Application of lactic acid bacteria for development of innovative potato protein-based yogurt alternatives

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APPLICATION OF LACTIC ACID BACTERIA FOR DEVELOPMENT OF INNOVATIVE POTATO PROTEIN-BASED YOGURT ALTERNATIVES

The experimental part of this Graduate thesis was conducted at the Technical University of Denmark, DTU Food - National Food Institute, Gut, Microbes and Health research group, Denmark. The thesis was made under the supervision of professor Egon Bech Hansen with the help of PhD student Lise Friis Christensen and under mentorship of professor Jasna Novak.

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PRIMJENA BAKTERIJA MLIJEČNE KISELINE U RAZVOJU INOVATIVNIH PROIZVODA SLIČNIH JOGURTU NA BAZI PROTEINA KRUMPIRA

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Sažetak: Cilj ovog rada bio je razvoj inovativnih fermentiranih proizvoda na bazi proteina krumpira kao alternativa jogurtu. Optimiran je sastav hranjivih medija s modificiranim izvorima dušika, prvenstveno porijeklom iz krumpira, te ugljika za odabir potencijalnih starter kultura u industrijskoj proizvodnji iz kolekcije mikroorganizama od ukupno 119 sojeva od kojih većina pripada rodovima bakterija mliječne kiseline (BMK), a preostali sojevima kvasca ili funga. Analizirani sojevi su dio mikrobne populacije biljnih mikrookoliša, prvenstveno krumpira i nusproizvoda poput kore krumpira kao sekundarne sirovine. U konačnici odabrana su 4 soja BMK, *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Leuconostoc mesenteroides* subsp. *mesenteroides i Leuconostoc pseudomesenteroides,* za proces fermentacije inovativnih prehrambenih proizvoda, te je definiran sastav prehrambenog matriksa kao temelj za fermentaciju. Rast sojeva praćen je određivanjem broja bakterijskih kolonija (CFU mL^{-1}) i optičke gustoće suspenzije stanica (OD600), te mjerenjem brzine zakiseljavanja u eksperimentalnim medijima pomoću iCinac uređaja. Inovativni proizvodi su razvijeni korištenjem dva različita koncentrata proteina krumpira komercijalnog naziva Protafy i KMC Food. Senzorsku analizu 4 inovativna proizvoda proveo je stručni panel. Ovaj rad je doprinos istraživanjima s ciljem održivosti proizvodnje krumpira primjenom BMK koji su među industrijski najznačajnijim mikroorganizmima.

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APPLICATION OF LACTIC ACID BACTERIA FOR DEVELOPMENT OF INNOVATIVE POTATO PROTEIN-BASED YOGURT ALTERNATIVES

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Abstract: The aim of this thesis was to develop innovative potato protein-based dairy fermented alternatives. The composition of nutrient media with modified sources of carbon and nitrogen, essentially from potato, has been optimized to select potential starter cultures in industrial production from a collection of 119 microorganisms, mostly lactic acid bacteria (LAB) and several strains of yeast and fungi. The tested strains were isolated from the plant microenvironments primarily potatoes or their peel as a sustainable source. Additionally, after defining the composition of the non food-grade media, a food-grade food matrix for the laboratory-scale fermentation process was developed. Finally, 4 LAB strains were selected, *Leuconostoc pseudomesenteroides*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Lactococcus lactis* subsp. *cremoris*, for the fermentation process. Strain growth was monitored by determining the number of bacterial colonies (CFU mL^{-1}) and the optical density of the cell suspension (OD600), and by measuring the acidification rate in the experimental media using iCinac instrument. Innovative products have been developed using two different potato protein concentrates, Protafy and KMC Food. Sensory analysis of 4 innovative products was conducted by an expert panel. This work is a contribution to research aimed at the sustainability of potato production using LAB, which are among the most industrially important microorganisms.

Keywords: dairy alternatives, lactic acid bacteria, innovative products, potato proteins, sustainability

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1. INTRODUCTION

LAB are well-known for their fastidious nutritional requirements that can differ between both species and strains (Teusink and Molenaar, 2017). Since nitrogen sources form a significant part of the average cost of the medium for LAB cultivation, there are continuous attempts to replace them with low-cost food by-products or agricultural wastes (Hayek et al., 2013). Potato protein concentrate is one such alternative source of plant protein. Potato proteins are extracted during potato starch production and are associated with several health-promoting and beneficial techno-functional properties (Levy et al., 2021; Waglay and Karboune, 2016). However, several studies showed that potato by-products or potato extracts could not completely replace expensive nitrogen sources (Liu et al., 2005).

Screening beneficial microorganisms for their suitability in plant-based food products is crucial since a mismatch between a substrate and microbial culture might result in lower biomass yields (Guo and Yang, 2015). Scientific and industrial stakeholders are particularly interested in LAB isolated from non-dairy foods due to their complex metabolic profile, unique flavour-forming abilities, and potential utilization as starter cultures. Moreover, several studies have demonstrated that LAB isolated originally from the same source are going to be an optimal starter culture for industrial fermentation of similar products (Jini et al., 2011). Still, the vast majority of plant-based fermented food products are still made with yogurt LAB starter cultures (Levy et al., 2021; Montemurro et al., 2021).

Plant-based yogurt-like food products have been extensively explored in recent years as a result of the growing demand for dairy alternatives, which is driven by health, ethical, and environmental concerns (Montemurro et al., 2021; Tangyu et al., 2019). Despite efforts, producers of dairy alternatives continue to encounter considerable issues related to appearance, textural properties, unbalanced nutritional profile, and the presence of antinutritional factors (Pontonio and Rizzello, 2021; Veber et al., 2021). Additionally, improving flavour of plant-based milk alternatives is crucial for product development. Besides nutritional and biopreservation effects, fermentation by LAB generates a diverse range of aromatic compounds giving food products distinct flavours and aromas (Masiá et al., 2020; Magala et al., 2015). Yet, there is a lack of knowledge regarding the sensory properties of fermented food products based on potato proteins.

The aim of this thesis was to develop innovative fermented potato protein-based dairy alternatives. Therefore, several growth media will be formulated for the selection of microorganisms capable of utilizing potato protein as a sole source of nitrogen. Microorganisms will be isolated from plant-based foods/wastes or industrial side streams with LAB strains isolated from potato wastes expected to have more competitive metabolic capacities that will support their growth. A diverse group of LAB strains will be tested in fermentation experiments. After the final selection, potato protein-based yogurt-like food products will be developed. The sensory properties of these novel fermented food products will be evaluated by a highly trained sensory panel.

2. THEORETICAL PART

2.1. GROWTH REQUIREMENTS OF LAB

LAB are a heterogeneous group of bacteria that are generally described as gram-positive, nonsporing, microaerophilic or anaerobic cocci, coccobacilli, or rods. They are characterized by the production of lactic acid as a significant end product during the fermentation of carbohydrates (De Vuyst and Leroy, 2020). Additionally, they are considered as fastidious bacteria that require a rich, complex growth medium (Hayek et al., 2019; Djeghri-Hocine et al., 2006). Optimization of the LAB growth medium is challenging since their nutritional requirements can vary widely depending on the species and even different strains of the same species (De Filippis et al., 2020). LAB cultivation medium usually requires several nitrogen sources (such as peptone, yeast extract, beef extract, or whey protein), carbohydrates (simple sugars such as dextrose, sucrose, maltose, or lactose), minerals (mainly Mn^{2+} and Mg^{2+} in trace amounts), and buffering agents (such as sodium acetate and di-sodium-glycerophosphate). Generally, LAB have specific amino acid and vitamin auxotrophies. Their growth on a rich and complex medium is based on particular auxotrophies that are associated with their microenvironment (Wegkamp et al., 2009).

Since LAB need a plethora of growth factors, nitrogen sources provide building blocks for amino acids, peptides, proteins, ribonucleic acids, minerals, and vitamins (Hayek et al., 2019). Also, LAB have a very limited capacity to synthesize amino acids from inorganic nitrogen sources which is why they depend on amino acids from these organic nitrogen sources (Hayek and Ibrahim, 2013). Although they are considered auxotrophic for several amino acids, it is considered that some residual genetic material for biosynthesis is still present since mutagenesis can make some lactobacilli prototrophic (Hébert et al., 2004a). While some amino acids can promote growth, it is important to remember that others can show inhibitory effects on the growth of certain LAB strains. For example, cysteine, arginine, histidine, and serine suppressed growth in several lactobacilli in a concentration-dependent manner with higher concentrations showing stronger suppression (Suzuki et al., 2014).

Minerals have a key role in microbial growth and can be used to optimize and control LAB enzymatic activity. Essential metal ions assist bacteria in a variety of ways: as enzyme activators or cofactors, in membrane transport, and as components of molecules or structural complexes (Hayek and Ibrahim, 2013). LAB require manganese for growth and metabolic activity. It is a key element in protecting some LAB from endogenous oxygen radicals and is

responsible for catalytic scavenging of O_2, which is required for anaerobic growth (Hayek et al., 2019). Likewise, magnesium can enhance the growth and improve survival of LAB and is usually added to LAB cultivation medium. It is also considered the only essential oligoelement for certain LAB strains in a chemically defined medium (Hayek et al., 2019; Hébert et al., 2004b).

Regarding vitamins, it is considered that individual strains need from one to four vitamins to achieve normal growth (Hayek and Ibrahim, 2013). Generally, vitamins have a catalytic role in the cells as coenzyme components or as enzyme prosthetic groups (Hébert et al., 2004a). Although requirements vary among LAB strains, pantothenic acid, riboflavin, and nicotinic acid are essential for most strains. However, some vitamins can be essential for certain strains, while others use them as stimulatory factors (Hayek and Ibrahim, 2013).

The pH value of a cultivation medium has a vital effect on the metabolic activity of LAB (Hayek and Ibrahim, 2013). Furthermore, buffering agents are required in LAB cultivation medium to help maintain the pH at optimum levels because of lactic acid production during bacteria growth (Hayek et al., 2019). In most cases, pH of 4.4 could inhibit or significantly hinder LAB growth rate (Hayek and Ibrahim, 2013).

And finally, surfactants such as Tween 80 and lecithin can be used to protect LAB cells against harsh conditions, improve their growth and enhance nutrient uptake (Hayek et al., 2019; Ibrahim et al., 2009). By supplementing LAB cultivation medium with Tween 80, oleic acid is incorporated into the cell membrane and later converted into cyclopropane fatty acids. This protects LAB strains from difficult environmental conditions such as low pH, oxygen effects, and extreme temperatures. Tween also helps in recovery ability, bile tolerance, and metabolic activity of LAB strains (Hayek and Ibrahim, 2013).

2.2. OPTIMIZATION OF GROWTH MEDIUM

The evaluation of selective LAB medium has been a topic of interest for many years. Among other specialized media, MRS and M17 remain particularly useful for a variety of LAB strains (Djeghri-Hocine et al., 2006). However, a variety of commonly available agricultural wastes, such as crop residues, woody materials, and food by-products are being explored for LAB cultivation due to their potential economic and environmental advantages (López-Gómez et al., 2020; Wang et al., 2015). These materials can be used for lactic acid (LA) and beneficial compounds production, cell mass production, or as an alternative to develop low-cost medium (Hayek et al., 2019).

2.2.1. Modifying nitrogen sources

Standard media like MRS and M17 always contain expensive nitrogen sources (different peptones, tryptone, beef extract, yeast extract) that make a noteworthy portion of medium costs. Hence, there have been many attempts to replace these expensive nitrogen sources with other low-cost options such as food by-products and agricultural wastes (López-Gómez et al., 2020). Mixtures of yeast and potato extracts, spent cabbage juice, pineapple peel juice, corn steep liquor, and sweet potatoes are some of the tested alternatives made to be used as a LAB cultivation medium (Hayek et al., 2013). Compared to cultivation in MRS, after growth in alternative media LAB strains have often shown similar LA production, biomass yields, and cell viability improvement (Hayek et al., 2019). Yet, to achieve growth levels in MRS, alternative low-cost nitrogen sources often had to be supplemented with external nitrogen sources (Hayek et al., 2013; Liu et al., 2005). Sometimes, a small supplementation of external nitrogen sources led to a significant improvement in LAB growth and functionality (Hayek et al., 2019). In general, multiple nitrogen components are preferably used to optimize LAB growth even in standard medium. One of the nitrogen sources often used to supplement alternative low-cost medium is yeast extract. Although less expensive than peptones, yeast extract still makes a significant portion of medium's cost. Therefore, implementing suitable alternatives is attractive. However, limitations are that using alternative nitrogen sources led to a decrease in LA production or the need in increasing the incubation period (Altaf et al., 2007). On the other hand, corn steep liquor can not only completely replace yeast extract as a source of nitrogen but also help improve LA production when combined with additional beneficial medium components (Yu et al., 2008). Additionally, both peptone and yeast extract were completely replaced with wheat bran, red lentil flour, and baker's yeast (Altaf et al., 2007). Further, horse bean extract was utilized as a nitrogen source instead of peptones and meat extract in a cultivation medium, and it proved to be suitable for growth of all tested LAB strains (Djeghri-Hocine et al., 2006).

2.2.2. Modifying carbohydrate sources

Although LAB are capable of using most sugars as a source of carbon and energy, glucose is still considered a preferred source of most LAB (Kim et al., 2009). The ability of LAB species or even strains to ferment different sugars varies, which may affect their growth and

functionality (Hayek and Ibrahim, 2013). For instance, in a study assessing the mode of carbohydrate metabolism by LAB in broth containing glucose, fructose, maltose, and sucrose, *Pediococcus pentosaceus* could not ferment sucrose but *Lactobacillus brevis* could only ferment maltose (Paramithiotis et al., 2007). Knowing the preferred sugar substrate makes it easier to achieve optimal growth and metabolic activity of the LAB strain (Hayek and Ibrahim, 2013).

Several LAB strains have both amylolytic and LA production capacity. LA is usually produced from substrates containing refined sugars which are relatively expensive. Utilizing starch, a readily available and less expensive substrate, is considered an attractive alternative (Altaf et al., 2007). Additionally, incorporating food-grade components into the medium can further boost productivity while lowering expenses (Boontun et al., 2021).

Some species of LAB appear to have adapted to plant-based habitats, where sucrose is typically the most abundant carbohydrate source (Prechtl et al., 2018). Sucrose is a widely available disaccharide easily available through food-grade sources (Boontun et al., 2021). Of particular interest is the synthesis of exopolysaccharide (EPS), e.g. dextran, from sucrose by LAB that express enzymes for sucrose metabolism (Prechtl et al., 2018). The majority of *Lactobacillus* spp. seem to have at least one, if not multiple, pathways for sucrose metabolism. Sometimes sucrose is one of the highly preferred substrates, as evidenced by the existence of two or more different routes of its metabolism (Gänzle and Follador, 2012). For example, sucrose was selected as the most cost-effective carbon source for both *Bifidobacterium animalis* subsp. *lactis* and *L. reuteri* since it facilitated bacterial growth that was comparable to that when grown on glucose (Boontun et al., 2021). Also, the growth of *L. plantarum* cultivated in food-grade MRS-based medium containing table sugar (sucrose) was similar to that observed in MRS medium (Sawatari et al., 2006).

2.2.3. Utilization of potato in modified growth medium

Potato residues have been recognized as promising raw materials for fermentation medium because of their availability and composition (Kot et al., 2020). The use of potato waste as medium component reduces the total production costs. However, potato extract could not completely replace the yeast extract from the growth medium for LAB. In some cases, partial substitution of yeast extract with potato extract was beneficial but varied depending on strains

and the sources of yeast extract. This was of economic interest since the use of some types of potato extracts can impact the reduction of 40 % in costs (Gaudreau et al., 2002). Studies have also reported the use of potato tubers unacceptable for food processing because of their size or damage by bruising or disease (discarded potatoes) as a substrate for the simultaneous production of nisin and LA. Despite the fact cull potato hydrolysate alone could not support the production of LA and nisin, it has been shown to have potential when supplemented with corn steep solid and soybean peptone (Liu et al., 2005). On top of that, liquid potato wastes from potato chips processing industry have been used as a nutritional source for the production of LA. Similarly, potato peel as a source of carbon has been shown as prominent in the production of highly active and stable amylolytic, cellulolytic, and ligninolytic enzymes (Kot et al., 2020). Finally, sweet potato medium that was not supplemented with external nitrogen sources showed only 1 to 2 log CFU mL^{-1} lower bacterial population of *Lactobacillus* strains than MRS. Yet, when sweet potato medium was supplemented with 4 g L^{-1} of beef extract and 4 g L^{-1} of yeast extract, there was similar growth and a somewhat higher bacterial population than MRS. To compare, the authors also found that supplementing their experimental medium with 8 g L^{-1} of beef extract and 8 g L^{-1} of yeast extract resulted in no significant difference. This means a sweet potato medium containing less than 50 % of the nitrogen content of MRS could use as an alternative to MRS for *Lactobacillus* cultivation (Hayek et al., 2013).

2.3. SCREENING OF MICROBIAL CULTURE

Traditionally, fermentation is a natural process carried out in different LAB fermented foods whose autochthonous microbial populations consist of a mixed culture of yeasts, bacteria, and fungi. Today, LAB, bacilli, and yeasts, especially *Saccharomyces*, are the most widely used microbes in terms of plant-based fermentation. They are proven to possess metabolic features that improve nutritional and/or sensory properties (Tangyu et al., 2019). LAB have been particularly popular to be used in food products due to the Generally Recognized as Safe (GRAS) status and are included in the Qualified Presumption of Safety (QPS) list (EFSA, 2021; Peyer et al., 2016). Some of them have already been used in plant-based fermented dairy substitutes, such as *L. delbrueckii, S. thermophilus, L. delbrueckii* subsp. *bulgaricus, B. longum* or *L. rhamnosus* (Masiá et al., 2020). In general, the variety of fermented foods is determined by the type of substrates and microbes used in the fermentation process (El Youssef et al., 2020). Therefore, many authors have studied the influence of mixed culture fermentation to

enhance product quality as a result of their synergistic effects (Beganović et al., 2011). A well-understood example is a mutualistic cooperation between *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* during yogurt fermentation. Yet, these strains might not be optimal to use to ferment plant-based materials. Mårtensson et al. (2000) tested different strains in an oat-based beverage and found that samples fermented with *S. thermophilus* and *L. bulgaricus* had low consumer acceptance, whereas samples fermented with *Leuc. mesenteroides* had pleasant flavours and a pleasant taste. On top of that, the data comparing the impact of mono vs mixed culture fermentation in plant-based milk alternatives on growth, nutrient value, anti-nutrient, and mineral availability, bioactive compounds and sensory values has been conflicting (Tangyu et al., 2019). In regards to sensory values, it seems that monoculture fermentation is as efficient as mixed culture fermentation in decreasing the content of n-hexanal and n-hexanol but mixed culture fermentation could be more successful in producing preferred flavour enhancers (Tangyu et al., 2019).

2.3.1. Unconventional sources of LAB

Much attention has been paid to unconventional sources used to isolate different LAB strains with potential beneficial health or functional properties (Zielińska and Kołożyn-Krajewska, 2018). Strains isolated from unconventional sources, such as raw fruits and vegetables, can produce bioactive metabolites that help improve the nutritional value and sensory profile of food products. Moreover, some of the strains showed antimicrobial capacity which points to their use as biopreservatives in food products (Pimental et al., 2021). There has also been a lot of interest in LAB isolated from non-dairy products because of their diverse metabolic profile, distinctive flavour-forming capabilities, and a possibility for use as starter cultures (Teneva-Angelova and Beshkova, 2015). LAB grow fast under moderately acidic and anaerobic conditions, making them well-suited for growth in fruit or vegetable-based foods (Fessard and Remize, 2019). Many studies have also tried isolating LAB from different food processing wastes. For instance, several LAB with proteolytic, lipolytic, and antibacterial properties were isolated from various freshwater fish wastes (Jini et al., 2011). Also, dairy industries have been increasingly interested in LAB isolated from non-dairy sources like decaying plant materials since they want to expand their culture collection and take advantage of existing biodiversity (Smid and Kleerebezem, 2014).

Not all yogurt starter cultures can fully utilize the components in soymilk and if there is a mismatch between the substrate and microbial culture, it could result in a lower specific growth

rate of microorganisms and consequently slower acidification of microenvironment (Guo and Yang, 2015). Also, a comparison of the genetic diversity of two dairy strains and one plant-derived *Lc. lactis* strain revealed that multiple genes required for growth in plant materials are not present in dairy species (Wegkamp et al., 2009). This could be evident in a study using horse bean extract as a replacement for standard nitrogen sources (peptones, meat extract) in multiple cultivation media. While the media supported the growth of all tested strains, it was more efficient for the growth of strains isolated from plant materials as compared to those derived from milk or human faeces. This difference was attributed by the authors to the plant origin of the nitrogen supplementation, which resulted in greater growth than that observed on MRS medium (Djeghri-Hocine et al., 2006). Several studies have found LAB isolated from the same source, as opposed to LAB isolated from other sources, as a superior starter culture for fermentation of comparable products (Jini et al., 2011). To add to that, the use of strains from unconventional sources in fermented food might decrease the fermentation time and improve the food products' characteristics such as creating a specific flavour (Pimental et al., 2021). In a study where a strain was isolated from spontaneous coffee fermentation, aroma-specific volatile compounds were increased during the coffee bean fermentation process which contributed to the production of high-quality coffee. In the end, the coffee beverage had enhanced sensory qualities because of stronger “vanilla” and “floral” aromas (Pereira et al., 2015). Another study isolated, identified, and analyzed different LAB from herbs. Some lactococci and lactobacilli they've found had better growth in a wide pH and temperature range and a high acid production. These strains have the potential of one day becoming starter cultures for new fermented products (Teneva-Angelova and Beshkova, 2015).

2.3.2. Safety aspect of microbial culture use in food products

Before microorganisms are allowed to be used as food or feed in the European market, EFSA (European Food and Safety Authority) evaluates their safety. They have introduced a concept of QPS which is supposed to avoid long and unnecessary inquiries into the safety of microorganisms that are known to be safe (EFSA, 2021). That is a generic harmonized approach for risk assessment that is somewhat similar to the GRAS assessment in USA. While microbial food cultures with a long history of consumption in the EU do not undergo a full safety assessment, others require pre-market approval that involves a safety assessment by European legislation (Herody et al., 2010). One of the requirements for the suitability of a microorganism for QPS status is an unambiguously defined taxonomy. For microorganisms that have not been

traditionally used in food, this implies the need for experimental data on the genetics of the taxonomic unit (Sybesma et al., 2006). That means a strain could be excluded from the QPS list if its taxonomic identity cannot be defined with current data which doesn't necessarily mean the strain is unsafe in food products (Herody et al., 2010). Other requirements are knowledge about the history of use, clinical aspects and industrial applications, safety concerns (possible presence of virulence that can contribute to their pathogenicity), and end-use. By these criteria, microorganisms such as *Enterococcus faecium* and *Escherichia coli* have not yet been granted QPS status (Herman et al., 2019). If a manufacturer wants to use a microbial culture that hasn't been granted QPS status, they must do a case-by-case safety evaluation by strain and/or by application purpose (Herody et al., 2010).

Moreover, there are LAB species that are resistant to certain antibiotics. For example, *Leuconostoc* and *Enterococcus* species are known to be vancomycin-resistant (Ino et al., 2016). The presence of virulence features in microorganisms utilized in food fermentation should be avoided (Bourdichon et al., 2012). However, many bacterial species such as *Lactobacillus* strains carry intrinsic resistance which means it is inherent to their species. That type of resistance is considered to have a minimal potential for horizontal spread, unlike acquired resistance that has a high potential for lateral spread (Herody et al., 2010). Still, even if strains belong to species with a long history of use, those that present unwanted properties should be subjected to a risk assessment (Bourdichon et al., 2012).

2.4. SENSORY PROFILE OF NON-DAIRY MILK ALTERNATIVES

Consumer demand for non-dairy milk substitutes with a high acceptance rate and functionality is increasing as a shift towards a more plant-based diet that might help to increase food production sustainability and reduce environmental impact (Levy et al., 2021; Nionelli et al., 2014). Figure 1 describes frequent technical, nutritional, and sensory quality criteria of plant-based dairy alternatives.

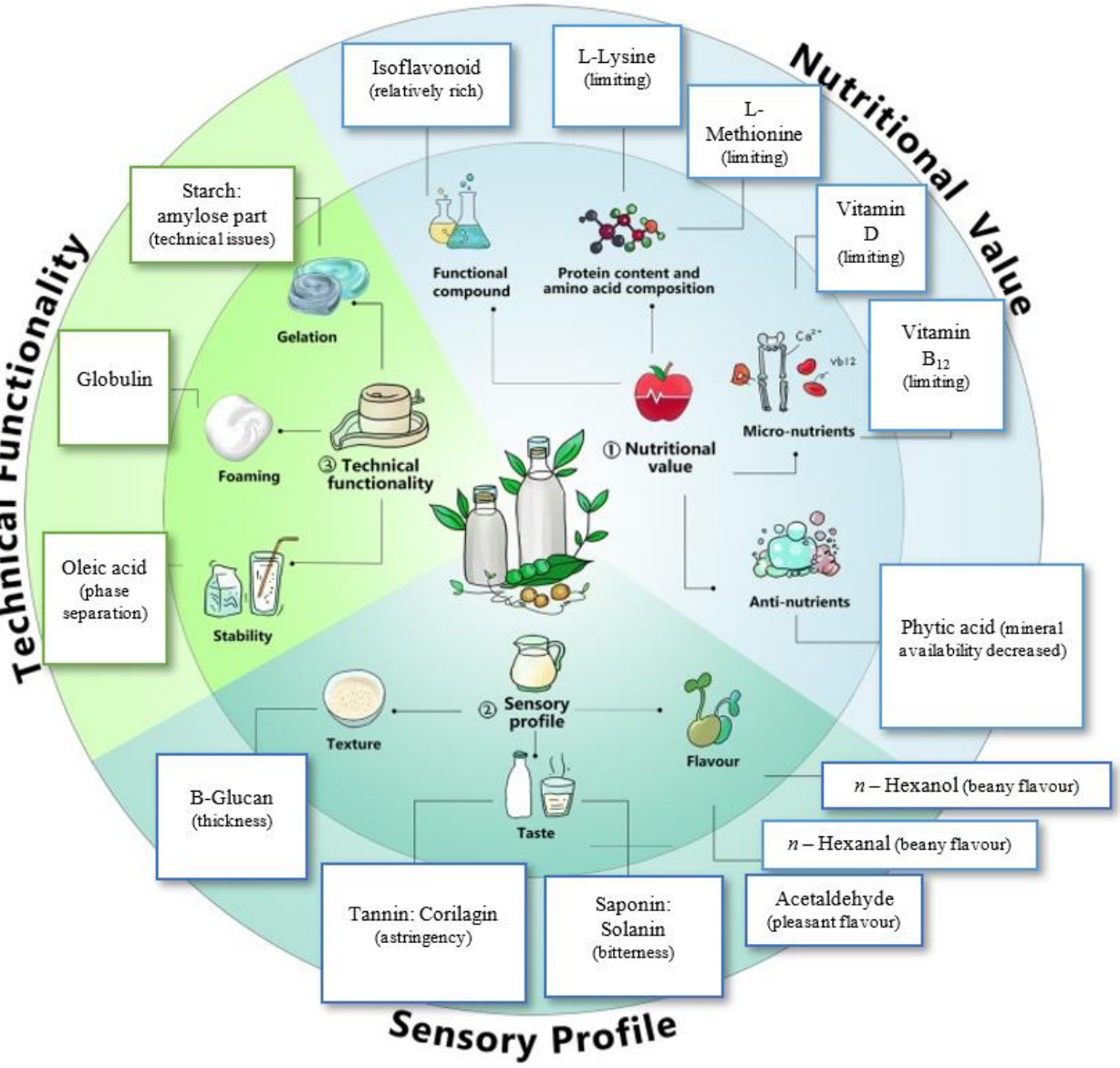


Figure 1. A visual representation showing the quality criteria of plant-based milk alternatives (Tangyu et al., 2019)

Although plant-based milk alternatives are designed to imitate animal milk in terms of color and texture, they frequently carry unpleasant off-flavours (Tangyu et al., 2019). To start with, raw cereals commonly have low levels of organoleptically active compounds which can give them a "green" or a flat, unpleasant odour and flavour. Additionally, they contain particular phenolic compounds that contribute to their bitterness and astringency which leads to a poor acceptance rate and negatively influences consumer's purchase willingness (Peyer et al., 2016). Similar challenges can also be noticed in legumes. For example, peas carry undesirable notes

described as “beany”, “green”, “hay-like” and “rancid” which limits their consumption. These off-flavour compounds are likely a product of a combination of inherent pea flavours and flavours produced during harvesting, processing, and storage (El Youssef et al., 2020). However, plant materials do possess components like soluble fiber that can improve both the texture and the mouthfeel of the products, but it is often the case that the insoluble particles create a thin mouthfeel and a chalky, sandy texture. Besides, some bioactive compounds such as isoflavonoids can be linked to an objectionable aftertaste (Tangyu et al., 2019). In some cases, lipid oxidation can contribute to an unwanted aftertaste. In an oat-based drink, volatile compounds produced through lipid oxidation, such as hexanal, pentanal, and some carbonyl compounds led to a rancid-like unpleasant aftertaste. Furthermore, the presence of long-chain hydroxy fatty acids caused bitter off-notes in oat flavour (Masiá et al., 2020; Nionelli et al., 2014). Oxidation of plant lipids can generate volatile compounds like n-hexanol and n-hexanal that are mainly responsible for a typical “earthy” or “beany” smell of plant-based milk alternatives (Tangyu et al., 2019). Furthermore, these limited techno-functional properties, off-flavour, and colour can often require the need of stabilizers, that have been indicated to have negative health effects or be negatively seen by consumers (Levy et al., 2021). To consumers, favorable organoleptic properties are dominating over sustainability or health concerns. In the end, meeting consumer’s expectations by improving sensory profiles is key when it comes to developing a successful plant-based milk alternative product (Masiá et al., 2020).

2.4.1. Optimization of fermentation for improved sensory profile

Fermentation has traditionally been used to naturally enhance and fortify a food substrate, removing the need for additives or preservatives. During LA fermentation nutrients in raw material are generated to a variety of antimicrobial compounds including organic acids, mainly LA, carbon dioxide, ethanol, bacteriocins, and other metabolites (Levy et al., 2021). It has been found to improve protein digestibility, enhance bioavailability of minerals and other micronutrients, reduce or remove antinutritional factors, and prolong shelf life (Peyer et al., 2016; Nionelli et al. 2014). It has also been used for centuries as a method to improve organoleptic properties (El Youssef et al., 2020). LAB can produce a diverse range of aromatic compounds, creating fermented products with distinct flavours and aromas originated from the breakdown of the primary macronutrients in the food matrix. Additionally, some LAB can generate EPS. These compounds are known to enhance the texture of fermented products by increasing their viscosity and improving their stability (Masiá et al., 2020). As a result, many

plant-based fermented foods are made with microbial communities due to their potential synergistic effects in helping to improve the quality of fermented products (El Youssef et al., 2020). Previous studies have shown that LA fermentation has reduced unpleasant "green/beany" descriptors connected to pea proteins (El Youssef et al., 2020; Tangyu et al., 2019) and the "soapy/beany" off-flavours in soybean milk. Moreover, a plant-based yogurt that satisfied organoleptic needs of consumers was produced with soy and coconut milk (Kosterina et al., 2020).

Texture and mouthfeel attributes are crucial factors in overall food acceptability (Greis et al., 2020). As the structure of food changes during mastication due to oral processing and chemical breakdown, it makes texture perception of semi-solid food gels a dynamic process. In some cases, food texture and mouthfeel can even result in food aversions (Greis et al., 2020; De Lavergne et al., 2015). In the case of dairy yogurts, sensations of creaminess and thickness have been preferred (Greis et al., 2020; Antmann et al., 2010). Starch and starch-related products have sometimes been used in dairy yogurts to provide stability to the final product (Luallen, 2004). Interestingly, they have also been found to improve creaminess perception as thickening agents. It has been suggested that alternative structural components like starch particles may add to the thickness and smoothness which correlates with creaminess in some studies (Greis et al., 2020).

Although no definitive conclusion has been reached, the most widely cited compounds responsible for adding preferred flavour to dairy yogurt are LA, acetaldehyde, diacetyl, acetoin, and 2-butanone. It is important to keep these metabolites in acceptable concentrations since some volatile compounds can contribute to undesirable flavours, particularly when the product is improperly stored (Chen et al., 2017). Additionally, in plant-based dairy alternatives, acetone and ethanol are also very important for sensory properties (Montemurro et al., 2021). In the case of non-dairy alternatives, the levels of ethanol, diacetyl, acetoin, acetaldehyde, and acetone have been significantly differing based on the nature of plant material used. For example, acetone levels ("fruity", apple flavour) were significantly higher in soy samples and ethanol levels were significantly higher in coconut samples (Masiá et al., 2020). Several compounds containing four carbon atoms are found to be responsible for the characteristic aroma of yogurt in fermented dairy products, as well as its butter-like flavour. These include diacetyl, acetoin, and 2,3-butanediol (Chen et al., 2017). During cereal-based fermentation, for instance, diacetyl is released which gives a butterscotch-like aroma (Tangyu et al., 2019). Acetoin is the reduced

form of diacetyl with a much milder flavour compared to diacetyl. However, acetoin is important for contributing to the mild creamy flavour and for lowering the strong aroma of diacetyl. 2,3-butanediol is the reduced form of acetoin and it has a small contribution to the creamy or buttery flavour of fermented products (Chen et al., 2017; Peyer et al., 2016). Further, acetaldehyde has been characterized as having a strong, “fruity” (green apples) taste with sweet undertones that has been shown to positively contributing to high acceptance of some plant-based alternatives (Peyer et al., 2016). LA, although not usually linked with yogurt's aroma profile, is a crucial component of the overall flavour profile (Pontonio et al., 2020). In LAB, amino acids have a critical function as flavour-forming substrates. Aside from having their own flavour (sweet, bitter, sulfurous, umami), amino acids serve as a substrate for Maillards reactions, which can produce organoleptically active carbonyl compounds, heterocycles, and melanoidins (Peyer et al., 2016).

2.5. UTILIZATION OF POTATO BY-PRODUCTS

Potatoes (*Solanum tuberosum* L.) are a vital crop for feeding the world’s population. They play a major role in the human diet because of their low price, low-fat content, good source of carbohydrates, high-quality proteins, fiber, and vitamins (Kim et al., 2012). It has been estimated that 20-50 % of raw potato products are discharged as waste, which poses a serious disposal concern for the potato industry. As a result, potato wastes have been investigated as a source of acetone, butanol, ethanol, and ethanol production and as a substrate for yeast, vitamin B12, LA, xanthan, and pullulan production by biotechnological methods (Saeedyzadeh et al., 2017; Zhang et al., 2007). Furthermore, potato peel waste has been used as a source of natural antioxidants for slowing down lipid oxidation in mackerel and meat. Potato starch waste has been used in a combination with concentrated red grape for making functional fermented beverages (Saeedyzadeh et al., 2017).

Regardless of their low protein content of less than 2 %, potato proteins have been recognized by some authors to be nutritionally equivalent to lysozyme, egg white protein (Waglay and Karboune, 2016). In comparison to other vegetable proteins, potato proteins are found to be more digestible, soluble, and nourishing. Besides, potato proteins are a source of lysine and have not been considered an allergy-causing food which makes them a decent alternative for preventing wheat allergies (Hussain et al., 2021). Among several associated health benefits of potato proteins, there are also some functional qualities being reported such as emulsification and foaming abilities (Levy et al., 2021; Waglay and Karboune, 2016). As a result, potato

proteins have been recently gaining popularity in the food industry (Hussain et al., 2021). As large amounts of potatoes are used to make potato starch, potato protein isolate is extracted from by-product potato juice after starch removal. This extraction is required to help mitigate both the economic effect as well as high polluting capacity of starch industry by-product (Levy et al., 2021).

2.6. PLANT-BASED YOGURT-LIKE PRODUCT DEVELOPMENT

Plant-based yogurt-like products are typically made by fermenting aqueous extracts from soy, cereals, legumes, oilseeds, etc (Grasso et al., 2020). Although they are processed in a similar way to traditional yogurt-making, their fermentation time is often longer and they have a lower protein content. Additionally, the acidification of plant-based proteins commonly results in a weak gel formation and phase separation which requires the addition of hydrocolloids (Sim et al., 2020). Attempts have been made to solve this obstacle by using novel technologies, namely (ultra) high-pressure homogenization. This technology could increase the protein content and physical stability of yogurt-like products but its influence on the sensory profile is not yet well-understood (Levy et al., 2021). Also, there are only a few studies where a pressure-induced non-soy plant protein-based emulsion gel was created and further compared to dairy yogurts (Sim et al., 2020).

Another common approach in the food industry is using a combination of gelling agents such as natural gums, starches, and proteins to create a gel-type product with a desirable texture. Further, it was shown that the presence of agar and rice starch has an important impact on the rheological properties of yogurt-like products (Grasso et al., 2020). Another study showed that the addition of 2 % kudzu starch and 0.5 % lecithin led to better texture and stability of yogurt-like products during storage. It also didn’t affect the final flavour of the yogurt-like product (Kosterina et al., 2020).

Figure 2 summarized the standard and new biotechnological options in the production of plant-based yogurt-like food product.

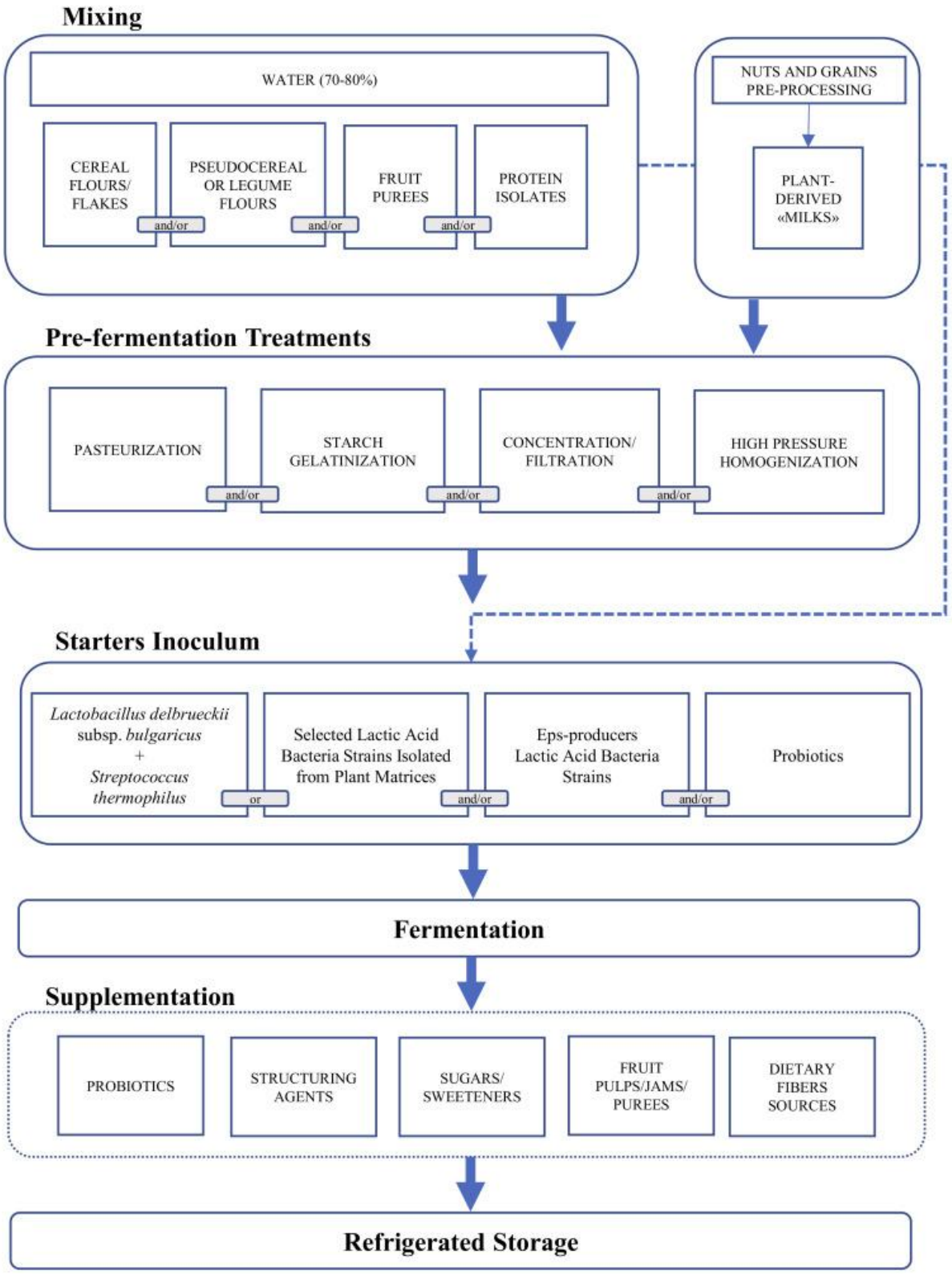


Figure 2. Flow chart of yogurt-like production protocol (Montemurro et al., 2021)

Fermentation time and temperature tends to significantly vary for fermented dairy substitutes. For example, white kidney bean-based beverage was fermented at 37 °C for 24 hours (Kosterina

et al., 2020), chickpea-based beverage was fermented at 42 °C for 16 hours (Wang et al., 2018) and coconut milk beverage was fermented for 48 hours where the optimal temperature was determined to be around 34-37 °C (Mauro and Garcia, 2019). In conventional dairy yogurts, the fermentation of cow milk lasts until the pH lower than 4.5 is reached and the final LAB density is higher than 8 $\log_{10}$ CFU mL^{-1}. Because of the differences in protein and carbohydrate content (lack of lactose), availability in micronutrients and possible inhibiting compounds (such as polyphenols), there are alterations in the fermentation process of dairy substitutes (Montemurro et al., 2021).

There's been several yogurt-like products created where some type of oil was incorporated. For example, in a study creating a potato protein isolate-based yogurt-like product, an emulsion was formed after 3 % of sunflower oil has been incorporated to the pre-treated solution (Levy et al., 2021). Another study analyzed the influence of oil addition on the physical properties of several plant-based yogurts based on different protein sources. Except for mung bean protein and pea protein, where gel strength increased with included oil, all plant protein samples showed no changes in viscoelastic characteristics after a small amount of sunflower oil was added. The authors proposed the difference in protein-protein and protein-lipid interactions among plant proteins as an explanation for these variations in viscoelastic properties (Sim et al., 2020). Other authors suggest the use of bovine milk fat globule in plant-based alternatives since it is an important contributor to the creaminess, texture, and flavour of dairy products. In the end, the fat phase of dairy alternatives is usually either added through different oils or fabrication of fat globules from plant sources (Dhankhar and Kundu, 2021).

One of the simplest, most convenient, and least time-consuming techniques of food preservation is freezing (Foote, 2016). Frozen yogurt is a dairy product available globally and is perceived as a healthier alternative compared to conventional ice cream. It is often produced with low acidity, which is a change from the high acidity of frozen yogurts in the late 1970s (Davidson et al., 2000). It combines the pleasant sensory qualities of a frozen dessert with nutritional advantages such as reduced fat content and the presence of viable (potentially probiotic) bacteria (Skryplonek et al., 2019). However, the freezing process and storage can pose a challenge for the viability of LAB cells since the frozen yogurt environment is not optimal for their survival (Davidson et al., 2000).

Non-dairy alternatives tend to be unstable during both manufacturing and storage. Some of the most common problems are phase separation and spoilage on long-term storage. Since

consumers prefer clean labels, the exclusion of stabilizers is a preferable solution (Dhankhar and Kundu, 2021).

Even though there are many plant-based frozen yogurt-like products on the market, there has been a lack of studies dedicated to this category of non-dairy products. For instance, several non-dairy frozen yogurt-like products by Yogurtland contain traditional yogurt cultures such as *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, but also a mixture of other cultures such as *L. casei*, *L. rhamnosus* and *Bifidobacterium* subsp. Additionally, they contain stabilizers, emulsifiers (vegetable monodiglycerides, guar gum, carrageenan), and caramel color (Fleming, 2020).

3. EXPERIMENTAL PART

3.1. MATERIALS

3.1.1. Instruments

The following instruments were used in this work:

- Convection oven (Rational Combi Steamer, Type CCM, Landsberg am Lech, Germany)
- iCinac instrument (Alliance Instruments, Frepillon, France)
- Autoclave (Systec V-Series, Holm & Halby, Brøndby, Denmark)
- pH meter (SevenCompact™, Mettler Toledo, Columbus, Ohio, USA)
- pH test strips (Macherey-Nagel GmbH & Co.KG , Düren, Germany)
- Magnetic stirrer (Variomag monotherm, Holm & Halby, Brøndby, Denmark)
- Analytical and technical scale (Mettler Toledo, Columbus, Ohio, USA)
- Spectrophotometer (Novaspec Visible, Biochrom, Cambridge, UK)
- Centrifuge (Eppendorf, Hamburg, Germany)
- Optical binocular microscope (Nikon Eclipse, Tokyo, Japan)
- Table-top electronic electronic colony counter (Stuart Digital Colony Counter with IQ/OQ Documents; 90 to 230 V, 50/60 Hz, Cole-Parmer, Vernon Hills, Illinois, USA)
- Laminar flow cabinet
- Fume hood
- Laboratory consumables - red cap and blue cap flasks, beakers, pipettes, pipette tips, inoculation loops, Eppendorf tubes, petri dishes, sterile nitrile gloves, microflex nitrile gloves, forceps, laboratory thermometer, laboratory funnel, graduated glass cylinders, laboratory stainless steel spatulas and spoons, test tubes, test tube racks, microscope glass slides

3.1.2. Microorganisms

A collection of 119 different strains included different strains of LAB and non-LAB, yeast, and fungi. Of these, 65 strains were from DTU strain collection (provided by PhD Claus Heiner Bang-Berthelsen) and selected based on bioinformatics analysis. The other 54 strains belonged to KMC Kartoffelmelcentralen, Denmark (provided by PhD Mathias Greve-Poulsen). The plant-based isolation sources included juice of potato flour which is left in pipes for several months or from the bottom of the ventilation tank, juice of fresh or old pulp of potato flour stored outside, potato on the surface of or inside a rotten potato pile, and damaged seed potatoes.

The strains were stored at -80 °C in a preferred nutrient broth medium supplemented with 30 % or 15 % glycerol (v/v). All strains used in this thesis are shown in the Supplements.

3.1.3. Chemicals

The chemicals used were purchased from Sigma-Aldrich Corporation (St. Louis, Missouri, USA), Oxoid Inc (Ogdensburg, New York, USA), Merck & Co. (Kenilworth, New Jersey, USA), BD Biosciences (San Jose, California, USA) and SSI Diagnostica (Hillerød, Denmark). Table 1 includes all chemicals and media used in this thesis and table 2 contains the composition of potato protein concentrate used (Protafy).

Table 1. List of chemicals and media used

Name	**Purity**	**Producer**
Magnesium sulfate heptahydrate	99.5 %	Merck
D-(+)-Glucose	≥ 99.5 %	Sigma-Aldrich
Manganese (II) sulfate hydrate	≥ 99.99 %	Sigma-Aldrich
di-Potassium hydrogen phosphate	≥ 99.0 %	Merck
Agar bacteriological (no. 1)	Purified	Oxoid
Tween Tween® 80 – Polysorbate 80	Oleic acid ≥ 58.0% (primarily linoleic, palmitic, and stearic acids)	Sigma-Aldrich
Sodium acetate	≥ 99.0 %	Sigma-Aldrich
D-(+)-Maltose monohydrate (from potato)	≥ 99.0 %	Sigma-Aldrich
D(+)-Saccharose (Sucrose)	≥ 99.5 %	Sigma-Aldrich
Starch, soluble		Sigma-Aldrich
BD Bacto™ yeast extract		BD Biosciences
Bacto™ Proteose Peptone		BD Biosciences
Bacto™ Tryptone		BD Biosciences
Sodium hydroxide		
Hydrochloric acid		
MRS medium (broth, agar)		SSI Diagnostica
M17 medium (broth, agar)		SSI Diagnostica
Blood medium (agar)		SSI Diagnostica
LB medium (agar)		SSI Diagnostica

Table 2. Composition of potato protein concentrate (**Protafy**)

Compounds	**Protafy**
Energy	1575 kJ / 371 kcal
Total fat / of which saturated	2.4 g / 1.6 g
Carbohydrates /of which sugars	1.8 g / 0 g
Dietary fiber	6.4 g
Protein	83 g
Salt	0.1 g

Protafy (KMC, Brande, Denmark) is a food-grade potato protein concentrate from heat/acid precipitation. **KMC Food** (KMC, Brande, Denmark) is a food-grade potato protein concentrate from non-denaturing extraction that has a protein content of 85 % (Kjeldahl-N x 6.25). A more detailed composition is currently unavailable.

Food-grade ingredients used to make final products were produced by Mamone (Denmark) and they include:

- Potato flour that contains potato starch as sole ingredient (Kartoffelmel)
- White sugar (Sukker)
- Baking powder (Natron)

3.2. METHODS

3.2.1. Whole genome sequencing (WGS)

WGS was performed for strain identification and to compare the results with the results of Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identification. The sequences were assembled and the contigs of each strain were stored in a FASTA database format. The sequences were annotated using RAST and stored in a GenBank format. *gyrB* and *dnaA* genes were used as phylogenetic markers. A phylogenetic tree showing the phylogenetic distances between the strains based on gyrase beta subunit was created. The phylogenetic tree included the matching database sequences. Only the most important results are shown.

3.2.2. Experimental media development and strain selection

Overall, 15 different experimental cultivation media were developed to select the most promising strains and to optimize the composition of food matrixes used for fermented product development. For the purpose of strain selection, the developed media contained standard MRS/M17 components such as yeast extract, sodium acetate, tween-80, di-potassium hydrogen phosphate, and minerals, together with a modified primary protein and/or carbon source in optimized amounts. The most promising strains were considered to be those capable of using potato protein as a primary source of nitrogen and potentially starch as a source of carbon. Growth was monitored on agar plates after 48 h incubation at 30 °C, determining the CFU mL^{-1} for selected strains. For food product development, a literature review was done by using EFSA's QPS database to determine strains safely applied in food products. Acidification rates for certain strains were recorded in experimental broth media. For the purpose of food matrix optimization, food-grade ingredients and their content was determined. Only the most important results are shown.

*3.2.2.1. Development of Potato protein glucose (**Non food-grade**) complex media + Control medium*

All of the components, excluding agar, were weighted in a total of 1 L volume of Mili-Q water and stirred using a magnetic stirrer. After the pH was adjusted to 7.4 with NaOH and HCl, 500

mL of media were placed in 1 L glass bottles because KMC Food 3.0 showed foaming properties, particularly when heated. After autoclaving, the media were stored at 4 °C in the cold room. Before using agar media to make plates, the media were heated for 45 minutes at 95-100 °C. They were then put in a water bath for 30 minutes to cool down to around 45 °C before use.

Both agar and broth media were created. After optimizing the media composition, the components of the developed media are shown in table 3.

Table 3. Composition of modified cultivation media with potato protein concentrate as the primary nitrogen source and composition of control media. Additionally, agar media were created containing 15 g L^{-1} of agar

	Protafy 1	**KMC Food 3.0**	**Potato control**
Medium composition (per L)	broth medium	broth medium	broth medium
Glucose solution 20 % (sterile filtered)	50 mL	50 mL	50 mL
Potato protein concentrate	25 g Protafy	25 g KMC Food	/
Yeast extract	0.025 g	0.025 g	0.025 g
Sodium acetate	1 g	1 g	1 g
Tween-80	1 mL	1 mL	1 mL
Dipotassium hydrogen phosphate	2 g	2 g	2 g
Magnesium sulfate heptahydrate	0.2 g	0.2 g	0.2 g
Manganese sulfate monohydrate	0.05 g	0.05 g	0.05 g
pH before autoclaving	7.4	7.4	7.4

*3.2.2.2. Development of Peptone starch (**Non food-grade**) complex media + Control medium*

Peptone starch medium, Mili-Q water was heated to around 50 °C before adding starch and stirred with a magnetic stirrer until it was dissolved. The rest of the components (excluding agar) were put in a 1 L glass cylinder containing Mili-Q water. The pH of Peptone starch medium, was adjusted with NaOH and HCl only after the solution reached room temperature. The pH was adjusted to 7.4 and media were put in 1 L glass bottles that contained agar and autoclaved. After autoclaving, the media were stored at 4 °C in the cold room.

Before using, Peptone starch media were heated for 135 minutes at 95-100 °C and Peptone glucose media were heated for 45 minutes at 95-100 °C. Peptone starch media were also shaken every 45 minutes to dissolve the starch. They were then put in a water bath for 30 minutes to cool down to around 45 °C before use. Composition of the developed media is shown in table 4.

Table 4. Composition of the experimental media used to create agar plates and determine if selected strains can be utilized in modified cultivation media with carbon source being primarily starch compared to the carbon source being primarily glucose (used as control)

	Peptone starch	**Peptone glucose**
Medium composition (per L)	agar medium	agar medium
Glucose solution 20 % (sterile filtered)	/	50 mL
Starch	20 g	/
Bacto Proteose Peptone	10 g	10 g
Bacto Tryptone	10 g	10 g
Yeast extract	0.025 g	0.025 g
Sodium acetate	1 g	1 g
Tween-80	1 mL	1 mL
Dipotassium hydrogen phosphate	2 g	2 g
Magnesium sulfate heptahydrate	0.2 g	0.2 g
Manganese sulfate monohydrate	0.05 g	0.05 g
Agar	15 g	15 g
pH before autoclaving	7.4	7.4

*3.2.2.3. Development of Peptone maltose and Peptone sucrose (**Non food-grade**) complex media*

All of the components (exlcuding agar) were put in a 1 L glass cylinder containing Mili-Q water. All media were stirred using a magnetic stirrer. The pH was adjusted with NaOH and HCl to 7.4 and media were put in 1 L glass bottles that contained agara and then autoclaved. After autoclaving, the media were stored at 4 °C. Composition of the developed media is shown in table 5.

Table 5. Composition of the experimental media used to create agar plates and determine alternative carbon sources that selected strains can utilize in a modified cultivation media

	Peptone maltose	**Peptone sucrose**
Medium composition (per L)	agar medium	agar medium
Maltose monohydrate	10.45 g	/
Sucrose	/	10 g
Bacto Proteose Peptone	10 g	10 g
Bacto Tryptone	10 g	10 g
Yeast extract	0.025 g	0.025 g
Sodium acetate	1 g	1 g
Tween-80	1 mL	1 mL
Dipotassium hydrogen phosphate	2 g	2 g
Magnesium sulfate heptahydrate	0.2 g	0.2 g
Manganese sulfate monohydrate	0.05 g	0.05 g
Agar	15 g	15 g
pH before autoclaving	7.4	7.4

*3.2.2.4. Development of Potato protein starch (**Non food-grade**) simple media*

Mili-Q water was heated to around 50 °C before adding starch. Starch was added slowly into the heated water and stirred with a magnetic stirrer until it was dissolved. The rest of the components were put in a 1 L glass cylinder containing Mili-Q water. They were stirred using a magnetic stirrer, KMC Food starch media also required additional manual stirring to dissolve. pH was adjusted only after the solution reached room temperature. The pH was adjusted to 7.4 and 500 mL of media were put in 1 L glass bottles because KMC Food starch medium showed foaming properties, particularly when heated. These were then autoclaved. After autoclaving, the media were stored at 4 °C.

Composition of the developed media is shown in table 6.

Table 6. Composition of the experimental media used to determine acidification rates in modified cultivation media containing solely potato protein as protein source and starch as carbon source

	Protafy starch	**KMC Food starch**
Medium composition (per L)	broth medium	broth medium
Protafy	50 g	/
KMC Food	/	50 g
Starch	20 g	20 g
pH before autoclaving	7.4	7.4

*3.2.2.5. Development of Potato protein starch sucrose (**Non food-grade**) simple media*

Mili-Q water was heated to around 50 °C before adding starch. Starch was added slowly into the heated water and stirred with a magnetic stirrer until it was dissolved. The rest of the components were put in a 1 L glass cylinder containing Mili-Q water. They were stirred using a magnetic stirrer, KMC Food starch media also required additional manual stirring to dissolve. pH was adjusted only after the solution reached room temperature. The pH was adjusted to 7.4 with NaOH and HCl. Afterwards, 500 mL of media were put in 1 L glass bottles because KMC Food starch medium showed foaming properties, particularly when heated. These were then autoclaved. After autoclaving, the media were stored at 4 °C in the cold room.
Before using, both media were heated to 95-100 °C for around 1 hour and shaken. KMC Food starch media had to be additionally heated for another hour, stirred and shaken to dissolve.
Composition of the developed media is shown in table 7.

Table 7. Composition of the experimental media used to determine acidification rates in modified cultivation media containing solely potato protein as protein source and starch with the addition of sucrose as carbon sources

	Protafy starch sucrose	**KMC Food starch sucrose**
Medium composition (per L)	broth medium	broth medium
Protafy	50 g	/
KMC Food	/	50 g
Starch	20 g	20 g
Sucrose	10 g	10 g
pH before autoclaving	7.4	7.4

*3.2.2.6. Development of food matrixes (**Food-grade**)*

Food matrixes were created with utensils and glassware that are exclusively used for sensory analysis. Tap water was heated to around 50 °C before adding starch. Starch was added slowly into the heated water while being stirred with a stirring rod until it dissolved. Afterwards, other components were added with additional stirring. 500 mL of food matrixes were put in 1 L glass bottles because KMC Food showed foaming properties, particularly when heated. These were then autoclaved. After autoclaving, the food matrixes were stored at 4 °C in the cold room. Before use, both media were heated to 100 °C for around 1 hour and shaken. KMC Food matrix had to be additionally heated for another hour, stirred and shaken to dissolve. Afterwards, their pH was adjusted using baking soda with pH test strips to be approximately 6.5. Composition of the developed food matrixes is shown in table 8.

Table 8. Composition of the experimental food matrixes used to create novel fermented yogurt-like food products

Composition (per L)	Protafy food matrix	KMC Food food matrix
Protafy	50 g	/
KMC Food	/	50 g
Starch	20 g	20 g
Sugar	10 g	10 g

To check for presence of yeast contamination in the fermented KMC Food matrix, LB and blood agar plates were inoculated with a loopful of the food matrix fermented by a mixed culture of *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) and *Lc. lactis* subsp. *lactis* (16.1 M17 RT) using a four-way streaking technique. Inoculated agar plates were incubated at 30 °C for 48 hours to check the yeast growth. Additionally, decimal dilutions (10^{-9}, 10^{-8}, 10^{-7}) were made and the presence of the yeast cells was checked with an optical microscope.

3.2.3. Fermentation conditions for **non food-grade** samples

Fermentation was conducted in several non food-grade media, including Potato protein glucose complex media (Protafy 1 and KMC Food 3.0), Potato protein starch simple media and Potato protein starch sucrose simple media. Overnight bacterial culture was inoculated in MRS/M17 + 0.5 % glucose media and incubated at 30 °C for 24 hours. The bacterial cells were centrifuged

for 15 minutes at 3220 x g at 4 °C and washed with a physiological solution (0.9 % NaCl) twice. Cell growth was monitored by measuring the optical density at 600 nm (OD600). The samples were inoculated with 1 % of the microbial culture. Fermentation was carried out at 30 °C in different intervals. Acidification of the samples was followed with an iCinac instrument to measure the pH, oxidation-reduction potential and temperature at the same time points, continuously for every minute. Initially, iCinac probes were disinfected using 70 % ethanol alcohol and calibrated using buffers of pH 4 and 7. Probes were inserted into the samples before fermentation took place.

3.2.4. Fermentation conditions for **food-grade** samples

Additionally, fermentation was conducted in the developed food matrixes. 1 % of inoculum was propagated in Protafy and KMC Food matrixes at 30 °C for 24 hours. Fermentation was carried out at 30 °C for 41 hours in a convection oven. pH was previously measured with iCinac for 41 hours. At the end of fermentation, the samples were cooled down to room temperature and thereafter stored in a freezer for approximately two weeks before the sensory analysis.

3.2.5. Sensory analysis

As textural properties in novel food categories have a crucial role in consumer acceptance, sensory analysis of potato-based yogurt-like products was performed. Preliminary sensory analysis of 4 fermented Protafy-based (KMC) products (P1- P8) was performed by a panel of 10 trained assessors. The samples were evaluated in duplicates, in a randomized order. Samples were kept at -30 °C for 2 weeks before the analysis took place and thawed on the day of the tasting. After defrosting, they were kept at 10 °C until serving. The first sessions were used to develop a series of words for attributes to describe the sensory characteristics describing the odour of the samples.

The sensory laboratory fulfilled the national standards and guidelines for the design and construction of sensory assessment rooms (ISO 8589, 2010; ISO 8586, 2014; NMKL Procedure No. 6, 2016). Noise levels were kept to a minimum; lighting was real daylight according to ISO standard (ISO 8589, 2010) with an intensity of 600 – 1500 LUX m^{-2}; the room was maintained odour-free by higher air pressure in the room than outside so the airflow is from inside the room and out. The temperature was kept constant at 20 °C. All equipment used for the sensory analysis was kept absolutely free of any odour or taste. All panel members had passed a test of

their capability to use their senses and to express the response. According to ISO 5496, 2006; ISO 8586, 2014; ISO 3972, 2001, and ISO/CD 13300, 2006, the tests contained training in detection and recognition of tastes and odours, sensitivity, ranking and/or triangle tests of basic tastes, odour, texture, and appearances tests, as well as scale and product training.

4. RESULTS AND DISCUSSION

4.1. DEVELOPMENT OF EXPERIMENTAL MEDIA

There is a constant global need to reorient the food system in a more sustainable direction. This has driven efforts to exploit alternative non-animal sources of food protein and functional food ingredients such as potato-derived peptides (Yesiltas et al., 2021). Therefore, the purpose of this thesis was to develop potato protein-based yogurt-like products to be applied as an alternative to dairy yogurts. The final goal is to contribute to the efforts to reinforce the use of potatoes as a sustainable food source.

To this date, only a few studies developed potato-based food products that would serve as fermented dairy alternatives (Levy et al., 2021; Saeedyzadeh et al., 2017). In this context, only a few studies used non-starter LAB strains isolated from plant-based sources to develop novel products (Bansal et al., 2016). Initially, to develop novel yogurt-like products, the most promising mono or mixed cultures had to be selected through the optimization of an appropriate nutrient medium. 119 different microbial strains, mostly belonging to LAB species, were screened in this thesis. To be able to select these strains and characterize their nutritional requirements, several experimental media had to be designed. For designing an optimal production medium, the most suitable fermentation conditions such as pH, temperature, agitation speed, and the appropriate medium components including carbon and nitrogen sources were defined and optimized accordingly. Therefore, after strain selection, a yogurt-like production protocol had to be created which included the duration of fermentation, temperature during fermentation, method of storing the products, ingredients, and quantities of ingredients used. Finally, challenges regarding potential yeast contamination and taxonomy had to be resolved. This also considered preliminary sensory analysis of developed products by a trained sensory panel. Due to a high number of strains tested in this thesis, the results related to the most promising strains are presented. Finally 4 different strains: *Lc. lactis* subsp. *cremoris* (16.3 MRS 30), *Lc. lactis* subsp. *lactis* (16.1 M17 RT), *Leuc. pseudomesenteroides* (96), and *Leuc. mesenteroides* subsp. *mesenteroides* (2.2 M17 30) were selected based on the use of potato protein as the sole nitrogen source and considering their QPS status.

A total of 15 different experimental cultivation media were developed. When analyzing the utilization of potato protein, complex experimental media contained only 1 % of glucose as the main carbohydrate source, as opposed to 2 % of glucose commonly included in MRS medium or experimental media with adjusted nitrogen content (Pathak and Martirosyan, 2012). However, a lower amount was successfully utilized in other studies (Djeghri-Hocine et al., 2006). Furthermore, when analyzing the utilization of alternative carbon sources such as maltose and sucrose, the same percentage was used to make it comparable. But when analyzing the utilization of starch as a carbon source, 2 % was used due to a lower conversion rate of starch by most microorganisms used in this thesis. The amounts of magnesium sulfate, dipotassium hydrogen phosphate, and tween 80 was as same as contained in most commercial MRS media (Hayek et al., 2019). A slightly higher amount of manganese sulfate was used since it has been reported to stimulate growth of certain LAB, mainly heterofermentative lactobacilli (Rabe and Hillier, 2003). The amount of sodium acetate was lowered from the standard 5 to only 1 g L^{-1} as it can negatively influence LA production (Klongklaew et al., 2021).

Additionally, the amount of yeast extract was significantly lowered to only 0.025 g from the standard 4-5 g L^{-1}. This was done since yeast extract is one of the main sources of essential vitamins, but also most commonly used source of nitrogen in LA fermentation process (Michalczyk et al., 2021). Thus, the amount of yeast extract was significantly minimized to promote the use of potato protein as a nitrogen source, but a very low amount was still left to be a source of necessary vitamins. The appereance of developed experimental media agar plates is shown in figure 3, while developed experimental media with examples after fermentation was conducted are shown at figure 4. Before autoclaving, pH was adjusted to 7.4. This was done because the high temperatures of autoclaving would cause a pH drop which would make the final pH approximately 6.5. This value was very similar to the final pH of commercial MRS broths (Hayek et al., 2019). However, pH level at and/or lower than 6.0 would not have been accepted due to the observed limitations of LA production at lower pH levels (Smerilli et al., 2015).

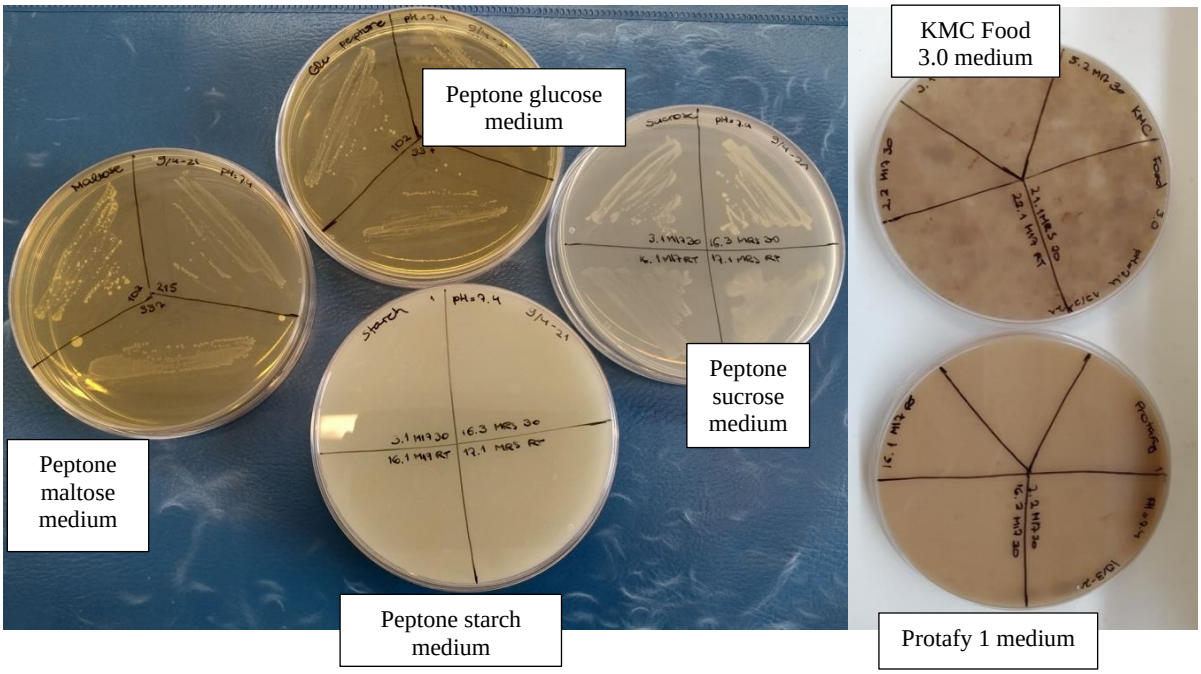


Figure 3. Developed nutrient agar media inoculated with several strains including *Leuc. pseudomesenteroides* (102), *Lc. lactis* subsp. *lactis* (215), *Lc. lactis* subsp. *lactis* (337), *Leuc. mesenteroides* subsp. *mesenteroides* (3.1 M17 30), *Lc. lactis* subsp. *cremoris* (16.3 MRS 30), *Lc. lactis* subsp. *lactis* (16.1 M17 RT), *Leuc. mesenteroides* subsp. *mesenteroides* (17.1 MRS RT), *Leuc. mesenteroides* subsp. *mesenteroides* (2.2 M17 30), *Leuc. mesenteroides* subsp. *mesenteroides* (21.1 MRS 30), *L. plantarum* (22.1 M17 RT) and *Leuc. pseudomesenteroides* (5.2 M17 30)

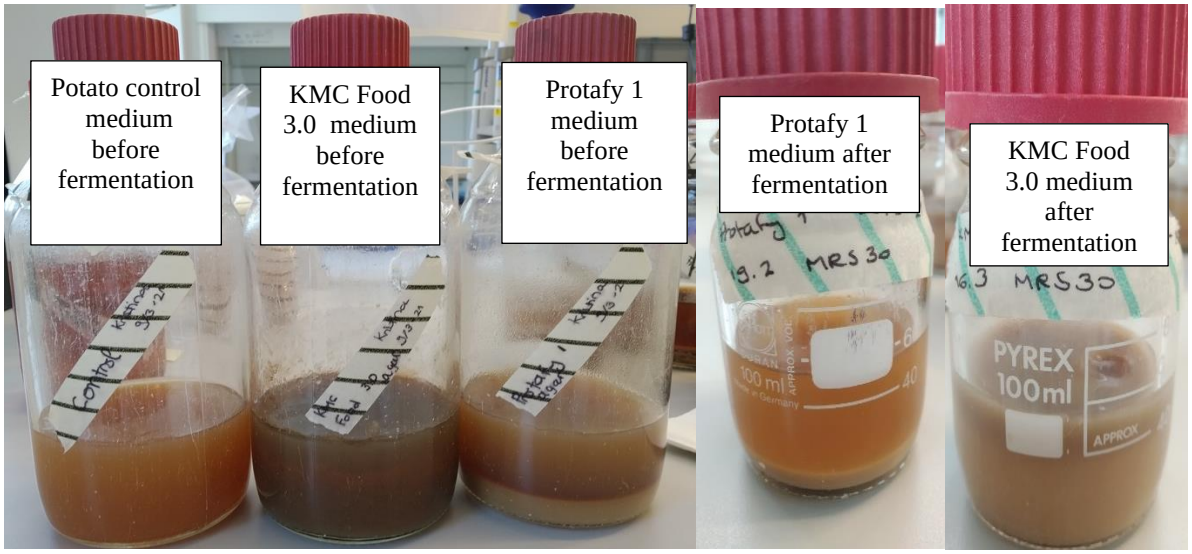


Figure 4. Developed media after autoclaving and before fermentation (the right side of figure) and the observed difference in texture and colour between KMC Food 3.0 and Protafy 1 media after fermentation (the left side of figure)

4.2. WGS

MALDI-TOF MS is a sophisticated analytic technique often used for the microbial identification (Rychert, 2019). However, inherent similarities between microorganisms and a limited number of spectra in the database can lead to poor discrimination between species resulting in misidentification. This omission can be overwhelmed with additional testing. Since genus and/or species of several strains used in this thesis were incorrectly identified by this technique, WGS analysis was used for their reclassification. Moreover, WGS can be used for phylogenetic tree analysis. There is an advantage of using this technique since large numbers of genetic characteristics spread throughout whole genomes can create very accurate phylogenies, especially for closely related clonal populations (He, 2015). A phylogenetic tree of several strains used in this thesis is shown in figure 5.

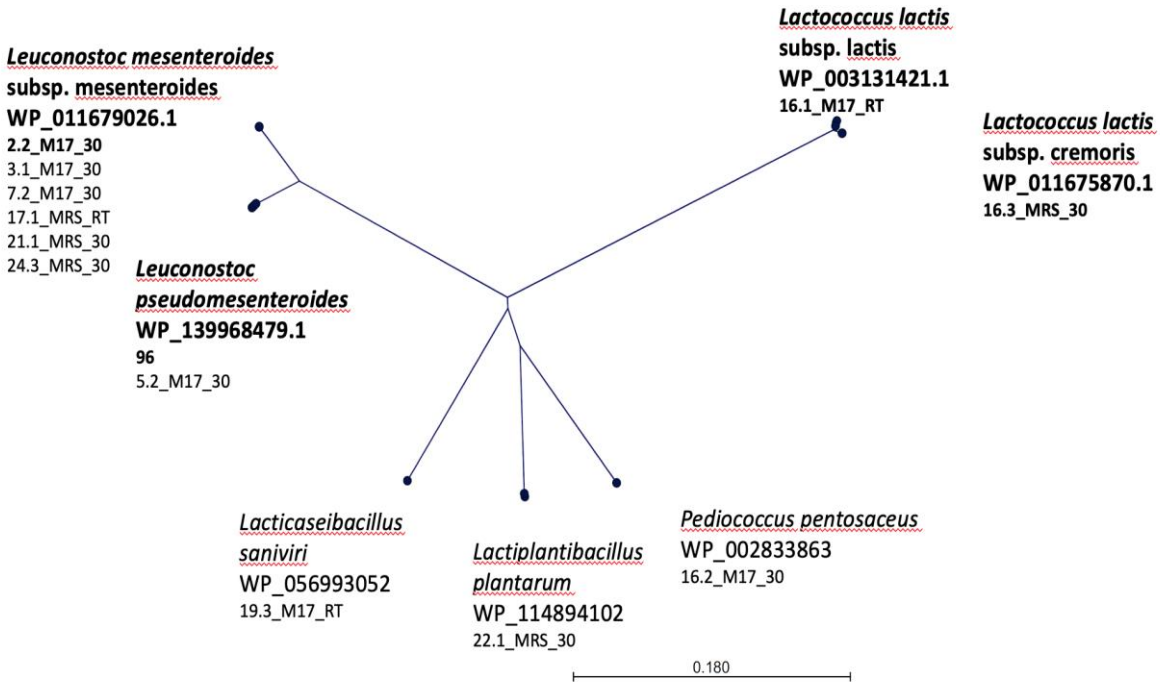


Figure 5. A phylogenetic tree showing the phylogenetic distances between the strains based on gyrase beta subunit with the bolded strains used in the final food product development

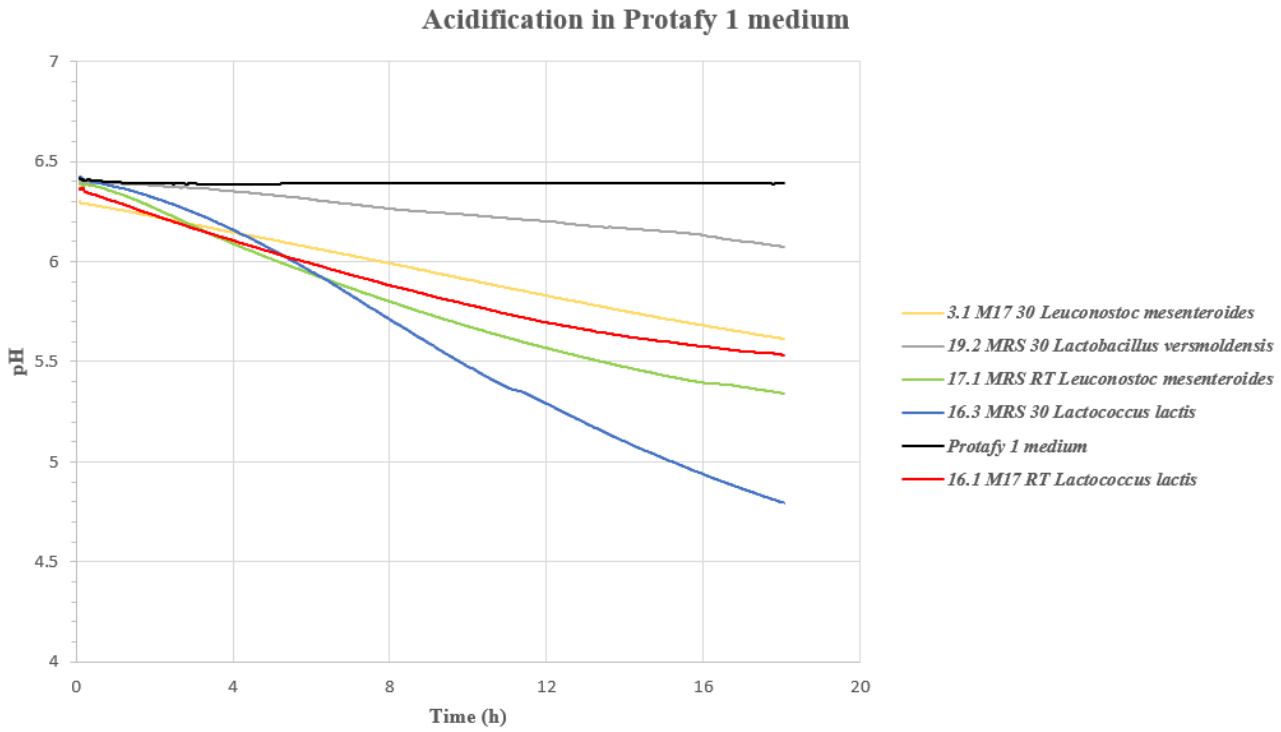
4.3. STRAIN SELECTION

Although initially some yeast and fungi strains were intended to be analyzed for food product development, the microbial collection included 119 strains, mostly from LAB genera. The main selection criterion was that the strain was granted the QPS status in order to be included in the sensory testing of developed products. However, not all studies that previously used microorganisms in food product development followed this criterion. For example, peanut-based yogurt-like product was developed and tested by a sensory panel using *E. faecalis*, a strain that has yet to be granted the QPS status (Bansal et al., 2016). To use such a strain, a producer would have to do a safety evaluation before applying it to food products (Herody et al., 2010).

In general, the ability to transform raw materials into lactic acid rapidly and effectively with low nutritional requirements is an important trait for an industrial LAB starter cultures. This is partially because rapid acidification of the substrate limits the undesirable microorganisms that can cause product spoilage. Moreover, it is necessary for the final aroma, texture, and flavour of the product (Akabanda et al., 2014). However, acidification and fermentation of plant proteins commonly lead to protein destabilization which results in weak gel formation and phase separation during storage (Montemurro et al., 2021). Still, as mentioned before, the optimization of texture is usually solved by structuring agents or by different processing methods. Finally, the starter cultures chosen for fermented dairy alternatives production besides providing rapid acidification that also prevents contamination, should carry out sufficient proteolysis that contributes to nutritional and sensory qualities, potentially enhance texture through EPS production, and survive at high cell density in storage conditions (Montemurro et al., 2021). After monitoring of the growth in developed medium and elimination of the strains without QPS status the microbial collection was reduced to 12 strains. Thus, experiments were conducted to analyze acidification rates of selected strains including *Leuc. mesenteroides* subsp. *mesenteroides* (3.1 M17 30), *Lc. lactis* subsp. *cremoris* (16.3 MRS 30), *Lc. lactis* subsp. *lactis* (16.1 M17 RT), *Leuc. mesenteroides* subsp. *mesenteroides* (17.1 MRS RT), *Leuc. mesenteroides* subsp. *mesenteroides* (2.2 M17 30), *Leuc. mesenteroides* subsp. *mesenteroides* (21.1 MRS 30), *L. plantarum* (22.1 M17 RT), *Leuc. pseudomesenteroides* (5.2 M17 30), *L.*

versmoldensis (19.2 MRS 30), *Leuc. pseudomesenteroides* (96), *P. pentosaceus* (16.2 M17 30), and *Lc. lactis* (215) (figure 6-figure 9).

a)



b)

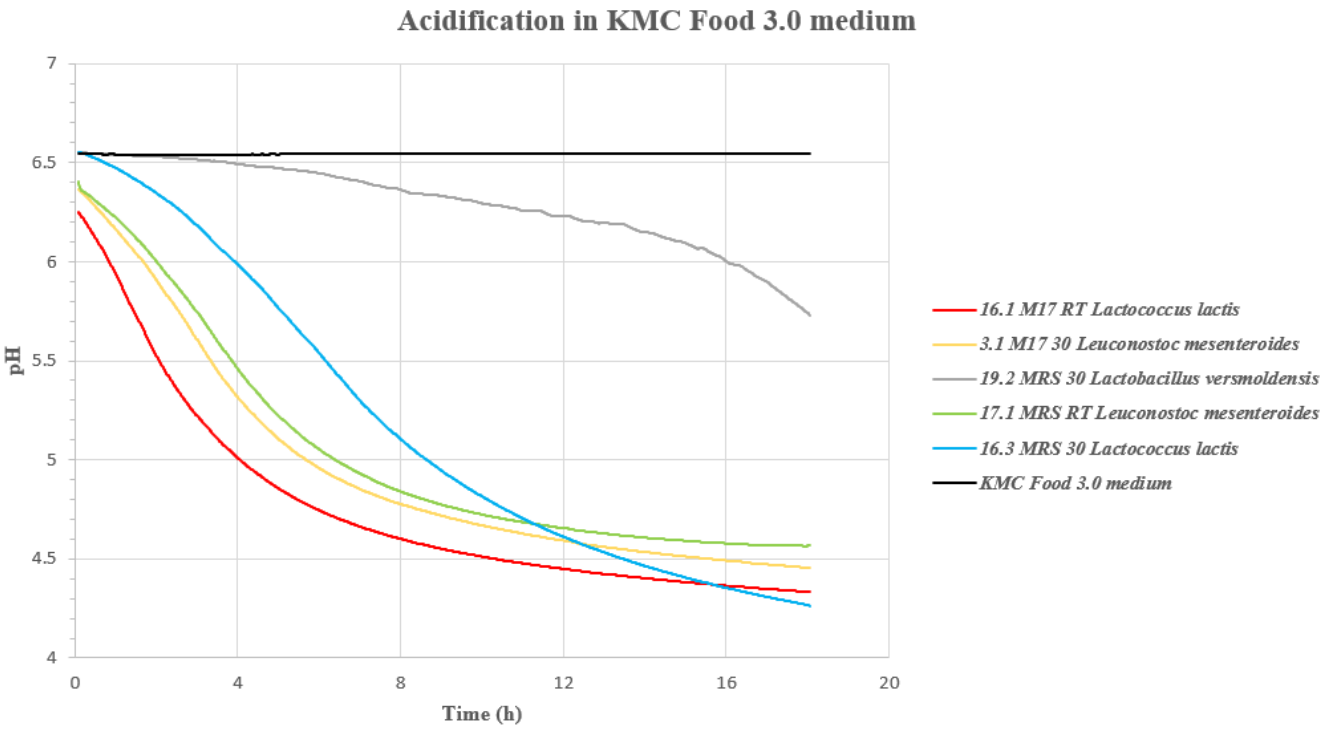


Figure 6. Acidification rates of different LAB strains in **a)** Protafy 1 medium **b)** in KMC Food 3.0 medium during the period of 18 hours at 30 °C

Although Protafy 1 and KMC Food 3.0 media only differ in potato protein concentrate used, the acidification rates of strains tested are notably different (figure 6). After growth in KMC Food 3.0 medium, most strains have reached pH levels below or approximately 4.5 after 18 hours with some of them seemed to have reached a plateau. Strains in Protafy 1 medium have not reached the levels of 4.5 and were still acidifying even after 18 hours. The promising result was that the appearance of KMC Food 3.0 medium after fermentation was noticed to be yogurt-like and smooth, but the consistency of Protafy 1 medium after fermentation could be described as two separated phases with visible precipitation. Similarly, Levy et al. (2021) used potato protein extract to develop a dairy-free yogurt-like fermented product. In their study, fermentation was conducted for 16 hours at 37 °C by yogurt LAB starter cultures which resulted in pH values 4.44 ± 0.07. Althought shortened fermentation time compared to the results of this work, Levy et al. (2021) used a potato protein isolate (90.5 % protein content), commercial yogurt LAB starter cultures, a higher temperature, and different processing methods. Still, their results are somewhat similar to the acidification rates of selected strains achieved in KMC Food 3.0.

In parallel, the strain growth was monitored by the determination of CFU mL^{-1} (table 9) and measurement of OD600 nm. OD600 nm of overnight cultures prepared with MRS or M17 media supplemented with 0.5 % glucose was measured before every fermentation experiment. These measurements were used as a confirmation of growth in MRS/M17 media and were not indicative of growth in experimental media which is why they were not included.

Table 9. Bacterial count expressed as CFU mL^{-1} (±SD) for selected LAB strains in Protafy 1 and KMC Food 3.0 media after 48 hours of incubation at 30 °C

LAB strain	Protafy 1 medium	KMC Food 3.0 medium	Potato control medium
	CFU mL^{-1} (±SD)		
***Leuc. mesenteroides* subsp. *mesenteroides* (3.1 M17 30)**	3 x 10^7 (±0.8165)	6.1 x 10^7 (±0.7454)	No growth
***Lc. lactis* subsp. *cremoris* (16.3 MRS 30)**	3 x 10^8 (±0.8292)	2.12 x 10^7 (±0.7483)	No growth
***Leuc. mesenteroides* subsp. *mesenteroides* (17.1 MRS RT)**	1.58 x 10^8 (±0.4714)	3.7 x 10^7 (±0.3727)	No growth
***Lc. lactis* subsp. *lactis* (16.1 M17 RT)**	8.2 x 10^7 (±0.4330)	4.4 x 10^7 (±0.8806)	No growth
L. versmoldensis* (19.2 MRS 30)	9.2 x 10^7 (±0.8660)	6.2 x 10^7 (±0.7638)	No growth

*Strain identification was determined by MALDI-TOF MS

As was to be expected, *Leuc. mesenteroides* subsp. *mesenteroides* (3.1 M17 30) and *Lc. lactis* subsp. *lactis* (16.1 M17 RT) strains showed modest growth on Protafy 1 and KMC Food 3.0 agar plates (10^7 CFU mL^{-1}) (table 9). Also, these strains showed slow acidification rates in Protafy 1 medium since the minimal pH level after 18 hours of acidifying reached by *Leuc. mesenteroides* subsp. *mesenteroides* (3.1 M17 30) and *Lc. lactis* subsp. *lactis* (16.1 M17 RT) were 5.61 and 5.53, respectively (figure 6). After all, strains used are non-starter LAB isolated from plant-based sources and potato protein concentrate has not been confirmed as an adequate LAB nitrogen source. However, certain strains such as *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) have been shown as promising strains to be used in potato protein-based fermented foods based on their acidification rates in both Protafy 1 (pH = 4.79) and KMC Food 3.0 medium (pH = 4.26) after 18 hours of acidifying (figure 6) together with their CFU mL^{-1} count (10^8 in Protafy 1 medium) (table 9). A study investigating *Lc. lactis* metabolism and gene expression during growth on plant tissues has shown that certain strains are well-adapted for growth on plant materials and have particular characteristics that are important for plant-based food and feed fermentations (Golomb and Marco, 2015). According to comparative genomics research, *Lc. lactis* species actually evolved in connection with plants, and individuals of this species adapted to the dairy niche as a result of anthropogenic pressures. Additionally, plant-isolated *Lc. lactis* have a higher genetic potential than their dairy-isolated counterparts, as evidenced by traits such as the ability to utilize a wider range of carbohydrates, particularly those derived from plants,

increased biosynthetic capacity for nonribosomal peptides, and fewer amino acid auxotrophies (Siezen et al., 2011).

Interestingly, strain *L. vermoldensis* (19.2 MRS 30) has shown acceptable growth in both Profaty 1 and KMC Food 3.0 agar plates as shown in table 9 (10^7 CFU mL^{-1}) but poor acidification rate in both Protafy 1 (pH = 6.07) and KMC Food 3.0 medium (pH = 5.72) after 18 hours of acidifying (figure 6). However, WGS afterwards revealed that the strain was incorrectly identified by MALDI-TOF ID, and since its taxonomic identification was not confirmed it was eliminated from further studies.

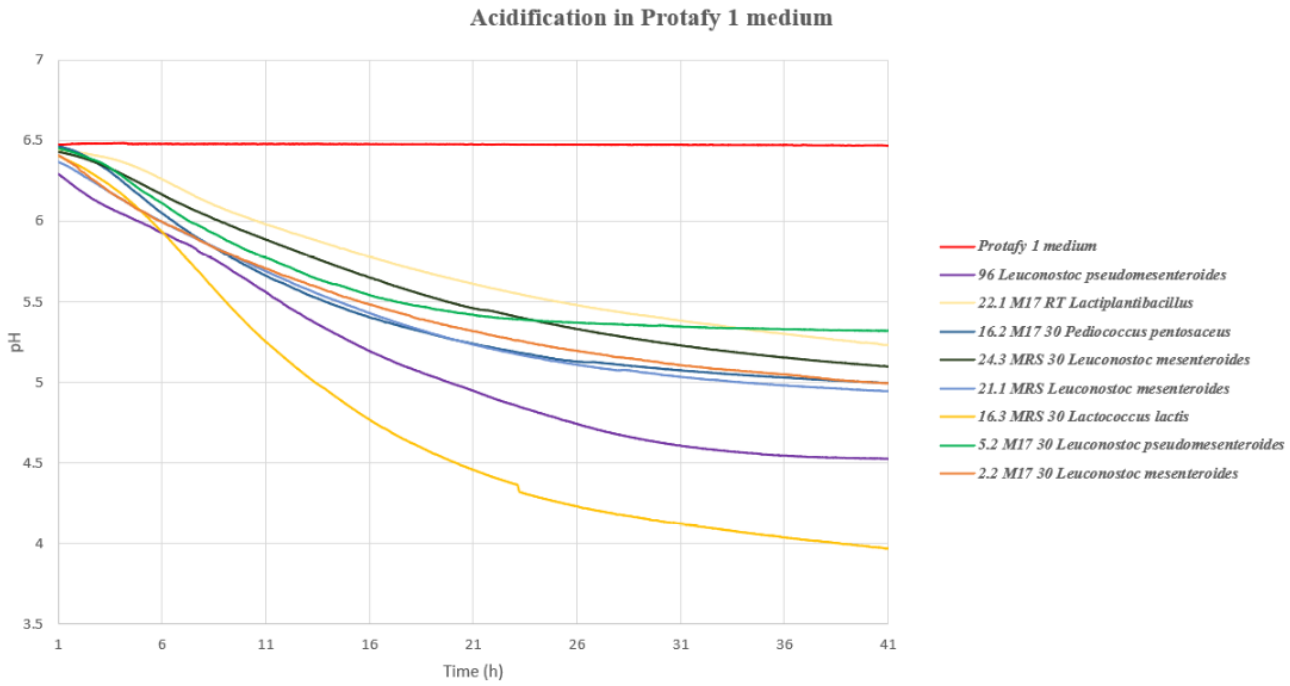
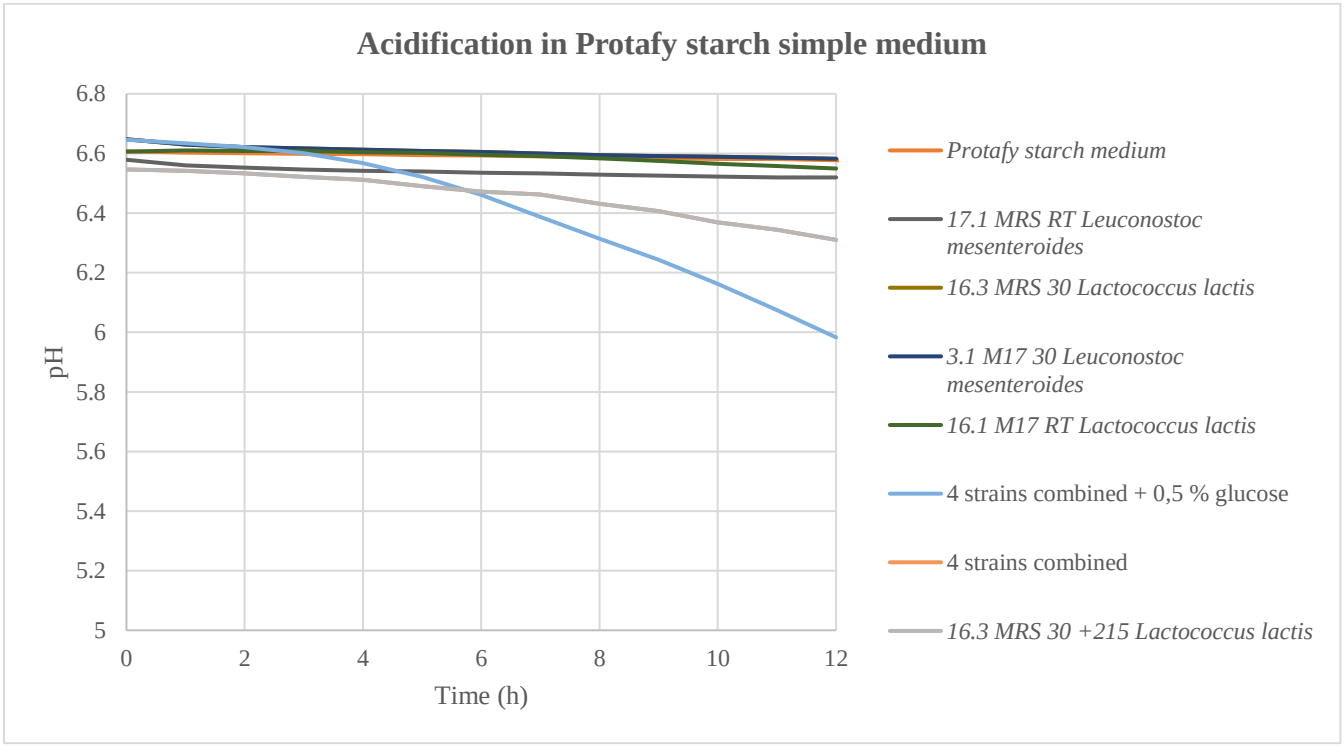


Figure 7. Acidification rates of certain strains in Protafy 1 medium determined with iCinac instrument during the 41 hours at 30 °C

To reach the similar acidification rates as after inoculation in KMC Food 3.0 medium, strains inoculated in Protafy 1 needed a longer incubation time of 41 hours of fermentation at 30 °C to reach pH levels of 4.52 and 3.93, respectively. These levels were achieved only by 2 strains tested, *L. pseudomesentoeroides* (96) and *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) (figure 7). The potato protein concentrate Protafy is a form of an insoluble potato protein concentrate which could explain the precipitation and a slower acidification rate since it might be more difficult to utilize as a nitrogen source. Potato protein is often extracted by heat/acid precipitation, which causes protein denaturation. Moreover, Protafy is a protein extract from

heat/acid precipitation, while KMC Food potato protein concentrate is a protein extract from non-denaturing extraction. It was reported that the protein content in both raw potato fruit juice and non-denatured protein extract was significantly higher than what was shown for heat/acid extracted protein. However, potato protein fractions called patatins are found to be significantly enriched in the heat/acid-precipitated protein extracts compared to non-denatured protein extracts (García-Moreno et al., 2020). Nevertheless, patatin proteins coagulate in an acidic environment and have a low denaturation temperature (50–55 °C). Additionally, the application of thermally coagulated potato proteins in food manufacturing is limited, due to their poor solubility, at times excessive glycoalkaloids concentrations, and undesirable organoleptic properties (Pęksa and Miedzianka, 2021). Still, more research has to be done on potato protein utilization by different LAB.

a)


Acidification in Protafy starch simple medium
pH
Time (h)
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
5.2
5
0
2
4
6
8
10
12
Protafy starch medium
17.1 MRS RT Leuconostoc mesenteroides
16.3 MRS 30 Lactococcus lactis
3.1 M17 30 Leuconostoc mesenteroides
16.1 M17 RT Lactococcus lactis
4 strains combined + 0,5 % glucose
4 strains combined
16.3 MRS 30 +215 Lactococcus lactis

b)

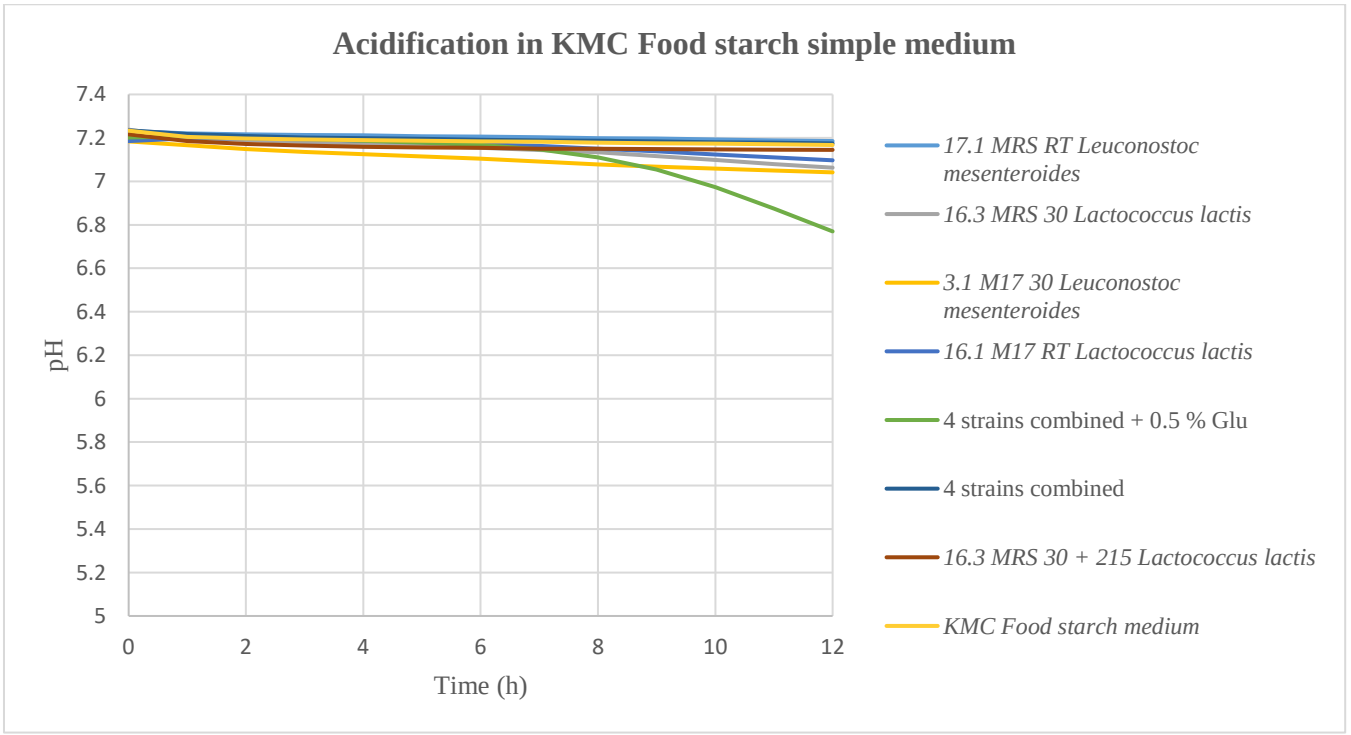
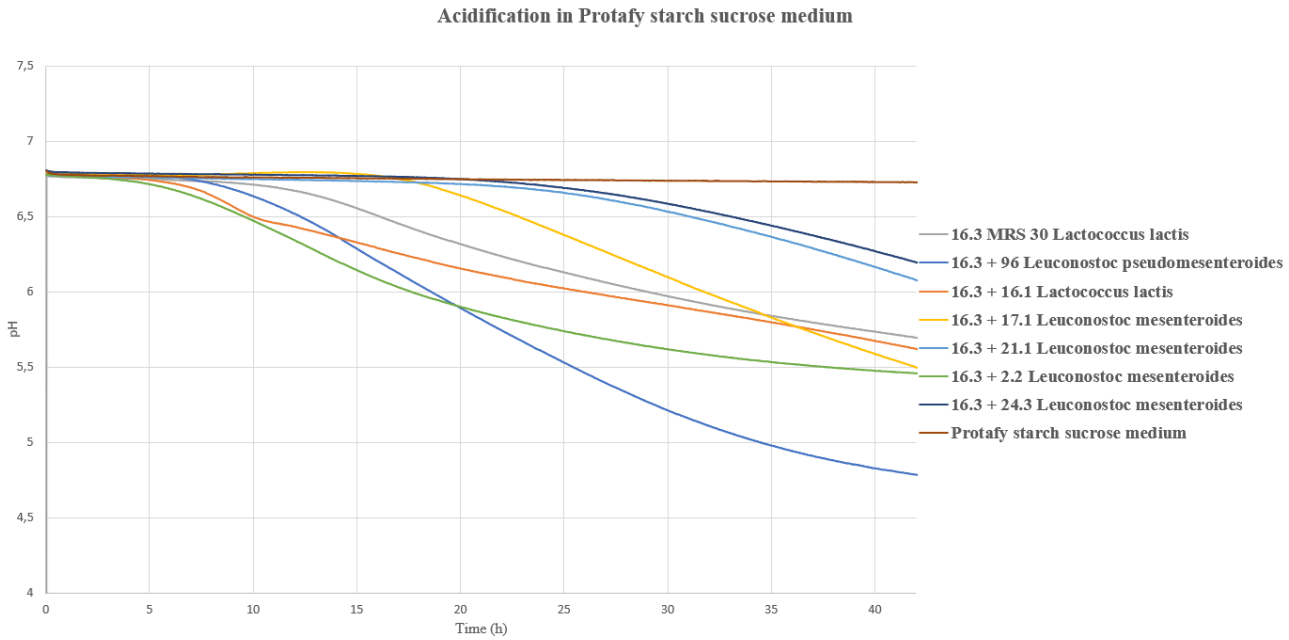

Acidification in KMC Food starch simple medium
pH
Time (h)
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
5.2
5
0
2
4
6
8
10
12
17.1 MRS RT Leuconostoc mesenteroides
16.3 MRS 30 Lactococcus lactis
3.1 M17 30 Leuconostoc mesenteroides
16.1 M17 RT Lactococcus lactis
4 strains combined + 0.5 % Glu
4 strains combined
16.3 MRS 30 + 215 Lactococcus lactis
KMC Food starch medium

Figure 8. Acidification rates of LAB strains in **a)** Protafy starch simple medium or **b)** KMC Food starch simple medium during the period of 12 hours at 30 °C

According to the results shown in figure 8 a) and b), starch cannot be used as the sole carbon source by examined LAB. Only slight acidification was observed in a co-culture of 4 strains when both media were supplemented with 0.5 % glucose. In Protafy starch medium, it is possible that *Lc. lactis* (215) contributed to better acidification compared to strain *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) alone (figure 8). However, pH levels still have not even reached the value of 6 after 12 hours of fermentation. Additionally, on Peptone starch agar plates, there was no or poor growth of all LAB strains tested. That confirmed the need to include an alternative carbon source for the growth of LAB. Previous research has shown that some LAB strains are capable of converting potato starch and potato residues into LA by direct fermentation (Smerilli et al., 2015). There are a lot of amylolytic LAB available, particularly in cereal-based fermented foods. However, many of them do not meet the criteria for efficient starch fermentation into LA. Also, the screening for suitable amylolytic LAB is both time-consuming and difficult (Petrova and Petrov, 2012). Further, certain strains capable of direct conversion of starch to LA such as *E. faecium* or *Geobacillus stearothermophilus* are not on a QPS list which was a selection criterion of this thesis (Smerilli et al., 2015). Consequentially, in plant-based dairy alternatives production, starch is often utilized as a gelling agent, not necessarily as a source of nutrients for the cultures used (Grasso et al., 2020).

a)



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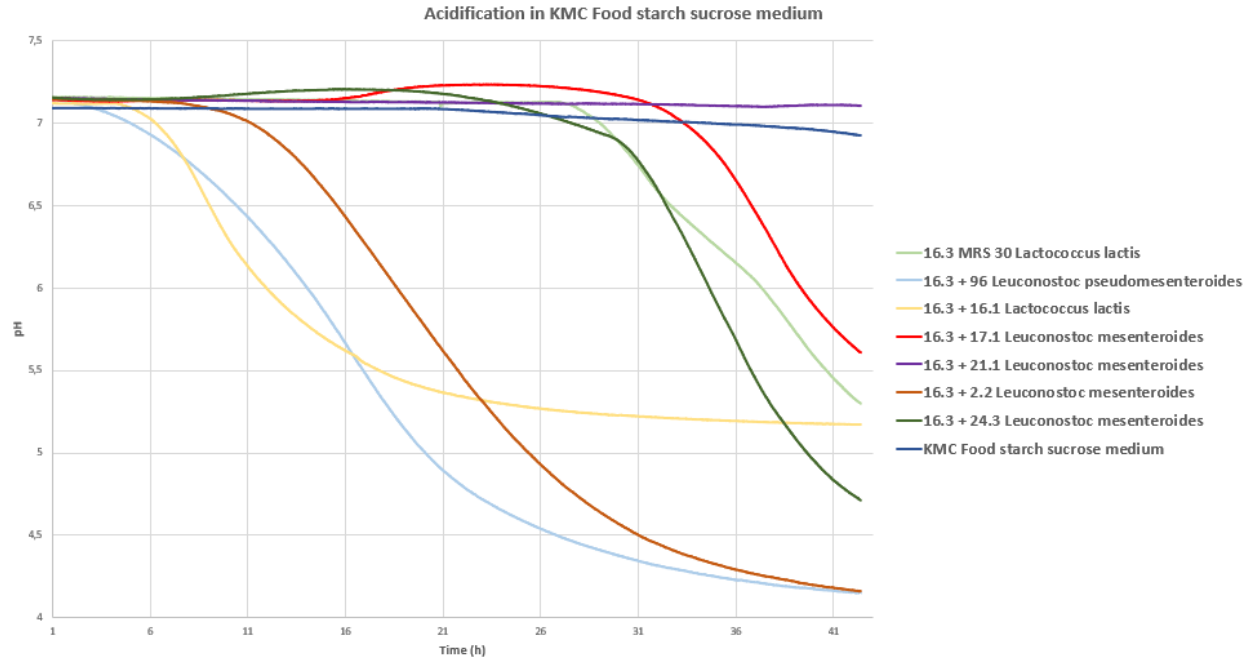

Figure 9. Acidification rates of certain strains in **a)** Protafy starch sucrose medium **b)** KMC Food starch sucrose medium during the period of 41 hours at 30 °C

The media used in figure 9 a) and b) were created by adding only potato protein concentrate (5 %), starch (2 %), and sucrose (1 %). Additionally, overnight culture was developed in these media as opposed to MRS/M17 supplemented with 0.5 % glucose which have been used previously. This was done since sensory testing required the use of only food-grade ingredients which included eliminating the use of MRS/M17 media. All this probably contributed to slower acidification rates shown by the strains. However, it can be seen that supplementation with 1 % sucrose made an important difference compared to media with starch as a sole carbon source. That was expected, as sucrose is a common nutrient for LAB that affects bacterial activity during fermentation. After the degradation of sucrose into LA during fermentation, vegetable protein coagulates due to a decrease in the pH value. This affects the yogurt's viscosity by forming a thick texture. Furthermore, sucrose addition of 8 % and 12 % showed a significant increase in the LAB population of butterfly pea yogurt, compared to the addition of 0 % and 4 % (Suharman et al., 2021). Sucrose is preferred over maltose as an alternative carbon source because it is one of the most cost-efficient carbon sources and is widely available (Boontun et al., 2021).

It can also be seen that autoclaving of simple media caused a smaller drop in pH compared to autoclaving complex media (figure 9). To make it more comparable, in food matrix pH was adjusted after autoclaving to be approximately 6.5 in both media.

It was noticed that certain mixed cultures reached a lower pH compared to monocultures (figure 9). The use of mixed cultures is desirable since synergistic effects on the fermentation process and the ultimate product quality can be achieved. An application of the combination of strains can stimulate each other's growth, acid production, and volatile compound formation (Tangyu et al., 2019). Members of the microbial consortium communicate through metabolite or molecular signals exchange and there appears to be a distribution of metabolic activities among included strains. Moreover, when compared with monocultures, complex microbial consortia conduct more complicated activities (versatility) and endure more environmental fluctuation (robustness) (Smid and Lacroix, 2012). Although the type of interaction among mixed cultures in this thesis is not known, it can be suggested that it is mutualistic or commensalistic relationship. For instance, a well-known example of commensalism in cheese is the one between protease-negative and protease-positive variants of *Lc. lactis* starter cultures where the protease-positive variants supply the protease-negative ones with peptides and amino acids (Smid and Lacroix, 2012).

Finally, 4 LAB strains were selected for application in product development (table 10). Strains were incubated at 30 °C for 48 hours in MRS/M17 agar plates supplemented with 0.5 % glucose before use. For the fermentation of final food products, 1 LAB monoculture and 3 LAB mixed cultures that are shown in table 11 were used.

Table 10. List of 4 LAB strains used in the final product development and their identification by WGS. All bacterial strains were stored as stock culture in 30 % (v/v) glycerol at -80 °C

WGS identification	Strain label	Medium	Origin
Leuc. pseudomesenteroides	96	MRS + 0.5 % glucose	Potato
Lc. lactis subsp. *cremoris*	16.3_MRS_30	MRS + 0.5 % glucose	Potato from inside a rotten potato pile
Leuc. mesenteroides subsp. *mesenteroides*	2.2_M17_30	M17 + 0.5 % glucose	Juice of potato flour which is left in pipes for several months
Lc. lactis subsp. *lactis*	16.1_M17_RT	M17 + 0.5 % glucose	Potato from inside a rotten potato pile

Table 11. LAB monoculture of *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) and combinations of the strains used in final food-grade product development

Product	Bacterial culture	Food matrix
Product 1	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30)	KMC food
Product 2	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30) + *Leuc. pseudomesenteroides* (96)	
Product 3	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30) + *Lc. lactis* subsp. *lactis* (16.1 M17 RT)	
Product 4	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30) + *Leuc. mesenteroides* subsp. *mesenteroides* (2.2 M17 30)	
Product 5	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30)	Protafy
Product 6	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30) + *Leuc. pseudomesenteroides* (96)	
Product 7	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30) + *Lc. lactis* subsp. *lactis* (16.1 M17 RT)	
Product 8	*Lc. lactis* subsp. *cremoris* (16.3 MRS 30) + *Leuc. mesenteroides* subsp. *mesenteroides* (2.2 M17 30)	

Due to an empirically known issue of yeast contamination, KMC Food matrix fermented by a mixed culture of *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) and *Lc. lactis* subsp. *lactis* (16.1 M17 RT) were checked for the presence of the yeast cells by analysis on LB and blood agar plates and incubated for 48 hours at 30 °C (figure 10). Yeasts are heterotrophic organisms that preferably metabolize sugars that are primarily converted to ethanol and carbon dioxide, but depending on the species can utilize use amino and organic acids, polyols, alcohols, fatty acids, and other substances (Tofalo and Suzzi, 2016). Although there was a yeast-like smell and taste of these samples, no yeast cells were found observed under an optical microscope. Cells observed were cocci and rod-shaped bacilli, and were most likely LAB.

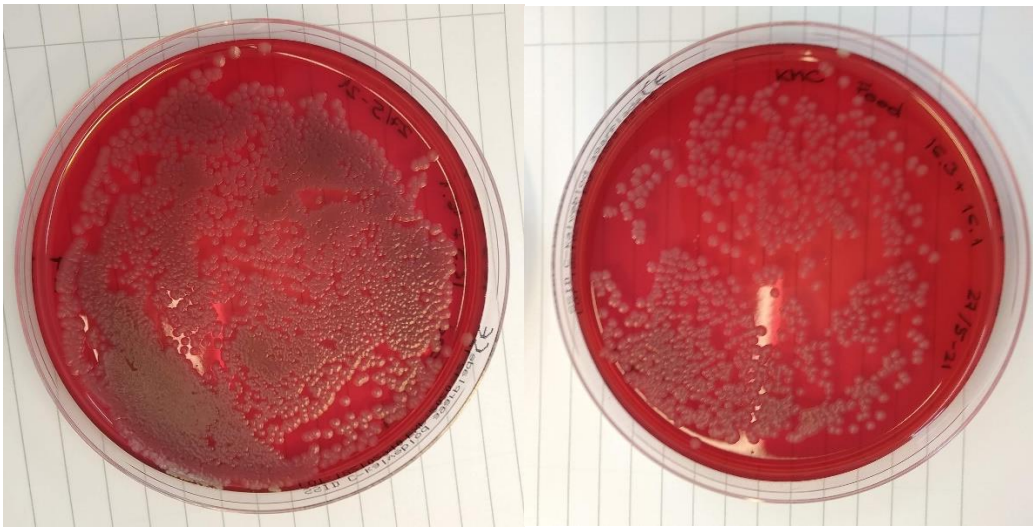

Figure 10. Food-grade KMC Food medium fermented by a mixed culture of *Lc. lactis* subsp. *cremoris* (16.3 MRS 30) and *Lc. lactis* subsp. *lactis* (16.1 M17 RT) inoculated on blood agar plates and incubated for 48 hours at 30 °C

4.4. SENSORY ANALYSIS

Only Protafy-based samples were tested due to a suspicion of yeast contamination in KMC Food-based samples in two panel sessions. Additionally, a preliminary sensory analysis was performed due to a possible contaminating strain found by WGS. Although the strains' identities confirmed that the applied strains are safe and that the contamination is absent, the sensory analysis could not have been continued because it was unknown if the food samples were still safe since their storage was prolonged due to the contamination issues. The appearance of developed potato protein-based food-grade innovative products can be seen in

figure 11. During the session, the expert sensory panel has developed the specified vocabulary which is presented in table 12.

Figure 11. Protafy (5 %)-based samples fermented by mono- or mixed non-starter LAB cultures *Lc. lactis* subsp. *cremoris* (16.3 MRS 30), *Leuc. pseudomesenteroides* (96), *Lc. lactis* subsp. *lactis* (16.1 M17 RT), and *Leuc. mesenteroides* subsp. *mesenteroides* (2.2 M17 30), being tested by the assessors after defreezing

Table 12. Vocabulary determined by the assessors after tasting all 4 food-grade innovative products a) in their first session b) in their second session

a)

SMELL	TASTE	TEXTURE
Cereal Fermented, sharp Coconut Sweet Cardboard Acidic Essence of almond/ synthetic Boiled cold potatoes	Cardboard Water (like in a spring or a fountain) Sweet Cereal Flour Beer-porridge Essence of almond/ synthetic	Sandy Divided into phases Lumpy Fluffy lumps Viscous Separated Astringent

b)

SMELL	TASTE	TEXTURE
Sweet Cereal Fermented Essence of almond (artificial sweet) Other things	Sweet Cereal Fermented Essence of almond (artificial sweet) Other things	Sandy Soft fluffy lumps Viscous Separated Astringent

LAB may influence the flavour of fermented foods in a variety of ways depending on the composition of raw material. For example, LAB significantly affected the sensory quality of lupine-based dairy analogues by decreasing "beany" notes. However, the effects were highly strain-dependent, with some strains resulting in products with too much acidity and sourness (Laaksonen et al., 2021). Other studies that were investigating sensory properties of plant-based fermented food products developed a somewhat similarly vocabulary like in table 12, including "astringency", "powdery/chalky" and "smoothness". Astringency creates a similar feeling as very unripe fruit and it indicates the samples dries out the mouth (Masiá et al., 2020). It is also related to an increased level of acetaldehyde that results in an astringent off-flavour yogurt (Chen et al., 2017). In a study that was comparing fermentation of soy, oat, and coconut samples, the latter showed a lower perception of astringency. This is because soy and oat are dryer and rougher raw materials with a lower fat content than coconut samples. Their high levels of polyphenols could have additionally contributed to the astringency and/or bitterness perception (Masiá et al., 2020; Peyer et al., 2016). Also, sweet, lemon, and sour taste were reported in all three plant-based samples, but with no significant difference (Masiá et al., 2020). However, most studies are conducted on legume or cereal-based dairy alternative beverages, so the vocabulary does tend to differ. For instance, in a pea-based beverage that was fermented with a mixed culture of LAB and yeasts, the panelists used descriptors as "green/vegetal", "leguminous", "beer/yeast" and "sparkling" (El Youssef et al., 2020).

Some LAB are able to enhance the viscosity of a liquid substrate by producing polysaccharides with high molecular weight. EPS are produced by polymerizing sugar subunits and can be made of repeated sugar subunits or two or more different subunits (Peyer et al., 2016). However, EPS production was not measured in this thesis so its impact on the sensory properties of the products is to be assesed. Since *Lc. lactis* subsp. *cremoris* is one of the strains extensively studied for EPS-producing capabilities one can assume that there is potential that it impacts the texture of the innovative products developed in this work (Mostefaoui et al., 2014). Besides viscosity, EPS-producing strains could have contributed to the improvement of other textural, sensory, nutritional, and functional characteristics of the yogurt-like products (Montemurro et al., 2021). Moreover, in a study evaluating chemical, rheological, and sensory properties of 6 commercial plant-based yogurts and dairy yogurt, plant-based yogurts had a similar texture and sensory properties to a dairy yogurt. However, plant-based yogurts contained hydrocolloids which significantly influenced the textural properties of these products, including firmness, consistency, cohesivenessm, and index of viscosity. In this study, both soy and coconut yogurts

were appreciated as the dairy yogurt (Grasso et al., 2020). Another study found that almond-based probiotic beverage was the most similar to probiotic milk beverages in terms of appearance, consistency, flavour, texture, and overall acceptability out of all tested plant-based samples (He and Hekmat, 2015). Some of the words that were used to describe the texture of samples were “separated” and “sandy”. This was expected, as producing a texture of yogurt-like alternatives comparable to that of dairy yogurts is both expensive and time-consuming due to the many factors that need to be considered. There is usually a lower amount of proteins in plant-based matrixes, they have different coagulation properties and there is almost always a need for the addition of structuring agents and emulsifiers (Montemurro et al., 2021).

The smell and taste of the products were described by the panelist as “sweet”, “cereal”, “fermented” and “essence of almond”. While the first 3 descriptors are relatively common for plant-based yogurt-like products, “essence of almond” or “artificial sweet” was peculiar. An off-flavour molecule responsible for such a description could be 2-hexenal (also described as “tea-like”, “green grass” or “juicy”) that has been found in other plant-based beverages (El Youssef et al., 2020). However, there are several other aldehydes that could be responsible such as furfural, benzaldehyde, or 3-methylbutanal (Chen et al., 2017). Unlike cow’s milk’s somewhat delicate buttery smell due to its particular aroma profile, plant-based alternatives often present unique aroma profiles depending on the protein source used (Grossmann et al., 2021).

Besides analysis performed in this thesis, additional analysis methods implemented for evaluation of developed fermented food products in future studies are desirable. Rheological and viscoelastic properties analysis, measurement of creaminess and lightness, texture profile analysis, and particle size distribution would support further characterization of fermented food products. Additionally, the fatty acid and amino acid composition of developed products would be of importance. Sensory evaluation could have included a consumer panel. Different processing methods could have been applied such as ultra-high-pressure homogenization or new ingredients or additives could have been included which could improve the nutritional and sensory qualities of developed products. Finally, it would have been interesting to include yeast or non-LAB strains into a mixed culture used for developing fermented products and compare results.

5. CONCLUSIONS

1. Among 119 tested strains, 4 strains granted with the QPS status were able to utilize potato protein as their primary source of nitrogen in media containing only 0.025 g L^{-1} of yeast extract as a secondary nitrogen source. None of the tested strains were able to utilize starch as a primary carbon source.

2. Non-food grade media for cultivation and food-grade matrixes for product development, both with potato protein concentrate, have been developed to select the most promising strains for the fermentation process.

3. Innovative potato protein-based yogurt-like products were produced by the efficient fermentation carried at 30 °C during 41 hours by inoculation of two different protein matrixes containing potato protein concentrates, starch and sucrose with mono- or mixed non-starter LAB cultures *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Leuc. pseudomesenteroides*, and *Leuc. mesenteroides* subsp. *mesenteroides*, all isolated from potato-based sources.

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7. SUPPLEMENTS:

Supplement 1. Table 13 and table 14 contain the identities of every strain used in this thesis

Table 13. Identification of 65 strains from the DTU strain collection (by courtesy of PhD Claus Heiner Bang-Berthelsen) that were selected based on a bioinformatic analysis and used in this thesis. MRS and M17 agar plates used for plating were supplemented with 0.5 % glucose.

MALDI-TOF ID	Number	Organism group	Plated on	Temperature	Origin
E. durans	1	LAB	M17	30 °C	Sourdough
E. faecium	5	LAB	M17	30 °C	Sourdough
E. faecium	6	LAB	M17	30 °C	Sourdough
E. durans	14	LAB	M17	30 °C	Sourdough
E. durans	16	LAB	M17	30 °C	Sourdough
E. durans	18	LAB	M17	30 °C	Sourdough
E. faecium	60	LAB	M17	30 °C	Sourdough
E. durans	63	LAB	M17	30 °C	Sourdough
E. durans	64	LAB	M17	30 °C	Sourdough
Lc. lactis	80	LAB	MRS	30 °C	Raspberry

Leuc. pseudomesenteroides	96	LAB	MRS	30 °C	Potato
Rhodotorula mucilaginosa	98	Yeast	MRS	30 °C	Potato
Leuc. pseudomesenteroides	99	LAB	MRS	30 °C	Apple
Rhodotorula mucilaginosa	100	Yeast	MRS	30 °C	Dill
Leuc. pseudomesenteroides	102	LAB	MRS	30 °C	Pumpkin
P. pentosaceus	103	LAB	MRS	30 °C	Pumpkin
Leuc. mesenteroides	115	LAB	M17	30 °C	Currant
Lysinibacillus fusiformis	119	Bacteria	M17	30 °C	Beetroot
E. durans	120	LAB	M17	30 °C	Jerusalem artichokes
E. casseliflavus	160	LAB	M17	30 °C	*Pisum Lollandske* raisins
E. casseliflavus	161	LAB	M17	30 °C	*Pisum Lollandske* raisins
E. casseliflavus	164	LAB	M17 (-MRS)	30 °C	*Pisum Lollandske* raisins
E. casseliflavus	165	LAB	M17	30 °C	*Petroselinum crispum*
E. casseliflavus	166	LAB	M17	30 °C	*Petroselinum crispum*
E. casseliflavus	168	LAB	M17	30 °C	*Beta vulgaris* sp, D
E. casseliflavus	175	LAB	M17	30 °C	*Daucus carota* subsp. *sativus*
E. casseliflavus	176	LAB	M17	30 °C	*Beta vulgaris* sp, C
E. casseliflavus	177	LAB	M17	30 °C	*Daucus carota* subsp. *sativus*

E. casseliflavus	178	LAB	M17	30 °C	*Daucus carota* subsp. *sativus*
Leuc. pseudomesenteroides	180	LAB	MRS	30 °C	*Beta vulgaris* sp, A
Leuc. pseudomesenteroides	181	LAB	MRS	30 °C	*Beta vulgaris* sp, A
Leuc. pseudomesenteroides	182	LAB	MRS	30 °C	*Beta vulgaris* sp, A
Leuc. mesenteroides	200	LAB	MRS/M17	30 °C	sea rocket
Leuc. pseudomesenteroides	202	LAB	MRS/M17	30 °C	sea rocket
Lc. lactis subsp. *lactis*	215	LAB	M17	30 °C	elderberry
Leuc. pseudomesenteroides	227	LAB	MRS/M17	30 °C	beach cochlearia
Leuc. pseudomesenteroides	228	LAB	MRS	30 °C	beach cochlearia
Leuc. mesenteroides	239	LAB	MRS	30 °C	sea rocket
Leuc. mesenteroides	240	LAB	MRS/M17	30 °C	beach cochlearia
Leuc. pseudomesenteroides	313	LAB	MRS	30 °C	*Beta vulgaris* sp, A
Leuc. pseudomesenteroides	314	LAB	M17	30 °C	*Dancus carotus* A
Leuc. pseudomesenteroides	322	LAB	Skim milk plate/MRS	30 °C	Eranthis – Flower with stem
Leuc. pseudomesenteroides	323	LAB	Skim milk plate/MRS	30 °C	Ivy leaf
Lc. lactis subsp. *lactis*	337	LAB	M17	30 °C	Sourdough
Lc. lactis	338	LAB	0	30 °C	0
Leuc. pseudomesenteroides	339	LAB	0	30 °C	0

E. casseliflavus	398	LAB	M17	30 °C	*Petroselinum crispum*
E. casseliflavus	400	LAB	M17	30 °C	*Pisum Lollandske* raisins
E. casseliflavus	404	LAB	M17	30 °C	unknown
E. casseliflavus	422	LAB	M17	30 °C	*Daucus carota* subsp. *sativus*
E. casseliflavus	423	LAB	M17	30 °C	*Daucus carota* subsp. *sativus*
not reliable identification	429	0	MRS	30 °C	unknown
E. casseliflavus	434	LAB	MRS/M17	30 °C	Sourdough
Carnobacterium maltaromaticum	476	LAB	M17	30 °C	Blackthorn (*Prunus spinosa*)
Bacillus mycoides	503	Bacteria	0	30 °C	Beech tree leaves
Enterobacter cloacae	520	Bacteria	Skim milk plate/M17	30 °C	Klæbeplante
Lysinibacillus sphaericus	529	Bacteria	M17	30 °C	Common juniper (*Juniperus communis*)
Bacillus pumilus	530	Bacteria	M17	30 °C	Common juniper (*Juniperus communis*)
Bacillus simplex	531	Bacteria	M17	30 °C	Common juniper (*Juniperus communis*)
Staphylococcus hominis	533	Bacteria	M17	30 °C	English Yew (*Taxus baccata*)
Rahnella aquatilis	544	Bacteria	M17	30 °C	*Dictyota dichotoma*
Serratia plymuthica	545	Bacteria	M17	30 °C	*Dictyota dichotoma*
Carnobacterium maltaromaticum	546	LAB	M17	30 °C	*Dictyota dichotoma*
not reliable identification	567	0	0	30 °C	0

Trichosporon loubieri	576	Fungi	MRS	30 °C	Brewers spent grain

Table 14. Identity of 54 strains from KMC (by Mathias Greve-Poulsen) that were used in this thesis. MRS and M17 agar plates used for plating were supplemented with 0.5 % glucose.

MALDI-TOF ID	Number	Organism group	Plated on	Temperature	Origin	Storage
L. brevis	1.1_MRS_RT	LAB	MRS	RT	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. plantarum	2.2_MRS_RT	LAB	MRS	RT	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. plantarum subsp. *argentoratensis*	1.3_MRS_RT	LAB	MRS	RT	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. sakei subsp. *sakei*	2.1_MRS_RT	LAB	MRS	RT	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
P. pentosaceus	3.2_MRS_RT	LAB	MRS	RT	Juice of potato flour from bottom of ventilation tank.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. plantarum	5.3_MRS_RT	LAB	MRS	RT	Fresh pulp of potato flour	Sample stored with 30 % (v/v)

					stored outside.	glycerol at -80 °C.
P. pentosaceus	7.2_MRS_RT	LAB	MRS	RT	Old pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. sakei	15.2_MRS_RT	LAB	MRS	RT	Potato on the surface of a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. mesenteroides subsp. *mesenteroides*	15.1_MRS_RT	LAB	MRS	RT	Potato on the surface of a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. sakei subsp. *sakei*	14.2_MRS_RT	LAB	MRS	RT	Potato on the surface of a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. brevis	16.2_MRS_RT	LAB	MRS	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. mesenteroides subsp. *mesenteroides*	17.1_MRS_RT	LAB	MRS	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. curvatus	17.3_MRS_RT	LAB	MRS	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. plantarum	17.2_MRS_RT	LAB	MRS	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. hilgardii	1.2_M17_RT	LAB	M17	RT	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. citreum	4.2_M17_RT	LAB	M17	RT	Juice of potato flour from bottom	Sample stored with 30 % (v/v) glycerol at -80 °C.

					of ventilation tank.	
Leuc. citreum	5.1_M17_RT	LAB	M17	RT	Fresh pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. sakei subsp. *sakei*	7.3_M17_RT	LAB	M17	RT	Old pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis	9.2_M17_RT	LAB	M17	RT	Fresh pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. citreum	9.3_M17_RT	LAB	M17	RT	Fresh pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis	12.3_M17_RT	LAB	M17	RT	Old pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
E. mundtii	15.2_M17_RT	LAB	M17	RT	Potato on the surface of a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Carnobacterium divergens	16.2_M17_RT	LAB	M17	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
E. casseliflavus	18.2_M17_RT	LAB	M17	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis subsp. *cremoris (*Later identified as *Lc. lactis* subsp. *lactis* by WGS)	16.1_M17_RT	LAB	M17	RT	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Clostridium bifermentans (Later identified as *Lacticaseibacillus saniviri*)	19.3_M17_RT	LAB	M17	RT	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.

E. italicus	21.3_M17_RT	LAB	M17	RT	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. mesenteroides subsp. *cremoris* (Later identified as *L. plantarum*)	22.1_M17_RT	LAB	M17	RT	Daucus carota subsp. sativus	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. alimentarius	1.2_MRS_30	LAB	MRS	30 °C	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. sakei	2.2_MRS_30	LAB	MRS	30 °C	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. plantarum	4.1_MRS_30	LAB	MRS	30 °C	Juice of potato flour from bottom of ventilation tank.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. sakei	7.1_MRS_30	LAB	MRS	30 °C	Old pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. fermentum	10.2_MRS_30	LAB	MRS	30 °C	Old pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis subsp. *cremoris*	12.2_MRS_30	LAB	MRS	30 °C	Old pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis subsp. *lactis*	12.3_MRS_30	LAB	MRS	30 °C	Old pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. paracasei subsp. *paracasei* (Later identified as *Lc.*	16.3_MRS_30	LAB	MRS	30 °C	Potato from inside a	Sample stored with 30 % (v/v)

lactis subsp. *cremoris* by WGS)					rotten potato pile.	glycerol at -80 °C.
L. plantarum subsp. *argentoratensis*	17.2_MRS_30	LAB	MRS	30 °C	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. versmoldensis	19.2_MRS_30	LAB	MRS	30 °C	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.
L. curvatus	20.1_MRS_30	LAB	MRS	30 °C	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis subsp. *hordniae*	20.2_MRS_30	LAB	MRS	30 °C	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. mesenteroides subsp. *dextranicum* (Later identified as *Leuc. mesenteroides* subsp. *mesenteroides*)	21.1_MRS_30	LAB	MRS	30 °C	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. mesenteroides subsp. *mesenteroides*	24.3_MRS_30	LAB	MRS	30 °C	Damaged seed potatoes.	Sample stored with 30 % (v/v) glycerol at -80 °C.
*Leuc. citreum (*Later identified as *Leuc. mesenteroides* subsp. *mesenteroides* by WGS)	2.2_M17_30	LAB	M17	30 °C	Juice of potato flour which is left in pipes for several months.	Sample stored with 30 % (v/v) glycerol at -80 °C.
*Lc. lactis (*Later identified as *Leuc. mesenteroides* subsp. *mesenteroides* by WGS)	3.1_M17_30	LAB	M17	30 °C	Juice of potato flour from bottom of ventilation tank.	Sample stored with 30 % (v/v) glycerol at -80 °C.
*P. pentosaceus (*Later identified as *Leuc. pseudomesenteroides* by WGS)	5.2_M17_30	LAB	M17	30 °C	Fresh pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.

L. plantarum subsp. *argentoratensis*	7.3_M17_30	LAB	M17	30 °C	Old pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
*Leuc. citreum (*Later identified as *Leuc. mesenteroides* subsp. *mesenteroides* by WGS)	7.2_M17_30	LAB	M17	30 °C	Old pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Carnobacterium maltaromaticum	8.3_M17_30	LAB	M17	30 °C	Old pulp of potato flour stored outside.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis subsp., *lactis*	9.3_M17_30	LAB	M17	30 °C	Fresh pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. citreum	10.2_M17_30	LAB	M17	30 °C	Old pulp of potato flour.	Sample stored with 30 % (v/v) glycerol at -80 °C.
E. casseliflavus	15.1_M17_30	LAB	M17	30 °C	Potato on the surface of a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Leuc. mesenteroides subsp. *dextranicum (*Later identified as *P. pentosaceus* by WGS)	16.2_M17_30	LAB	M17	30 °C	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
E. mundtii	17.4_M17_30	LAB	M17	30 °C	Potato from inside a rotten potato pile.	Sample stored with 30 % (v/v) glycerol at -80 °C.
Lc. lactis subsp. *tructae*	4.4_M17_30	LAB	M17	30 °C	Potato.	Sample stored with 30 % (v/v) glycerol at -80 °C.

STATEMENT OF ORIGINALITY

This is to certify, that the intellectual content of this thesis is the product of my own independent and original work and that all the sources used in preparing this thesis have been duly acknowledged.

Name of student