
	P + LAWPC	8.68 ± 0.01^{x}	n. d.	6.28 ± 0.16^{Y}	$7.83 \pm 0.02^{X*}$	n. d.	$6.81 \pm 0.05^{Yy*}$
	P + LAWPC + L. p	8.47 ± 0.01^{Yz}	6.29 ± 0.40	6.20 ± 0.05^{Y}	$7.74 \pm 0.08^{X*}$	6.46 ± 0.01^{X}	$7.01 \pm 0.11^{Xx*}$
45	R (C)	8.82 ± 0.08^{A}	n. d.	n. d.	$7.20 \pm 0.24^{B*}$	n. d.	n. d.
	R + LAWPC	8.84 ± 0.04^{A}	n. d.	n. d.	$7.47 \pm 0.19^{B*}$	n. d.	n. d.
	R + LAWPC + L. p	8.88 ± 0.02^{A}	7.59 ± 0.08^{A}	n. d.	7.45 ± 0.15*	$5.88 \pm 0.22^{A*}$	n. d.
	P (C)	8.77 ± 0.01^{X}	n. d.	6.97 ± 0.03^{Xx}	$7.06 \pm 0.05^{Y*}$	n. d.	6.98 ± 0.07^{Xy}
	P + LAWPC	8.75 ± 0.07	n. d.	7.06 ± 0.15^{Xx}	$7.31 \pm 0.08^{Y*}$	n. d.	7.16 ± 0.02^{Xx}
	P + LAWPC + L. p	8.74 ± 0.05^{X}	6.00 ± 0.01	6.46 ± 0.10^{Xy}	$7.38 \pm 0.20^{Y*}$	$5.02 \pm 0.15^{Y*}$	$6.11 \pm 0.03^{Yz*}$

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11. Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among R cheese samples, and different lowercase letters (x–z) indicate significant differences ($p < 0.05$) among P cheese samples. Different uppercase letters (A–C) indicate significant differences ($p < 0.05$) among R cheese storage times within the same treatment, while different uppercase letters (X–Z) indicate significant differences ($p < 0.05$) among P cheese storage times within the same treatment. Asterisks (*) mark significant differences (paired t-test, $p < 0.05$) between values before and after *in vitro* digestion. Results are presented as mean of three replicates ± SD.

After 45 days of cheese ripening, LAB counts before digestion increased significantly ($p < 0.05$) in all regular cheese samples and reached approximately 8.85 $\log_{10}$ CFU/g. In addition, *L. paracasei* counts increased significantly ($p < 0.05$) to 7.59 $\log_{10}$ CFU/g, while *L. plantarum* remained still undetectable before and after digestion. *In vitro* digestion resulted in a significant reduction in LAB counts in all regular cheese samples (paired t-test, $p < 0.05$), as well as a significant decrease ($p < 0.05$) between days in the R (C) and R + LAWPC samples. *L. paracasei* counts increased significantly when comparing among days ($p < 0.05$), but decreased significantly after digestion ($p < 0.05$), reaching 5.88 $\log_{10}$ CFU/g.

In the probiotic cheese group on day 45, LAB counts increased significantly ($p < 0.05$) in the P (C) and P + LAWPC + L. p samples compared with values before cheese ripening. *L. paracasei* counts remained constant at 6.00 $\log_{10}$ CFU/g in the P + LAWPC + L. p sample, although *L. plantarum* counts increased significantly ($p < 0.05$) in all probiotic cheese samples compared with day 1. Significantly higher *L. plantarum* counts ($p < 0.05$) were observed in the P + LAWPC and P (C) samples (7.06 and 6.97 $\log_{10}$ CFU/g, respectively) than in the P + LAWPC + L. p sample (6.46 $\log_{10}$ CFU/g). Following *in vitro* digestion, LAB counts decreased significantly in in all probiotic cheese samples (paired t-test, $p < 0.05$), with a significant decrease also observed between days ($p < 0.05$). In the P + LAWPC + L. p sample, both storage time ($p < 0.05$) and *in vitro* digestion (paired t-test, $p < 0.05$) significantly reduced *L. paracasei* counts, which reached 5.02 $\log_{10}$ CFU/g. The counts of *L. plantarum* increased significantly ($p < 0.05$) in the P (C) and P + LAWPC samples compared with day 1, with the highest counts observed in the P + LAWPC sample (7.16 $\log_{10}$ CFU/g). Meanwhile, the P + LAWPC + L. p sample exhibited the lowest *L. plantarum* counts (6.11 $\log_{10}$ CFU/g) compared with the other probiotic cheese samples, showed a significant decrease ($p < 0.05$) relative to day 1, and displayed a digestion-related reduction (paired t-test, $p < 0.05$).

The relative abundance of dominating (Fig. 3.8.1.1a) and non-dominating genera (Fig. 3.8.1.1b) in coated regular and probiotic Edam-type cheeses was evaluated on days 1 and 45 of ripening.

In both cheese types, the microbiota was dominated by the starter lactic acid bacteria (SLAB) culture *Lactococcus*, followed by *Streptococcus* (Fig. 3.8.1.1a).

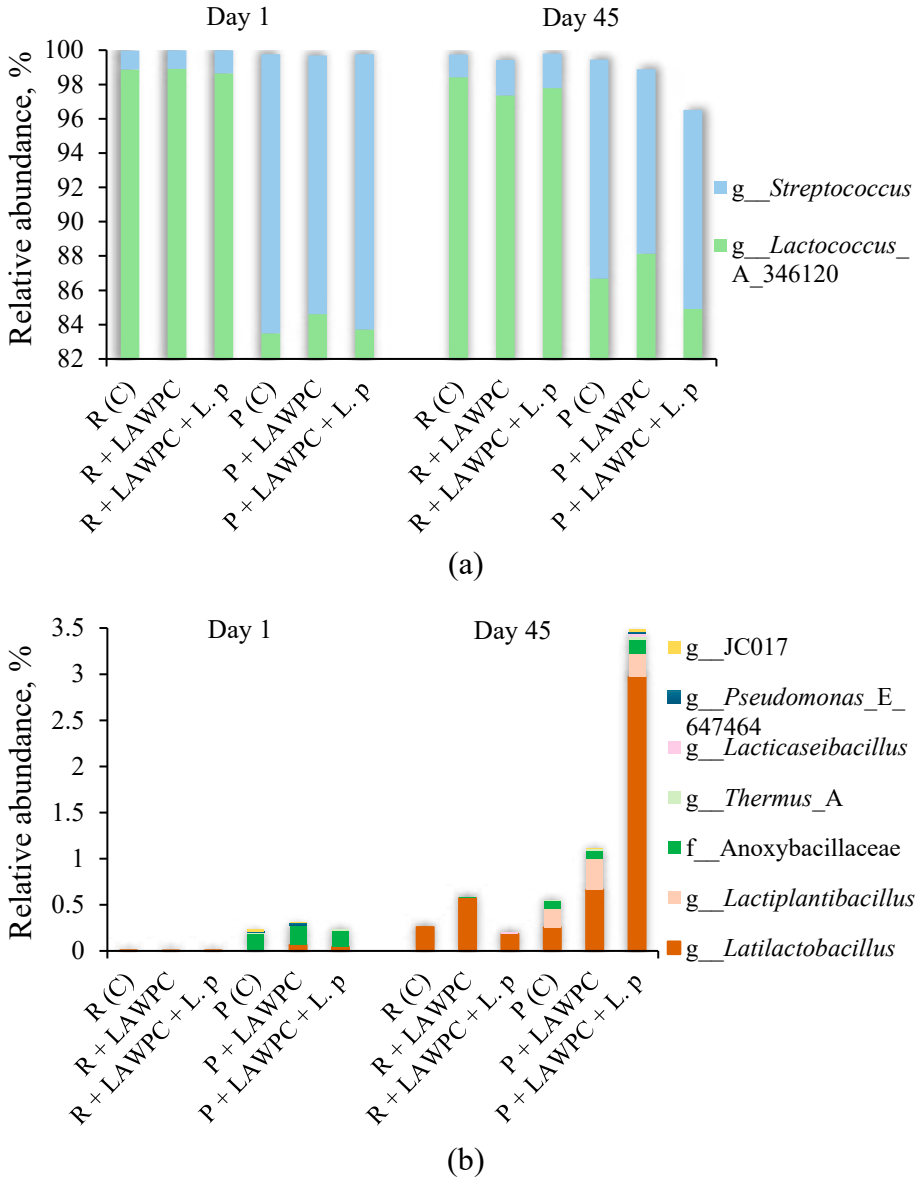


Fig. 3.8.1.1. *Relative abundance and diversity of dominating genera (a) and non-dominating genera (b) of coated regular and probiotic Edam-type cheeses after in vitro digestion, before and after ripening*

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11; Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Results are presented as mean of duplicates.

In regular cheese, *Lactococcus* represented more than 97% of the microbiota at both sampling times, while *Streptococcus* remained at low abundance. After cheese ripening (day 45), a slight increase in *Streptococcus* relative abundance was observed in coated samples, accompanied by a corresponding decrease in *Lactococcus*. However, Kruskal-Wallis test showed no significant differences among samples and days ($p > 0.05$).

In probiotic cheese, *Lactococcus* also remained the dominant genus, but at a lower proportion (approximately 85.26%) compared with regular cheese. Conversely to regular cheese, *Streptococcus* slightly decreased after ripening. Nevertheless, no significant differences among samples and days were detected (Kruskal-Wallis test, $p > 0.05$).

Several low-abundance non-dominating genera were also detected, including *JC017*, *Pseudomonas E647464*, *Lacticaseibacillus*, *Thermus A*, *Anoxybacillaceae, Lactiplantibacillus, and Latilactobacillus* (Fig. 3.8.1.1b).

In regular cheese, most minor genera remained below 1% throughout ripening. Only *Anoxybacillaceae* exhibited a significant difference between ripening days (Kruskal–Wallis test, $p < 0.05$), while the remaining genera did not differ significantly among samples at either day 1 or day 45 ($p > 0.05$). No significant differences among samples or between days were observed for the other non-dominant minor genera ($p > 0.05$).

In probiotic cheese, the relative abundance of non-dominating genera was generally higher and variable, especially after ripening, where *Latilacto-bacillus* increased in coated samples. However, none of the detected genera differed significantly among samples and days according to the Kruskal-Wallis test ($p < 0.05$).

The genera classified as *Lacticaseibacillus* and *Lactiplantibacillus* most likely correspond to the bacteria incorporated into the coating and adjunct culture, respectively, based on sequence identity and experimental design. Both remained at low abundance (< 1%) and did not become dominant during ripening. Confirmation at the strain level would require whole-genome sequencing.

Principal component analysis (PCA) revealed clear clustering of regular and probiotic Edam-type cheese samples on days 1 and 45 (Fig. 3.8.1.2). Regular cheese samples formed a compact cluster (green color), with a visible shift between day 1 and day 45, indicating changes in microbial community structure during ripening. In contrast, probiotic cheese samples were more dispersed (blue color) and did not show a consistent separation between samples or sampling days.

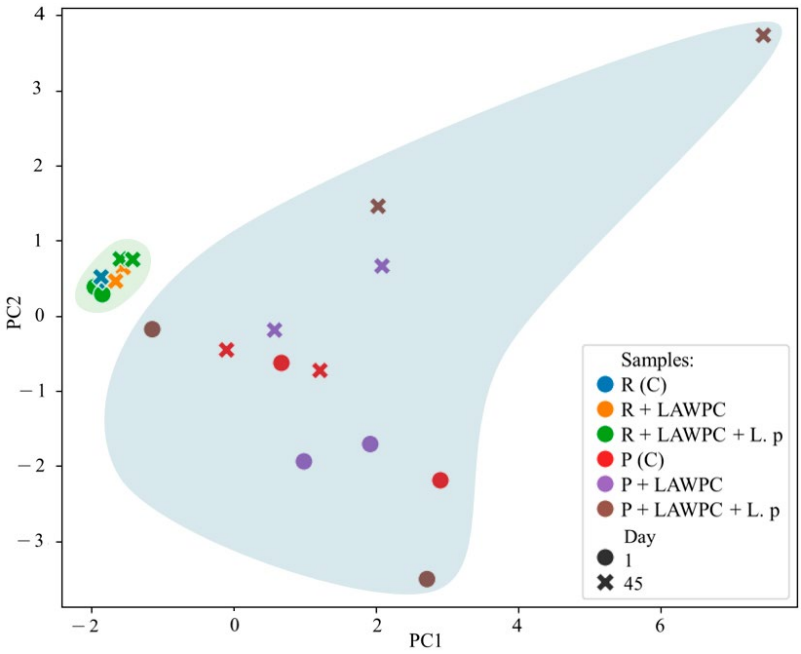


Fig. 3.8.1.2. *Bacterial community clustering using principal component analysis (PCA) in coated regular and probiotic Edam-type cheeses before and after ripening*

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11; Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Results are presented in duplicates. Colors indicate samples and symbols indicate sampling time.

Within regular cheese samples, PERMANOVA confirmed a significant effect of ripening time on bacterial community composition ($p < 0.05$), whereas sample type had no significant effect ($p > 0.05$). A significant sample × day interaction was observed ($p < 0.05$). However, pairwise PERMANOVA comparisons showed no significant differences between individual samples at either sampling day ($p > 0.05$).

In probiotic cheese, microbial community structure was not significantly affected by sample type, ripening day, and their interaction (PERMAVOVA, $p > 0.05$).

3.8.2. Protein digestibility of Edam-type cheese

The different complexity of coated regular and probiotic Edam-type cheese samples is reflected in variations in protein solubilization and digestibility

during *in vitro* digestion after 45 days of ripening and the results are presented in Figure 3.8.2.1.

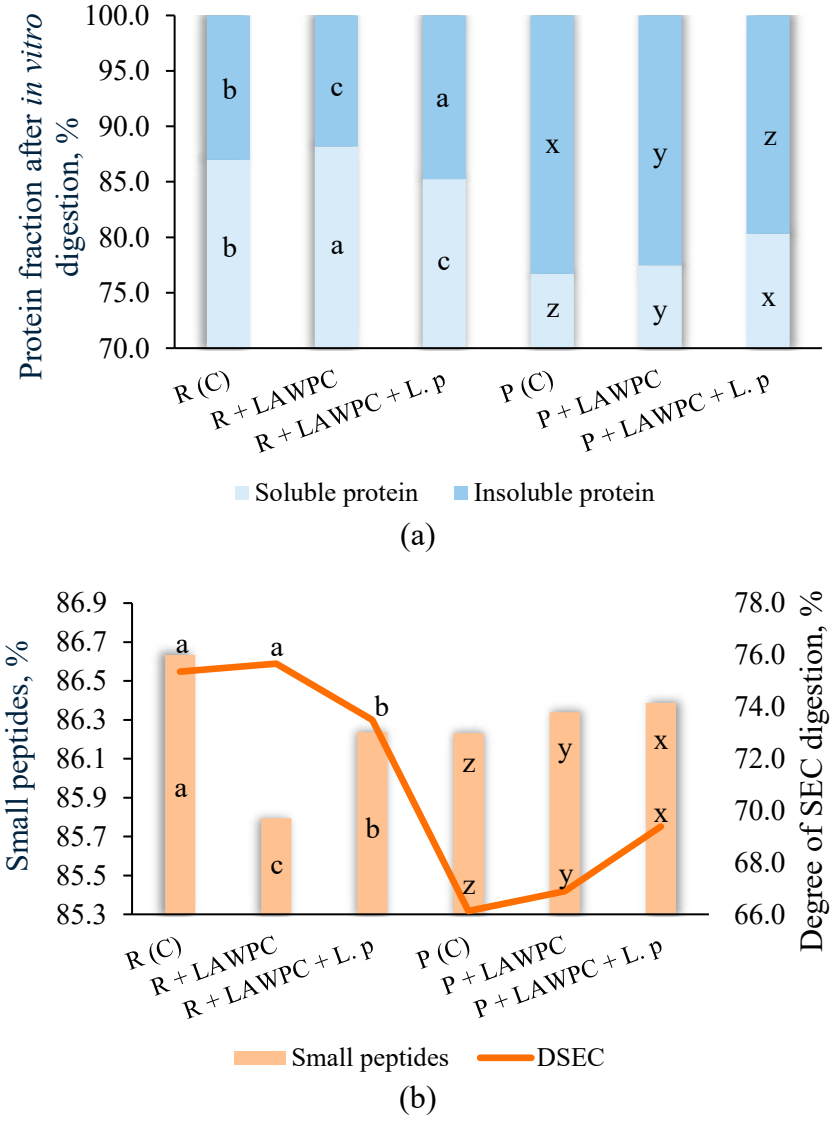


Fig. 3.8.2.1. *Protein fractions (a) and in vitro digestion efficiency (b) of regular and probiotic Edam-type cheese after ripening*

LAWPC – liquid acid whey protein concentrate; R – regular cheese; P – probiotic cheese; C – control; L. p – *Lacticaseibacillus paracasei* A11; D_{SEC} – digestibility based on size exclusion chromatography. Because different milk batches were used, R and P cheeses were analyzed and interpreted independently, and no direct statistical comparison was made between them. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) among R cheese samples, and different lowercase letters (x–z) indicate significant differences ($p < 0.05$) among P cheese samples. Results are presented as mean of duplicates.

In regular cheese samples, protein solubilization increased significantly ($p < 0.05$) in R + LAWPC (88.2%), whereas a decrease was observed in R + LAWPC + L. p (85.2%), compared with the control R (C) (87.0%) (Fig. 3.8.2.1a). Conversely, the probiotic cheese samples the soluble protein fraction was significantly highest ($p < 0.05$) in P + LAWPC + L. p (80.3%), followed by P + LAWPC (77.5%) and P (C) (76.7%).

Among the regular cheese samples, the control R (C) showed the significantly highest small peptide content (86.6%, $p < 0.05$). Application of the LAWPC + L. p coating significantly decreased ($p < 0.05$) the value to 86.2%, and the LAWPC coating reduced it further to 85.8% compared to control (Fig. 3.8.2.1b). In contrast, in the probiotic cheese samples, a significant increase ($p < 0.05$) in small peptide content was observed in P + LAWPC (86.3%) and P + LAWPC + L. p (86.4%) compared with P (C) (86.2%).

In the regular cheese samples, digestibility based on size exclusion chromatography (D_{SEC}) decreased significantly ($p < 0.05$) in R + LAWPC + L. p (73.5%) compared with R + LAWPC (75.7%) and R (C) (75.4%). As opposite, in probiotic cheese samples edible coatings significantly increased ($p < 0.05$) D_{SEC}, with the highest value observed in P + LAWPC + L. p (69.4%), followed by P + LAWPC (66.9%) and P (C) (66.1%).

4. DISCUSSION

4.1. Screening and functional characterization of lactobacilli for incorporation into edible coatings

Many LAB strains have been classified as probiotics and fermented vegetables, meat and milk products are an excellent source of such LAB [7,233]. However, probiotic properties are strain-specific and cannot be assumed at the species level [234]. Only selected strains are able to tolerate gastrointestinal conditions, bile salts and show antimicrobial activity [235]. For this reason, INFOGEST *in vitro* digestion protocol was applied in our study as a selective tool to isolate acid and bile-tolerant, potential probiotic lactobacilli from fermented milk, ensuring that only strains capable of surviving simulated gastrointestinal conditions were selected for further evaluation and potential application in edible coatings. From 20 processed samples, 22 colonies were grown, of which more than half showed phenotypic characteristics typical of *Lactobacillus*. Molecular identification confirmed four isolates as *Lactobacillus* spp., including three *Lacticaseibacillus paracasei* and one *Lactiplantibacillus plantarum*, indicating that fermented milk remains a reliable source of technologically relevant potential probiotics. The predominance of *L. paracasei* among the identified isolates is consistent with previous studies reporting its isolation from fermented food products, including fermented milk and cheese [236].

Although probiotic bacteria have demonstrated health benefits, the use of newly isolated microorganisms may lead to undesirable effects, such as antibiotic resistance and hemolytic activity [237,238]. In this study, γ-hemolysis among all tested lactobacilli (*L. paracasei* A161-1, *L. paracasei* A11, *L. paracasei* A173-2, and *L. plantarum* A154-d1) confirms their suitability for food-related applications. Reported studies showed that most of the isolates from various dairy matrices were also γ-hemolytic [239–241]. Antibiotic susceptibility profiling supported strain-specific differences in safety potential. All of the tested strains were sensitive to ampicillin, erythromycin, clindamycin, streptomycin, gentamycin, kanamycin, and tetracycline. However, all strains exhibited resistance to vancomycin above the breakpoint established by EFSA (2012). The intrinsic resistance to vancomycin observed in all isolates is well documented in lactobacilli and is attributed to the intrinsic resistance mechanisms [64,242]. In addition, *L. paracasei* A161-1 and *L. paracasei* A173-2 exceeded cut-off values for chloramphenicol, a finding that is consistent with reports documenting chloramphenicol resistance among lactobacilli strains [243,244]. Based on

these safety assessments, only *L. paracasei* A11 and *L. plantarum* A154-d1 were selected for further experiments.

Certain enzymes produced by microorganisms can affect human health. For instance, β-glucuronidase and β-glucosidase convert aromatic hydrocarbons and amines into active carcinogenic compounds, thereby increasing the risk of colon cancer [245]. In this study, enzymatic profiling showed no activity of β-glucuronidase, though *L. plantarum* A154-d1 was found to produce strong β-glucosidase activity. Other studies reported β-glucuronidase and β-glucosidase activity in 99 tested LAB strains [246]. In contrast, both isolates exhibited strong leucine and valine arylamidase activity, with higher activity observed in *L. paracasei* A11. Such aminopeptidase activity is considered desirable for starter or adjunct applications, as these enzymes release free amino acids during cheese ripening, which can be further metabolized by bacterial enzymes and contribute to flavor development [247]. Given that the lactobacilli were screened for incorporation into edible coatings subsequently applied to Edam-type cheese, the enzymatic profile of *L. paracasei* A11 was particularly relevant.

LAB possess enzymatic and metabolic activities that are important for dairy applications, including proteolysis, lipolysis, and acidification, and the technological profile of *L. paracasei* A11 indicates its suitability when specific biochemical activity is required. Positive caseinolytic activity of the tested strain is considered an important technological trait intended for cheese production, as it enables the breakdown of milk proteins into peptides and amino acids that support bacterial metabolism and contribute to flavor development during ripening [248]. The strain showed negative extracellular proteolytic and lipolytic activities, similar to those results obtained by other authors [249]. Lipolytic activity in certain LAB enables the hydrolysis of glycerides and FA, which can influence flavor development in dairy products [250]. Since oils are included in edible coatings mainly for their hydrophobic properties, the absence of lipolytic activity in *L. paracasei* A11 indicates that the strain is unlikely to hydrolyze lipids in the coating matrix, thereby reducing the risk of lipid-derived off-flavors such as bitterness. In addition, the moderate-to-high acidifying activity of the strain represents another desirable trait since this attribute may contribute to inhibition of spoilage or undesirable microorganisms through a reduction pH [251].

The ability of LAB to ferment carbohydrates is essential, since energy generation in these microorganisms depends largely on carbohydrate metabolism [252]. Results showed that *L. paracasei* A11 was capable to catabolize fifteen carbohydrates, which is consistent with results reported by other researchers [253]. The ability of the strain to ferment lactose, glucose, and galactose is particularly relevant for dairy applications, as these

carbohydrates are commonly present in milk and cheese [254]. As lactose is a major carbohydrate component of whey and whey permeates [255], this fermentation profile indicates that *L. paracasei* A11 is well suited for incorporation in whey-based edible coatings.

Microbial contamination of cheese surface by spoilage microorganisms and fungi can cause undesirable changes in appearance, texture, flavor, and odor [167,256]. Because of that, different food spoilage microorganisms, pathogenic bacteria, yeasts, and molds were selected to assess antibacterial and antifungal activity of *L. paracasei* A11. The results showed strong antibacterial activity of *L. paracasei* A11 against foodborne pathogen *L. monocytogenes*, a major safety concern in the ready-to-eat dairy products [257], supporting the potential use of this lactobacilli strain in edible coatings as a bioprotective culture. The pronounced inhibitory activity against *D. hansenii* is noteworthy, as this yeast is frequently associated with semi-hard cheese surface spoilage and has been reported to negatively affect the organoleptic properties of fully ripened Raclette cheese [258,259]. Moderate inhibition by *L. paracasei* A11 of microorganisms such as *P. fluorescens*, *B. thermosphacta*, *E. coli*, *P. aeruginosa*, *B. cereus*, and *A. versicolor* are particularly relevant to the cheese industry due to their association with food spoilage, hygiene issues, and food safety concerns in dairy products [260–262]. Low or no inhibitory effects were observed against fungi such as *C. herbarum*, *Y. lipolytica*, *C. glabrata*, *P. commune*, *C. albicans*, *M. racemosus* and for bacteria including *S.* Typhimurium and *S. aureus*. These findings are consistent with those reported by Ramos-Pereira et al. (2021), where only seven out of 56 tested strains exhibited antifungal activity, with *L. paracasei* showing the strongest effect [263]. In contrast, other studies have described strong antifungal activity of *L. paracasei* against *Mucor racemosus* [264], an effect that was not observed in the present study, highlighting the strain-dependent nature of antifungal activity [265]. Furthermore, LAB exposed to stress were found to be associated with FA, amino acids, and carbohydrates content, which may play an important role in antibacterial and antifungal behavior, suggesting that lactobacilli could express stronger properties in real food matrices than under *in vitro* conditions [266].

In humans, gastric acid pH typically ranges from 1.5 to 2.0 [267], and after passage through the stomach, microorganisms are exposed to bile concentrations of 0.3 – 0.5% in the upper intestine [268]. Therefore, the ability to survive under acidic conditions and in the presence of bile salts is considered a key functional property of probiotic microorganisms [269]. Our study showed that *L. paracasei* A11 expressed acid resistance up to 100%, which is consistent with previous reports showing *L. paracasei* high resistance to acidity [237,270] and supports its potential to remain viable during

digestion. Interestingly, research shows that *L. paracasei* strains isolated from dairy samples exhibited the highest tolerance to bile salts, with survival rates of at least 50% [271]. Therefore, *L. paracasei* A11 was expected to display the same result. Although exposure to higher bile salts concentrations reduced the viable counts, the survival rate reached 73%. Overall, the high resistance of *L. paracasei* A11 to both bile salts and acidic conditions indicates strong gastrointestinal resilience. This outcome supports the application of the INFOGEST *in vitro* digestion protocol to fermented cow's milk prior to strain isolation as an effective screening strategy for selecting potential probiotic strains, while significantly reducing the number of isolates and subsequent analytical workload.

4.2. Cultivation behavior of *L. paracasei* A11 in whey-based media

MRS broth is widely used for the cultivation of LAB due to its rich nutrient composition, although its relatively high cost and time-consuming biomass processing procedures limit its application to specific laboratory settings [272,273]. In contrast, whey-derived products such as LAWPC and LAWP represent sustainable cultivation media for certain acid-tolerant LAB and enable the valorization of acid whey, which is frequently disposed as waste [274]. Flask cultivation was used to assess the biomass yield of *L. paracasei* A11 in different whey-based media, while bioreactor cultivation was used to evaluate the growth kinetics of the strain and suitability for controlled and large-scale technological applications [275]. In both cultivation systems, the growth behavior of *L. paracasei* A11 reflected variations in nutrient availability and buffering capacity of the tested media. As expected, MRS broth supported rapid growth and strong acidification, which is rich in nutrients, required for the growth of lactobacilli strains [276]. Conversely, the limited growth of *L. paracasei* A11 in LAWP further underscores the importance of nutrient composition for LAB proliferation [277]. Even though LAWP contains lactose as an energy source, it is deficient in nitrogenous compounds, peptides, and growth factors compared with LAWPC, which likely limited *L. paracasei* A11 growth under these conditions. On the other hand, LAWPC supported slower but stable growth and limited acidification, but still resulted a gradual increase in viable counts due to high content of vitamins, minerals, and lactose [132]. It has been reported that LAB growth in acid whey can be enhanced by supplementation with 0.5% milk phospholipids [278], 50% dairy ingredients [279], and low levels of yeast extract [280,281]. In our case, LAWPC was not enriched with growth promoting additives, yet supported *L. paracasei* A11 cultivation. This behavior was confirmed under bioreactor conditions, demonstrating its potential as sustainable and ready to

use medium for LAB growth that can be applied in subsequent technological processes.

The metabolic activity of LAB in cultivation media is largely governed by carbohydrate utilization and organic acid production, which determine bacterial growth, acidification behavior, and technological functionality [4,282]. The differences in carbohydrate utilization and metabolite production of *L. paracasei* A11 in MRS broth, LAWPC and LAWP indicate a significant influence of substrate composition on bacterial metabolism. In previous studies, MRS broth was supplemented with carbohydrates to evaluate their effects on LAB growth and lactic acid production, and glucose, fructose and certain disaccharides appeared to be the most effective substrates [283]. In our study, glucose was already present in MRS broth, which supported rapid sugar utilization and high production of D/L lactic acid and D/L lactate. In contrast, whey-based media contained lower glucose and galactose levels but higher lactose levels. Despite this difference, LAWPC supported active cultivation and high production of D/L lactic acid and D/L lactate, indicating that *L. paracasei* A11 can efficiently utilize whey-derived substrates. The lower organic acid production observed in LAWP is consistent with reduced nutrient availability, particularly carbohydrate deficiency.

4.3. Influence of *L. paracasei* A11 on the properties of whey-based edible coating solutions and films

LAWPC and LAWP were used in edible coating formulations to compare the properties of different whey products and evaluate their suitability for coating development. Four coating formulations were prepared: LAWPC (C), a liquid acid whey protein concentrate-based control coating; LAWP (C), a liquid acid whey permeate-based control coating; LAWPC + L. p, a liquid acid whey protein concentrate-based coating containing the incorporated *L. paracasei* A11; and LAWP + L. p, a liquid acid whey permeate-based coating containing the incorporated *L. paracasei* A11.

Analysis of the rheological properties of edible coating solutions is important since viscosity and flow properties determine the coating application, spreading behavior, and uniformity of the resulting film on food surfaces [284,285]. All film-forming solutions exhibited shear-thinning behavior that are favorable for practical application [286]. The lower shear stress values of LAWP compared to LAWPC solutions indicated lower viscosity, making them suitable for spraying [287]. In contrast, LAWPC control solutions were the most viscous and better suited for product immersion due to the higher solids content of the total solution. The addition of *L. paracasei* A11 to LAWPC solution resulted in a decrease in viscosity, as indicated by lower shear stress

values at all shear rates compared to LAWPC control solution. This indicates that LAB affects the viscosity of the film-forming solutions, most likely by modifying the structural network of the solution. These results are consistent with other studies, which observed that LAB encapsulated in gelatin film-forming solutions also significantly reduced viscosity [288].

It is necessary to evaluate the physicomechanical and optical properties of edible films, as they can directly affect the food product [289,290]. The observed differences between LAWP and LAWPC films reflect variations in base composition and bacterial incorporation. Films produced from LAWPC were thicker and had lower moisture content, resulting in reduced flexibility than those prepared from LAWP, due to higher dry matter of the solution. This behavior can be attributed to the formation of a more compact matrix in the LAWPC film, resulting from the interaction of proteins, polysaccharides, and other solid particles, which increased the overall structural density of the film [291]. However, the addition of *L. paracasei* A11 to the LAWPC-based film improved the elasticity, possibly due to enzymes produced by LAB, such as proteases or glycosidases, that partially alter proteins or polysaccharides in the film matrix, leading to a more flexible and less compact network structure [40,292]. Optical properties were also affected by the composition of the film. LAWPC films showed higher opacity and lower lightness than LAWP, which was expected since the thickness of the LAWPC films was higher. Nevertheless, the incorporated LAB strain notably reduced the opacity of the LAWPC film compared to control. In contrary, Ebrahimi et al. (2017) and Sanchez-Gonzalez et al. (2014) reported that encapsulation of live microorganisms in films can increase light scattering, thereby increasing opacity [232,293]. In our study, the presence of *L. paracasei* A11, cultivated in LAWPC likely impacted the film transparency due to the physical presence of bacterial cells and metabolites that interact with matrix components such as proteins and polysaccharides, altering light transmission. Moreover, the lower a* and higher b* color coordinates of LAWPC films compared with LAWP films can be attributed to the content of solid particles and the yellow color of LAWPC.

SEM analysis was performed to examine the microstructure of the LAWPC and LAWP film samples. Results showed that the LAWPC-based films exhibited irregular, uneven surfaces with crystallized areas, indicating an excess of solid particles in the composition. In contrast, the LAWP-based films, especially the control film sample, exhibited surface cavities that were more prominent than the LAWP + L. p sample. LAB are known to form biofilms, structured communities of microorganisms embedded in a self-produced extracellular polymeric matrix [294]. This biofilm formation may result in a smoother surface of the whey-pectin-based film, reducing the size

of the observed cavities. In addition, EPS produced by LAB may interact with the film material and alter its surface structure and morphology [89].

Evaluation of elemental composition is important for understanding the chemical nature and uniformity of edible films. EDS results confirmed that all films were dominated by carbon and oxygen, which are the components of both edible films and LAB biomass. Carbohydrates and lipids in the edible films are mainly composed by hydrogen, carbon, and oxygen [295], while nucleic acids such as DNA and RNA contain hydrogen, carbon, oxygen, phosphorus, and sodium [296]. Therefore, differences in elemental composition between films may be influenced by the presence of *L. paracasei* A11, whose metabolic activity may affect the distribution of elements in the matrix. Furthermore, interactions between LAB and other film components, including pectin, proteins, and minerals, may altered the spatial distribution or chemical state of these elements within the film structure [297].

Evaluation of LAB viability during film drying process and storage is essential because the efficacy of edible coatings containing live bacteria depends on their ability to maintain viable cell populations over time. The differences observed between LAWPC and LAWP films indicate that film composition had an important role in maintaining *L. paracasei* A11 viability during drying and storage. The higher survival of the strain in LAWPC films compared with LAWP films after drying indicates a protective effect associated with the higher nutrient availability of the LAWPC matrix. The higher protein and total dry matter content of LAWPC, including fermentable sugars, likely contributed to the better stability of LAB during the drying process [298]. Similar protective effects of protein-based films on LAB survival have been reported in whey protein-based [299] and whey protein isolate-based films [300]. In our study, during refrigerated storage, LAB viability declined in both film formulations, which is consistent with previous studies reporting gradual loss of bacterial viability over time in dried samples due to storage stress [301]. However, the higher survival rate in LAWPC-based films throughout the storage period suggests that the presence of nutrients and the associated moisture retention contributed to the better stability of bacterial cells [24]. In contrast, the faster degradation observed in LAWP-based films may be related to their lower protein content and reduced ability to protect cells from oxidative and desiccation stress, as observed in other whey permeate-based films [302].

4.4. Effects of edible coating with *L. paracasei* A11 on Edam-type cheese quality

The pH of the cheese curd matrix is a key parameter in cheese production, influencing processing steps, microstructure, rheological properties, microbiological changes, and overall product quality and safety [303]. During the experiment, across ripening and storage, pH changes developed similarly in regular and probiotic Edam-type cheeses. The main difference between the two groups of cheeses was the presence of *L. plantarum* Tensia as an adjunct culture in the probiotic cheeses. In the present study, both cheese groups showed a gradual rise in pH as ripening progressed, indicating ongoing ripening, consistent with the findings of Sabikhi et al. (2013), who reported that the pH of both control and probiotic Edam-type cheeses increased progressively and more or less uniformly throughout the ripening period of 3 months [304]. Nevertheless, our results also revealed that R (C) and P (C) consistently exhibited higher pH than the LAWPC-coated samples, especially LAWPC + L. p-coated samples, in both regular and probiotic groups. Similar findings have been reported by other authors, who observed lower pH in ripened cheeses treated with probiotic coatings compared with uncoated controls [209,305]. This outcome could be explained by the low pH of the coatings, which is caused by the acidic nature of whey and the active metabolism of LAB that convert lactose into lactate, thereby producing lactic acid [160,163,306]. This explanation is further supported by earlier findings from *L. paracasei* A11 cultivation studies in dairy byproducts, where the strain demonstrated effective lactose fermentation and acid production.

A_w measurement is essential in food products, as it is directly related to practical aspects and microbiological safety [307]. The average a_w in semi-hard cheeses, including Gouda and Edam is 0.95 – 0.96, but this value depends on the exact position within cheese and it changes during brining and ripening due to salt and water migration [307–309]. Through ripening and storage the decrease in a_w in both regular and probiotic Edam-type cheeses can be attributed by the addition of salt and its penetration into cheese, hydrolysis of proteins, amino acids, and triglycerides [310,311]. In our research, differences among samples further shaped a_w changes: the particularly low a_w on day 45 in regular and probiotic cheese samples covered with LAWPC + L. p suggested that microbial metabolism or interactions between bacteria cells and coating components reduced the moisture retention capacity of the coating [310,312,313]. By the end of storage, coating composition influenced moisture redistribution differently in the two cheese groups, reflected in higher a_w in R + LAWPC + L. p sample in regular cheese group and higher a_w in P + LAWPC sample in probiotic cheese group. The reasons for these group-

specific differences have not been clearly established and may be related to several interacting factors during ripening and storage.

The observed DM patterns reflected how each sample influenced moisture during ripening and storage, rather than showing absolute differences between coated and uncoated cheeses. In the regular cheese group, the higher DM content in R (C) sample at the end of ripening indicated greater moisture loss compared with coated samples, which retained more water due to the barrier properties of the applied coatings [314,315]. By the end of storage, the higher DM in the R + LAWPC + L. p sample indicated that prolonged storage modified moisture migration within the coated cheese samples. In the probiotic cheese group, the opposite dynamics, with higher DM on day 45 in P + LAWPC, followed by higher DM in P (C) on day 135, demonstrated that moisture redistribution did not follow a uniform pattern. This phenomenon appears to be sample-specific and may reflect the combined effects of the interaction of ripening time, coating presence, and cheese matrix, rather than a single controlling factor.

The optical transparency of edible coatings is important for maintaining the natural appearance of food products [316]. In this study, the absence of significant ΔE changes in regular cheese group indicates that the LAWPC coating did not alter the surface color, which is consistent with studies showing that protein-based edible coatings are generally transparent and have no or little influence on optical properties [317–319]. Meanwhile, ΔE increased during storage in probiotic cheeses, a pattern consistent with reports that color changes in ripened cheeses naturally progress due to biochemical and environmental factors rather than coatings [11]. The lack of a significant sample × day interaction further indicates that LAWPC coatings, with or without *L. paracasei* A11, did not differentially influenced probiotic cheese color changes.

FA are known to significantly influence the bioactivity, aroma, and flavor of ripened cheese [320]. Since regular and probiotic Edam-type cheeses were made from different milk batches and contained different microbial cultures, they represent two distinct biochemical systems. As a result, the LAWPC coatings, with or without *L. paracasei* A11, influenced FA profiles and LQI differently in regular and probiotic cheese groups during ripening and storage. On day 1, the absence of differences between samples in both cheese groups was expected, as FA composition reflects milk origin and initial processing conditions [321].

In the regular cheese group on day 45, most FA groups and LQI changed within treatments, and trans FA content was significantly higher in R (C) compared with R + LAWPC sample. Whey-protein-based coatings can function as effective gas barriers under low humidity and also limit the

transfer of aromatic compounds and oils, which may contribute to these observed differences [322]. By day 135, the sample coated with LAWPC + L. p coating exhibited the most pronounced changes, characterized by reduced SFA and increased UFA compared to R (C), indicating improved lipid ratio [323]. These FA shifts were also accompanied by lower AI and higher DFA, both of which are considered favorable indicators of lipid quality [321,324]. The strong leucine and valine arylamidase activities of *L. paracasei* A11 (API ZYM score 5) indicate active proteolysis, which can influence the ripening microenvironment by altering pH, redox conditions, and peptide availability [325–328]. Such microenvironmental changes may indirectly modulate lipolysis and FA oxidation, contributing to UFA preservation and supporting the observed changes in AI and DFA [329]. The reduction in LCFA and SCFA in the LAWPC + L. p-coated sample compared with the control may reflect shifts in lipolysis linked to coating matrix, as also observed by Saravani et al. (2019), where coated Cheddar cheese showed lower SCFA content compared to control due to more intensive lipolysis [330]. The esterase activity profile of *L. paracasei* A11 (C4 = 2; C8 = 1; C14 = 0) further suggest moderate activity toward short-chain substrates, which can shift the SCFA/LCFA balance in the product [331]. The higher n6/n3 ratio and trans FA in the LAWPC + L. p sample at the end of storage may be also related to interactions between the coating components in incorporated bacteria [332].

In the probiotic cheeses, both coatings resulted higher SFA and LCFA and lower MUFA and UFA on day 45 compared with P (C), whereas PUFA, MCFA, and SCFA remained unchanged. These differences indicate that the coatings did not affect all FA groups uniformly but mainly influenced the balance between saturated and unsaturated fractions. The probiotic cheese contained *L. plantarum* Tensia as an adjunct culture, and this species is known to metabolize linoleic acid and produce UFA-derived metabolites, including CLA isomers, in cheese [333,334]. To increase coating surface hydrophobicity, sunflower oil was added to the composition, which provided an additional source of linoleic acid [316,335]. PUFA from plant oils are prone to oxidation and isomerization during storage, particularly at the cheese surface, where moisture, oxygen availability, and microbial metabolism interact [336,337]. Such activity can divert unsaturated FA toward alternative pathways rather than increasing their total concentration, which may explain the lower MUFA and UFA detected in coated samples despite microbial activity [338,339]. Also, these processes may account for the slightly higher omega-6 and lower omega-3 contents, and for the higher n6/n3 ratio, AI, and TI values in coated cheeses. By day 135, the probiotic control cheese showed increasing SFA, TI, and n6/n3 and declining MUFA, UFA, and omega-3, indicating progressive oxidation during ripening. In contrast, coated probiotic cheeses

showed reduced LCFA and increased SCFA, suggesting surface-level shifts in lipolysis influenced by the coating matrix, consistent with studies showing that whey-protein coatings can modify oxygen transfer and alter lipid-related reactions at the cheese surface [160]. The trans FA increased in all samples during storage, which is more consistent with oxidation-related isomerization rather than treatment effects.

The microbiota of cheese contributes significantly to the development of its visual characteristics, texture, flavor, and aroma [340]. For this reason, microbial behavior in regular and probiotic Edam-type cheeses was examined during ripening and storage. Microbial patterns developed differently in the cheese matrix and at the surface, reflecting the distinct environmental conditions and the influence of the coatings.

At the beginning of ripening, TBC and LAB counts were higher in regular and probiotic cheese matrix coated with LAWPC + L. p, which is attributed to the direct introduction of viable cells from the coating and their initial transfer into the cheese matrix, rather than to microbial growth. *L. plantarum* was detected only in probiotic cheese samples, at approximately 6.16 $\log_{10}$ CFU/g, representing the initial level of adjunct culture in the cheese matrix. After 45 days of ripening, LAB counts decreased in regular cheeses matrix but increased in probiotic cheeses matrix. On the same day, coated samples showed higher TBC than controls, but by day 135, the highest counts were observed in the control samples, which is consistent with research showing non-starter lactic acid bacteria (NSLAB) gradual increase in control cheese by the third month of ripening [341]. By the end of storage, TBC in the cheese matrix increased steadily in both cheese groups, which is consistent with typical ripening dynamics where NSLAB gradually dominate as ripening process progresses [342]. LAB counts in the probiotic cheese matrix had decreased, while *L. plantarum* remained detectable only in probiotic cheeses and increased over time. This trend agrees with reports showing that starter LAB increase during production and early ripening of Cheddar cheese, after which their numbers decline as lactose is utilized [341], allowing *L. plantarum* and other NSLAB to become dominant [343].

On day 1, microbial development on the cheese surface differed markedly between samples. TBC, LAB and *L. paracasei* counts were detected only on the surfaces of regular and probiotic cheeses coated with LAWPC + L. p. These counts correspond to the initial inoculated level and early survival of the incorporated strain on the cheese surface. By day 45, surface TBC increased in all cheeses, with the highest counts observed in R (C) and P (C), contrary to cheese matrix results, which is consistent with findings that cheese rind supports microbial proliferation due to higher pH and oxygen exposure compared to cheese core [344]. LAB and *L. paracasei* counts increased and

were only detected in regular and probiotic cheese samples covered with LAWPC + L. p coating. By day 135, surface TBC and LAB counts were lower in R (C) and P (C). However, on the same samples, including regular cheese coated with LAWPC coating, showed detectable mold and yeast growth. In contrast, no yeast and mold counts were detected on the surface of either regular or probiotic cheeses coated with LAWPC + L. p, in which *L. paracasei* counts increased during storage. Similar results have been reported by other researchers, who showed that whey coatings containing probiotic cells delayed visible fungal growth on whole Gruyere cheeses until day 60 [345] and effectively prevented microbial spoilage in semi-hard Flamengo cheese for at least 30 days [177]. In addition, antifungal effect observed in our study may be attributed to the activity of *L. paracasei* incorporated into the coating, as this species is known to inhibit yeasts and fungi through the production of antimicrobial metabolites such as lactic acid [346]. This explanation is further evidenced by the antifungal properties of *L. paracasei* A11, determined during strain characterization experiments *in vitro*.

Edible coatings provide several functional advantages in cheese production. They can prevent moisture loss, improve physicochemical and optical properties, provide a protective shield against microbial contamination [161,347]. In addition, they can contribute to the sensory attributes of cheese.

Sensory analysis demonstrated that edible coatings and incorporated *L. paracasei* A11 influenced the overall sensory quality of both regular and probiotic Edam-type cheeses during ripening and storage. At the end of ripening (day 45), cheeses coated with LAWPC + L. p received the highest scores for flavor and overall acceptability in both cheese groups. LAB are known to produce different metabolites that can modify structure and flavor of food products [25], and in the present study the presence of *L. paracasei* A11 likely contributed to the sensory differences. In regular cheeses, the LAWPC + L. p coating increased the sensory acceptance by adding mild acidity, which complemented the typically mild flavor profile of Edam-type cheese. In contrast, other studies have reported no significant sensory changes when probiotic bacteria were incorporated into whey-based coatings, such as the addition of *Bifidobacterium animalis* subsp. *lactis* BB-12 to Mozzarella cheese [348], indicating that sensory effects depend on the strain used and the cheese type. In our study, probiotic cheeses were described as relatively salty, which may reflect differences in salt perception between consumer groups, which have been reported previously [349,350]. In this context, cheeses coated with LAWPC + L. p received higher flavor and overall acceptability scores. The whey-protein coating containing *L. paracasei* A11 may have contributed additional surface acidity, which is known to balance salt perception and reduce perceived saltiness, thereby improving overall

flavor acceptance. After prolonged storage (day 135), the positive effect of the LAWPC + L. p coating was more pronounced. In regular cheeses, R + LAWPC + L. p resulted in higher scores for flavor, appearance, and overall acceptability compared with R (C), while LAWPC-coated samples showed improved body and texture despite a general decline in appearance between days. Similar effects have been reported for edible coatings, which potentially help to stabilize food products by maintaining the structural integrity of the food [322]. In probiotic cheeses, P + LAWPC + L. p received the highest scores for flavor, appearance, body and texture, and overall acceptability, and was the only sample without a significant decline in appearance by the end of storage.

4.5. Microbiome behavior and proteolytic changes in coated Edam-type cheese

The behavior of LAB, *L. paracasei* A11, and *L. plantarum* Tensia in regular and probiotic Edam-type cheeses showed that both the LAWPC coating and the added probiotic strains influenced microbial dynamics during ripening and digestion. The increases in LAB and *L. paracasei* during 45 days of ripening, together with the stable survival of *L. plantarum* in probiotic cheeses, match observations that LAB remain active throughout the maturation of semi-hard cheeses, contributing to ongoing metabolic activity and microbial stability during ripening [25]. After digestion, reductions in LAB and *L. paracasei* correspond to known sensitivity of LAB to acidic and bile conditions [235,351]. Although *L. paracasei* A11 itself is acid- and bile-tolerant, its viability decreased when incorporated into the LAWPC coating. This aligns with findings that whey-based films can present a more challenging environment due to lower moisture and higher oxygen exposure compared with the cheese matrix [100,312]. Nevertheless, despite the reduction in viable cells, *L. paracasei* remained detectable after digestion, supporting its ability to persist during gastrointestinal transit. In contrast, *L. plantarum* survived well in the cheese matrix, reflecting the buffering capacity and higher nutrient availability of semi-hard cheese, which provides more affective protection during digestion [352–354].

Whey-based edible coatings used in cheese are formulated primarily to extend shelf life and provide barrier properties [25], and therefore they generally have a limited effect on cheese microbiota, which is mainly governed by the activity of SLAB and NSLAB [190]. Accordingly, the relative abundance results were consistent with the general microbial patterns observed in Edam-type cheeses. *Lactococcus* remained the dominant genus in all samples, which agrees with findings showing that *Lactococcus* typically maintains

a strong competitive advantage due to its rapid growth and acidification capacity during early cheese development [7,355]. The small shifts observed in *Streptococcus*, such as the slight increase in regular coated cheeses and the small decrease in probiotic cheeses were minor and did not change the overall community composition. The non-dominant genera remained below 1%, which is consistent with published cheese-microbiome studies showing that NSLAB, initially present at low levels, typically proliferate during ripening and become dominant in aged cheeses [356]. In our case, the Edam-type cheese is relatively young and mild, which explains why NSLAB levels were still low at the end of ripening. The presence of *Lacticaseibacillus* and *Lactiplantibacillus* at very low abundance corresponds to their intentional introduction through the coating or adjunct culture. The absence of significant differences among samples or days further supports that the coatings and probiotics did not disrupt the core microbiota and were specific to certain samples [357]. The single significant change observed for *Anoxybacillaceae* in regular cheese appears to represent an isolated fluctuation rather than a trend.

The PCA results showed that regular cheese samples clustered tightly and shifted clearly between day 1 and day 45, indicating a consisted ripening-related evolution of the microbial community. This trend is consistent with earlier findings in Edam-type cheeses, where microbial development during ripening follows a structured trajectory affected by time rather than sample differences and dominated by SLAB [358]. The significant effect of ripening day in regular cheeses confirmed that time was the main factor of microbial change, while the lack of differences between samples indicates that the LAWPC + L. p coating did not disrupt the community dynamics. Conversely, the probiotic cheese samples showed a more dispersed PCA pattern and did not significantly differ between days or samples. Similar to our findings, Bettera et al. (2023) reported that adjunct cultures increased microbial abundance in semi-hard cheeses but did not lead to consistent or statistically significant differences during ripening [359].

Proteins in semi-hard and hard cheeses are slower to digest than those in soft cheeses due to their lower moisture content and more compact protein matrix [360]. Technological interventions, such as edible coatings, may therefore influence proteolysis and protein digestibility in these cheeses. In the present study, application of the LAWPC coating in regular cheeses increased protein solubilization, whereas adding *L. paracasei* A11 to the coating lowered solubilization, small peptide content, and D_{SEC} compared to R (C). These findings indicate that incorporation of potential probiotic culture into LAWPC coating did not alter the primary proteolytic pathway of Edam-type cheese, consistent with established proteolytic mechanisms in cheese

[361,362]. This outcome is consistent with the *in vitro* characterization of *L. paracasei* A11, which showed an absence of extracellular proteolytic activity, as also reported by other authors [363]. In probiotic cheeses, the coating with *L. paracasei* A11 increased soluble protein, small peptide content, and D_{SEC}, suggesting enhanced protein digestibility. *L. plantarum* Tensia is known to express proteolytic properties *in vitro*, including the ability to release bioactive dipeptides with ACE-inhibitory potential [364]. Although *L. paracasei* A11 lacked extracellular proteolytic activity in the *in vitro* strain characterization experiments, its positive caseinolytic activity suggests a potential contribution to casein degradation [365,366]. This profile may explain why *L. paracasei* A11 alone did not enhance protein digestibility in regular cheese yet contributed positively when combined with *L. plantarum* in probiotic cheese. In this setting, *L. plantarum* Tensia is likely to remain the main contributor to protein breakdown. The effects observed for *L. paracasei* A11 on solubilization and digestibility may be associated with its caseinolytic activity, as reported for *Lactobacillus* strains isolated from fermented dairy products [229]. However, further research is required to clarify these findings.

CONCLUSIONS

1. A total of 22 colonies were isolated from 20 fermented cow's milk samples that were digested using the harmonized INFOGEST *in vitro* digestion method:
   1.1. Out of 22 isolated colonies, 4 strains (18%) were identified as *Lactobacillus* spp., including *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*.
   1.2. Among these 4 tested strains, *L. paracasei* A11 showed the most suitable safety, technological, and probiotic properties. These include absence of hemolytic activity, sensitivity to tested antibiotics (with expected intrinsic resistance to vancomycin), strong tolerance to acid and bile salts (100% survival at pH 2.5; 73% at 1% bile), and antimicrobial activity against spoilage and pathogenic microorganisms, with inhibition zones up to 25 mm against yeasts and 15 mm against *Listeria monocytogene*s in *in vitro* experiments.
2. Cultivation of selected protective, potential probiotic lactobacilli in dairy industry by-products demonstrated their suitability as growth media:
   2.1. *L. paracasei* A11 showed stable growth in LAWPC, reaching high biomass yield (8.3 $\log_{10}$ CFU/g), compared to lower growth in LAWP.
   2.2. The strain actively utilized available carbohydrates and produced lactic acid and lactate, indicating efficient metabolic activity in LAWPC.
   2.3. LAWP was less suitable for cultivation due to lower nutrient content, resulting in lower LAB counts (6.30 $\log_{10}$ CFU/g) compared to LAWPC.
3. Protective edible coatings based on dairy industry by-products and cultivated lactobacilli were successfully developed and characterized:
   3.1. Incorporation of *L. paracasei* A11 reduced the viscosity of LAWPC film-forming solutions and significantly improved film elasticity while reducing opacity ($p < 0.05$).
   3.2. LAB counts in LAWPC-based coatings reached 7.67 $\log_{10}$ CFU/g after drying and remained at 5.21 $\log_{10}$ CFU/g after 21 days of storage at 4 °C, indicating higher viability compared to LAWP-based coatings.

4. Application of edible coatings on regular and probiotic Edam-type cheeses improved their safety and quality during ripening and storage:
   4.1. Coated cheeses showed significantly lower pH ($p < 0.05$) and differences in a_w and DM compared to control samples, while no significant differences in ΔE were observed.
   4.2. Edible coatings influenced the FA profile of cheeses. In regular cheeses, coatings containing *L. paracasei* A11 resulted in lower SFA and higher UFA, along with improved LQI (AI and DFA) at the end of storage ($p < 0.05$). In probiotic cheeses, the effect was less consistent, with changes in FA composition showing different trends compared to regular cheeses.
   4.3. *L. paracasei* counts on cheese surfaces increased during storage, reaching up to 6.70 $\log_{10}$ CFU/g, supporting survival of incorporated bacteria.
   4.4. Coatings containing *L. paracasei* A11 effectively inhibited spoilage microorganisms in both regular and probiotic cheeses, as mold and yeast (2.30–2.97 $\log_{10}$ CFU/g) were detected only in control and LAWPC-coated samples at the end of storage (day 135), while no growth was observed in LAWPC + L. p samples.
   4.5. Sensory evaluation showed that cheeses coated with LAWPC + L. p had significantly higher ($p < 0.05$) scores for flavor and overall acceptability (up to 85.96 points) compared with control samples.
5. Simulated gastrointestinal digestion demonstrated the functional potential of coated cheese:
   5.1. *In vitro* digestion significantly reduced LAB counts ($p < 0.05$) in all cheese samples. However, *L. paracasei* remained detectable after *in vitro* digestion (5.02–6.46 $\log_{10}$ CFU/g), indicating persistence under simulated gastrointestinal conditions.
   5.2. Edible coatings with incorporated *L. paracasei* A11 did not significantly affect the cheese microbiota composition ($p > 0.05$), which remained dominated by *Lactococcus* spp. throughout ripening.
   5.3. The probiotic cheese matrix supported the presence of both *L. plantarum* (administered as the probiotic adjunct culture *L. plantarum* Tensia) and *L. paracasei* (*L. paracasei* A11 incorporated into the edible coating), indicating their potential coexistence within the cheese matrix. However, confirmation at the strain level requires whole-genome sequencing.
   5.4. Protein digestibility was influenced by coating composition. In probiotic cheeses, coatings containing *L. paracasei* A11

were associated with higher digestibility, reaching up to 69.4% ($p < 0.05$), whereas in regular cheeses no improvement in digestibility was observed compared to control samples.

RECOMMENDATIONS

1. The use of an *in vitro* digestion model prior to strain isolation can be applied as a tool for the selection of potential probiotic lactobacilli in further studies.
2. LAWPC is recommended as a suitable dairy industry by-product for both lactobacilli cultivation and edible coating formulation.
3. The strain *L. paracasei* A11 can be used in edible coatings without additional protective components, simplifying coating formulation and application in cheese production.
4. Edible coatings containing protective, potential probiotic lactobacilli may be applied in cheese production to control spoilage microorganisms and extend product shelf life.
5. The composition of edible coatings should be optimized, particularly the type of oil used, in order to ensure consistent improvement of FA profile in cheese.
6. When applying edible coatings with incorporated lactobacilli to probiotic cheeses, interactions between coating components and adjunct cultures should be considered, as they may influence protein digestibility and other quality parameters.

SANTRAUKA

Problemos aktualumas ir svarba

Pastaraisiais metais vis daugiau dėmesio skiriama natūralioms maisto produktų saugos ir kokybės gerinimo strategijoms [1]. Maisto saugos kontekste vis dažniau vartojama „švarios etiketės“ koncepcija, apibūdinanti natūralios kilmės arba minimaliai apdorotas medžiagas, užtikrinančias mikroorganizmų augimo kontrolę, taip mažinant sintetinių cheminių priedų naudojimą. Tokios priemonės atliepia augantį vartotojų poreikį saugesniems maisto produktams [2,3]. Tarp jų ypatingas dėmesys skiriamas vietinės kilmės mikroorganizmams, ypač pieno rūgšties bakterijoms (PRB) [4].

PRB yra neatsiejama fermentuotų maisto produktų, ypač pieno produktų, dalis. Šios bakterijos dalyvauja konservavimo procesuose, formuoja skonį, gerina tekstūrą ir prisideda prie produkto stabilumo [5]. Sūrių gamyboje PRB, slopinančios nepageidaujamų mikroorganizmų augimą, svarbios ne tik fermentacijos ir brandinimo metu, bet ir užtikrinant produkto saugą [6]. Šį poveikį lemia konkurencinė mikroorganizmų sąveika bei antimikrobinių junginių, tokių kaip organinės rūgštys ir bakteriocinai, gamyba [7].

PRB reikšmė ypač svarbi vertinant sūrių kokybę ir saugą, kurios glaudžiai susijusios su produkto mikrobiologiniu stabilumu [8,9]. Nustatyta, kad Europoje prarandama arba iššvaistoma iki 20 proc. pieno produktų [10]. Laikymo metu sūriai ypač jautrūs bakterijų, mielių ir pelėsių dauginimuisi [11]. Šių mikroorganizmų augimas lemia įvairius defektus – paviršiaus pokyčius, pigmentaciją, nepageidaujamus kvapus ir skonius, taip pat dėl toksinių metabolitų susidarymo gali kelti pavojų vartotojų sveikatai [12,13].

Siekiant sumažinti mikrobiologinės taršos riziką ir prailginti produktų galiojimo laiką, vis daugiau dėmesio skiriama valgomosioms dangoms ir plėvelėms. Tai tiesiogiai ant maisto produkto paviršiaus formuojami ploni maistinių biopolimerų sluoksniai, prisidedantys prie jo saugos užtikrinimo [14]. Tokios dangos veikia kaip barjeras deguoniui, drėgmei ir dujų apykaitai, todėl lėtina oksidacinius bei mikrobiologinius procesus ir prailgina produktų galiojimo laiką [15,16]. Be to, šios dangos gali būti naudojamos kaip funkciniai nešikliai, leidžiantys įterpti antioksidantus, antimikrobines medžiagas, aromatinius junginius ar probiotikus [17,18].

Valgomųjų dangų kaip probiotikų nešiklių naudojimas sudaro galimybes kurti funkcinius maisto produktus [14]. Sūrių technologijoje vis didesnę reikšmę įgyja probiotinės starterinės kultūros, didinančios produkto maistinę vertę ir galinčios turėti teigiamą poveikį vartotojų sveikatai [19]. Tačiau, nors probiotikai dažniausiai įterpiami tiesiogiai į sūrio matricą, vis dar trūksta

duomenų apie į valgomąsias dangas įterptų, papildomomis apsauginėmis ir probiotinėmis savybėmis pasižyminčių PRB gyvybingumą ir jų poveikį produktui.

Vienas pagrindinių iššūkių, naudojant valgomąsias dangas su įterptomis PRB, yra probiotinių bakterijų gyvybingumo išsaugojimas. Ant sūrio paviršiaus esančias bakterijas veikia įvairūs aplinkos veiksniai, tokie kaip deguonis, sumažėjęs drėgnis ir temperatūros svyravimai [20,21], galintys neigiamai paveikti jų išgyvenamumą tiek laikymo, tiek virškinimo metu. Siekiant padidinti probiotikų stabilumą, taikomi įvairūs technologiniai sprendimai, įskaitant prebiotikų (oligosacharidų, polifenolių) įterpimą [22] bei sudėtingesnes technologijas, tokias kaip mikrokapsuliacija [23].

Nepaisant didėjančio mokslinių tyrimų skaičiaus, dauguma darbų nagrinėja valgomąsias dangas, papildytas įvairiais bioaktyviais komponentais – eteriniais aliejais, fermentais, polisacharidais ar mikroorganizmų metabolitais [24,25]. Tuo tarpu tyrimų, kuriuose PRB būtų naudojamos kaip pagrindinis apsauginis ir funkcinis komponentas, ypač sūriuose, vis dar trūksta. Taip pat nepakankamai ištirtas ryšys tarp dangų sudėties, mikroorganizmų sąveikos, sūrio kokybės pokyčių laikymo metu ir procesų, vykstančių virškinimo metu.

Darbo tikslas ir uždaviniai

Darbo tikslas: sukurti ir įvertinti apsauginės valgomosios dangos, įterpiant apsauginėmis ir probiotinėmis savybėmis pasižyminčią laktobacilą, poveikį Edam tipo sūrio saugai ir kokybei.

Tyrimo uždaviniai:

1. Iš rauginto pieno, taikant *in vitro* virškinimo metodą, išskirti pieno rūgšties bakterijas, pasižyminčias probiotinėmis savybėmis, identifikuoti ir charakterizuoti laktobacilas bei atrinkti saugias, apsauginėmis ir probiotinėmis savybėmis pasižyminčias padermes.
2. Kultivuoti atrinktą apsauginėmis ir probiotinėmis savybėmis pasižyminčią laktobacilų padermę įvairiose antrinėse pieno pramonės žaliavose ir pagal biomasės prieaugį atrinkti tinkamiausią žaliavą.
3. Sukurti apsauginę valgomąją dangą iš antrinių maisto pramonės žaliavų ir laktobacilų kultivatų bei ištirti jos fizikines ir chemines savybes.
4. Taikyti apsauginę valgomąją dangą įprastiems ir probiotiniams Edam tipo sūriams bei įvertinti jų saugą ir kokybę.
5. Įvertinti *in vitro* virškinimo poveikį įprastų ir probiotinių Edam tipo sūrių, padengtų valgomosiomis dangomis su įterptomis laktobacilomis, mikrobiotai ir baltymų virškinamumui brandinimo metu.

Tyrimo naujumas ir reikšmė

Atliktas tyrimas yra pirmasis, kuriame standartizuotas INFOGEST *in vitro* virškinimo metodas buvo taikytas rauginto karvės pieno mėginiams prieš laktobacilų izoliavimą, o bakterijos buvo izoliuojamos iš suvirškintų mėginių. Toks metodinis sprendimas leido ankstyvoje stadijoje atrinkti rūgščiai atsparias ir probiotinį potencialą turinčias padermes bei sumažinti tolesniems tyrimams reikalingų izoliatų skaičių.

Tyrime taip pat pagrįstas pieno pramonės šalutinių produktų – skysto rūgščių išrūgų baltymų koncentrato (SRIBK) ir skysto rūgščių išrūgų permeato (SRIP) – panaudojimas: tiek probiotinių savybių laktobacilų kultivavimui, tiek kaip pagrindinių komponentų valgomųjų dangų sudėtyje. Toks sprendimas prisideda prie tvarių maisto technologijų plėtros, mažos vertės žaliavų panaudojimo ir žiedinės ekonomikos principų įgyvendinimo.

Skirtingai nei daugumoje ankstesnių tyrimų, šiame darbe sukurtose valgomosiose dangose PRB buvo naudojamos kaip pagrindinis antimikrobinis ir funkcinis komponentas, nenaudojant papildomų apsauginių priedų. Tai rodo galimybę kurti paprastesnes, lengviau pramonėje pritaikomas dangų kompozicijas, galinčias pakeisti tradicinius konservantus.

Nustatyta, kad *L. paracasei* rūšies bakterijos išlieka gyvybingos sūrio matricoje ir po *in vitro* virškinimo net ir nenaudojant papildomų apsauginių komponentų ar mikrokapsuliacijos. Šis rezultatas rodo, kad probiotinių savybių bakterijos gali būti sėkmingai įterpiamos į valgomąsias dangas taikant paprastesnius technologinius sprendimus ir kuriant mažiau sudėtingas valgomąsias dangas.

Taip pat atliktas išsamus tyrimas, apimantis valgomųjų dangų sudėties, mikroorganizmų aktyvumo, sūrio kokybinių rodiklių ir virškinimo rezultatų vertinimą. Toks kompleksinis požiūris leido įvertinti probiotinių dangų veikimą viso proceso metu – nuo sūrių brandinimo ir laikymo iki virškinimo – bei geriau suprasti dangų sudėties, mikroorganizmų aktyvumo ir produkto kokybės tarpusavio sąveiką.

Gauti rezultatai rodo, kad PRB praturtintos valgomosios dangos gali būti efektyvi ir praktiškai pritaikoma priemonė sūrių saugai ir kokybei gerinti nenaudojant sintetinių priedų. Tokia priemonė gali slopinti gedimą sukeliančių mikroorganizmų augimą, prailginti produktų galiojimo laiką ir padėti išsaugoti juslines savybes, taip mažinant maisto nuostolius ir didinant produktų tvarumą.

Be to, nustatytas probiotinių bakterijų išgyvenamumas *in vitro* virškinimo metu rodo, kad valgomosios dangos gali veikti kaip probiotikų pernašos sistema ir didinti maisto produktų funkcinę vertę, atitinkančią šiuolaikinių vartotojų lūkesčius.

Tyrimo metodika

Tyrimų laikas ir vieta. Tyrimai buvo atlikti 2022 – 2026 metais Lietuvos sveikatos mokslų universitete Veterinarijos akademijoje (LSMU VA) Maisto saugos ir kokybės katedroje. Atskirų tyrimo etapų metu bendradarbiauta su Minjo universitetu (Portugalija), Latvijos gyvybės mokslų ir technologijų universitetu, BioCC Biokompetencijos centru (Estija) bei Norvegijos maisto, žuvininkystės ir akvakultūros tyrimų institutu (Nofima).

Mėginiai ir tyrimų medžiagos, mikroorganizmų kultūros. PRB išskyrimui naudoti ekologiško karvės pieno mėginiai, kurie iki tolesnių tyrimų buvo laikomi 4 °C temperatūroje.

Bakterijų kultivavimui bei valgomųjų dangų gamybai naudoti pieno pramonės šalutiniai produktai – SRIBK ir SRIP (Pieno žvaigždės, Lietuva). Dangų sudėčiai formuoti naudotas glicerolis (99 proc. grynumo) kaip plastifikatorius, saulėgrąžų aliejus (*Helianthus annuus*) kaip hidrofobinė fazė ir polisorbatas (Tween 80) kaip paviršiaus aktyvioji medžiaga emulsijos stabilizavimui, kurie įsigyti iš Sigma-Aldrich (Darmštatas, Vokietija). Cukrinių runkelių pektinas (54 proc. esterifikacija) kaip struktūrinė medžiaga įsigyta iš Herbstreith & Fox (Neuenbürg, Vokietija).

Šviežio sūrio mėginiai buvo gauti iš Estijos sūrio ir sviesto gamintojo „E-Piim“.

Antibakterinio aktyvumo tyrime buvo naudoti mikroorganizmai: *Listeria monocytogenes* ATCC 35152, *Staphylococcus aureus* ATCC 9144, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* NCTC 6750, *Bacillus cereus* ATCC 11778, *Salmonella* Typhimurium ATCC 13311, *Pseudomonas fluorescens* ATCC 13525 ir *Brochothrix thermosphacta* ATCC 11509, gauti iš LSMU VA Maisto saugos ir kokybės katedros laboratorijos kolekcijos.

Priešgrybelinio aktyvumo tyrime naudoti mikroorganizmai: *Debaryomyces hansenii* (BTL, M-31/Deb. 4 (T)), *Yarrowia lipolytica* (BTL, M-48/C.6.1), *Candida glabrata* (BTL, 8B), *Candida albicans* (BTL, M-7/C.17), *Mucor racemosus* (BTL, G-134/1064), *Cladosporium herbarum* (BTL, G-86/KŠ-2/7), *Penicillium commune* ML 21-3 ir *Aspergillus versicolor* (CBS 113090), gauti iš Gamtos tyrimų centro Botanikos instituto (Vilnius, Lietuva).

PRB izoliavimas ir laktobacilų atranka bei jų saugos ir funkcinių savybių charakterizavimas. PRB išskyrimas. Šviežio ekologiško karvės pieno mėginiai buvo rauginti 37 °C temperatūroje 96 valandas, o vėliau suvirškinti taikant harmonizuotą INFOGEST *in vitro* virškinimo metodą, pritaikytą maisto produktams [194]. Šis metodas buvo pasirinktas kaip potencialus būdas išskirti rūgščiai atsparias, probiotinių savybių turinčias laktobacilas, skirtas įterpti į apsaugines valgomąsias dangas. Minėtas metodas imituoja burnos, skrandžio ir plonosios žarnos virškinimo fazes, naudojant standarti-

zuotus virškinimo skysčius ir fermentus. Visi fermentai ir tulžis buvo įsigyti iš „Sigma-Aldrich“ (St. Louis, JAV). Mėginiams taikytas *in vitro* virškinimas, imituojant burnos, skrandžio ir plonosios žarnos virškinimo fazes. Virškinimas atliktas dviem pakartojimais kiekvienam mėginiui. Po plonosios žarnos fazės dalis mėginio pasėta ant MRS agaro (Biolife, Milanas, Italija) ir inkubuota 37 °C temperatūroje anaerobinėmis sąlygomis. Po inkubavimo iš kiekvieno mėginio atrinktos reprezentatyvios kolonijos ir išgrynintos pakartotinai persėjant ant MRS agaro. Išgrynintos kolonijos dažytos Gramo būdu. Gramteigiamos PRB kolonijos iki tolesnių tyrimų laikytos MRS sultinyje (Biolife, Milanas, Italija), papildytame 30 proc. gliceroliu, −80 °C temperatūroje. Prieš tolesnius eksperimentus padermės atgaivintos MRS agare, 48 val. inkubuojant 37 °C temperatūroje anaerobinėmis sąlygomis.

PRB padermių identifikavimas. Bakterijų DNR išskirta naudojant „GenElute Bacterial Genomic DNA Kit“ (Sigma-Aldrich), vadovaujantis gamintojo instrukcijomis, skirtomis gramteigiamoms bakterijoms. *Lactobacillus* genties bakterijų identifikavimui taikytas polimerazės grandininės reakcijos (PGR) metodas. Rūšių identifikavimui naudoti specifiniai pradmenys. PGR sąlygos ir pradmenys parinkti remiantis Torriani ir kt. (2001) [195], Ventura ir kt. (2003) [196] ir Gaspar ir kt. (2019) [197] metodikomis. PGR produktai atskirti 2 proc. agarozės gelyje, naudojant 100 bp DNR dydžio žymenį (Thermo Fisher Scientific, Vilnius, Lietuva), ir vizualizuoti dažant etidžio bromidu (Sigma-Aldrich).

Atsparumo antibiotikams įvertinimas. Atsparumas antibiotikams buvo įvertintas naudojant minimalią slopinančią koncentraciją (MSK) nustatančias juosteles (Liofilchem), vadovaujantis gamintojo pateiktomis instrukcijomis. Atsparumas tirtas šiems antibiotikams: chloramfenikoliui, klindamicinui, streptomicinui, gentamicinui, tetraciklinui, eritromicinui ir ampicilinui. MSK nustatyta pagal juostelių skalę ir išreikšta μg/ml.

Hemolizinis aktyvumas. Hemolizinis aktyvumas nustatytas naudojant kraujo agarą su 5 proc. avies kraujo (E & O Laboratories Ltd., Jungtinė Karalystė). Po 48 val. inkubavimo 37 °C temperatūroje rezultatai vertinti pagal hemolizės tipą: β-, α- arba γ-hemolizė.

Fermentinis aktyvumas. Fermentinis aktyvumas įvertintas naudojant API ZYM testų rinkinį (bioMérieux, Prancūzija). Rezultatai interpretuoti pagal gamintojo instrukcijas, vertinant spalvos intensyvumą 0 – 5 balų skalėje.

Ekstraląstelinis proteolitinis aktyvumas. Proteolitinis aktyvumas vertintas naudojant nugriebto pieno agarą. Ant terpės užtaškuota 2 μl bakterijų kultūros. Po 4 parų inkubavimo 30 °C temperatūroje aktyvumas nustatytas pagal skaidrių zonų susidarymą aplink kolonijas.

Kazeinolitinis ir lipolitinis aktyvumas. Tyrimai atlikti remiantis Pisano ir kt. (2015) metodika [201]. Kazeinolitinis aktyvumas vertintas naudojant PCA

(Liofilchem) agarą, praturtintą 10 proc. nugriebto pieno priedu. Lipolitiniam aktyvumui nustatyti naudotas MRS agaras su 0,01 proc. kalcio chloridu ir 0,1 proc. Tween 80.

Rūgštinamasis aktyvumas. Ultra aukštoje temperatūroje (UAT) sterilizuotas 1,5 proc. pienas inokuliuotas atgaivinta tiriama bakterijų kultūra, o pH vertės buvo matuojamos po 6 ir 24 val. inkubavimo. Pienas be pridėtinės kultūros buvo naudojamas kaip kontrolinis mėginys.

Angliavandenių fermentacijos profilis. Angliavandenių fermentacijos profilis buvo įvertintas naudojant API 50 CH testą („BioMerieux“, Marcy-l'Etoile, Prancūzija) ir laikantis gamintojo instrukcijų. Inkubacijos metu dėl angliavandenių fermentacijos susidarančios organinės rūgštys lėmė terpės pH pokyčius, kurie nustatyti pagal indikatoriaus spalvos pokytį. Rezultatai interpretuoti taip: stipri fermentacija – geltona spalva, vidutinė fermentacija – žalia, silpna fermentacija – tamsiai žalia, fermentacijos nebuvimas – mėlyna spalva.

Antibakterinis ir priešgrybelinis aktyvumas. Antibakterinis ir priešgrybelinis aktyvumas vertintas taikant agaro taškavimo (*angl. agar spot*) metodą. Atgaivinta bakterijų kultūra (3 µl) užlašinta ant MRS agaro paviršiaus ir inkubuota anaerobinėmis sąlygomis 37 °C temperatūroje 48 val. Antibakteriniam aktyvumui įvertinti po inkubavimo lėkštelės padengtos 7 ml 0,7 proc. agaro sluoksniu, kuriame buvo 100 µl indikatorinės mikroorganizmų kultūros suspensijos, ir toliau inkubuotos 24 val. aerobinėmis sąlygomis, esant optimaliai indikatorinių mikroorganizmų augimo temperatūrai. Priešgrybeliniam aktyvumui įvertinti lėkštelės padengtos 5 ml Sabūro dekstrozės agaro (SDA; Oxoid, Basingstokas, Jungtinė Karalystė) sluoksniu, kuriame buvo 10^5 pelėsių sporų arba mielių ląstelių 1 ml. Lėkštelės inkubuotos 24 val. aerobinėmis sąlygomis, esant optimalioms grybelinių mikroorganizmų augimo sąlygoms. Naudoti mikroorganizmai pateikti metodikos skyriuje „Mėginiai ir tyrimų medžiagos, mikroorganizmų kultūros“. Antibakterinis ir priešgrybelinis aktyvumas vertintas matuojant skaidrios inhibicijos zonos skersmenį aplink tiriamų padermių kolonijas.

Atsparumas rūgštinei terpei ir tulžies druskai. Atsparumo rūgštinei terpei tyrimui į 9 ml PBS („Biolife“, Milanas, Italija), kurio pH buvo pakoreguotas iki 2,5 naudojant 5 M HCl buvo pridėta 1 ml atgaivintos atrinktos tiriamos kultūros ir inkubuojama 37 °C temperatūroje. Gyvybingų ląstelių skaičius buvo suskaičiuotas po 0 ir 3 inkubacijos valandų, atliekant pasėlius ant MRS agaro. Atsparumo tulžies druskai tyrimui į 9 ml MRS sultinio, kurio sudėtyje buvo 0,3 proc., 0,5 proc. ir 1 proc. tulžies druskos, pridėta 1 ml atgaivintos tiriamos kultūros ir inkubuota 37 °C temperatūroje. Gyvybingos kolonijos suskaičiuotos ant MRS agaro po 0 ir 24 val. inkubavimo.

***L. paracasei* A11 padermės kultivavimas antrinėse pieno pramonės žaliavose.** Padermė buvo kultivuota SRIBK ir SRIP terpėse, o MRS sultinys naudotas kaip kontrolinė terpė. Kultivavimo procesas siekiant įvertinti biomasės prieaugį ir augimo kinetiką atliktas termostate ir realaus laiko bioreaktoriuje (RTS-1C, Biosan, Ryga, Latvija). Padermės augimas stebėtas realiuoju laiku pagal gamintojo instrukcijas. Prieš kultivavimą padermė atgaivinta MRS agare, inkubuojant 48 val. 37 °C temperatūroje anaerobinėmis sąlygomis. SRIBK ir SRIP terpės pasterizuotos 93–95 °C temperatūroje 10 min. ir atvėsintos iki 37 °C. Tuomet 29 ml terpės ir 1 ml *L. paracasei* A11 suspensijos (sureguliuotos iki 1 McFarland vieneto) perkelta į 30 ml „TubeSpin“ mėgintuvėlius, kurie patalpinti į bioreaktorių esant 37 °C temperatūrai 96 val. aerobinėmis sąlygomis. Optinis tankis (OD600) registruotas kas 10 min. esant 110 aps./min. maišymo greičiui, o augimo greitis apskaičiuotas automatiškai. Po kultivavimo gyvybingų bakterijų skaičius nustatytas atliekant pasėlius ant MRS agaro ir inkubuojant anaerobinėmis sąlygomis 37 °C temperatūroje 0, 24 ir 48 val. Kiekvieno termostate esančio mėginio pH matuotas tiesiogiai naudojant pH matavimo prietaisą („Sartorius Professional“, Vokietija). Cukrų kiekis fermentuotuose SRIBK ir SRIP terpėse bei MRS sultinyje nustatytas taikant skysčių chromatografijos metodą pagal ISO 22184:2021 standartą [203]. Pieno rūgšties ir laktato kiekis įvertintas fermentiniu metodu pagal ISO 8069:2005 standartą [204].

***L. paracasei* A11 poveikis valgomųjų dangų savybėms.** Valgomųjų dangų ir plėvelių paruošimas. Keturių skirtingų valgomųjų dangų receptūros pateiktos 1 lentelėje. Sausieji komponentai (cukrinių runkelių pektinas, Tween 80, saulėgrąžų aliejus ir glicerolis) ištirpinti receptūroje numatyto 50 proc. SRIBK arba SRIP kiekio, siekiant paruošti pradinį dangos tirpalą. Gautas mišinys homogenizuotas 3 min. 10 000 aps./min. greičiu, pasterizuotas 93–95 °C temperatūroje 10 min. ir atvėsintas iki 35 °C. *L. paracasei* A11 padermė atskirai kultivuota pasterizuoto SRIBK terpės dalyje (50 proc.). Gautas SRIBK kultivatas, kuriame bakterijų kiekis siekė 8,3 $\log_{10}$ KSV/g, įmaišytas į pradinį dangos tirpalą, siekiant gauti eksperimentines valgomąsias dangas, kurių galutinė *L. paracasei* A11 padermės koncentracija buvo apie 7 $\log_{10}$ KSV/g. Bakterijų koncentracija įvertinta naudojant densitometrą (DEN-600, Biosan, Latvija). Kontrolinių dangų paruošimui į pradinį tirpalą papildomai įterpta likusi SRIBK dalis, tačiau bakterijų kultūra nebuvo pridėta.

1 lentelė. *Valgomųjų dangų receptūros*

Valgomosios dangos	Pektinas (proc. m/m)	Saulėgrąžų aliejus (proc. v/v)	Glicerolis (proc. v/v)	Tween 80 (proc. v/v)	SRIP (proc. m/m viso tirpalo)	SRIBK (proc. m/m viso tirpalo)
SRIBK (K)	2	2	5	0.2	–	90.8
SRIBK + L. p		2			–	90.8
SRIP (K)		1			50	41.8
SRIP + L. p		1			50	41.8

SRIBK – skystas rūgščių išrūgų baltymų koncentratas; SRIP – skystas rūgščių išrūgų permeatas; K – kontrolė; L. p – *Lacticaseibacillus paracasei* A11.

Plėvelės formuotos 150 mm skersmens Petri lėkštelėse, įpilant po 28 ml valgomosios dangos, ir džiovintos 37 °C temperatūroje esant 50 ± 5 proc. santykinei oro drėgmei 12 val. Išdžiovintos plėvelės laikytos 21 dieną 4 ± 1 °C temperatūroje, siekiant įvertinti *L. paracasei* A11 išgyvenamumą laikymo metu.

Valgomųjų dangų ir plėvelių savybių charakterizavimas. Reologiniai matavimai atlikti remiantis Vieira ir kt. (2021) metodika [205]. Plėvelę formuojančių tirpalų reologinės savybės vertintos reometru AR1500ex (TA Instruments, JAV), naudojant nerūdijančio plieno kūgio plokštelės geometriją (6,0 cm skersmens, 2° kampo, 67 μm tarpo aukščio). Visi matavimai atlikti esant 25 °C temperatūrai. Srauto kreivės nustatytos esant skirtingiems šlyties įtampiams, taikant dinaminį matavimo režimą, esant šlyties greičiui nuo 0 iki 300 s^{-1}. Gauti duomenys aprašyti Niutono (1 lygtis) ir galios dėsnio (2 lygtis) modeliais, siekiant įvertinti tirpalų reologines savybes:

$$\sigma = \eta \times \gamma \tag{1}$$

$$\sigma = k \times \gamma^{n} \tag{2}$$

kur σ – šlyties įtempis (Pa), η – klampa (Pa.s), k – konsistencijos indeksas (Pa.s^n), $\dot{\gamma}$ – šlyties greitis (s^{-1}), n – takumo indeksas.

Plėvelių spalva vertinta naudojant spalvos matuoklį Minolta CR-400 (Konica Minolta, Japonija), taikant CIE 1976 L*, a*, b* spalvų sistemą. Šioje sistemoje L* parodo šviesumą (0 – juoda, 100 – balta), a* nusako raudonos–žalios, o b* – geltonos–mėlynos spalvų ašis. Matavimams naudota balta etaloninė plokštelė (L* = 97,6; a* = 0,01; b* = 1,60). Kiekvienam mėginiui matavimai atlikti trimis pakartojimais.

Plėvelių šviesos pralaidumas nustatytas taikant Hunter Lab metodiką. Matavimai atlikti trimis pakartojimais, o skaidrumas apskaičiuotas pagal lygtį:

$$Šviesos\ pralaidumas\left(proc.\right) = \left(Y_b / Y_w\right) \times 100 \tag{3}$$

kur Y_b ir Y_w – mėginio šviesos pralaidumas atitinkamai matuojamas ant juodos ir baltos etaloninių plokštelių [206].

Plėvelių storis matuotas naudojant skaitmeninį mikrometrą (Mitutoyo, Japonija), kurio tikslumas – 0,001 mm. Kiekvienam mėginiui atlikta po 10 matavimų atsitiktinai parinktose plėvelės vietose. Apskaičiuotos vidutinės storio reikšmės naudotos tolesniems skaičiavimams, vertinant mechanines plėvelių savybes.

Tempimo stipris (TS) ir pailgėjimas iki plyšimo (EAB) nustatyti naudojant universalų tempimo aparatą Instron (Model 4500, Instron Corporation, JAV), vadovaujantis ASTM D 882-91 standartu. Pradinė griebtuvų tarpusavio distancija buvo 30 mm, o kryžminės galvutės judėjimo greitis – 5 mm/min. TS apskaičiuotas didžiausią apkrovą (N) dalijant iš mėginio skerspjūvio ploto (m^2) ir išreikštas MPa. EAB išreikštas procentais, palyginant mėginio ilgį lūžio metu su pradiniu ilgiu (30 mm). Kiekvienam mėginiui atlikti penki pakartojimai.

Plėvelių drėgmės kiekis ir tirpumas vandenyje nustatyti pagal Ortiz de Elguea-Culebras ir kt. (2019) [207] metodiką. Drėgmės kiekis nustatytas gravimetriniu metodu, remiantis masės pokyčiu. 2 cm skersmens plėvelių pavyzdžiai buvo pasverti, džiovinti 105 °C temperatūroje 24 valandas ir vėl pasverti. Drėgmės kiekis apskaičiuotas pagal lygtį:

$$Drėgmės\ kiekis\ \left(proc.\right) = \left(\left(M_0 - M\right) / M_0\right) \times 100 \tag{4}$$

kur M_0 – pradinė mėginio masė, M – masė po džiovinimo.

Vandens tirpumas nustatytas išdžiovintus plėvelių mėginius panardinant į 50 ml distiliuoto vandens ir kratant 24 val. 150 aps./min. greičiu. Po inkubavimo netirpios plėvelės dalys buvo atskirtos ir džiovintos 105 °C temperatūroje 24 val. Vandens tirpumas apskaičiuotas pagal lygtį:

$$Tirpumas\ \left(proc.\right) = \left(\left(S_0 - S\right) / S_0\right) \times 100 \tag{5}$$

kur S_0 – pradinė sauso mėginio masė, S – netirpios dalies masė.

Vandens garų pralaidumas (WVP) nustatytas gravimetriniu metodu, vadovaujantis ASTM E96/E96M-16 (2016) standartu. Išdžiovinti plėvelių diskai (45 mm skersmens) sandariai uždengė indų, pripildytų distiliuoto vandens, viršų. Mėginiai laikyti desikatoriuje su silikageliu. Masės pokyčiai registruoti kas 2 val. 10 val. laikotarpiu. WVP apskaičiuotas pagal lygtį:

$$WVP = \left(WVTR \times L\right) / \Delta P \tag{6}$$

kur $WVTR$ – vandens garų perdavimo greitis, L – plėvelės storis (m), ΔP – dalinio vandens garų slėgio skirtumas (2337 Pa).

Plėvelių mikrostruktūra tirta skenuojančios elektroninės mikroskopijos (SEM) metodu, naudojant „Mira3“ mikroskopą (Tescan Orsay Holding, Čekija). Mėginiai paruošti išpjaunant 0,4 × 0,4 cm dydžio fragmentus ir tvirtinant juos ant silicio pagrindo. Tyrimai atlikti aukšto vakuumo sąlygomis, naudojant antrinių elektronų (SE) ir atgalinių sklaidos elektronų (BSE) detektorius.

Elementinė sudėtis nustatyta energijos dispersinės rentgeno spektroskopijos (EDS) metodu, naudojant INCA X-act detektorių (Oxford Instruments, Jungtinė Karalystė). Matavimai atlikti esant 15 mm darbinio atstumo sąlygoms, o elementų pasiskirstymas vertintas sudarant elementinius žemėlapius.

Gyvybingų PRB kiekis dangose ir plėvelėse nustatytas naudojant selektyvias mitybines terpes, atliekant tris pakartojimus. Dangų tirpalai tirti 0 ir 1 laikymo dieną, o plėvelės – 0, 1, 3, 7, 14 ir 21 dienomis. Bendras mezofilinių pieno rūgšties bakterijų skaičius nustatytas pagal ISO 15214:1998 [208].

Valgomųjų dangų pritaikymas Edam tipo sūriams gamybinėmis sąlygomis. Dviejų tipų nebrandinto Edam tipo sūrio blokai – įprastas ir probiotinis sūris su starterine *Lactiplantibacillus plantarum* Tensia (DSM 21380) kultūra – steriliomis sąlygomis supjaustyti vienodais 100 ± 5 g masės gabalais. Paruoštos šešios sūrio mėginių grupės (po tris pakartojimus):

1. Į (K) – kontrolinis įprastas sūris be dangos;
2. Į + SRIBK – įprastas sūris su SRIBK danga;
3. Į + SRIBK + L. p – įprastas sūris su SRIBK danga ir *L. paracasei* A11;
4. P (K) – kontrolinis probiotinis sūris be dangos;
5. P + SRIBK – probiotinis sūris su SRIBK danga;
6. P + SRIBK + L. p – probiotinis sūris su SRIBK danga ir *L. paracasei* A11.

Sūrio mėginiai panardinti į valgomąsias dangas, 1 val. džiovinti ir vakuumuoti naudojant vakuuminio pakavimo įrenginį (Status d.o.o., Slovėnija). Mėginiai laikyti brandinimo kameroje 12 °C temperatūroje, esant 80 proc. santykinei oro drėgmei, 45 dienas, po to papildomai laikyti 4 °C temperatūroje 90 dienų. Kadangi tyrimuose naudotos skirtingos pieno partijos, įprasto ir probiotinio sūrio mėginių rezultatai analizuoti atskirai, jų tiesiogiai statistiškai nelyginant.

Sūrio mėginių pH nustatytas kambario temperatūroje naudojant kalibruotą laboratorinį pH matavimo prietaisą (Mettler Toledo JENWAY3510, Jungtinė Karalystė). Analizei 10 g sūrio mėginio homogenizuota su 30 ml distiliuoto vandens, o pH išmatuotas iš karto po homogenizavimo.

Vandens aktyvumas (a_w) nustatytas naudojant „LabSwift-aw“ matuoklį (Novasina AG, Šveicarija). Prieš matavimą sūrio mėginiai susmulkinti ir perkelti ant matavimo plokštelės.

Sausųjų medžiagų (SM) kiekis nustatytas pagal ISO 5534:2004 standartą [210].

Sūrio mėginių spalva vertinta naudojant spalvos matuoklį Minolta CR-400 (Konica Minolta, Japonija), taikant CIE 1976 L*, a*, b* spalvų sistemą. Šioje sistemoje L* parodo šviesumą (nuo 0 – juoda iki 100 – balta), a* nusako raudonos–žalios, o b* – geltonos–mėlynos spalvų ašis. Remiantis išmatuotomis spalvų koordinatėmis, bendras spalvos pokytis (ΔE) apskaičiuotas pagal lygtį [211]:

$$\Delta E^* = \sqrt{\left(L^* - L_0\right)^2 + \left(a^* - a_0\right)^2 + \left(b^* - b_0\right)^2} \quad (7)$$

kur L_0, a_0 ir b_0 – pradinės spalvos koordinatės, o L^*, a^* and b^* – spalvos koordinatės laikotarpiu tiriamuoju laikotarpiu.

Mikrobiologiniai tyrimai atlikti trimis pakartojimais, siekiant nustatyti gyvybingų mikroorganizmų skaičių. Mėginiai tirti 1, 45 ir 135 laikymo dienomis, naudojant selektyvias mitybines terpes. Mikroorganizmų kiekis nustatytas tiek sūrio paviršiuje, tiek viduje ir išreikštas $\log_{10}$ KSV/g. Vertinti šie mikrobiologiniai rodikliai: bendras aerobinių mikroorganizmų skaičius pagal ISO 4833-1:2013 standartą [212], mezofilinių PRB skaičius pagal ISO 15214:1998 standartą [208], enterobakterijų skaičius pagal ISO 21528-2:2017 standartą [213] ir mielių bei pelėsių skaičius pagal ISO 6611:2004 standartą [214]. Mėginiai nuo paviršiaus ir iš sūrio matricos buvo paimti pagal ISO 18593:2018 standartą [215].

Riebalų rūgščių (RR) sudėčiai sūrio mėginiuose nustatyti 1 g sūrio homogenizuotas su 2 ml III tipo vandens. Mėginiai 1 val. laikyti kambario temperatūroje, periodiškai maišant, po to centrifuguoti 6 min. esant 4000 × g greičiui kambario temperatūroje. Į 1 ml gauto supernatanto pridėta 100 µl 0,6 M oksalo rūgšties tirpalo, mėginiai išmaišyti ir pakartotinai centrifuguoti. RR nustatytos dujų chromatografijos metodu, naudojant dujų chromatografą „Agilent 6890A“ su kapiliarine kolonėle CP-Wax 52 CB (30 m × 0,25 mm, 0,25 µm) ir liepsnos jonizacijos detektoriumi (FID), nustatytu 280 °C temperatūrai. Krosnelės temperatūros programa: pradinė temperatūra – 175 °C (1 min.), vėliau temperatūra mažinta 10 °C/min. greičiu iki 115 °C ir palaikyta 3 min., po to didinta 20 °C/min. greičiu iki 190 °C ir palaikyta 5 min. Kiekvienos RR kiekis išreikštas g/100 g bendro RR kiekio. Riebalų rūgštys suskirstytos į šias grupes: sočiąsias riebalų rūgštis (SFA), mononesočiąsias riebalų rūgštis (MUFA), polinesočiąsias riebalų rūgštis (PUFA), nesočiąsias riebalų rūgštis (UFA), ilgos grandinės riebalų rūgštis (LCFA), vidutinės grandinės riebalų rūgštis (MCFA), trumpos grandinės riebalų rūgštis (SCFA), omega-6 polinesočiąsias riebalų rūgštis, omega-3 polinesočiąsias riebalų rūgštis, trans riebalų rūgštis. Riebalų kokybės rodikliai – aterogeniškumo,

trombogeniškumo, hipocholesteroleminių ir hipercholesteroleminių RR santykis (h/H) bei pageidaujamų RR kiekis (DFA) – apskaičiuoti pagal lygtis [216,217]:

$$Aterogeniškumo\ indeksas\ (AI) = \frac{(C12:0 + (4\,x\,C14:0) + C16:0)}{(PUFA + MUFA)} \tag{8}$$

$$Trombogeniškumo\ indeksas\ (TI) = \\ = \frac{(C12:0 + C16:0 + C18:0)}{(0.5\,xMUFA) + (0.5\,xn6PUFA) + (3\,xn3PUFA) + (n3PUFA / n6PUFA)} \tag{9}$$

$$h / H = \frac{(C18:1 + PUFA)}{(C14:0 + C16:0)} \tag{10}$$

$$DFA = UFA + C18:0 \tag{11}$$

Po 45 dienų brandinimo atlikta juslinė analizė. Vertinime dalyvavo septyni apmokyti vertintojai (abiejų lyčių, 20–50 metų amžiaus), o vertinimas atliktas specialiai tam skirtoje juslinėje patalpoje. Vertintojai atrinkti ir apmokyti vadovaujantis ISO 8586:2023 reikalavimais [218]. Prieš vertinimą mėginiai pateikti kambario temperatūroje ir užkoduoti atsitiktiniais trijų skaitmenų kodais. Vertintojams atsitiktine tvarka pateiktos vienodos plastikinės lėkštės su šešiais 2 × 2 × 2 cm dydžio sūrio kubeliais. Juslinis vertinimas atliktas taikant 100 balų vertinimo sistemą pagal Bodyfelt ir kt. (1988) [219], skiriant 50 balų skoniui, 40 balų tekstūrai ir konsistencijai bei 10 balų išvaizdai.

Sūrio mėginių *in vitro* virškinimas. Siekiant įvertinti su sūriu susijusių mikroorganizmų (bendro PRB skaičiaus, *L. paracasei* A11 ir *L. plantarum* Tensia) išgyvenamumą virškinimo metu, taikytas anksčiau aprašytas standartizuotas INFOGEST *in vitro* virškinimo modelis. Prieš virškinimą sūrio mėginiai buvo homogenizuoti ir veikiami burnos fazės sąlygų. Virškinimas atliktas dviem pakartojimais kiekvienam sūrio tipui, įtraukiant tuščią kontrolę (be sūrio). Plonosios žarnos fazės pabaigoje gauti mėginiai pasėti ant MRS agaro (pH 5,7) ir MRS agaro su manitoliu vietoje gliukozės (pH 5,7), po to inkubuoti anaerobinėmis sąlygomis 30 °C temperatūroje. MRS terpė su manitoliu naudota siekiant sudaryti palankesnes sąlygas *L. paracasei* augimui, lyginant su kitomis PRB. Vis dėlto tikslus kiekybinis šios rūšies įvertinimas išliko sudėtingas dėl kitų PRB buvimo. *L. paracasei* kolonijos identifikuotos MALDI-TOF MS metodu (Bruker). Palyginimui, PRB kiekis sūryje prieš virškinimą nustatytas analogiškai, prieš tai mėginius homogenizavus buferiniame peptono vandenyje.

Sūrio baltymų virškinamumas vertintas po 45 dienų brandinimo, *in vitro* virškinimo metu. Po virškinimo fermentai inaktyvuoti kaitinant verdančio vandens vonelėje 10 min., o mėginiai atskirti į supernatantą ir nuosėdas centrifuguojant (4000 aps./min., 10 min.). Baltymų koncentracija sūrio mėginiuose prieš ir po virškinimo (supernatante ir nuosėdose) nustatyta Dumas metodu, naudojant „Vario EL cube“ analizatorių („Elementar“), kaip aprašyta Rieder ir kt. (2021) [220]. Plonosios žarnos virškinimo fazės supernatantas analizuotas dydžių išskyrimo chromatografijos (SEC) metodu, siekiant nustatyti peptidų molekulinės masės pasiskirstymą ir apskaičiuoti baltymų virškinamumą (D_{SEC}) (Wubshet ir kt., 2017) [221]. D_{SEC} apskaičiuota pagal Rieder ir kt. (2021) [220] metodiką, vertinant tirpių mažos molekulinės masės peptidų dalį bendro baltymų kiekio atžvilgiu. Virškinamumas apskaičiuotas pagal lygtį:

$$Virškinamumas_{SEC}(D_{SEC})(\text{proc.}) = \\ = \left(\frac{Tirpūs\ baltymai}{Bendras\ baltymų\ kiekis} \times \frac{SEC\ ploto\, 9.4-15\ \text{min}}{SEC\ ploto\, 5-15\ \text{min}} \right) \times 100 \qquad (12)$$

Sūrio mikrobiota analizuota mėginiuose prieš virškinimą, siekiant įvertinti brandinimo trukmės (1 ir 45 d.), dangų ir *L. paracasei* A11 poveikį tiek įprasto, tiek probiotinio sūrio mikrobiotai, taip pat siekiant aptikti *L. paracasei* ir *L. plantarum*. Bakterijų DNR išskirta iš nuosėdų, gautų homogenizavus sūrį buferiniame peptono vandenyje, naudojant „DNeasy PowerSoil 96 Pro Kit“ (Qiagen), vadovaujantis gamintojo instrukcijomis. Mikrobiota analizuota taikant 16S rRNR geno amplikonų sekoskaitą (2×150 bp), nukreiptą į V4 regioną (Caporaso ir kt., 2012) [222]. Sekoskaita atlikta „Illumina MiSeq“ platformoje, naudojant PGR produktus iš trijų nepriklausomų reakcijų kiekvienam mėginiui su individualiais koduotais pradmenimis. PhiX Control v3 sudarė 10 proc. visų skaitymų.

Sekoskaitos duomenys apdoroti naudojant atvirojo kodo bioinformatikos platformą QIIME2 (2023.5) [223], taikant DADA2 algoritmą. Taksonominė klasifikacija atlikta naudojant Greengenes2 (2022.10) duomenų bazę [224], kiekvieną unikalų vienetą apibrėžiant kaip amplikono sekos variantą (ASV). Mikrobiotos struktūros palyginimui atlikta beta įvairovės analizė, o rezultatai vizualizuoti pagrindinių koordinačių analize (PCoA), paremta svertine UniFrac metrika. Taksonominiai duomenys pateikti genties lygiu. *L. paracasei* ir *L. plantarum* identifikacija patvirtinta NCBI BLAST 16S rRNR duomenų baze, nustatant 100 proc. sekų atitikimą.

Statistinė duomenų analizė. Statistinė analizė atlikta naudojant SPSS programinę įrangą (24 versija, SPSS Inc., Armonk, NY, USA). Aprašomoji

statistika gauta naudojant „Explore“ funkciją. Atsižvelgiant į eksperimento dizainą ir siekiant įvertinti apdorojimo veiksnių ir laikymo trukmės poveikį taikyta vienfaktorė arba dvifaktorė dispersinė analizė (ANOVA), po kurios atliktas Tukey post hoc testas. Priklausomiems matavimams taikytas porinis t-testas, o ryšiams tarp kintamųjų – Pirsono koreliacijos analizė. Skirtumai laikyti statistiškai reikšmingais, kai $p < 0,05$.

Mikrobiotos sekoskaitos duomenys analizuoti naudojant Python (3.12) programinę įrangą ir bibliotekas „pandas“, „NumPy“, „scikit-bio“, „scikit-learn“ ir „matplotlib“. Mikrobiotos sudėties skirtumai vertinti taikant permutacinę daugiamatę dispersinę analizę (PERMANOVA), paremtą Bray–Curtis disimiliarumo matrica. Poriniai PERMANOVA palyginimai atlikti siekiant įvertinti skirtumus tarp eksperimentinių grupių kiekvieną mėginių ėmimo dieną. Atskirų genčių santykinės gausos skirtumai analizuoti taikant Kruskal–Wallis testą.

REZULTATAI

Taikant INFOGEST *in vitro* virškinimo modelį, iš rauginto ekologiško karvių pieno buvo išskirtos rūgščiai atsparios, antimikrobinėmis savybėmis pasižyminčios probiotinių savybių PRB, tinkamos panaudoti valgomųjų dangų kūrimui rūgščių išrūgų pagrindu. Iš viso *in vitro* buvo suviršinta 20 mėginių, iš kurių atlikus pasėlius išaugintos 22 bakterijų kolonijos tolesnei analizei. Fenotipinės analizės metu nustatyta, kad 12 kolonijų (55 proc.) pasižymėjo *Lactobacillus* genties bakterijoms būdingomis savybėmis. PGR metodu patvirtinta, kad iš jų 4 kolonijos (18 proc.) priklausė *Lactobacillus* spp., iš kurių trys identifikuotos kaip *Lacticaseibacillus paracasei*, o viena – kaip *Lactiplantibacillus plantarum*.

Visos išskirtos padermės buvo įvertintos saugos požiūriu. Nustačius atrinktų padermių hemolizinį aktyvumą paaiškėjo, kad visos bakterijos pasižymėjo γ-hemolize, būdinga saugioms, maistui tinkamoms bakterijoms.

Padermių atsparumo antibiotikams tyrimai atskleidė, kad visos padermės buvo jautrios daugumai tirtų antibiotikų (ampicilinui, eritromicinui, klindamicinui, streptomicinui, gentamicinui, kanamicinui ir tetraciklinui), tačiau visos pasižymėjo įgimtu atsparumu vankomicinui. Nustatyta, kad dvi padermės pasižymėjo didesniu atsparumu chloramfenikoliui nei rekomenduojama EMST, todėl tolesniems tyrimams atrinktos tik *L. paracasei* A11 ir *L. plantarum* A154-d1 padermės.

Storojoje žarnoje esančios bakterijų padermės, įskaitant kai kurias laktobacilas, pasižyminčios dideliu β-gliukuronidazės arba β-gliukozidazės aktyvumu, gali turėti neigiamą poveikį vartotojų sveikatai [227, 228]. Fermentinių savybių tyrimai parodė, kad nei viena iš dviejų tirtų laktobacilų neparodė

šarminės fosfatazės, lipazės, tripsino, α-galaktozidazės, β-gliukuronidazės, α-manozidazės ir α-fukozidazės fermentinio aktyvumo. Priešingai, abi padermės parodė stiprias leucino aril amidazės ir valino aril amidazės fermentines reakcijas. *L. paracasei* A11 taip pat pasižymėjo stipriu α-gliukozidazės aktyvumu, o *L. plantarum* A154-d1 stipriu β-gliukozidazės aktyvumu, dėl kurio ši padermė nebuvo pasirinkta tolesniems tyrimams.

Tiriant atrinktos *L. paracasei* A11 padermės technologines savybes nustatyta, kad ši padermė geba hidrolizuoti kazeiną, o tai rodo jos potencialą dalyvauti pieno produktų baltymų skaidyme. Tuo tarpu ekstraląstelinis proteolitinis ir lipolitinis aktyvumas nenustatytas. Padermė pasižymėjo vidutiniu rūgštinimo aktyvumu – per 6 valandas pH sumažėjo 1,48 ± 0,01 vieneto, o per 24 valandas – 2,46 ± 0,01 vieneto.

Angliavandenių fermentacijos tyrimai parodė, kad *L. paracasei* A11 geba selektyviai fermentuoti įvairius angliavandenius. Nustatytas teigiamas daugelio monosacharidų (gliukozės, galaktozės, fruktozės, manozės ir kt.) bei disacharidų (laktozės, maltozės, celobiozės, trehalozės) fermentavimas. Taip pat padermė gebėjo fermentuoti trisacharidą melezitozę ir kai kuriuos glikozidus. Kita vertus, dauguma kitų tirtų angliavandenių nebuvo fermentuojami arba buvo fermentuojami silpnai, kas rodo selektyvų šios padermės metabolinį profilį.

Įvertinus antimikrobinį aktyvumą nustatyta, kad *L. paracasei* A11 pasižymėjo skirtingo stiprumo inhibiciniu poveikiu įvairiems mikroorganizmams. Stipriausias slopinimas nustatytas prieš *Debaryomyces hansenii* ir *Listeria monocytogenes*. Vidutinis slopinimo poveikis nustatytas prieš *Pseudomonas fluorescens*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* ir kai kurias pelėsių rūšis. Silpnesnis aktyvumas nustatytas prieš kai kurias mieles ir bakterijas, o prieš *Candida albicans* ir *Mucor racemosus* slopinimo nenustatyta.

Probiotinės savybės buvo vertinamos nustatant *L. paracasei* A11 atsparumą rūgščiai ir tulžies druskoms. Esant žemam pH (2,5), bakterijų gyvybingumas išliko nepakitęs net po 3 valandų inkubacijos, o tai rodo didelį atsparumą rūgštinei terpei. Tulžies druskų poveikio tyrimai parodė, kad net esant didžiausiai (1 proc.) jų koncentracijai bakterijų kiekis sumažėjo, tačiau išliko pakankamai didelis (6,66 $\log_{10}$ KSV/g), o išgyvenamumas siekė apie 73 proc. Šie rezultatai rodo, kad *L. paracasei* A11 yra gerai prisitaikiusi prie virškinamojo trakto sąlygų.

Įvertintas *L. paracasei* A11 augimas skirtingose terpėse – MRS sultinyje, taip pat SRIBK ir SRIP terpėse. Nustatyta, kad augimo terpė ir inkubacijos trukmė turėjo statistiškai reikšmingą įtaką tiek pH pokyčiams, tiek bakterijų augimui ir gyvybingų ląstelių skaičiui ($p < 0,05$). MRS sultinyje nustatytas intensyvus rūgštėjimas: pH per 48 valandas sumažėjo nuo 6,25 iki 3,46 viene-

tų. SRIBK terpėje pH pokyčiai buvo minimalūs ir vėliau išliko stabilūs (nuo 4,70 iki 4,49 per 48 val.). SRIP terpėje pH reikšmės viso auginimo laikotarpiu iš esmės nekito (nuo 4,36 iki 4,25 per 48 val.), o tai rodo mažą fermentacijos aktyvumą. Tyrimų metu nustatyta, kad bakterijų augimas reikšmingai priklausė nuo terpės sudėties ($p < 0,05$). MRS sultinyje stebėtas intensyviausias augimas – per 48 valandas bakterijų skaičius padidėjo nuo 5,51 ± 0,02 iki 9,23 ± 0,03 $\log_{10}$ KSV/g, o didžiausias augimo šuolis nustatytas per pirmąsias 24 valandas. SRIBK terpėje augimas buvo lėtesnis, tačiau tolygus – bakterijų skaičius per tą patį laikotarpį padidėjo iki 8,30 ± 0,02 $\log_{10}$ KSV/g. Tuo tarpu LAWP terpėje augimas buvo ribotas – pradžioje bakterijų skaičius sumažėjo, vėliau nežymiai padidėjo iki 6,3 ± 0,03 $\log_{10}$ KSV/g, kas rodo mažą šios terpės tinkamumą bakterijų dauginimuisi.

Vertinant metabolinį aktyvumą nustatyta, kad jis reikšmingai priklausė nuo terpės sudėties ($p < 0,05$). MRS sultinyje stebėtas intensyvus gliukozės sunaudojimas – jos koncentracija sumažėjo nuo 1,67 iki 0,15 proc. Tuo tarpu SRIBK terpėje nustatyti nedideli gliukozės kiekio pokyčiai (sumažėjo nuo 0,66 iki 0,57 proc.), o SRIP terpėje – sumažėjo reikšmingai (nuo 0,45 iki 0,02 proc.) ($p < 0,05$), nors tai nebuvo susiję su intensyviu bakterijų augimu ir gali būti paaiškinama mažesniu pradiniu angliavandenių kiekiu šioje terpėje. SRIBK terpėje nustatytas nežymus, tačiau statistiškai reikšmingas galaktozės ir laktozės koncentracijos sumažėjimas ($p < 0,05$), tuo tarpu SRIP terpėje reikšmingų šių cukrų pokyčių nenustatyta ($p > 0,05$). Rūgščių susidarymas atitiko šias tendencijas: MRS sultinyje nustatytas didžiausias pieno rūgšties (nuo 34,45 iki 1462,95 mg/100 g) ir laktato (nuo 34,06 iki 1442,17 mg/100 g) kiekio padidėjimas; SRIBK terpėje šie procesai vyko nuosaikiau (pieno rūgštis padidėjo nuo 847,01 iki 1030,23 mg/100 g, o laktato kiekis didėjo nuo 837,20 iki 1019,09 mg/100 g); SRIP terpėje buvo mažiausiai intensyvūs (pieno rūgštis padidėjo nuo 120,0 iki 431,0 mg/100 g, o laktato kiekis padidėjo nuo 113,01 iki 416,01 mg/100 g). Tai rodo, kad fermentacijos aktyvumas tiesiogiai priklausė nuo terpės maistinių medžiagų sudėties.

Tiriant bakterijų augimą bioreaktoriuje patvirtintos anksčiau nustatytos tendencijos – SRIP terpėje bakterijų augimas nenustatymas, kas gali būti siejama su ribotu maistinių medžiagų kiekiu. Tuo tarpu MRS sultinyje ir SRIBK terpėje nustatytas aktyvus bakterijų augimas. MRS sultinyje vyko intensyvus eksponentinis augimas, o SRIBK terpėje nustatytas diauksinis augimas – pirmajai fazei būdingas didesnis augimo intensyvumas, o antrojoje fazėje augimas sulėtėjo, siejant tai su skirtingų substratų panaudojimu. Atsižvelgiant į gautus rezultatus ir SRIBK terpėje nustatytą aktyvų bakterijų augimą, bakterijų kultivavimui buvo pasirinkta ši terpė, kuri vėliau panaudota valgomųjų dangų kūrimui.

Tyrimo metu paruoštos keturios valgomųjų dangų kompozicijos, naudojant SRIBK ir SRIP pagrindus su ir be įterptos *L. paracasei* A11 padermės. Iš šių kompozicijų paruošti tirpalai ir plėvelės buvo tiriami siekiant įvertinti išrūgų produktų tinkamumą kompozicijų kūrimui bei padermės įtaką jų savybėms.

Reologiniai tyrimai parodė, kad valgomųjų dangų tirpalų klampa priklausė nuo jų sudėties – SRIP pagrindu sudarytų dangų tirpalai pasižymėjo mažesne klampa (19,64 Pa) nei SRIBK pagrindu paruošti tirpalai (37,58 Pa), o *L. paracasei* A11 įterpimas reikšmingai sumažino SRIBK tirpalų klampą – apie 13,24 Pa ($p < 0,05$).

Vertinant valgomųjų dangų plėvelių fizines ir optines savybes nustatyta, kad jų sudėtis ir įterpta padermė turėjo įtakos daugumai tiriamų parametrų. Plėvelių storis priklausė nuo pagrindo – SRIP pagrindu pagamintos plėvelės buvo plonesnės (apie 0,13 μm), tuo tarpu SRIBK pagrindu pagamintos kontrolinės plėvelės – storesnės (0,28 μm), o įterpta padermė reikšmingai sumažino jų storį iki 20 μm ($p < 0,05$). Nors tempimo stipris tarp skirtingų plėvelių tipų reikšmingai nesiskyrė ($p > 0,05$), elastingumo rodikliai (pailgėjimas iki plyšimo) skyrėsi reikšmingai – SRIP pagrindu pagamintos plėvelės pasižymėjo didesniu tamprumu (apie 28,75 proc.), palyginti su SRIBK kontrolinėmis plėvelėmis (9,36 proc.) ($p < 0,05$). Tačiau *L. paracasei* A11 įterpimas į SRIBK plėveles reikšmingai padidino jų elastingumą – nuo 9,36 iki 23,87 proc. ($p < 0,05$). Vertinant plėvelių drėgmės kiekį nustatyta, kad SRIP plėvelėse jis buvo reikšmingai didesnis (apie 32,91 proc.) nei SRIBK plėvelėse (iki 22,84 proc.), kas siejama su skirtingu sausųjų medžiagų kiekiu kompozicijose. Tuo tarpu plėvelių vandens garų pralaidumas ir tirpumas vandenyje tarp skirtingų kompozicijų reikšmingai nesiskyrė ($p > 0,05$). Optinių savybių analizė parodė, kad plėvelių šviesos pralaidumas ir spalviniai parametrai priklausė nuo jų sudėties. SRIP plėvelės pasižymėjo didesniu šviesos pralaidumu ir šviesumu (apie 12,23 proc. ir L* apie 92,84) nei SRIBK kontrolinės plėvelės (21,59 proc. ir L* 84,34), tačiau *L. paracasei* A11 įterpimas reikšmingai padidino SRIBK plėvelių šviesos pralaidumą ir šviesumą (17,09 proc. ir L* 88,23) ($p < 0,05$). Be to, nustatyta, kad plėvelių kompozicija turėjo įtakos spalviniams parametrams – SRIBK plėvelės pasižymėjo mažesnėmis a* ir didesnėmis b* reikšmėmis, palyginti su SRIP plėvelėmis. Šiems skirtumams įtakos galėjo turėti skirtingas sausųjų medžiagų kiekis SRIP ir SRIBK kompozicijose.

Morfologinė analizė parodė plėvelių struktūros skirtumus – SRIBK plėvelės pasižymėjo nevienalyčiu paviršiumi su kristalinėmis struktūromis, o SRIP plėvelės buvo porėtesnės. Elementinės sudėties tyrimai parodė, kad pagrindiniai plėvelių komponentai buvo anglis ir deguonis, o kitų elementų aptikta tik nedideliais kiekiais.

Vertinant *L. paracasei* A11 išgyvenamumą plėvelėse, nustatyta, kad džiovinimo procesas, priklausomai nuo plėvelių sudėties, skirtingai veikė PRB išgyvenamumą. SRIBK plėvelėse po džiovinimo gyvybingų PRB skaičius reikšmingai padidėjo (nuo 7,16 iki 7,67 $\log_{10}$ KSV/g), tuo tarpu SRIP plėvelėse nustatytas nedidelis, tačiau reikšmingas sumažėjimas (nuo 7,60 iki 7,53 $\log_{10}$ KSV/g) ($p < 0,05$). Laikymo metu (4 °C, 21 d.) abiejų kompozicijų plėvelėse nustatytas reikšmingas gyvybingų PRB skaičiaus mažėjimas, tačiau jis buvo ryškesnis SRIP plėvelėse. SRIBK plėvelėse bakterijų skaičius sumažėjo nuo 7,67 iki 5,21 $\log_{10}$ KSV/g per 21 dieną, o SRIP plėvelėse – nuo 7,53 iki 4,76 $\log_{10}$ KSV/g per 14 dienų ($p < 0,05$). Lyginant PRB išgyvenamumą nustatyta, kad SRIBK pagrindu sudarytose plėvelėse bakterijų gyvybingumas viso laikymo laikotarpiu buvo reikšmingai didesnis nei SRIP plėvelėse ($p < 0,05$).

Atsižvelgiant į ankstesnių tyrimų rezultatus, tolesniems eksperimentams buvo atrinkta SRIBK pagrindu pagaminta valgomoji danga, su ir be įterptos *L. paracasei* A11 padermės, kuri buvo aplikuota ant įprasto (Į) ir probiotinio (P – su *Lactiplantibacillus plantarum* Tensia) Edam tipo sūrio prieš brandinimą. Sūrių kokybės, cheminiai ir mikrobiologiniai rodikliai buvo vertinti 1, 45 ir 135 laikymo dienomis, atitinkamai prieš brandinimą, po brandinimo ir laikymo pabaigoje. Kadangi įprastų ir probiotinių sūrių gamybai buvo panaudotos skirtingos pieno partijos, šių sūrių rezultatai buvo analizuojami ir interpretuojami atskirai, jų tarpusavyje nelyginant.

Vertinant fizikinius ir cheminius rodiklius nustatyta, kad pH, a_w ir SM kiekis reikšmingai priklausė nuo mėginio tipo ir laikymo trukmės ir šių veiksnių sąveikos abiejų tipų sūriuose ($p < 0,01$). Įprastuose sūriuose laikymo metu pH didėjo ir laikymo pabaigoje siekė iki 5,51 kontroliniuose mėginiuose, tuo tarpu dengtuose mėginiuose buvo reikšmingai mažesnis (apie 5,29–5,36) ($p < 0.05$). A_w laikymo metu mažėjo, tačiau laikymo pabaigoje Į + SRIBK + L. p mėginiuose išliko reikšmingai didesnis (0,943), palyginti su kontroliniais įprasto sūrio mėginiais (0,939) ($p < 0,05$). SM kiekis kito priklausomai nuo mėginio – laikymo pabaigoje didžiausias nustatytas Į + SRIBK + L. p mėginyje (53,72 proc.). ΔE įprastuose sūriuose laikymo metu reikšmingai nesiskyrė. Probiotiniuose sūriuose nustatytos panašios tendencijos. pH laikymo metu didėjo (iki 5,33 kontrolėje), tačiau dengtuose mėginiuose buvo stebimi didesni svyravimai. A_w mažėjo, o mažiausia reikšmė buvo P + SRIBK + L. p mėginyje brandinimo metu (0,927) ($p < 0,05$). SM kiekis reikšmingai padidėjo P + SRIBK mėginyje po brandinimo (57,25 proc.), tačiau laikymo pabaigoje sumažėjo iki 53,13 proc. ir buvo mažesnis nei kituose mėginiuose ($p < 0,05$). Skirtingai nei įprastuose sūriuose, probiotiniuose sūriuose laikymo trukmė turėjo reikšmingą poveikį ΔE, kas rodo, kad spalvos pokyčiai laikui bėgant

didėjo. Tačiau reikšmingos mėginio ir laikymo dienų sąveikos nenustatyta, todėl galima teigti, kad dangos neturėjo poveikio spalvos kitimui.

RR analizė parodė, kad sūrių brandinimo pradžioje reikšmingų skirtumų tarp įprastų ir probiotinių sūrio mėginių nebuvo. Laikymo pabaigoje mažiausias sočiųjų riebalų rūgščių kiekis nustatytas Į + SRIBK + L. p mėginyje (68,66 g/100 g bendro RR kiekio), o didžiausias – įprasto sūrio kontroliniame mėginyje (68,92 g/100 g bendro RR kiekio) ($p < 0.05$). Nesočiųjų riebalų rūgščių ir DFA kiekis buvo didžiausios Į + SRIBK + L. p mėginyje 31,34 g/100 g bendro RR kiekio ir 40,14 g/100 g bendro RR kiekio, atitinkamai ($p < 0.05$). Tuo tarpu, AI laikymo metu mažėjo ir buvo reikšmingai mažiausias Į + SRIBK + L. p mėginyje ($p < 0,05$). Taip pat nustatyta, kad laikymo metu valgomosiomis dangomis padengtuose įprastų sūrių mėginiuose sumažėjo ilgos grandinės ir padidėjo trumpos grandinės riebalų rūgščių kiekis ($p < 0,05$). Omega-3 ir omega-6 riebalų rūgščių kiekis laikymo metu reikšmingai nesikeitė, tačiau n-6/n-3 santykis didėjo ir buvo didžiausias Į + SRIBK + L. p mėginyje ($p < 0,05$). Trans RR kiekis laikymo metu didėjo visuose mėginiuose, o laikymo pabaigoje buvo didžiausias Į + SRIBK + L. p mėginyje (3,22 g/100 g bendro riebalų rūgščių kiekio), palyginti su Į + SRIBK mėginiu, kuriame nustatyta reikšmingai mažiausia reikšmė – 3,19 g/100 g ($p < 0,05$). Probiotiniuose sūriuose po brandinimo (45 dieną) valgomosiosmis dangomis dengtuose mėginiuose nustatytas didesnis sočiųjų RR kiekis, o kontroliniuose – didesnis mononesočiųjų ir bendras nesočiųjų RR kiekis ($p < 0,05$). Taip pat nustatyta, kad P + SRIBK + L. p mėginyje omega-6 kiekis buvo didžiausias (2,07 g/100 g bendro RR kiekio), o omega-3 – mažiausias (0,80 g/100 g bendro RR kiekio), palyginti su kontroliniu mėginiu (atitinkamai 2,04 ir 0,83 g/100 g bendro RR kiekio) ($p < 0,05$), todėl kontrolėje nustatyti mažesni n-6/n-3 santykis bei lipidų kokybės rodikliai (AI, TI). Laikymo pabaigoje (135 dieną) kontroliniuose probiotinio sūrio mėginiuose nustatytas sočiųjų RR, n-6/n-3 santykio ir trombogeniškumo indekso padidėjimas, o mononesočiųjų RR, bendras nesočiųjų RR rūgščių ir omega-3 kiekis sumažėjo ($p < 0,05$). Dengtuose mėginiuose nustatyta priešinga tendencija – sumažėjo ilgos grandinės ir padidėjo trumpos grandinės RR kiekis ($p < 0,05$).

Mikrobiologiniai tyrimai parodė, kad valgomosios dangos turėjo reikšmingą poveikį mikroorganizmų pasiskirstymui tiek įprastuose, tiek probiotiniuose sūriuose. Abiejose sūrių grupėse laikymo pradžioje bendras bakterijų skaičius (BBS) sūrio matricoje buvo didesnis valgomosiomis dangomis padengtuose mėginiuose (apie 8,43 $\log_{10}$ KSV/g), palyginti su kontroliniais (apie 7,50 $\log_{10}$ KSV/g) ($p < 0,05$). Po brandinimo (45 dieną) abiejose sūrių grupėse BBS reikšmingai padidėjo ir siekė apie 8,79 $\log_{10}$ KSV/g valgomosiomis dangomis padengtuose mėginiuose bei apie 7,79 $\log_{10}$ KSV/g kontroliniuose sūrių mėginiuose ($p < 0,05$). Laikymo pabaigoje didžiausias

BBS nustatytas kontroliniuose mėginiuose, tuo tarpu dangomis padengtuose mėginiuose jis buvo reikšmingai mažesnis ($p < 0,05$). PRB skaičius įprastuose sūriuose laikymo metu reikšmingai mažėjo ($p < 0,05$), tuo tarpu probiotiniuose sūriuose kito priklausomai nuo laikymo trukmės ($p < 0,05$), tačiau nuoseklios mažėjimo tendencijos nenustatyta. Be to, probiotinių sūrių matricoje nustatyta įterptų probiotinių *L. plantarum* augimo tendencija – šių bakterijų kiekis laikymo metu reikšmingai didėjo ir laikymo pabaigoje siekė iki 7,30 $\log_{10}$ KSV/g ($p < 0,05$).

Sūrio paviršiaus mikrobiologinė analizė parodė, kad mikroorganizmų pasiskirstymą reikšmingai veikė *L. paracasei* A11 įterpimas į valgomąją dangą. Laikymo pradžioje BBS ir PRB kiekis sūrio paviršiuje nustatytas tik abiejų sūrių grupių mėginiuose, padengtuose SRIBK pagrindu suformuota danga su *L. paracasei* A11. Abiejų mikroorganizmų grupių koncentracija buvo panaši ir siekė 5,99 $\log_{10}$ KSV/g. Šiuose mėginiuose taip pat nustatytas *L. paracasei* kiekis, siekęs apie 5,63 $\log_{10}$ KSV/g. Po brandinimo (45 dieną) abiejų sūrių grupių mėginių paviršiuje nustatytas reikšmingas BBS padidėjimas ($p < 0,05$), didžiausias reikšmes fiksuojant kontroliniuose mėginiuose – iki 8,79 $\log_{10}$ KSV/g įprastuose ir 8,54 $\log_{10}$ KSV/g probiotiniuose sūriuose. PRB aptiktos tik SRIBK + L. p danga padengtų mėginių paviršiuje, o jų kiekis statistiškai reikšmingai didėjo ($p < 0,05$) ir siekė 6,58 $\log_{10}$ KSV/g įprastuose bei 6,76 $\log_{10}$ KSV/g probiotiniuose sūriuose. Atitinkamai *L. paracasei* kiekis taip pat didėjo ir pasiekė 6,52 $\log_{10}$ KSV/g įprastuose bei 6,27 $\log_{10}$ KSV/g probiotiniuose mėginiuose ($p < 0,05$). Laikymo pabaigoje (135 dieną) nustatyta, kad BBS ir PRB kiekiai abiejose sūrių grupėse tarp mėginių reikšmingai skyrėsi ($p < 0,05$). Mažiausios šių rodiklių reikšmės nustatytos kontrolinių mėginių paviršiuje, o didžiausios – SRIBK pagrindu suformuotomis dangomis padengtuose mėginiuose. *L. paracasei* kiekis laikymo metu toliau didėjo ir laikymo pabaigoje siekė 6,70 $\log_{10}$ KSV/g įprastuose bei 6,57 $\log_{10}$ KSV/g probiotiniuose sūriuose ($p < 0,05$). Pelėsiai ir mielės laikymo pradžioje nenustatyti, tačiau laikymo pabaigoje aptikti tik kontroliniuose ir SRIBK danga be bakterijų padengtuose mėginiuose (iki 2,30–2,97 $\log_{10}$ KSV/g). Tuo tarpu mėginiuose, padengtuose SRIBK + L. p danga, šių mikroorganizmų per visą laikymo laikotarpį nenustatyta, patvirtinant dangos su *L. paracasei* A11 potencialų antimikrobinį poveikį.

Juslinė analizė parodė, kad valgomosios dangos su *L. paracasei* A11 turėjo reikšmingą poveikį sūrių kokybei abiejose sūrių grupėse. Po brandinimo (45 dieną) didžiausias bendro priimtinumo įvertinimas nustatytas Į + SRIBK + L. p įprasto sūrio mėginiuose (85,54 ± 7,14), jis buvo reikšmingai didesnis nei kontrolinių mėginių (61,52 ± 4,32) ($p < 0,05$). Analogiška tendencija nustatyta ir probiotiniuose sūriuose, kuriuose P + SRIBK + L. p mėginiai pasižymėjo reikšmingai aukštesniais skonio ir išvaizdos rodikliais

($p < 0,05$). Laikymo pabaigoje (135 dieną) valgomosiomis dangomis padengti mėginiai su *L. paracasei* A11 abiejose sūrių grupėse pasižymėjo reikšmingai aukštesniais skonio, tekstūros ir bendro priimtinumo įvertinimais, lyginant su kontroliniais mėginiais ($p < 0,05$). Tuo tarpu kai kuriuose mėginiuose nustatytas reikšmingas išvaizdos rodiklių sumažėjimas laikymo metu ($p < 0,05$), tačiau SRIBK + L. p danga padengtuose abiejų sūrių grupių mėginiuose reikšmingų šio parametro pokyčių nenustatyta. Gauti rezultatai rodo, kad *L. paracasei* A11 įterpimas į valgomąją dangą turėjo teigiamą poveikį juslinėms savybėms ir užtikrino jų išsaugojimą bei stabilumą laikymo metu.

Atsižvelgiant į valgomųjų dangų su *L. paracasei* A11 ir probiotinės starterinės kultūros *L. plantarum* Tensia taikymą, buvo vertintas PRB, *L. paracasei* ir *L. plantarum* kiekis įprastuose ir probiotiniuose Edamo tipo sūriuose prieš ir po *in vitro* virškinimo, taikant INFOGEST virškinimo modelį, 1 ir 45 brandinimo dienomis.

Nustatyta, kad tiek įprastuose, tiek probiotiniuose sūriuose PRB kiekis po *in vitro* virškinimo reikšmingai sumažėjo ($p < 0,05$), kas rodo virškinimo proceso neigiamą poveikį PRB gyvybingumui. Įprastuose sūriuose PRB kiekis 1 dieną prieš virškinimą siekė 8,47–8,69 $\log_{10}$ KSV/g ir po virškinimo sumažėjo, skirtumui siekiant 0,50–0,96 $\log_{10}$ KSV/g ($p < 0,05$). *L. paracasei* nustatyta tik Į + SRIBK + L. p mėginiuose, o jos kiekis po virškinimo reikšmingai nekito (apie 5,06 $\log_{10}$ KSV/g; $p > 0,05$), kas rodo šios rūšies bakterijų stabilumą virškinimo sąlygomis. *L. plantarum* bakterijos įprastų sūrių mėginiuose nenustatytos. Tuo tarpu probiotiniuose sūriuose PRB kiekis prieš virškinimą buvo panašus (8,47–8,68 $\log_{10}$ KSV/g), tačiau po virškinimo taip pat reikšmingai sumažėjo, skirtumui siekiant 0,68–0,86 $\log_{10}$ KSV/g ($p < 0,05$). *L. plantarum* kiekis po virškinimo reikšmingai padidėjo, pokyčiui siekiant 0,48–0,81 $\log_{10}$ KSV/g ($p < 0,05$), kas gali būti siejama su šių bakterijų buvimu sūrio matricoje sudėtine starterinės kultūros dalimi. *L. paracasei* bakterijų kiekis po virškinimo reikšmingai nekito (apie 6,46 $\log_{10}$ KSV/g; $p > 0,05$).

Po 45 dienų brandinimo įprastuose sūriuose PRB kiekis prieš virškinimą reikšmingai padidėjo iki 8,82–8,88 $\log_{10}$ KSV/g ($p < 0,05$), tačiau po virškinimo sumažėjo, kas atspindi bendrą PRB skaičiaus mažėjimo tendenciją virškinimo sąlygomis. *L. paracasei* kiekis prieš virškinimą padidėjo iki 7,59 $\log_{10}$ KSV/g, o po virškinimo sumažėjo iki 5,88 $\log_{10}$ KSV/g ($p < 0,05$), kas rodo, kad šių bakterijų kiekis didėjo brandinimo metu, tačiau sumažėjo po *in vitro* virškinimo. Probiotiniuose sūriuose po brandinimo nustatytas reikšmingas *L. plantarum* kiekio padidėjimas (6,97 $\log_{10}$ KSV/g kontroliniuose mėginiuose ir 7,06 $\log_{10}$ KSV/g P + SRIBK mėginiuose; $p < 0,05$). Tuo tarpu P + SRIBK + L. p mėginiuose šių bakterijų kiekis buvo mažesnis (apie 6,11 $\log_{10}$ KSV/g; $p < 0,05$). Po *in vitro* virškinimo visų mėginių PRB kiekis sumažėjo, o *L. paracasei* kiekis sumažėjo iki 5,02 $\log_{10}$ KSV/g ($p < 0,05$).

In vitro virškinimas turėjo reikšmingą įtaką *L. plantarum* bakterijų kiekiui, kuris sumažėjo P + SRIBK + L. p mėginiuose (6,11 $\log_{10}$ KSV/g; $p < 0,05$), ir būtent šiuose mėginiuose nustatytas mažiausias šių bakterijų kiekis, palyginti su kitais mėginiais. Tai gali rodyti galimą mikroorganizmų tarpusavio sąveiką sūrio matricoje.

Sūrių mikrobiotos analizė parodė, kad abiejų sūrių tipuose dominavo *Lactococcus* genties bakterijos, kurių santykinė gausa viršijo 97 proc. įprastuose ir siekė apie 85 proc. probiotiniuose sūriuose, kas rodo starterinės kultūros dominavimą formuojant mikrobiotos struktūrą. Dangų tipas reikšmingos įtakos dominuojančių genčių pasiskirstymui neturėjo ($p > 0,05$). Nedominuojančių mikroorganizmų įvairovė buvo didesnė probiotiniuose sūriuose, tačiau reikšmingų skirtumų tarp mėginių ar brandinimo trukmės nenustatyta ($p > 0,05$). *Lacticaseibacillus* ir *Lactiplantibacillus* gentims priskiriamų mikroorganizmų santykinė gausa išliko maža (< 1 proc.), rodančia, kad įterptos padermės netapo dominuojančia mikrobiotos dalimi.

Pagrindinių komponentų analizė parodė skirtingą įprastų ir probiotinių sūrių mėginių išsidėstymą. Įprastų sūrių mėginiai sudarė kompaktišką klasterį, o tarp 1 ir 45 brandinimo dienų stebėtas reikšmingas poslinkis (PERMANOVA; $p < 0,05$), rodantis mikrobiotos struktūros pokyčius brandinimo metu. Mėginio tipas reikšmingos įtakos mikrobiotai neturėjo (PERMANOVA; $p > 0,05$), tačiau nustatyta reikšminga mėginio tipo ir brandinimo trukmės sąveika (PERMANOVA; $p < 0,05$). Tačiau poriniai PERMANOVA palyginimai neparodė reikšmingų skirtumų tarp atskirų mėginių nė vienu brandinimo laikotarpiu ($p > 0,05$), todėl reikšmingo valgomųjų dangų poveikio mikrobiotos struktūrai nenustatyta. Probiotinių sūrių mėginiai pasižymėjo didesniu išsisklaidymu ir neparodė nuoseklaus grupavimosi nei tarp skirtingų mėginių, nei tarp brandinimo laikotarpių, o reikšmingų mikrobiotos struktūros pokyčių nenustatyta ($p > 0,05$).

Siekiant įvertinti valgomųjų dangų su *L. paracasei* A11 ir probiotinės starterinės kultūros *L. plantarum* Tensia poveikį baltymų hidrolizės ir virškinamumo procesams, buvo analizuojamas baltymų tirpumas, mažos molekulinės masės peptidų kiekis ir baltymų virškinamumas po *in vitro* virškinimo.

Įprastuose sūriuose po 45 dienų brandinimo didžiausias baltymų tirpumas nustatytas Į + SRIBK mėginiuose (88,2 proc.), o mažiausias – Į + SRIBK + L. p mėginiuose (85,2 proc.), palyginti su kontroliniais mėginiais (87,0 proc.) ($p < 0,05$). Tuo tarpu probiotiniuose sūriuose didžiausias tirpių baltymų kiekis nustatytas P + SRIBK + L. p mėginiuose (80,3 proc.) ($p < 0,05$), lyginant su P + SRIBK + L. p (77,5 proc.) ir probiotinių sūrių kontroliniais mėginiais (P (C); 76,7 proc.).

Įprastuose sūriuose mažos molekulinės masės peptidų kiekis buvo didžiausias kontroliniuose mėginiuose (86,6 proc.), o valgomųjų dangų tai-

kymas buvo susijęs su reikšmingai mažesnėmis šio rodiklio reikšmėmis ($p < 0,05$). Probiotiniuose sūriuose nustatytas priešingas pasiskirstymas – didesnis šių peptidų kiekis nustatytas mėginiuose su valgomosiomis dangomis (86,3–86,4 proc.) ($p < 0,05$).

Įvertintas baltymų virškinamumas, kuris įprastuose sūriuose buvo mažesnis Į + SRIBK + L. p mėginiuose (73,5 proc.), palyginti su kontroliniais įprasto sūrio mėginiais (75,4 proc.; $p < 0,05$). Probiotiniuose sūriuose nustatytas priešingas pasiskirstymas – didžiausias virškinamumas nustatytas P + SRIBK + L. p mėginiuose (69,4 proc.), lyginant su P + SRIBK (66,9 proc.) mėginiais ir probiotinio sūrio kontroliniais mėginiais (66,1 proc.; $p < 0,05$). Gauti rezultatai rodo, kad valgomųjų dangų poveikis baltymų hidrolizės ir virškinamumo rodikliams priklausė nuo sūrio tipo ir *L. paracasei* A11 įterpimo į dangą, tačiau šių skirtumų mechanizmui išsiaiškinti reikalingi tolesni tyrimai.

IŠVADOS

1. Taikant standartizuotą INFOGEST *in vitro* virškinimo metodą iš 20 raugintų karvės pieno mėginių buvo išaugintos 22 bakterijų kolonijos:
   1.1. Iš jų 4 padermės (18 proc.) identifikuotos kaip *Lactobacillus* spp., įskaitant *Lacticaseibacillus paracasei* ir *Lactiplantibacillus plantarum* rūšis.
   1.2. Iš tirtų padermių *L. paracasei* A11 pasižymėjo tinkamiausiomis saugos, technologinėmis ir probiotinėmis savybėmis. Nustatyta, kad ši padermė neturėjo hemolizinio aktyvumo, buvo jautri daugumai tirtų antibiotikų (išskyrus įgimtą atsparumą vankomicinui), pasižymėjo dideliu atsparumu rūgščiai ir tulžies druskoms (100 proc. išgyvenamumas esant pH 2,5 ir 73 proc. – esant 1 proc. tulžies koncentracijai), taip pat slopino gedimą sukeliančių ir patogeninių mikroorganizmų augimą: mielėms nustatytos iki 25 mm, o *Listeria monocytogenes* – iki 15 mm slopinimo zonos.
2. Atrinktos probiotinių savybių *L. paracasei* A11 padermės auginimas antrinėse pieno pramonės žaliavose patvirtino šių terpių tinkamumą bakterijų kultivavimui:
   2.1. *L. paracasei* A11 augimas buvo stabilesnis SRIBK terpėje, kurioje nustatytas didesnis biomasės kiekis (8,3 $\log_{10}$ KSV/g), lyginant su SRIP terpe.
   2.2. Nustatyta, kad padermė aktyviai metabolizavo angliavandenius, o pieno rūgšties ir laktato koncentracijų pokyčiai patvirtino efektyvų metabolinį aktyvumą SRIBK terpėje.

2.3. SRIP terpė buvo mažiau tinkama bakterijų augimui dėl mažesnio maistinių medžiagų kiekio, todėl nustatytas mažesnis bakterijų biomasės kiekis (6,30 $\log_{10}$ KSV/g).

3. Sukurtos ir įvertintos valgomosios dangos, pagamintos iš antrinių pieno pramonės žaliavų, į kurių sudėtį įterptos probiotinių savybių laktobacilos:
   3.1. *L. paracasei* A11 įterpimas į SRIBK pagrindu pagamintus valgomųjų dangų tirpalus sumažino jų klampumą bei reikšmingai padidino plėvelių elastingumą ir skaidrumą ($p < 0,05$).
   3.2. Po džiovinimo SRIBK pagrindu pagamintose plėvelėse nustatytas PRB skaičius siekė 7,67 $\log_{10}$ KSV/g, kuris po 21 dienos laikymo 4 °C temperatūroje sumažėjo iki 5,21 $\log_{10}$ KSV/g, tačiau išliko didesnis nei SRIP pagrindu pagamintose plėvelėse.

4. Įprastų ir probiotinių Edam tipo sūrių padengimas valgomosiomis dangomis pagerino jų saugą ir kokybę brandinimo bei laikymo metu:
   4.1. Valgomosiomis dangomis dengtuose sūriuose nustatytos reikšmingai mažesnės pH reikšmės ($p < 0,05$), taip pat nustatyti a_w ir SM kiekio skirtumai, o ΔE tarp mėginių reikšmingai nesiskyrė.
   4.2. Valgomosios dangos turėjo įtakos RR sudėčiai. Įprastuose sūriuose dangos su *L. paracasei* A11 buvo susijusios su sumažėjusiu sočiųjų ir padidėjusiu nesočiųjų RR kiekiu bei palankesniais lipidų kokybės rodikliais (AI ir DFA) laikymo pabaigoje ($p < 0,05$), o probiotiniuose sūriuose šios tendencijos buvo mažiau nuoseklios.
   4.3. *L. paracasei* kiekis sūrio paviršiuje laikymo metu didėjo ir laikymo pabaigoje siekė 6,70 $\log_{10}$ KSV/g, patvirtinant šios rūšies bakterijų išgyvenamumą.
   4.4. Dangos su *L. paracasei* A11 veiksmingai slopino gedimą sukeliančių mikroorganizmų augimą – pelėsiai ir mielės (2,30–2,97 $\log_{10}$ KSV/g) nustatyti tik kontroliniuose ir SRIBK dangomis be bakterijų padengtuose mėginiuose, tuo tarpu mėginiuose su SRIBK + L. p. dangomis jų nenustatyta.
   4.5. Juslinė analizė parodė, kad SRIBK + L. p. dangomis padengti sūriai pasižymėjo reikšmingai geresnėmis skonio ir bendro priimtinumo savybėmis, o bendro priimtinumo įvertinimas siekė iki 85,96 balo, palyginti su kontroliniais mėginiais ($p < 0,05$).

5. *In vitro* virškinimas parodė valgomosiomis dangomis padengtų sūrių funkcinį potencialą:
   5.1. *In vitro* virškinimas reikšmingai sumažino PRB skaičių visuose mėginiuose ($p < 0,05$), tačiau *L. paracasei* rūšies bakterijos išliko

aptinkamos (5,02–6,46 $\log_{10}$ KSV/g), patvirtinant jų gebėjimą išlikti virškinimo sąlygomis.

5.2. Valgomosios dangos su *L. paracasei* A11 neturėjo reikšmingos įtakos sūrių mikrobiotai ($p > 0,05$), kuri brandinimo metu išliko dominuojama starterinių *Lactococcus* genties bakterijų.

5.3. Probiotinio sūrio matricoje aptiktos *L. plantarum* (naudota kaip probiotinė starterinė kultūra *L. plantarum* Tensia) ir *L. paracasei* (*L. paracasei* A11 įterpta į valgomąją dangą), kas rodo jų gebėjimą vienu metu išlikti toje pačioje sūrio matricoje. Vis dėlto jų išlikimas padermių lygmeniu gali būti patvirtintas tik atlikus viso genomo sekoskaitos analizę.

5.4. Baltymų virškinamumui įtakos turėjo valgomosios dangos ir į jas įterpta *L. paracasei* A11: probiotiniuose sūriuose dangos su *L. paracasei* A11 buvo susijusios su didesniu baltymų virškinamumu (iki 69,4 proc.; $p < 0,05$), tuo tarpu įprastuose sūriuose nustatyti skirtumai nebuvo susiję su baltymų virškinamumo padidėjimu ($p < 0,05$).

REKOMENDACIJOS

1. *In vitro* virškinimo modelio taikymas prieš bakterijų išskyrimą gali būti veiksminga priemonė atrenkant rūgščiai atsparias, probiotinio potencialo turinčias laktobacilų padermes.
2. SRIBK rekomenduojamas kaip tinkama antrinė pieno pramonės žaliava, tinkama tiek laktobacilų kultivavimui, tiek valgomųjų dangų kūrimui.
3. *L. paracasei* A11 padermė gali būti naudojama valgomosiose dangose be papildomų apsauginių komponentų, taip supaprastinant dangų sudėtį ir jų pritaikymą sūrių gamyboje.
4. Valgomosios dangos su apsauginėmis probiotinių savybių laktobacilomis gali būti taikomos sūrių gamyboje siekiant kontroliuoti gedimą sukeliančių mikroorganizmų augimą ir prailginti produktų galiojimo laiką.
5. Valgomųjų dangų sudėtis turėtų būti optimizuojama, ypatingą dėmesį skiriant naudojamų aliejų tipui ir siekiant užtikrinti sūriuose nuoseklius riebalų rūgščių profilio pokyčius.
6. Taikant valgomąsias dangas su įterptomis laktobacilomis probiotiniuose sūriuose, būtina įvertinti dangos sudedamųjų dalių ir starterinių kultūrų sąveiką, nes ji gali turėti įtakos baltymų virškinamumui ir kitiems kokybės rodikliams.

REFERENCES

1. Teshome E, Forsido SF, Rupasinghe HPV, Olika Keyata E. Potentials of Natural Preservatives to Enhance Food Safety and Shelf Life: A Review. Sci World J. 2022;2022:9901018. https://doi.org/10.1155/2022/9901018
2. Kirby R, Teixeira P. Consumer food trends and food safety: Challenges in modern food systems. Trends Food Sci Technol. 2025;166:105398. https://doi.org/10.1016/j.tifs.2025.105398
3. Rahman MS, Soltani S, LaPointe G, Karboune S, Fliss I. Lactic acid bacteria: beyond fermentation to bio-protection against fungal spoilage and mycotoxins in food systems. Front Microbiol. 2025;16. https://doi.org/10.3389/fmicb.2025.1580670
4. Anumudu CK, Miri T, Onyeaka H. Multifunctional Applications of Lactic Acid Bacteria: Enhancing Safety, Quality, and Nutritional Value in Foods and Fermented Beverages. Foods. 2024;13:3714. https://doi.org/10.3390/foods13233714
5. Cai H, Tao L, Zhou X, Liu Y, Sun D, Ma Q, et al. Lactic acid bacteria in fermented fish: Enhancing flavor and ensuring safety. J Agric Food Res. 2024;16:101206. https://doi.org/10.1016/j.jafr.2024.101206
6. Falih MA, Altemimi AB, Hamed Alkaisy Q, Awlqadr FH, Abedelmaksoud TG, Amjadi S, et al. Enhancing safety and quality in the global cheese industry: A review of innovative preservation techniques. Heliyon. 2024;10:e40459. https://doi.org/10.1016/j.heliyon.2024.e40459
7. Coelho MC, Malcata FX, Silva CCG. Lactic Acid Bacteria in Raw-Milk Cheeses: From Starter Cultures to Probiotic Functions. Foods. 2022;11. https://doi.org/10.3390/foods11152276
8. Primavilla S, Roila R, Rocchegiani E, Blasi G, Petruzzelli A, Gabucci C, et al. Assessment of the Microbiological Safety and Hygiene of Raw and Thermally Treated Milk Cheeses Marketed in Central Italy between 2013 and 2020. Life. 2023;13:2324. https://doi.org/10.3390/life13122324
9. Eugénio A, Ramos R, Barreto IR, Carriço R, Marcos J, Camelo A, et al. Cheese: mere indulgence or part of a healthy diet? Front Nutr. 2025;12:1649432. https://doi.org/10.3389/fnut.2025.1649432
10. Kechagias EP, Gayialis SP, Panayiotou N, Papadopoulos GA. A Holistic Framework for Evaluating Food Loss and Waste Due to Marketing Standards across the Entire Food Supply Chain. Foods. 2024;13:3273. https://doi.org/10.3390/foods13203273
11. Ferraz AR, Pintado CS, Serralheiro ML. A Global Review of Cheese Colour: Microbial Discolouration and Innovation Opportunities. Dairy. 2024;5:768–85. https://doi.org/10.3390/dairy5040056
12. Martin NH, Torres-Frenzel P, Wiedmann M. *Invited review:* Controlling dairy product spoilage to reduce food loss and waste. J Dairy Sci. 2021;104:1251–61. https://doi.org/10.3168/jds.2020-19130
13. Choi K-H, Lee H, Lee S, Kim S, Yoon Y. Cheese Microbial Risk Assessments — A Review. Asian-Australas J Anim Sci. 2016;29:307–14. https://doi.org/10.5713/ajas.15.0332
14. Tasnim M, Islam MdN. Active edible coatings for smart food preservation and sustainability: A review. Carbohydr Polym Technol Appl. 2026;13:101061. https://doi.org/10.1016/j.carpta.2025.101061
15. Gupta D, Lall A, Kumar S, Patil TD, Gaikwad KK. Plant-based edible films and coatings for food-packaging applications: recent advances, applications, and trends. Sustain Food Technol. RSC; 2024;2:1428–55. https://doi.org/10.1039/D4FB00110A

16. Guimarães A, Abrunhosa L, Pastrana LM, Cerqueira MA. Edible Films and Coatings as Carriers of Living Microorganisms: A New Strategy Towards Biopreservation and Healthier Foods. Compr Rev Food Sci Food Saf. 2018;17:594–614. https://doi.org/10.1111/1541-4337.12345
17. Salama HH, Trif M, Rusu AV, Bhattacharya S. Application of Functional and Edible Coatings and Films as Promising Strategies for Developing Dairy Functional Products—A Review on Yoghurt Case. Coatings. 2022;12:838. https://doi.org/10.3390/coatings12060838
18. Aranda C, Rodriguez R, Fernández-Baldo MA, Durán P. Mycotoxins in Cheese: Assessing Risks, Fungal Contaminants, and Control Strategies for Food Safety. Foods. 2025;14:351. https://doi.org/10.3390/foods14030351
19. Garbowska M, Berthold-Pluta A, Stasiak-Różańska L, Pluta A. The Impact of the Adjunct Heat-Treated Starter Culture and *Lb. helveticus* LH-B01 on the Proteolysis and ACE Inhibitory Activity in Dutch-Type Cheese Model during Ripening. Anim 2021;11:2699. https://doi.org/10.3390/ani11092699
20. Sionek B, Szydłowska A, Trząskowska M, Kołożyn-Krajewska D. The Impact of Physicochemical Conditions on Lactic Acid Bacteria Survival in Food Products. Fermentation. 2024;10:298. https://doi.org/10.3390/fermentation10060298
21. Peruzzolo M, Ceni GC, Junges A, Zeni J, Cansian RL, Backes GT. Probiotics: Health benefits, microencapsulation, and viability, combination with natural compounds, and applications in foods. Food Biosci. 2025;66:106253. https://doi.org/10.1016/j.fbio.2025.106253
22. You S, Ma Y, Yan B, Pei W, Wu Q, Ding C, et al. The promotion mechanism of prebiotics for probiotics: A review. Front Nutr. 2022;9:1000517. https://doi.org/10.3389/fnut.2022.1000517
23. Shu D, Zhang T, Dong Y, Xu J, Yuan Y. Encapsulation of probiotics to improve their survival: A focus on *Bifidobacterium animalis* subsp. *lactis* BB-12. Food Chem X. 2026;35:103802. https://doi.org/10.1016/j.fochx.2026.103802
24. Silva SPM, Teixeira JA, Silva CCG. Recent advances in the use of edible films and coatings with probiotic and bacteriocin-producing lactic acid bacteria. Food Biosci. 2023;56:103196. https://doi.org/10.1016/j.fbio.2023.103196
25. Pereira CD, Hodowaniec K, Kucz K, Szkolnicka K, Gomes D, Pires A. Evaluation of the Impact of Whey Edible Coatings with Bioprotective Cultures and Thyme Essential Oil Applied to Cheese. Dairy. 2025;6. https://doi.org/10.3390/dairy6050056
26. Zapaśnik A, Sokołowska B, Bryła M. Role of Lactic Acid Bacteria in Food Preservation and Safety. Foods. 2022;11:1283. https://doi.org/10.3390/foods11091283
27. Mokoena MP. Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Mol J Synth Chem Nat Prod Chem. 2017;22:1255. https://doi.org/10.3390/molecules22081255
28. De Angelis M, Gobbetti M. *Lactobacillus* SPP.: General Characteristics. Ref Module Food Sci. 2016. https://doi.org/10.1016/B978-0-08-100596-5.00851-9
29. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int J Syst Evol Microbiol. Microbiology Society,; 2020;70:2782–858. https://doi.org/10.1099/ijsem.0.004107
30. Aguirre-Garcia YL, Nery-Flores SD, Campos-Muzquiz LG, Flores-Gallegos AC, Palomo-Ligas L, Ascacio-Valdés JA, et al. Lactic Acid Fermentation in the Food Industry and Bio-Preservation of Food. Fermentation. 2024;10:168. https://doi.org/10.3390/fermentation10030168

31. Voidarou C, Antoniadou M, Rozos G, Tzora A, Skoufos I, Varzakas T, et al. Fermentative Foods: Microbiology, Biochemistry, Potential Human Health Benefits and Public Health Issues. Foods. 2020;10:69. https://doi.org/10.3390/foods10010069
32. Loo JS, Oslan SNH, Mokshin NAS, Othman R, Amin Z, Dejtisakdi W, et al. Comprehensive Review of Strategies for Lactic Acid Bacteria Production and Metabolite Enhancement in Probiotic Cultures: Multifunctional Applications in Functional Foods. Fermentation. 2025;11:241. https://doi.org/10.3390/fermentation11050241
33. Bintsis T. Lactic acid bacteria: their applications in foods. J Bacteriol Mycol. 2018;6. https://doi.org/10.15406/jbmoa.2018.06.00182
34. Abdul Hakim BN, Xuan NJ, Oslan SNH. A Comprehensive Review of Bioactive Compounds from Lactic Acid Bacteria: Potential Functions as Functional Food in Dietetics and the Food Industry. Foods. 2023;12:2850. https://doi.org/10.3390/foods12152850
35. Tang H, Huang W, Yao Y-F. The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. Microb Cell Graz Austria. 2023;10:49–62. https://doi.org/10.15698/mic2023.03.792
36. Campagnollo FB, Margalho LP, Kamimura BA, Feliciano MD, Freire L, Lopes LS, et al. Selection of indigenous lactic acid bacteria presenting anti-listerial activity, and their role in reducing the maturation period and assuring the safety of traditional Brazilian cheeses. Food Microbiol. 2018;73:288–97. https://doi.org/10.1016/j.fm.2018.02.006
37. Rolim FRL, dos Santos KMO, de Barcelos SC, do Egito AS, Ribeiro TS, da Conceição ML, et al. Survival of *Lactobacillus rhamnosus* EM1107 in simulated gastrointestinal conditions and its inhibitory effect against pathogenic bacteria in semi-hard goat cheese. Food Sci Technol. 2015;63:807–13. https://doi.org/10.1016/j.lwt.2015.05.004
38. Chen Z, Jiang X, Li L, Liu D, Zhao F, Liu Y, et al. Bacteriocinogenic *Lacticaseibacillus paracasei* strains from Inner Mongolian fermented milk efficiently control pathogenic bacteria in model cheddar-like cheese. Food Biosci. 2024;57:103516. https://doi.org/10.1016/j.fbio.2023.103516
39. Zhang K, Zhang T-T, Guo R-R, Ye Q, Zhao H-L, Huang X-H. The regulation of key flavor of traditional fermented food by microbial metabolism: A review. Food Chem X. 2023;19:100871. https://doi.org/10.1016/j.fochx.2023.100871
40. Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, et al. Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. Front Bioeng Biotechnol. 2021;9:612285. https://doi.org/10.3389/fbioe.2021.612285
41. Castellone V, Bancalari E, Rubert J, Gatti M, Neviani E, Bottari B. Eating Fermented: Health Benefits of LAB-Fermented Foods. Foods. 2021;10:2639. https://doi.org/10.3390/foods10112639
42. Pal M, Molnár J. Fermented Dairy Products and Their Place in International Gastronomy. Int J Food Sci Agric. 2022;6:1–3. https://doi.org/10.26855/ijfsa.2022.03.001
43. Widyastuti Y, Rohmatussolihat R, Febrisiantosa A. The Role of Lactic Acid Bacteria in Milk Fermentation. Food Nutr Sci. 2014;05:435–42. https://doi.org/10.4236/fns.2014.54051
44. Ağagündüz D, Şahin TÖ, Ayten Ş, Yılmaz B, Güneşliol BE, Russo P, et al. Lactic acid bacteria as pro-technological, bioprotective and health-promoting cultures in the dairy food industry. Food Biosci. 2022;47:101617. https://doi.org/10.1016/j.fbio.2022.101617
45. Nuñez IM, Verni MC, Argañaraz Martinez FE, Babot JD, Terán V, Danilovich ME, et al. Novel Lactic Acid Bacteria Strains from Regional Peppers with Health-Promoting Potential. Fermentation. 2024;10:209. https://doi.org/10.3390/fermentation10040209

46. Huligere SS, Chandana Kumari VB, Alqadi T, Kumar S, Cull CA, Amachawadi RG, et al. Isolation and characterization of lactic acid bacteria with potential probiotic activity and further investigation of their activity by α-amylase and α-glucosidase inhibitions of fermented batters. Front Microbiol. 2023;13:1042263. https://doi.org/10.3389/fmicb.2022.1042263
47. Uhegwu C, Anumudu C. Probiotic Potential of Traditional and Emerging Microbial Strains in Functional Foods: From Characterization to Applications and Health Benefits. Microorganisms. 2025;13:2521. https://doi.org/10.3390/microorganisms13112521
48. Kouadri Boudjelthia N, Belabbas M, Bekenniche N, Monnoye M, Gérard P, Riazi A. Probiotic Properties of Lactic Acid Bacteria Newly Isolated from Algerian Raw Cow's Milk. Microorganisms. 2023;11:2091. https://doi.org/10.3390/microorganisms11082091
49. Zhang B, Wang Y, Tan Z, Li Z, Jiao Z, Huang Q. Screening of Probiotic Activities of Lactobacilli Strains Isolated from Traditional Tibetan Qula, A Raw Yak Milk Cheese. Asian-Australas J Anim Sci. 2016;29:1490–9. https://doi.org/10.5713/ajas.15.0849
50. Barbosa JP, Trindade DP de A, Martins EMF, Tette PAS. Lactic Acid Bacteria Isolated from Fruits: A Review on Methods for Evaluation of Probiotic Potential. J Food Nutr Res. 2022;10:711–26. https://doi.org/10.12691/jfnr-10-10-9
51. Sliti A, Kim R-H, Shin J-H. Screening and characterization of probiotic lactic acid bacteria from Korean fermented foods for functional food development. Appl Food Res. 2026;6:101787. https://doi.org/10.1016/j.afres.2026.101787
52. Bin Masalam MS, Bahieldin A, Alharbi MG, Al-Masaudi S, Al-Jaouni SK, Harakeh SM, et al. Isolation, Molecular Characterization and Probiotic Potential of Lactic Acid Bacteria in Saudi Raw and Fermented Milk. Evid-Based Complement Altern Med ECAM. 2018;2018:7970463. https://doi.org/10.1155/2018/7970463
53. Ruiz-Moyano S, Martín A, Benito MJ, Nevado FP, de Guía Córdoba M. Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in Iberian dry fermented sausages. Meat Sci. 2008;80:715–21. https://doi.org/10.1016/j.meatsci.2008.03.011
54. Jacobsen NMY, Caglayan I, Caglayan A, Bar-Shalom D, Müllertz A. Achieving delayed release of freeze-dried probiotic strains by extrusion, spheronization and fluid bed coating - evaluated using a three-step *in vitro* model. Int J Pharm. 2020;591:120022. https://doi.org/10.1016/j.ijpharm.2020.120022
55. Mesquita MC, dos Santos Leandro E, de Alencar ER, Botelho RBA. Survival of *Lactobacillus paracasei* subsp. *paracasei* LBC 81 in Fermented Beverage from Chickpeas and Coconut in a Static *In Vitro* Digestion Model. Fermentation. 2021; 7:3. https://doi.org/10.3390/fermentation7030135
56. Silva M, Tagliapietra B, Flores V, Silvia Pereira dos Santos Richards N. *In Vitro* test to evaluate survival in the gastrointestinal tract of commercial probiotics. Curr Res Food Sci. 2021;4. https://doi.org/10.1016/j.crfs.2021.04.006
57. Chen C-T, Chao W-Y, Lin C-H, Shih T-W, Pan T-M. Comprehensive Safety Assessment of *Lacticaseibacillus paracasei* subsp. *paracasei* NTU 101 Through Integrated Genotypic and Phenotypic Analysis. Curr Issues Mol Biol. 2024;46:12354–74. https://doi.org/10.3390/cimb46110734
58. Shah AB, Baiseitova A, Zahoor M, Ahmad I, Ikram M, Bakhsh A, et al. Probiotic significance of *Lactobacillus* strains: a comprehensive review on health impacts, research gaps, and future prospects. Gut Microbes. 16:2431643. https://doi.org/10.1080/19490976.2024.2431643
59. Binda S, Hill C, Johansen E, Obis D, Pot B, Sanders ME, et al. Criteria to Qualify Microorganisms as "Probiotic" in Foods and Dietary Supplements. Front Microbiol. 2020;11. https://doi.org/10.3389/fmicb.2020.01662

60. Rychen G, Aquilina G, Azimonti G, Bampidis V, Bastos M de L, Bories G, et al. Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA J. 2018;16:e05206. https://doi.org/10.2903/j.efsa.2018.5206
61. Chiș AA, Rus LL, Morgovan C, Arseniu AM, Frum A, Vonica-Țincu AL, et al. Microbial Resistance to Antibiotics and Effective Antibiotherapy. Biomedicines. 2022;10:1121. https://doi.org/10.3390/biomedicines10051121
62. Gueimonde M, Sánchez B, G. de los Reyes-Gavilán C, Margolles A. Antibiotic resistance in probiotic bacteria. Front Microbiol. 2013;4:202. https://doi.org/10.3389/fmicb.2013.00202
63. Anisimova E, Gorokhova I, Karimullina G, Yarullina D. Alarming Antibiotic Resistance of Lactobacilli Isolated from Probiotic Preparations and Dietary Supplements. Antibiotics. 2022;11:1557. https://doi.org/10.3390/antibiotics11111557
64. Campedelli I, Mathur H, Salvetti E, Clarke S, Rea MC, Torriani S, et al. Genus-Wide Assessment of Antibiotic Resistance in *Lactobacillus* spp. Appl Environ Microbiol. 2018;85:e01738-18. https://doi.org/10.1128/AEM.01738-18
65. Arellano K, Vazquez J, Park H, Lim J, Ji Y, Kang H-J, et al. Safety Evaluation and Whole-Genome Annotation of *Lactobacillus plantarum* Strains from Different Sources with Special Focus on Isolates from Green Tea. Probiotics Antimicrob Proteins. 2020;12:1057–70. https://doi.org/10.1007/s12602-019-09620-y
66. Egervärn M, Roos S, Lindmark H. Identification and characterization of antibiotic resistance genes in *Lactobacillus reuteri* and *Lactobacillus plantarum*. J Appl Microbiol. 2009;107:1658–68. https://doi.org/10.1111/j.1365-2672.2009.04352.x
67. Lee J, Kim S, Kang C-H. Screening and Probiotic Properties of Lactic Acid Bacteria with Potential Immunostimulatory Activity Isolated from Kimchi. Fermentation. 2023;9:4. https://doi.org/10.3390/fermentation9010004
68. Sharma P, Tomar SK, Sangwan V, Goswami P, Singh R. Antibiotic Resistance of *Lactobacillus* sp. Isolated from Commercial Probiotic Preparations. J Food Saf. 2016;36:38–51. https://doi.org/10.1111/jfs.12211
69. Anisimova E, Yarullina D. Characterization of Erythromycin and Tetracycline Resistance in *Lactobacillus* fermentum Strains. Int J Microbiol. 2018;2018:3912326. https://doi.org/10.1155/2018/3912326
70. Hirad AH, Ahmad J, Alkhedhairy AA, Bahkali AH, Khan ST. Bacterial isolates exhibiting multidrug resistance, hemolytic activity, and high 16S rRNA gene similarity with well-known pathogens found in camel milk samples of Riyadh region. APMIS. 2018;126:215–26. https://doi.org/10.1111/apm.12802
71. González-Rodríguez N, Santos JA, Otero A, García-López M-L. Cell-associated hemolytic activity in environmental strains of Plesiomonas shigelloides expressing cell-free, iron-influenced extracellular hemolysin. J Food Prot. 2007;70:885–90. https://doi.org/10.4315/0362-028x-70.4.885
72. Di Vincenzo F, Del Gaudio A, Petito V, Lopetuso LR, Scaldaferri F. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. Intern Emerg Med. 2024;19:275–93. https://doi.org/10.1007/s11739-023-03374-w
73. Martín R, Miquel S, Ulmer J, Kechaou N, Langella P, Bermúdez-Humarán LG. Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. Microb Cell Factories. 2013;12:71. https://doi.org/10.1186/1475-2859-12-71
74. Fang F, O'Toole PW. Genetic tools for investigating the biology of commensal lactobacilli. Front Biosci. 2009;14:3111–27. https://doi.org/10.2741/3439
75. Gao W, Hernandez-Garcia L, Castagnini JM, Pallares N, Barba FJ, Culebras PVM. Identification, safety evaluation and probiotic potential of lactic acid bacteria isolated from wheat. Int J Food Microbiol. 2026;444:111436. https://doi.org/10.1016/j.ijfoodmicro.2025.111436

76. Baek J, Kim BS, Kim Y, Bai J. Safety, Antagonistic Activity, and Probiotic Properties of Lactic Acid Bacteria Isolated from Jeotgal, Korean Fermented Seafoods. J Microbiol Biotechnol. 2024;35:e2411055. https://doi.org/10.4014/jmb.2411.11055
77. Roldán-Pérez S, Gómez Rodríguez SL, Sepúlveda-Valencia JU, Ruiz Villadiego OS, Márquez Fernández ME, Montoya Campuzano OI, et al. Assessment of probiotic properties of lactic acid bacteria isolated from an artisanal Colombian cheese. Heliyon. 2023;9:e21558. https://doi.org/10.1016/j.heliyon.2023.e21558
78. da Silva LA, Lopes Neto JHP, Cardarelli HR. Safety and probiotic functionality of isolated goat milk lactic acid bacteria. Ann Microbiol. 2019;69:1497–505. https://doi.org/10.1007/s13213-019-01533-z
79. Nawaz Z, Zahoor MK, Shafique M, Athar R, Yasmin A, Zahoor MA. *In vitro* assessment of probiotic properties of lactic acid bacteria isolated from camel milk: enhancing sustainable foods. Front Sustain Food Syst. 2024;8. https://doi.org/10.3389/fsufs.2024.1437201
80. Fachrial E, Ismawati, Jati AP, Nugroho TT, Saryono. Isolation and Characterization of Lactic Acid Bacteria From "Trites" Having the Ability to Produce α-Glucosidase Inhibitors. Int J Microbiol. 2025;2025:8864668. https://doi.org/10.1155/ijm/8864668
81. Jiang Y, Wu J, Tian L, Liu Y, Zhao F, He Z, et al. The therapeutic effects of fermented milk with lactic acid bacteria from traditional Daqu on hypertensive mice. J Dairy Sci. 2024;107:742–58. https://doi.org/10.3168/jds.2023-23858
82. Elsaadany K, EL-Sayed AIM, Awad S. Identification, Safety Assessment, and Antimicrobial Characteristics of Cocci Lactic Acid Bacteria Isolated from Traditional Egyptian Dairy Products. Foods. 2024;13:3059. https://doi.org/10.3390/foods13193059
83. Kamiloğlu A. Functional and technological characterization of lactic acid bacteria isolated from Turkish dry-fermented sausage (sucuk). Braz J Microbiol. 2022;53:959–68. https://doi.org/10.1007/s42770-022-00708-2
84. Shi X, Li Y, Cheng Y, Liu W, Li Z, Jin H, et al. Emerging roles of lactic acid bacteria in health management: Insights from fermented foods to microbiota. Food Biosci. 2026;75:108088. https://doi.org/10.1016/j.fbio.2025.108088
85. Bintsis T. Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. AIMS Microbiol. 2018;4:665–84. https://doi.org/10.3934/microbiol.2018.4.665
86. Wang Y, Han J, Wang D, Gao F, Zhang K, Tian J, et al. Research Update on the Impact of Lactic Acid Bacteria on the Substance Metabolism, Flavor, and Quality Characteristics of Fermented Meat Products. Foods. 2022;11:2090. https://doi.org/10.3390/foods11142090
87. Li Y, Zhang W, Pan X, Xiao Y, Liu L, Ding F, et al. Recent advances in exopolysaccharides from lactic acid bacteria: Acquisition, characterization, bioactivities, and applications. Food Biosci. 2026;79:108830. https://doi.org/10.1016/j.fbio.2026.108830
88. Korcz E, Varga L. Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. Trends Food Sci Technol. 2021;110:375–84. https://doi.org/10.1016/j.tifs.2021.02.014
89. Jurášková D, Ribeiro SC, Silva CCG. Exopolysaccharides Produced by Lactic Acid Bacteria: From Biosynthesis to Health-Promoting Properties. Foods. 2022;11:156. https://doi.org/10.3390/foods11020156
90. Ruiz Rodríguez LG, Mohamed F, Bleckwedel J, Medina R, De Vuyst L, Hebert EM, et al. Diversity and Functional Properties of Lactic Acid Bacteria Isolated From Wild Fruits and Flowers Present in Northern Argentina. Front Microbiol. 2019;10. https://doi.org/10.3389/fmicb.2019.01091

91. Ibrahim SA, Ayivi RD, Zimmerman T, Siddiqui SA, Altemimi AB, Fidan H, et al. Lactic Acid Bacteria as Antimicrobial Agents: Food Safety and Microbial Food Spoilage Prevention. Foods. 2021;10:3131. https://doi.org/10.3390/foods10123131
92. de Melo Pereira GV, de Carvalho Neto DP, Maske BL, De Dea Lindner J, Vale AS, Favero GR, et al. An updated review on bacterial community composition of traditional fermented milk products: what next-generation sequencing has revealed so far? Crit Rev Food Sci Nutr. 2022;62:1870–89. https://doi.org/10.1080/10408398.2020.1848787
93. Darbandi A, Asadi A, Mahdizade Ari M, Ohadi E, Talebi M, Halaj Zadeh M, et al. Bacteriocins: Properties and potential use as antimicrobials. J Clin Lab Anal. 2021;36:e24093. https://doi.org/10.1002/jcla.24093
94. Hernández-González JC, Martínez-Tapia A, Lazcano-Hernández G, García-Pérez BE, Castrejón-Jiménez NS. Bacteriocins from Lactic Acid Bacteria. A Powerful Alternative as Antimicrobials, Probiotics, and Immunomodulators in Veterinary Medicine. Anim. 2021;11:979. https://doi.org/10.3390/ani11040979
95. Campion A, Casey PG, Field D, Cotter PD, Hill C, Ross RP. In vivo activity of Nisin A and Nisin V against *Listeria monocytogenes* in mice. BMC Microbiol. 2013;13:23. https://doi.org/10.1186/1471-2180-13-23
96. Zhang Y-M, Jiang Y-H, Li H-W, Li X-Z, Zhang Q-L. Purification and characterization of *Lactobacillus plantarum*-derived bacteriocin with activity against *Staphylococcus argenteus* planktonic cells and biofilm. J Food Sci. 2022;87:2718–31. https://doi.org/10.1111/1750-3841.16148
97. Yi L, Li X, Luo L, Lu Y, Yan H, Qiao Z, et al. A novel bacteriocin BMP11 and its antibacterial mechanism on cell envelope of *Listeria monocytogenes* and *Cronobacter sakazakii*. Food Control. 2018;91:160–9. https://doi.org/10.1016/j.foodcont.2018.03.038
98. Vera-Pingitore E, Jimenez ME, Dallagnol A, Belfiore C, Fontana C, Fontana P, et al. Screening and characterization of potential probiotic and starter bacteria for plant fermentations. Food Sci Technol. 2016;71:288–94. https://doi.org/10.1016/j.lwt.2016.03.046
99. Campana R, van Hemert S, Baffone W. Strain-specific probiotic properties of lactic acid bacteria and their interference with human intestinal pathogens invasion. Gut Pathog. 2017;9:12. https://doi.org/10.1186/s13099-017-0162-4
100. Özlük G, Krausová G. Gastric survival of lactic acid bacteria in probiotic-labelled products from the Turkish market: An *in vitro* study. Czech J Food Sci. 2025;43. https://doi.org/10.17221/36/2025-CJFS
101. Javanshir N, Hosseini GNG, Sadeghi M, Esmaeili R, Satarikia F, Ahmadian G, et al. Evaluation of the Function of Probiotics, Emphasizing the Role of their Binding to the Intestinal Epithelium in the Stability and their Effects on the Immune System. Biol. 2021;23:23. https://doi.org/10.1186/s12575-021-00160-w
102. Aziz G, Fakhar H, Rahman S ur, Tariq M, Zaidi A. An assessment of the aggregation and probiotic characteristics of *Lactobacillus* species isolated from native (desi) chicken gut. J Appl Poult Res. 2019;28:846–57. https://doi.org/10.3382/japr/pfz042
103. Moens F, Van den Abbeele P, Basit AW, Dodoo C, Chatterjee R, Smith B, et al. A four-strain probiotic exerts positive immunomodulatory effects by enhancing colonic butyrate production *in vitro*. Int J Pharm. 2019;555:1–10. https://doi.org/10.1016/j.ijpharm.2018.11.020
104. Zare D, Aryaee H, Mirdamadi S, Shirkhan F. The Benefits and Applications of *Lactobacillus plantarum* in Food and Health: A Narrative Review. Iran J Public Health. 2024;53:2201–13. https://doi.org/10.18502/ijph.v53i10.16698

105. Al-Ghamdi SB. Probiotics as Renal Guardians: Modulating Gut Microbiota to Combat Diabetes-Induced Kidney Damage. Biology. 2025;14:2. https://doi.org/10.3390/biology14020122
106. Kezer G, Paramithiotis S, Khwaldia K, Harahap IA, Čagalj M, Šimat V, et al. A comprehensive overview of the effects of probiotics, prebiotics and synbiotics on the gut-brain axis. Front Microbiol. 2025;16. https://doi.org/10.3389/fmicb.2025.1651965
107. Arroyo-López FN, Blanquet-Diot S, Denis S, Thévenot J, Chalancon S, Alric M, et al. Survival of pathogenic and lactobacilli species of fermented olives during simulated human digestion. Front Microbiol. 2014;5. https://doi.org/10.3389/fmicb.2014.00540
108. Khushboo, Karnwal A, Malik T. Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. Front Microbiol. 2023;14:1170725. https://doi.org/10.3389/fmicb.2023.1170725
109. Park J-M, Moon J-W, Zhang B-Z, An B-K. Antioxidant Activity and Other Characteristics of Lactic Acid Bacteria Isolated from Korean Traditional Sweet Potato Stalk Kimchi. Foods. 2024;13:3261. https://doi.org/10.3390/foods13203261
110. Alp D, Kuleaşan H. Adhesion mechanisms of lactic acid bacteria: conventional and novel approaches for testing. World J Microbiol Biotechnol. 2019;35:156. https://doi.org/10.1007/s11274-019-2730-x
111. Zhou Y, Xue K, Li Y, Huang H. Various strategies for intelligent intestinal protection, adhesion, and survival of probiotics. Food Wellness. 2026;2:100047. https://doi.org/10.1016/j.foodw.2026.100047
112. Maione A, Imparato M, Buonanno A, Salvatore MM, Carraturo F, de Alteriis E, et al. Evaluation of Potential Probiotic Properties and *In Vivo* Safety of Lactic Acid Bacteria and Yeast Strains Isolated from Traditional Home-Made Kefir. Foods. 2024;13:1013. https://doi.org/10.3390/foods13071013
113. Darmastuti A, Hasan PN, Wikandari R, Utami T, Rahayu ES, Suroto DA. Adhesion Properties of *Lactobacillus plantarum* Dad-13 and *Lactobacillus plantarum* Mut-7 on Sprague Dawley Rat Intestine. Microorganisms. 2021;9:2336. https://doi.org/10.3390/microorganisms9112336
114. Sim EA, Kim S-Y, Kim S, Mun E-G. Probiotic Potential and Enhanced Adhesion of Fermented Foods-Isolated Lactic Acid Bacteria to Intestinal Epithelial Caco-2 and HT-29 Cells. Microorganisms. 2025;13:32. https://doi.org/10.3390/microorganisms13010032
115. Meng Z, Huang X, Qiao M, Song L, Liu Y, Hai D. Lactic Acid Bacteria Surface Proteins in the Mechanisms of Cell Adhesion and Immunoregulation. Food Sci Nutr. 2024;12:10148–63. https://doi.org/10.1002/fsn3.4517
116. Atanasova J, Moncheva P, Ivanova I. Proteolytic and antimicrobial activity of lactic acid bacteria grown in goat milk. Biotechnol Biotechnol Equip. 2014;28:1073–8. https://doi.org/10.1080/13102818.2014.971487
117. Kalathinathan P, Sain A, Pulicherla K, Kodiveri Muthukaliannan G. A Review on the Various Sources of β-Galactosidase and Its Lactose Hydrolysis Property. Curr Microbiol. 2023;80:122. https://doi.org/10.1007/s00284-023-03220-4
118. Chengolova Z, Shentov P, Ivanova R, Syarova R. How to Keep Lactose Avoiders Healthy. Dairy. 2024;5:702–26. https://doi.org/10.3390/dairy5040052
119. Dhall RK. Advances in Edible Coatings for Fresh Fruits and Vegetables: A Review. Crit Rev Food Sci Nutr. 2013;53:435–50. https://doi.org/10.1080/10408398.2010.541568
120. Karnwal A, Kumar G, Singh R, Selvaraj M, Malik T, Al Tawaha ARM. Natural biopolymers in edible coatings: Applications in food preservation. Food Chem X. 2025;25:102171. https://doi.org/10.1016/j.fochx.2025.102171
121. Perez-Vazquez A, Barciela P, Carpena M, Prieto MA. Edible Coatings as a Natural Packaging System to Improve Fruit and Vegetable Shelf Life and Quality. Foods. 2023;12:3570. https://doi.org/10.3390/foods12193570

122. Falguera V, Quintero JP, Jiménez A, Muñoz JA, Ibarz A. Edible films and coatings: Structures, active functions and trends in their use. Trends Food Sci Technol. 2011;22:292–303. https://doi.org/10.1016/j.tifs.2011.02.004
123. Sanjaykumar GS, Malviya R, Sundram S, Sridhar SB, Singh DP. Polysaccharide-based Edible Film Coatings for Preservation and Packaging of Food. Nat Prod J. 2025;15. https://doi.org/10.2174/0122103155327879240903102010
124. Cruz-Monterrosa RG, Rayas-Amor AA, González-Reza RM, Zambrano-Zaragoza ML, Aguilar-Toalá JE, Liceaga AM. Application of Polysaccharide-Based Edible Coatings on Fruits and Vegetables: Improvement of Food Quality and Bioactivities. Polysaccharides. 2023;4:99–115. https://doi.org/10.3390/polysaccharides4020008
125. Hashemi SMB, Kaveh S, Abedi E, Phimolsiripol Y. Polysaccharide-Based Edible Films/Coatings for the Preservation of Meat and Fish Products: Emphasis on Incorporation of Lipid-Based Nanosystems Loaded with Bioactive Compounds. Foods. 2023;12:3268. https://doi.org/10.3390/foods12173268
126. Milani J, Nemati A. Lipid-Based Edible Films and Coatings: A Review of Recent Advances and Applications. J Packag Technol Res. 2022;6. https://doi.org/10.1007/s41783-021-00130-3
127. Devi LS, Jaiswal AK, Jaiswal S. Lipid incorporated biopolymer based edible films and coatings in food packaging: A review. Curr Res Food Sci. 2024;8:100720. https://doi.org/10.1016/j.crfs.2024.100720
128. Mihalca V, Kerezsi AD, Weber A, Gruber-Traub C, Schmucker J, Vodnar DC, et al. Protein-Based Films and Coatings for Food Industry Applications. Polymers. 2021;13:769. https://doi.org/10.3390/polym1305076
129. Mohanty B, Hauzoukim, Swain S. Functionality of protein-Based edible coating-review. J Entomol Zool Stud. 2020;8:1432–40.
130. Dhumal CV, Sarkar P. Composite edible films and coatings from food-grade biopolymers. J Food Sci Technol. 2018;55:4369–83. https://doi.org/10.1007/s13197-018-3402-
131. Karimi Sani I, Masoudpour-Behabadi M, Alizadeh Sani M, Motalebinejad H, Juma ASM, Asdagh A, et al. Value-added utilization of fruit and vegetable processing by-products for the manufacture of biodegradable food packaging films. Food Chem. 2023;405:134964. https://doi.org/10.1016/j.foodchem.2022.134964
132. Mutaliyeva B, Turkeyeva E, Madybekova G, Živković L, Talluri VP, Issayeva A, et al. Acid whey valorization: novel approaches for probiotic and functional products development. Front Nutr. 2025;12:1630925. https://doi.org/10.3389/fnut.2025.1630925
133. Mishra A, Mann B, Poonia A, Rai D. Milk Protein-Based Edible Coatings: Properties and Applications. 2022. https://doi.org/10.1007/978-981-16-2383-7_11
134. Rosseto M, Toniciolli Rigueto C, Alessandretti I, Oliveira R, Wohlmuth D, Loss R, et al. Whey-based polymeric films for food packaging applications: a review of recent trends. J Sci Food Agric. 2022;103. https://doi.org/10.1002/jsfa.12310
135. Punia Bangar S, Chaudhary V, Thakur N, Kajla P, Kumar M, Trif M. Natural Antimicrobials as Additives for Edible Food Packaging Applications: A Review. Foods. 2021;10:2282. https://doi.org/10.3390/foods10102282
136. Erazo Anacona D, Neira Garzón D, Rivera AMP, Ramírez-Toro C, Bolívar Escobar G. Efficacy of EPS Gel Coating and Lactic Acid Bacteria in Preserving Strawberry Postharvest Quality. Gels. 2026;12:341. https://doi.org/10.3390/gels12040341
137. Bambace MF, Alvarez MV, Moreira MDR. Novel functional blueberries: Fructo-oligosaccharides and probiotic lactobacilli incorporated into alginate edible coatings. Food Res Int. 2019;122:653–60. https://doi.org/10.1016/j.foodres.2019.01.040

138. Odila Pereira J, Soares J, J P Monteiro M, Gomes A, Pintado M. Impact of whey protein coating incorporated with *Bifidobacterium* and *Lactobacillus* on sliced ham properties. Meat Sci. 2018;139:125–33. https://doi.org/10.1016/j.meatsci.2018.01.016
139. Concha-Meyer A, Schöbitz R, Brito C, Fuentes R. Lactic acid bacteria in an alginate film inhibit *Listeria monocytogenes* growth on smoked salmon. Food Control. 2011;22:485–9. https://doi.org/10.1016/j.foodcont.2010.09.032
140. Silva SPM, Ribeiro SC, Teixeira JA, Silva CCG. Application of an alginate-based edible coating with bacteriocin-producing *Lactococcus* strains in fresh cheese preservation. LWT. 2022;153:112486. https://doi.org/10.1016/j.lwt.2021.112486
141. Soukoulis C, Singh P, Macnaughtan W, Parmenter C, Fisk ID. Compositional and physicochemical factors governing the viability of *Lactobacillus rhamnosus* GG embedded in starch-protein based edible films. Food Hydrocoll. 2016;52:876–87. https://doi.org/10.1016/j.foodhyd.2015.08.025
142. Khodaei D, Hamidi-Esfahani Z, Lacroix M. Gelatin and low methoxyl pectin films containing probiotics: Film characterization and cell viability. Food Biosci. 2020;36:100660. https://doi.org/10.1016/j.fbio.2020.100660
143. Shahrampour D, Khomeiri M, Razavi S, Kashiri M. Development and characterization of alginate/pectin edible films containing *Lactobacillus plantarum* KMC 45. LWT. 2019;118:108758. https://doi.org/10.1016/j.lwt.2019.108758
144. Akman P, Bozkurt F, Dogan K, Tornuk F, Tamturk F. Fabrication and characterization of probiotic *Lactobacillus plantarum* loaded sodium alginate edible films. J Food Meas Charact. 2021;15. https://doi.org/10.1007/s11694-020-00619-6
145. Li S, Ma Y, Ji T, Sameen D, Ahmed S, Qin W, et al. Cassava starch/carboxymethylcellulose edible films embedded with lactic acid bacteria to extend the shelf life of banana. Carbohydr Polym. 2020;248:116805. https://doi.org/10.1016/j.carbpol.2020.116805
146. Paulo A, Baú T, Ida E, Shirai M. Edible coatings and films with incorporation of prebiotics —A review. Food Res Int. 2021;148:110629. https://doi.org/10.1016/j.foodres.2021.11062
147. Davachi SM, Pottackal N, Torabi H, Abbaspourrad A. Development and characterization of probiotic mucilage based edible films for the preservation of fruits and vegetables. Sci Rep. 2021;11:16608. https://doi.org/10.1038/s41598-021-95994-5
148. Rahma LD, Srisa A, Wongphan P, Nakphaichit M, Sablani SS, Harnkarnsujarit N. Pregelatinized Starch-Based Edible Films as Effective Carriers for *Bacillus coagulans*: Influence of Starch Type on Film Properties and Probiotic Viability. Foods. 2025;14:2424. https://doi.org/10.3390/foods14142424
149. Deol IS, Kaur J, Singh U, Bajaj K, Gill PS. Sodium alginate-based edible coatings enriched with bioactive additives: an innovative strategy for preserving quality and extending postharvest life of fruits. Food Res Int. 2026;233:119009. https://doi.org/10.1016/j.foodres.2026.11900
150. Senthilkumar S, Mundas G, Ravi H, C K S, Venkatachalapathy N. Edible coatings in food: Specific materials and their impact on techno-functional properties and sustainable applications. Food Biomacromolecules. https://doi.org/10.1002/fob2.70057
151. Pellegrino M, Elechi JOG, Plastina P, Loizzo MR. Application of Natural Edible Coating to Enhance the Shelf Life of Red Fruits and Their Bioactive Content. Appl Sci. 2024;14:4552. https://doi.org/10.3390/app14114552
152. Hashemi TS, Celikin M, McCarthy HO, Levingstone TJ, Dunne NJ. Influence of coating layers and immersion time on adhesion force and corrosion resistance of DCPD coatings on pure magnesium. Surf Interfaces. 2025;77:107941. https://doi.org/10.1016/j.surfin.2025.107941

153. Andrade RD, Skurtys O, Osorio FA. Atomizing Spray Systems for Application of Edible Coatings. Compr Rev Food Sci Food Saf. 2012;11:323–37. https://doi.org/10.1111/j.1541-4337.2012.00186.x
154. Krishnan R, Misra M, Subramanian J, Mohanty A. Emerging Trends and Application of Edible Coating as a Sustainable Solution for Postharvest Management in Stone Fruits: A Comprehensive Review. Compr Rev Food Sci Food Saf. 2025;24:e70179. https://doi.org/10.1111/1541-4337.7017
155. Zhong Y, Cavender G, Zhao Y. Investigation of different coating application methods on the performance of edible coatings on Mozzarella cheese.Food Sci Technol. 2014;56:1–8. https://doi.org/10.1016/j.lwt.2013.11.006
156. Deddefo A, Mamo G, Asfaw M, Amenu K. Factors affecting the microbiological quality and contamination of farm bulk milk by *Staphylococcus aureus* in dairy farms in Asella, Ethiopia. BMC Microbiol. 2023;23:65. https://doi.org/10.1186/s12866-022-02746-0
157. Jafarzadeh S, Salehabadi A, Mohammadi Nafchi A, Oladzadabbasabadi N, Jafari SM. Cheese packaging by edible coatings and biodegradable nanocomposites; improvement in shelf life, physicochemical and sensory properties. Trends Food Sci Technol. 2021;116:218–31. https://doi.org/10.1016/j.tifs.2021.07.021
158. Lorenzo JM, Munekata PE, Dominguez R, Pateiro M, Saraiva JA, Franco D. Main Groups of Microorganisms of Relevance for Food Safety and Stability. Innov Technol Food Preserv. 2018;53–107. https://doi.org/10.1016/B978-0-12-811031-7.00003-0
159. Bagheripoor N, Khoshgozaran S, Sohrabvandi S, Khorshidian N, Mortazavian A, Mollakhalili N, et al. Application of Active Edible Coatings to Improve the Shelf-life of Cheese. Food Sci Technol Res. 2018;24:949–62. https://doi.org/10.3136/fstr.24.94
160. Ramos ÓL, Pereira JO, Silva SI, Fernandes JC, Franco MI, Lopes-da-Silva JA, et al. Evaluation of antimicrobial edible coatings from a whey protein isolate base to improve the shelf life of cheese. J Dairy Sci. 2012;95:6282–92. https://doi.org/10.3168/jds.2012-5478
161. El-Sayed SM, Youssef AM. Emergence of cheese packaging by edible coatings for enhancing its shelf-life. J Food Meas Charact. 2024;18:5265–80. https://doi.org/10.1007/s11694-024-02564-0
162. Özer B, Kirmaci HA, Şenel E, Atamer M, Hayaloğlu A. Improving the viability of *Bifidobacterium bifidum* BB-12 and *Lactobacillus acidophilus* LA-5 in white-brined cheese by microencapsulation. Int Dairy J. 2009;19:22–9. https://doi.org/10.1016/j.idairyj.2008.07.001
163. Ceylan HG, Atasoy AF. Optimization and characterization of prebiotic concentration of edible films containing *Bifidobacterium animalis* subsp. *lacti*s BB-12® and its application to block type processed cheese. Int Dairy J. 2022;134:105443. https://doi.org/10.1016/j.idairyj.2022.105443
164. Kure CF, Skaar I. The fungal problem in cheese industry. Curr Opin Food Sci. 2019;29:14–9. https://doi.org/10.1016/j.cofs.2019.07.003
165. Cabañas CM, Hernández León A, Ruiz-Moyano S, Merchán AV, Martínez Torres JM, Martín A. Biocontrol of Cheese Spoilage Moulds Using Native Yeasts. Foods. 2025;14:2446. https://doi.org/10.3390/foods14142446
166. Souza LV, Martins E, Moreira IMFB, de Carvalho AF. Strategies for the Development of Bioprotective Cultures in Food Preservation. Int J Microbiol. 2022;2022:6264170. https://doi.org/10.1155/2022/6264170
167. Garnier L, Valence F, Mounier J. Diversity and Control of Spoilage Fungi in Dairy Products: An Update. Microorganisms. 2017;5:42. https://doi.org/10.3390/microorganisms5030042

168. Antoszewska A, Maćkiw E, Kowalska J, Patoleta M, Ławrynowicz-Paciorek M, Postupolski J. Microbiological Risks of Traditional Raw Cow's Milk Cheese (Koryciński Cheeses). Foods. 2024;13:1364. https://doi.org/10.3390/foods13091364
169. Pedroso MAP, Oliveira W de C, Felice AG, Cunha SCS, Oliveira MBPP, Richards NSP dos S. Pathogenic and Harmful Bacteria in Dairy Technology: Genomic Characterization and Its Correlation with Physicochemical Parameters of Sheep's Cheese Sold in Southern Brazil. Fermentation. 2024;10:397. https://doi.org/10.3390/fermentation10080397
170. Shamloo E, Hosseini H, Abdi Moghadam Z, Halberg Larsen M, Haslberger A, Alebouyeh M. Importance of *Listeria monocytogenes* in food safety: a review of its prevalence, detection, and antibiotic resistance. Iran J Vet Res. 2019;20:241–54.
171. Durango-Zuleta MM, Fuentes-Vanegas M, Sepúlveda-Valencia JU, Moreno Herrera CX. Isolation, identification, and antimicrobial activity of lactic acid bacteria associated with two traditional Colombian types of cheese: Quesillo and double-cream cheese. LWT. 2022;171:114119. https://doi.org/10.1016/j.lwt.2022.11411
172. Shehata MG, Alsulami T, El-Aziz NMA, Abd-Rabou HS, El Sohaimy SA, Darwish AMG, et al. Biopreservative and Anti-Mycotoxigenic Potentials of *Lactobacillus paracasei* MG847589 and Its Bacteriocin in Soft White Cheese. Toxins. 2024;16:93. https://doi.org/10.3390/toxins16020093
173. Remini H, Remini-Sahraoui Y, Benbara T, Sadoun D. From farm to cheeseboard: Harnessing the biopreserving performance and enhancing safety of *Lactococcus lactis* KJ660075 in goat's milk cheese. Int Dairy J. 2024;157:105977. https://doi.org/10.1016/j.idairyj.2024.105977
174. Medved'ová A, Koňuchová M, Kvočiková K, Hatalová I, Valík L. Effect of Lactic Acid Bacteria Addition on the Microbiological Safety of Pasta-Filata Types of Cheeses. Front Microbiol. 2020;11. https://doi.org/10.3389/fmicb.2020.612528
175. Benítez-Serrano JC, Hernández-Castro R, Martínez-Pérez L, Palomares-Resendiz G, Díaz-Aparicio E, Suárez-Güemes F, et al. Effect of the *Lacticaseibacillus paracasei* JLM Strain Against *Brucella abortus* Strains in Ripened Cheese. Foodborne Pathog Dis. 2023;20:169–76. https://doi.org/10.1089/fpd.2022.0063
176. Leyva Salas M, Thierry A, Lemaître M, Garric G, Harel-Oger M, Chatel M, et al. Antifungal Activity of Lactic Acid Bacteria Combinations in Dairy Mimicking Models and Their Potential as Bioprotective Cultures in Pilot Scale Applications. Front Microbiol. 2018;9:1787. https://doi.org/10.3389/fmicb.2018.01787
177. Guimarães A, Ramos O, Cerqueira M, Venâncio A, Abrunhosa L. Active Whey Protein Edible Films and Coatings Incorporating *Lactobacillus buchneri* for *Penicillium nordicum* Control in Cheese. Food Bioprocess Technol. 2020;13. https://doi.org/10.1007/s11947-020-02465-2
178. Dong J, Wang S, Li M, Liu J, Sun Z, Mandlaa, et al. Application of a Chitosan-based Active Packaging Film Prepared with Cell-free Supernatant of *Lacticaseibacillus paracasei* ALAC-4 in Mongolian Cheese Preservation. J Food Prot. 2023;86:100158. https://doi.org/10.1016/j.jfp.2023.100158
179. Ledina T, Đorđević J, Glišić M, Čobanović N, Kovandžić M, Bulajić S. Technological and Functional Characteristics of Lactic Acid Bacteria from Traditional Serbian Cheeses. Foods. 2025;14:38. https://doi.org/10.3390/foods14010038
180. Castro JM, Tornadijo ME, Fresno JM, Sandoval H. Biocheese: A Food Probiotic Carrier. BioMed Res Int. 2015;2015:723056. https://doi.org/10.1155/2015/723056
181. Ahmed A, Abdelsater O, Abdelal M, Hamouda M, Don P. Cheese as a Functional Matrix for Probiotics: From Strain Selection to Clinical Benefit. Pak J Nutr. 2025;24:62–73. https://doi.org/10.3923/pjn.2025.62.73

182. Coronado K, García-Torres S, Caldas-Cueva J, Ludeña-Urquizo F. Physicochemical, textural properties, and lactic acid bacteria counts of Peruvian fresh cheese with added probiotic cultures. Braz J Food Technol. 2025;28. https://doi.org/10.1590/1981-6723.05424
183. Wedajo Lemi B. Lactic Acid Bacteria: Benefits, Selection Criteria and Probiotic Potential in Fermented Food. J Probiotics Health. 2015;03. https://doi.org/10.4172/2329-8901.100012
184. Wilkinson MG, LaPointe G. *Invited review:* Starter lactic acid bacteria survival in cheese: New perspectives on cheese microbiology. J Dairy Sci. 2020;103:10963–85. https://doi.org/10.3168/jds.2020-18960
185. Misra S, Pandey P, Dalbhagat CG, Mishra HN. Emerging Technologies and Coating Materials for Improved Probiotication in Food Products: a Review. Food Bioprocess Technol. 2022;15:998–1039. https://doi.org/10.1007/s11947-021-02753-5
186. Agriopoulou S, Tarapoulouzi M, Varzakas T, Jafari SM. Application of Encapsulation Strategies for Probiotics: From Individual Loading to Co-Encapsulation. Microorganisms. 2023;11:2896. https://doi.org/10.3390/microorganisms11122896
187. Sbehat M, Mauriello G, Altamimi M. Microencapsulation of Probiotics for Food Functionalization: An Update on Literature Reviews. Microorganisms. 2022;10:1948. https://doi.org/10.3390/microorganisms10101948
188. Gbassi GK, Vandamme T. Probiotic Encapsulation Technology: From Microencapsulation to Release into the Gut. Pharmaceutics. Molecular Diversity Preservation International; 2012;4:149–63. https://doi.org/10.3390/pharmaceutics401014
189. Kumar L, Tyagi P, Lucia L, Pal L. Innovations in Edible Packaging Films, Coatings, and Antimicrobial Agents for Applications in Food Industry. Compr Rev Food Sci Food Saf. 2025;24:e70217. https://doi.org/10.1111/1541-4337.70217
190. Neviani E, Gatti M, Gardini F, Levante A. Microbiota of Cheese Ecosystems: A Perspective on Cheesemaking. Foods. 2025;14:830. https://doi.org/10.3390/foods14050830
191. Gbassi G, Vandamme T, Yolou F, Marchioni E. *In vitro* effects of pH, bile salts and enzymes on the release and viability of encapsulated *Lactobacillus plantarum* strains in a gastrointestinal tract model. Int Dairy J. 2011;21:97–102. https://doi.org/10.1016/j.idairyj.2010.09.006
192. Angiolillo L, Conte A, Faccia M, Zambrini AV, Del Nobile MA. A new method to produce synbiotic Fiordilatte cheese. Innov Food Sci Emerg Technol. 2014;22:180–7. https://doi.org/10.1016/j.ifset.2013.09.010
193. Sáez-Orviz S, Puertas C, Marcet I, Rendueles M, Díaz M. Bioactive synbiotic coatings with lactobionic acid and *Lactobacillus plantarum* CECT 9567 in the production and characterization of a new functional dairy product. J Funct Foods. 2020;75:104263. https://doi.org/10.1016/j.jff.2020.104263
194. Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, et al. A standardised static *in vitro* digestion method suitable for food - an international consensus. Food Funct. 2014;5:1113–24. https://doi.org/10.1039/c3fo60702j
195. Torriani S, Felis GE, Dellaglio F. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by recA Gene Sequence Analysis and Multiplex PCR Assay with recA Gene-Derived Primers. Appl Environ Microbiol. 2001;67:3450–4. https://doi.org/10.1128/AEM.67.8.3450-3454.2001
196. Ventura M, Canchaya C, Meylan V, Klaenhammer TR, Zink R. Analysis, Characterization, and Loci of the tuf Genes in *Lactobacillus* and *Bifidobacterium* Species and Their Direct Application for Species Identification. Appl Environ Microbiol. 2003;69:6908–22. https://doi.org/10.1128/AEM.69.11.6908-6922.2003

197. Gaspar C, Palmeira-de-Oliveira R, Martinez-de-Oliveira J, Neves J das, Pestana PG, Rolo J, et al. Development and validation of a new one step Multiplex-PCR assay for the detection of ten *Lactobacillus* species. Anaerobe. 2019;59:192–200. https://doi.org/10.1016/j.anaerobe.2019.06.004
198. European Food Safety Authority. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal. 2012; 10(6):1–10. 2012.
199. Maragkoudakis PA, Mountzouris KC, Psyrras D, Cremonese S, Fischer J, Cantor MD, et al. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. Int J Food Microbiol. 2009;130:219–26. https://doi.org/10.1016/j.ijfoodmicro.2009.01.027
200. Kondrotiene K, Lauciene L, Andruleviciute V, Kasetiene N, Serniene L, Sekmokiene D, et al. Safety Assessment and Preliminary *In Vitro* Evaluation of Probiotic Potential of *Lactococcus lactis* Strains Naturally Present in Raw and Fermented Milk. Curr Microbiol. 2020;77:3013–23. https://doi.org/10.1007/s00284-020-02119-8
201. Pisano MB, Fadda M, Melis R, Ciusa M, Viale S, Deplano M, et al. Molecular identification of bacteriocins produced by *Lactococcus lactis* dairy strains and their technological and genotypic characterization. Food Control. 2015;51. https://doi.org/10.1016/j.foodcont.2014.11.005
202. Thirabunyanon M, Boonprasom P, Niamsup P. Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. Biotechnol Lett. 2009;31:571–6. https://doi.org/10.1007/s10529-008-9902-3
203. International Organization for Standardization. ISO 22184:2021: Milk and milk products—Determination of the sugar contents—High performance anion exchange chromatography with pulsed amperometric detection method (HPAEC-PAD). Geneva: ISO; 2021.
204. International Organization for Standardization. ISO 8069:2005: Dried milk—Determination of content of lactic acid and lactates. Geneva: ISO; 2005.
205. Vieira JM, Andrade CCP, Santos TP, Okuro PK, Garcia ST, Rodrigues MI, et al. Flaxseed gum-biopolymers interactions driving rheological behaviour of oropharyngeal dysphagia-oriented products. Food Hydrocoll. 2021;111:106257. https://doi.org/10.1016/j.foodhyd.2020.106257
206. Costa M, Cerqueira M, Ruiz H, Fougnies C, Richel A, Vicente A, et al. Use of wheat bran arabinoxylans in chitosan-based films: Effect on physicochemical properties. Ind Crops Prod. 2015;66:305–11. https://doi.org/10.1016/j.indcrop.2015.01.003
207. Ortiz de Elguea-Culebras G, Bourbon AI, Costa MJ, Muñoz-Tebar N, Carmona M, Molina A, et al. Optimization of a chitosan solution as potential carrier for the incorporation of *Santolina chamaecyparissus* L. solid by-product in an edible vegetal coating on '*Manchego*' cheese. Food Hydrocoll. 2019;89:272–82. https://doi.org/10.1016/j.foodhyd.2018.10.054
208. International Organization for Standardization. ISO 15214:1998: Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of mesophilic lactic acid bacteria—Colony-count technique at 30 °C. Geneva: ISO; 1998.
209. Aleksandrovas E, Vasiliauskaitė A, Milerienė J, Muizniece-Brasava S, Ciprovica I, Songisepp E, et al. The Study of Combination of Biodegradable Packaging and Biocoating with Lactic Acid Bacteria as a Green Alternative for Traditional Packaging in Gouda Cheese. Coatings. 2024;14. https://doi.org/10.3390/coatings14070886
210. International Organization for Standardization. ISO 5534:2004: Cheese and processed cheese—Determination of the total solids content. Geneva: ISO; 2004.

211. Xing S, Liu L, Zhang X, Guan H, Gong H, Li H, et al. A mathematical model to predict the color change of fresh dough sheets under fluctuation temperatures. LWT. 2022;162:113447. https://doi.org/10.1016/j.lwt.2022.113447
212. International Organization for Standardization. ISO 4833-1:2013: Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30 °C by the pour plate technique. Geneva: ISO; 2013.
213. International Organization for Standardization. ISO 21528-2:2017: Microbiology of the food chain—Horizontal method for the detection and enumeration of Enterobacteriaceae—Part 2: Colony-count technique. Geneva: ISO; 2017.
214. International Organization for Standardization. ISO 6611:2004: Milk and milk products—Enumeration of colony-forming units of yeasts and/or molds—Colony-count technique at 25 °C. Geneva: ISO; 2004.
215. International Organization for Standardization. ISO 18593:2018: Microbiology of the food chain—Horizontal methods for surface sampling. Geneva: ISO; 2018.
216. Pilarczyk R, Wójcik J, Sablik P, Czerniak P. Fatty acid profile and health lipid indices in the raw milk of Simmental and Holstein-Friesian cows from an organic farm. South Afr J Anim Sci. 2015;45. https://doi.org/10.4314/sajas.v45i1.4
217. Mileriene J, Serniene L, Kondrotiene K, Lauciene L, Kasetiene N, Sekmokiene D, et al. Quality and nutritional characteristics of traditional curd cheese enriched with thermo-coagulated acid whey protein and indigenous *Lactococcus lactis* strain. Int J Food Sci Technol. 2021;56:2853–63. https://doi.org/10.1111/ijfs.14922
218. International Organization for Standardization. ISO 8586:2023: Sensory analysis—Selection and training of sensory assessors. Geneva: ISO; 2023.
219. Bodyfelt FW, Tobias J, Trout GM. The sensory evaluation of dairy products. Van Nostrand Reinhold; 1988. 598 p.
220. Rieder A, Afseth NK, Böcker U, Knutsen SH, Kirkhus B, Mæhre HK, et al. Improved estimation of *in vitro* protein digestibility of different foods using size exclusion chromatography. Food Chem. 2021;358:129830. https://doi.org/10.1016/j.foodchem.2021.129830
221. Wubshet SG, Måge I, Böcker U, Lindberg D, Knutsen SH, Rieder A, et al. FTIR as a rapid tool for monitoring molecular weight distribution during enzymatic protein hydrolysis of food processing by-products. Anal Methods. 2017;9:4247–54. https://doi.org/10.1039/C7AY00865A
222. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6:1621–4. https://doi.org/10.1038/ismej.2012.8
223. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37:852–7. https://doi.org/10.1038/s41587-019-0209-9
224. McDonald D, Jiang Y, Balaban M, Cantrell K, Zhu Q, Gonzalez A, et al. Greengenes2 unifies microbial data in a single reference tree. Nat Biotechnol. 2024;42:715–8. https://doi.org/10.1038/s41587-023-01845-1
225. Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous Probiotic *Lactobacillus* Isolates Presenting Antibiotic like Activity against Human Pathogenic Bacteria. Biomedicines. 2017;5:31. https://doi.org/10.3390/biomedicines5020031
226. Lee J, Jo J, Seo H, Han S-W, Kim D-H, Lee J, et al. The Probiotic Properties and Safety of *Limosilactobacillus mucosae* NK41 and *Bifidobacterium longum* NK46. Microorganisms. 2024;12. https://doi.org/10.3390/microorganisms12040776
227. Mroczyńska M, Libudzisz Z. Beta-glucuronidase and beta-glucosidase activity of *Lactobacillus* and *Enterococcus* isolated from human feces. Pol J Microbiol. 2010;59:265–9.

228. Rzepkowska A, Zielińska D, Ołdak A, Kołożyn-Krajewska D. Safety assessment and antimicrobial properties of the lactic acid bacteria strains isolated from polish raw fermented meat products. Int J Food Prop. 2017;20:2736–47. https://doi.org/10.1080/10942912.2016.1250098
229. Novak J. Caseinolytic proteases of *Lactobacillus* and *Lactococcus* strains isolated from fermented dairy products. Mljekarstvo. 2021;72:11–21. https://doi.org/10.15567/mljekarstvo.2022.0102
230. Wiktorczyk-Kapischke N, Grudlewska-Buda K, Budzyńska A, Chomacka-Gollus K, Gospodarek-Komkowska E, Skowron K, et al. Antimicrobial Action of *Lactobacillus* spp. Isolated from Yoghurt against *Escherichia coli*, *Salmonella Enteritidis* and *Listeria monocytogenes*: A Pilot Study. Appl Sci; 2024;14. https://doi.org/10.3390/app14199010
231. Souza LV, Rodrigues da Silva R, Falqueto A, Fusieger A, Martins E, Caggia C, et al. Evaluation of antifungal activity of lactic acid bacteria against fungi in simulated cheese matrix. LWT. 2023;182:114773. https://doi.org/10.1016/j.lwt.2023.114773
232. Sánchez-González L, Quintero Saavedra JI, Chiralt A. Antilisterial and physical properties of biopolymer films containing lactic acid bacteria. Food Control. 2014;35:200–6. https://doi.org/10.1016/j.foodcont.2013.07.001
233. Parlindungan E, Lugli GA, Ventura M, van Sinderen D, Mahony J. Lactic Acid Bacteria Diversity and Characterization of Probiotic Candidates in Fermented Meats. Foods. 2021;10:1519. https://doi.org/10.3390/foods10071519
234. Azaïs-Braesco V, Bresson JL, Guarner F, Corthier G. Not all lactic acid bacteria are probiotics, …but some are. Br J Nutr. 2010;103:1079–81. https://doi.org/10.1017/S0007114510000723
235. Tumbarski Y, Peykova-Shapkova I, Ivanova M, Cholakov R, Dutkiewicz A, Grzymajło K. Characterization and Selection of *Lactobacillus* Strains with Potential Probiotic Applications. Appl Sci; 2025;15. https://doi.org/10.3390/app15062902
236. Smokvina T, Wels M, Polka J, Chervaux C, Brisse S, Boekhorst J, et al. *Lactobacillus paracasei* Comparative Genomics: Towards Species Pan-Genome Definition and Exploitation of Diversity. PLoS ONE. 2013;8:e68731. https://doi.org/10.1371/journal.pone.0068731
237. Lee HB, Bang WY, Shin GR, Jeon HJ, Jung YH, Yang J. Isolation, Characterization, and Safety Evaluation of the Novel Probiotic Strain *Lacticaseibacillus paracasei* IDCC 3401 via Genomic and Phenotypic Approaches. Microorganisms. 2023;12:85. https://doi.org/10.3390/microorganisms12010085
238. Yasmin I, Saeed M, Khan WA, Khaliq A, Chughtai MFJ, Iqbal R, et al. *In Vitro* Probiotic Potential and Safety Evaluation (Hemolytic, Cytotoxic Activity) of *Bifidobacterium* Strains Isolated from Raw Camel Milk. Microorganisms. 2020;8:354. https://doi.org/10.3390/microorganisms8030354
239. Nami Y, Haghshenas B, Vaseghi Bakhshayesh R, Mohammadzadeh Jalaly H, Lotfi H, Eslami S, et al. Novel autochthonous lactobacilli with probiotic aptitudes as a main starter culture for probiotic fermented milk. LWT. 2018;98:85–93. https://doi.org/10.1016/j.lwt.2018.08.035
240. Bujnakova D, Strakova E. Safety, probiotic and technological properties of Lactobacilli isolated from unpasteurised ovine and caprine cheeses. Ann Microbiol. 2017;67:813–26. https://doi.org/10.1007/s13213-017-1310-2
241. Mangia N, Saliba L, Deiana P. Functional and safety characterization of autochthonous *Lactobacillus paracasei* FS103 isolated from sheep cheese and its survival in sheep and cow fermented milks during cold storage. Ann Microbiol. 2018;69. https://doi.org/10.1007/s13213-018-1416-1

242. Pisano MB, Viale S, Conti S, Fadda ME, Deplano M, Melis MP, et al. Preliminary Evaluation of Probiotic Properties of *Lactobacillus* Strains Isolated from Sardinian Dairy Products. BioMed Res Int. 2014;2014:286390. https://doi.org/10.1155/2014/286390
243. Erginkaya Z, Turhan EU, Tatlı D. Determination of antibiotic resistance of lactic acid bacteria isolated from traditional Turkish fermented dairy products. Iran J Vet Res. 2018;19:53–6.
244. Anisimova EA, Yarullina DR. Antibiotic Resistance of *LACTOBACILLUS* Strains. Curr Microbiol. 2019;76:1407–16. https://doi.org/10.1007/s00284-019-01769-7
245. Drago L. Probiotics and Colon Cancer. Microorganisms. 2019;7:66. https://doi.org/10.3390/microorganisms7030066
246. Muñoz-Atienza E, Gómez-Sala B, Araújo C, Campanero C, del Campo R, Hernández PE, et al. Antimicrobial activity, antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC Microbiol. 2013;13:15. https://doi.org/10.1186/1471-2180-13-15
247. Abarquero D, Bodelón R, Flórez AB, Fresno JM, Renes E, Mayo B, et al. Technological and safety assessment of selected lactic acid bacteria for cheese starter cultures design: Enzymatic and antimicrobial activity, antibiotic resistance and biogenic amine production. LWT. 2023;180:114709. https://doi.org/10.1016/j.lwt.2023.114709
248. Toe CJ, Foo HL, Loh TC, Mohamad R, Abdul Rahim R, Idrus Z. Extracellular Proteolytic Activity and Amino Acid Production by Lactic Acid Bacteria Isolated from Malaysian Foods. Int J Mol Sci. 2019;20:1777. https://doi.org/10.3390/ijms20071777
249. Nespolo CR, Brandelli A. Production of bacteriocin-like substances by lactic acid bacteria isolated from regional ovine cheese. Braz J Microbiol. 2010;41:1009–18. https://doi.org/10.1590/S1517-838220100004000020
250. Guan C, Tao Z, Wang L, Zhao R, Chen X, Huang X, et al. Isolation of novel *Lactobacillus* with lipolytic activity from the vinasse and their preliminary potential using as probiotics. AMB Express. 2020;10:91. https://doi.org/10.1186/s13568-020-01026-2
251. Atasoy M, Álvarez Ordóñez A, Cenian A, Djukić-Vuković A, Lund PA, Ozogul F, et al. Exploitation of microbial activities at low pH to enhance planetary health. FEMS Microbiol Rev. 2023;48:fuad062. https://doi.org/10.1093/femsre/fuad062
252. Gunkova P, Buchilina A, Maksimiuk N, Bazarnova Y, Girel K. Carbohydrate Fermentation Test of Lactic Acid Starter Cultures. IOP Conf Ser Earth Environ Sci. 2021;852:012035. https://doi.org/10.1088/1755-1315/852/1/012035
253. Kowalczyk M, Radziwill-Bienkowska JM, Marć MA, Jastrząb R, Mytych J, Siedlecki P, et al. Screening for probiotic properties and potential immunogenic effects of lactobacilli strains isolated from various food products. Front Microbiol. 2024;15. https://doi.org/10.3389/fmicb.2024.1430582
254. Cebeci A, Yaman M, Yalcin B, Gunes F. Determination of carbohydrate amounts of various cheese types presented to sale in the market. 2020;2455–4898.
255. Panesar PS, Kennedy JF, Gandhi DN, Bunko K. Bioutilisation of whey for lactic acid production. Food Chem. 2007;105:1–14. https://doi.org/10.1016/j.foodchem.2007.03.035
256. Proulx J, Hsu LC, Miller BM, Sullivan G, Paradis K, Moraru CI. Pulsed-light inactivation of pathogenic and spoilage bacteria on cheese surface. J Dairy Sci. 2015;98:5890–8. https://doi.org/10.3168/jds.2015-9410
257. Ribeiro AC, Almeida FA de, Medeiros MM, Miranda BR, Pinto UM, Alves VF. *Listeria monocytogenes*: An Inconvenient Hurdle for the Dairy Industry. Dairy. 2023;4:316–44. https://doi.org/10.3390/dairy4020022
258. Bintsis T. Yeasts in different types of cheese. AIMS Microbiol. 2021;7:447–70. https://doi.org/10.3934/microbiol.2021027

259. Karanth S, Feng S, Patra D, Pradhan AK. Linking microbial contamination to food spoilage and food waste: the role of smart packaging, spoilage risk assessments, and date labeling. Front Microbiol. 2023;14:1198124. https://doi.org/10.3389/fmicb.2023.1198124
260. Martin N, Murphy SC, Ralyea R, Wiedmann M, Boor K. When cheese gets the blues: *Pseudomonas fluorescens* as the causative agent of cheese spoilage. J Dairy Sci. 2011;94:3176–83. https://doi.org/10.3168/jds.2011-4312
261. Liu Y, Zhang B, Wang S, Yang Z, Yuan L. Different risk potential of *Bacillus cereus* to the dairy industry − keeping up with the old story. Food Sci Anim Prod. 2024;2:9240085. https://doi.org/10.26599/FSAP.2024.9240085
262. Barbosa V, Leniza L, Marina R, Joao B, Amilton P, Maria M, et al. Assessment of survival *Escherichia coli* in rennet cheese manufactured from experimentally contaminated milk. Afr J Microbiol Res. 2017;11:1230–4. https://doi.org/10.5897/AJMR2016.8005
263. Ramos-Pereira J, Mareze J, Fernández D, Rios EA, Santos JA, López-Díaz T-M. Antifungal activity of lactic acid bacteria isolated from milk against *Penicillium commune*, *P. nordicum*, and *P. verrucosum*. Int J Food Microbiol. 2021;355:109331. https://doi.org/10.1016/j.ijfoodmicro.2021.109331
264. Fernandez B, Vimont A, Desfossés-Foucault É, Daga M, Arora G, Fliss I. Antifungal activity of lactic and propionic acid bacteria and their potential as protective culture in cottage cheese. Food Control. 2017;78:350–6. https://doi.org/10.1016/j.foodcont.2017.03.007
265. Tremonte P, Pannella G, Succi M, Luca T, Sturchio M, Coppola R, et al. Antimicrobial activity of *Lactobacillus plantarum* strains isolated from different environments: A preliminary study. Int Food Res J. 2017;24:852–9.
266. Parlindungan E, Jones OAH. Using metabolomics to understand stress responses in Lactic Acid Bacteria and their applications in the food industry. Metabolomics. 2023;19:99. https://doi.org/10.1007/s11306-023-02062-2
267. Fujimori S. Gastric acid level of humans must decrease in the future. World J Gastroenterol. 2020;26:6706–9. https://doi.org/10.3748/wjg.v26.i43.6706
268. Paongphan P, Ditudompo S, Vitheejongjaroen P, Pachekrepapol U, Taweechotipatr M. Selected lactobacilli isolated from Thai foods for production of fermented dairy products with cholesterol lowering potential. NFS J. 2023;33:100151. https://doi.org/10.1016/j.nfs.2023.100151
269. Fijałkowski K, Peitler D, Rakoczy R, Żywicka A. Survival of probiotic lactic acid bacteria immobilized in different forms of bacterial cellulose in simulated gastric juices and bile salt solution. Food Sci Technol. 2016;68:322–8. https://doi.org/10.1016/j.lwt.2015.12.038
270. Reale A, Di Renzo T, Rossi F, Zotta T, Iacumin L, Preziuso M, et al. Tolerance of *Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus rhamnosus* strains to stress factors encountered in food processing and in the gastro-intestinal tract. Food Sci Technol. 2015;60:721–8. https://doi.org/10.1016/j.lwt.2014.10.022
271. Sieladie D, Francois Z, Marie K, Cresci A, Florence F. Probiotic Properties of Lactobacilli Strains Isolated from Raw Cow Milk in the Western Highlands of Cameroon. Innov Romanian Food Biotechnol. 2011;9:12–28.
272. Hayek SA, Gyawali R, Aljaloud SO, Krastanov A, Ibrahim SA. Cultivation media for lactic acid bacteria used in dairy products. J Dairy Res. 2019;86:490–502. https://doi.org/10.1017/S002202991900075X
273. Kirmaci H, Ozer B, Akçelik M, Akcelik N. Identification and characterisation of lactic acid bacteria isolated from traditional Urfa cheese. Int J Dairy Technol. 2015;69. https://doi.org/10.1111/1471-0307.12260

274. Deshmukh N, Rao PS, Sharma H, M.h. SK, N. LN, C.t. MK. Waste to nutrition: The evolution of whey, a byproduct to galactooligosaccharides production. Food Chem Adv. 2024;4:100642. https://doi.org/10.1016/j.focha.2024.100642
275. Troiani L, Levante A, Russmayer H, Marx H, Neviani E, Bernini V, et al. Screening and bioreactor cultivation of wild-type lactic acid bacteria for high purity D-lactic acid production. New Biotechnol. 2025;90:48–55. https://doi.org/10.1016/j.nbt.2025.08.005
276. Gökmen GG, Sarıyıldız S, Cholakov R, Nalbantsoy A, Baler B, Aslan E, et al. A novel *Lactiplantibacillus plantarum* strain: probiotic properties and optimization of the growth conditions by response surface methodology. World J Microbiol Biotechnol. 2024;40:66. https://doi.org/10.1007/s11274-023-03862-3
277. Garrote Achou C, Cantalejo Díez MJ, Diaz Cano J, Molinos Equiza X. Evaluation of Different Nutritional Sources in Lactic Acid Bacteria Fermentation for Sustainable Postbiotic Production. Foods. 2025;14:649. https://doi.org/10.3390/foods14040649
278. Zhang L, Garcia-Cano I, Jiménez-Flores R. Effect of milk phospholipids on the growth and cryotolerance of lactic acid bacteria cultured and stored in acid whey-based media. JDS Commun. 2020;1:36–40. https://doi.org/10.3168/jdsc.2020-0007
279. Skryplonek K, Dmytrów I, Mituniewicz-Małek A. Probiotic fermented beverages based on acid whey. J Dairy Sci. 2019;102:7773–80. https://doi.org/10.3168/jds.2019-16385
280. Dudkiewicz M, Berlowska J, Kregiel D. Acid whey as a medium for cultivation of conventional and non-conventional yeasts. Biotechnol Food Sci. 2016;80:75–82.
281. Mondragón-Parada ME, Nájera-Martínez M, Juárez-Ramírez C, Galíndez-Mayer J, Ruiz-Ordaz N, Cristiani-Urbina E. Lactic acid bacteria production from whey. Appl Biochem Biotechnol. 2006;134:223–32. https://doi.org/10.1385/abab:134:3:223
282. Abedi E, Hashemi SMB. Lactic acid production – producing microorganisms and substrates sources-state of art. Heliyon. 2020;6:e04974. https://doi.org/10.1016/j.heliyon.2020.e04974
283. Morales-Landa JL, Lazcano-Díaz E, Escamilla-García E, Pérez-De la Rosa AG, Luiz-Santos N. Comparative study of L/D lactic acid production in cultures of probiotic strains supplemented with carbohydrates from agave and other agroindustrial resources. Front Sustain Food Syst. 2025;9. https://doi.org/10.3389/fsufs.2025.1613809
284. Chen Y, Zhu H, Feng Y, Wang Y, Wang X, Zhang W, Luo Y. Rheological analysis in food processing: factors, applications, and future outlooks with machine learning integration. RSC Adv. 2026;16:5040–63. https://doi.org/10.1039/d5ra07499a
285. Janowicz M, Sitkiewicz I, Ciurzynska A, Galus S. Rheological Properties of Film-Forming Dispersions of Selected Biopolymers Used for Packaging Films or Food Coating. Coatings. 2022;12:11. https://doi.org/10.3390/coatings12111704
286. Sun G, Qian C, Li Z, Wang Q. Optimizing Powder-to-Liquid Ratios in Lost Foam Casting Coatings: Impacts on Viscosity, Shear Thinning Behavior, Coating Weight, and Surface Morphology. Coatings. 2024;14:1089. https://doi.org/10.3390/coatings14091089
287. Bremenkamp I, Sousa Gallagher MJ. Edible Coatings for Ready-to-Eat Products: Critical Review of Recent Studies, Sustainable Packaging Perspectives, Challenges and Emerging Trends. Polymers. 2025;17:376. https://doi.org/10.3390/polym17030376
288. García-Argueta I, Dublán-García O, Quintero-Salazar B, Dominguez-Lopez A, Gómez-Oliván LM, Salem A-FZ. Effect of lactic acid bacteria on the textural properties of an edible film based on whey, inulin and gelatin. Afr J Biotechnol. 2013;12.
289. Herrera-Vázquez SE, Dublán-García O, Arizmendi-Cotero D, Gómez-Oliván LM, Islas-Flores H, Hernández-Navarro MD, et al. Optimization of the Physical, Optical and Mechanical Properties of Composite Edible Films of Gelatin, Whey Protein and Chitosan. Molecules. 2022;27:869. https://doi.org/10.3390/molecules27030869

290. Singh GP, Bangar SP, Yang T, Trif M, Kumar V, Kumar D. Effect on the Properties of Edible Starch-Based Films by the Incorporation of Additives: A Review. Polymers. 2022;14:10. https://doi.org/10.3390/polym14101987
291. Sartika M, Rambe F, Parinduri S, Harahap H, Nasution H, Lubis M, et al. Tensile properties of edible films from various types of starch with the addition of glycerol as plasticizer: a review. IOP Conf Ser Earth Environ Sci. 2022;1115:012075. https://doi.org/10.1088/1755-1315/1115/1/012075
292. Kieliszek M, Pobiega K, Piwowarek K, Kot AM. Characteristics of the Proteolytic Enzymes Produced by Lactic Acid Bacteria. Molecules. 2021;26:1858. https://doi.org/10.3390/molecules26071858
293. Ebrahimi B, Mohammadi R, Rouhi M, Mortazavian A, Shojaee-Aliabadi S, Koushki M. Survival of probiotic bacteria in carboxymethyl cellulose-based edible film and assessment of quality parameters. Food Sci Technol. 2017;87. https://doi.org/10.1016/j.lwt.2017.08.066
294. Salas-Jara MJ, Ilabaca A, Vega M, García A. Biofilm Forming *Lactobacillus*: New Challenges for the Development of Probiotics. Microorganisms. 2016;4:35. https://doi.org/10.3390/microorganisms4030035
295. Morris AL, Mohiuddin SS. Biochemistry, nutrients. Treasure Island (FL): StatPearls; 2024./
296. Ateto A. The structure of DNA and RNA. In: Molecular biology of the gene. James D. Watson; 2002. p. 33.
297. Sedó Molina GE, Shetty R, Xiao H, Wätjen AP, Tovar M, Bang-Berthelsen CH. Development of a novel lactic acid bacteria starter culture approach: From insect microbiome to plant-based fermentations. 2022;167:113797. https://doi.org/10.1016/j.lwt.2022.113797
298. de Oliveira KÁR, Fernandes KFD, de Souza EL. Current Advances on the Development and Application of Probiotic-Loaded Edible Films and Coatings for the Bioprotection of Fresh and Minimally Processed Fruit and Vegetables. Foods. 2021;10:2207. https://doi.org/10.3390/foods10092207
299. Odila Pereira J, Soares J, Sousa S, Madureira AR, Gomes A, Pintado M. Edible films as carrier for lactic acid bacteria. LWT. 2016;73:543–50. https://doi.org/10.1016/j.lwt.2016.06.060
300. Odila Pereira J, Soares J, Costa E, Silva S, Gomes A, Pintado M. Characterization of Edible Films Based on Alginate or Whey Protein Incorporated with *Bifidobacterium animalis* subsp. *lactis* BB-12 and Prebiotics. Coatings. 2019;9:493. https://doi.org/10.3390/coatings9080493
301. Orozco-Parra J, Mejía CM, Villa CC. Development of a bioactive synbiotic edible film based on cassava starch, inulin, and *Lactobacillus casei*. Food Hydrocoll. 2020;104:105754. https://doi.org/10.1016/j.foodhyd.2020.105754
302. Mukherjee P, Raj N, Sivaprakasam S. Harnessing valorization potential of whey permeate for D-lactic acid production using lactic acid bacteria. Biomass Convers Biorefinery. 2023;13:15639–58. https://doi.org/10.1007/s13399-023-05038-3
303. Bansal V, Nagaraj V. Understanding the role of pH in cheese manufacturing: general aspects of cheese quality and safety. J Food Sci Technol. 2022;61. https://doi.org/10.1007/s13197-022-05631-w
304. Sabikhi L, Kumar MHS, Mathur BN. *Bifidobacterium bifidum* in probiotic Edam cheese: influence on cheese ripening. J Food Sci Technol. 2014;51:3902–9. https://doi.org/10.1007/s13197-013-0945-7
305. Olivo P, Scapim M, Maia L, Miazaki J, Rodrigues B, Madrona G, et al. Probiotic Coating for Ripened Cheeses With *Lactobacillus Acidophilus* and *Lactobacillus Helveticus* Inclusion. J Agric Stud. 2020;8:152. https://doi.org/10.5296/jas.v8i3.16052

306. Abedi E, Hashemi SMB. Lactic acid production – producing microorganisms and substrates sources-state of art. Heliyon. 2020;6:e04974. https://doi.org/10.1016/j.heliyon.2020.e04974
307. Trmčić A, Ralyea R, Meunier-Goddik L, Donnelly C, Glass K, D'Amico D, et al. Consensus categorization of cheese based on water activity and pH—A rational approach to systemizing cheese diversity. J Dairy Sci. 2017;100:841–7. https://doi.org/10.3168/jds.2016-11621
308. Wemmenhove E, Beumer RR, van Hooijdonk ACM, Zwietering MH, Wells-Bennik MHJ. The fate of *Listeria monocytogenes* in brine and on Gouda cheese following artificial contamination during brining. Int Dairy J. 2014;39:253–8. https://doi.org/10.1016/j.idairyj.2014.06.002
309. Sakkas L, Moschopoulou E, Moatsou G. Influence of Salting and Ripening Conditions on the Characteristics of a Reduced-Fat, Semi-Hard, Sheep Milk Cheese. Foods. 2023;12. https://doi.org/10.3390/foods12244501
310. Ziarno M, Bryś J, Kowalska E, Cichońska P. Effect of metabolic activity of lactic acid bacteria and propionibacteria on cheese protein digestibility and fatty acid profile. Sci Rep. 2023;13:15363. https://doi.org/10.1038/s41598-023-42633-w
311. Aljewicz M, Cichosz G, Nalepa B, Kowalska M. Influence of the Probiotic *Lactobacillus acidophilus* NCFM and *Lactobacillus rhamnosus* HN001 on Proteolysis Patterns of Edam Cheese. Food Technol Biotechnol. 2014;52:439–47. https://doi.org/10.17113/ftb.52.04.14.3659
312. Sogut E, Filiz BE, Seydim AC. Whey protein isolate- and carrageenan-based edible films as carriers of different probiotic bacteria. J Dairy Sci. 2022;105:4829–42. https://doi.org/10.3168/jds.2021-21245
313. Lu J, Lu Y, Chang C, Wu J. Novel probiotic fermented edible film based on gum arabic/whey protein isolate/isomalt/glycerol incorporated with *Lactobacillus rhamnosus*: Physical, antibacterial, and fresh pork preservation properties. Food Hydrocoll. 2025;161:110875. https://doi.org/10.1016/j.foodhyd.2024.110875
314. Amer DA, Albadri AAM, El-Hamshary HA, Nehela Y, Makhlouf AH, El-Hawary MY, et al. Changes in Sensory Properties, Physico-Chemical Characteristics, and Aromas of Ras Cheese under Different Coating Techniques. Foods. 2023;12. https://doi.org/10.3390/foods12102023
315. Siriwardana J, Wijesekara I. Analysis of the Effectiveness of an Antimicrobial Edible Coating Prepared from Sweet Whey Base to Improve the Physicochemical, Microbiological, and Sensory Attributes of Swiss Cheese. Adv Agric. 2021;2021:5096574. https://doi.org/10.1155/2021/5096574
316. Weng Y, Zhu Y, Ren Y, Oguzlu H, Shewan H, Baldelli A. Transparent and water-resistant edible coatings for sustainable post-harvest preservation of banana. Sustain Food Technol. 2026; https://doi.org/10.1039/D5FB00804B
317. Kandasamy S, Yoo J, Yun J, Kang H-B, Seol K-H, Kim H-W, et al. Application of Whey Protein-Based Edible Films and Coatings in Food Industries: An Updated Overview. Coatings. 2021;11. https://doi.org/10.3390/coatings11091056
318. Nemati V, Hashempour-Baltork F, Gharavi-Nakhjavani MS, Feizollahi E, Júnior LM, Alizadeh AM. Application of a Whey Protein Edible Film Incorporated with Cumin Essential Oil in Cheese Preservation. Coatings. 2023;13. https://doi.org/10.3390/coatings13081470
319. Murrieta-Martínez CL, Soto-Valdez H, Pacheco-Aguilar R, Torres-Arreola W, Rodríguez-Felix F, Márquez Ríos E. Edible protein films: Sources and behavior. Packag Technol Sci. 2018;31:113–22. https://doi.org/10.1002/pts.2360

320. Ali AH, Khalifa SA, Gan R-Y, Shah N, Ayyash M. Fatty acids, lipid quality parameters, and amino acid profiles of unripened and ripened cheeses produced from different milk sources. J Food Compos Anal. 2023;123:105588. https://doi.org/10.1016/j.jfca.2023.105588
321. Rashidimehr A, Mohammadi-Nasrabadi F, Alhouei B, Khoshtinat K, Esfarjani F. Fatty acid composition of dairy products and their impact on atherogenicity and thrombogenicity. Sci Rep. 2025;15:43613. https://doi.org/10.1038/s41598-025-27445-4
322. Pires AF, Díaz O, Cobos A, Pereira CD. A Review of Recent Developments in Edible Films and Coatings-Focus on Whey-Based Materials. Foods. 2024;13:2638. https://doi.org/10.3390/foods13162638
323. Chen J, Liu H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review. Int J Mol Sci. 2020;21. https://doi.org/10.3390/ijms21165695
324. Paszczyk B, Polak-Śliwińska M, Łuczyńska J. Fatty Acids Profile, Trans Isomers, and Lipid Quality Indices in Smoked and Unsmoked Cheeses and Cheese-Like Products. Int J Environ Res Public Health. 2019;17. https://doi.org/10.3390/ijerph17010071
325. Ribeiro SC, Coelho MC, Todorov SD, Franco BDGM, Dapkevicius MLE, Silva CCG. Technological properties of bacteriocin-producing lactic acid bacteria isolated from Pico cheese an artisanal cow's milk cheese. J Appl Microbiol. 2014;116:573–85. https://doi.org/10.1111/jam.12388
326. Sousa Gallagher MJ, Ardö Y, McSweeney P. Advances in the study of proteolysis during cheese ripening. Int Dairy J. 2001;11:327–45. https://doi.org/10.1016/S0958-6946(01)00062-0
327. Coppola F, Fratianni F, Bianco V, Wang Z, Pellegrini M, Coppola R, et al. New Methodologies as Opportunities in the Study of Bacterial Biofilms, Including Food-Related Applications. Microorganisms. 2025;13. https://doi.org/10.3390/microorganisms13051062
328. Park YW. Proteolysis and Lipolysis of Goat Milk Cheese. J Dairy Sci. 2001;84:E84–92. https://doi.org/10.3168/jds.S0022-0302(01)70202-0
329. Mordvinova V, Topnikova E, Danilova E, Ostroukhova I. Impact of changes in the fat phase on the peculiarities of the formation of quality indicators of semi-hard and hard cheeses. Food Syst. 2023;5:361–8. https://doi.org/10.21323/2618-9771-2022-5-4-361-368
330. Saravani M, Ehsani A, Aliakbarlu J, Ghasempour Z. Gouda cheese spoilage prevention: Biodegradable coating induced by *Bunium persicum* essential oil and lactoperoxidase system. Food Sci Nutr. 2019;7:959–68. https://doi.org/10.1002/fsn3.888
331. Disca V, Carrà F, Stampini M, Arlorio M, Locatelli M. Treatments of food and food by-products to enhance gut microbiota short-chain fatty acids production. Food Biosci. 2025;72:107435. https://doi.org/10.1016/j.fbio.2025.107435
332. Sikosana MKLN, Ruland A, Werner C, Renner LD. Combining Microscopy Assays of Bacteria-Surface Interactions To Better Evaluate Antimicrobial Polymer Coatings. Appl Environ Microbiol. 88:e02241-21. https://doi.org/10.1128/aem.02241-21
333. Govari M, Vareltzis P. Conjugated linoleic acid in cheese: A review of the factors affecting its presence. J Food Sci. 2025;90:e70021. https://doi.org/10.1111/1750-3841.70021
334. Ribeiro SC, Stanton C, Yang B, Ross RP, Silva CCG. Conjugated linoleic acid production and probiotic assessment of *Lactobacillus plantarum* isolated from Pico cheese. LWT. 2018;90:403–11. https://doi.org/10.1016/j.lwt.2017.12.065
335. Nakonechna K, Ilko V, Berčíková M, Vietoris V, Panovská Z, Doležal M. Nutritional, Utility, and Sensory Quality and Safety of Sunflower Oil on the Central European Market. Agriculture. 2024;14. https://doi.org/10.3390/agriculture14040536

336. Zhang M, Fu J, Mao J, Dong X, Chen Y. Storage evolution in non-volatile and volatile lipid oxidation products of salted large yellow croaker based on targeted lipidomics and GC–MS analysis. Food Chem. 2025;486:144389. https://doi.org/10.1016/j.foodchem.2025.144389
337. Choi J, Lee SI, Rackerby B, Goddik L, Frojen R, Ha S-D, et al. Microbial communities of a variety of cheeses and comparison between core and rind region of cheeses. J Dairy Sci. 2020;103:4026–42. https://doi.org/10.3168/jds.2019-17455
338. Khan A, Nadeem M, Al-Asmari F, Imran M, Ambreen S, Rahim MA, et al. Effect of *Lactiplantibacillus plantarum* on the Conversion of Linoleic Acid of Vegetable Oil to Conjugated Linoleic Acid, Lipolysis, and Sensory Properties of Cheddar Cheese. Microorganisms. 2023;11. https://doi.org/10.3390/microorganisms11102613
339. Iorizzo M, Martino CD, Letizia F, Thomas W. Crawford J, Paventi G. Production of Conjugated Linoleic Acid (CLA) by *Lactiplantibacillus plantarum*: A Review with Emphasis on Fermented Foods. Foods. 2024;13. https://doi.org/10.3390/foods13070975
340. Korena K, Klimesova A, Florianova M, Krzyzankova M, Karasova D, Babak V, et al. Microbial Profiling of Smear-Ripened Cheeses: Identification of Starter Cultures and Environmental Microbiota. Appl Sci. 2025;15. https://doi.org/10.3390/app15073787
341. Stefanovic E, Kilcawley KN, Roces C, Rea MC, O'Sullivan M, Sheehan JJ, et al. Evaluation of the Potential of *Lactobacillus paracasei* Adjuncts for Flavor Compounds Development and Diversification in Short-Aged Cheddar Cheese. Front Microbiol. 2018;9. https://doi.org/10.3389/fmicb.2018.01506
342. Briggiler-Marcó M, Capra ML, Quiberoni A, Vinderola G, Reinheimer JA, Hynes E. Nonstarter *Lactobacillus* Strains as Adjunct Cultures for Cheese Making: *In Vitro* Characterization and Performance in Two Model Cheeses. J Dairy Sci. 2007;90:4532–42. https://doi.org/10.3168/jds.2007-0180
343. Flórez AB, Vázquez L, Rodríguez J, Mayo B. Phenotypic and Safety Assessment of the Cheese Strain *Lactiplantibacillus plantarum* LL441, and Sequence Analysis of its Complete Genome and Plasmidome. Int J Mol Sci. 2022;24:605. https://doi.org/10.3390/ijms24010605
344. Korena K, Krzyzankova M, Florianova M, Karasova D, Babak V, Strakova N, et al. Microbial Succession in the Cheese Ripening Process—Competition of the Starter Cultures and the Microbiota of the Cheese Plant Environment. Microorganisms. 2023;11:1735. https://doi.org/10.3390/microorganisms11071735
345. Moreno A, Calpe J, Dopazo V, Luz C, Quiles JM, Meca G. Development of a Natural Coating Based on Fermented Milk Whey for Biopreservation of Cheese. Foods. 2025;14. https://doi.org/10.3390/foods14132149
346. Guan Y, Lv H, Wu G, Chen J, Wang M, Zhang M, et al. Effects of Lactic Acid Bacteria Reducing the Content of Harmful Fungi and Mycotoxins on the Quality of Mixed Fermented Feed. Toxins. 2023;15:226. https://doi.org/10.3390/toxins15030226
347. Erceg T, Šovljanski O, Tomić A, Aćimović M, Stupar A, Baloš S. Comparison of the Properties of Pullulan-Based Active Edible Coatings Implemented for Improving Sliced Cheese Shelf Life. Polymers. 2024;16:178. https://doi.org/10.3390/polym16020178
348. Sharafi H, Moradi M, Amiri S. Application of Cheese Whey Containing Postbiotics of *Lactobacillus acidophilus* LA5 and *Bifidobacterium animalis* BB12 as a Preserving Liquid in High-Moisture Mozzarella. Foods. 2022;11:3387. https://doi.org/10.3390/foods11213387
349. Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ. ; 2013;3:e003733. https://doi.org/10.1136/bmjopen-2013-003733

350. Tan S-Y, Sotirelis E, Bojeh R, Maan I, Medalle M, Chik XSF, et al. Is dietary intake associated with salt taste function and perception in adults? A systematic review. Food Qual Prefer. 2021;92:104174. https://doi.org/10.1016/j.foodqual.2021.104174
351. Rudzka Nee Dudkiewicz A, Masmejean L, Arnaud C, Onarinde DB, Sundara R, Hossein Pour A, et al. Approaches for Improvement in Digestive Survival of Probiotics, a Comparative Study. Pol J Food Nutr Sci. 2020;265–73. https://doi.org/10.31883/pjfns/120184
352. Sumeri I, Adamberg S, Uusna R, Sarand I, Paalme T. Survival of cheese bacteria in a gastrointestinal tract simulator. Int Dairy J. 2012;25:36–41. https://doi.org/10.1016/j.idairyj.2011.12.016
353. Leeuwendaal NK, Hayes JJ, Stanton C, O'Toole PW, Beresford TP. Protection of candidate probiotic lactobacilli by Cheddar cheese matrix during simulated gastrointestinal digestion. J Funct Foods. 2022;92:105042. https://doi.org/10.1016/j.jff.2022.105042
354. Leeuwendaal N, Stanton C, O'Toole PW, Beresford TP. The potential of non-starter lactic acid bacteria from Cheddar cheese to colonise the gut. J Funct Foods. 2021;83:104425. https://doi.org/10.1016/j.jff.2021.104425
355. Kondrotiene K, Zavistanaviciute P, Aksomaitiene J, Novoslavskij A, Malakauskas M. *Lactococcus lactis* in Dairy Fermentation—Health-Promoting and Probiotic Properties. Fermentation. 2023;10. https://doi.org/10.3390/fermentation10010016
356. Kaushik R, Anand S. Studying the Population Dynamics of NSLAB and Their Influence on Spores During Cheese Ripening. Appl Microbiol. 2025;5. https://doi.org/10.3390/applmicrobiol5030065
357. Blaya J, Barzideh Z, LaPointe G. Symposium review: Interaction of starter cultures and nonstarter lactic acid bacteria in the cheese environment1. J Dairy Sci. Elsevier; 2018;101:3611–29. https://doi.org/10.3168/jds.2017-13345
358. Nalepa B, Ciesielski S, Aljewicz M. The Microbiota of Edam Cheeses Determined by Cultivation and High-Throughput Sequencing of the 16S rRNA Amplicon. Appl Sci. 2020;10. https://doi.org/10.3390/app10124063
359. Bettera L, Dreier M, Schmidt RS, Gatti M, Berthoud H, Bachmann H-P. Selective enrichment of the raw milk microbiota in cheese production: Concept of a natural adjunct milk culture. Front Microbiol. 2023;14. https://doi.org/10.3389/fmicb.2023.1154508
360. Nasim I, Faisal Z, Akhtar A, Maqsood S, Khalid N, Barrow CJ. A review of *In vitro* digestion models and studies for soft/semisoft and hard/semihard cheeses. Int Dairy J. 2025;169:106335. https://doi.org/10.1016/j.idairyj.2025.106335
361. Baptista DP, Gigante ML. Bioactive peptides in ripened cheeses: release during technological processes and resistance to the gastrointestinal tract. J Sci Food Agric. 2021;101:4010–7. https://doi.org/10.1002/jsfa.11143
362. Azambuja N, Moreno I, Gallina D, Spadoti L, Lima M. de Queiroz A, Pacheco MT, et al. Effect of Adjunct Culture *Lactobacillus helveticus* (B02) on the Composition, Proteolysis, Free Amino Acids Release and Sensory Characteristics of Prato Cheese. Food Nutr Sci. 2017;8:512–25. https://doi.org/10.4236/fns.2017.85035
363. Ahmed HAA, Khalil RAM, AbouEINour AM, El-Sisi AS, Ibrahim A. Ultrasonication of Lactobacilli Cultures on Edam Cheese Quality during Ripening. J. Food Nutr. Res. 2023;11:7. doi: 10.12691/jfnr-11-7-3
364. Sharafedtinov KK, Plotnikova OA, Alexeeva RI, Sentsova TB, Songisepp E, Stsepetova J, et al. Hypocaloric diet supplemented with probiotic cheese improves body mass index and blood pressure indices of obese hypertensive patients - a randomized double-blind placebo-controlled pilot study. Nutr J. 2013;12:138. https://doi.org/10.1186/1475-2891-12-138

365. Bintsis T, Vafopoulou-Mastrojiannaki A, Litopoulou-Tzanetaki E, Robinson R k. Protease, peptidase and esterase activities by lactobacilli and yeast isolates from Feta cheese brine. J Appl Microbiol. 2003;95:68–77. https://doi.org/10.1046/j.1365-2672.2003.01980.x
366. Valdiviezo-Marcelo J, Arana-Torres NM, Vega-Portalatino EJ, Ruiz-Flores LA, Tamariz-Angeles C, Olivera-Gonzales P, et al. Technological potential of native lactic acid bacteria isolated from Swiss-type artisanal cheese (Ancash, Peru) for their application in food. Front Sustain Food Syst. 2023;7. https://doi.org/10.3389/fsufs.2023.1212229

LIST OF PUBLICATIONS

1. **Vasiliauskaitė, A.,** Milerienė, J., Songisepp, E., Rud, E., Muizniece-Brasava, S., Ciprovica, I., Axelsson, L., Lutter, L., Aleksandrovas, E., Tammsaar, E., Šalomskienė, J., Šernienė, L., & Malakauskas, M. (2022). Application of edible coating based on liquid acid whey protein concentrate with indigenous *Lactobacillus helveticus* for acid-curd cheese quality improvement. *Foods*. Basel: MDPI, 2022, Vol. 11, No. 21., 1-13. https://doi.org/10.3390/foods11213353 [Citav. rodiklis: 5.2, bendr. cit. rod.: 5.3, kvartilis: Q1 (2022. InCites JCR SCIE)]
2. **Vasiliauskaitė, A.,** Milerienė, J., Kasparavičienė, B., Aleksandrovas, E., Songisepp, E., Rud, I., Axelsson, L., Muizniece-Brasava, S., Ciprovica, I., Paškevičius, A., Aksomaitienė, J., Gabinaitienė, A., Uljanovas, D., Baliukonienė, V., Lutter, L., Malakauskas, M., & Šernienė, L. (2023). Screening for Antifungal Indigenous Lactobacilli Strains Isolated from Local Fermented Milk for Developing Bioprotective Fermentates and Coatings Based on Acid Whey Protein Concentrate for Fresh Cheese Quality Maintenance. *Microorganisms*. Basel: MDPI, 2023, Vol. 11, No. 3., 1-16. https://doi.org/10.3390/microorganisms11030557 [Citav. rodiklis: 4.1, bendr. cit. rod.: 4.327, kvartilis: Q2 (2023. InCites JCR SCIE)]
3. **Vasiliauskaitė, A.,** Aleksandrovas, E., Martins, J. T., Vieira, J. M., Vicente, J. M. V. A. A., Radenkovs, V., Rud, I., Malakauskas, M., & Šernienė, L. (2025). Characterization of Dairy Industry Secondary Material-Based Edible Films: Effect of Incorporated Lactic Acid Bacteria. *Food and Bioprocess Technology*, 18 (6), 5332-5345. https://doi.org/10.1007/s11947-025-03764-2 [Citav. rodiklis: 5.8, bendr. cit. rod.: 5.331, kvartilis: Q1 (2024. InCites JCR SCIE)]

LIST OF SCIENTIFIC CONFERENCE ABSTRACTS

1. **Vasiliauskaitė, A.,** Aleksandrovas, E., Klupšaitė, D., Mockus, E., Bartkienė, E., Rud, I., Malakauskas, M., & Šernienė, L. (2025). Impact of Whey Protein-Based Coating on Biogenic Amine Content and Microbial Activity in Edam-Type Cheese. EFFoST 2025 International Conference "Fostering the Transition to Sustainable Food Systems: Embracing Novelty and Overcoming Challenges" : 17-19 November 2025, Porto, Portugal : Abstract book, p. 124 - 124. https://hdl.handle.net/20.500.12512/262120
2. **Vasiliauskaitė, A.,** Aleksandrovas, E., Rud, I., Laučienė, L., Malakauskas, M., & Šernienė, L. (2025). Enhancing probiotic viability: edible coatings from dairy by-products for functional food applications. Veterinarija Ir Zootechnika: One Health: Challenges for Food Safety: Abstracts, 83 (Suppl. 1), 5-5. https://hdl.handle.net/20.500.12512/256519
3. **Vasiliauskaitė, A.,** Aleksandrovas, E., Rud, I., Malakauskas, M., & Šernienė, L. (2025). Effect of probiotic edible coatings on the nutritional and biochemical properties of edam-type cheese. AGRISCI2025: The 14th International Conference of the Young Scientists for Advance of Agriculture: Abstracts, 41-41. https://hdl.handle.net/20.500.12512/256627
4. Rud, I., Tzimorotas, D., Axelsson, L., Songisepp, E., **Vasiliauskaitė, A.,** & Šernienė, L. (2025). Probiotic survival and impact on gut microbiota and metabolites during *in vitro* fermentation. 2nd Forum on Fermented Foods : February 5-7, 2025 : School of Medicine, University of Málaga : Book of abstracts, p. 113-113, ISBN 978-84-1335-424-8. https://hdl.handle.net/20.500.12512/261141
5. **Vasiliauskaitė, A.,** Aleksandrovas, E., Martins, J. T., Vieira, J. M., Vicente, A. A., Malakauskas, M., & Šernienė, L. Structural characterization of edible films with incorporated lactic acid bacteria from dairy by-products. (2025) The 6th International Electronic Conference on Foods "Future Horizons in Foods and Sustainability" : 28–30 October 2025, Online : Program and Abstract Book, p. 116-116. https://hdl.handle.net/20.500.12512/261140
6. **Vasiliauskaitė, A.,** Aleksandrovas, E., Martins, J. T., Vieira, J. M., Vicente, A. A., Malakauskas, M., & Šernienė, L. (2024). The Impact of Lactic Acid Bacteria on Rheological Properties of Whey-Pectin-Based Edible Coatings. The 5th International Electronic Conference on Foods: 28 – 30 October 2024, Online: Program and Abstract Book, 87-87. https://hdl.handle.net/20.500.12512/250562

7. **Vasiliauskaitė, A.,** Aleksandrovas, E., Radenkovs, V., Muizniece-Brasava, S., Ciprovica, I., Malakauskas, M., & Šernienė, L. (2024). Optical attributes of films derived from dairy by-products – influence of lactic acid bacteria. 3rd International PhD Student's Conference at the University of Life Sciences in Lublin, Poland: ENVIRONMENT–PLANT–ANIMAL–PRODUCT: 24 April 2024 Editors: Katarzyna Ognik, Anna Stępniowska, 1-1. https://doi.org/10.24326/ICDSUPL3.T006
8. **Vasiliauskaitė, A.,** Aleksandrovas, E., Rud, I., Laučienė, L.,Malakauskas, M., & Šernienė, L. (2024). *In vitro* study of fermented bovine milk digestion: Validated method for the isolation of indigenous probiotics. The 13th International Conference of Young Scientists "Young Scientists for Advance of Agriculture" AGRISCI2024 : Abstracts, 48-48. https://hdl.handle.net/20.500.12512/248222
9. **Vasiliauskaitė, A.,** Milerienė, J., Kasparavičienė, B., Aleksandrovas, E., Songisepp, E., Rud, I., Axelsson, L., Muizniece-Brasava, S., Ciprovica, I., Lutter, L., Bankovsky, V., Malakauskas, M., & Šernienė, L. (2023). Low-cost culture medium for biomass production of indigenous lactic acid bacteria by using bioreactor. 37th EFFoST International Conference "Sustainable Food and Industry 4.0: Towards the 2030 Agenda": 6-8 November 2023, Valencia, Spain : Abstract Book, 463-463. https://hdl.handle.net/20.500.12512/244955
10. **Vasiliauskaitė, A.,** Milerienė, J., Kasparavičienė, B., Aleksandrovas, E., Songisepp, E., Rud, I., Axelsson, L., Muizniece-Brasava, S., Ciprovica, I., Lutter, L., Malakauskas, M., & Šernienė, L. (2023). Fermentates and coatings with *L. paracasei* for control of fresh cheese spoilage. 2nd International PhD Student's Conference Environment-Plant-Animal-Product (ICDSUPL) at the University of Life Sciences in Lublin: 19-20 April 2023, Lublin, Poland : Volume 2 Editors: Kararzyna Ognik, Anna Stępniowska; Doctoral School of the University of Life Sciences in Lublin. Doctoral Students Council of the University of Life Sciences in Lublin. Lublin : Publishing House of the University of Life Sciences in Lublin, 2023. ISBN 9788372593900., 1-1. https://doi.org/10.24326/ICDSUPL2

PATENTS

1. Milerienė, J. (aut., išrad.), Šernienė, L. (aut., išrad.), Malakauskas, M. (aut., išrad.), **Vasiliauskaitė, A.** (aut., išrad.). Lietuvos sveikatos mokslų universitetas (fotogr.), Norwegian Institute of Food, Fisheries and Aquaculture Research (fotogr.). Valgoma apsauginė danga (LT 7052 B) 2024. https://hdl.handle.net/20.500.12512/242770. Lietuvos Respublikos patentų duomenų bazė.

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2017–2018	Supplementary (bridging) studies in Veterinary Food Safety for admission to Master’s studies, Lithuanian University of Health Sciences
2014–2017	Professional Bachelor’s degree in Nutrition, qualification of Dietitian, Klaipėda State College

Professional activity:

2025 11–2026 06	Junior Assistant, Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences
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2024	Award for the Best Oral Presentation in the Food Safety and Veterinary Medicine section at the conference “Young Scientists for Agricultural Progress”, organized by the Lithuanian Academy of Sciences
2023	Award for the Best Presentation in the Food Technology Sciences section at the 2nd International PhD Students’ Conference “Environment–Plant–Animal–Product”, University of Life Sciences in Lublin, Poland

PADĖKA

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