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Effects of fruit juices and carbohydrates on the fermentation of egg white drink by probiotic bacteria

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LIST OF ABBREVIATIONS

4 EW: 2 PI a mixture of egg white and pineapple juice in a 4:2 ratio
4 EW: 2 ST a mixture of egg white and strawberry juice in a 4:2 ratio
4 EW: 3 PI a mixture of egg white and pineapple juice in a 4:3 ratio
4 EW: 3 ST a mixture of egg white and strawberry juice in a 4:3 ratio
4 EW:1 PI a mixture of egg white and pineapple juice in a 4:1 ratio
4 EW:1 ST a mixture of egg white and strawberry juice in a 4:1 ratio
a*: Redness, Green
b*: Yellowness and blueness
CFU: Colony Forming Unit
EW Egg white drink
EW: PE a mixture of egg white and peach juice in a 3:1 ratio
EW: PI a mixture of egg white and pineapple juice in a 3:1 ratio
EW: ST a mixture of egg white and strawberry juice in a 3:1 ratio
FOS Fructo-oligosaccharides
GOS Galacto-oligosaccharides
K: consistency coefficient
L*: Lightness
L.C EW: PI a mixture of egg white and pineapple juice in a 3:1 ratio fermented by *L. casei* 01
L.C EW: ST a mixture of egg white and strawberry juice in a 3:1 ratio fermented by *L. casei* 01
L.S EW: PI a mixture of egg white and pineapple juice in a 3:1 ratio fermented by *L. salivarius* CRL 1328.
L.S EW: ST a mixture of egg white and strawberry juice in a 3:1 ratio fermented by *L. salivarius* CRL 1328.
L.C EW: PI a mixture of egg white and pineapple juice in a 3:1 ratio fermented by *L. casei* 01
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L.S EW: PI a mixture of egg white and pineapple juice in a 3:1 ratio fermented by *L. salivarius* CRL 1328.
MRS De Man, Rogosa, Sharpe media
n: flow behaviour index
Pa Pascal
PE peach juice
PI pineapple juice
ST strawberry juice
TPY Tryptone Peptone Yeast Extract
USDA United States Department of Agriculture
XOS Xylo-oligosaccharides
$\tau 0$ indicates the yield stress
τ refers to shear stress (Pa)
$\dot{\gamma}$ is the shear rate (1/s)
η apparent viscosity

1 Introduction

Functional beverages have been becoming more prevalent over the past few years. This is due to a rapidly expanding vegetarian market and a growing need for beverages that suit individuals who have particular dietary restrictions. These include those who are hypersensitive to lactose, diabetic, athletes, or sensitive to milk proteins. Consumers are also becoming more conscious of how their food habits influence their health and general life expectancy.

Extensive research definitively confirms that the inclusion of functional foods, particularly probiotics, in our diets can bring a multitude of positive effects to our bodies. This is as long as they are consumed in the appropriate amounts. These incredible benefits include significant improvement in the gastrointestinal and immune systems, as well as relief from symptoms typically associated with lactose intolerance, such as bloating, diarrhea, gas, and nausea.

It's important to recognize that including prebiotics in probiotic products can greatly improve the probiotic ability to survive, increase the absorption of calcium and magnesium, and stimulate the production of short-chain fatty acids that are extremely beneficial for the body.

However, these beneficial microorganisms are typically found in dairy products, which are only consumed by certain segments of the population. Additionally, individuals with special diets may need to exclude these beneficial products from their diet and seek alternative options.

For the first decade of human existence on Earth till now, eggs have been the primary source of nutrition. They provide essential nutrients, particularly functional protein, which is mainly found in egg white those proteins including ovalbumin, ovomucoid, lysozyme, ovo-transferrin, and ovo-flavoprotein. Additionally, egg white is free of cholesterol and contains low levels of carbohydrates. ToTu drink, which is the subject of my study, is mainly made of egg white and it is produced by separating the yolk and then concentrating and homogenising the egg white by enzymatic treatment, it is considered to be a lactose-free product. Although egg white is highly perishable and ToTu drink shelf life is only two days after opening, fermentation of egg white can prolong its shelf life and boost its nutritional benefits, furthermore, as the starting culture is the main determinant of the final quality and flavour of the fermented product thus, it is essential to choosing the most probiotics bacteria which suitable to grow in egg white drink and doesn’t affect the sensory characteristics of the final product.

Although egg whites drink might be a suitable matrix for probiotics, it does not provide the necessary carbohydrates that these microorganisms require to thrive. This can significantly impede their growth, as they heavily rely on these nutrients for their survival.

It is imperative to consume fruits regularly in order to maintain a healthy diet. Fruits are enriched with vital vitamins, minerals, phenolic compounds, and antioxidants, which are essential for optimal health. Numerous studies have clearly linked consistent fruit intake to a significant reduction in the possibility of developing chronic diseases, particularly those caused by chronic inflammation. Therefore, it is vital to prioritize fruit consumption as a key component of a healthy and balanced lifestyle.

Fruit juices are essentially a concentrated form of the original fruit, containing similar substances that make them equally beneficial to the host. A thorough study conducted by Ruxton et al. (2006) found no significant differences between the health benefits of fruits and fruit juices. Additionally, the attractive taste, colour, and aroma of fruit juices make them an appealing choice for individuals of all ages, as they are free of allergenic substances and safe for consumption. However, they are also a great source of carbohydrates, which can affect the propagation of probiotics on the medium and making them an ideal vehicle for probiotic bacteria. On the other hand, there is still limited research on the use of fruit juices or prebiotics in egg white fermentation. Also, the changes in protein and phenolic compounds, as well as the growth and viability of probiotics during storage and fermentation, have yet to be extensively documented.

In addition, probiotic fermentation can generate exopolysaccharides and accumulate lactic acid in the medium as well as other organic compounds, which could impact the rheological characteristics of the end product. It is also essential to investigate how the fermentation process affects sensory properties and consumer preference.

2 Objectives

Daily probiotic consumption has been linked to a variety of advantages, involving strengthened immune and digestive systems. In addition, it is possible to relieve lactose intolerance symptoms such as diarrhea and bloating using probiotics. In the case of protein allergies, probiotic fermentation can also reduce some allergen factors in the final product. Even though probiotics typically exist in dairy-based foods and have limited availability in vegetarian drinks, those who are unable to consume milk or dairy products miss out on the benefits of probiotics. Egg white has a variety of functional proteins that may be utilized as a vehicle for probiotics, resulting in an innovative probiotic egg white drink that satisfies the vegetarian market's expanding demands.

My present study mainly focuses on the influence of several mono and mixed culture probiotic starters -lactic acid bacterial and bifidobacterial- on the fermentability of ToTu drink. The major goal is to extend its shelf life and generate a probiotic fermented egg white beverage that may be used as a dairy substitute for individuals who are lactose intolerant, allergic to milk proteins, or follow an ovo-vegetarian or paleo diet. Furthermore, this beverage is highly recommended for athletes and others who rely on a high-protein diet.

The specific objectives were the following:

- To investigate the ability of probiotics to grow in egg white (ToTu drink)
  - without any other additives.
  - in the presence of varied carbohydrate sources such as mono-, di-, and oligo-saccharides.
  - with different fruit juices such as pineapple, strawberry, and peach.
- To compare the mono and mixed culture fermentation after selection the most promising strains.
- To examine the viability of the probiotics in fermented egg white beverages during refrigerated storage at 4 °C to determine the shelf life.
- In addition, the cells growth, the effect of fermentation by probiotic bacteria on the microbial, physicochemical and rheological properties of the final products were aimed to be studied based on the following:
  - pH
  - protein profile
  - protein content
  - colour

- ❖ viscosity
- ❖ storage stability
- ❖ phenolic content
- ❖ lactic acid content
- ❖ antagonistic activity

➢ Sensory evaluation of the selected product.

3 Literature overview

3.1 Functional food

Currently, a growing number of individuals are becoming more aware of the association between what they consume and their state of health (Begum et al., 2017), hence food producers supply alternative food standards that fulfil their desires for healthy living. Functional foods have a special significance in this scenario, as the functional food market has expanded dramatically in Europe, Germany, France, the United Kingdom, and the Netherlands (Annunziata & Vecchio, 2011), (*Figure* 1).

These food items are designed to satisfy hunger, to provide humans with necessary nourishment, and to help prevent diet-related diseases while improving customers' physical and mental health (Menrad, 2003). Functional food involves biological substances that are either naturally or technologically produced in order to boost the quantity of biologically active ingredients. Biologically active compounds are dietary components that have a positive effect on vital body functions that are essential for health. They minimize the risk of acquiring diseases including atherosclerosis, hypertension, myocardial infarction, and diabetes. Fresh fruits, vegetables, and tea (particularly green tea) are among the most important sources of these compounds, which have good health benefits due to their high polyphenol, flavone, and catechin content (Butnariu & Sarac, 2019). Due to their effectiveness in relieving various types of diarrheal diseases, modulating the immune response, preventing colon cancer, and treating other chronic gastrointestinal inflammatory disorders (Begum et al., 2017). Probiotics have been proven as potential food components with functional properties, resulting in the growing recognition of probiotic supplemented foods. Prebiotics can also be described as functional substances prevalent in many fruits and vegetables that possess valuable technical advantages in many different food applications. Besides their health benefits, especially enhancing sensory attributes such as flavour and texture as well as raising the stability of foams, emulsions, and mouth feel in dairy products and bread are important (Alsheraji et al., 2013). Synbiotics are a combination of probiotics and prebiotics that have synergistic effects in food industries (Alsheraji et al., 2013). For instance, the incorporation of prebiotics such as RaftiloseP95 into yogurt boosted the survival rate of *Lactobacillus rhamnosus*, *Bifidobacterium spp*., *Lactobacillus acidophilus*, and *Lactobacillus casei* over a period of four weeks in cold storage (Capela et al., 2006).

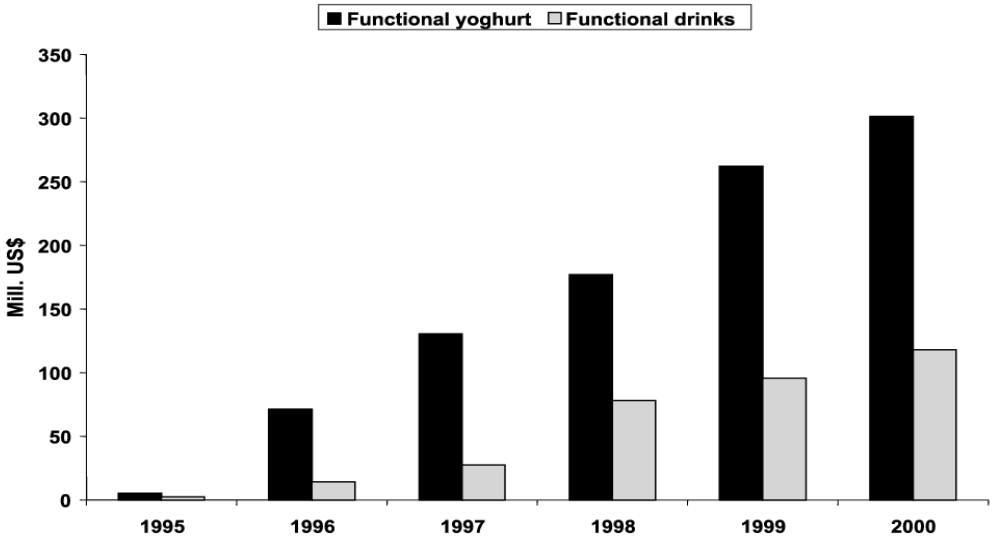


***Figure* 1. Functional dairy products development in Germany Market**

Source (Menrad, 2003).

3.2 Probiotics

Probiotics are known as live microorganisms that supply a substantial nutritional advantage on the host when provided in appropriate doses (FAO, 2001). The cultivation of probiotic in foods has grown in popularity due to its role in maintaining excellent overall wellness/health (Desmond et al., 2001). These beneficial microorganisms attach to the epithelium of the intestines (Salminen et al., 1998) and compete with undesirable microbes for nutrients, and famished them (Mombelli & Gismondo, 2000). To ensure that the probiotic product offers the best advantages to the consumers the choice of probiotic strain should take into account technological parameters (Alakomi et al., 2005). Such as being able to spread swiftly via storage in the product while maintaining an acceptable standard of viability and stability. When mixed with food, the strains must not result in strange tastes or textures. They ought to be biologically active against particular targets and metabolically active within the gastrointestinal tract. Additionally, possess better sensory qualities and be phage-resistant (Thantsha et al., 2012).

In modern times, there are various microorganisms that can be used as probiotics particularly bacteria belonging to genera *Lactobacillus* and *Bifidobacterium* are the most commonly used (Mombelli & Gismondo, 2000). *Lactobacilli* species include *L. acidophilus*, *L. rhamnosus*, *L. casei*, *L. delbrueckii ssp*. *L. bulgaricus*, *L. johnsonii*, *L. reuteri*, *L. brevis, L. cellobiosus, L. curvatus, L. fermentum, L. gasseri* and *L. plantarum* (Krasaekoopt et al., 2003; Meurman & Stamatova, 2007). In contrast, the most common bifidobacteria species used are *Bifidobacterium breve, B. animalis subsp lactis* and *B. longum* biotypes *infantis* and *longum* (Masco et al., 2005).

Probiotics are additionally categorized as functional foods because they influence specific processes in the body, resulting in a positive health impact. They are readily accessible in the form of conventionally fermented foods as well as supplements. Likewise, most probiotics are included in dairy products such as milk powders, yogurt, soft-, semi-hard, and hard cheeses, and ice cream (Thantsha et al. 2012), which counted as a suitable vehicle for probiotic viability and growth (Özer et al., 2009). However, the demand for different types of probiotic-supplemented foods, such as malt-based drinks and fruit juices, continues to rise as a significant percentage of the population is sensitive to dairy products (Sheehan et al., 2006; Rozada-Sanchez et al., 2008; Champagne et al., 2008).

Lactic acid bacteria (LAB) play a major part in the food, agricultural, and pharmaceutical sectors. As they rely primarily on LAB's rapid growth and metabolic functions that are affected by biochemical and biophysical conditions. The biochemical conditions might be established, but there are many obstacles and problems in regulating and improving them. Desirable metabolic activities require the correct strain in addition to the optimization and management of affordable resources, such as carbohydrates, peptides, free amino acids, minerals, and vitamins, as well as buffering agents. As a consequence, a substantial study was conducted to understand how easily available nutrients influenced the development and metabolic activities of LAB. Nevertheless, only a limited number of dietary parameters could be changed simultaneously while other variables remained steady. On the other hand, the dietary factors may interact, yielding incorrect outcomes. Additional limitations and difficulties in this area have been caused by LAB features such as their demanding nutritional requirements, ability to synthesize acids and antibacterial compounds, and the diversity in nutritional requirements among strains (Hayek & Ibrahim, 2013).

Bifidobacteria were once believed to be essential for human health, with the first discovery occurring in the stool of a breastfed newborn. These microorganisms are spore-forming, non-motile, heterofermentative, and gram-positive. Since bifidobacteria produce lactic acid as one of their main fermentation end products, they are often classified as part of the lactic acid bacteria group.

In recent years, mixed bacterial cultures have attracted a lot of attention as it has been proven to be beneficial for several fermentations (Shalin et al., 2012). Mixed-culture fermentations were defined as an inoculum composed of two or more organisms. They can be made up entirely of recognized species or unknown species. They also may be entirely of one microbial group or they may be a mixture of organisms from fungi and bacteria, fungi and yeasts, or other irrelevant

mixtures (Clifford, 1992). Bacterial growth and product yield can both be increased. Mixtures often complement each other and drive out unwanted microbes. The substrate for fermented foods is always a complex combination of carbohydrates, proteins, and lipids. Mixed cultures have a wider range of enzymes and can attack a greater number of substances. Similarly, enhances the ability to eliminate hazardous or unpleasant chemicals found in the fermentation substrate (Clifford, 1992).

3.2.1 Carbohydrate metabolism

As carbohydrate is broken down, there are two primary pathways in LAB and each process releases a unique set of by-products (Kandler, 1983; Von Wright & Axelsson, 2011). In accordance, homofermentative LAB converts one molecule of hexose carbohydrates such as glucose into two molecules of lactic acid and two molecules of ATP, as a conversion rate of lactic acid is around 85% from one molecule of glucose (Axelsson, 2004; Von Wright & Axelsson, 2011). Heterofermentative LAB converts one molecule of glucose into one molecule of lactic acid, one molecule of ethanol/acetate, one molecule of CO_2, and one molecule of ATP, creating a 50% lactic acid (Axelsson, 2004). Moreover, each LAB strain demonstrated a preference for a unique carbohydrate type. Carvalho and coworkers (2004) investigated the effect of adding several carbohydrate sources to a cultivation medium on the thermotolerance and proliferation of *Lactobacillus bulgaricus,* throughout storage after freeze-drying, as *L. bulgaricus* cells maintained higher growth when propagated on fructose, lactose, or mannose rather than glucose. Further, in the presence of lactose, the cells exhibited the highest level of heat resistance. Similarly, carbohydrate concentration influences probiotic growth. In this regard, Suharman et al., (2021) assessed three sucrose concentrations (4%, 8%, and 12%) and their effects on *Lactobacillus bulgaricus* and *Streptococcus thermophilus* growth in butterfly pea (*Clitoria ternatea L.*) mixed with yogurt. The results revealed that raising the sugar content causes an increase in cell population as well as lactic acid production. Also, Al-Kaf et al., (2021) found out that *Lactobacillus acidophilus* growth was enhanced by the addition of non-digestible carbohydrates including barley, sweet potatoes, yams, garlic, and bananas compared to non-added carbon control.

Bifidobacteria can also metabolize several kinds of dietary carbohydrates at the same time various bifidobacterial strains may have different carbohydrate-using capacities (Pokusaeva et al., 2011) which might be responsible for their abundance in various environments for instant *B. longum subsp. infantis* can thrive in the infant gut because it is capable of using specific oligo-saccharides present in breast milk (Egan and Van Sinderen, 2018).

3.2.2 Protein metabolism

LAB cells have received a lot of research interest due to their proteolytic potential, which is especially crucial in the ripening and enzyme manipulation of diverse food items like cheese. The process of proteolysis, which is carried out by proteinases and peptidases that are either intracellularly inside the cell or extracellularly discharged, breaks down proteins into polypeptides, amino acids, and peptides (Hayek & Ibrahim., 2013) (*Figure* 2). For instance, methionine, leucine, phenylalanine, isoleucine, tyrosine, tryptophan, valine, and threonine are essential amino acids that are released during milk proteins breaking down, further improving their digestibility, thus, increase their nutritional value (Sharma et al., 2020).

The proteolytic system in a LAB is extremely important for producing proteins, peptides, and amino acids as well as providing cells with the nitrogen source needed for flourishing (Kieliszek et al., 2021). It also helps fermented foods improve their rheological and organoleptic qualities. A lot of research has been done on proteolysis in milk, where lactobacilli and lactococci are mostly in charge of taste creation while making cheese (Bintsis et al., 2003; Liu et al., 2010). Additionally, proteinase reduces the allergenic potential of milk and milk products for newborns. LAB proteolytic systems, involve peptide transporters that transport peptides into the cell, cell-wall bound proteinases start the breakdown of extracellular protein into oligopeptides, and intracellular peptidases break down peptides into shorter peptides and amino acids (Liu et al., 2010). Aldehydes, alcohols, and esters are examples of taste substances that can be formed from amino acids (Liu et al., 2008). The ideal pH range for the development and proliferation of proteolytic bacteria in milk is between 7 and 7.5. Furthermore, temperature affects both the growth and development of bacteria as well as their enzymatic activity. Proteolytic enzymes quickly lose their ability to function as temperature rise above those needed for bacterial development (Worsztynowicz et al., 2020; Ji et al, 2021; Linares-Morales et al., 2020). Noteworthy, thermophilic *lactobacilli* species have higher proteolytic capacity than rods or streptococci, and each species has strains with significantly varying activity in this regard. For example, the lactobacilli species with the highest capacity for proteolytic activity are *Lacticaseibacillus casei*, *Lactobacillus delbrueckii subsp*., *L. bulgaricus*, *L. helveticus*, and *L. acidophilus*, while *Lactiplantibacillus plantarum* has the lowest levels of activity (Kieliszek et al., 2021). LAB's ability to generate energy in a limited nutrient environment is also believed to be significantly influenced by their ability to metabolize amino acids. Furthermore, it was shown that the distribution of peptidases, proteinase, and the oligopeptide transport system differed throughout LAB strains, most likely due to the presence or absence of plasmids encoding them (Liu et al., 2010).

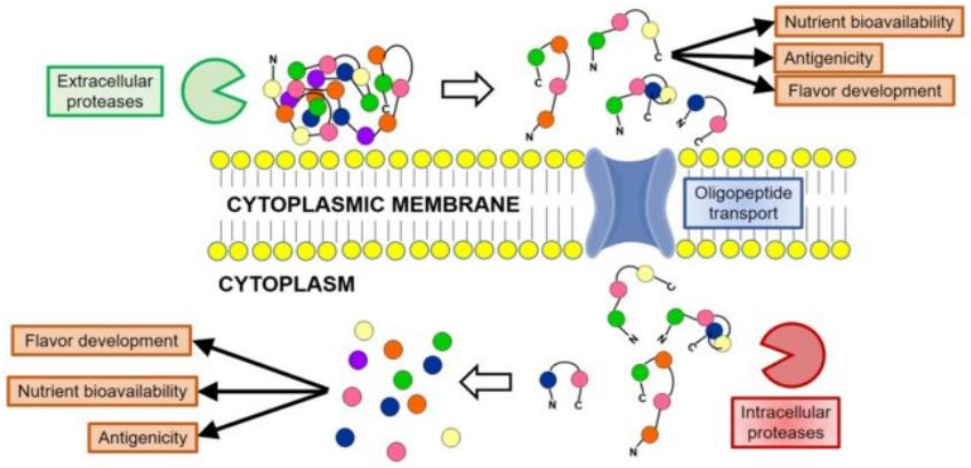


***Figure* 2: The LAB proteolytic system** (Garcia-Cano et al., 2020).

3.2.3 Functional compound production in fermented foods

As well as the rapid production of lactic acid, LAB can also produce antimicrobial chemicals like bacteriocin, hydrogen peroxide, carbon dioxide, and diacetyl which prevent the growth of harmful bacteria (Cintas et al., 2001). Furthermore, fermented foods differ in taste, astringency, and colour because of the different metabolic activities of LAB strains and their capacity to break down organic materials (Rodríguez et al., 2009). Terpenoids, carotenoids, sterols, polyphenols, and isoflavones are among the many of the complex and simple functional compounds that LAB breaks down into smaller metabolites throughout food fermentation or in the gut, which are absorbed by the host organism (Rodríguez et al., 2009; Chen et al., 2012; Hayek & Ibrahim., 2013).

According to Bartowsky and Henschke (2004), a fundamental taste component of butter, dairy products, wine (produced from grapes), brandy, roasted coffee, and many other fermented foods is diacetyl. When milk's citric acid is turned into pyruvate, which is subsequently transformed into α-acetolactate and the precursor for diacetyl, diacetyl is created. The majority of LAB strains possess the enzyme α-acetolactate decarboxylase, which allows them to decarboxylate α-acetolactate into acetoin and aromatic compounds. However, some LAB strains lack this enzyme, which leads to the anticipation of α-acetolactate and increased production of diacetyl in dairy products (Hayek & Ibrahim, 2013). Donkor et al. (2007) assessed the chemical composition and rheological properties of probiotic soy yogurt supplemented with inulin and raffinose. According to their findings, probiotic bacteria metabolized more aldehyde than yoghurt culture, which is the primary component that gives the yoghurt its beany flavour. As a result, the beaniness of the soy yoghurt was significantly reduced. Probiotic soy yoghurts also showed higher levels of viscosity

and pseudoplasticity, as well as higher lactic acid content and a one-log cycle increase in the bacterial population.

Also, acetaldehyde is one of the primary sources of the taste in dairy products (Cintas et al., 2001). Exopolysaccharides (EPS) are another metabolite that can be produced during LAB fermentation, they are high molecular weight and biodegradable polymers that fall into two categories homopolysaccharides and heteropolysaccharides. Homopolysaccharides are made up of the same monosaccharide, while heteropolysaccharides are made up of at least two or more different types of monosaccharides. EPSs are not used as a source of energy and are responsible for fermented products' optimal rheology, texture, and mouth feel (Sanlibaba & Çakmak, 2016). EPS has been used in the production of several fermented foods as thickeners, stabilizers, emulsifiers, and water-binding or gelling agents. Their health benefits include anticancer properties, boosting immunity, and lowering cholesterol (Sanlibaba & Çakmak, 2016). Several factors affect the formation of EPS, including the strain type, growth medium acidity, oxygen, and carbon supplies, as well as incubation temperature, and duration (Petry et al., 2000). EPS is used by a number of industries, including the food, pharmaceutical, cosmetic, medical, and beauty product sectors. Particularly as thickeners, suspending agents, and coating agents it has been also used to modulate and modify flow and viscosity, and to produce lower-calorie foods (Bajpai et al., 2016). Extran, xanthan, pullulan, gellan, curdlan, and scleroglucan are among of the primary EPS used in dairy and fermented food quality enhancement (Suryawanshi et al., 2022).

3.2.4 Antagonistic activity

It has been established that lactic acid bacteria preserve food by generating a variety of chemical molecules that are hazardous to other pathogens. In this way, *L. acidophilus* has been confirmed to alleviate diarrhea caused by *Salmonella* or *Shigella*. Also, *L. casei* was shown to have therapeutic effects on illnesses caused by *Salmonella typhimurium* and *E. coli*.

Bacteriocins are classified as a bio preservative and are generated by certain strains of LAB. They are antimicrobial peptides that are capable of eliminating Gram-positive foodborne pathogens including *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Clostridium perfringens* spores (Saranya & Hemashenpagam, 2011). Furthermore, *L. salivarius* L61 has been reported to produce a bacteriocin-like activity that inhibits *Enterobacter* sp., *Shigella* sp., and *Staphylococcus aureus* (Piyadeatsoontorn & Sornplang, 2015). while *L. salivarius* CECT5713 was found to be protected significantly against *Salmonella* (Abramov et al., 2023). *Lactobacillus acidophilus* PTCC 1643 and *Lactobacillus fermentum* PTCC 1744 were evaluated in fermented peach juice by (Hashemi et al., 2021) for antibacterial activity against *Shigella flexneri* PTCC

1865. The results showed that *Lactobacillus* strains effectively inhibited the development of *S. flexneri* during the peach juice's fermentation. Furthermore, the beverage's anti-inflammatory, ferrous reducing power, and superoxide anion antiradical properties all rose during the fermenting process.

3.2.5 The beneficial effect of probiotics

Probiotics are a significant therapeutic tool for immune system function, metabolic balance, and gastrointestinal health (Gul & Durante-Mangoni, 2024). They have been shown to positively impact the bioavailability, amount, and digestibility of certain nutrients (Parvez et al., 2006). Calcium, iron, manganese, copper, and phosphorus are better absorbed when probiotics are present (Alm, 1982), producing free amino acids and short-chain fatty acids by enzymatic hydrolysis of protein and fat. Generating organic acids such as acetate and lactate during fermentation lowers the pH of intestinal contents, consequently, creating an unpleasant environment for infectious bacteria (Parvez et al., 2006). It is recognized for its ability to alleviate lactose intolerance, allergies, and diarrheal disorders, lower serum cholesterol, minimize mutagenicity and carcinogenicity, and strengthen immunity. For instance, *L. bulgaricus* and *Streptococcus thermophilus* can help in improving lactose tolerance by producing β-galactosidase. Probiotics can also relieve constipation including *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, and *Propionibacterium freudenreichii* (Thantsha et al., 2012). *Bifidobacterium* spp (Guandalini et al., 2000), *L. rhamnosus* GG, and *L. bulgaricus* (Goldin, 1998; Sazawal et al., 2006) have a positive effect on reducing diarrhea.

Probiotics also serve as a treatment for inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). Ulcerative colitis, Crohn's disease, and pouchitis an example of IBD-related conditions that are identified by repetitive inflammation, ulceration, and abnormal narrowing of the gastrointestinal tract, resulting in discomfort in the abdominal area, diarrhea, and bleeding (Marteau et al., 2001; Hanauer, 2006). IBS symptoms include stomach discomfort, gas, irregular bowel movements, and bloating (Madden & Hunter, 2002). *Bifidobacterium longum* (Furrie et al., 2005), *Lactobacillus rhamnosus* GG (Gupta et al., 2000), and *Lactobacillus acidophilus* and *Bifidobacterium infantis* mixture (Hoyos, 1999) showed a positive effect against IBD. Additional evidence of the beneficial benefits of probiotics includes the ability of *Lactobacillus johnsonii* and *L. reuterii* to lower blood cholesterol levels, which in turn lowers the risk of cardiovascular disease (Fooks et al., 1999; Mombelli & Gismondo, 2000). It has also been demonstrated that *Lactobacillus acidophilus* and *Bifidobacterium bifidum* reduce the risk of cancer (Thantsha et al., 2012).

According to meta-analysis, probiotic products are safe for use during pregnancy and lactation, involving preventing gestational diabetes, mastitis, constipation, and postpartum depression. Likewise, some probiotic products may affect breastmilk microbial composition, consequently, improving the gut health of newborn infants (Sheyholislami, & Connor, 2021). In addition, probiotic supplement showed a positive effect in reducing body mass index (BMI), and blood glucose changes in patients with hypertension (Chi et al., 2020). Furthermore, a meta-analysis including 38 studies found that the usage of probiotics lowered the average blood glucose (sugar) levels during the previous two to three months, but did not approach the significance level and had no effect on LDL-cholesterol. However, its ingestion reduced levels of fasting blood glucose, total cholesterol, triacylglycerol, and insulinaemia and boosted HDL-cholesterol levels (Bock et al., 2021).

3.3 Prebiotics

Prebiotics are a nutrient category that is consumed by the gut microbiota and selectively support the growth and activity of probiotic bacteria. It is also characterized as a nonviable food element that provides health advantages to the host via modulating the microbiota (Wang et al., 2020). A prebiotic claim is any statement, suggestion, or implication that a prebiotic food possesses certain qualities related to its origin, nutritional value, attributes, and health. However, the EU does not yet authorize health claims for probiotics or prebiotics. They need sufficient scientific support for health advantages; also, producers cannot claim that prebiotics promote health, but may emphasize their existence as a component (Loveren et al., 2012).

Over the years, there has been an increased interest in their association with human health. Besides contributing to support the intestinal microbiota, prebiotics also produce short-chain fatty acids (acetate, propionate, and butyrate) that reach the bloodstream and can affect not just the gastrointestinal tract, but also other organs throughout the body. To be relevant to diverse food items, prebiotics must exhibit the following features; they can endure gastric acidity, resist hydrolysis by enzymes in the human body, and be absorbed in the upper gastrointestinal tract; easily metabolize by microorganisms in the intestines; selectively encourage the proliferation of specific beneficial bacteria in the intestines, and contributing to positive health effects and overall well-being (Rawi et al., 2020; Slavin, 2013; Gibson et al., 1995). Furthermore, prebiotics are increasingly being utilized as advantageous compounds in food manufacturing. They are found in a variety of products, including probiotic yogurts, symbiotic products, milk, fruit juices, coffee, cocoa, tea, soft drinks, alcoholic beverages, desserts (including jellies, puddings, and fruit-flavoured ice cream), biscuits, breakfast cereals, chocolate, breads, pasta, meat products (such as fish paste), and tofu. They are also present in cosmetics, pharmaceuticals, and diabetic care items

(Murari, 2014). Prebiotics are available in a variety of forms, many of which are oligo-saccharides, a sort of carbohydrate. Fructo-oligosaccharides and galacto-oligosaccharides are among the most important groups of prebiotics (Davani-Davari et al., 2019).

3.3.1 Fructans

Fructans include inulin and fructo-oligosaccharides (FOS), sometimes known as oligofructose (Rawi et al., 2020, Davani-Davari et al., 2019). Previous research has shown that fructans specifically induce lactic acid bacteria. However, a recent investigation shows that the length of the fructan chain is critical in deciding which bacteria are capable of fermenting it (Scott et al., 2014). Inulin, a soluble dietary fibre made up of individual fructose molecules, is well-studied and found in plants. Chicory is high in inulin and is commonly used in industrial methods to extract fructan chains (Torres et al., 2010). Fructo-oligosaccharides are a mixture of different chain length oligosaccharides, they are naturally found in many therapeutic plants, including onion, garlic, asparagus, bananas, and artichokes. FOS have a variety of medicinal properties such as reducing blood pressure, cholesterol, and blood sugar levels. They also improve calcium and magnesium absorption while inhibiting reductase enzyme formation, both of which contribute to cancer development. FOS are increasingly being used in food items especially infant formulas due to their immune-boosting benefits and inducement of the establishment of healthy intestinal microbiota, specially bifidobacteria (Murari, 2014).

3.3.2 Galacto-oligosaccharides

Galacto-oligosaccharides (GOS), also commonly known as oligo-galactosyllactose, oligogalactose, oligolactose, or transgalacto-oligosaccharides, is an indigestible carbohydrate that resists intestinal digestive enzyme action and has fibre-like characteristics (Tomal et al. 2019). These carbohydrates are prevalent in both cow's and human milk and are extensively utilized in the dairy industry. GOS is created by glycoside hydrolases, which use lactose as their substrate. Glycoside hydrolase enzymes transform lactose into GOS, leading to a combination of GOS with variable degrees of polymerization (DP), unaffected lactose, and monomeric sugars involving glucose and galactose (Torres et al., 2010). GOS incorporates physicochemical features such as water solubility, colourlessness, and stability at 160 ℃ for 10 minutes at pH 7. It is also stable at 100 ℃ for 10 minutes at pH 2 and may tolerate 37 ℃ at pH 2 for several months. In addition, GOS is capable of decreasing the freezing point of food products (Sangwan et al., 2011). It has been used in a variety of food manufacturing, including infant formula, drinks, bread, pet feeds, and confectionery (Tomal et al., 2019).

3.3.3 Xylo-oligosaccharides

Xylo-oligosaccharides (XOS) are sugar oligomers generated mostly from agricultural feedstock. They are popularly referred to as "emerging prebiotic" compounds (Precup et al., 2022) and are known for their numerous health benefits, notably in the treatment of gastrointestinal diseases. XOS can be manufactured from lignocellulosic resources. XOS occurs naturally in honey, bamboo shoots, fruits, and vegetables (Samanta et al., 2015), though the levels discovered in these sources are relatively small. As a result, researchers have resorted to food waste to produce XOS via enzymatic, thermal, or chemical processes (Farias et al., 2019; Lelia & Suharoschi, 2022). In contrast to other non-digestible oligo-saccharides like FOS and inulin, XOS is stable throughout a wide pH range (2.5-8.0), even within the acidic environment of gastric juice and at temperatures up to 100 °C (Bhat, 1998). Added to that, XOS has been demonstrated to increase the persistence of *Bifidobacteria* in the gut, which leads to improved health. Adding XOS to baked products can improve their physicochemical properties (Precup et al., 2022).

3.4 Hen egg

Eggs have been an essential part of the human diet since ancient times, not only because of their high protein content but also because of their easily digested nutrients, which are necessary for daily growth. As stated by Belitz et al. (2009), egg white is an aqueous, gel-like liquid composed of four distinct layers with varying viscosities: the outer thin layer next to the shell membrane, the gluey outer thick layer, the inner thin white, and the so-called (chalaziferous) inner thick layer (*Figure* 3). According to Burley and Vadehra (1989), the percentages of each layer are 23.3%, 57.3%, 16.8%, and 2.7%, respectively, and they vary based on the breed of hens, their surroundings, the size of the eggs, and the pace of production (Mine, 2008). It was also discovered that there was more ovomucin in the thick albumin.

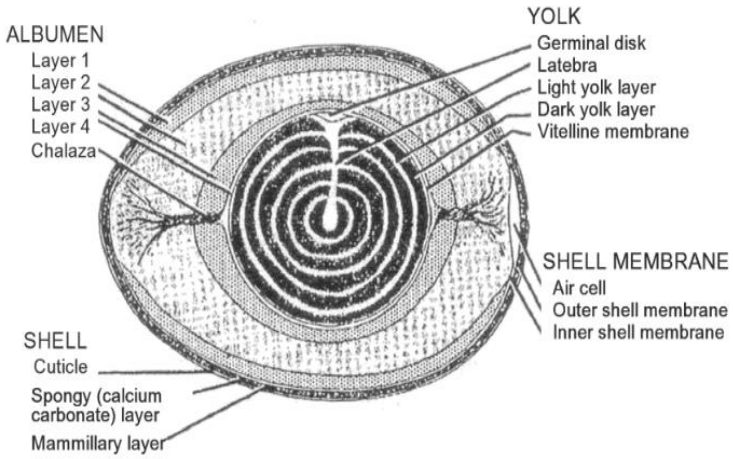


***Figure* 3. Egg parts** (Source: Mine, 2008).

3.5 Egg white protein

Egg white involves mainly of water (88%), protein (11%), trace amounts of carbohydrates, ash, and lipids (1%). Egg white protein includes primarily egg albumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3%), and ovomucin (3%), avidin (0.05%), cystatin (0.05%), ovomacroglobulin (0.5%), ovo-flavoprotein (0.8%), ovo-glycoprotein (1.0%), and ovoinhibitor (1.5%) are minor proteins (Kovacs-Nolan et al., 2005), these proteins are known for their highly functional properties (Abeyrathne et al., 2013).

3.5.1 Ovalbumin (albumin)

Molecular weight of ovalbumin is 45 kDa (Abeyrathne et al., 2013), the most prevalent protein in egg white which constitutes around 50% of total egg white protein since it is a monomer of phospho-glycoprotein, and due to the difference in the phosphorylation level it divides into 3 types of ovalbumin A1, A2, and A3. Moreover, it has a heterogenous carbohydrate chain including a monosaccharide, disaccharide, and polysaccharide. Ovalbumin contains 3 cysteine residues and they react differently to different chemical reagents after denaturation by heat, cold, and surface treatments. The determination of cysteine groups is considered to be an efficient method to evaluate the degree of ovalbumin denaturation. Heating ovalbumin solution results in a turbid suspension as the turbidity depends on protein concentration, pH, and ionic strength, additionally when the pH is acidic or alkalic a transparent solution is obtained (Damodaran, 2017).

3.5.2 Ovo-transferrin

Ovo-transferrin (conalbumin) is the second major protein in egg white (12%) (Abeyrathne et al., 2013) and it is made of a single polypeptide chain with 78 kDA, it is also a monomeric glycoprotein and it belongs to the transferrin family which is known for its capacity binding Fe^{3+} ion per molecule, easy to denture during heating, the denaturation temperature is 60 °C, as its binding metal ion increases its stability (Damodaran, 2017). It was reported that ovo-transferrin terminates the growth of *Pseudomonas sp., Escherichia coli*, and *Streptococcus* mutans (Valenti et al, 1982), besides, *E. coli* O157:H7 and *Listeria monocytogens* (Ko et al, 2008). Moreover, OTAP-92 peptides derived from ovotransferrin are capable of eliminating bacteria by damaging their cell membrane (Ibrahim et al, 2000). Ovo-transferrin can be used for iron supplementation. It binds strongly and readily to iron and releasing it at a pH less than 4.5 (Abeyrathne et al., 2013).

3.5.3 Lysozyme

Lysozyme compromise 3.5 % of total egg white protein. There are many types of lysozyme and C lysozyme is the most common type in hen egg white and the most soluble and stable in comparison to other egg white proteins (Abeyrathne et al., 2013). It has four disulfide bonds with no free

sulphydryl group, the denaturation temperature is 70°C. Lysozyme stays stable at room temperature for 6 months with a pH range of 3.4-9.1 which is related to intramolecular disulfide bonds (Damodaran, 2017). Due to its antimicrobial activity against Gram-negative bacteria and a limited number of Gram-positive bacteria, it is used as a preservative and therapeutic factor. Additionally, it is effective against foodborne pathogens particularly *Listeria monocytogens* and *Clostridium botulinum* which cause most food safety problems (Cegielska-Radziejewska et al., 2008).

3.5.4 Ovomucoid

Ovomucoid constitutes around 10% of total egg white protein with a molecular weight of 28 kDa, and 20-25% of ovomucoid is a carbohydrate. It is a series of asparagine-linked carbohydrate chains consisting of mannose, galactose, and n-acetyl galactosamine.

3.5.5 Ovomucin

A highly glycosylated protein, ovomucin, is majorly comprised of 2 types of subunits; α-ovomucin is homogeneous, whereas β-ovomucin is heterogeneous. α-Ovomucin has 2 subunits called α1 and α2, which have less carbohydrate group than β-ovomucin. In general, 33% of ovomucin is carbohydrate (Abeyrathne et al., 2013). Mine (2008) has reported that at least 3 types of carbohydrate chains were observed in ovomucin as the chain consist of galactosamine, sialic acid, and sulphate with a molecular ratio of 1:1:1:1. Ovomucin inhibits *E. coli*, *Bacillus sp*., and *Pseudomonas sp.,* in addition, it has antimicrobial properties against foodborne pathogens (Omana et al., 2010) thus, it can be used as a food preservative, moreover, it has a strong emulsifying and foaming characteristics which attributed to improving food texture (Abeyrathne et al., 2013). It's worth noting that ovomucin can be counted as a good source of 2 important nutrients, protein, and carbohydrates (Abeyrathne et al., 2013).

3.5.6 Avidin

Molecular weight of avidin is 68.3 kDa, a tetramer made up of four corresponding subunits, each of which binds one mole of biotin. At pH 5.0, the dissociation of the avidin-biotin combination is stable, and its antimicrobial properties are maintained (Belitz et al., 2009).

3.6 Egg white fermentation

In general, the microorganism remains scarcely utilized in egg manufacturing (Nahariah et al., 2019), however, the most frequent utilization of egg white is as a side dish or in baking. Since it wasn't processed yet in order to produce a drink (Milawati et al., 2020). In this regard, Jiang and colleagues (2020) utilized *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to ferment

egg whites for 0, 3, 6, and 9 hours. Based on their findings, 6 hours of fermentation affects the molecular structure of egg whites by increasing surface hydrophobicity and decreasing free sulfhydryl groups. It also improves the rheological properties of egg whites by increasing foaming activity and lowering apparent viscosity, all of which can improve the texture and quality of egg products. When the apparent viscosity of egg white is reduced, it flows more easily. This allows for easier incorporation of air bubbles, resulting in a larger foam ability (Bovšková & Míková 2011). Furthermore, Li and coworkers (2013) investigated the potential of minimizing IgE binding ability after lactic acid fermentation. *Lactobacillus sanfranciscensis*, *Lactobacillus sakei*, and *Lactobacillus delbrueckii subsp*. *delbrueckii* were chosen for this investigation. *L. sanfranciscensis* and *L. sakei* attained pH 5 within 48 hours, while *L. delbrueckii subsp. delbrueckii* achieved that after 72 hours. Although, the SDS-PAGE protein profile of fermented egg white proteins revealed no substantial degradation, *L. delbrueckii subsp. delbrueckii* was the only strain tested that reduced IgE binding in fermented egg white by 50%. This may be connected to ovomucoid, the main egg allergen. Subsequently, they concluded that egg white fermentation with *L. delbrueckii subsp. delbrueckii* lowers the risk to egg allergy. The same prospect was proven by Gazme and coworkers (2022) as they stated that microbial fermentation and Maillard processes can lower the amount of allergenicity in egg white, especially by lowering the IgE binding activity of egg white proteins.

Egg white fermentation with different percentages of milk (0, 2, 4, and 6%) was investigated using mixed LAB bacteria (*L. bulgaricus*, *L. acidophilus*, and *Streptococcus thermophilus*). After 12 hours of fermentation with 4% milk, the quantity of LAB strains flourished, whereas the pH level decreased. Furthermore, fermentation and milk addition didn't affect the total acidity in egg whites (Nahariah et al., 2019). However, when *L. plantarum* FNCC 0027 was propagated in egg white for varying fermentation times (18, 24, and 30 hours) the total cell count, and total acidity increased while the pH level decreased, since the cell concentration reached its highest point of 6.13 Log10 CFU/mL, and the pH level reached 6.4 after 30 hours suggesting that 24 hours of egg white fermentation enhances the ability of bacteria to grow Nahariah et al., (2013). Milawati et al. (2020) examine the organoleptic quality of fermented egg white, including aroma, taste, preference, and colour changes, and concluded that egg white fermentation can enhance its aroma and flavour while also conferring a desired yellowish-white hue. Lin and Cunningham (1984) studied the possibility of egg white fermentation using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* with varying percentages of skim milk, gums (guar gum, carboxymethyl cellulose, or xanthan gum), soymilk, and glucose. The most favourable results were obtained when 47.4% egg white was mixed with 28.4% alkali-treated soymilk, 19.0% skim milk, 1.9% glucose, 2.8%

sucrose, 0.5% xanthan gum, and 0.01% vanilla extract. The final product had 7.52% protein, 0.57% fat, and 62 calories. Furthermore, the product was free of pathogens and had a longer shelf life at refrigeration temperature.

In addition, fermented egg white powder was evaluated at different drying temperatures of 45, 50, and 55 °C and over periods of 30, 39, and 48 hours to determine its most effective functional characteristics. The results showed that 50 °C for 39 hours drying increased the egg white powder's solubility and coagulation time while 45 °C for 48 hours increased the egg white powder's foaming capacity and stability (Nahariah et al., 2018). Furthermore, Pratama et al. (2019) examined the effect of fermentation time and starter concentration on the physicochemical and functional attributes of egg white powder. Three different concentrations of *Kluyveromyces lactis* (1.0%, 2%, and 0.6%) were tested, as well as two different fermentation times (12 and 24 hours). The results revealed that, in addition to fermentation time, the concentration of the added starter has a significant effect on the physicochemical and functional properties (pH, yield, solubility, water, ash, and carbohydrate content, as well as colours (L*, a*, b*)) of the egg white powder, nevertheless increases its protein content. Egg white powder's physicochemical and functional qualities were optimized by fermenting with 0.6% stater for 24 hours before drying. A liquid egg white (v/v) was also incorporated into yogurt in various ratios of 15%, 30%, and 45% (v/v). After 3 hours of fermentation, 45% of the egg white addition increased protein content by 8.50% as it recorded acceptable sensory characteristics (Gogo, 2012). Similarly, Bouhadi and coworkers (2021) examined the impact of different egg white inclusion levels (1%, 2%, 3%, 4%, and 5%) on the production of yogurt. During a 28-day storage period, an increase in dry matter, protein, viscosity, and density was observed, as 2 and 3% are suitable to produce egg white yogurt with no discernible negative effects on its physicochemical, microbial, or sensory properties.

3.7 Fruit juice

Fruits and vegetables are plentiful in a variety of biologically active substances that improve overall health and lower the risk of chronic diseases involving cardiovascular disease. Further, they are the most common source of phenolic substances as well as polyphenols, that have antioxidative, immunomodulatory, and antibacterial properties (Henning et al., 2017). Moreover, they also include a lot of fermentable fibre with a prebiotic effect considering that high fibre consumption is linked to a lower risk of heart disease, type 2 diabetes, and certain cancers (Dahl et al., 2017). They also contain oligo-saccharides which survive during digestion in the small intestine and are transferred to the colon where they serve as nourishment for the bacteria in the

gut (Simpson & Campbell, 2015). Additionally, lactose intolerant and non-dairy food consumers may choose fruit drinks containing probiotics (Antunes et al. 2013; Martins et al. 2013).

Fruit juices are popular because they have a distinctive taste that attracts all ages, and they are refreshing drinks wealthy in nutrients that are favourable for health, such as vitamins, minerals, dietary fibre, and antioxidants. Additionally, they lack the allergenic factors that are found in dairy products (Pimentel et al., 2019). Although they are rich in nutrients and do not contain starter cultures that compete for nutrients with probiotics, they might be an alternative vehicle for incorporating probiotics (Henning et al., 2017; Pimentel et al., 2019). Consumers are becoming more and more interested in functional foods that contain bioactive ingredients like fibre, oligosaccharides, or probiotic microbes. Furthermore, several foods that include probiotics or prebiotics are milk based, however producers and consumers are also interested in non-dairy products. Fruit juices that can be consumed by wide segments of populations and have been considered to be healthful have gained greater interest these days (Horackova et al., 2018). Fruit juices supplemented with probiotic varieties are, however, limited by certain factors (Perricone et al. 2015). These factors include low protein and amino acid content as well as low pH due to high levels of phenolic substances, flavonoids, and organic acids; these foods may not be the best for probiotic purposes and may even inhibit bacterial growth (Nualkaekul et al. 2013). Ascorbic acid, which lowers redox potential (Antunes et al. 2013), saccharides, or organic acids suitable as a carbon source (Nualkaekul & Charalampopoulos 2011) are examples of substances that conversely may enable bacteria to grow in fruit juices. The microbial strain and its inoculum, the juice's composition, which determines pH, oxygen concentration, the presence of antimicrobial compounds, natural dyes and flavours, and the production processes and subsequent treatment (pasteurization, storage temperature, and packaging material used) are all factors that interfere with the viability of probiotics in fruit matrices (Perricone et al. 2015).

In contrast, many researchers have proven that some fruit juices might be a good carrier for probiotics, such as, Worku and coworkers (2019) investigated the growth of *Lactobacillus acidophilus* in three different fruit juices (orange, banana, and apple) using five different sugar types (table sugar Brix 11, table sugar Brix 15, glucose, fructose, and sucrose) for 36 hours. The findings demonstrated that all studied fruit juice was a suitable matrix for propagating *Lactobacillus acidophilus* since their pH levels dropped and titratable acidity rose with extended incubation time. Brix 15, and Brix 11 were superior for probiotic bacteria growth more than when glucose, fructose, and sucrose were added. Similarly White and Hekmta's (2018) study demonstrated the applications of probiotic fruit juices as a possible dairy product alternative. The study examined the growth of *Lactobacillus rhamnosus* GR-1 in three different fruit juices (apple

cider, orange, and grape) fortified with either short-chain or long-chain inulin fibre at a rate of 4% over 72 hours and 30 days of refrigeration. The results showed that the average cell population reached at least 10^7 CFU/mL, with the apple cider juice containing the highest sensory score. Tomato, orange, and grape juices were also found to be effective carriers for both *L. plantarum* and *L. acidophilus*; however, during the first 24 hours, *L. acidophilus* was seen to consume the sugar at a higher rate than *L. plantarum*. since the pH was low and the acidity was high in both cultures (Nagpal et al., 2012). Thakur and Joshi (2017) also reported that apple juice might be a promising probiotic carrier for *L. plantarum* and *S. thermophilus,* as antioxidant activity, antibacterial activity, and viable cell counts increased after 72 hours of fermentation, whereas vitamin C and total phenol levels decreased. Furthermore, the number of coliform, yeast, and mould colonies reduced as antibacterial activity rose. Upon four weeks of cold storage, the amount of lactic acid bacteria in the apple juices remained within the required range ($4.76\text{-}6.00*10^6$ CFU/ml). Meanwhile, fermentation didn't affect the attractive characteristics of apple juices.

It was found that combining whey with pineapple juice (65:35) made a satisfactory probiotic beverage since, after 24 hours, the *Lactobacillus acidophilus* cell count grew to $8.38*10^8$ CFU/ml while the total acidity reduced to 0.93 g/100ml. A, exhibiting a shelf life of 24 days at 5°C and 48 hours at 30°C (Shukla & Emire, 2013). Priya and Vasudevan (2016) assessed whether papaya juice was suitable for the preparation of probiotic juice with *L. plantarum* and *L. acidophilus*. The juice was fermented with a variety of inoculum sizes (1-3%) and time durations (24–72 hours). The optimal conditions for papaya juice production were 48 hours of fermentation and a 3% inoculum size.

Moreover, probiotic guava juice was produced using *Lactobacillus acidophilus* (MTCC no. 10307) at a concentration of $10^8\text{-}10^9$CFU/ml, and a pH 3.99. Over four weeks of cold storage, *Lactobacillus* viability sustained higher than 8 log CFU/ml and the yeast and mould counts were acceptable for the whole eight-week storage period. Overall, probiotic guava juice was found to be highly acceptable (Natt & Katyal., 2022). Over and over, a mixture of purple cabbage, tomato, and carrot was used to cultivate *Lactobacillus plantarum.* During the 48-hour fermentation period, the amount of lactic acid, titratable acid, glucose, and pH all dropped, and the culture of *Lactobacillus plantarum* increased to 9.13 log CFU/mL. Furthermore, there has been a minor decrease in oxidation activity (Yang et al., 2020).

3.7.1 Pineapple juice

The pineapple (*Ananas comosus (L.) Merrill*) is the third most important tropical fruit crop, behind bananas and mangoes as it belongs to the order *Bromeliales*, family *Bromeliaceae*, subfamily

Bromelioideae. According to FAO (2005), 15,287413 tons of pineapples were produced globally; over half of the world's output was accounted for by pineapples grown in Thailand, the Philippines, Brazil, China, and India. Processed pineapple, such as canned chunks, spears, slices, juice, and concentrates, makes up the vast majority of pineapple exported internationally. Pineapples are an excellent source of carbohydrates, fibre, citric acid, copper, magnesium, manganese, and vitamins A, C, and B group as they are greatly influenced by geographic and climatological conditions for plant growth (Sun et al., 2016; Ancos et al., 2016).

The worldwide pineapple juice industry has grown fourfold since 1984. The United States and the European Union account for more than 90% of the global pineapple juice and concentrate market (Khalid et al., 2016) due to their high nutritional value, as these compositions vary based on geography, culture, harvest season, and processing time.

100 mL of pineapple juice includes 4.1 g sucrose, 2.5 g fructose, 2.3 g glucose, 124–130 mg potassium, 12–15.4 mg magnesium, 3.1–8.0 mg phosphorus, 0.2-0.31 mg iron, 0.3-0.99 mg manganese and 9.2 - 93.8 mg vitamin C (Krueger et al., 1992; Cárnara et al., 1995; Elkins et al., 1997; Kabasakalis et al., 2000; Luximon-Ramma et al., 2003; Khalid et al., 2016). In addition, 100 g of pineapple juice contains 50 kcal, 0.14 g fat, 12.2 g carbohydrate, and 0.36 g protein (USDA, 2019).

The primary amino acids that are present in pineapple juice are asparagine, proline, aspartic acid, serine, glutamic acid, tyrosine, valine, and isoleucine as tyrosine and tryptophan are the two major aromatic amino acids of pineapple (Gawler, 1962). 100 mL of pineapple juice contain 3.6 mg tyrosine, 1.8 mg serotonin, and 2.2 mg tryptophan (Khalid et al., 2016), Additionally, Mohamed et al., (2014) reported the following amino acid concentrations in pineapple juice: asparagine 1.8-5.3 mmol/L, methionine 0.2-0.6 mmol/L, histidine 0.1-0.2 mmol/L, and lysine 0.1-0.2 mmol/L, which can vary based on their origin. At pH less than 7, the amino acids and sugars produce hydroxy methyl furfural in the Maillard reaction which is an important quality indicator in the preparation of pineapple juice (Khalid et al., 2016).

Pineapple juice is also rich in antioxidant components that are related mostly to phenolic compounds without any considerable variances in the total polyphenol content of natural fresh pineapple juice and commercial pineapple juice (Mahdavi et al., 2010), since the total phenolic content in fresh pineapple juice reached 36.2 mg GAE/100mL (Mohamed et al., 2014). P-coumaric acid, caffeic acid, ferulic acid, sinapic acid, p-coumaroylquinic acid, feruloyl glucose, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, and syringic acid are among the phenolic acids

found in pineapple juice, in addition to phenolic content, pineapple also rich in carotenoids such as violaxanthin, leuteoxanthin, beta-carotene, and neoxanthin (Khalid et al., 2016).

Numerous studies have examined the possibility of fermenting pineapple juice such as Hossain and coworkers (2016) who studied the development of *Lactobacillus fermentum* and *Lactobacillus desidiosus* in pineapple juice over 48 hours, as both strains were able to develop meanwhile with a decrease in pH and an increase in acidity via consuming the fruit juices. Following three weeks of storage under refrigeration, the strains' viability remained at 10^7 CFU/100 mL.

Utilizing different probiotic strains including *L. plantarum* 299v, *L. acidophilus* La5, and *Bifidobacterium lactis* Bb-12 for pineapple juice fermentation was studied by Nguyen et al. (2019) with and without the presence of fructose or FOS. Their findings showed that the tested strains flourished without the need to supplement pineapple juice with extra nutrients. After 24 hours of fermentation, the bifidobacteria cell counts increased to 10^9 CFU/mL, while the lactobacilli cell counts surpassed $5*10^9$ CFU/mL. In addition, *L. plantarum* 299v had the highest volumetric productivity. All studied strains preferred fructose as a sugar source. In addition, the total phenolic content and antioxidant capacity increased somewhat but declined during storage as the number of microorganisms did not vary much throughout the first month of storage. Also, Costa et al. (2013) evaluated *Lactobacillus casei* NRRL B442 as a starting culture for sonicated pineapple juice fermentation. After 24 hours of fermentation, the cell count reached 8.7 Log CFU/mL, and sucrose declined while glucose and fructose increased. For the storage examination, the juice was categorized into sweetened samples with 10% w/v sucrose and non-sweetened samples. The microbiological viability was 6.03 Log CFU/mL in the non-sweetened sample and 4.77 Log CFU/mL in the sweetened sample after 42 days of refrigeration additionally, the pH of both samples decreased when lactic acid accumulated during storage and there was no browning, and the juice preserved its characteristic colour throughout its shelf life. Gangwar and coworkers (2016) developed a yogurt-fruit juice blend. Different fruit juice ratios (5%, 10%, and 15%) from various fruits (pineapple, apple, and sweet lemon) were mixed into milk and cultured with a mixed culture including *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Overall, yogurts supplemented with 5% and 10% pineapple juice and apple juice performed well. Yogurts containing 10% pineapple juice had more moisture, less total solid content, and less protein, fat, and ash than plain yogurt.

3.7.2 Strawberry juice

Strawberry (*Fragaria sp*.) is a perennial herbaceous plant belonging to the Rosaceae family. With grapes, it is the most generally accessible fruit. It is grown all over the world, but mainly in tropical

and subtropical areas -the United States, Japan, Mexico, Italy, and Lebanon - because of their low demand for cooling.

Glucose is the major carbohydrate in strawberries, accounting for more than half of their carbohydrates. Anthocyanin is the pigment that provides the red colour. The fruit taste is related to a high concentration of aromatic esters. The strawberry is a fruit with low-calorie carbohydrate content, more fibre and more vitamin C than oranges. Strawberry's essential ingredients include vitamin C (64.0 mg/100 g), water (91.75 g/100 g), protein (0.61 g/100 g), fat (0.37 g/100 g), carbohydrate (7.02 g/100 g), fibre (2.3 g/100 g), calcium (14.0 mg/100 g), potassium (166.0 mg/160 g), vitamin A 27 IU, and total anthocyanin content ranges between 200 and 600 mg/kg, with pelargonidin-3-glucoside constituting 77–90% of the anthocyanins in the strawberry extracts followed by pelargonidin-3-rutinoside (6–11%) and cyanidin-3-glucoside (3–10%) (Silva et al., 2007). The low pH affects colour stability. The acidity ranges from 0.58 to 1.35%, with citric and malic acids being the most prominent organic acids that contribute to its flavour (Ayub et al., 2010). Strawberries also contain the flavonoid quercetin, a natural anti-inflammatory agent that minimizes atherosclerosis risk. Further, its high concentration of anthocyanin and polyphenols reduces the risk of a heart attack, besides, it's fibre and potassium content that have beneficial effects on the heart. Because of their antioxidant action against free radicals, they prevent tumour formation and reduce inflammation in the body. They additionally contribute to regular bowel movements (Nishu et al., 2021).

However, because of their fast respiration rate and high-water content, strawberries are a particularly sensitive fruit that can be harmed by mechanical stress, water loss, and microbial infections while stored (Sanz et al., 1999; Perkins, 2010). The fruit must be treated carefully during transportation to the distant market since it is fragile, and it should be consumed as quickly as possible. Strawberry is one of the first fresh fruits available on the market in the spring. Consumer demand for the fruit is expanding because of its amazing flavour, attractive colour, and structure (Ayub et al., 2010). European strawberry production has been on the rise to keep up with growing consumer interest (Hernández-Martínez et al., 2023). As strawberries are prized for their quality and freshness in Europe, local farmers supply most of the market. However, there's still a need for imported strawberries, especially when they are not in season locally (Carpineti et al., 2024)

Fermentation might extend their shelf life since many studies have been conducted to investigate the possibility of strawberry juice fermentation. In this instance, Zhao and coworkers (2021) examined how strawberry fermentation influenced the biological and sensory qualities since the starting culture included a combination of *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus bulgaricus*. The juice had a primary fermentation for 30 days at 25 ˚C and then

performed a mature fermentation for a total of 6 months at 16 ˚C. Their findings showed an increase in overall antioxidant capacity and a decrease in the content of phenols and flavonoids. According to a sensory investigation, the average ratings for colour, appearance, and taste were higher in fermented beverages but flavour and acceptability were lowered. In addition, Fang and coworkers (2019) reported that the fermentation of strawberry juice by *Lactobacillus delbrueckii subsp. bulgaricus* F17 and *Leuconostoc lactis* H52 can reduce the loss caused by strawberry decaying. Furthermore, Cataldo et al. (2020) proposed that strawberry juice could be used as a great plant-based carrier for *Levilactobacillus brevis* CRL 2013 as after 168 hours of fermentation the final product had a high gamma-aminobutyric acid (GABA) that possesses anti-inflammatory, anti-hypertensive, and antidepressant properties.

Moreover, the effects of Brix (9,11,13), pH (pH 3 and pH 4), time (0h, 7h, 14h, 21h, 28h), and bacteria (*Lactobacillus casei* and *Lactobacillus plantarum*) on the manufacturing of probiotic strawberry juice were assessed by Berimavandi and Fadaei Noghani (2018). Their findings have shown that while pH, vitamin C, total soluble solids, and bacterial population decreased during storage, acidity increased. Also, by rising Brix, there were rises in soluble solids, microbial numbers, and acidity, however, vitamin C levels decreased. As pH rose, vitamin C and bacterial count increased, while acidity lowered. The results showed that pH 4 and Brix 13 had the highest probiotic bacteria survival and that *Lactobacillus casei* had greater growth.

As well as the study by Nualkaekul et al. (2012), probiotic cells may be effectively delivered by instant juice powders, such as strawberry powder, which can serve as a substitute for very acidic fruit drinks. In their investigation, *Lactobacillus plantarum* was mixed with several fruit powders (cranberry, blackcurrant, strawberry, and pomegranate) and stored for over a year in addition to being mixed with water on monthly intervals. Two amounts of inulin and gum arabic (10% and 20% w/w) were added to the instant fruit powders. Cranberry powder had the lowest rates of cell survival over a 12-month storage period, whereas blackcurrant powder and strawberries had the greatest rates. Gum arabic and inulin did not significantly affect the dried fruit powder's ability to reconstitute.

Also, strawberry puree was supplemented with varied levels of soy milk (10, 20, 30, and 40%) along with sugar addition and using a *Streptococcus thermophilus* and *Lactobacillus bulgaricus* for fermentation. It was found that adding soy milk at a 20% level improved the colour and appearance levels of the final product slightly. However, as the soy milk incorporation ratio increased flavour, texture, sweetness, and overall acceptability reduced. In conclusion, the 20% soy milk addition enhanced the moisture and protein content of strawberry puree (Devi et al., 2018).

3.7.3 Peach juice

The peach (*Prunus persica L.*) is a member of the subgenus *Amygdalus* within the family *Rosaceae* (Hancock et al., 2008). Besides being rich in organic acids and natural sugars, peaches are also high in potassium and vitamin A. These ingredients enhance the nutritional value of peaches. Consuming peaches has therapeutic effects because they reduce the development of reactive oxygen compounds in human blood plasma (Dinkecha & Setu, 2021). Peach fruits have laxative properties (Mrázová et al 2021) and are often utilized to alleviate duodenal ulcers and prevent constipation. Climate, agronomic approaches, and cultivar variances all have an impact on fruit phytochemical composition (Tsantili et al., 2010). A 100g serving of peach fruit contains 42 kcal, 0.91 g protein, 0.27 g fat, 10.1 g carbohydrate, 1.5 g fibre as the insoluble dietary fibre is higher than soluble fibre, and minerals such as calcium (4 mg), iron (0.34 mg), magnesium (8 mg), phosphorus (22 mg), potassium (122 mg), sodium (13 mg), zinc (0.23 mg), copper (0.078 mg), selenium (2.1 µg), vitamin C (4.1 mg), vitamin A (24 µg), vitamin K (3 µg) (Grigelmo-Miguel et al., 1999; USDA, 2020).

The main ones responsible for the sweetness and/or sourness taste in peaches are sugars and organic acids while phenolic compounds, add bitterness or astringency. Many soluble sugars and sugar alcohols may be found in peach fruit; the most prevalent ones are sucrose, glucose, fructose, and sorbitol. Sucrose makes up 40 to 85% of the total sugar content of mature peach. Glucose and fructose, in varying amounts, contribute 10 to 25 %, while sorbitol makes up less than 10%. Mature peach fruit contains additional sugars such as xylose, trehalose, isomaltose, raffinose, and galactinol, along with polyols galactinol, glycerol, myoinositol, and maltitol (Cirilli et al., 2016) since low-quality peaches contain higher levels of sorbitol and glucose and less fructose (Cirilli et al., 2016).

Because peaches contain a lot of flesh, which makes it difficult to extract the juice, most peaches are processed into puree. This puree is then used as the starting point for producing peach juice products. Peach juice puree contains 0.5 g/100 g of organic acids, mostly citric and malic acids as well as 10 mg/100 g of hydroxycinnamic acids. Hydroxycinnamic acids, β-carotene, vitamin E, and copper are key micronutrients in peach juice products (Khomich et al., 2019).

Pakbin and colleagues (2014) investigated the possibility of utilizing peaches as a raw material for lactic acid bacteria to generate probiotic peach juice, using *Lactobacillus plantarum* DSMZ 20179, *L. delbrueckii* DSMZ 15996, and *L. casei* DSMZ 20011. Following a 48-hour fermentation period at 30°C, *L. delbrueckii* exhibited good growth in peach juice, reaching $10*10^9$ CFU/mL. Conversely, the cell concentration of *L. casei* was approximately 10^9 CFU/mL. As the bacteria

consumed more sugar and produced more lactic acid it lowered the pH. Upon four weeks of refrigeration, the viable cell counts of *L. delbrueckii* in fermented peach juice were $1.72*10^7$ CFU/mL. they also stated that the conditions of cold were not suitable for *L. casei*. Further research on peach-based probiotic drinks has been conducted by Parveen and Ain (2023). Peach pulp was fermented with probiotic *Lactobacillus casei* for 3 to 10 days at different temperatures (15, 20, 25, 30, and 35°C). While acidity, cell populations, and total probiotic count grew greatly over 3 weeks of storage, the pH, total soluble solids and total sugars declined markedly. The probiotic density varied in range from 8.29 to 12.68 Log CFU/mL respectively. Colour, taste, flavour, odour, and overall appeal all substantially decreased after storage, with fermented samples at 20 °C receiving the greatest ratings in terms of sensory attributes.

4 Materials and methods

4.1 Materials

4.1.1 Egg white drink (ToTu drink)

Caprivous LTD (Budapest, Hungary) provided fresh egg-white drink (ToTu) bottles, which were kept cooled at 4±1°C until usage. Egg white drink is made by separating egg yolk, then the egg white is heated to about 80 °C and the pH is adjusted to around 6.5. After enzymatic treatment, the dry matter was adjusted to 6. *Table* 1 shows it also has a low calorie and high protein content, being lactose, milk, fat, and preservative-free. The product has a texture, colour, and taste analogous to milk.

***Table* 1: Nutritional contents of egg white drink (ToTu)**

Carbohydrate	Protein	Dry matter	Energy in 100 g
0.1 g/100 g dry matter	5.6 g /100 g dry matter	6 g /100 g	23 kcal dry matter

Source: (Caprivous LTD, 2023)

4.1.2 Carbohydrate sources

- Glucose and sucrose were purchased from Reanal, Hungary.
- D (-)-Fructose was purchased from Reanal Finomvegyszergyár ZRT.
- Fructo-oligosaccharides were brought from Orafti®Inulin HSI, which contains 88% inulin, DP ≥ 23.
- Galacto-oligosaccharides were brought from DMO®Vivinal ® GOS powder, Netherlands.
- Xylo-oligosaccharides were brought from LONGLIVE, 95P.

4.1.3 Fruit Juices

- Pineapple and strawberry juice (25% fruit) (Hey-Ho, Budapest, Hungary) were commercially purchased from the local market. The pH of fruit juices was adjusted to pH 6.1-6.5 before fermentation.
- 100% pineapple fruit juice (Happy Day juice, Rauch Hungaria, Hungary) was bought commercially at the local market. Before fermentation, the pineapple juice's pH had been modified to 6.5 through the addition of 4N NaOH.
- Strawberries were purchased from the local market. They were then pressed to extract the juice, followed by thermal pasteurization and pH adjustment to pH 6-7 using 4N NaOH before fermentation.

4.1.4 Bacterial strain

Bacterial strains were purchased according to *Table* 2 then, they were activated by adding them to the MRS liquid media in the case of *Lactobacillus* strains and TPY liquid media in the case of *Bifidobacterium* and incubating them under aerobic conditions in the case of *Lactobacillus* and anaerobic condition in the case of *Bifidobacterium* for 24 hours at a temperature of 37 °C degrees and then used to inoculate the egg white products. The initial cell counts of the probiotic strains used were approximately 10^9 CFU/mL.

***Table* 2. Probiotic strains applied and their source**

Strain	**Source**
Lactiplantibacillus plantarum 299v	Christian Hansen
Lacticaseibacillus casei 01	Christian Hansen
Lactobacillus helveticus R-52	Lallemand Health Solutions
Lacticaseibacillus rhamnosus Rosell-11	Lallemand Health Solutions
Ligilactobacillus salivarius CRL 1328	Probiotical
Lactobacillus acidophilus 150	Christian Hansen
Levilactobacillus brevis HA-112	Lallemand Health Solutions
Limosilactobacillus fermentum HA-179	Lallemand Health Solutions
Lactobacillus helveticus Lafti RL10	Lallemand Health Solutions
Limosilactobacillus reuteri HA-188	Lallemand Health Solutions
Lacticaseibacillus rhamnosus HA-111	Lallemand Health Solutions
Ligilactobacillus salivarius HA-118	Lallemand Health Solutions
Lactobacillus crispatus LCRO1	Probiotical S.p.A.
Lacticaseibacillus rhamnosus GG ATCC53103	Probiotical S.p.A.
Limosilactobacillus fermentum LF08	Probiotical S.p.A.
Lactobacillus acidophilus La-5	Christian Hansen
Bifidobacterium longum DSM 16603	Probiotical
Bifidobacterium longum Bb46	Christian Hansen
Bifidobacterium bifidum Rosell-71	Lallemand Health Solutions
Bifidobacterium lactis Lafti^{R}B94	Lallemand Health Solutions
Bifidobacterium longum Rosell-175	Lallemand Health Solutions
Bifidobacterium lactis Bb12	Christian Hansen

4.1.5 MRS media

MRS (De Man, Rogosa, Sharpe) media is a selective culture medium used to isolate and count lactic acid bacteria. MRS agar was prepared by combining the appropriate amount of the contents as in Table 3 with distilled water and 15g agar and autoclaving for 15 minutes at 121 °C.

***Table* 3. Composition of the MRS medium (Corry et al., 2003).**

Components	Quantity
Glucose	20 g
Proteose peptone	10 g
Beef extract	8 g
Yeast extract	4 g
Sodium acetate	5 g
Tri ammonium citrate	2 g
Dipotassium hydrogen phosphate	2 g
Tween 80	1 ml
Magnesium sulphate	0.2 g
Manganese sulphate	0.05 g
Distilled water	1000 ml

4.1.6 TSA media

Tryptic Soy Agar (TSA) was prepared by combining the appropriate amount of the contents as in *Table* 4 with distilled water and autoclaving for 15 minutes at 121 °C.

***Table* 4. Composition of the TSA media (VWR, Leuven, Belgium)**

Components	Quantity
Pancreatic digest of casein	17 g
Papaic digest of soyabean meal	3 g
Sodium chloride	5 g
Dextrose	2.5 g
Dibasic potassium phosphate	2.5 g
Agar	15g
Distilled water	1000 ml

4.1.7 TPY media

TPY **(**Tryptone Peptone Yeast Extract**)** was used for selective isolation of *Bifidobacterium.* TPY agar was prepared by combining the appropriate amount of the contents as in *Table* 5 with distilled water and 15 g agar and autoclaving for 15 minutes at 121 °C.

***Table* 5. Composition of the TPY media** (Gorgun and Ersan, 2019).

Components	Quantity
Proteose Peptone	10 g
Phytone Peptone	5g
Glucose	5 g
Yeast extract	2.5 g
Potassium hydrogen phosphate	2g
Tween 80	1 ml
Cysteine HCl	0.5 g
Magnesium dichloride hexahydrate	0.5 g
Zinc sulphate	0.25 g
Calcium chloride	0.15g
Ferric chloride hexahydrate	0.03g
Distilled water	1000 ml

4.1.8 Saline solution

0.85% w/v NaCl solution was prepared in the laboratory by adding 8.5 g of NaCl to 1000 mL of distilled water and has been distributed 4.5 mL to tubes and sterilized by autoclave at 121 °C for 15 minutes.

4.2 METHODS

4.2.1 Fermentation of egg white drink fortified with different carbohydrate source

A 1 % (v/v) inoculum of *Lactobacillus* and *Bifidobacterium* strains was mixed into 100 ml of egg white drink. Sequentially, distinct carbohydrate solutions (fructo-oligosaccharides, xylo-oligosaccharides, galacto-oligosaccharides, fructose, glucose, and sucrose) at a concentration of 20% (w/v) each were individually incorporated, aiming to achieve a 2% (w/v) sugar concentration in the resultant products. Control samples, devoid of sugar supplementation, were employed for comparative purposes. Replication was undertaken three times for each experimental condition. Incubation was carried out at a constant temperature of 37 °C for a duration of 24 hours.

Subsequent evaluation encompassed key parameters, namely pH, cell growth, viscosity, and quantification of protein content. The results were evaluated using multivariate analysis of variance (MANOVA) and mean comparisons by post hoc test including Tukey HSD test, and Games Howell.

4.2.2 Fermentation of egg white drink fortified with different fruit juices

Egg white (EW) drink and fruit juices - peach (PE), pineapple (PI), and strawberry (ST) - were mixed separately in 3:1 ratio (v/v) as EW: PE represents a mixture of egg white drink with peach juice, EW: PI represents a mixture of egg white drink with pineapple juice, EW: ST represents a mixture of egg white drink with strawberry juice. Three replications were made for each formula. The fermentations were initiated with 1% (v/v) of inoculum using *L. casei* 01 and *L. salivarius* CRL 1328. The incubation temperature was 37°C for 24 hours. During fermentation time the changes in the pH value and the total cell count were monitored for 4, 8, 16 and 24 hours internally. Additionally, the final products were tested for the following parameters: probiotics viability, pH value, viscosity, protein profile by SDS PAGE, colour measurements as well as sensory characteristics of EW: PI, and EW: ST.

4.2.3 Fermentation of egg white drink fortified with different ratios of fruit juice

L. casei 01, and *L. salivarius* CRL 1328 lyophilizate were grown separately in MRS broth before 24 hours of the fermentation, then they were mixed separately with egg white drink, and pineapple/strawberry juice in (4:1, 4:2, and 4:3) (v/v) ratios egg white drink to pineapple/strawberry juice. Three replications were made for each formula, samples without fruit juice were served as control samples. The incubation temperature was 37 °C for 16 hours, after which the final products were tested for the following parameters: pH value, probiotic cell count, viscosity, total protein content, total phenolic content, protein profile, and colour.

4.2.4 Fermentation of egg white drink by a mixed culture

Both *L. casei* 01 and *L. salvarius* CRL 1328 lyophilizate were separately grown in MRS broth prior to the 24-hour fermentation period. After that, an equal amount of the two inoculums was mixed, and 1% (v/v) of the combined strains was added to the egg white drink samples without any extra additions, and to a combination of strawberry juice and egg white in a 3:1 EW: ST (v/v) ratio.

4.2.5 Determination of the pH

The pH levels of the samples were assessed using a digital pH meter (Mettler-Toledo GmbH, Switzerland) following calibration with standard solutions. The pH measurements were taken at

various time points (0, 4, 8, 16, and 24 hours) during the fermentation process, as well as throughout the shelf-life experiment at (1, 2, and 3 weeks) time points for fermented egg white drink samples with different carbohydrate sources. Measurements were also taken at (4 and 8 weeks) for fermented egg white drink samples mixed with different fruit juices.

4.2.6 Determination of the cell count

Tenfold serial dilution of fermented egg white drink with a 0.85% (w/v) NaCl saline solution followed by a pour plate method was used to determine the cell count. After the dilution procedure, 0.1 ml of the suitable dilutions were added to Petri dishes and MRS or TPY agar (45–46 °C) was transferred using pour plates. After a natural cooling process to room temperature and solidification of the agar, the plates were incubated for 72 hours at a constant 37°C, or until colonies were clearly visible. The average colony count has been multiplied by the reciprocal of the sample volume to calculate the visible colonies on the plates accounting for the proper dilution factor. The incubation was carried out aerobically for *Lactobacillus* and anaerobically for *Bifidobacterium* (Boczek et al., 2015).

4.2.7 Determination of specific growth rate and generation time

The specific growth rate (μ) of the cell populations during the exponential phase was measured graphically, as it represents the slope of the growth curve at various time points of fermentation (Perni et al., 2005). The generation time (t_g) is defined as the time taken for the organism to divide in a culture that includes necessary nutrients in an available form and all other environmental factors that are suitable. Generation time was measured using equation (1) according to (Cajal-Medrano & Maske, 1999)

$$t_g = 0.693/ \mu \qquad (1)$$

Where

t_g is the generation time (hour)

μ is the specific growth rate (1/hour)

4.2.8 Determination of volumetric productivity

The volumetric productivity of *Lactobacillus* and *Bifidobacterium* strains was measured using equation (2) (Nguyen et al., 2021).

Volumetric productivity (CFU/mL.h)= Total cell count after 24 hours of fermentation (CFU/mL) - initial cell count (CFU/mL) /fermentation time (hours) (2)

4.2.9 Antagonistic activity

Antagonistic effects of the fermented product were determined by well-diffusion agar assay. After cultivating over an entire night, a pathogenic strain that included *Escherichia coli* 8739, *Escherichia coli* 0157: H7, *Enterobacter cloacae*, *Listeria*, and *Enterococcus faecalis* was carefully mixed with 100 mL of TSA (Tryptic Soy Agar) medium that had been melted and tempered at 45°C before being transferred into Petri dishes. A sterilized cork borer was used to create agar wells (8 mm in diameter) in each of these plates. A 150 µl of the studied samples were pipetted into each well, followed by keeping Petri plates for an hour at 4°C, then an overnight incubation at 37°C, afterward the clear inhibition zones around the well were visually detected (Hossain et al., 2022).

4.2.10 Viscosity measurement

The viscosity of each egg white drink sample was measured by MCR 92 rheometer (Anton Paar, France), in rotational mode equipped with a concentric cylinder (cup diameter 28.920 mm, bob diameter 26.651 mm, bob length 40.003 mm, active length 120.2 mm, positioning length 72.5 mm). Anton Paar RheoCompass software (version 1.21.852) was used to control the equipment. Readings were taken at constant time intervals with progressively increasing shear rate (10 – 500/mins at T=20^0 C. Flow curves were plotted with the values obtained from intervals. Herschel—Bulkley model was successfully fitted to describe the flow curve of the samples based on Abbasnezhad and coworkers (2015) publication.

$$\tau=\tau 0+K\dot{\gamma}^{n} \quad (3)$$

where τ—refers to shear stress (Pa); τ0—indicates the yield stress (Pa); $\dot{\gamma}$—is the shear rate (1/s), K—refers to the consistency coefficient (Pa·sn), and n—is the flow behaviour index (dimensionless).

4.2.11 Total protein content determination

The protein measurement was performed based on the Bradford method (Bradford,1976), a widely used protein analytical method based on dye binding. The principle is that the protein and the colouring substances create a blue complex, as the dye binds to arginyl and lysyl side chains. The maximum absorbance of the complex can be measured at 595 nm. For the calibration curve (*Figure* 4), a tenfold serial dilution was prepared from bovine serum albumin (BSA) with a concentration of 10 mg/ml then 6 tubes including different concentrations from BSA were prepared according to *Table* 6. For example, to prepare tube 2 with a final protein concentration of 0.38 mg/mL, 30 mL of a 10 mg/mL BSA solution was combined with 10 mL of distilled water. Then, to prepare tube

4, 20 mL from tube 2 was mixed with 20 mL of distilled water, resulting in a total protein concentration of 0.19 mg/mL. Based on this, the calculation the samples were diluted to 10^1-10^2 mg/mL using distilled water in order to obtain relevant, evaluable results.

To determine the protein content, 10 µl of the appropriate dilution of the samples was pipetted onto a microplate and reacted with 200 µl of Bradford reagent. After 1 minute, the absorbance of the samples was measured at 595 nm.

***Table* 6. Standard curve preparation method**

Tube	Source of the protein standard	Protein standard volume (ml)	Distilled water Volume (ml)	Final protein concentration mg/mL
1	10 mg/mL BSA	20	0	0.5
2	10 mg/mL BSA	30	10	0.38
3	10 mg/mL BSA	20	20	0.25
4	Tube2	20	20	0.19
5	Tube3	20	20	0.13
6	Tube5	20	20	0.07
7	Tube6	20	20	0.04

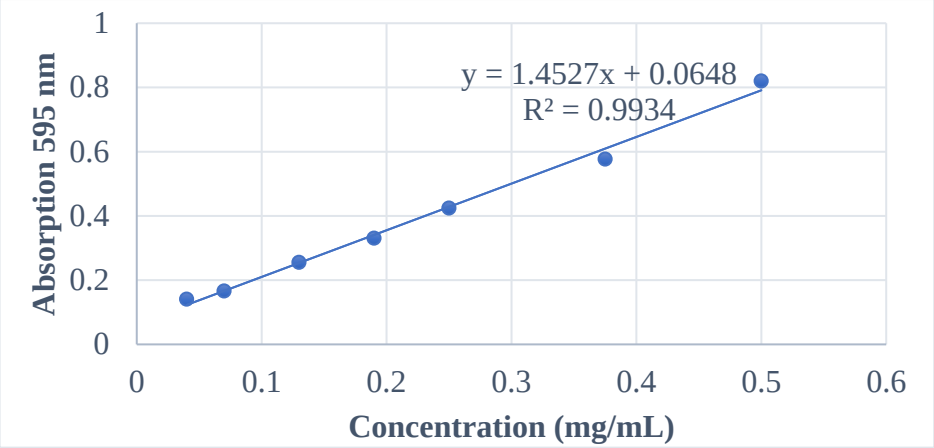


***Figure* 4. Calibration line of protein content determination**

4.2.12 Protein profile

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) was performed to determine the protein profile of egg white drink and fermented egg white drink samples according to protein size (mass). Gels were cast in the laboratory using unpolymerized monomer and buffer components, the stacking gel was 4% pH=6.8, 15% resolving gel pH=8.8 according to *Table* 7. For better results, the protein samples on the same gel were loaded with different protein standards. The protein samples were heated for 1 min at 100 °C in the presence of SDS and β-

mercaptoethanol, and bands were stained with Coomassie Brilliant Blue (R- 250) before loading (Laemmli, 1970). After the electrophoresis process was completed (parameters), the gels were washed with 20% trichloroacetic acid for 20 minutes. They were then rinsed in 850 ml of distilled water, 50 ml of acetic acid, and 100 ml of ethanol for 30 minutes. Next, a mixture of 0.2g Coomassie, 50 ml distilled water, 10 ml acetone, and 50 ml ethanol was used to colour the gel for 30 minutes. The gels were then left overnight in a 10% acetic acid solution using a vortex mixer. To analyse the gels, a digital picture was captured using the Biorad ChemDoc XRS imaging system (USA) and Quantity One software. Finally, the gels were analysed using Image Lab version 5.1 software.

***Table* 7. Sodium dodecyl sulphate-polyacrylamide gel preparation method**

	Resolving gel	**Sacking gel**
Acryl (29:1 Acryl/Bis)	6 ml	0.75 ml
Tris 2M	2.7 ml	-
Tris 0.5 M	-	495 ul
10% SDS solution	75 ul	42 ul
Distilled water	3.09	2.4 ml
TEMED	9 ul	4.5 ul
Ammonium persulphate	75 ul	37.5 ul

4.2.13 Total phenolic content determination

Total phenolic content was determined by the Folin–Ciocalteu method. The samples were prepared according to Koren and coworkers (2021) with some modifications. A 1 mL of fermented egg white drink was centrifuged at 3000 rpm for 2 min in Eppendorf tube, then 10 µl of the supernatant was collected and added to 1250 µl from a mix of ten times diluted Folin-Ciocalteu reagent and 240 µl methanol:water (4:1) solvent. Afterward, the mixture was homogenized and allowed to react for 1 min, then 1 cm^3 of 0.7 M Na_2CO_3 was added, vortexed, and was allowed to stand for 5 min at 50 °C in a water bath before measurement. The absorbance was measured at λ = 765 nm. Gallic acid was utilized as a standard to create a calibration curve. A dilution series was prepared from gallic acid solution with a concentration of 2 mg/mL. The total phenolic content was then calculated as mg gallic acid per millilitre, as shown in *Figure* 5.

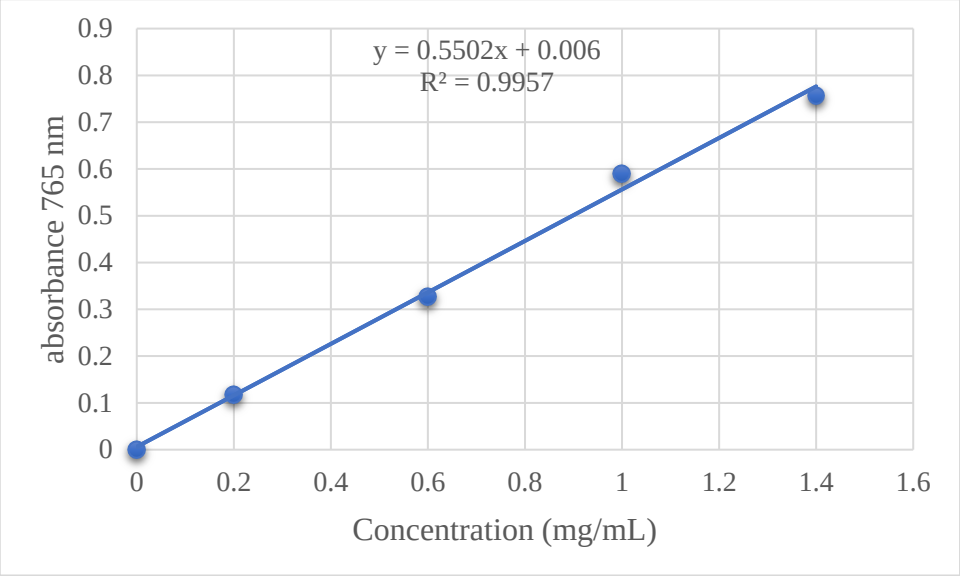


***Figure* 5: Calibration curve of gallic acid**

4.2.14 Titratable acidity determination

The samples were titrated to a pH of 8.2 using 0.1N NaOH. The following formula was used to calculate titratable acidity (Iland et al., 2000):

$$\text{Titratable acidity (Lactic acid g/100 mL)} = V_{NaOH} * C_{NaoH} * E_{C_3H_6O_3} / V_{sample} \quad (4)$$

V_{NaOH}: the consumed volume of titrant to adjust the pH (mL), C_{NaoH}: concentration of titrant (normality), $E_{C_3H_6O_3}$: equivalent weight of lactic acid g/ mol, V_{sample}: volume of sample (mL)

4.2.15 Colour measurements

The colour of the samples was measured with a Konica-Minolta CR-400 chromameter (Konica Minolta Sensing Inc., Osaka, Japan) at room temperature. L * value indicates the lightness ranging from blackness (0) to whiteness (100). a* value ranged from (-60) greens to redness (+60), and b* value ranged from blueness (-60) to yellowness (+60) (Dimitrellou et al., 2020; Chen et al., 2023). Calibration was performed with a calibration tile before starting the measurements. The brightness of materials is determined by the L* parameter, which has a range of 0 to 100.

4.2.16 Sensory evaluation

Besides the fermentation procedure, the sensory analysis of fermented beverages was done. One day prior to evaluation, samples were refrigerated at 4°C. Ten participants conducted the sensory analysis in a laboratory for sensory analysis. At 4 ±1 °C, samples were served in plastic cups that were coded with three-digit random numbers that were written in a random order. During the testing process, people were given water to drink and bread to eat to clean their mouths. Under

bright light, each test was done on a separate table. The tests were conducted using Addinsoft's XLSTAT Sensory Solution (Pro module) software (Kókai et al., 2003).

4.2.17 Determine the shelf life of the products

The viability evaluation procedure included examining bacterial cell counts and pH values to determine the survival rate of bacteria over the course of storage. Fermented samples using *L. plantarum* 299v, *L. casei* 01 with fructose and FOS, egg white pineapple/strawberry juice mixture fermented by *L. casei* 01 and *L. salivarius* CRL 1328 samples, as well as fermented egg white drink with different ratios of strawberry juice were subjected to this process. The time intervals for this analysis were as follows: samples containing fruit juices were analysed every two, four, six, and eight weeks, whereas samples containing diverse sources of carbohydrates were analysed every one, two, and three weeks. Three independent duplicates of each sample were used, and they were kept in a refrigerator at a temperature of 4±1 °C.

4.2.18 Statistical methods

All determinations were made in triplicates for each sample, and data were analysed by multivariate analysis of variance (MANOVA) and mean comparisons by post hoc test including Tukey HSD test, and Games Howell (when homogeneity was violated). A one-way analysis of variance (ANOVA) and pairwise significant differences were used for sensory evaluation tests. Also, two-way analysis of variance (ANOVA) and Tukey HSD test in the case of significant differences were used in the experiments of screening of different lactic acid bacteria and bifidobacteria for the fermentation of egg white drink. All statistical procedures were conducted using the software IBM SPSS27 (IBM Corp, 2020).

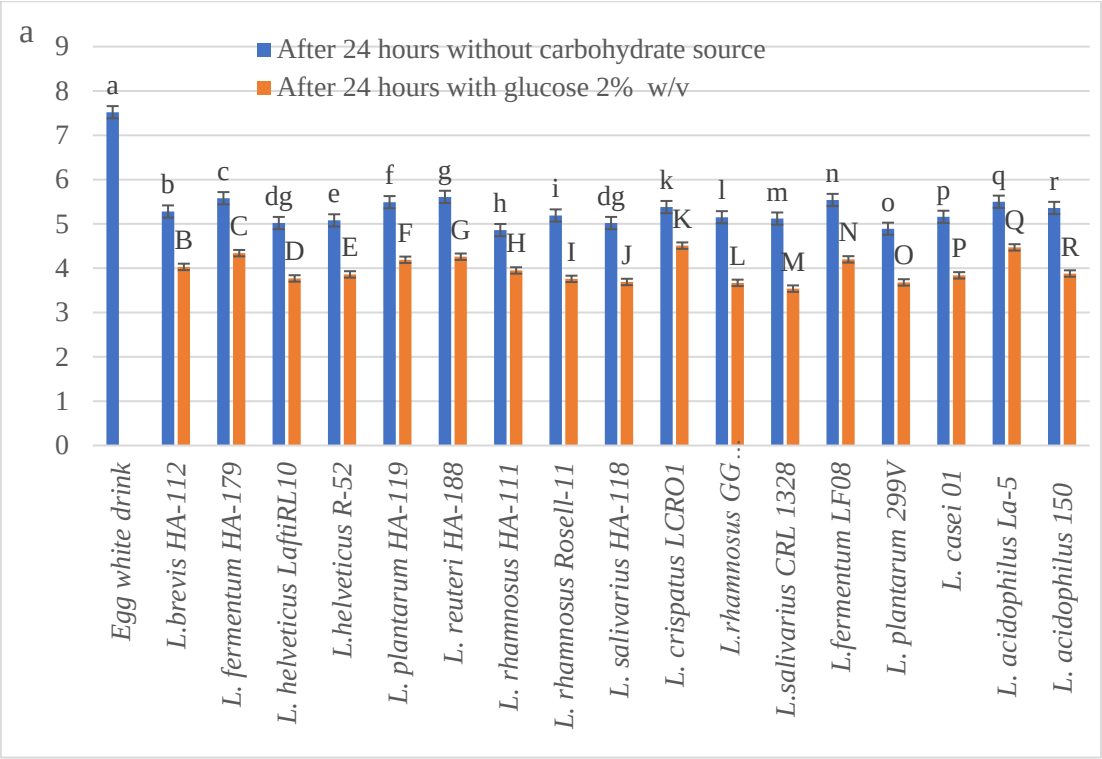
5 Results and discussion

5.1 Screening of different lactic acid bacteria and bifidobacteria for the fermentation of egg white drink

5.1.1 Changes in the pH values during egg white fermentation with and without carbohydrate source

Seventeen *Lactobacillus* strains and seven *Bifidobacterium* strains were cultivated in an egg white drink to assess their ability to grow in this medium and to identify the most suitable strains. *Figure* 6. illustrates the relationship between the strains used and the pH value of the fermented egg white drink, both with and without the addition of 2% (w/v) glucose. It was observed that probiotics could grow in the egg white drink regardless of sugar addition. However, samples with added sugar showed a significant decrease in pH compared to those without sugar. As noted by Mis Solval et al. (2019), egg white hydrolysates promote the growth of probiotic lactic acid bacteria in fermentation media.

These results align with the findings of Suzuki et al. (2004), who studied the effect of adding hydrolysed hen egg white to skim milk on the growth of *L. delbrueckii subsp. bulgaricus* SU-3, *L. casei* SBR 1202, *Bifidobacterium breve* SBR 3213, *B. adolescentis* N-3, and *B. adolescentis* N-4. Their study suggested that adding hydrolysed egg white to milk before fermentation significantly promoted the growth of these strains.



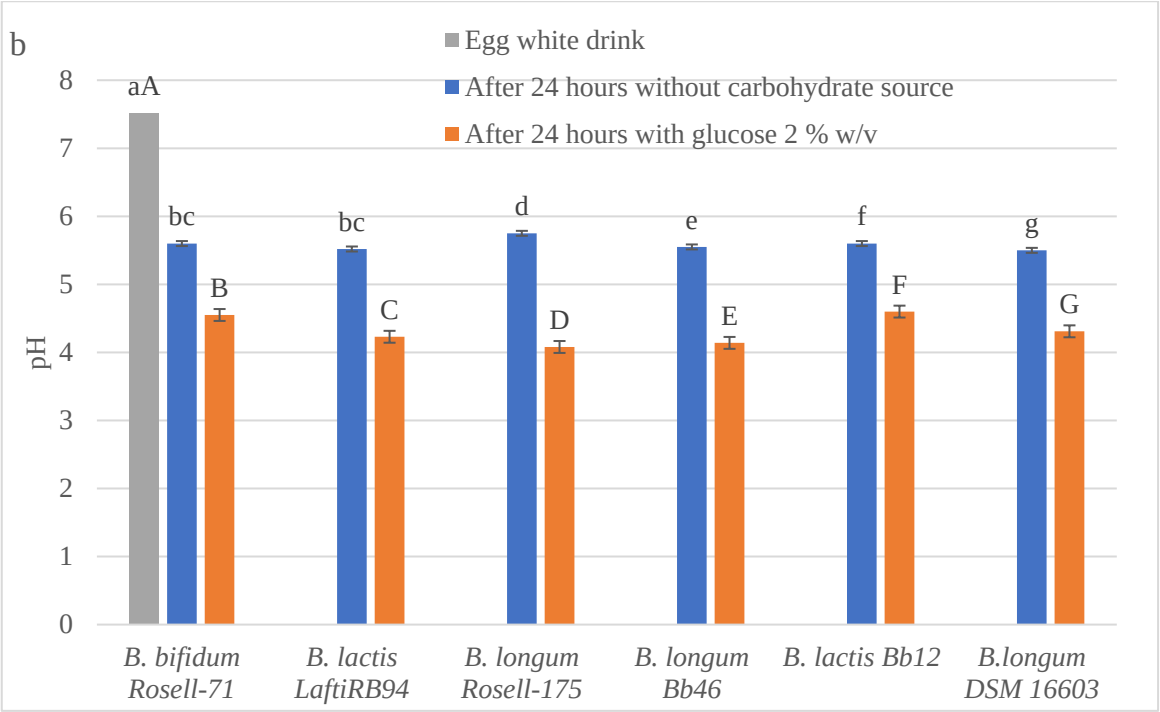


***Figure* 6. The pH of fermented egg white drink after 24 hours with and without carbohydrate source 2.0% (w/v) using *Lactobacillus* (a) and *Bifidobacterium* (b).** Higher case letters indicate the difference between probiotic strains in the presence of glucose 2% (w/v), Lowercase letters indicate the difference between probiotic strains without glucose addition.

5.1.2 Growth of probiotics in egg white drink in the presence of glucose

From ancient times on, fermentation has been used as an economical, efficient, and effective method of food preservation. In preliminary experiments including 17 strains of *Lactobacillus* and 6 strains of *Bifidobacterium,* four *Lactobacillus* strains (*L. acidophilus* 150, *L. plantarum* 299v, *L. casei* 01, and *L. salivarius* CRL 1328), and two *Bifidobacterium* strains (*B. longum* DSM 16603 and, *B. longum* Bb46) were selected based on the pH reduction to monitore the cell growth, along with the pH of medium over 24 hours of fermentation in the presence of glucose 2.0 % (w/v) as a carbohydrate source which is one of the main nutrients that are required to be present in the environment to promote the growth of probiotics. The study found that the growth of all the probiotic strains increased significantly over time, while the pH level decreased due to an increase in the production of organic acids, which accumulated in the medium. All the probiotic strains had a cell number higher than 8 Log_{10} CFU/mL, indicating that egg white drink provides a suitable medium for probiotics to grow in the presence of 2.0% (w/v) glucose.

Based on *Figures* 7a, and 8a it was observed that *L. plantarum* 299v had the highest growth rate (9.26 Log_{10} CFU/mL) and the lowest pH value (3.61) compared to the other samples. The growth rate was found to be approximately 2 Logs higher than the minimum recommended dose for

probiotic bacteria. After 20 hours of fermentation, there was no significant difference between the samples with *L. plantarum* 299v and *L. casei* 01. On the other hand, *L. salivarius* CRL 1328 had the weakest growth in egg white drink, with a growth rate of 8.29 Log_{10} CFU/mL and a pH level of 4.04. After 24 hours of fermentation, the cell counts of *L. acidophilus* 150 increased by 1.95 Logs, *L. casei* 01 by 2.1 Logs, *L. acidophilus* 150 by 2.45 Logs and *L. salivarius* CRL 1328 by 1.91 Logs.

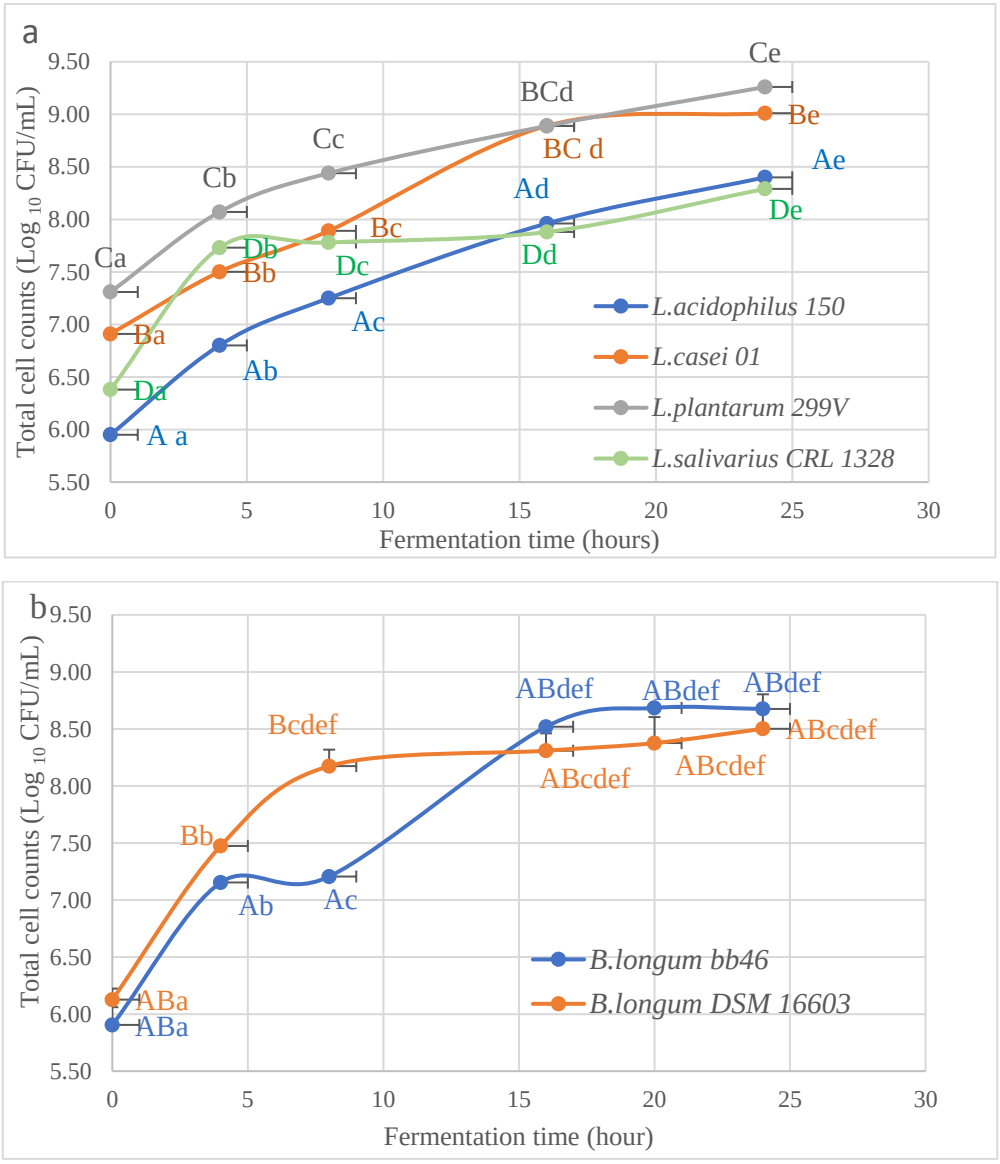


***Figure* 7. The growth of probiotic strains in egg white drinks with glucose 2% (w/v) during 24 hours of fermentation time. Using *Lactobacillus* strain (a), and *Bifidobacterium* (b).** Higher case letters represent the difference between strains at the same period, and lowercase letters show the significant distinct over fermentation time for the same strain.

These results were consistent with the findings of Nahariah and coworkers (2013), who reported an increase in the growth rate of *L. plantarum* FNCC 0027 and a decrease in the pH level of egg white over time. However, the growth rate of *L. plantarum* FNCC 0027 in their study was lower

than ours as it was 6.04 Log_{10} CFU/mL after 24 hours of fermentation due to different fermentation conditions, as they conducted the experiment without adding carbohydrates. Furthermore, LAB has limited growth in egg white drinks due to their low carbohydrate content (0.4-0.9%) as reported by (Nahariah et al. 2019).

Regarding the growth of *Bifidobacterium*, (*Figures* 7b and 8b) *B. longum* Bb46 showed an increase of 2.78 Logs, while *B. longum* DSM 16603 increased by 2.37 Logs. The growth of the two tested strains were not significantly different after 24 hours of fermentation. *B. longum* Bb46 reached its highest cell concentration after 16 hours, while *B. longum* DSM 16603 peaked at 8 hours of fermentation with considerably lower cell concentration compared to *B. longum* Bb46.

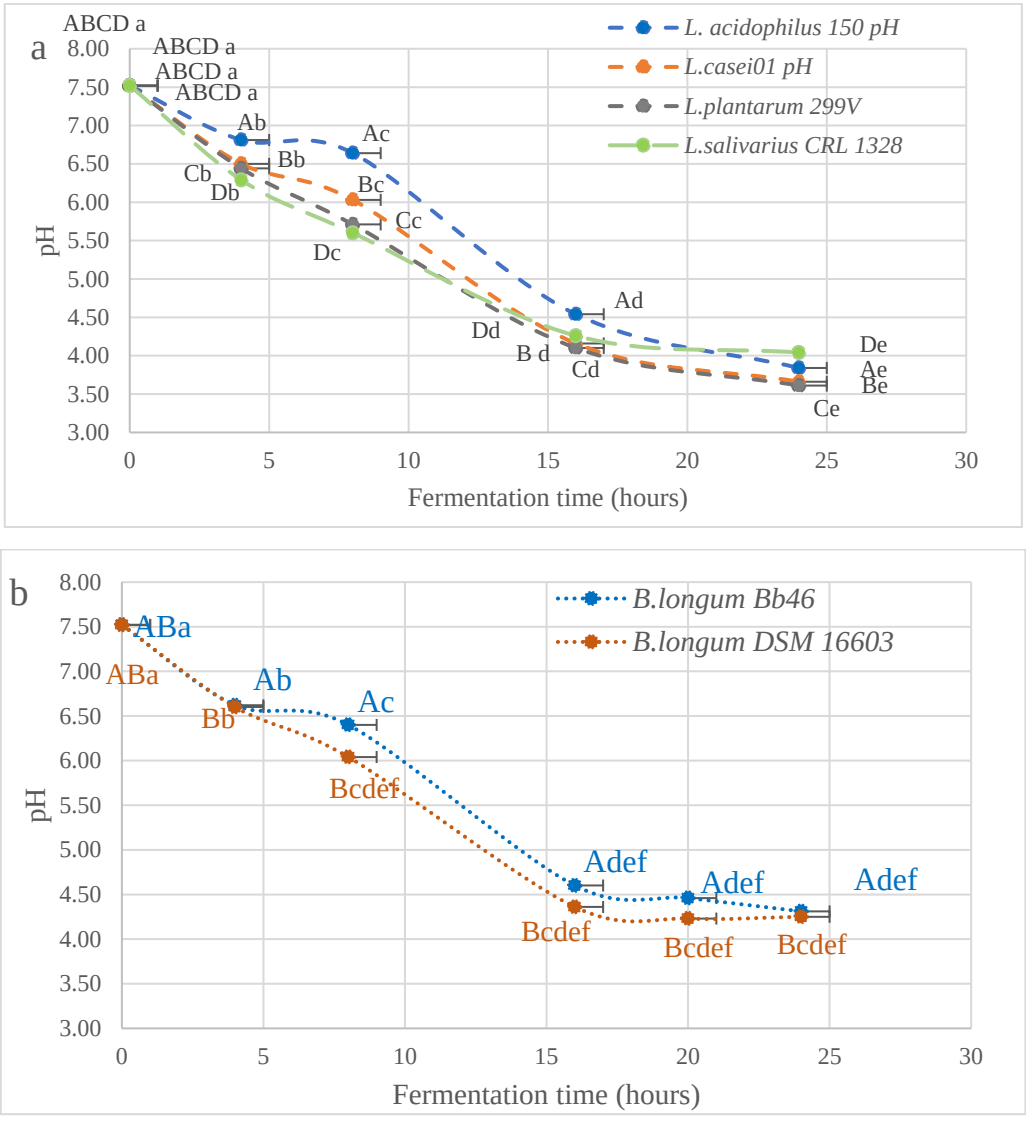


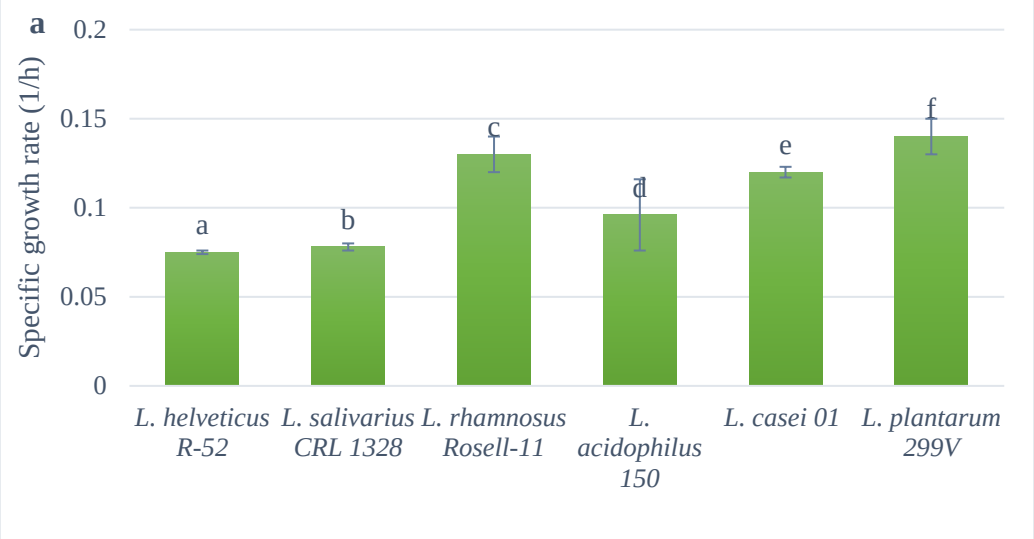
***Figure* 8. The pH of fermented egg white drinks with glucose 2% (w/v) during 24 hours of fermentation time using *Lactobacillus* strain (a), and *Bifidobacterium* (b).** Higher case letters represent the difference between strains at the same period, and lowercase letters show the significant distinct over fermentation time for the same strain.

In general, the pH reached 4.30 after 24 hours of fermentation. Our results were higher than those obtained by Chou and Hou (2000) for growth of *Bifidobacteria* in soy milk. In their study, the cell counts of *B. infantis* CCRC 14633 reached 7.57 Log_{10} CFU/mL, and *B. longum* B6 was at 5.96 Log_{10} CFU/mL in the presence of glucose 1.0% (w/v).

5.1.3 The specific growth rate of selected *Lactobacillus* and *Bifidobacterium* strains in egg white drink with glucose

The specific growth rate (μ) is defined as the relative change of microbial biomass per unit of time (Fecskeová et al., 2021). It indicates how quickly the microorganisms are growing and is crucial for understanding microbial kinetics and optimizing bioprocesses. In addition to medium composition, the growth of *Lactobacillus* spp. can be influenced by various conditions such as temperature, pH, oxygen concentration, and water activity, as well as fermentation medium composition (Śliżewska & Chlebicz-Wójcik, 2020).

Figure 9 illustrates the specific growth rate of different *Lactobacillus* strains (*L. helveticus* R-52, *L. acidophilus* 150, *L. plantarum* 299v, *L. rhamnosus* Rosell-11, *L. casei* 01, *L.salivarius* CRL1328) and *Bifidobacterium* strains (*B.longum* Bb46, *B.longum* DSM 16603) in egg white drink after 24 hours of the cultivation.



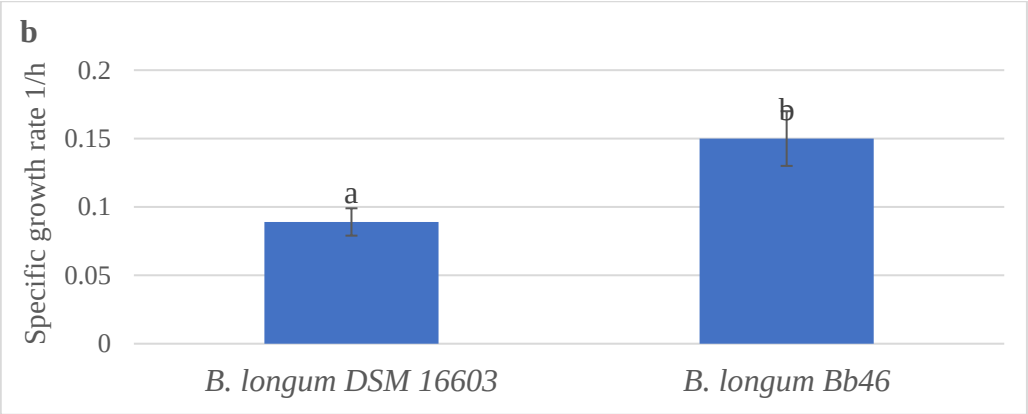


***Figure* 9. The specific growth rate of the selected probiotic strains in egg white drink containing glucose 2% (w/v). *Lactobacillus* (a), *Bifidobacterium* (b).** Lowercase letters show the significant differences between the strains used in egg white drink fermentation.

In *Figure* 9a, it is evident that the cell population of *L. plantarum* 299v exhibited the fastest growth with a specific growth rate value of 0.14 1/h, while *L. helveticus* R-52 had the slowest growth with a specific growth rate value of 0.075 1/h. This result was lower than that of Passos et al., (2003) as *L. plantarum* had a specific growth rate of 0.20 1/h in a medium containing yeast extract, trypticase, and ammonium sulphate. It was relatively similar to the results of Mesquita et al., (2017), who found that *L. plantarum* LFBM 02 and *L. rhamnosus* ATCC 9595 01 exhibited specific growth rates of 0.17 and 0.20, respectively, when grown in MRS. In the case of *Bifidobacterium* strains, *B. longum* DSM 16603 showed better results than *B. longum* Bb46 after 24 hours of fermentation. In general, *Bifidobacteria* grew better than *Lactobacilli* (*Figure* 9b).

5.1.4 The generation time of selected *Lactobacillus* and *Bifidobacterium* strains in egg white drink with glucose

The generation time, defined as the duration required for the initial bacterial population to double during the exponential growth phase (Delignette-Muller, 1998), holds considerable importance in optimizing processing conditions to achieve maximal efficiency in production. In industrial fermentation processes, for instance, the utilization of faster-growing strains can enhance productivity and reduce the production timeline required to attain the desired product.

Table 8 displays the generation times of various *Lactobacillus* (*L. helveticus* R-52, *L. acidophilus* 150, *L. plantarum* 299v, *L. rhamnosus* Rosell-11, *L. casei* 01, *L. salivarius* CRL1328) and *Bifidobacterium* strains (*B. longum* Bb46, *B. longum* DSM 16603) cultivated in an egg white drink supplemented with 2% glucose. The findings reveal that *L. plantarum* 299v exhibited the shortest generation time at 4.95, while *L. helveticus* R-52 demonstrated the longest, requiring more time to

double. Notably, *B. longum* Bb46 demonstrated quicker propagation in the glucose-enriched egg white drink compared to *B. longum* DSM 16603.

Our results notably contrasted with those reported by Śliżewska and Chlebicz-Wójcik (2020), where *L. plantarum* ŁOCK 0860 exhibited a generation time of 2.06 hours and *L. rhamnosus* ŁOCK 1087 exhibited a time of 2.61 hours, utilizing MRS as the growth medium.

***Table* 8. The generation time of selected probiotic strains grow in egg white drink containing glucose** 2.0 % (w/v)

Strain	**Generation time (h)**
Lactobacillus	
***L. helveticus* R-52**	9.24 ± 0.21^{a}
***L. salivarius* CRL 1328**	8.88 ± 0.02^{b}
***L. rhamnosus* Rosell-11**	5.33 ± 0.001^{c}
***L. acidophilus* 150**	7.22 ± 0.03^{d}
***L. casei* 01**	5.78 ± 0.003^{e}
***L. plantarum* 299v**	4.95 ± 0.02^{f}
Bifidobacterium	
***B. longum* DSM 16603**	7.79 ± 0.2^{g}
***B. longum* Bb46**	4.62 ± 0.2^{h}

Lowercase letters indicate the significant differences between the used strains in egg white drink fermentation.

5.2 Study the effect of incorporating mono-, di- and polysaccharides into egg white drink fermentation

On the basis of the previous investigations, the probiotics *L. acidophilus* 150, *L. casei* 01, and *L. plantarum* 299v were chosen to be studied for their ability to grow in egg white drink when monosaccharides (glucose and fructose), disaccharides (sucrose), and oligo-saccharides (fructo-oligosaccharides, galacto-oligosaccharides, and xylo-oligosaccharides) were inserted at a concentration of 2% (w/v). Control samples were made without the addition of sugar. After 24 hours of fermentation, the final product's total cell numbers, pH, and rheological characteristics were measured.

5.2.1 Growth of probiotics

Figure 10, shows the growth of probiotic strains on various carbon sources. The strain utilized was an isolated culture of *L. acidophilus* 150 with an initial cell counts of $1.46*10^{7}$ CFU/mL, *L.*

casei 01 4.35*10^6 CFU/mL, and *L. plantarum* 299v 1.22*10^7 CFU/mL. Fermentation studies showed that probiotic bacteria could be cultivated in egg white drink without the addition of a carbohydrate source. The initial cell counts increased significantly after 24 hours of fermentation. This might be because probiotics are capable of survival by using free sugars (glucose form) in albumin, as well as the existence of glycoproteins that consist of mannose and galactose units (Lin, 1982).

In line with *Figure* 10, a XOS addition to the egg white drink samples revealed no discernible differences in the cell populations of *L. acidophilus* 150 (8.70 Log_{10} CFU/mL), *L. casei* 01 (8.86 Log_{10} CFU/mL), and *L. plantarum* 299v (8.81 Log_{10} CFU/mL). Also, *L. casei* 01 had the strongest preference for FOS and fructose among other studied carbohydrate sources, as it had much higher proliferation, reaching 9.33-9.55 Log_{10} CFU/mL, correspondingly. Moreover, its development in the presence of glucose or sucrose was comparable to that of *L. acidophilus* 150, while having a substantially greater cell counts than *L. plantarum* 299v. Similarly, *L. plantarum* 299v was not a suitable starter culture to be used when a monosaccharide source was added. This finding is consistent with a previous study by Lin (1982) which indicated that *L. plantarum* ATCC 21088 did not grow well in liquid egg white.

In addition, *L. acidophilus* 150 displayed a higher preference for mono- and disaccharides over oligo-saccharides. These findings are consistent with Lin's (1982) study, which concluded that adding 2% glucose enhances the growth of *Lactobacillus* bacteria.

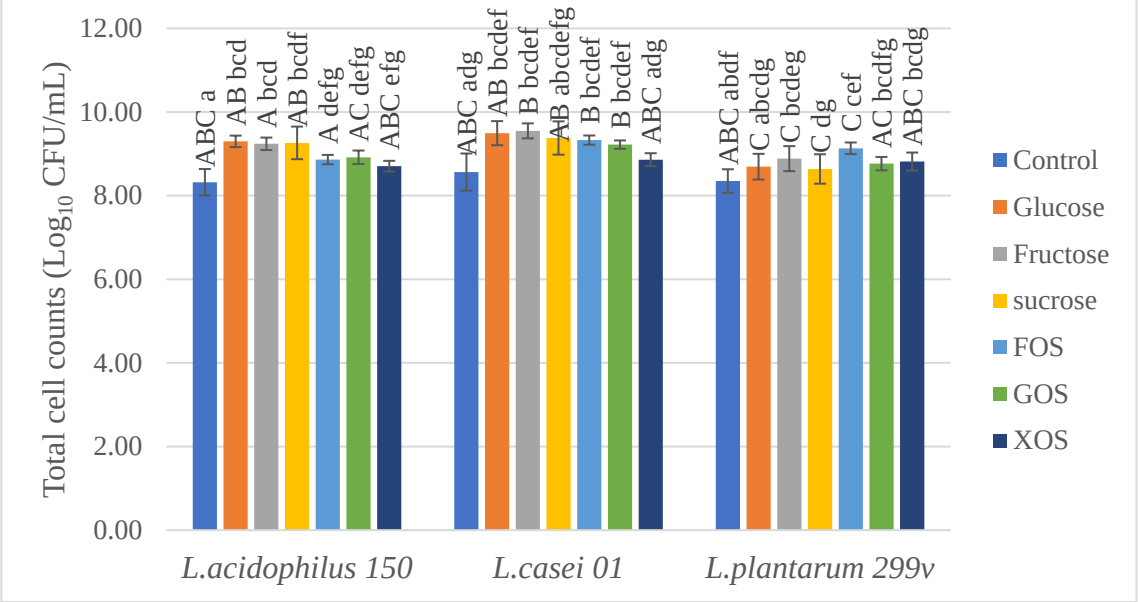


***Figure* 10. Probiotic growth in egg white drink using 2% (w/v) different types of carbohydrate sources after 24 hour fermentation.** Higher case letters (from A to C) indicate the difference when different probiotic strains were used with the same carbohydrate source, and

lowercase letters (from a to g) show a significant difference when different carbohydrate sources were added with the same strain. Control: fermented egg white drink without carbohydrate source, glucose: fermented egg white drink with glucose solution 2% (w/v), fructose: fermented egg white drink with fructose solution 2% (w/v), sucrose: fermented egg white drink with sucrose 2%, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% (w/v), GOS: fermented egg white drink with galacto-oligosaccharides solution 2% (w/v), XOS: fermented egg white drink with xylo-oligosaccharides solution 2% (w/v).

5.2.2 Changes in the pH values of egg white drink fermented by selected *Lactobacillus* strains

As indicated in *Table* 9, after 24 hours of fermentation, the studied *Lactobacillus* strains utilized the nutrients present in egg white drink and produced organic acids released into the medium, thus decreasing the initial pH value (pH 7) of EW drink at different rates; however, the pH reduction was higher in samples with carbohydrate source due to conversion the sugars into lactic acid. Based on Nahariah and coworkers (2019) have studied the fermentation of egg white by a mixed culture of *L. bulgaricus, L. achidopilus, and Streptococcus thermophilus* with various ratios of milk (0, 2, 4, and 6%). These results support their findings that increasing the milk ratio reduces the pH by increasing the sugar content of the milk in the form of galactose and glucose, as the pH value of the samples without milk reached 6.57 after 24 hours of fermentation, which is close to that of the control samples. Since, egg white completed with fructose, FOS, GOS, or XOS reacted differently when a different strain was used for instance the pH value of samples with *L. casei* 01 decreased significantly compared to other samples reached a value of pH 3.73, pH 3.97, pH 4.05, pH 4.92 with fructose, FOS, GOS, and XOS, respectively.

***Table* 9. The pH value of fermented egg white drink using different probiotic strains and various carbohydrate sources.**

Carbohydrate source	***Strain***		
	L. acidophilus 150	*L. casei* 01	*L. plantarum* 299 v
Control	6.02 ± 0.12^{aAC}	$6.31\pm0.08^{a\,B}$	6.04 ± 0.10^{aAC}
Glucose	$3.49\pm0.04^{bc\,A}$	$3.62\pm0.06^{bc\,B}$	$4.05\pm0.17^{bcd\,C}$
Fructose	$3.53\pm0.05^{bc\,A}$	3.73 ± 0.0006^{bcB}	$4.09\pm0.1^{bcd\,C}$
Sucrose	$3.90\pm0.005^{d\,A}$	$4.17\pm0.05^{df\,BC}$	$4.28\pm0.18^{bcde\,BC}$
FOS	$4.52\pm0.04^{\,e\,A}$	$3.97\pm0.02^{ef\,B}$	$4.36\pm0.0005^{de\,C}$

GOS	$4.76\pm0.0004^{f\,A}$	$4.05\pm0.08^{def\,B}$	$5.07\pm0.03^{f\,C}$
XOS	$5.25\pm0.07^{g\,AC}$	$4.92\pm0.04^{g\,B}$	$5.26\pm0.004^{g\,f\,AC}$

Small letters indicate the difference in pH value when a different type of sugar was used (reads vertically), and big letters indicate the difference in pH value when a different strain was used (reads horizontally). Control: fermented egg white drink without carbohydrate source, glucose: fermented egg white drink with glucose solution 2% (w/v) , fructose: fermented egg white drink with fructose solution 2% (w/v), sucrose: fermented egg white drink with sucrose 2%, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% (w/v), GOS: fermented egg white drink with galacto-oligosaccharides solution 2%, XOS: fermented egg white drink with xylo-oligosaccharides solution 2% (w/v).

Whereas the pH value of fermented EW by *L. acidophilus* 150 was the lowest when glucose or sucrose was added. Distinctly control samples had significantly the highest pH value in comparison with the other samples with carbohydrate sources followed by, *L. plantarum* 299v, *L. acidophilus* with incorporated XOS, and *L. plantarum* 299v with GOS consecutively, considering that XOS was not the best carbohydrate source compared to others carbohydrate sources. In the case of *L. casei* 01 using FOS was not significantly different from GOS regarding the pH level, however, the difference when adding fructose or glucose was also not substantial in all samples regardless of the probiotic strain.

5.2.3 Changes in the rheological properties of fermented egg white drink by selected *Lactobacillus* strains

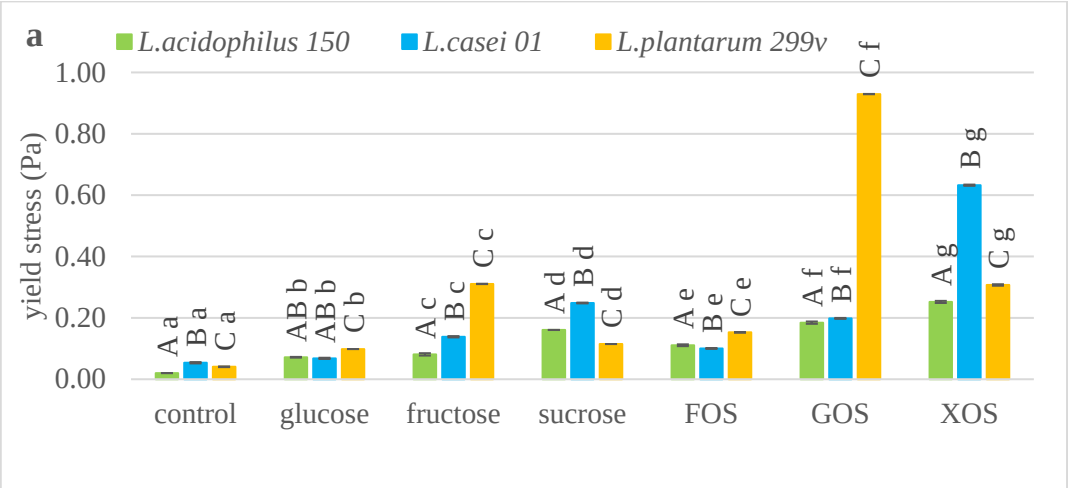
The study of the rheological properties of fluid foods and their variations in temperature and concentration has been an important part of the industrialization of food technology worldwide. It is important for designing a transport system, designing equipment (heat exchangers and evaporators), and determining pump capacity as well as power requirements for mixing (Keshani *et al*., 2012).

After 24 hours of fermentation, the rheological properties of the EW drink with various carbohydrate sources were investigated. The drink was fermented by three probiotic lactic acid bacteria, *L. acidophilus* 150, *L. casei* 01, and *L. plantarum* 299v.

The yield stress of a substance is an essential measurement that describes the lowest amount of stress required to induce flow or deformation. Yield stress is important in a variety of applications, including pumping system design and optimizing the processing of complicated fluids. It is useful in applications requiring extremely accurate material flow and deformation surveillance, for instance, manufacturing and processing. According to *Figure* 11a, all studied samples had stress

yield values higher than 0, in addition, adding a carbohydrate source to an EW drink during fermentation substantially raised the yield stress from 0.02-0.05 Pa in the control samples and to 0.07-0.9 Pa in carbohydrate source samples; furthermore, using different strains in the fermentation represented a remarkable variance among all samples utilizing the same sugar type except for the case of glucose addition fermented samples by *L. casei* 01 and *L. acidophilus* 150 were not significantly different in yield stress. It is clear that fermented EW samples with *L. plantarum* 299v and GOS addition were stronger and more resistant to deformation, as they required significantly more power to start flowing than other investigated samples. Furthermore, when glucose, fructose, and FOS were incorporated into egg white beverage, *L. plantarum* 299v as a starter culture exhibited a larger stress yield than *L. casei* 01 and *L. acidophilus* 150. Meanwhile, fermented samples of *L. casei* 01 had greater stress yield compared to other samples with sucrose and XOS inclusion as they reached 0.05, 0.25, 0.63 Pa, respectively.

The consistency coefficient defines the relation between shear stress and shear rate in a non-Newtonian fluid. A higher K means a thicker fluid, whereas a lower K suggests a less stiff or resistant fluid. The study found that adding FOS or not adding any sugar did not significantly affect the K values of *L. acidophilus* 150 samples (0.004 Pa.s^n) (*Figure* 11b). However, adding GOS resulted in a higher K value compared to samples with other carbohydrate sources. Likewise, the K value of the samples containing XOS and *L. casei* 01 was greater, reaching 0.009 Pa.s^n. However, out of all the samples examined, those that had fructose or FOS addition together with



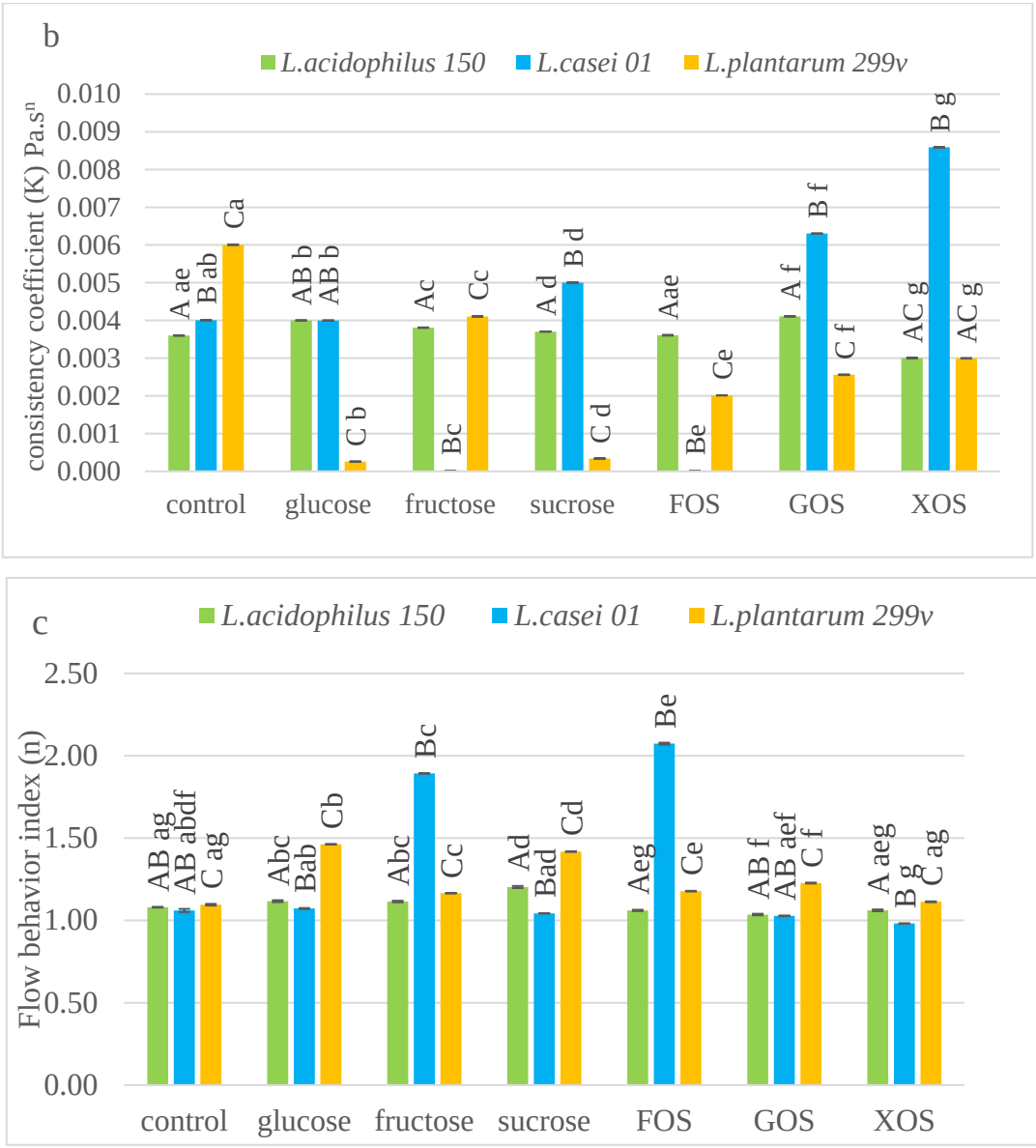


***Figure* 11. Rheological characteristics of a fermented egg white beverage including several probiotic strains and sources of carbohydrates. (a) yield stress, (b) consistency coefficient, (c) flow behaviour index.** Uppercase letters show the significant difference between when different strains were used with the same carbohydrate source, lowercase letters indicate the significant difference when different carbohydrate sources were examined with the same strain. Control: fermented egg white drink without carbohydrate source, glucose: fermented egg white drink with glucose solution 2% (w/v), fructose: fermented egg white drink with fructose solution 2% (w/v), sucrose: fermented egg white drink with sucrose 2%, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% (w/v), GOS: fermented egg white drink with galacto-oligosaccharides solution 2% (w/v), XOS: fermented egg white drink with xylo-oligosaccharides solution 2% (w/v).

L. casei 01 had the lowest K values. Additionally, samples with *L. plantarum* 299v and glucose addition had the highest K value compared to fermented samples with other sugar sources.

According to *Figure* 11c, the flow behaviour index of the examined materials was more than one, indicating dilatant shear thickening, except in samples containing XOS, where it was 0.98, indicating shear thinning or pseudoplastic behaviour. Shear thinning is a time-independent flow in which the apparent viscosity decreases as the shear rate rises. Paints, concentrated polymer solutions, and dispersed systems such as inks exhibit this behaviour. This type of behaviour occurs when a system has a structure that can be reversibly broken down when stressed. A shear thickening (dilatant) fluid exhibits a reversible increase in apparent viscosity as the shear rate increases (Braun & Rosen, 1999). Additionally, samples of *L. casei* 01 with fructose and FOS added exhibit strong dilatant properties. In the case of control samples and samples with GOS, the flow behaviour index of *L. casei* 01 and *L. acidophilus* 150 did not differ from each other. Meanwhile adding glucose, sucrose, GOS, and XOS to fermented samples by *L. plantarum* 299v presented a higher n value compared to other strains when the same sugar type was tested.

5.3 The effect of cold storage on the fermented egg white drink using *L. plantarum* 299v

The study assessed the fermentation of egg white drink by *L. plantarum* 299v utilizing fructose and FOS (fructo-oligosaccharides) as carbohydrate sources. Following fermentation, the changes in viability of *L.plantarum* 299v, pH, protein profile, and rheological properties of the final products were monitored during cold storage. Measurements were obtained at weekly intervals for a total of three weeks (weeks 1, 2, and 3).

5.3.1 Changes in viability

Figure 12 indicates that after a 24-hour fermentation process and three weeks of refrigeration, the samples without a carbohydrate source had a significantly lower cell counts compared to the samples that contained one. The total cell counts of the control samples were not notably different from the samples that were stored in the first and second weeks of refrigeration ($p>0.05$). However, the viable cell counts decreased to 8.12 ± 0.19 Log_{10} CFU/mL in the third week. During storage, samples with fructose as a carbon source had consistent cell counts ranging from 9.27 to 9.44 Log_{10} CFU/mL. Also, samples containing FOS had relatively stable cell counts until the second week of storage, when they greatly increased to 9.73 ± 0.09 Log_{10} CFU/mL due to the metabolism of sugar by *L. plantarum* 299v. Compared to the fermented samples with fructose the samples with FOS showed no significant difference in the cell counts in the first two weeks but were higher than those with fructose in the third week. Since Martins *et al*., (2013) mentioned that the minimum required cell number is 10^6 CFU/mL in fermented food products by probiotics to have an advantageous health effect on the human body and that was compatible with our results where the CFU/mL was higher than 10^7 during storage time if carbohydrate sources were added or not. Temperature of refrigeration also played a major role in maintaining the viability of the fermented

product. Yoon and coworkers (2006) had the same findings, in their study, they evaluated the viability of probiotics in cabbage juice during storage in the refrigerator. The results showed that *L. plantarum* and *L. delbrueckii* could maintain their bioavailability at 4 °C for several weeks.

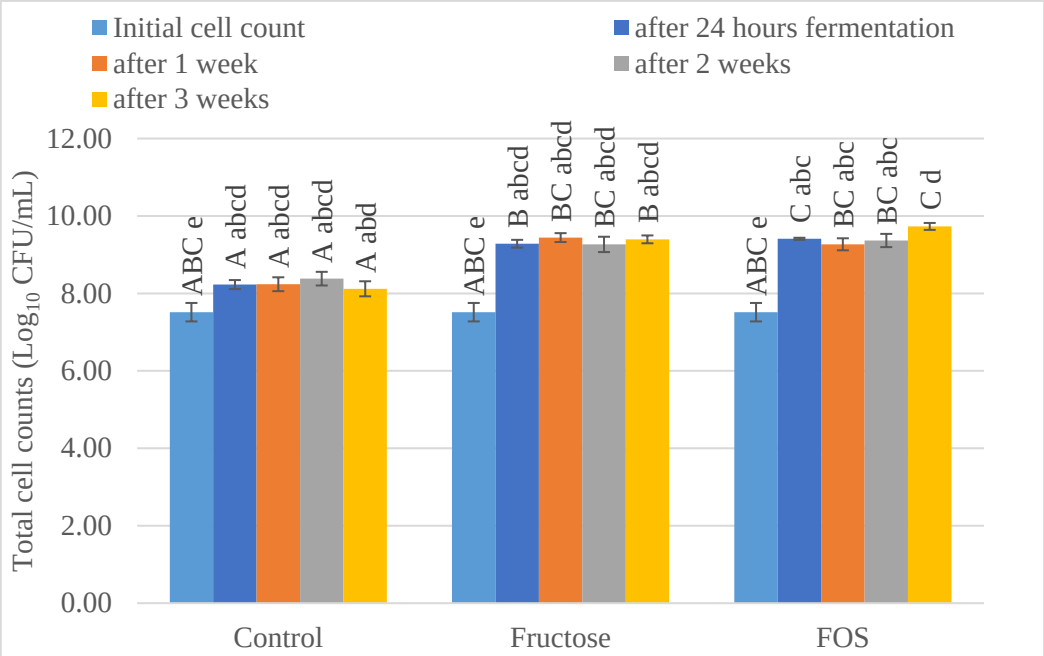


***Figure* 12. The survivability of *L. plantarum* 299v in fermented egg white drink during storage time**. Higher case letters express the significant difference when a different sugar type was used at the same period, Lowercase letters indicate the significant difference when the same carbohydrate sources were utilized at a different storage period. Control: fermented egg white drink without carbohydrate source using *L.plantarum* 299v, fructose: fermented egg white drink with fructose solution 2% using *L.plantarum* 299v, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% using *L.plantarum* 299v.

5.3.2 Changes in the pH value

Figure 13 shows the pH values of a fermented egg white drink using *L. plantarum* 299v with different carbohydrate sources during 3 weeks of refrigeration. The control samples had the highest pH value compared to the others with sugar addition at any period of storage. This was due to the lower cell concentration of *L. plantarum* 299v as a result of the lack of carbohydrates, and consequently lower lactic acid release. However, the pH values of samples in the first and second week of storage were lower (pH 5.9) compared to samples after 24 hours of fermentation (pH 6.15) and samples in the third week of storage (pH 6.08).

Additionally, the pH levels of samples containing fructose and FOS did not differ significantly from each other over time. In the first week of storage, the pH value of fermented samples with fructose dropped significantly. However, in the second week, it decreased even further to pH 3.69.

This was likely due to the activity of *L. plantarum* 299v in the egg white drink caused by the consumption of sugar and the production of lactic acid and acetic acid (Bahrami et al., 2019). Later, the pH value increased and reached pH 3.81. Similarly, the pH value of fermented EW samples with FOS increased significantly in the third week to 3.83, which could be attributed to the fact that when microorganisms lack the required nutrients in the medium, they start consuming organic acids instead. As a result, the pH increases.

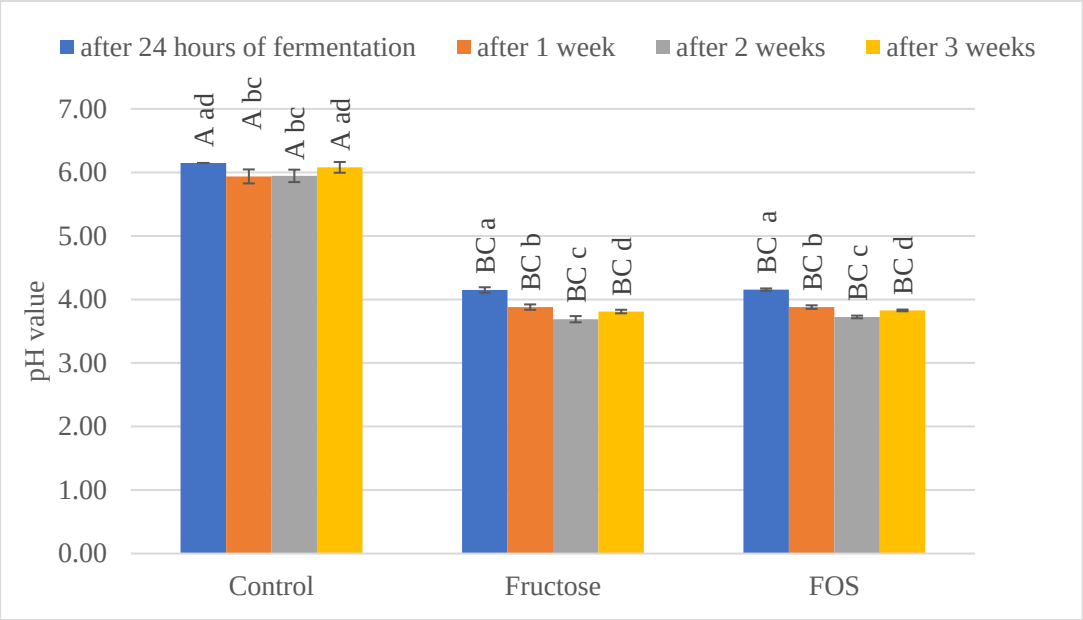


***Figure* 13. The pH value of fermented egg white drinks during storage time using *L. plantarum* 299v.** The higher case letters express the significant difference in the pH value when a different carbohydrate source was used at the same storage period, and lowercase letters indicate the significant difference when the same carbohydrate source was used over the storage period. Control: fermented egg white drink without carbohydrate source using *L.plantarum* 299v, fructose: fermented egg white drink with fructose solution 2% using *L.plantarum* 299v, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% using *L.plantarum* 299v.

5.3.3 Changes in the rheological properties

Table 10a displays the changes in yield stress of fermented EW samples using fructose and FOS as a sugar source over 3 weeks of cold storage. After 24 hours of fermentation, control samples had the lowest yield stress and closer to a 0 when compared to samples with carbohydrate sources, and it remained stable over refrigeration for up to 3 weeks. EW samples with FOS had higher yield stress than fructose samples after 24 hours of fermentation and over 2 weeks of storing then it decreased in the third week as they required less energy to move the samples' structures. Also, the control samples exhibited a higher consistency coefficient (K value) after 24 hours of fermentation compared to samples containing fructose and FOS, (*Table* 10 b). However, there was

no significant difference in the first week. Then, in the second and third weeks, the k value was higher than fructose samples, with a slight distinction from FOS samples.

***Table* 10. The changes in the rheological characteristics, yield stress (a), consistency coefficient (b), flow behaviour index (c) of a fermented egg white beverage by *L. plantarum* 299 v and different sources of carbohydrates during refrigeration**

(a)

Yield stress (Pa)	**Control**	**Fructose**	**FOS**
After 24 hours	0.002±0.000006 $^{A\ abcd}$	0.31±0.00001 $^{B\ a}$	0.34±0.00001 ^{Cab}
After 1 week	0.00202±0.00001 ^{Aabcd}	0.09±0.00001 ^{Bb}	0.34±0.001 ^{Cab}
After 2 weeks	0.00203±0.00001 ^{Aabcd}	0.1±0.00001 ^{Bc}	0.20±0.002 ^{Cc}
After 3 weeks	0.002±0.00001 ^{Aabcd}	0.08±0.00001 ^{Bd}	0.014±0.0001 ^{Cd}

(b)

Consistency coefficient (K) Pa.s^n	**Control**	**Fructose**	**FOS**
After 24 hours	0.008±0.0001 ^{Aab}	0.0001±0.00005 ^{BCa}	0.000002±0.0000002 ^{BCabcd}
After 1 week	0.042±0.03 ^{ABCbcd}	0.0029±0.0001 ^{ABbc}	0.000003±0.000001 $^{AC\ abcd}$
After 2 weeks	0.0041±0.0002 ^{ACbcd}	0.0031±0.000001 ^{BCbc}	0.0022±0.002 ^{ABCcd}
After 3 weeks	0.0038±0.0001 ^{ACbcd}	0.0033±0.000001 ^{BCd}	0.019±0.03 ^{ABCcd}

(c)

Flow behaviour index (n)	**Control**	**Fructose**	**FOS**
After 24 hours	0.99±0.001 ^{Aa}	1.74±0.001 ^{Ba}	2.40±0.003 ^{Cab}
After 1 week	1.01±0.0003 ^{Ab}	1.12±0.002 ^{Bbc}	2.39±0.001 ^{Cab}
After 2 weeks	1.048±0.001^{Ac}	1.12±0.002 ^{Bbc}	1.56±0.001 ^{Cc}
After 3 weeks	1.068±0.002^{Ad}	1.10±0.002 ^{Bd}	1.06±0.001 ^{Cd}

Higher case letters indicate the difference when different carbohydrate sources were applied at the same period, and lowercase letters refer to the difference over time when the same carbohydrate source was utilized. (a) yield stress, (b) consistency coefficient, (c) flow behaviour index. Control: fermented egg white drink without carbohydrate source using *L.plantarum* 299v, fructose: fermented egg white drink with fructose solution 2% using *L.plantarum* 299v, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% using *L.plantarum* 299v.

Generally, the K values for control and FOS samples remained constant throughout the study period. In contrast, fructose samples showed an increase in the third week, reaching 0.0033 Pa. s^n compared to 0.019 for FOS samples and 0.038 for control samples. Singh and coworkers (2011) pointed out that storage time could produce different physical, chemical, and biological changes in a food product, that lead to changes in rheological attributes, additionally, in their study they confirmed that eggs decay fast when stored at room temperature, but at a slower rate when stored at cold temperatures.

Examining the flow behaviour index, *Table* 10c revealed shear thickening or dilutant properties, as indicated by n values exceeding 1 since the viscosity increases with the increasing shear rate. Moreover, the n values of control increased over time, transitioning from 0.99 to 1.068. This suggests an augmentation of dilutant characteristics over time. Conversely, samples containing fructose experienced a decrease in the first week, maintained stability until the second week, and then exhibited a decline after three weeks. Similarly, the n value of samples with FOS stayed constant until the first week, then significantly decreased. These findings differ from those of Singh and colleagues (2011), who found that the rheological properties of the liquid whole egg maintained at room and refrigerator temperatures exhibited pseudoplastic and time-dependent behaviour which may be related to a different experimental condition especially the temperature, or it could be related to the enzymatic treatment during the processing of egg white drink prior to fermentation.

5.4 The effect of cold storage on the fermented egg white drink using *L. casei* 01

Fermented EW drink by *L. plantarum* 299v, with fructose, and FOS as a carbohydrate source was monitored during cold storage by taking measurements at weekly intervals (after 1, 2, and 3 weeks).

5.4.1 Changes in viability

A 24-hour egg white fermentation with FOS and fructose was carried out with *L. casei* 01 before storage. Following *Figure*14, a fermentation of EW by *L. casei* 01 resulted in the initial cell concentration increasing from 6.7 to 8.98 Log_{10} CFU/mL in samples with FOS, and 9.04 Log_{10}CFU/mL in samples with fructose, while in control samples it was significantly lower cell concentration reached a value of 8.04 Log_{10} CFU/mL. These phenomena could be explained by the high nutrient requirements of *Lactobacillus* to propagate. In our case, although *L. casei* 01 was able to grow in egg white drink without carbohydrate sources by using the carbohydrate bound to protein in the glycoprotein present in egg white drink, however, its growth was better when carbohydrate sources were added. Additionally, fructose can be used by a wide range of

microorganisms because it is a monosaccharide and easily fermentable. After 3 weeks, there were no noticeable distinction in cell counts between the egg white samples containing fructose and FOS, on the other hand, the survivability was extremely stable as Daneshi (2012) indicated that cold storage maintains the cell populations of *L. casei* 01. Moreover, the probiotics count was higher than 8.4 Log_{10} CFU/mL throughout storage time and it was in agreement with the results of

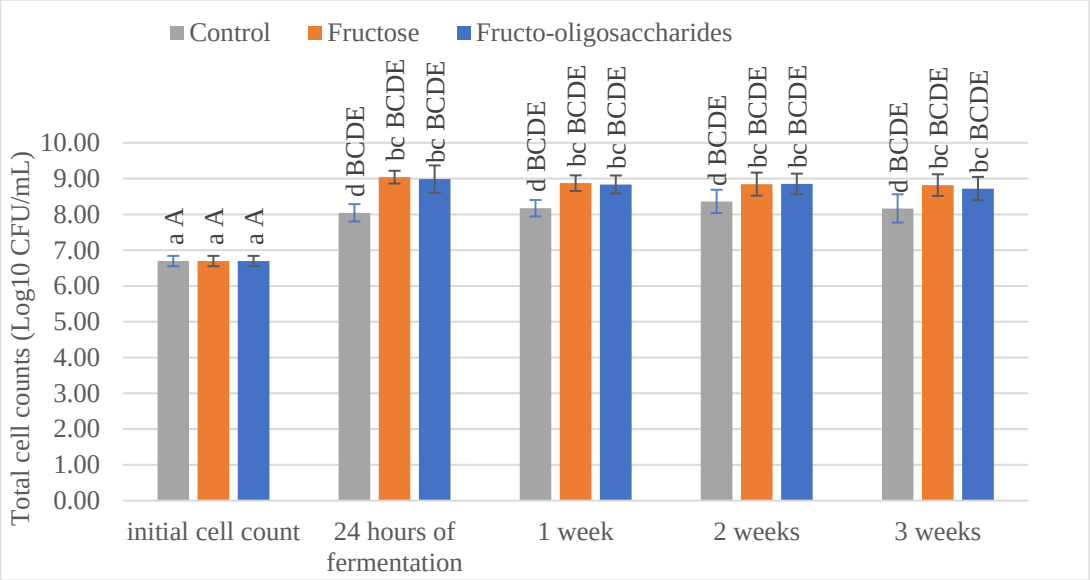


***Figure* 14. The viability of *L. casei* 01 in fermented egg white drink with different carbohydrates throughout cold storage.** Higher case letters indicate the difference during the time using the same sugar. Lowercase letters indicate the difference between sugar types in the same storage period. Control: fermented egg white drink without carbohydrate source using *L.casei* 01, fructose: fermented egg white drink with fructose solution 2% using *L.casei* 01, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% using *L.casei* 01.

Nighswonger and coworkers (1996) as the viability of *L. casei* GG in cultivated buttermilk and yogurt was stable upon 28 days of refrigeration. These results also agreed with Łopusiewicz and coworkers (2019) who studied the survivability of *Lactobacillus* along 21 days of storage at 6°C in fermented flaxseed cake, which showed that the bacterial counts were maintained higher than 10^7 CFU/mL. Correspondingly, 3 weeks of cold storage did not affect the growth of probiotics, additionally, the survivability of *L. casei* 01 was not greatly different when using fructose or FOS. However, it was better to extend the storage for a few months to specify the ideal time of storage.

5.4.2 Changes in the pH values

In compliance with *Figure*15, the initial pH value of the egg white drink (pH 6.80) dropped significantly after 24 hours of fermentation reaching a level of pH 5.91 It was completely higher compared to samples with carbohydrate sources because of the higher growth in samples with

carbohydrates that consequently lead to increased production of organic acids. Meantime, control samples were not considerably different in the first and third weeks, the pH value was around 5.8 in both. Moreover, the pH values of samples with FOS were greatly different as they decreased over time, after 1 weeks reduced to pH 3.8, and dropped on the 21 days of storage. Yeo and Liong (2010) reported an increase in lactic acid production when fermented soy drink by *Lacticaseibacillus casei* ATCC 393 was supplemented with FOS.

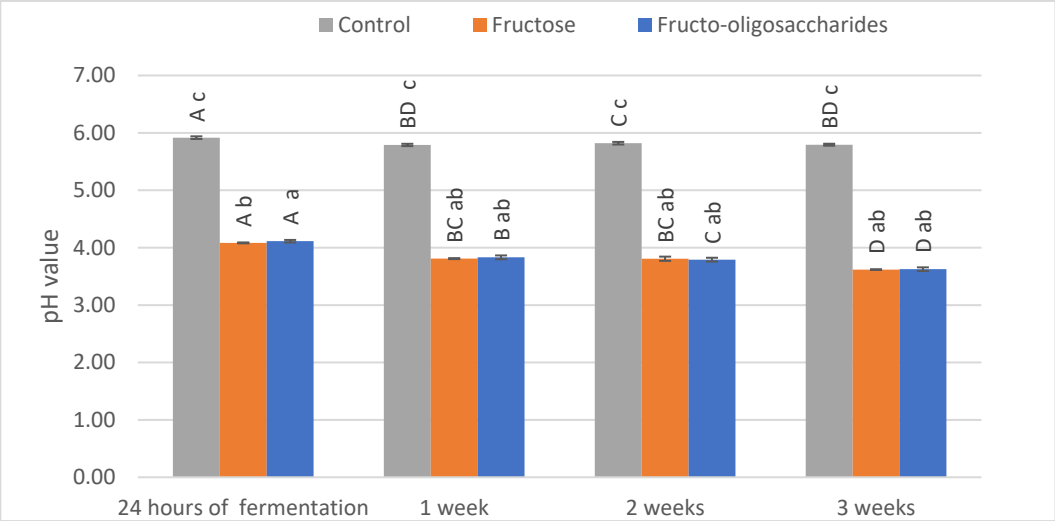


***Figure* 15. The pH value of fermented egg white beverages by *L. casei* 01 with different carbohydrate types during cold storage.** Lowercase letters indicate the difference between sugar types in the same storage period, and higher case letters indicate the difference during the time using the same sugar. Control: fermented egg white drink without carbohydrate source using *L.casei* 01, fructose: fermented egg white drink with fructose solution 2% using *L.casei* 01, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% using *L.casei* 01.

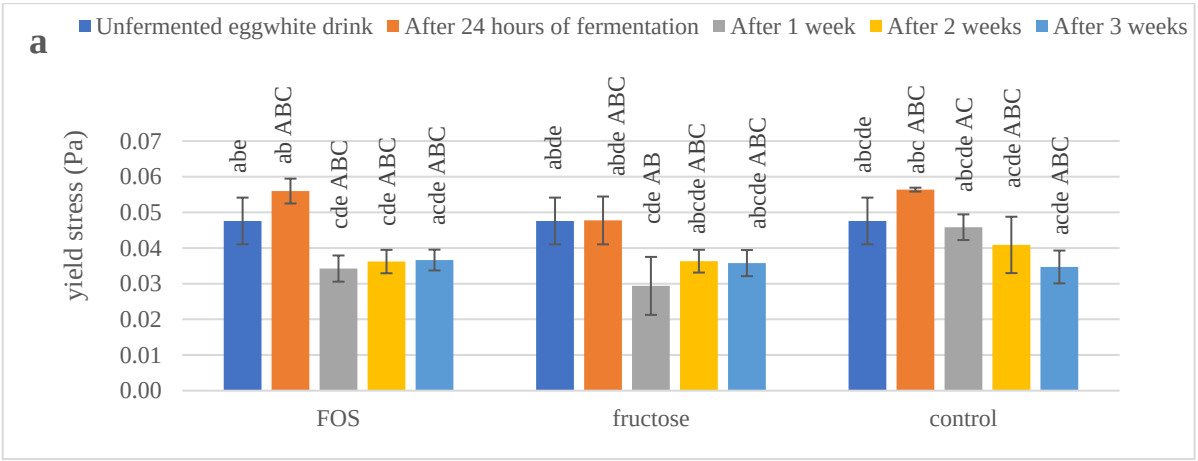
In contrast, fructose samples during the first and second weeks were not considerably different from each other as they dropped substantially in the third week of storage to pH 3.62 which may have been caused by the proliferation of microorganisms. After the third week of storage, there were no considerable changes in pH between the fermented samples with fructose and FOS. Thus, the higher acidity of the probiotic egg white drink can affect positively the microbiological stability of the product consequently extending its shelf life from two days to over 3 weeks.

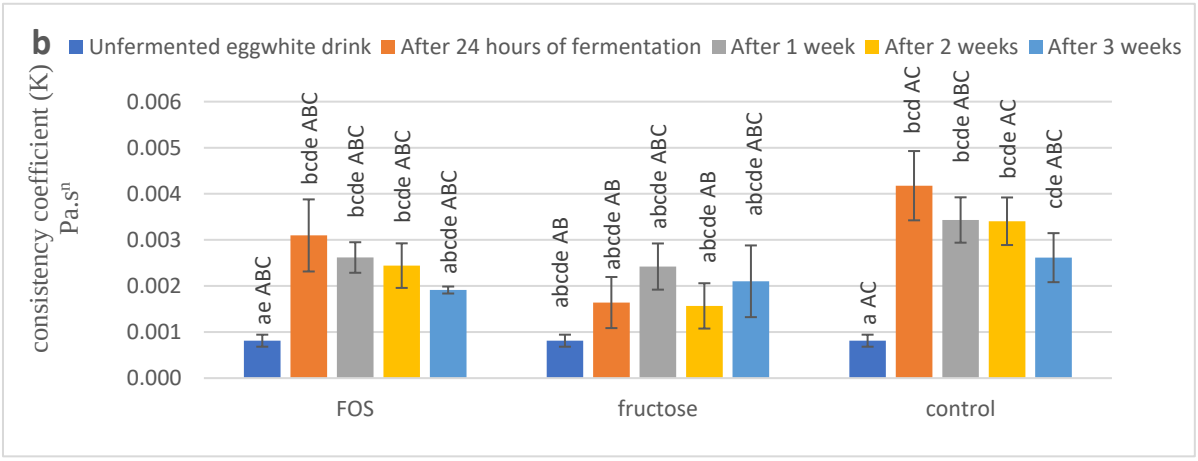
5.4.3 Changes in the rheological properties

In accordance with the yield stress (*Figure* 16a) the control samples slightly raised after 24 hours of the fermentation in comparison with the fresh samples thus, they required a higher force to move their structure before starting the flow. Later on, it significantly decreased in the second and third weeks of cold storage in the case of control samples. After the first week, fructose samples

showed a dramatic drop in yield stress, followed by a slight increase without any significant difference from fresh samples. As compared to control samples in the same storage period, samples with fructose required less force to remove. In the case of samples with FOS, the yield stress did not significantly differ throughout storage time, but they were quite lower values compared to fermented samples after 24 hours of fermentation.

It is important to note that the yield stress values were higher than 0 in all studied samples and storage periods. Undeniably the consistency coefficient (K) of egg white drink (*Figure* 16b) increased significantly after the fermentation from 0.0008 to 0.004 Pa.s^n in control samples, and to 0.003 Pa.s^n in samples with FOS since K values varied from 0.0008 to 0.004 Pa.s^n. That means fermentation raises the strength of the structure against breakdown by increasing water retention and protein aggregation. On the other hand, samples containing fructose did not vary significantly from fresh samples throughout the time. After 3 weeks of storage, the K values were not remarkably different in samples involving fructose and FOS, since they were both equivalent to fresh samples. These results were the same as the results of Kumbár et al. (2015a) since an increase in K value was found during the cold storage of liquid egg white drink in the first three weeks.





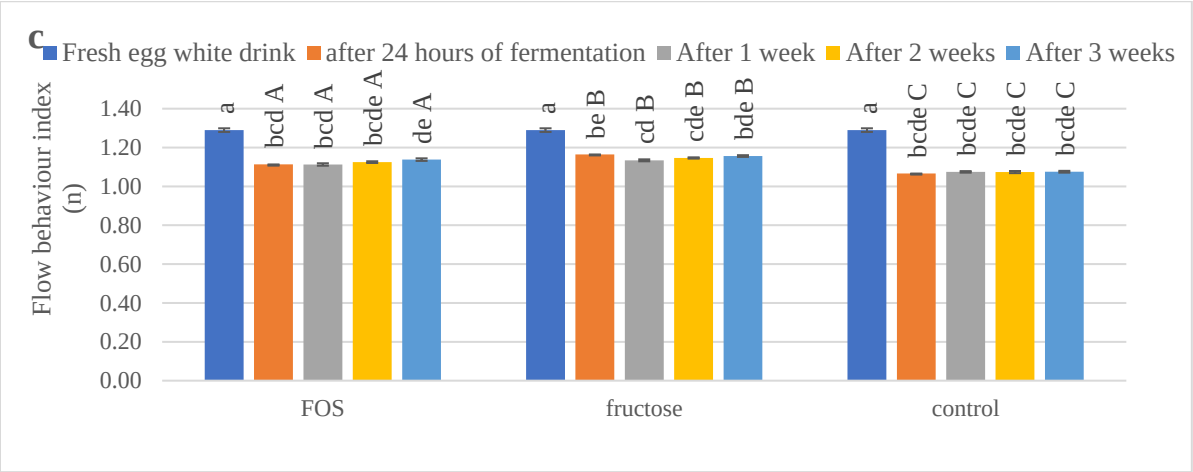


***Figure* 16. The rheological parameters for fermented egg white drink during cold storage. (a) yield stress, (b) consistency coefficient, (c) flow behaviour index.** Higher case letters indicate the difference over the cold storage when the same carbohydrate source was used. Lowercase letters indicate the difference when different carbohydrate source was used in the same storage period. Control: fermented egg white drink without carbohydrate source using *L.casei* 01, fructose: fermented egg white drink with fructose solution 2% using *L.casei* 01, FOS: fermented egg white drink with fructo-oligosaccharides solution 2% using *L.casei* 01.

Studying the flow behaviour index values which were higher than 1 (*Figure* 16c) expressed a non-Newtonian fluid that has shear thickening behaviour, additionally, fresh samples had significantly the highest n value. The reduction in n value and the increase in K could be attributed to the dissociation of the protein network due to the shearing (Aboulfazli et al., 2015). Our findings contradict those of Kumbár and coworkers (2015 b) who reported that fresh EW exhibited shear thinning behaviour. Additionally, it was different from Varga-Tóth et al. (2023) findings who observed a pseudoplastic behaviour in EW drink enriched with mixed berries and bovine collagen peptides.

5.5 Development and evaluation of probiotic fermented egg white drink fortified with different fruit juices

Pineapple and strawberry juices are highly favoured for their vibrant colours and rich content of carbohydrates and biological compounds. Consequently, they were chosen to be mixed with egg white drink in a 3:1 ratio of egg white drink to fruit juices. This mixture was then fermented for 24 hours using *L. casei* 01 and *L. salivarius* CRL 1328 separately. The selection of these strains was based on the previous investigation where *L. casei* 01 was able to thrive in egg white even in cold conditions, whereas *L. salivarius* CRL 1328 showed weaker growth in the presence of glucose 2% w/v. Therefore, adding fruit juices could potentially enhance the growth of *L. salivarius* CRL 1328.

5.5.1 Growth of probiotics during 24 hours of fermentation

The viable cell counts of the tested probiotic strains in egg white drink mixed with different fruit juices after 24 hours of fermentation are shown in Figure 20**.** Based on the results presented in *Figure* 17a, it was observed that extending the fermentation time over 24 hours played a significant role in promoting the growth of *L. casei* 01 when peach juice was added. However, the growth rate did not differ significantly when pineapple and strawberry were added separately, even after 16 and 24 hours of fermentation. After 4 hours, *L. casei* 01 entered the lag phase to adapt to the fermentation medium, and after 8 hours, the cells began multiplying quickly, therefore initiating the logarithmic growth phase. Over time, the cell counts in EW: PE samples increased significantly, reaching 9.06 Log_{10} CFU/mL after 24 hours. Similarly, the cell counts in EW: PI and EW: ST reached 8.97 and 8.83 Log_{10} CFU/mL respectively, with no significant differences among them. It is worth noting that fruit juice has been reported as a suitable matrix for probiotics (Perricone et al., 2015). Additionally, the fermentable sugar in fruit juices affects *Lactobacillus* cell viability, which contributes to the significant growth of the bacterial strain viability (Hossain et al., 2020). According to Li and coworkers (2020) 58.26–77.1% of peach juice's sugar content is derived from sucrose; however, the carbohydrate types in pineapple juice are glucose, fructose, and sucrose in an average ratio of 1:1:16 (Ivanova et al., 2019) whereas fructose, glucose, and sucrose make up the total sugar content in strawberry varying from 5.35 g/100 mL to 10.96 g/100 mL based on the soluble solids (Brix) (Kallio et al., 2000). Also, Costa and coworkers (2013) studied the growth of *L. casei* NRRL B-442 in pineapple juice as it reached 8.34 Log_{10} CFU/mL after 24 hours of fermentation in our case it is slightly higher (8.97 Log_{10} CFU/mL). As shown in *Figure* 17b, the initial cell counts of *L. salivarius* CRL 1328 in the egg white drink increased significantly after 4 hours of fermentation. There was no considerable difference observed when peach, pineapple, or strawberry were added separately to the drink. After 8 hours of incubation, the concentration of probiotic cells in the EW:PE samples did not differ significantly between 16 and 24 hours, reaching approximately 8 Log_{10} CFU/mL.

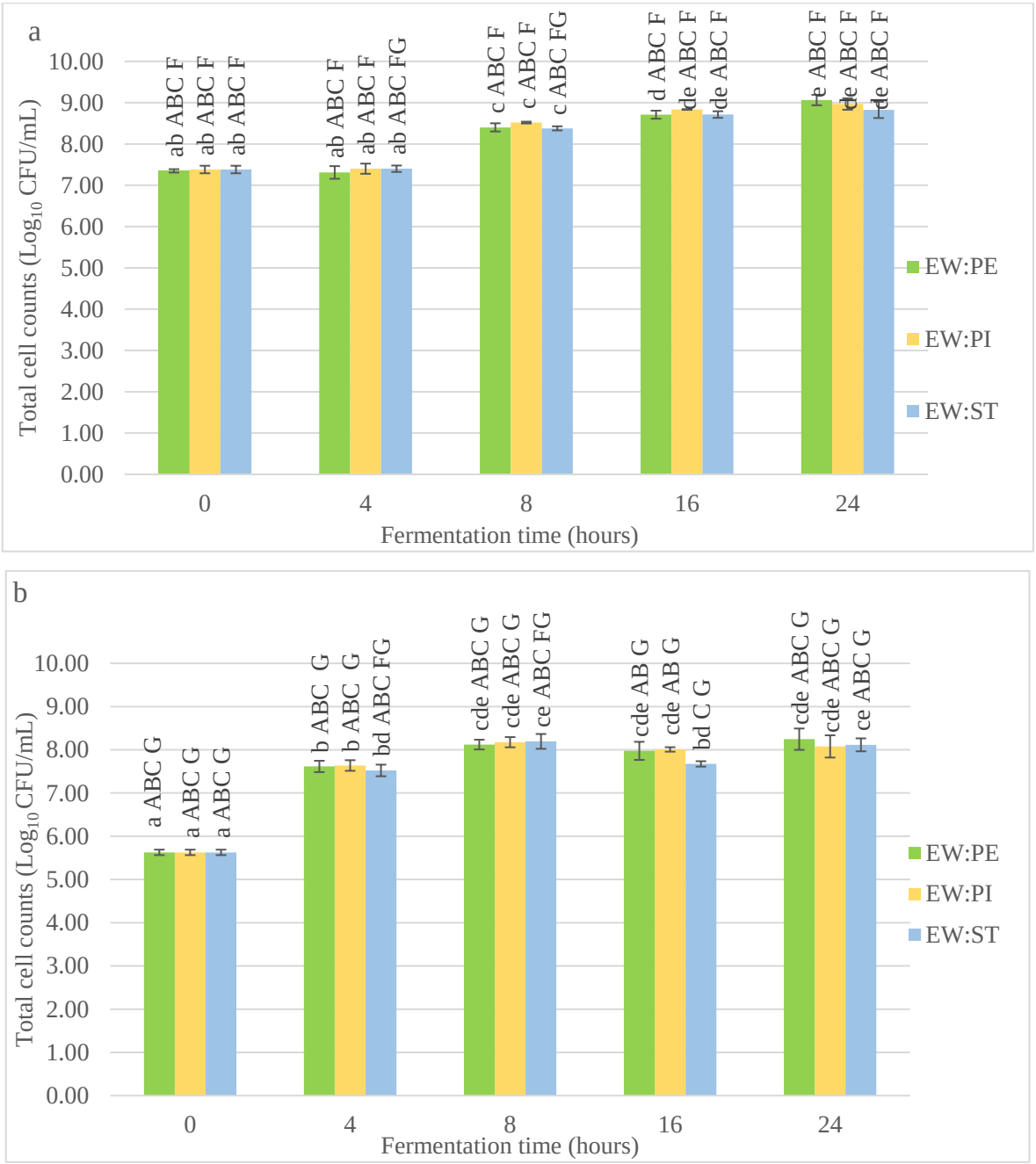


***Figure* 17. The viable cell counts of probiotics strains in egg white drink mixed with different fruit juices during fermentation time (a): *L. casei* 01, (b): *L. salivarius* CRL 1328.** Higher case letters from A to C indicate the effect of adding different fruit juice using the same strain at the same interval. Higher case letters from G to F indicate the significant difference between *L. casei* 01 and *L. salivarius* CRL 1328 (the same fruit juice, the same interval), and lowercase letter indicates the effect when the same fruit juices were added using the same strains over fermentation time with a 95% confidence level ($p < 0.05$). EW:PE fermented egg white drink mixed with peach juice. EW:PI fermented egg white drink mixed with pineapple juice. EW:ST fermented egg white drink mixed with strawberry juice.

Also, the viable cell counts in EW: PI (8.18 Log_{10} CFU/mL) and EW: ST (8.19 Log_{10} CFU/mL) reached its peak after 8 hours. The addition of various fruit juices to the egg white drink did not affect the cell concentration of *L. salivarius* CRL 1328 during the first 8 hours of fermentation. However, after 16 hours of fermentation, EW: ST samples showed significantly lower cell counts compared to the other samples. It's worth noting that after 8 hours of fermentation, all samples increased by approximately 3 Logs over the initial cell count, which is in line with the daily recommended therapeutic dose of probiotics. Additionally, it was observed that our results were significantly lower than those reported by Busaga and coworkers (2022), who found that *L. salivarius* [DBE1] [MR2] spp. could be grown in clementine juice after 24 hours of fermentation, reaching a 9.3 Log_{10} CFU/mL.

L. casei 01 and *L. salivarius* CRL 1328 were also compared using the same juice and time intervals, as illustrated in *Figures* 17a, 17b. After 4 hours of fermentation, *L. salivarius* acclimated faster than *L.casei* 01 to the egg white drink containing EW:PE and EW:PI or EW:ST samples. However, *L. casei* 01 exhibited a considerably greater total cell counts than *L. salivarius* CRL 1328 after 8 hours and up to 24 hours, except for EW:ST samples, there was no significant difference between *L. casei* 01 and *L. salivarius* CRL 1328 growth at the 4 and 8-hour intervals.

5.5.2 Changes in the pH values

During the fermentation process, the pH value was continuously monitored and it was observed that the pH decreased significantly over time, as seen in *Figure* 18 a. The ST fermented drink made with *L. casei* 01 had the highest pH value of 3.86 after 24 hours, compared to other samples made with different fruit juices. This may be because of a higher initial pH of the EW: ST sample. Additionally, the pH values of the fermented drink with *L. salivarius* CRL 1328, as shown in *Figure* 18 b, also decreased over time due to the breakdown of carbohydrate molecules into lactic acids and other by-products. İçier et al. (2015) mentioned that adding apple juice to soy milk provided probiotics like *L. acidophilus* with an additional carbohydrate source, which increased their metabolic activity and resulted in a decrease in pH. The pH value remained constant over the 16 and 24 hours of EW fermentation, except for when strawberry juice (EW: ST) was added. This was because the probiotic bacteria had reached the stationary phase, which is characterized by a slowing of the growth rate and a reduction in lactic acid production. According to Pakbin and coworkers (2014), the development of probiotic bacteria and its lactic acid production can also be impacted by the low pH and high acidity, oxygen tension of fruit juices, and water activity.

The pH value of fermented egg white drink samples with pineapple, peach, and strawberry by *L. salivarius* CRL 1328 (*Figure* 18 b) showed significant differences during fermentation time.

Although the pH value of EW: PI was the highest throughout 8, 16, and 24 hours, they achieved the lowest acidity after 4 hours of fermentation. Conversely, for *L. casei* 01, *Figure* 18a the highest pH value over fermentation time belonged to EW: ST samples. This may be due to the higher initial pH value of strawberry juice compared to pineapple and peach.

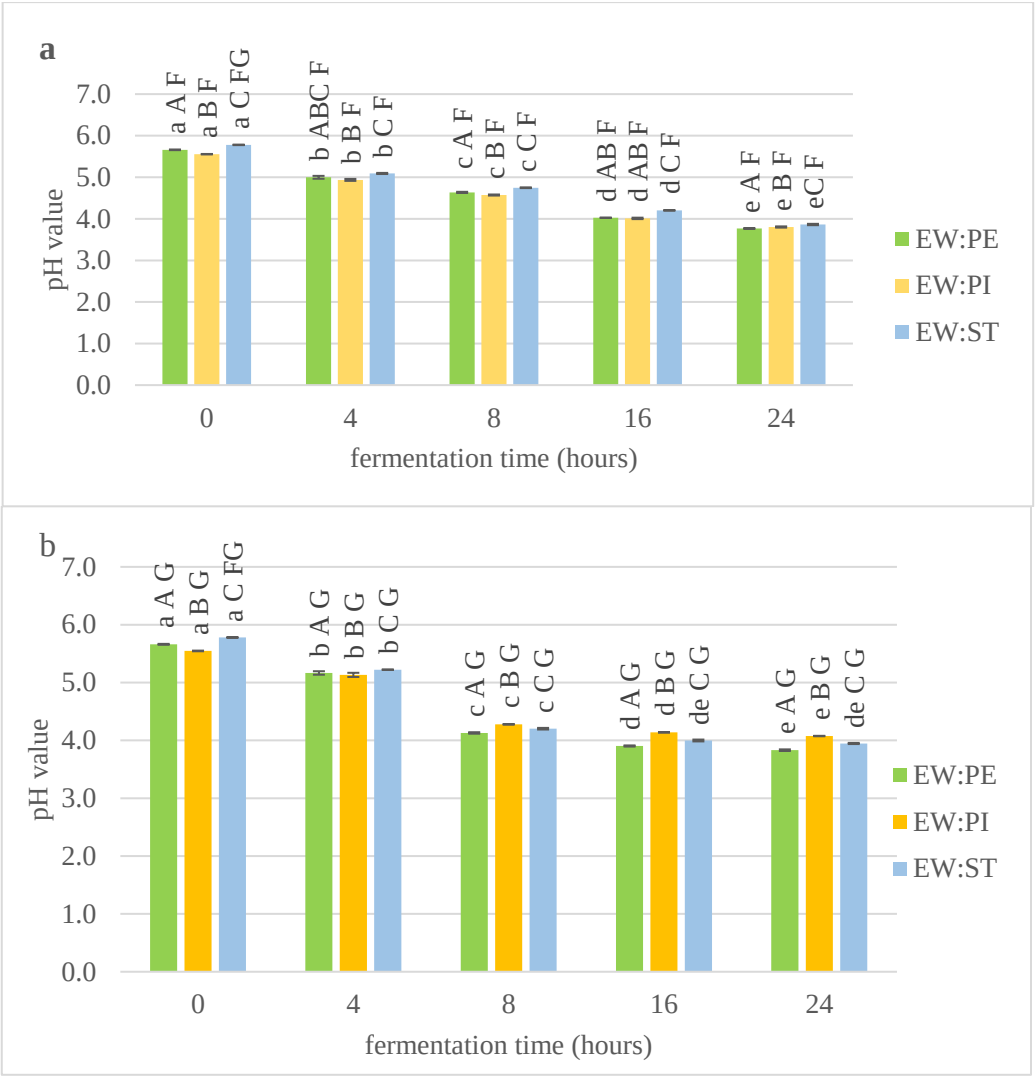


***Figure* 18. The changes in the pH value of egg white drink mixed with fruit juices during 24 hours of fermentation by *L. casei* 01 (a), and *L. salivarius* CRL 1328 (b).** Higher case letters from A to C indicate the effect of adding different fruit juice using the same strain at the same interval. Higher case letters from G to F indicate the significant difference between *L. casei* 01 and *L. salivarius* CRL 1328 (the same fruit juice, the same interval), and lowercase letter indicates the effect when the same fruit juices were added using the same strains over fermentation time with a 95% confidence level ($p < 0.05$). EW:PE fermented egg white drink mixed with peach juice. EW:PI fermented egg white drink mixed with pineapple juice. EW:ST fermented egg white drink mixed with strawberry juice.

Our findings confirmed those of Pakbin and coworkers (2014), who found that fermented peach juice by *L. casei*, *L. plantarum*, and *L. delbrueckii* separately had a pH of less than 4.4 in all samples while *L. delbrueckii* was the most suitable strain to grow in peach juice. They also agree with the study results of Scibisz and coworkers (2019), who studied the effects of utilizing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* to produce fruit yogurt (strawberry, blueberry, and cherry) individually. The strawberry yogurts had a lower acidity and a higher pH following production. Furthermore, lactic acid was produced faster by *L. casei* 01 than by *L. salivarius* CRL 1328 (*Figures* 18a, and 18b) since *L. salivarius* CRL 1328 samples had significantly higher pH values compared to *L .casei* 01 samples after 24 hours of fermentation, as the pH level of EW:PE, EW:PI, and EW: ST reached (pH 3.77, pH 3,80, pH 3.86) respectively in samples with *L. casei* 01 and (pH 3.83, pH 4.08, pH 3.95) respectively in *L. salivarius* CRL 1328 samples which is not so far from the results of Chen and coworkers (2023). In their study the fermentation of strawberry juice by *L. acidophilus* and *L. plantarum* resulted in a cell counts of 8.5 Log_{10} CFU/mL after 24 hours of fermentation, in general, the pH value reached pH 3.30. As well as the findings of Busaga and coworkers (2022), in their study on the fermentation of clementine juice by *L. salivarius spp salivarius* CECT 4063 with and without trehalose addition, the maximum growth occurred after 24 hours of fermentation approximately 9 log CFU/mL and the pH reached 4.37.

5.5.3 Changes in the colour parameters of fermented egg white drink with different fruit juices after 16 hours of fermentation

Colour, being one of the first features experienced by the senses, is critical in assessing food quality and has a substantial impact on customer acceptability. The colour parameters of fermented EW with fruit juices were analysed by Hunter Lab. According to *Table* 11 and *Figure* 19 the EW:PI samples in both strains had the greatest brightness, reaching 88% of the brightness range, which might be attributable to the green colour owing to the natural presence of iron and sulphur molecules in egg white components. However, the a* and b* values were significantly lower, indicating a bluish hue when compared to other fermented egg whites containing peach and strawberry juice. The yellow hue of the fermented sample containing pineapple juice is related to the high content of beta-carotene, the main pigment in pineapple juice which is responsible for its yellow colour (Barretto et al., 2013). When *L. casei* 01 or *L. salivarius* CRL 1328 was chosen as a starter culture, the a* value of the EW:ST samples was notably the greatest, while L* recorded the lowest. Additionally, the b* value raised to 7.68, indicating that the addition of strawberry juice produced a colour profile that was more reddish and yellowish. Strawberries include a form of flavonoid called anthocyanins, which is responsible for the red colour, while flavanols, another

type of flavonoid, are responsible for the yellow colour (Jeong, et al., 2012). Likewise, as reported by Chen and coworkers (2023), the colour profile of strawberry juice changed significantly after fermentation, with the juice treated with *Lactobacillu*s becoming more orange. Additionally, the differences in the colour parameters, when different fruit juice was added, may assign to different content of the anthocyanins (Scibisz et al., 2019) for instance peach is a source of cyanidin which is in charge of reddish-purple pigment (Swami et al., 2020). Although using both strains in EW:PE fermentation didn't affect the colour parameters, however, fermented samples *L. casei* 01 recorded higher b* values (5.67, 7.68) in EW:PI, and EW:ST respectively compared to *L. salivarius* CRL 1328 (5.37, 7.04).

***Table* 11. The colour parameters of fermented egg white drink with added fruit juice after 16 hours of fermentation by probiotics**

	Probiotic strain	EW:PE	EW:PI	EW:ST
L*	*L. casei* 01	85.92 ± 0.19^{Aab}	88.22 ± 0.14^{Bab}	82.37 ± 0.14^{Cab}
	L. salivarius CRL 1328	86.09 ± 0.07^{Aab}	88.40 ± 0.19^{Bab}	82.63 ± 0.17^{Cab}
a*	*L. casei* 01	-1.87 ± 0.20^{Aab}	-2.48 ± 0.07^{Ba}	3.68 ± 0.15^{Cab}
	L. salivarius CRL 1328	-1.66 ± 0.05^{Aab}	-2.18 ± 0.12^{Bb}	3.35 ± 0.27^{Cab}
b*	*L. casei* 01	12.49 ± 0.60^{Aab}	5.67 ± 0.14^{Ba}	7.68 ± 0.13^{Ca}
	L. salivarius CRL 1328	11.67 ± 0.42^{Aab}	5.37 ± 0.10^{Bb}	7.04 ± 0.11^{Cb}

Higher case letters indicate the difference when different juice was incorporated utilizing the same strain, lowercase letter indicates the differences when the same fruit juice was incorporated with the same probiotic strain. EW:PE fermented egg white drink mixed with peach juice. EW:PI fermented egg white drink mixed with pineapple juice. EW:ST fermented egg white drink mixed with strawberry juice.

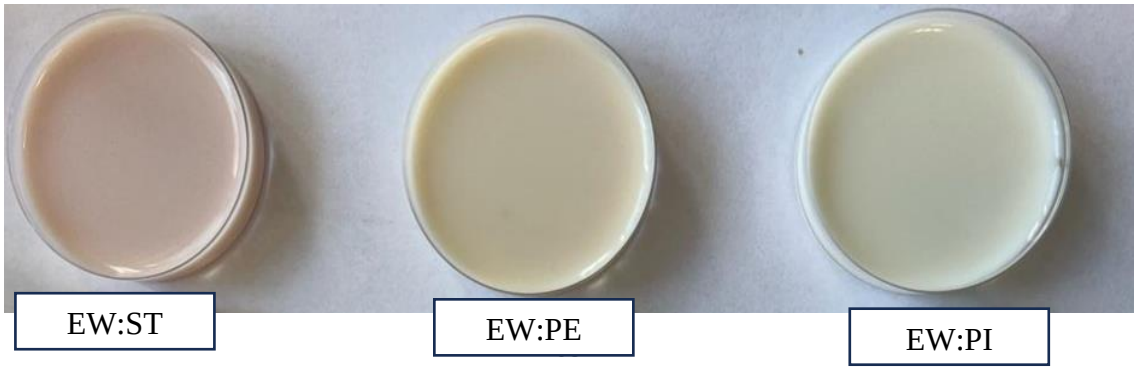


***Figure* 19. Fermented egg white drink (EW) samples with different fruit juices.**

(strawberry : ST, peach : PE and pineapple :PI)

5.5.4 Protein profile by SDS-PAGE

Different fruit juices (pineapple, strawberry, and peach) were used to study the changes in protein profiles of fermented egg white drinks. The samples were fermented by *L. casei* 01 and *L. salivarius* CRL 1328 for 16 hours. These changes were demonstrated in *Figure* 20. Several protein patterns were detected across the gels, according to the findings. Five significant protein bands were identified on the gels, including ovo-transferrin at 81.2-89.8 kDa, ovalbumin at 44.4-47.6 kDa, ovomucoid at 35.3-36.6 kDa, ovo-flavoprotein at 12.4-13.1 kDa, and lysozyme at 14.4 kDa.

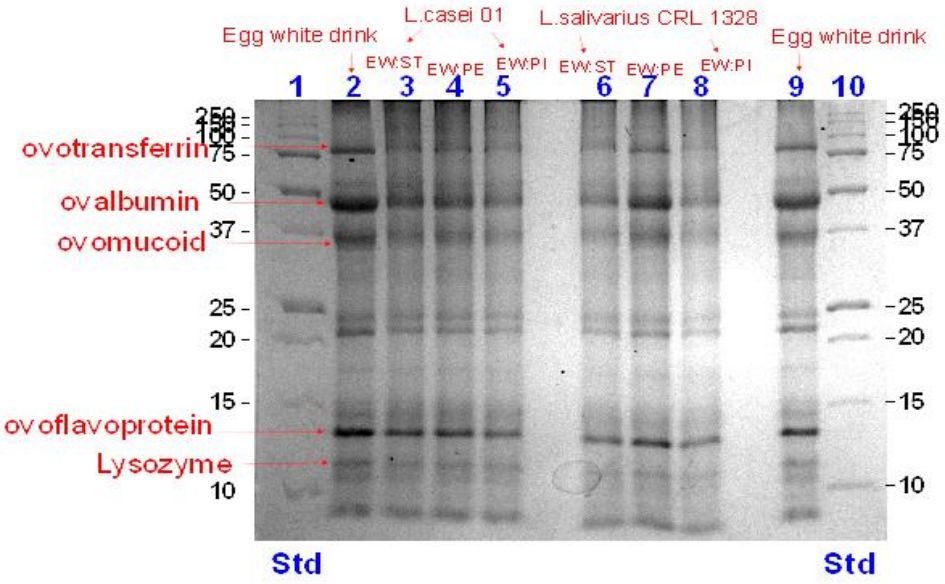


***Figure* 20. The protein profile of fermented egg white drink with added fruit juices.** 1, 10: Mw standard, kDa, 2, 9:fresh egg white drink, 3: a fermented mixture of EW drink and strawberry juice by *L. casei* 01, 4: a fermented mixture of EW drink and peach juice by *L. casei* 01, 5: a fermented mixture of EW drink and pineapple juice by *L. casei* 01, 6: a fermented mixture of EW drink and strawberry juice by *L. salivarius* CRL 1328, 7: a fermented mixture of EW drink and peach juice by *L. salivarius* CRL 1328, 8: a fermented mixture of EW drink and pineapple juice by *L. salivarius* CRL 1328.

According to a densitometric study and following 16 hours of fermentation, the density of some protein bands reduced due to the probiotics' proteolytic activity, which led the protein to degrade into amino acids and peptides in order to proliferate. For instance, ovalbumin bands were clearly apparent on the gel, although the strength was greater in fresh egg white drink samples than in

others. Peach juice, when used in egg white drink fermentation with *L. salivarius* CRL 1328, led to significantly stronger density bands of ovalbumin, ovo-flavoprotein, ovomucoid, and ovo-transferrin in egg whites compared to pineapple and peach juices. According to Kieliszek et al. (2021), pH, temperature, and dissolved oxygen content are the main elements that impact the proteolytic activity of the LAB. It could also be related to the fact that peach fruit contains more protein (0.91g/100g) than strawberry and pineapple (0.67-0.54g/100g), respectively (USDA, 2020). Thus, when peach was included, the decline in protein content following fermentation by *L. salivarius* CRL 1328 was smaller. However, due to the strong proteolytic system that *L. casei* 01 possesses (Kieliszek et al., 2021), the bands related to ovalbumin were fainter in the case of *L. casei* 01 compared to those of samples with *L. salivarius* CRL 1328 when the same fruit juice was added. Although lysozyme was not considerably different in fermented samples, they were higher in the fresh egg white drink which could be due to the addition the juices in a 3:1 ratio egg white drink: fruit juices. These results are similar with Matsuoka and coworkers (2019) they found a reduction in lysozyme bands after fermentation in their investigation of the effect of lactic acid bacteria fermented egg white on lowering belly fat in rats.

5.5.5 Sensory evaluation

In this study, four samples of fermented EW drinks, including fruit juices (strawberry and pineapple), were evaluated by ProfiSens using *L. casei* 01 and *L. salivarius* CRL 1328 individually as starter cultures. The number of tested attributes was 17 (colour hue, viscosity, prickliness, mouthfeel, medicinal flavour, acetic taste, egg flavour, sour taste, bitter taste, sweet test fruity flavour, overall flavour intensity, pungent odour, acidic odour, sweet odour, fruity odour, and overall odour intensity), as it is shown in *Figure* 21.

The results indicated that the strain used and the type of fruit juice incorporated significantly affected the sensory characteristics of the final product. These differences in sensory parameters and drink flavour may be due to the production of certain substances, such as fatty acids, diacetyl, volatile esters, terpenes, and bacteriocins, during fermentation (Sousa et al., 2020). According to *Figure* 24. although the colour hue of EW:PI fermented either by *L. casei* 01 or *L. salivarius* CRL 1328 did not show any significant difference, the drink fermented by *L. casei* 01 had a markedly less fruity smell, a lower level of sweetness, and a higher level of sourness. This can be attributed to the substantial production of lactic acid during the fermentation process. In contrast, *L. salivarius* CRL 1328 produced a higher fruity odour and lower acetic tastes when used in the fermentation of EW: PI samples compared to *L. casei* 01.

The study also found that all samples had low levels of egg flavour, medicinal flavour, and bitterness, but L.C EW:ST had slightly higher levels. This particular sample also had a higher score for mouthfeel and a pungent flavour. It was similar to the findings of Luckow and coworkers (2006) and Ranadheera and coworkers (2014). In their study, fruit juices, specifically pineapple juice, at a concentration of 10% (v/v), could be added to the fermentation process to enhance the flavour and aroma, while covering any undesirable medicinal taste that consumers might detect. To sum up, the majority of the panel members stated that EW:PI fermented by *L. casei* 01 had a sour taste, which is supported by the drink's pH of 3.8. Additionally, an egg taste was quite obvious in EW:ST samples fermented with *L. casei* 01, however, its texture was the most viscous. The viscosity score of all the studied samples was moderate, which varied from 38% to 46% while samples with strawberry juices recorded its highest. Furthermore, EW: ST samples fermented with *L. salivarius* CRL 1328 recorded the highest overall score while the fruity smell and odour were favourable.

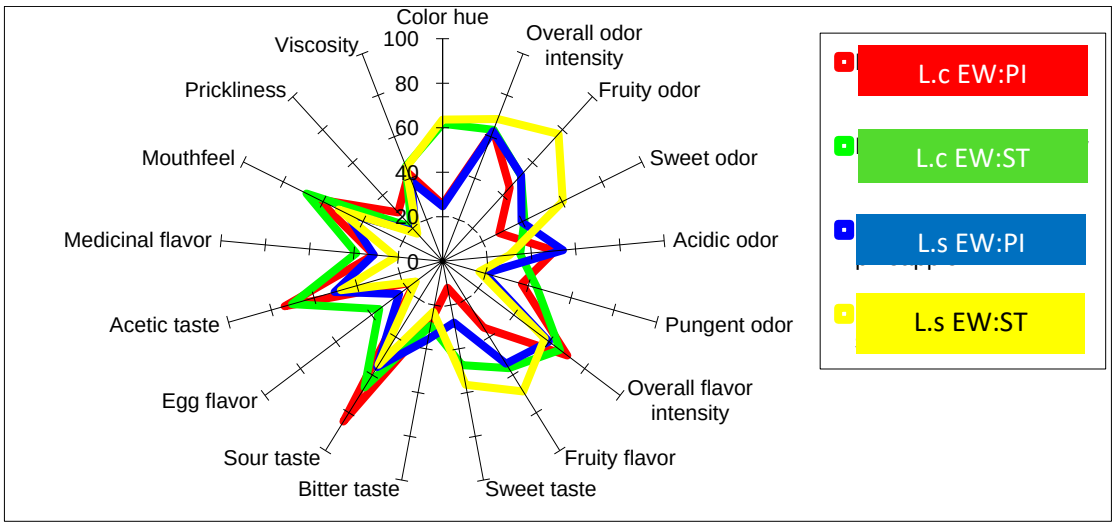


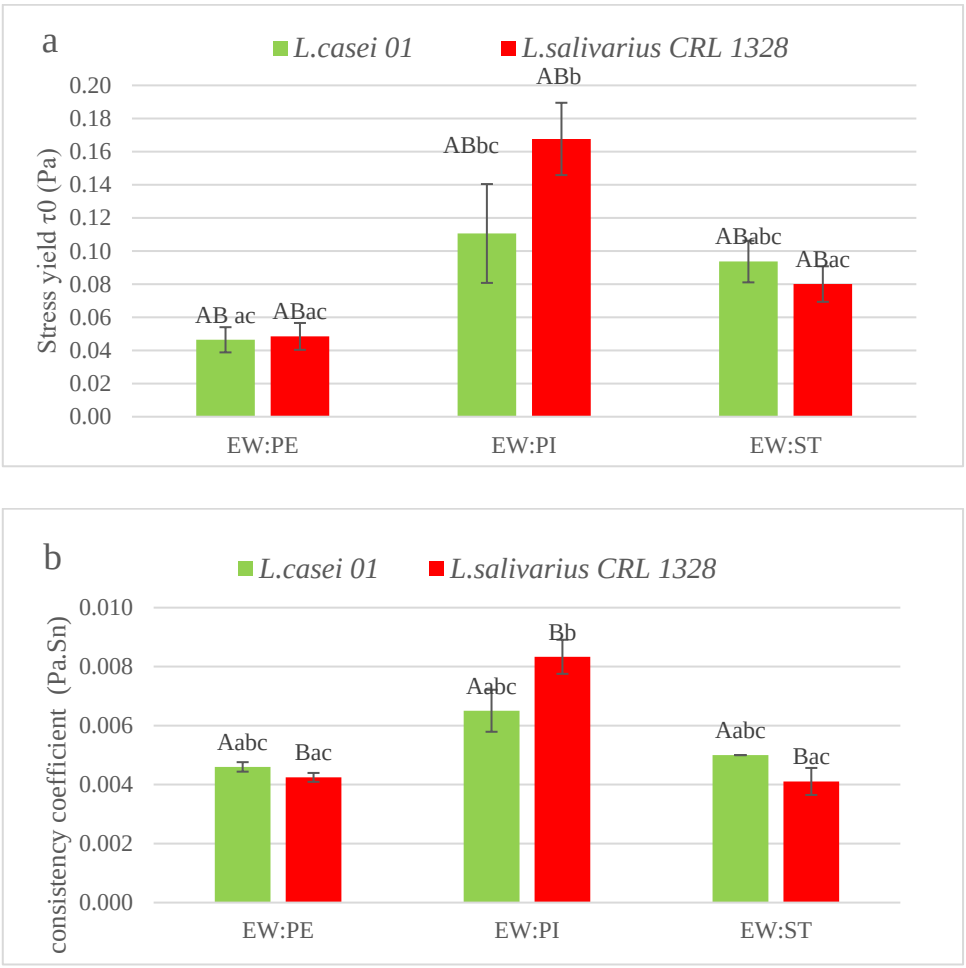
***Figure* 21. Quantitative sensory characteristics of probiotic fermented egg white drink samples with different fruit juice.** L.c EW:PI - fermented egg white drink mixed with pineapple juice and *L. casei* 01, L.s EW:PI - fermented egg white drink mixed with pineapple juice and *L. salivarius* CRL 1328, L.c EW:ST - fermented egg white drink mixed with strawberry juice and *L. casei* 01, L.s EW:ST - fermented egg white drink mixed with strawberry juice and *L. salivarius* CRL. 1328.

5.5.6 Rheological parameters of fermented egg white drink with fruit juices

Rheology is the study of material movement and deformation. It has significance for the production of fruit juice and especially for handling and processing of food, such as pasteurization, concentration, and dehydration (Dak et al., 2006). When the same fruit juices were combined into egg white fermentation with various probiotic strains (*L. casei* 01 or *L. salivarius* CRL 1328), the

stress yield did not change noticeably *Figure* 22a. However, the type of juice used affected the stress yield value since EW: PI samples in the *L. salivarius* CRL 1328 samples were entirely greater than the EW:PE and EW:ST samples. On the opposite, *L. casei* 01 EW:ST did not differ substantially from EW:PI and EW:PE. Generally, stress yield values were greater than 0. However, the stress yield values of EW: PE were the lowest.

Earle (2013) reported that increasing the flow resistance leads to an increase in the consistency coefficient and a decrease in the flowing rate in the pipes, following *Figure* 22b the highest K value when *L. salivarius* CRL 1328 was used as a starter culture was achieved in EW: PI (0.008 $Pa.S^n$) samples, while EW:ST and EW:PE were not significantly different from each other (0.004 $Pa.S^n$). In the case of *L. casei* 01, there was no considerable difference in K value when different fruit juices were added to an egg white drink. Furthermore, the fermentation of samples with *L. casei* 01 resulted in higher K values compared to those with *L. salivarius* CRL 1328 except in EW:PI samples.



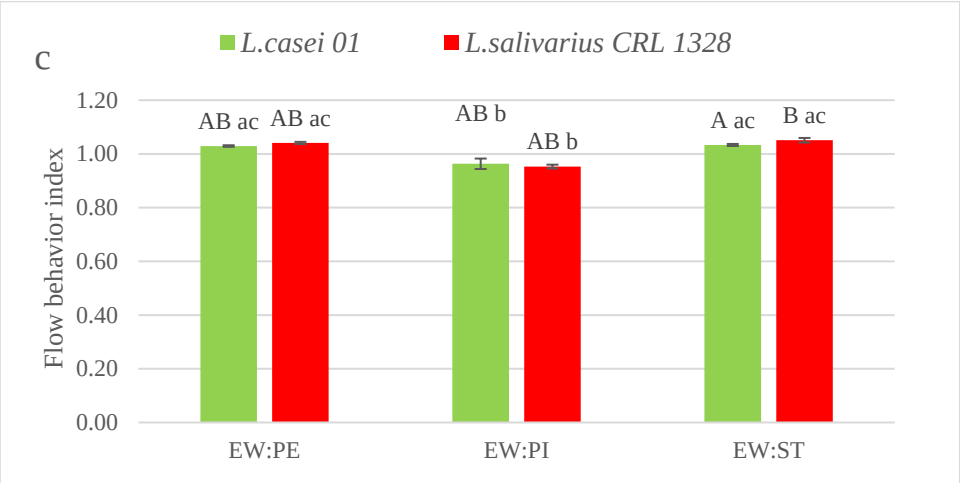


***Figure* 22. Herschel Bulkley parameters of fermented egg white drink with fruit juices, (a) stress yield (τ_0), (b) consistency coefficient (K), and (c) flow behaviour index (n).**

Higher case letters indicate the difference between *L. casei* 01 and *L. salivarius* CRL 1328, lowercase letters present the difference when different fruit juices were added using the same fermentation strain. (EW:PE fermented egg white drink with added pineapple juice, EW:PI fermented egg white drink with added pineapple juice EW: ST fermented egg white drink with added strawberry juice).

As shown in *Figure* 22c, a non-newtonian behaviour was indicated due to the nonlinear relationship between shear rate and shear stress among all studied samples, as well as the flow behaviour index values (were not equal to 1) which is similar to Egea et al. (2022) of a non-newtonian behaviour of fermented soy drink mixed with kefir. Furthermore, the n-values in EW:PI samples were less than 1 in the case of *L. casei* 01 (0.96) and *L. salivarius* CRL 1328 (0.95), thus, they support the pseudoplasticity and they presented a shear thinning behaviour which is in consistent with Egea et al. (2022) results. However, the n-value of EW:PE and EW:ST samples were higher than 1 as a shear thickening or dilutant behaviour was noticed particularly when *L. salivarius* CRL 1328 was used in the fermentation thus, they had higher dilutant characteristics compared to others with *L. casei* 01 since EW:ST with *L. salivarius* was considerably higher n-value (more viscous) compared to others.

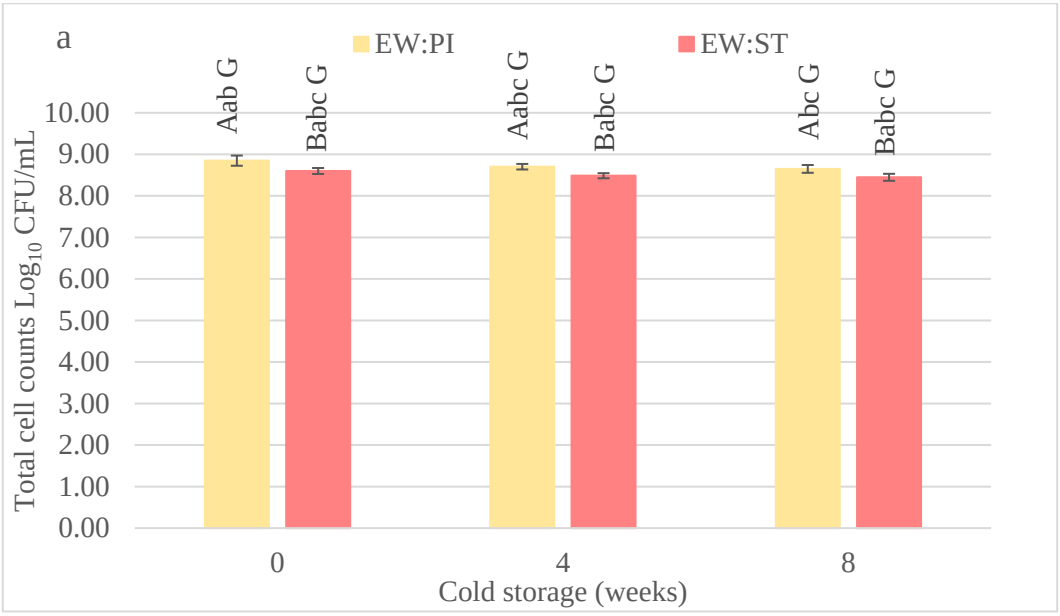
5.5.7 Effect of cold storage on cell viability

As stated by Sun and coworkers (2023), the survival of a probiotic is heavily regulated by manufacturing, storage, and digestion conditions. Therefore, the proliferation of *L. casei* 01 and *L. salivarius* CRL 1328 in a mixture of egg white drink with pineapple juice and strawberry juice was studied during 8 weeks of refrigeration storage

Fermented samples with peach juice were not included in this study due to primary sensory tests conducted in the laboratory, which showed unpleasant odour and taste for these drinks. In agreement with *Figure* 23a. the cell concentration of *L. casei* 01 was higher than 8.40 Log_{10} CFU/mL in EW: ST samples and stayed constant over 8 weeks of cold storage through the analysis period (higher than 10^6 CFU/mL at the end of shelf life). According to Patel, (2017) this value is a crucial standard that has to be obtained in probiotics products. He also mentioned that some food components particularly protein and dietary fibre protect the probiotic cells from acid stress at low pH, in our case egg white drink compromise of protein additionally, *L. casei* is considered acid tolerant and able to survive in fruit (Peres et al., 2012). Similar findings were obtained for EW: PI samples, where the cell concentration of *L. casei* 01 remained constant up to 8 weeks (8.65 Log_{10} CFU/mL), contrary to observations of Costa and coworkers (2013) that the viability of *L. casei* in pineapple juice decreased over time during refrigeration, reaching 6.3 Log CFU/mL after 42 days for unsweetened pineapple juice and 4.8 Log CFU/ml for sweetened pineapple juice. However, our findings were in line with Zheng and coworkers (2014) as *L. casei* was able to produce lactic acid in litchi juice even at cold storage.

Furthermore, the cell concentration of *L. salivarius* CRL 1328 *Figure* 23b. reduced with time, from 8.31 to 4.62 Log_{10} CFU/mL in EW: PI and from 8.21 to 5.44 Log_{10} CFU/mL in EW: ST, thus, resulting in a loss of 3.69 and 2.77 Logs, respectively, in the fourth week of cold storage. It is noteworthy that after 4 weeks of storage, *L. salivarius* CRL 1328 was unable to survive in cold conditions, as the total cell counts were not detected when pineapple or strawberry was added. Busaga and coworkers (2022) reported a reduction in *L. salivarius* spp' cell counts in clementine juice from the seventh day of cold storage because of the exhaustion of nutrients and oxygen and/or the secretion of organic acids and other biochemical contaminants into the medium.

However, *L. casei* 01 were able to survive in EW: PI drink after 4 weeks at a cell counts of 8.7 Log_{10} CFU/mL which is higher around 1 Log compared to results of Pakbin and coworkers (2014) since *L. delbrueckii* reached 7 Log_{10} CFU/mL in the fourth week of storage. Our results are in agreement with Acevedo-Martínez et al. (2018). In their study of growing *L. paracasei*, *L. casei,* and *L. rhamnosus* in mango beverages and storing at 4 °C, *L. casei* was the most stable strain, however, after 20 days of cold storage the cell counts were 6.5 Log CFU/mL.



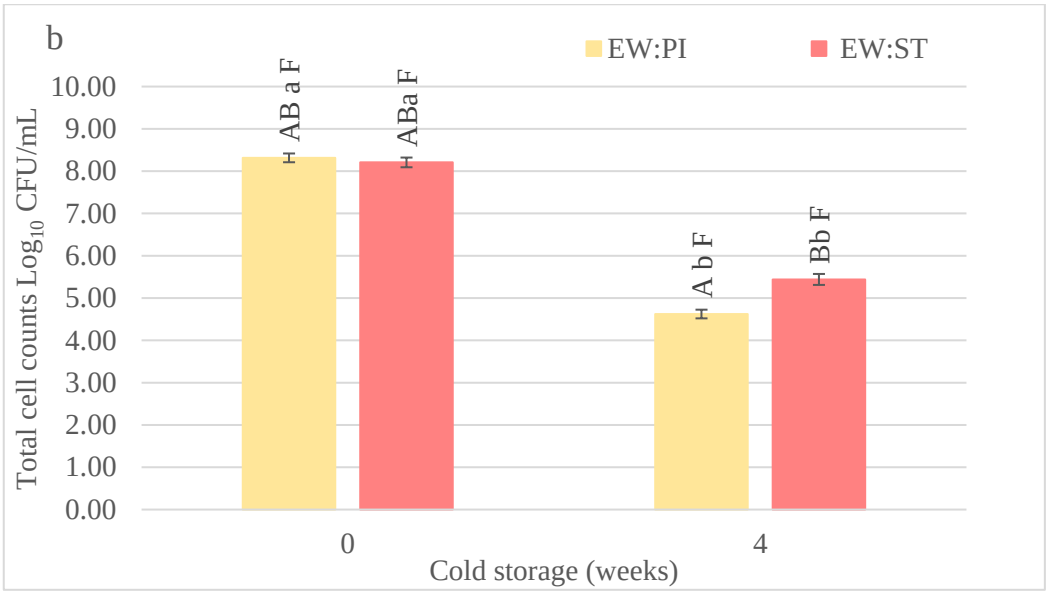


***Figure* 23. The growth of probiotic strains in a blend of egg white drink with different fruit juices during time storage. Using *L. casei* 01strain (a), and *L. salivarius* CRL 1328 (b).**

Higher case letters from A to B indicate the significants differences when strawberry or pineapple juice was added (the same strain, the same time), higher case letters from G to F indicate the significant difference between *L. casei* 01 and *L. salivarius* CRL 1328 (the same fruit juice, the same time), and lowercase letters from a to c indicate the significant differences during storage time(the same strain, the same juice). EW:PI - fermented egg white drink mixed with pineapple juice, EW:ST - fermented egg white drink mixed with strawberry juice.

Davis (2014) asserted that during storage, the temperature has an important role in maintaining the viable cell counts of probiotic bacteria in vegetable and dairy products. On the other hand, after 4 weeks of cold storage the cell counts of *L. casei* 01 were higher around 3 Logs compared to *L.*

salivarius CRL 1328 reaching 8.48, 5.44 Log_{10} CFU/mL) respectively, when they grew in a mix of egg white drink with strawberry juice. In addition to storage temperature, the viability is also a strain dependent property since *L. salivarius* CRL 1328 couldn't maintain its viability due to cold storage.

5.5.8 Effect of cold storage on the pH value

The pH of fermented egg white drink with pineapple juice and strawberry juice using *L. casei* 01 and *L. salivarius* CRL 1328 as a starter culture was monitored during 8 weeks of refrigeration storage.

The pH level of the fermented egg white drink by *L. casei* 01 was initially higher when strawberry juice was added (as shown in *Figure* 24a). However, in the 4^{th} and 8^{th} weeks, the pH level was significantly lower compared to when pineapple juice was incorporated. As a result of lactic acid accumulation over cold storage, the pH of *L. casei* 01 samples decreased until 4 weeks, after which the pH increased in the 8^{th} week. It might be due to dying cells.

Similarly, in case of *L. salivarius* CRL 1328 (*Figure* 24b) EW: ST samples had a much lower pH level compared to EW: PI samples after 4 weeks of storing, although there was no significant difference between them. In a comparable manner between strains, fermented samples by *L. casei* 01 had a higher pH level of 3.98 (EW: PI) and 3.96 (EW: ST) after 4 weeks of refrigeration, when pineapple and strawberry juice were added. Our results were slightly lower compared to those obtained by Parra et al (2013), who tested the utilization of pigeon pea (*Cajanus cajan*) as a suitable substrate for the development of a legume-based fermented product with *Lactobacillus acidophilus* ATCC 314 and *Lactobacillus casei* ATCC 393, where after 28 days of cold storage the pH level of final products dropped approximately to a value of 4. Furthermore, the viability decreased to 7.47 Log CFU/g in samples with banana sauce 20% (w/w) sugar, 7.23 Log CFU/g in samples with strawberry sauce 20% sugar, and 6.21 Log CFU/g in control samples (without further addition).

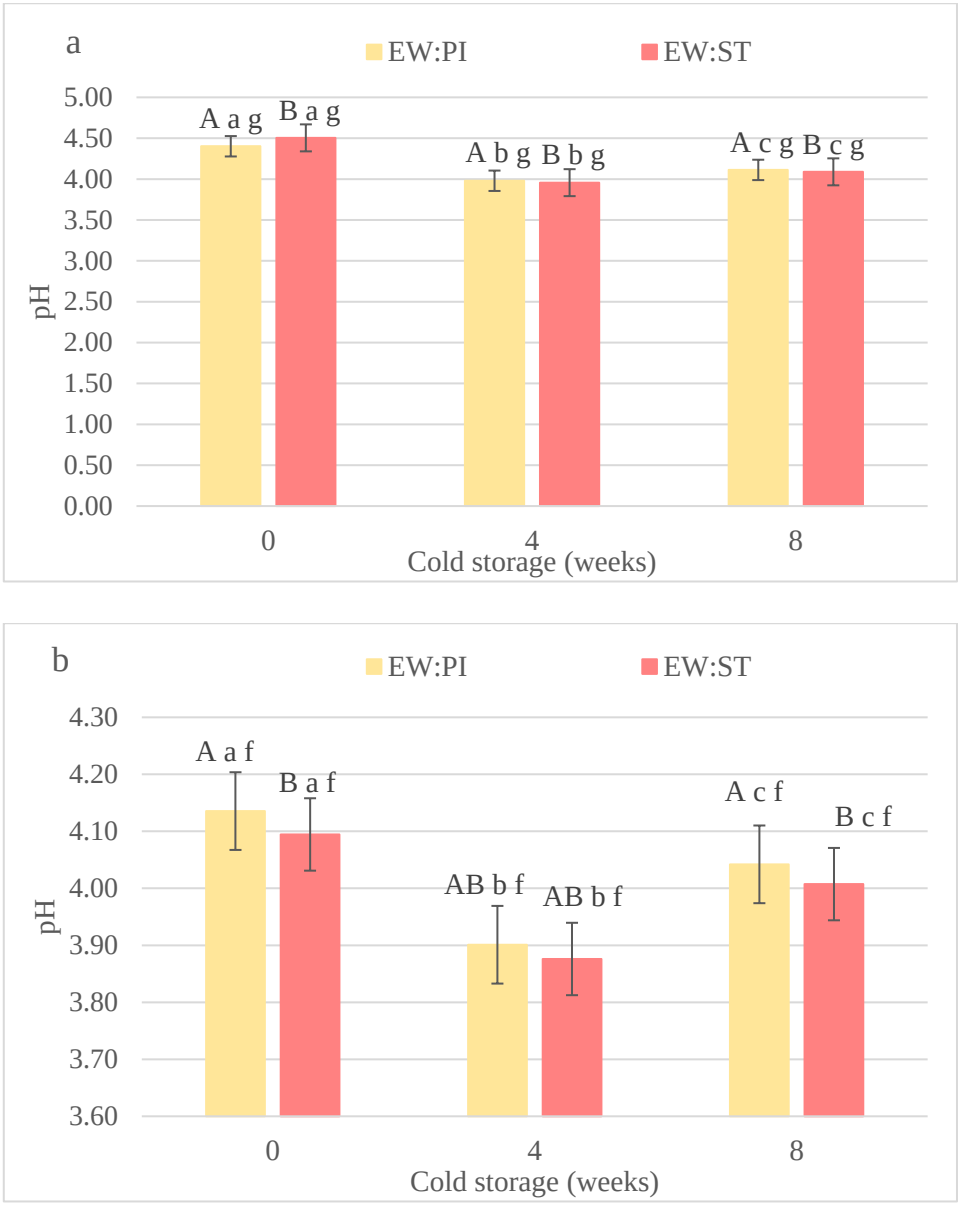


***Figure* 24. The changes in pH values of fermented egg white drink with different fruit juices by *L. casei* 01 (a) and *L. salivarius* CRL 1328 (b) during storage.** Small letters from a to c indicate the significant differences during storage time, (the same strain, the same juice), higher case letters A to B indicate the effect of juice, (the same strain, the same time). Higher case letters from G to F indicate the significant difference between *L. casei* 01 and *L. salivarius* CRL 1328 (the same fruit juice, the same time). EW:PI - fermented egg white drink mixed with pineapple juice, EW:ST - fermented egg white drink mixed with strawberry juice.

5.6 Fermentation of different egg white : pineapple juice ratios by probiotics

Lactobacillus species require fermentable carbohydrates, B-complex vitamins, nucleic and amino acids, and various minerals for effective growth, as they cannot synthesize B-group vitamins and amino acids on their own. Egg white lacks sufficient carbohydrates for probiotic needs, so adding pineapple juice compensates by providing the necessary sugars. Increasing the pineapple juice

ratio could enhance probiotic growth due to higher sugar content. This study investigates the growth of two probiotic strains, *L. casei* 01 and *L. salivarius* CRL 1328, in different ratios of egg white and pineapple juice 4:1, 4:2, and 4:3 (v/v) EW:PI.

5.6.1 Growth of probiotics and volumetric productivity

The proliferation of probiotic bacteria in different mixtures of egg white drink and pineapple juice is shown in *Figure* 25. After 16 hours of fermentation, *L. casei* 01 and *L. salivarius* CRL 1328 could grow in egg white drink with or without adding juice since the initial cell counts of *L. salivarius* CRL 1328 increased considerably from 5.02 Log_{10} CFU/mL to not less than 7.7 Log_{10} CFU/mL, while the initial cell counts of *L. casei* 01 were intensified from 7.7 to 8.16 Log_{10} CFU/mL. These results meet the conclusion of Jiang and coworkers (2020) that lactic acid bacteria could grow in egg white without extra addition. However, samples with pineapple juice contained significantly higher probiotics growth than the control samples due to the availability of carbohydrates in the juice where the probiotics consumed some of the initial glucose and fructose during fermentation (Palachum et al., 2021). In addition, the combination of egg white drink and pineapple juice provided the free amino acids and peptides required by lactic acid bacteria, particularly the proteins found in egg whites, as well as the vitamins and fermentable carbohydrates found in pineapple juice (Klaver *et el*., 1993).

Moreover, a significantly higher cell counts were observed after 16 hours of fermentation for egg white drink by *L. casei* 01 with or without pineapple juice as compared to *L. salivarius* CRL 1328. The cell counts of ratio 4:1, 4:2, and 4:3 egg white drink to pineapple juice formulas fermented by *L. salivarius* CRL 1328 greatly increased from 7.7 Log_{10} CFU/mL to 8.3, 8.2, and 8.2 Log_{10} CFU/mL, respectively. Although, there was no significant difference in *L. salivarius* CRL 1328 growth when different pineapple juice ratios were added.

A total cell counts of 9.17 Log_{10} CFU/mL were achieved in the 4:3 EW: PI and *L. casei* 01 combination, while the ratios of 4:1 and 4:2 EW: PI did not considerably differ in the total cell counts. Both samples reached 8.6 Log_{10} CFU/mL but differed significantly from control samples. To summarize, pineapple juice helps enhance the proliferation of tested probiotic bacteria. This is consistent with the results of Adebayo and Akpeji (2016), who studied the viability, physicochemical, and sensory characteristics of stored fermented pineapple juice using *Pediococcus pentosaceus* LaG1, *Lactobacillus rhamnosus* GG, and *Pediococcus pentosaceus* LBF2 - as a mono and mixed starter. The researchers concluded that pineapple juice enhances the viability of these probiotics.

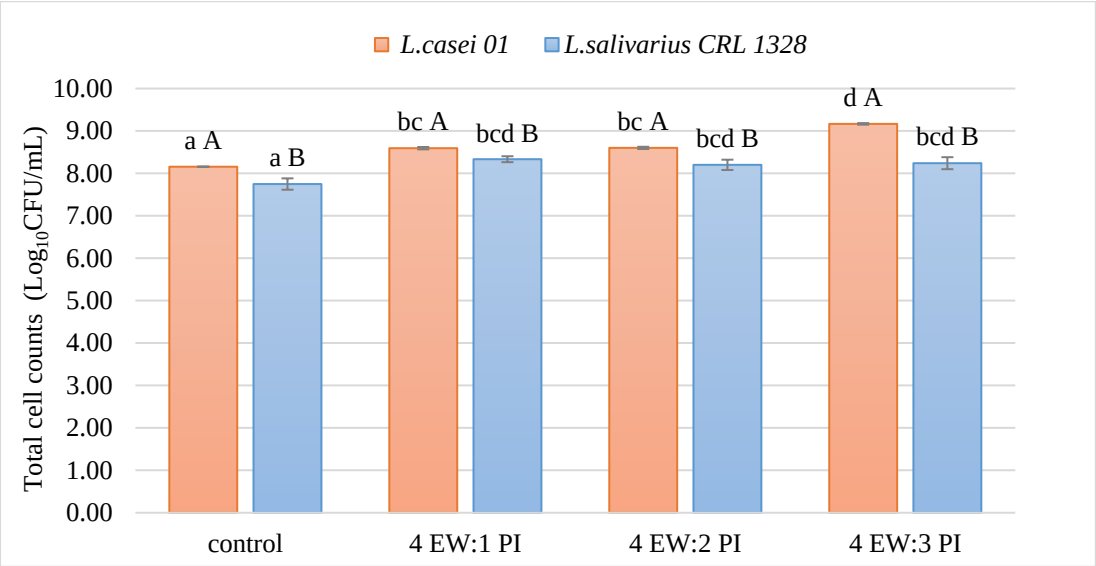


***Figure* 25. The growth of the probiotics in a combination of fermented egg white drink and different pineapple juice ratios**

Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 when using the same formula, and lowercase letters, indicate the difference when different formula was used with the same strain.

The cell yield of probiotic strains was also determined and presented in *Figure* 26, based on the control, the cell yield for *L. salivarius* CRL and *L. casei* 01 was nearly the same ($1*10^6$ CFU*mL^{-1}*h^{-1}). However, when different formulas (4:1, 4:2, and 4:3) EW:PI was applied, *L. casei* 01 recorded a much higher cell yield than *L. salivarius* CRL 1328. In sum, pineapple juice supplementation dramatically increased *L. casei* 01 growth, reaching $5.87*10^7$CFU*mL^{-1}*h^{-1} (equal to 7.77 Log_{10} CFU*mL^{-1}*h^{-1}) in the combination of 4 EW: 3 PI, which is in agreement with a study of Bujna and coworkers (2018) the volumetric productivity of *L. casei* 01 in fermented apricot juice was 7.06 ± 0.29 Log CFU *mL^{-1}*h^{-1}.

Additionally, the level of cell yield in fermented samples by *L. salivarius* CRL 1328 in 4:1 EW: PI samples was not considerably different from 4:3 EW: PI since they reached a level of $1.36*10^7$ CFU* mL^{-1}*h^{-1}.

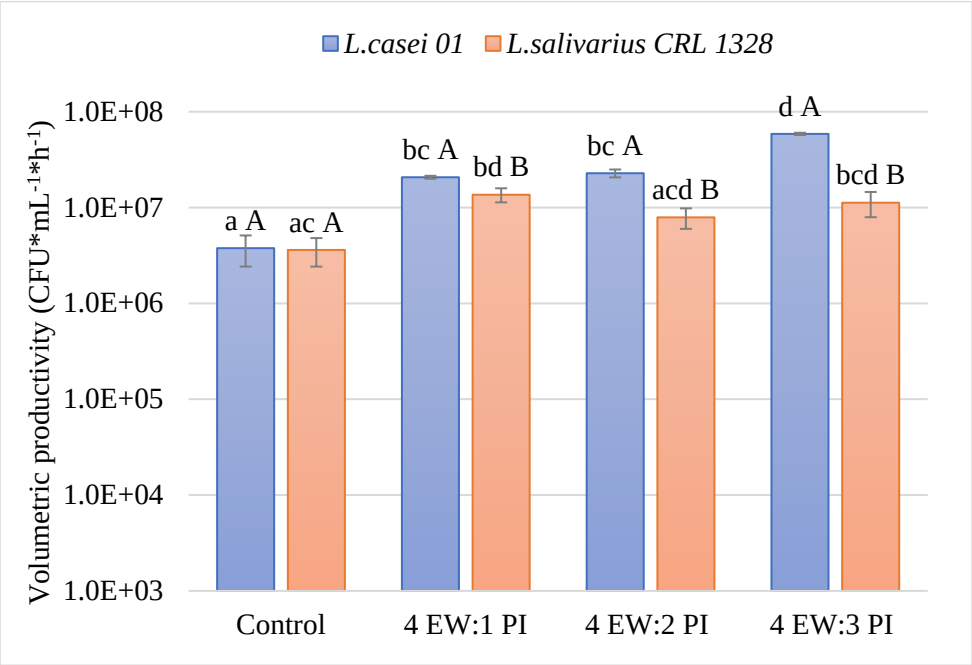


***Figure* 26. The cell yield of probiotics strains in fermented egg white drink with pineapple juice.** Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 when the in the same formula was utilized, and lowercase letters, indicate the difference different formula was used with the same strain.

5.6.2 Changes in the pH values

In addition to increasing acidity, fermentation reduces pH through the production of organic acids such as lactic acid, acetic acid, and other volatile acids. *Figure* 27. shows the pH value of different egg white:pineapple juice blends after 16 hours of fermentation. The pH of fresh unfermented egg white drink was not significantly different, compared to the pH values of control samples after 16 hours of fermentation. As the fermentation time might be insufficient to cultivate the probiotic strains in control samples since probiotic strains require carbohydrate sources to grow and release organic acids, which lower the pH level of the medium.

However, a significant difference was observed when PI was added to *L. salivarius* CRL 1328 and *L.casei* 01 samples reaching a pH value of 4 compared to control samples (pH 6.41), considering that different ratios of the juice did not significantly differ in pH when *L. salivarius* CRL 1328 was used as a starter strain. As stated in the study of Amadou and coworkers (2016) who incorporated pineapple puree in yogurt preparation, fruits are rich in fermentable carbohydrates, which increase the activity of lactic acid bacteria and consequently the formation of lactic acid, which is responsible for reducing yogurt acidity.

The reduction in the pH value is highly important to maintain a good quality of the probiotic fermented product (Viander et al., 2003). However, the results were incompatible with Jiang and coworkers (2020) findings, where a significant reduction in the pH value was noted in fermented egg white solution by probiotic bacteria without further addition. Further, the results were similar to the finding of Palachum and coworkers (2021) as the pH of fermented pineapple juice by *Lactobacillus plantarum* WU-P19 after 12 hours were around pH 4.1, also in the study of Akubor (2016) had a pH value of 4.22 of supplemented yogurt with pineapple juice.

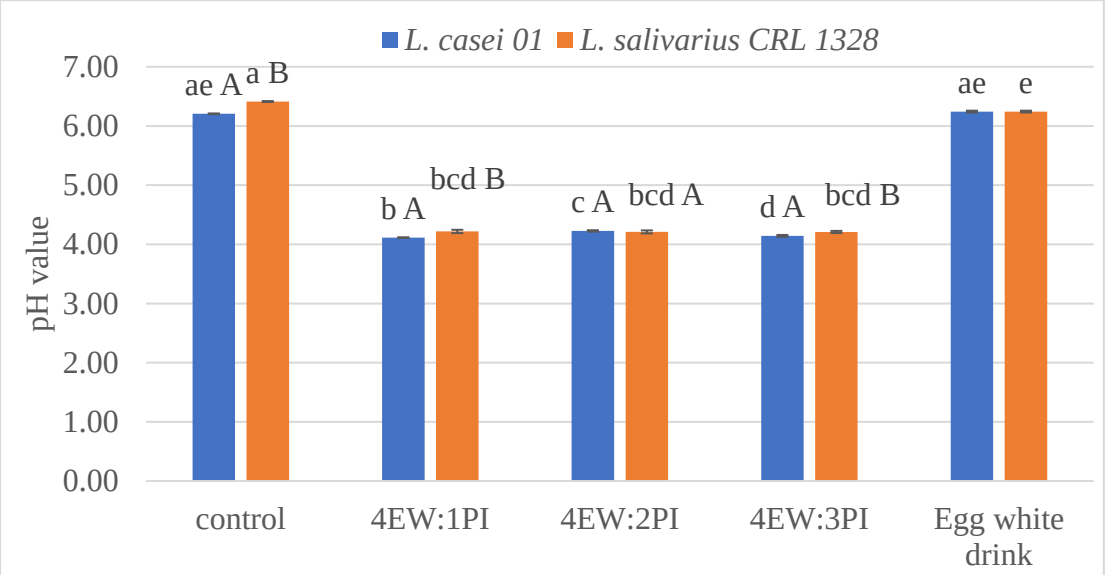


***Figure* 27. The pH values of egg white and pineapple juice mixtures after 16 hours of fermentation**

Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 when the in the same formula was utilized, and lowercase letters, indicate the difference different formula was used with the same strain.

5.6.3 The changes in total protein content and protein profile SDS-PAGE

Quantifying protein content is an important part of food industry practices (Martina and Vojtech, 2015), particularly in egg white or albumin since water and protein constitute the majority of the composition, followed by carbohydrates, ash, and trace amounts of lipids (1%) (Nurliyani et al., 2023). Following *Figure* 28 fresh unfermented egg white drink is a rich source of protein (13.3 mg/mL) which significantly decreased after the fermentation because of the probiotics strains that consumed amino acids required to grow and propagate. Additionally, *L. casei* 01 and *L. salivarius* CRL1328 have an appropriate proteolytic activity (Jiang et al., 2020). There was a significant

difference in the total protein content of the fresh unfermented samples and samples after 16 hours of fermentation time as it decreased from 13.3 mg/mL in the unfermented egg white drink to 7 mg/mL in all samples, that may be attributed to the consumption of protein by probiotic bacteria which is essential to growing. These findings are the same as the results of Oyewole and Ayo Odunfa (1989), they reported a 20% reduction in protein content after cassava fermentation. In addition, Arora and coworkers (2010) indicated a decrease in crud protein after probiotic fermentation of barley-based food mixes.

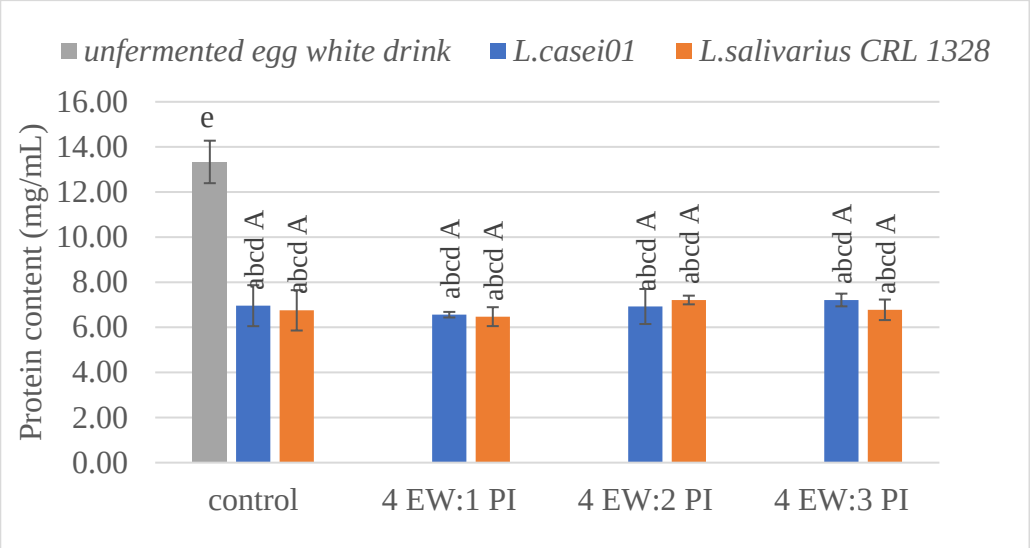


***Figure* 28. The total protein content of fermented egg white drink : pineapple juice mixture after 16 hours of fermentation**

Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 when the in the same formula was utilized, and lowercase letters, indicate the difference different formula was used with the same strain.

Based on the result of SDS-PAGE 6 polypeptide component in the egg white drink fraction were presented in *Figure* 29. The presence of ovalbumin was detected using standards as a comparison. Additionally, it appeared as the largest band on the gel, since it accounts for 54% of egg white protein content (Abeyrathne et al., 2013), the other proteins were white ovo-transferrin (12%), ovomucoid (11%), lysozyme (3.5%), ovo-flavoprotein (0.8%), avidin (0.5%) as the estimation was based on their relative proportions in egg (Stefanova et al., 2021). Also, control samples with *L. salivarius* CRL 1328 without any addition, showed a higher intensity of ovo-transferrin at 75 kDa and avidin at 54.3 kDa than samples containing pineapple juice as the strength of their bands decreased but was still obvious in the gel, this may be related to the proteolytic activity of *L.*

salivarius CRL1328, which increased when pineapple juice was added due to its carbohydrate content or because of the reduction in the egg white drink percentage when higher juice ratios were added. From another point of view, fermentation could change the physicochemical and functional characteristics of proteins in the secondary protein structure, particularly the percentage of alpha-helices and random coils (Gharbi, & Labbafi, 2019). Moreover, ovo-transferrin bands were slightly higher in the control samples compared to fresh egg white drink because ovo-transferrin can bind the metal and turn it into a complex that's resistant to proteolytic activity (Alleoni, 2006). In general, in the egg white drink: pineapple juice combinations, the ovo-transferrin intensity decreased as a result of the decreasing egg white drink ratio. In addition, control samples had a higher level of ovalbumin wherever *L. casei 01* or *L. salivarius* CRL1328 was used compared to samples with pineapple juice.

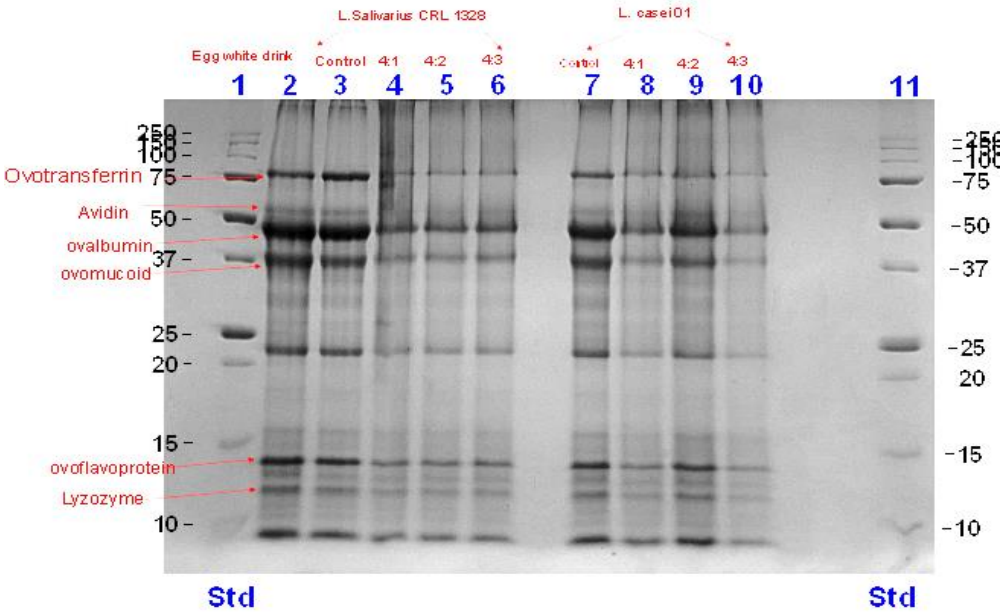


***Figure* 29. The protein profile of fermented egg white drink samples with/without pineapple juice.** 1, 11: Mw. standard, kDa, 2: fresh unfermented egg white drink., 3: 100% fermented egg white drink with *L. salivarius* CRL 1328, 4: a fermented combination of 4:1 egg white drink to pineapple juice using *L. salivarius* CRL 1328, 5: fermented combination of 4:2 egg white drink to pineapple juice using *L. salivarius* CRL 1328, 6: fermented combination of 4:3 egg white drink to pineapple juice using *L. salivarius* CRL 1328, 7: 100% fermented egg white drink using *L. casei* 01, 8: fermented combination of 4:1 egg white drink to pineapple juice using *L. casei* 01, 9: fermented combination of 4:2 egg white drink to pineapple juice using *L. casei* 01, 10: fermented combination of 4:3 egg white drink to pineapple juice using *L. casei* 01.

With regards to *L. salivarius* CRL 1328, there was no significant difference in ovalbumin intensity when pineapple juice was added at different ratios (4:1, 4:2, and 4:3 EW:PI) in addition, they were

lower ovalbumin content compared to 4:2 samples fermented by *L. casei* 01. On the other hand, Alleoni (2006) mentioned that ovalbumin contains 3.5% carbohydrates which may explain the decrease in ovalbumin intensity in control samples compared to fresh EW drink, as probiotic bacteria require carbohydrate source to grow. Ovomucoid at 37 kDa was higher in unfermented samples and control samples compared to samples with pineapple juice since ovomucoid structure consists of 25% carbohydrate (Mills and Tatham, 2003) that may be consumed by the probiotic bacteria to supply their nutritional requirements.

Further, ovo-flavoprotein intensity between 13 and 15 kDa decreased after the fermentation by *L. salivarius* CRL 1328 with different pineapple juice ratios. Moreover, lysozyme intensity decreased in the gel in the case of 4:3 samples fermented with *L. casei* 01.

5.6.4 Changes in the total phenolic content

It is well documented that polyphenols play an essential role in human health due to their antioxidant activity in chelating redox-active metal ions, inactivating lipid free radical chains, and preventing hydroperoxide transformation into reactive oxyradicals (Alias and Abbas, 2017). The total phenolic content (TPC) of unfermented/fermented egg white drink samples with/without PI was measured and presented in *Figure* 30. Although control samples did not differ significantly from unfermented samples, fermented beverages containing pineapple juice, had a slightly higher level of the phenolic content of 0.1 mg GAE /mL versus 0.09 mg GAE /mL in unfermented samples, which could be due to the excellent hydrogen donation properties of phenolic groups. Several studies have demonstrated that phenolic compounds may interact with proteins in multiple ways, both irreversibly and reversibly which affects phenolic recovery and activity (Dimitrellou et al., 2020).

In the 4EW:1PI, and 4EW:3PI combinations, there was no significant difference in TPC between *L. casei* 01 and *L. salivarius* CRL. However, fermented 4EW:2 PI combination by *L. salivarius* CRL 1328 samples were special with different characteristics as they showed higher TPC than *L. casei* 01 samples. Fermented egg whites with *L. salivarius* CRL 1328 had a significantly greater phenolic content in ratios 4:2 and 4:3 EW: PI compared to the control and 4:1 samples.

In the case of *L. casei* 01, the higher juice ratio did not affect the phenolic content as much as in the 4:1 sample and that may be assigned to *L. casei* 01 which was not able to produce antioxidant agents during fermentation (Bujna et al., 2018). Similarly to Nguyen and coworkers (2019), a slight increase in antioxidant activity and phenolic content was detected in the pineapple juice fermented by *Bifidobacterium lactis* Bb12, *Lactobacillus plantarum* 299v, and *Lactobacillus acidophilus* La5. Also, Mostafa and coworkers (2020) indicated an increase in the TPC in the ice

cream after supplementing it with pineapple juice, it reached a value of 198 mg/100g gallic acid when 20 % of the juice was added.

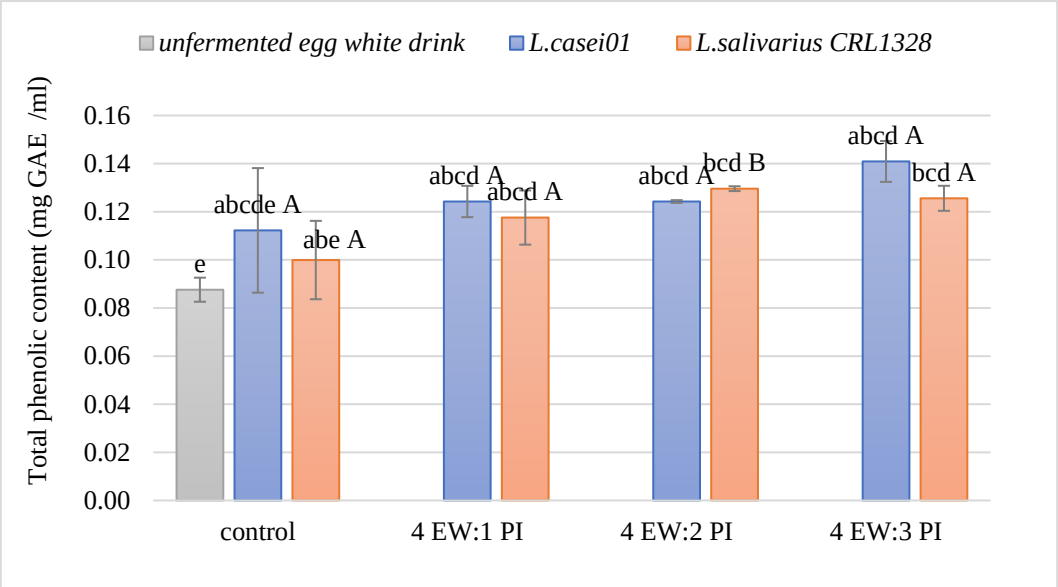


***Figure* 30. The total phenolic content of egg white drink and pineapple juice mixture after 16 hours of fermentation** Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 when the in the same formula was utilized, and lowercase letters, indicate the difference when different formula was used with the same strain.

5.6.5 Changes in the rheological properties of egg white drink and pineapple juice mixture after 16 hours of fermentation

The yield stress (τ0) or the force required to move the network structure of the studied material before starting the flow was presented in *Table* 12. The τ0 values of fermented samples were greater than 0 at the same time it was significantly higher in the fermented samples with *L. casei* 01 compared to the fresh egg white drink. In the case of samples with *L. salivarius* CRL 1328, the control samples were not significantly different from the fresh egg white drink. However, adding pineapple juice to egg white drink fermentation increased the stress yield, as the 4EW:2PI combination required the highest power to move their structure. Mostafa and coworkers (2020) studied the rheological characteristics of the ice cream supplemented with different ratios of pineapple juice (10, 15, and 20%). In their results, the yield stress increased and the apparent viscosity decreased with increasing pineapple juice ratio. Contrastingly, *L. salivarius CRL* 1328 samples had a higher τ0 compared to *L. casei* 01 samples thus, they needed a higher force to move

the network structure, while control samples were not significantly different in τ_0 from unfermented fresh egg white drink.

***Table* 12: The Herschel—Bulkley model parameters for fermented egg white drink: pineapple juice mixture after 16 hours of fermentation**.

		Unfermented EW	Control	4 EW:1 PI	4 EW:2 PI	4 EW:3 PI
L.casei 01	τ_0 (Pa)	0.05 ± 0.007^{e}	0.02 ± 0.002^{Aa}	0.11 ± 0.003^{Abd}	0.17 ± 0.002^{Ac}	0.12 ± 0.003^{Abd}
	n	1.28 ± 0.02^{e}	1.56 ± 0.03^{Aad}	$1.39\pm0.02^{A\,b}$	1.74 ± 0.01^{Ac}	1.59 ± 0.04^{Aad}
	K (Pa.S^{n})	0.0008 ± 0.00008^{e}	$0.00012\pm0.00001^{A\,ad}$	$0.00045\pm0.00001^{A\,b}$	0.000066 ± 0.00004^{Ac}	0.00013 ± 0.00003^{Aad}
L.salivarius CRL1328	τ_0 (Pa)	0.05 ± 0.007^{ae}	$0.06\pm0.01^{B\,ae}$	$0.21\pm0.13^{B\,bd}$	0.23 ± 0.04^{Bc}	0.21 ± 0.05^{Bbd}
	n	1.28 ± 0.02^{e}	1.45 ± 0.05^{Ba}	2.54 ± 0.05^{Bb}	2.75 ± 0.04^{Bc}	2.34 ± 0.02^{Bb}
	K (Pa.S^{n})	0.0008 ± 0.00008^{e}	$0.00027\pm0.00089^{B\,a}$	$0.0000007\pm0.000006^{B\,bc}$	$0.0000028\pm0.00004^{B\,bcd}$	$0.0000029\pm0.0000005^{Bcd}$

Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, read horizontally indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 in the same juice formula, lowercase letters, read vertically indicate the difference between pineapple juice ratios using the same strain. Data are presented with mean ± standard deviation. K: consistency coefficient, n: flow behaviour index, τo: stress yield.

The initial consistency coefficient (K) indicates the resistance to the deformation. As it decreased remarkably after the fermentation, adding juice also reduced the K value significantly compared to control samples, in the case of *L. salivarius* CRL 1328, 4EW:2PI samples were not considerably different from 4EW:1PI, or 4EW:3PI blends. However, 4EW:3PI samples had a significantly higher K value (0.000003) compared to 4EW:1PI (0.000001), on the other hand, using *L. casei* 01 indicated different results, where 4EW:2PI samples recorded the lowest K value (0.00007). Moreover, the consistency coefficient of *L. salivarius* CRL 1328 samples was significantly lower than *L. casei* 01 when pineapple juice was added. Jiang et al., (2020) reported that fermentation could change the molecular structure, surface activity, and rheological properties of egg white by changing the secondary and tertiary structures of egg white protein, reducing the apparent viscosity

of egg white dispersion, which could be attributed to the degradation of albumen proteins (especially ovomucin), in addition to enhanced surface characteristics.

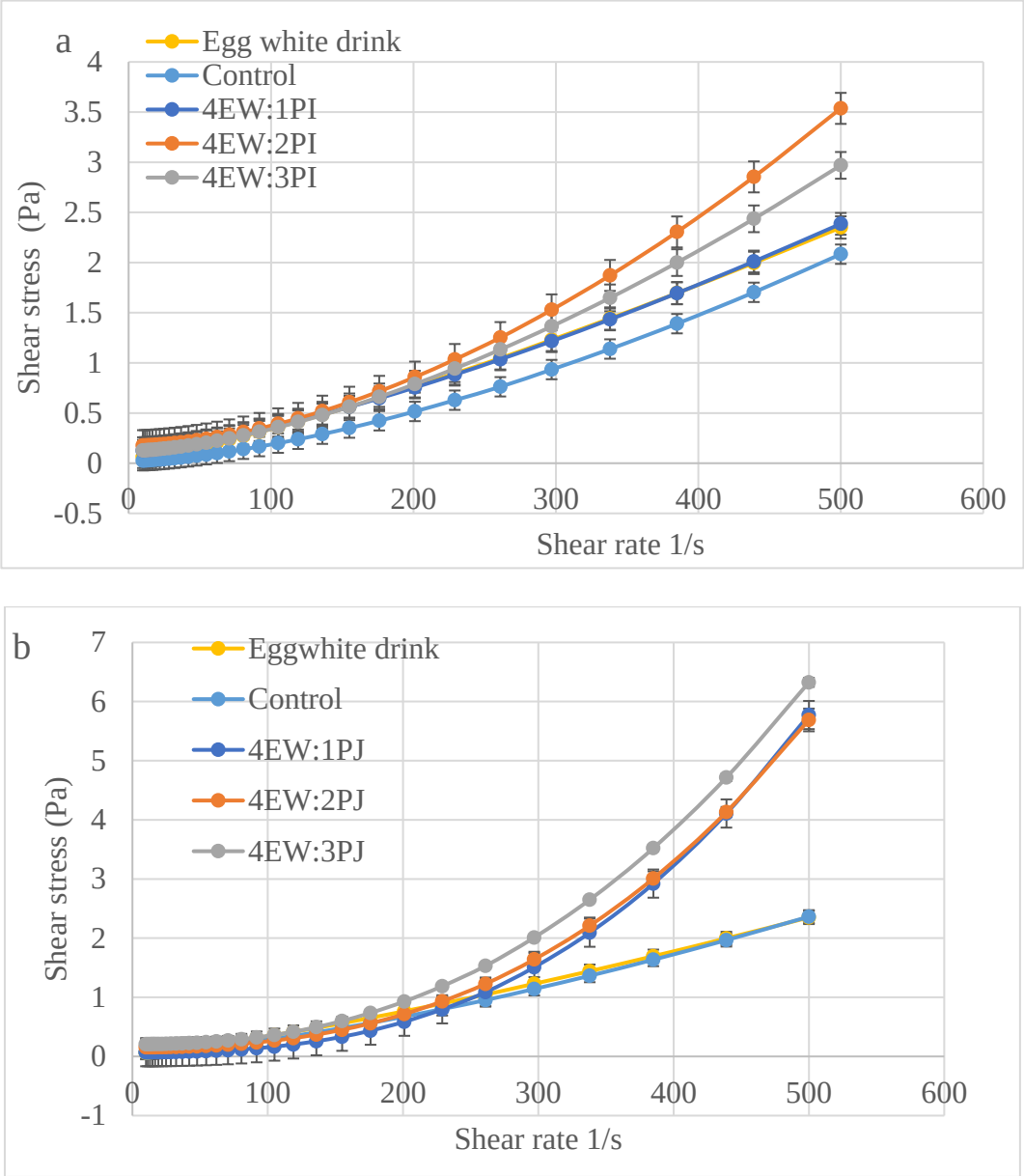


***Figure* 31. Flow curves of fermented egg white samples with different pineapple juice ratios using *L. casei* 01 (a) and *L. salivarius* CRL 1328 (b).** Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice).

Further, the flow behaviour index (n) indicated that all samples had a dilatant or shear thickening behaviour as n values were greater than 1, which is increased after the fermentation in the case of *L. casei* 01, the highest value was detected in 4EW:2PI. In general, fermented samples by *L. salivarius* CRL 1328 had considerably higher dilatant properties in comparison with samples with

L. casei 01. Moreover, as presented in *Figures* 31a and b, adding the juice indicated a rise in the shear stress since 4EW:2PI in *L. casei* 01 and 4EW:3PI in *L. salivarius* CRL 1328 samples recorded the higher shear stress at any shear rate point compared to when another ratio was used with the same strain. This also suggested that the fermented beverages had a shear thickening behaviour which means the viscosity of the studied samples increased with increasing the shear rate, where a high concentration of small solid particulate is suspended within the liquid. However, these results contradict the results of Jiang et al. (2020) who found that fermented egg white drink had a pseudoplastic behaviour. This difference could be explained by the enzymatic treatment that occurred during egg white drink production which caused dissociation of some proteins or might be related to different experiment conditions.

5.6.6 Changes in the colour parameters of egg white and pineapple juice mixture after 16 hours of fermentation

Among the first sensory attributes consumers observe is the colour of food (Dimitrellou et al., 2020). This study examined the colour change parameters in an egg white drink fermented by *L. casei* 01 and *L. salivarius* CRL 1328 with pineapple juice added. The colour changes were measured as L*, a*, and b* and can be seen in *Figure* 32.

The colour parameters were affected significantly ($p < 0.05$) by an increase in the concentration of fruit juices since b* (greenness values) were increasing, L* (lightness value) and a* (blue to yellow value) were decreasing, with increasing pineapple juice ratio in all studied samples expect 4EW:3PI samples fermented by *L. casei* 01 L* were significantly higher than 4EW:2PI samples, Also, b* values of 4EW:2PI, and 4EW:3PI fermented by *L.salivarius* CRL 1328 were not significantly different.

These results also indicated that the colours of the final products became darker, greener, and intensely yellowish as a result of adding the juice due to an increase in the carotenoid percentage with increasing the addition ratio (Khalid et al., 2016). Zhu and coworkers (2022) found that fermentation at a high temperature (25°C, 30°C, and 35°C) resulting a reduction in the lightness. Additionally, our results were synergistic with the results of Mostafa and coworkers (2020) who reported a reduction in L* value and increasing in b* value when different pineapple juice ratios were added to ice cream. Over and above that, samples with *L. casei* 01 were significantly darker compared to samples with *L. salivarius* CRL 1328 since fermented samples with *L. salivarius* recorded higher a* value while b* values were not noticeably different except for 4EW:3PI, it was higher in fermented samples by *L. casei* 01 than *L. salivarius* CRL 1328.

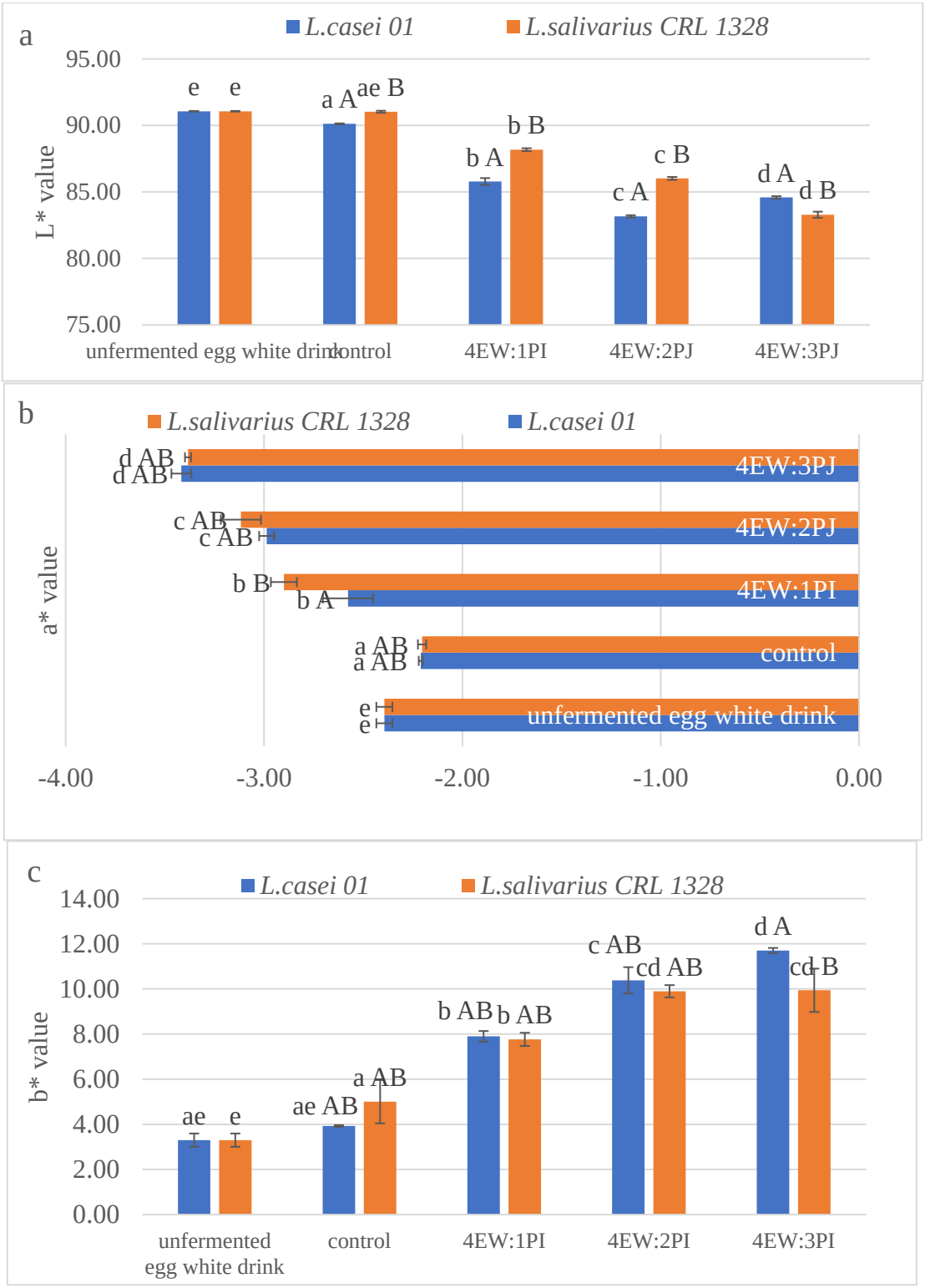


***Figure* 32. Colour parameters of fermented egg white drink samples mixed with different pineapple juice ratios. a* (a), b* (b), and L* values (c).** Control: 100% fermented egg white drink, 4 EW: 1 PI (4:1 egg white drink to pineapple juice), 4 EW: 2 PI (4:2 egg white drink to pineapple juice), 4 EW: 3 PI (4:3 egg white drink to pineapple juice), Higher case letters, indicates the difference ($p<0.05$) between *L. salivarius* CRL 1328 and *L. casei* 01 when the in the same formula was utilized, and lowercase letters, indicate the difference different formula was used with the same strain.

5.7 Studying the effect of adding strawberry juice in different ratios using *L. casei* 01 and *L. salivarius* CRL 1328

L. casei 01 and *L. salivarius* CRL 1328 were grown in EW drink mixed with different strawberry juice ratios including 4:1, 4:2, and 4:3 egg white drink to strawberry juice. During 24 hours of fermentation, the total cell counts and pH were monitored and the color parameters of the final products were determined.

5.7.1 Growth of probiotics

Figure 33a summarizes the findings of a 24-hour investigation on the development of *L. casei* 01 bacterium in various EW: strawberry formulations. The initial cell counts grew by roughly 1 Log in the control samples, but by up to 2 Logs in the strawberry juice samples. This reveals that adding strawberry juice improves the bacterial growth process greatly. After 24 hours of fermentation, the samples with the 4EW:2ST formula had the greatest population of cells (9.61 Log_{10} CFU/mL). The cell counts in samples with the 4EW:3ST formula were 9.33 Log_{10} CFU/mL, whereas samples with the EW to strawberry ratio 4:1 had an average cell counts of 9.04 Log_{10} CFU/mL. Moreover, after 24 hours of fermentation in the 4EW:1ST formula, *L. casei* 01 growth rose significantly, reaching 9.04 Log_{10} CFU/mL. Furthermore, there was no noticeable shift in *L. casei* 01 cell growth when different strawberry juice was added after 8 hours of fermentation time.

Figure 33b shows that after 24 hours of fermentation, the populations of *L. salivarius* CRL 1328 in the control and 4EW:1ST samples were notably the lowest, as they were not significantly different from each other. In contrast, the total cell counts in the 4EW:2ST and 4EW:3ST samples were considerably the highest, reaching 8.6 Log_{10} CFU/mL because the carbohydrate source in the strawberry juice encourages *L. salivarius* CRL 1328 growth. In addition, after 16 hours of fermentation in control samples (7.70 Log_{10} CFU/mL), 8 hours in 4EW:3ST samples (8.61 Log_{10} CFU/mL), and in 4 EW:1 ST samples (8.70 Log_{10} CFU/mL), *L. salivarius* CRL 1328 attained its highest level.

Comparatively speaking, *L. casei* 01 had better growth than *L. salivarius* CRL 1328 after 24 hours of fermentation, regardless of whether strawberry juice was added or not. Since *L. casei* 01 was higher by 1 Log compared to *L. salivarius* CRL 1328. Turgut and Cakmakci (2018) investigated the development of *L. acidophilus* and *B. bifidum* in a yogurt and strawberry marmalade mixture. The two bacteria were examined both as monoculture and mixed culture. The strawberry marmalade to yogurt ratio was 15 g to 100 g. When the pH of all samples reached 4.7, the counts of *L. acidophilus* were found to be the greatest in both mono and mixed cultures, reaching roughly 7 Log CFU/g, while *B. bifidum* recorded 6 Log CFU/g, which was lower than the values reported

in our study. This might be because of the increased proportion of added culture, which in our instance is 4%.

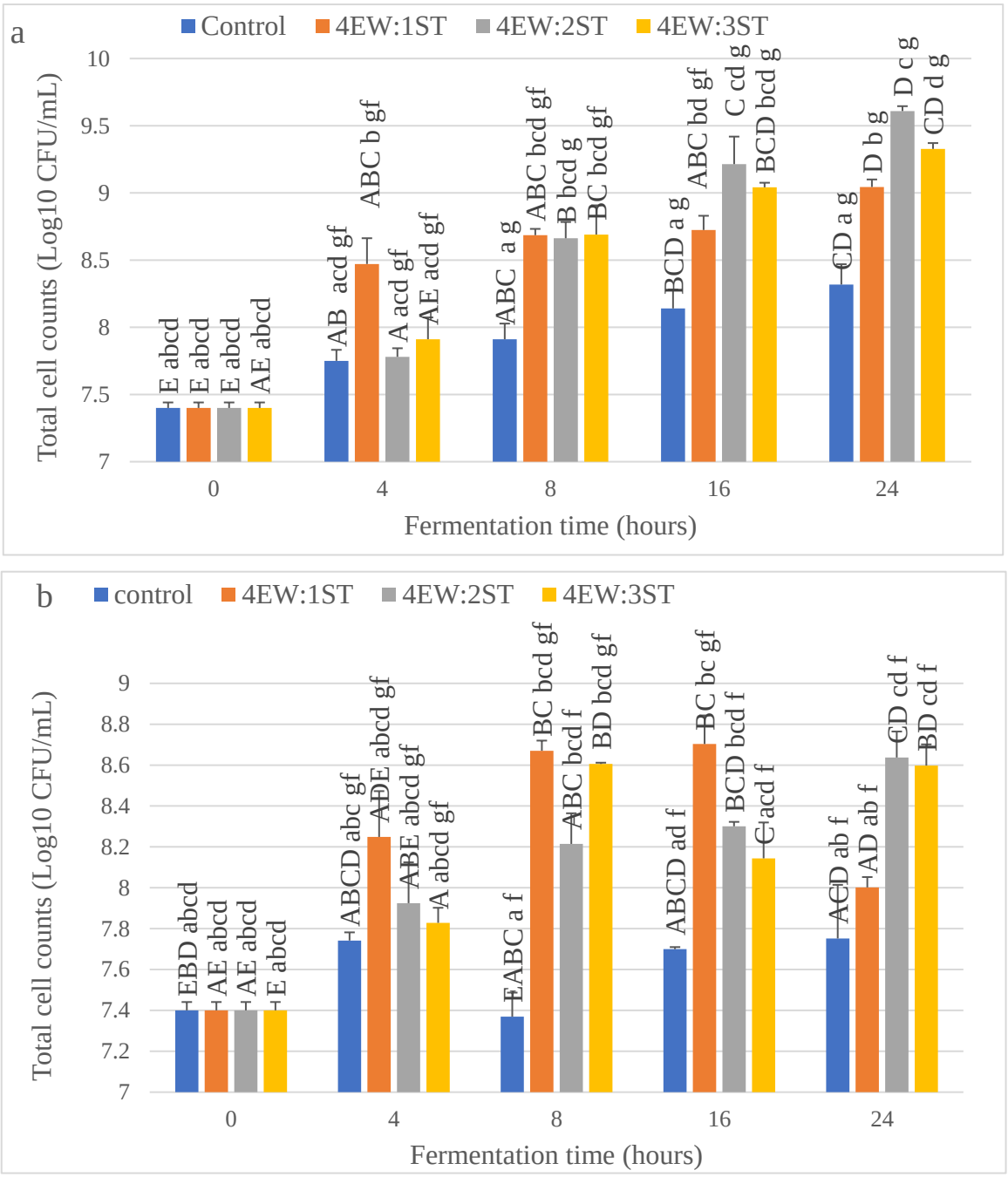
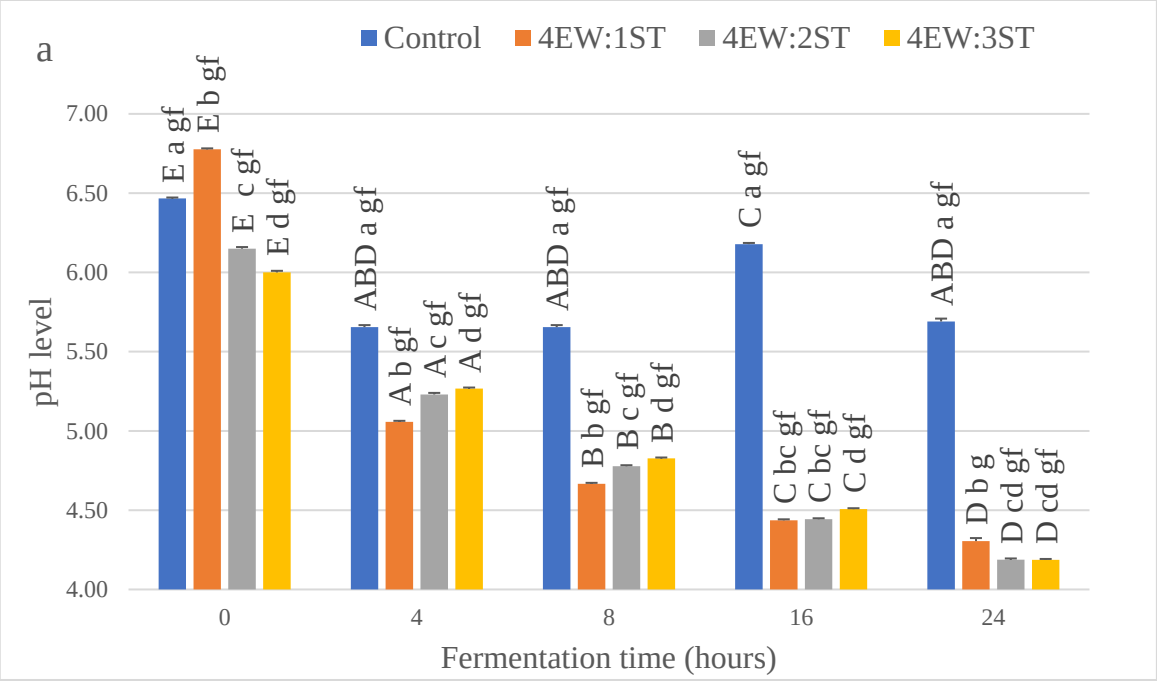


***Figure* 33. The growth of probiotic strains during 24 hours of fermentation in a mixture of egg white drink with different strawberry juice ratios, *L. casei* 01 (a), *L. salivarius* CRL 1328 (b).** Control: 100% fermented egg white drink, 4 EW: 1 ST (4:1 egg white drink to strawberry juice), 4 EW: 2 ST (4:2 egg white drink to strawberry juice), 4 EW: 3 ST (4:3 egg white drink to strawberry juice). Uppercase letters from A to E show the difference in fermentation time for the same strain and formula, whereas lowercase letters from a to d indicate the difference between various formulas for the same strain and time, and letters from g to f represent the difference between strains for the same time and formula.

5.7.2 Changes in the pH values

Figure 34 a and b expresses a reduction in the pH value over 24 hours of fermentation time. The pH level of fermented beverage with *L. casei* 01 was not significantly different from those with *L. salivarius* CRL 1328. In general samples with strawberry juice recorded a lower pH value compared to control samples as a result of increasing the production of organic acid due to the presence of carbohydrates in the strawberry juice.

The initial pH level of control samples decreased dramatically after 4 hours of fermentation reaching a value of pH 5.65 without any significant difference after 8 hours. Further, a 24-hour fermentation period revealed no significant pH difference between the 4:2 and 4:3 formulations. Both exhibited lower pH values (4.18) compared to the 4:1 formulation. This decrease in pH correlates with an increased strawberry juice ratio, suggesting a consequent enhancement in lactic acid production. Turgut and Cakmakci (2018) had a similar finding in their study of investigating a yogurt strawberry marmalade mixed as the pH of samples with *B. bifidum* reached 4.6, and 4.3 when *L. acidophilus* was used as a started culture.



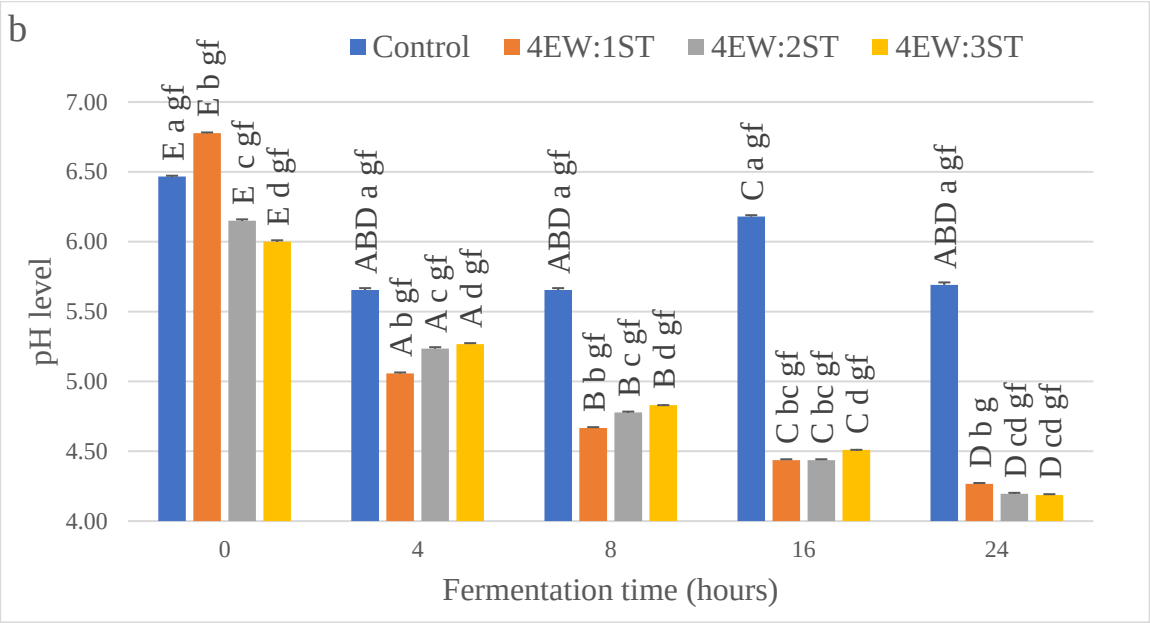


***Figure* 34. The changes in pH level during fermentation of EW drink mixed with different ratios of strawberry juice by *L. casei* 01 (a), *L. salivarius* CRL 13238 (b),** Control: 100% fermented egg white drink, 4 EW: 1 ST (4:1 egg white drink to strawberry juice), 4 EW: 2 ST (4:2 egg white drink to strawberry juice), 4 EW: 3 ST (4:3 egg white drink to strawberry juice). Uppercase letters show the difference in the same fermentation time for the same strain and formula, whereas lowercase letters indicate the difference between various formulas for the same strain and time, and letters from g to f represent the difference between strains for the same time and formula.

5.7.3 Changes in the colour parameters of fermented egg white drink and different strawberry juice ratios mixture

Many factors influence food colour, including anthocyanin, which gives strawberries their colour, according to Scibisz and colleagues (2019). 79% of the anthocyanin in strawberries is pelargonidin-3-glucoside, followed by pelargonidin-3-malonyl-glucoside, pelargonidin-3-rutinoside, and cyanidin-3-glucoside in trace amounts. Furthermore, based on anthocyanin concentration, fruit yogurt can range from red to blue-purple. Furthermore, the food matrix's pH affects the pigments' stability and colour, which appear red at pH 3 (Heredia et al., 1998).

As shown in *Figure* 35a and *Figure* 36, the L* value, which represents the brightness, decreased significantly from 88.86 and 91.43 in control samples with *L. casei* 01 and *L. salivarius* CRL 1328respectively to around 74 in 4EW:3ST samples due to the presence of anthocyanin in the strawberry. Meanwhile, a* value *Figure* 35b, which represents the green-red axis, increased substantially, reaching its highest in 4EW:3ST samples. This means that control samples tend to have more green characteristics, while samples with added strawberry juice are more reddish.

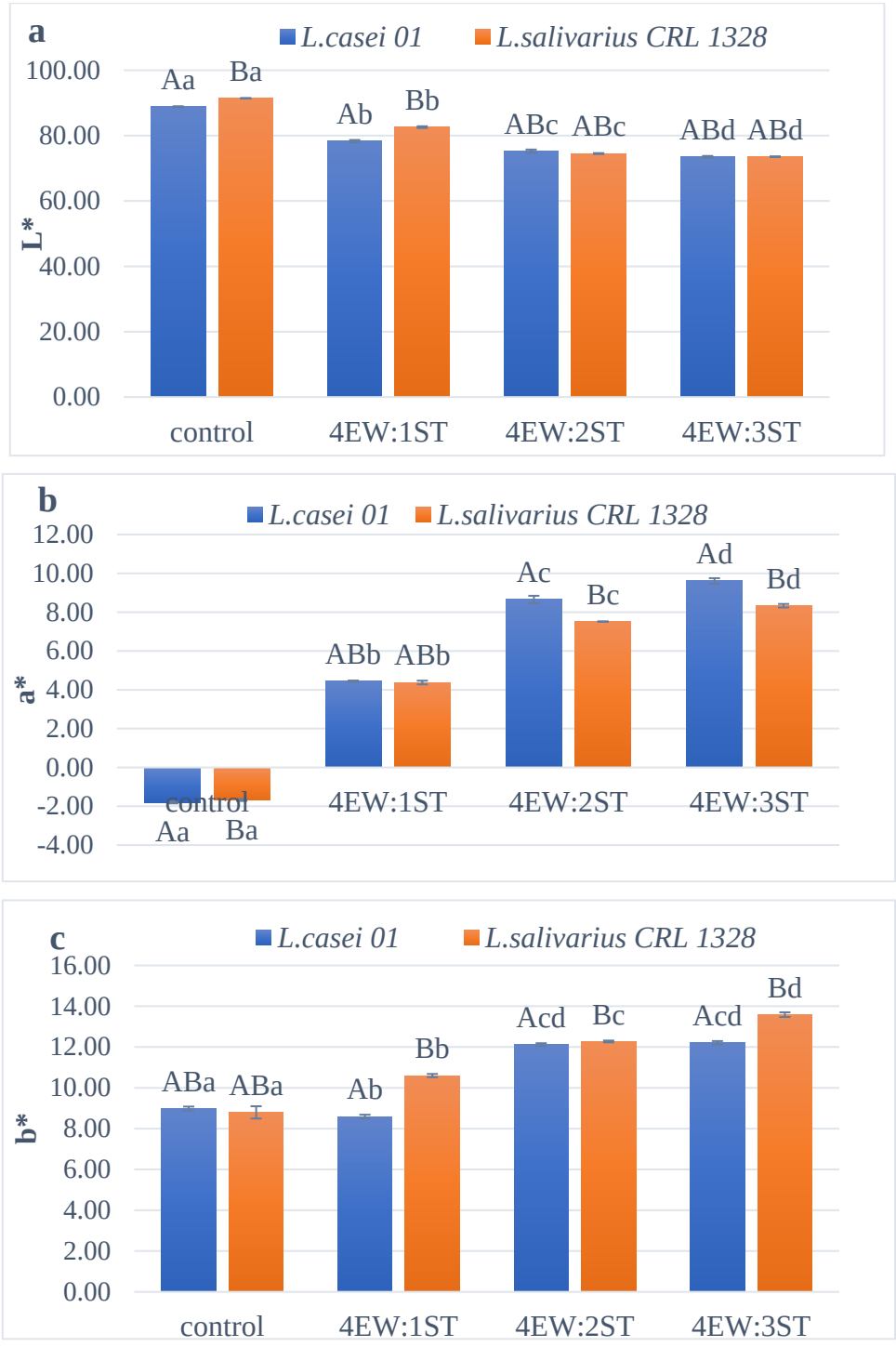


***Figure* 35. Colour parameters of fermented egg white drink by *L. casei* 01 and *L. salivarius* CRL 1328 as a monoculture fermentation using different strawberry juice ratios a (L*), b (a*), c (b*)** Control: 100% fermented egg white drink, 4 EW: 1 ST (4:1 egg white drink to strawberry juice), 4 EW: 2 ST (4:2 egg white drink to strawberry juice), 4 EW: 3 ST (4:3 egg white drink to strawberry juice). Uppercase letters indicate the significant difference when different strain was used with the same strawberry juice ratio, lowercase letters indicate the significant difference when different strawberry juice was incorporated using the same strain.

Upon observing *Figure* 35c, it is evident that the b* value which indicates the blue-yellow axis increased significantly with the increasing ratio of strawberry juice, resulting in the samples appearing more yellowish compared to the control samples. The colour parameters of fermented samples by *L. casei* 01 were significantly different from those of samples with *L. salivarius* CRL 1328. This difference may be due to the varying acid production of both strains. Higher acid production caused a change in pH, which in turn altered the anthocyanin colour. Additionally, the distinct microbial growth, metabolic by-products, and enzymatic activity of both strains may have contributed to the differences observed.

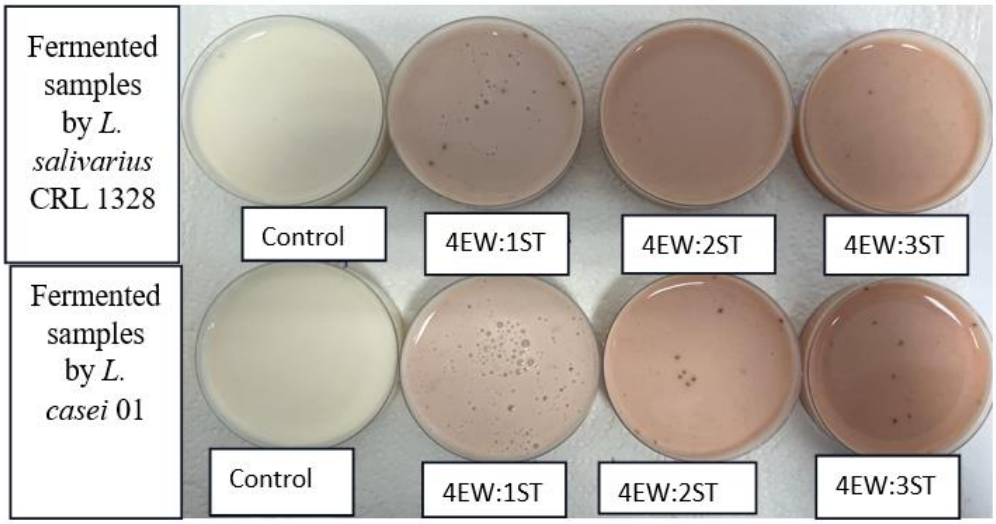


***Figure* 36. Fermented egg white drink with different strawberry juice ratios**

Our observations were consistent with those of Verma and colleagues (2019), who reported a reduction in L* and increasing in a* values with an increase in the ratio of strawberry puree to soy beverages. In contrast to our findings, they also reported a decrease in the b* value.

5.8 Study the effect of mixed culture (*L. casei* 01 and *L. salivarius* CRL 1328) in the fermentation of egg white drink with and without strawberry juice

Recent approaches have suggested the use of mixed-culture fermentations, which involve two or more microbial species, to enhance the fermentation process and the quality of the final product (Tangyu et al., 2019). In this study, mixed culture fermentation with *L. casei* 01 and *L. salivarius* CRL 1328 was employed to boost product quality, maximize the health benefits of both strains and improve technological aspects. These cultures were grown in an egg white drink both with and without a strawberry puree mixed in a 3:1 ratio of egg white drink to strawberry puree.

5.8.1 Growth of probiotics and the changes in the pH value

Figure 37a. shows the growth and pH value after mono and mixed culture fermentations involving *L. casei* 01 and *L. salivarius* CRL 1328 in egg white drink without further addition. According to *Figure* 37b, the pH value of the egg white drink was significantly higher compared to the fermented samples after 24 hours. Moreover, the samples with mixed culture fermentation achieved a pH of 5.34, which was higher than the samples fermented with *L. salivarius* CRL 1328 alone. The total cell counts in the mixed culture samples were higher, recording a value of 7.94, compared to 7.78 in the samples with *L. salivarius* CRL 1328, but there was no significant difference compared to the samples with *L. casei* 01.

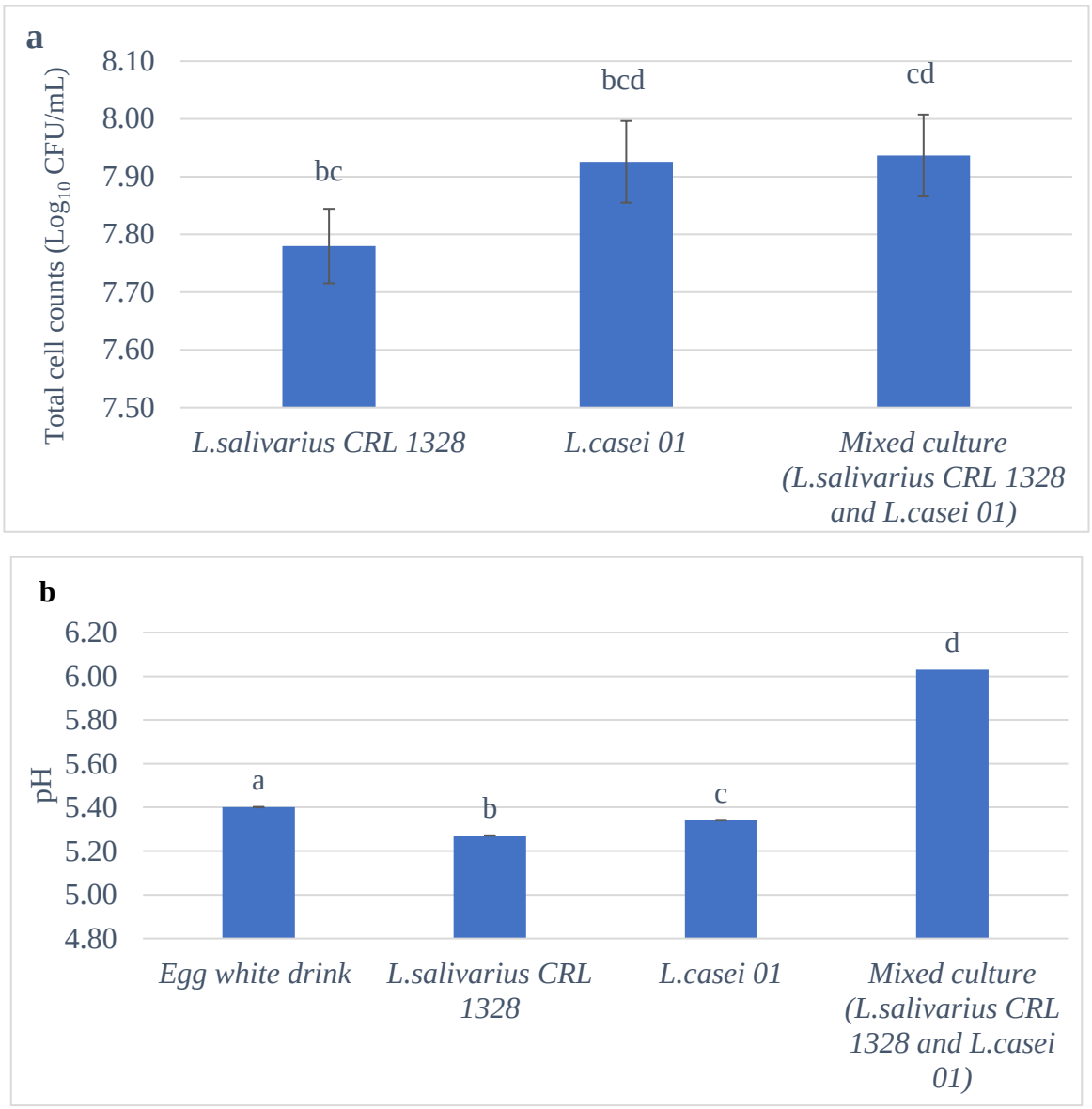


***Figure* 37. Mono and mixed culture fermentation of egg white drink**. (a) total cell counts, and (b) pH value. Lowercase letters indicate the significant differences.

5.8.2 Lactic acid content

Lipolysis, proteolysis, and carbohydrate breakdown are three primary processes contributing to fermentation. During the breakdown of carbohydrates, lactic acid is generated, which results in acidifying of the product (Harper et al., 2022).

As demonstrated in *Figure* 38, adding strawberry juice to the fermentation of egg white drinks increased the lactic acid content dramatically, from 0.04 to 0.07 g/100 g in samples with *L. casei* 01 and from 0.02 to 0.08 g/100 g in fermented samples with mixed culture. This was likely due to the strawberry's ability to increase the growth rate of Lactic acid strains due to its carbohydrate content. However, samples with *L. salivarius* CRL 1328 showed the highest lactic acid content regardless of whether strawberry juice was incorporated or not. In comparison to sour Boza, a traditional Turkish drink made by fermenting millet, cooked maize, wheat, or rice semolina using yeast and lactic acid bacteria, our results were lower. Since the titrable acidity was estimated to be between 0.5-1% expressed as lactic acid g/100g (Arici and Daglioglu, 2002). Furthermore, our findings were similar to those of Deziderio and colleagues (2023), who examined fermented plant-based beverages made from hydro-soluble extracts of oats, and rice and fermented by lactic cultures of *Bifidobacterium lactis* BB-12, *Lactobacillus acidophilus* LA-5, and *Streptococcus thermophilus* at 37 °C for 12 hours as the titrable acidity reached 0.02% in rice extract and 0.15% in oat extract. In contrast, it was significantly lower compared with Turgut and Cakmakci, (2018), as the titrable acidity reached 0.7% in samples with *B. bifidum* and 0.9 in samples fermented by *L. acidophilus.*

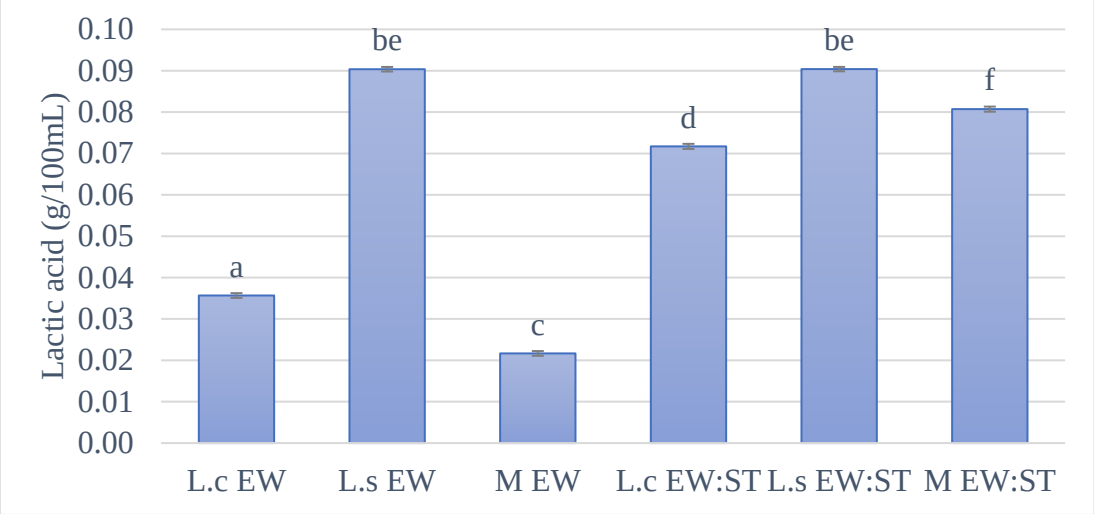


***Figure* 38. The lactic acid content of fermented egg white drink with/without strawberry puree.** Lowercase letters indicate the significant difference, $p<0.05$. L.c EW- fermented egg white drink with *L. casei* 01, L.s EW- fermented egg white drink with *L. salivarius* CRL 1328, M EW- fermented egg white drink using a mixed culture of *L. salivarius* CRL 1328 and *L. casei* 01, L.c

EW:ST- fermented egg white drink mixed with strawberry juice and *L. casei* 01, L.s EW:ST- fermented egg white drink mixed with strawberry juice and *L. salivarius* CRL 1328, M EW:ST- fermented egg white drink mixed with strawberry juice using a mixed culture of *L. salivarius* CRL 1328 and *L. casei* 01.

5.8.3 Changes in the total phenolic content

It was found that after fermenting the egg white drink for 24 hours with strawberry puree in a 3:1 ratio EW: ST using a mono and mixed starter of *L. casei* 01 and *L. salivarius* CRL 1328, the total phenolic compounds expressed as Gallic acid mg/mL (*Figure* 39), increased significantly from 0.08 to 0.2 mg/mL in the fermented samples. Similarly to Adebo and Medina Meza (2020), in their study on the fermentation of whole grain cereals, they found an increase in total phenolic content and antioxidant activity. This is because when the cereal cell wall is broken down subsequent enzymatic activity releases phenolic compounds thus increasing antioxidant activity. Moreover, there was no significant difference in the phenolic content if monoculture or mixed starter culture was utilized. On top of that, adding strawberry juice in a 1:3 ratio to egg white drink slightly enhanced the total phenolic content as the final product had higher total phenolic content in comparison to samples without further addition, possibly belonging to the anthocyanin group, which is the dominant phenolic group in strawberry (Yu et al., 2011). Our findings were substantially greater than those of Verma and colleagues (2019), who found that the phenolic content in nonfermented soy milk blended with strawberry juice at 5, 10, 15, and 20% ratios of soy drink was around 0.007 mg gallic acid eq./mL.

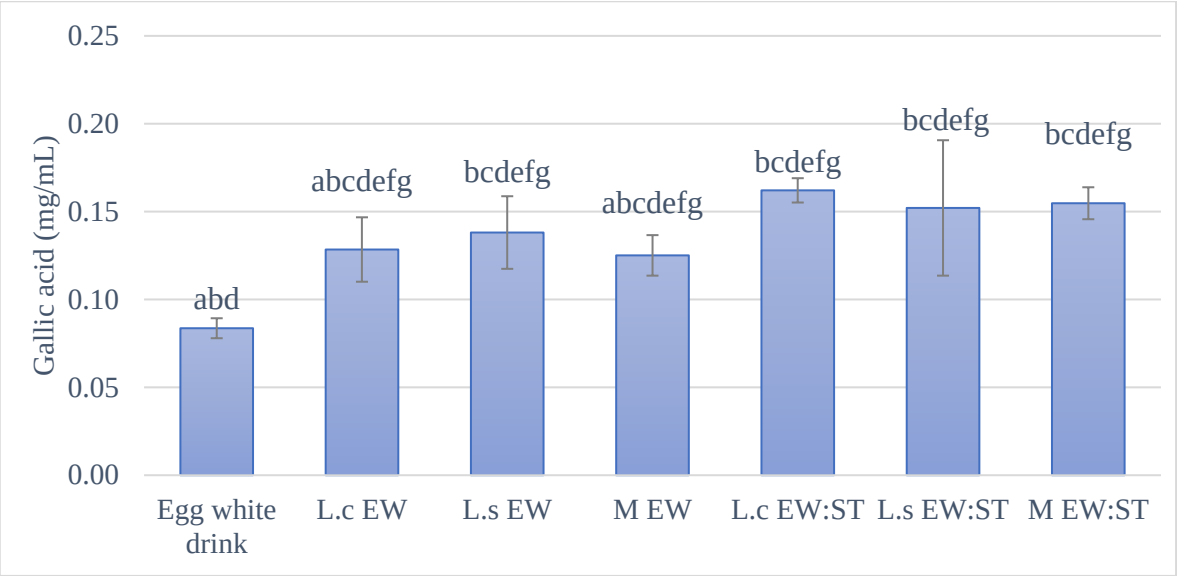


***Figure* 39. Total phenolic content of egg white drink mixed with strawberry puree and fermented by mono and mixed culture of lactic acid bacteria.** Lowercase letters indicate the significant difference, $p<0.05$. L.c EW- fermented egg white drink with *L. casei* 01, L.s EW-

fermented egg white drink with *L. salivarius* CRL 1328, M EW- fermented egg white drink using a mixed culture of *L. salivarius* CRL 1328 and *L. casei* 01, L.c EW:ST- fermented egg white drink mixed with strawberry juice and *L. casei* 01, L.s EW:ST- fermented egg white drink mixed with strawberry juice and *L. salivarius* CRL 1328, M EW:ST- fermented egg white drink mixed with strawberry juice using a mixed culture of *L. salivarius* CRL 1328 and *L. casei* 01.

5.8.4 The antagonistic activity of fermented egg white drink with and without strawberry juice against pathogens

The antimicrobial properties of *L. casei* 01 and *L. salivarius* CRL 1328 were evaluated using the agar diffusion method. The probiotic strains were cultured as single strains or in combination, in both MRS media and fermented egg white drink with and without strawberry juice. The antimicrobial activity was assessed after 24 hours of fermentation. *Table* 13 shows that all fermented beverages have antimicrobial activity against *E. coli* 8739, especially when EW is mixed with strawberry, regardless of the strain and whether it was a mixed or monoculture fermentation. As a result of low pH as well as fermentation by-products which present an antimicrobial activity. Also, fresh EW drink samples showed antagonistic activity. According to Legros and coworkers (2021), egg whites possess several natural antimicrobial properties due to ovo-transferrin. In addition, fermented EW: ST samples by *L. casei* 01 or *L. salivarius* CRL 1328 individually showed a larger clear zone than others, which indicates an enhanced antimicrobial effect against *E. coli* 0157: H7. Interestingly, all samples have antimicrobial activity against *E. cloacae* but the clarity zone size varies from sample to sample since it is smaller in fermented samples by a monoculture fermentation and samples without strawberry puree but expanded when strawberry juice was incorporated. *Listeria innocua* and *Enterococcus faecalis* showed similar effects when strawberry juice was added, as adding it enhanced antagonistic activity.

According to studies (Cárdenas-Valdovinos et al., 2018; Liya and Siddique, 2018), strawberries contain phenolic compounds that have remarkable antimicrobial properties. An antagonistic effect against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, and *Bacillus subtilis* ATCC6633 was also observed in a study by Zhao and coworkers (2021), who examined the antimicrobial activity of fermented strawberry juice by using starter strains of *Lactobacillus plantarum* and *Lactobacillus bulgaricus* and yeast.

***Table* 13. Antagonistic activity of fermented egg white drink using mono or mixed culture fermentation with and without strawberry puree**.

Samples		Agar diffusion test				
	Pathogen	*E. coli* 8739	*E. coli* 0157: H7	*E. cloacae*	*Listeria innocua*	*E. faecalis*
MRS media		**MRS media**				
MRS media	*L. casei* 01	2 mm	3mm	2mm	1mm	2mm
	L. salivarius CRL 1328	2 mm	3mm	2mm	1mm	2mm
	Mixed culture	3mm	3mm	3mm	3mm	3mm
Egg white	(without fermentation)	2mm	3mm	2mm	2mm	1mm
		Fermented EW beverages				
Egg white	*L. casei* 01	3mm	1mm	1mm	2mm	2mm
	L. salivarius CRL 1328	3mm	2mm	2mm	2mm	1mm
	Mixed culture	3mm	3mm	3mm	3mm	2mm
Egg white with strawberry	*L. casei* 01	3mm	3mm	3mm	3mm	3mm
	L. salivarius CRL 1328	4 mm	3mm	3mm	3mm	3mm
	Mixed culture	4 mm	2mm	3mm	2mm	3 mm

What is more, when the studied strains were growing in MRS broth, they exhibited antagonistic activity *Table* 13. against the tested pathogen, both when mixed or used separately. However, the diameter of the clear zone increased when strains were mixed, while the results were similar when the test was conducted on *E. coli* 0157:H7. As noted by Shukla and Sharma (2015), *L. casei* supplements can potentially be utilized as a health-enhancing agent as well as a defence against hazardous pathogenic microbes. Because it established an inhibitory zone over *P. aeruginosa* MTCC-103, *S. aureus* MTCC-740, *E. coli* MTCC-1652. Also, Mulla et al. (2021) confirmed that the probiotic *Lactobacillus salivarius* at 50 mg/mL is an effective method of protection against peri-implant infections such *P. gingivalis*, *P. intermedia*, *S. salivarius*, and *S. aureus*. For the reason that *L. salivarius* has antagonistic abilities that may strengthen host immunity and inhibit the progression of an infection.

6 Conclusion and recommendations

Egg white drink is high in functional proteins and is also free of cholesterol and fat, making it an excellent replacement for those who want to avoid dairy drinks. It can also serve as a vehicle for probiotics. The final characteristics of a fermented beverage are highly dependent on the type of starter culture used, whether it is a mixed or monoculture fermentation. It is also important to consider the conditions of the medium in which fermented beverages are produced, as each strain reacts differently to different environments. To ensure that the final product meets consumer demands, it is crucial to optimize the conditions of the fermentation process, including fermentation time, probiotic strain, and type of carbohydrate source, to achieve the desired properties.

After screening different probiotic lactic acid bacteria and bifidobacterial for the fermentation of egg white, six distinct strains of lactic acid bacteria including *L. helveticus* R52, *L. acidophilus* 150, *L. rhamnosus* Rosell 11, *L. casei* 01, *L. salivarius* CRL 1328, and *L. plantarum* 299v and 2 *Bifidobacterium* strains (*B. longum* Bb46, and *B. longum* DSM 16603) were chosen for further studies. It can be concluded that these strains are able to thrive in egg white drink with a 2% addition of glucose after 24 hours of fermentation time. The cell counts were greater than 7 Log_{10} CFU/mL, indicating that the growth was effective. Besides, *L. casei* 01 and *L. plantarum* 299v, recorded the highest cell concentration and smallest pH levels. In contrast, *L. helveticus* R52 had the lowest total cell counts and the highest pH level. In addition, no substantial variances between *Bifidobacterium longum* DSM 16603 and *Bifidobacterium longum* Bb46 growth were found, although, *Bifidobacterium longum* Bb46 had less generation time.

To investigate the influence of various carbohydrate sources, including monosaccharides (glucose and fructose), disaccharides (sucrose), and oligosaccharides (FOS, GOS, and XOS), *L. acidophilus* 150, *L. plantarum* 299v, and *L. casei* 01 were selected as the most promising. The results demonstrated that *L. casei* 01 had the greatest growth in the free carbohydrate sources condition, as well as in samples with fructose, FOS, and GOS. Also, *L. plantarum* 299v growth showed no significant difference between control samples, samples with glucose, and samples with sucrose. However, the cell counts were considerably higher in samples with FOS compared to other carbohydrate sources for this strain. Furthermore, when polysaccharides were included in *L. casei* 01 fermented samples, the pH level was the lowest. In addition, the pH level of *L. plantarum* 299v fermented samples was not substantially changed whether mono or disaccharide was added.

The viscosity of a fermented egg white drink can vary depending on several factors such as the strain used, whether a carbohydrate source was added before fermentation, and the type of sugar

added. For example, when monosaccharides (such as glucose or fructose) or oligosaccharides (such as FOS or GOS) were added before fermentation, the strain *L. plantarum* 299v had the highest yield stress. On the other hand, when sucrose was added, it had the lowest yield stress. Another strain, *L. casei* 01, required more energy to start flowing its structure when sucrose and XOS were added to the egg white drink. On the contrary, it needed less power in the case of FOS addition. Overall, during the fermentation process, *L. acidophilus* 150 had the lowest stress yield without any additional supplements, as well as in samples with fructose, GOS, and XOS. The consistency coefficient (K) of both control and egg white samples with fructose fermented by *L. plantarum* 299v indicated a higher viscosity compared to samples with *L. acidophilus* 150 and *L. casei* 01. Additionally, the K value of fermented egg white beverages by *L. casei* 01 was at its lowest when fructose and FOS were added. The flow behaviour index of the fermented samples represented a shear thickening behaviour, as the viscosity increased with increasing the shear rate. In general, samples with *L. plantarum* 299v recorded the highest n value when different carbohydrate sources were added, except for *L. casei* 01 samples with fructose and FOS, which showed the greatest n value.

Studying microbial stability during refrigeration is crucial, particularly for probiotics. It is advised that the probiotics population shouldn't be less than 10^6-10^7 CFU/mL at a consuming time. The ability of probiotic bacteria to survive in cold environments may also be affected. As a result, *L. casei* 01 and *L. plantarum* 299v were chosen for this investigation in fermented egg white beverages. For three weeks of cold storage, fructose and FOS were utilized as distinct sources of carbohydrates. The total cell counts of control samples fermented by *L. plantarum* 299v were higher than 8 Log_{10} CFU/mL, and 9 Log_{10} CFU/mL in samples with fructose and FOS respectively. The refrigeration helped to keep the cell population steady over 3 weeks. The fermented beverage with FOS had a significantly higher cell population in the third week compared to samples with fructose. In the case of *L. casei* 01 survivability, the total cell counts did not reduce to less than 8.04 Log_{10} CFU/mL in control samples and 8.71 Log_{10} CFU/mL in samples with carbohydrate sources. They were stable over the studied refrigeration time without any significant difference between the two types of beverages with fructose and FOS. During the storage period, the pH levels in the control samples were higher compared to samples with carbohydrates. This was due to the lower cell counts and, consequently, less organic acid production. In the third week of storage, the pH levels in the control samples remained relatively stable, while the pH levels in samples with carbohydrates decreased. There was no significant difference between the fermented egg white samples containing fructose and FOS. A similar trend was observed in *L. casei* 01 samples, with a reduction in pH levels observed in all samples without any significant difference

between fructose and FOS samples. It is worth noting that fermented beverages with *L. plantarum* 299v had lower pH levels compared to *L. casei* 01 samples at the end of the storage period. In general, the fermentation process extended the shelf life of egg white from 2 days to at least 3 weeks of cold storage.

The stress yield of control samples fermented by *L. plantarum* 299v did not change with storage time but it reduced in samples containing carbohydrate sources. Furthermore, it was substantially different between EW completed with fructose and FOS; the viscosity of control samples remained constant from the first week until the end of the study period, but it increased in EW with fructose samples in the third week and in FOS samples in the second week. Furthermore, the shear thickening characteristic remains in all fermented beverages throughout time. Moreover, the stress yield of *L. casei* 01 control samples declined in the third week of storage and remained constant for fructose and FOS samples, with no significant difference from control samples. K value didn't change when carbohydrate was added to the fermentation of the EW drink, while it decreased in the 3rd week in control samples. Also, a shear thickening behaviour was noticed over time.

Due to the nutritional benefits and carbohydrate content present in fruit juices, their incorporation into the fermentation process of egg whites enhances the overall nutritional value and supports the growth of probiotics. This study focused on investigating the growth of *L. casei* 01 and *L. salivarius* CRL 1328 in a combination of egg white drink and various fruit juices (peach, pineapple, and strawberry) at a 3:1 ratio of egg white to fruit juice. Results revealed that, after 24 hours of fermentation, there was no significant difference in total cell counts when different fruit juices were added. However, the growth of *L. casei* 01 was notably higher by 1 Log compared to *L. salivarius* CRL 1328. Moreover, in samples fermented by *L. casei* 01 and containing either pineapple or strawberry juice, the difference in cell counts after 16 and 24 hours was minimal. This suggests that a 16-hour fermentation period is sufficient to obtain the final product. In contrast, when peach juice was used, a 24-hour fermentation time was necessary to reach the highest cell counts. Additionally, EW samples mixed with peach, strawberry, or pineapple juice fermented by *L. salivarius* CRL 1328 did not significantly differ in cell counts after 8, 16, and 24 hours. Therefore, an 8-hour fermentation time is recommended to complete the fermentation process in these cases. The pH of the fermented sample exhibited a significant decrease throughout the fermentation period. After 24 hours, *L. salivarius* CRL 1328 displayed consistently higher pH levels compared to *L. casei* 01 samples, with those mixed with pineapple juice recording the highest pH value. In the case of EW with strawberry juice fermented by *L. casei* 01, the pH level was higher compared to when pineapple or peach was added. As a result of colour analysis, the L* value was at its peak, and the a* value was at its lowest when pineapple juice was incorporated.

Conversely, the L* value reached its lowest, and the a* value was at its highest in samples with strawberry juice. Additionally, the b* value of peach juice samples was the highest, followed by samples with strawberry juice.

The protein profile of fermented EW drink mixed with fruit juices did not change much in terms of the protein concentration, but the bands remained recognizable on the gel. Meanwhile, peach samples showed enhanced ovalbumin bands, especially when fermented by *L. salivarius* CRL 1328. Based on the results of sensory evaluation, *L. salivarius* CRL 1328 samples with strawberry juice had the strongest fruity odour and the least prominent egg flavour consequence, it obtained the highest overall sensory assessment score.

The stress yield of fermented samples containing pineapple juice and *L. casei* 01 was considerably higher than that of samples containing other fruit juices, with no statistically significant variance in stress yield value in samples including peach or strawberry. As soon as samples fermented by *L. salivarius* CRL 1328 were compared to *L. casei* 01 samples using the same fruit juice, the results were not significantly different. The flow behaviour index showed that adding pineapple juice to EW fermentation consequently impacts its flow behaviour as they had pseudoplastic characteristics while they exhibited a shear thickening behaviour once peach or strawberry juice was added. Also, the K value of *L. salivarius* CRL 1328 did not differ when various fruit juices were added while in the case of *L. casei* 01 pineapple samples were the highest. Examining the viability over the course of eight weeks under cold storage revealed that, the cell counts of *L. casei* 01 in the fourth week, was approximately three Logs higher than that of *L. salivarius* CRL 1328, likewise, the pH levels of the fermented samples by *L. casei* 01 were significantly higher. Besides, during the eighth week of storage, the cell counts of *L. casei* 01 had around 8 Log_{10} CFU/mL, which is higher than the minimal probiotic dosage that is advised. Also, *L. casei* 01 maintains a higher cell counts with pineapple juice addition, but *L. salivarius* CRL 1328 retains a better viability with strawberry juice addition.

Incorporating varying percentages of pineapple juice (100%) into egg white drink resulted in a significant rise in the *L. casei* 01 population and a decrease in the pH level of the 4:3 EW: PI samples. However, the EW: PI in ratio 4:2 samples did not exhibit a substantial difference from the 4:1 sample. Simultaneously, the survivability of *L. salivarius* CRL 1328 was notably higher compared to control samples. Nevertheless, increasing the ratio of pineapple juice from 4:1 to 4:3 in the EW: PI mixture did not lead to a significant impact on the total cell counts or the pH level. Also, the pH value of fermented samples by *L. casei* 01 was greater than those with *L. salivarius* CRL 1328 except in 4:2 samples they were not significantly different. The results of investigating the impact of adding different ratios of pineapple juice to egg white drink fermentation on the total

protein content (TPC) and SDS-PAGE protein profiles suggest that the addition of pineapple juice did not affect the TPC. Moreover, the protein profiles of samples with ratios of 4:1, 4:2, and 4:3 EW: PI were comparable to each other, although they showed less intensity compared to the control samples. These results indicate that increasing the pineapple juice addition ratio to egg white drink fermentation doesn't compromise its protein content. When pineapple juice was added to egg white drink in a 4:2 and 4:3 ratio, there was a significant increase in the phenolic content when *L. salivarius* CRL 1328 was used as a starter culture. Increasing the percentage of pineapple juice in egg white drink caused a rise in the stress yield of 4:1 and 4:2 EW: PI samples. The consistency coefficient of *L. salivarius* CRL 1328 samples was significantly lower than *L. casei* 01. Fermented egg white drink samples combined with pineapple juice 100% in various ratios showed a dilutant behaviour. The determination of colour parameters indicated that increasing the incorporation ratio of pineapple juice resulted in an increase in a* and b* values and a decrease in the L* value.

The study of the effect of different ratios of strawberry puree on the fermentation of egg white drink by *L. casei* 01 and *L. salivarius* CRL 1328 found that adding strawberry puree to the drink enhanced the growth of the bacteria. The samples with strawberry juice had a significantly higher total cell counts compared to the control samples, with the highest cell populations observed in the 4:2 EW: ST and 4:3 EW: ST samples after 24 hours of fermentation by *L. casei* 01. In the case of *L. salivarius* CRL 1328, the highest growth was observed in 4:1 EW: ST samples after 16 hours of fermentation. The pH level of the samples with strawberry juice was found to be significantly different from the control samples. The 4:1 EW: ST sample had a higher value compared to the 4:3 samples, with no significant difference between the 4:2 and 4:3 samples in the case of *L. casei* 01 and *L. salivarius* CRL 1328. The study also examined the colour parameters of the fermented egg white drink with different ratios of strawberry juice after 24 hours of fermentation. It was found that increasing the strawberry juice ratio led to an increase in the L*, b*, and a* values.

During the fermentation process of a mixture of egg white drink to strawberry puree in a ratio of 3:1 using a mixed culture of *L. casei* 01 and *L. salivarius* CRL 1328 did not have any effect on the total phenolic content, as it reached 0.2 expressed as Gallic acid mg/mL. Notabely, the lactic acid content was higher in samples fermented with the mixed culture compared to those fermented with *L. casei* 01 as a monoculture fermentation. In addition, samples that were fermented with mixed culture without strawberry puree addition recorded the lowest percentage of lactic acid. On the other hand, the lactic acid content of fermented samples by *L. salivarius* CRL 1328 did not significantly differ, whether the ST puree was added or not, as they recorded the highest value. Samples of mixed and monoculture-fermented EW drinks with and without ST puree were found to possess antimicrobial activity against *Escherichia coli* 8739, *Escherichia coli* 0157:H7,

Enterobacter cloacae, *Listeria innocua*, and *Enterococcus faecalis*. A greater inhibition zone was observed in samples containing ST puree.

In conclusion, the selection of bacterial strains for fermenting egg white beverages is dependent on the desired final product. For plain, low-sugar egg white drinks fermented by *L. casei* 01 is demonstrably the most suitable choice. Otherwise, a combination of *L. plantarum* 299v and 2% of fructo-oligosaccharides can also be used, with a recommended shelf life of three weeks. For the fermentation of egg white beverages incorporating fruit components, the optimal bacterial strain and processing parameters vary based on the specific fruit. When utilizing strawberry juice in a 3:1 ratio (egg white: juice), *L. salivarius* CRL 1328 is strongly recommended. This fermentation process typically requires eight hours and the resulting beverage should be stored for no longer than four weeks. For pineapple juice additions, a 4:3 ratio and 4:2 ratio are recommended (egg white: pineapple juice) when using *L. casei* 01 and *L. salivarius* CRL 1328 for fermentation, respectively. Finally, the inclusion of strawberry puree necessitates a 4:1 ratio (egg white: puree) and a 16-hour fermentation period when utilizing *L. salivarius* CRL 1328. Alternatively, a 4:2 ratio and 24-hour fermentation period can be used with *L. casei* 01. Summarising, fermentation is a good way to produce probiotic egg white based beverages with health promoting effects.

This study examined the effects of specific probiotic strains fermented with mono-, di-, and oligo-saccharides or specific fruit juices on the microbiological, chemical, and sensory characteristics of egg white drinks. However, these findings may not apply to a broader context. Future research could investigate the impacts of a wider range of factors. For example, research could explore the effects of mixed culture fermentation of egg white drinks, including strains such as *Lactobacillus* and Bifidobacteria. Furthermore, assessing the impact of fermenting with different fruit juices and varying the ratios of egg white drinks to fruit juice would be valuable.

In addition, further research efforts should be directed towards improvements to make the product marketable as well as sensory evaluation for egg white drinks fermented with Bifidobacteria strains, prebiotic concentration quantification, phenolic content assessment, and bacterial viability of mixed culture fermentation during storage.

7 New scientific results

1. It was stated, that a variety of microorganisms were able to multiply in egg white drink after 24 hours including *Lactobacillus helveticus* R52, *L.acidophilus* 150, *L.rhamnosus Rosell* 11, *L.casei* 01, *L.salivarius* CRL 1328, *L.plantarum* 299v and *Bifidobacterium longum* Bb46, however, 2% of glucose addition is also beneficial to reach Log_{10} CFU/mL >7. The greatest cell counts and lowest pH were observed in *L.casei* 01 and *L.plantarum* 299v after a 24-hour fermentation period.
2. It can be concluded, that the application of different carbohydrate sources (glucose, fructose, sucrose, fructo-oligosacchrides, galacto-oligosaccharides, and xylo-oligosaccharides) to the fermentation of EW drink by *L. acidophilus* 150, *L. plantarum* 299v, and *L. casei* 01 showed differences in carbohydrate preference. *L. casei* 01 had better growth when fructose, fructo-oligosacchrides, and galacto-oligosaccharides were added to the egg white drink. *L. plantarum* 299v preferred fructo-oligosacchrides for growth and its pH level was not affected by mono- or disaccharides. However, *L. casei* 01 had the lowest pH with polysaccharides.
3. The viscosity of a fermented egg white beverage is greatly influenced by the strains used and the type of carbohydrate source. The ToTu drink samples exhibited shear thickening behaviour when fermented with *L. plantarum* 299v, *L. casei* 01, or *L. acidophilus* 150 in the presence of these carbohydrates.
4. During 3-week cold storage at 4°C, the viable cell counts of *L. plantarum* 299v and *L. casei* 01 in a fermented egg white drink with fructose and fructo-oligosacchrides in 2% (w/v) concentration was greater than 10^7 CFU/mL.
5. The shelf life of the ToTu drink was extended from two days to at least three weeks by the fermentation process with *L. plantarum* 299v and *L. casei* 01, while maintaining its shear thickening behaviour.
6. Fermentation of egg white combining peach, pineapple, or strawberry juice (25% fruit) in a ratio of 1:3 by *L.salivarius* CRL 1328 or *L.casei* 01 boosts nutritional content and promotes probiotic development. The highest total cell counts were obtained by *L.casei* 01 among all fruit juices, surpassing *L.salivarius* CRL 1328. *L.casei* 01 grew to its maximum in 16 hours with pineapple or strawberry juice but required 24 hours to reach the greatest cell counts with strawberry. *L.salivarius* CRL 1328 cell counts did not change significantly after 8 hours of fermentation, indicating that an 8-hour fermentation may be effective.
7. It was found, that the flow behaviour index at 20° C showed pseudoplastic attributes when pineapple juice 25% in a 3:1 was added to egg white fermented by *L.salivarius* CRL1328

or *L.casei* 01; nevertheless, when strawberry and peach juices (25%) were used, they maintained shear thickening properties.

8. It was observed that the addition of different ratios of 100% pineapple or strawberry juice (4:1, 4:2, and 4:3 EW: PI, 4:2 and 4:3 EW: ST) to the fermentation of egg white by *L. casei* 01 and *L. salivarius* CRL 1328, respectively resulted in an increase in their growth after 24 hours.
9. The total phenolic content of egg white: pineapple in the ratio 4:2 and 4:3 fermented by *L. salivarius* CRL 1328 can be considerably increased by 18.18% compared to control samples.

8 Summary

Lately, the industry has been driven by a growing urge to innovate with a new non-dairy beverage, in order to fulfil various customer segments' requirements such as those with lactose intolerance who are unable to consume milk products and have to eliminate all of its health benefits, that involves probiotics, which can be vital in alleviating the unpleasant symptoms associated with their conditions. Egg white which constitutes the majority of ToTu drink could be an optimal alternative considering its substantial protein content and absence of cholesterol and fat, nevertheless, it is highly perishable. Supplementing it with probiotics might prolong its shelf life and enhance its nutritional and sensory properties. It could also be suitable for athletes, those on special diets, and individuals allergic to milk proteins. Interestingly, optimizing the conditions of processing, particularly selecting the suitable probiotic strains, is critical since its byproducts may impact the acceptability of the end product; also, its viability must be at an appropriate level at the time of consumption so that customers get its advantages. It is worth mentioning that LAB and *Bifidobacterium* strains possess extensive nutritional requirements including the presence of carbohydrates, which they cannot get from egg white drink, thus it could be added to the fermentation process as a carbohydrate solution or fruit juices which are considered to be rich in carbohydrates further it is highly phenolic and antioxidant compounds such as strawberry, pineapple and peach juice.

The purpose of the research is to investigate the ability of probiotics to flourish in egg white beverages without any other additives and in the presence of varied carbohydrate sources such as mono-, di-, and oligosaccharides, as well as fruit juices such as peach, pineapple, and strawberry in different ratios and concentrations. Additionally, the aim was to select the most appropriate probiotic bacteria and assess the impact of mixed culture fermentation. The study also analysed the effect of these probiotic bacteria on the microbial, physiochemical, rheological, and sensory characteristics of the final products.

The egg white drink beverage was supplied by the production line of Capriovus Ltd (Szigetcsép, Hungary), and tested its fermentability of different probiotic lactic acid bacteria and bifidobacterial strains. After strain selection, the drink was then incubated with *L. helveticus* R52, *L. acidophilus* 150, *L. rhamnosus* Rosell 11, *L. casei* 01, *L. salivarius* CRL 1328, and *L. plantarum* 299v 1% (v/v) as a monoculture fermentation. Glucose, fructose, sucrose, FOS, GOS, and XOS solution were individually added to the drink in a concentration of 2% (w/v) in the final product. Additionally, peach, strawberry, and pineapple juice (25% v/v fruit) were mixed with the egg white drink in a ratio of 3:1 (v/v%) egg white drink to fruit juice, and separately incubated with *L. casei* 01 and *L. salivarius* CRL 1328. Similarly, pineapple and strawberry juice (100% fruit juice) were

incorporated into the egg white drink in ratios of 4:1, 4:2, and 4:3 (v/v). Furthermore, a mixed culture of *L. casei* 01 and *L. salivarius* CRL 1328 1% (v/v) were added to the egg white drink with and without strawberry juice in a ratio of 3:1 EW: ST.

Following each treatment, the samples were stored in a refrigeration room at 4°C for approximately 8 weeks. During this time, pH, colour parameters, apparent viscosity, and microbiological properties were studied. The incubation temperature was 37°C for 24 hours for all experiments. Samples without carbohydrate or fruit juice addition were served as control. After 24 hours of fermentation in the presence of glucose 2% (w/v) all the studied probiotics could propagate in egg white drink, as *L. plantarum* 299v and *L. casei* 01 increased by 2 Logs while 1 log in the case of *L. rhamnosus* Rosell 11 and *L. helveticus* R52. In addition, the greatest pH level was achieved in samples with *L. helveticus* R52, contrary to *L. plantarum* 299v and *L. casei* 01 samples which had the lowest value. Also, *Bifidobacterium* strain thrived in egg white drink with glucose 2% since *B. longum* Bb46 growth was not considerably different compared to *B*. *longum* DSM. Studying the specific growth rate (1/h) and generation time (h) showed that *L. plantarum* 299v exhibited the fastest growth with a specific growth rate value of 0.14 h^{-1} and the shortest generation time at 4.95 h. *L. helveticus* R-52 had the slowest growth and the longest generation time. *B. longum* Bb46 demonstrated quicker propagation in the glucose-enriched egg white drink compared to *B. longum* DSM 16603. In general, *Bifidobacteria* grew better than *Lactobacilli*.

When different carbohydrate sources were included in the fermentation, it was observed that *L. acidophilus* 150 showed the highest preference for glucose, fructose, and sucrose, while *L. casei* 01 preferred glucose and fructose, On the other hand, *L. plantarum* 299v favoured FOS. However, no discernible difference was observed between the three strains when xylo-oligosaccharides were utilized in egg white fermentation. The pH level of fermented samples containing carbohydrate sources was notably lower than that of the control samples. However, the samples that contained XOS and either *L. acidophilus* 150 or *L. plantarum* 299v had the highest pH value. Rheological characteristics indicated an increase in stress yield when different carbohydrate sources were added, compared to the control samples. In addition, the viscosity increased with an increasing shear rate, reflecting a dilutant behaviour. Furthermore, the K value of *L. casei* 01 with GOS or XOS showed the highest value. The study also found that the fermented samples containing *L. casei* 01 and *L. plantarum* 299v remained viable for over three weeks with and without fructose or FOS during storage at refrigerated conditions. The total cell counts in the presence of a carbohydrate source was higher than 8 Log_{10} CFU/mL and the pH levels were above 3.6. Additionally, the yield stress of samples containing fructose and *L. plantarum* 299v had a significantly higher value compared to the control and FOS samples during the third week.

However, for *L. casei* 01 samples, there were no significant differences when different sugar solutions were added.

The incorporation of peach, strawberry, or pineapple juice into egg white fermentation by *L. casei* 01 and *L. salivarius* CRL 1328 supported their growth with minimal variation in cell counts when various juices were included, as *L. casei* 01 samples had a significantly higher population and a lower pH level than *L. salivarius* CRL 1328 samples. EW with strawberry juice samples were the darkest and most red, indicating the best overall acceptability when fermented by *L. salivarius* CRL 1328. Additionally, samples with a 3:1 EW to pineapple juice ratio exhibited pseudoplastic behaviour after 24 hours of fermentation. The cold storage over 8 weeks demonstrated that samples containing *L. casei* 01 with strawberry or pineapple juice stayed steady, with their proliferation remaining greater than 8.44 Log_{10} CFU/mL. In contrast, *L. salivarius* decreased to 4.62 Log_{10} CFU/mL after the fourth week of storage.

As long as the EW drink was mixed with different proportions of 100% fruit pineapple juice (4:1, 4:2, and 4:3) EW to PI, the total cell counts of *L. casei* 01 increased as a result, with the 4:3 formula producing the highest number of cells. However, there were no discernible changes in *L. salivarius* CRL 1328 when the fruit proportion was increased. Because of the dilution effect, the protein bands in the juice-containing samples were less visible than those in the control samples. However, they were still visible on the gel. With a 4:3 EW: PI ratio, the total phenolic content was 0.14 mg/mL gallic acid. The colour parameter determination showed that as the pineapple juice ratio was raised, b* increased while L* and a* dropped.

Examining the development of *L. casei* 01 and *L. salivarius* CRL 1328 in various mixture ratios of strawberry puree and EW drink (4:1, 4:2, and 4:3) EW: ST over the course of a 24-hour fermentation period revealed that *L. salivarius* CRL 1328 exhibited the highest total cell counts after 16 hours of fermentation in the 4:1 EW: ST formula, whereas *L. casei* 01 showed greater development in the 4:2 and 4:3 EW: ST samples following a 24-hour fermentation period. Utilizing a mixed culture of *L. casei* 01 and *L. salivarius* CRl 1328 in the fermentation of a mixture of EW drink with strawberry puree in a 3:1 ratio EW to ST did not affect the total phenolic content (0.2 Gallic acid mg/mL). However, lactic acid content was higher in mixed culture samples compared to *L. casei* 01 monoculture fermentation. Mixed and monoculture-fermented EW drinks exhibited antimicrobial activity against *Escherichia coli* 8739, *Escherichia coli* 0157:H7, *Enterobacter cloacae*, *Listeria*, and *Enterococcus faecalis*.

Overall, this research demonstrates the feasibility of producing probiotic beverages using egg white with various carbohydrates and fruit juices. The choice of probiotic strain and processing

parameters can affect the viability, functionality, and sensory properties of the final product. This work paves the way for the development of a novel, nutritious, and shelf-stable probiotic beverage derived from egg white, suitable for lactose-intolerant consumers and those seeking a protein-rich functional drink.

9 Appendices

Annex 1

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Annex 2

Tables summarize the applied measurements for each experiment.

***Table* 14. The applied measurements in the screening of different *Lactobacillus* and bifidobacteria for the fermentation of egg white drink experiment.**

Probiotic strains used in egg white drink fermentation	**Experimental parameters**			
	Microbiology	pH	Specific growth rate	Generation time
L. helveticus R52	+	+	+	+
L. acidophilus 150	+	+	+	+
L. rhamnosus Rosell 11	+	+	+	+
L. casei 01	+	+	+	+
L. salivarius CRL 1328	+	+	+	+
L. plantarum 299v	+	+	+	+
B. longum DSM 16603	+	+	+	+
B. longum Bb46	+	+	+	+
B. bifidum Rosell-71	/	+	/	/
B. lactis Lafti^{R}B94	/	+	/	/
B. longum Rosell-175	/	+	/	/
B. lactis Bb12	/	+	/	/
L. brevis **HA-112**	/	+	/	/
L. fermentum **HA-179**	/	+	/	/
L. helveticus **Lafti^{R}L10**	/	+	/	/
L. plantarum **HA-119**	/	+	/	/
L. reuteri **HA-188**	/	+	/	/
L. rhamnosus **HA-111**	/	+	/	/
L. salivarius **HA-118**	/	+	/	/
L. crispatus **LCR01**	/	+	/	/
L. rhamnosus **GG ATCC53103**	/	+	/	/
L. fermentum **LF08**	/	+	/	/
L. acidophilus **La-5**	/	+	/	/

***Table* 15. The applied measurements for analyzing the impact of mono-, di-, and polysaccharide incorporation on the fermentation of egg white beverages.**

Probiotic strain used in the fermentation	Carbohydrate source type	Experimental parameters			
		Total cell numbers	pH	Viscosity	Shelf life
***L. acidophilus* 150**	Control (without)	+	+	+	/
	Glucose	+	+	+	/
	Fructose	+	+	+	/
	Sucrose	+	+	+	/
	Fructo-oligosaccharides	+	+	+	/
	Galacto-oligosaccharides	+	+	+	/
	Xylo-oligosaccharides	+	+	+	/
***L. casei* 01**	Control	+	+	+	+
	Glucose	+	+	+	/
	Fructose	+	+	+	+
	Sucrose	+	+	+	/
	Fructo-oligosaccharides	+	+	+	+
	Galacto-oligosaccharides	+	+	+	/
	Xylo-oligosaccharides	+	+	+	/
***L. plantarum* 299v**	Control	+	+	+	+
	Glucose	+	+	+	/
	Fructose	+	+	+	+
	Sucrose	+	+	+	/
	Fructo-oligosaccharides	+	+	+	+
	Galacto-oligosaccharides	+	+	+	/
	Xylo-oligosaccharides	+	+	+	/

***Table* 16. Analytical methodologies employed for the assessment of probiotic egg white beverages enriched with various fruit juices**

		Probiotic strain used in the fermentation					
		L. casei 01			*L. salivarius* CRL 1328		
Fermentation medium	**EW: fruit juice**	EW:PE	EW:ST	EW:PI	EW:PE	EW:ST	EW:PI
Experimental parameters	Microbiology	+	+	+	+	+	+
	pH	+	+	+	+	+	+
	Viscosity	+	+	+	+	+	+
	Protein profile SDS-PAGE	+	+	+	+	+	+
	Colour parameters	+	+	+	+	+	+
	Sensory evaluation	/	+	+	/	+	+
	Shelf life	/	+	+	/	+	+

***Table* 17. Experimental parameters for evaluating the impact of varying pineapple and strawberry juice proportions on egg white beverage fermentation by *L. casei* 01 and *L. salivarius* CRL 1328 were determined.**

			Probiotic strain used in the fermentation					
			L. casei 01			*L. salivarius* CRL 1328		
Fermentation medium		**EW: fruit juice 4:1, 4:2, and 4:3 (v:v)**	Control	EW:ST	EW:PI	Control	EW:ST	EW:PI
Experimental parameters		Microbiology	+	+	+	+	+	+
		pH	+	+	+	+	+	+
		Viscosity	+	/	+	+	/	+
		Protein profile SDS-PAGE	+	/	+	+	/	+
		Colour parameters	+	+	+	+	+	+
		Total phenolic content	+	/	+	+	/	+
		Total protein content	+	/	+	+	/	+

***Table* 18. Analytical methods were utilized to examine the influence of a mixed culture of *L. casei* 01 and *L. salivarius* CRL 1328 on the fermentation of egg white beverage with and without strawberry juice addition.**

Probiotic strain used in the fermentation		*L. casei* 01		*L. salivarius* CRL 1328		*L. salivarius* CRL 1328 and *L. casei* 01	
Fermentation medium		Egg white drink	EW:ST 3:1 (v:v)	Egg white drink	EW:ST 3:1 (v:v)	Egg white drink	EW:ST 3:1 (v:v)
Experimental parameters	Total cell counts	+	/	+	/	+	/
	pH	+	/	+	/	+	
	Lactic acid content %	+	+	+	+	+	/
	Total phenolic acid	+	+	+	+	+	+
	Antagonistic activity	+	+	+	+	+	+

10 Publication list

Journal articles

1. **Mourad, R.,** Csehi, B., Friedrich, L., Nguyen, Q. D., & Bujna, E. (2023). Investigating the shelf-life of probiotics fermented egg white-based beverage using prebiotics. *Progress in Agricultural Engineering Sciences*, 19(S1), 105–111. https://doi.org/10.1556/446.2023.00088
2. **Mourad, R.,** Tóth, Zs., Csehi, B., & Bujna, E. (2023). A bifidobacterium fermented egg white drink with different carbohydrate sources. *Review on Agriculture and Rural Development*, 12(3-4), 44-49. https://doi.org/10.14232/rard.2023.3-4.44-49

Conference proceedings

1. **Mourad, R.,** Csehi, B., & Bujna, E. (2022). *L. plantarum* 299V as a starter probiotic in fermented egg white drink. In *Proceedings of the International Symposium on Analytical and Environmental Problems* (Vol. 28, pp. 296–300). https://acta.bibl.u-szeged.hu/78542/

Poster presentations

2. **Mourad, R.,** Csehi, B., & Bujna, E. (2021). The effect of adding different sugar types on the properties of fermented egg milk product. In *International Conference on Agricultural and Food Chain Safety Development*, Budapest, Hungary.
3. **Mourad, R.,** Csehi, B., & Bujna, E. (2021). Fermentation of egg white milk by probiotic bacteria. In *The 4th International Conference on Biosystems and Food Engineering* (E425, p. 1). http://www.biosysfoodeng.hu/2021/USB/pdf/E425.pdf
4. **Mourad, R.,** Csehi, B., Németh, Cs., & Bujna, E. (2021). The effect of adding different sugar types on the properties of fermented egg milk product. In *Proceedings of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting* (p. 90). https://lov.uni-mate.hu/documents/270121/0/lippai-ormos-vas-konferencia-%C3%96sszefoglal%C3%B3.pdf/981d5e90-61e2-3980-000e-635126cc94f7?t=1652421506337
5. **Mourad, R.,** Csehi, B., & Bujna, E. (2022). Studying the shelf-life of a probiotic fermented egg white milk product. In *4th International Conference on Food Science and Technology* (p. 21). http://www.foodconf.hu/files/BOA2022.pdf
6. **Mourad, R.,** Csehi, B., & Bujna, E. (2022). Probiotic fermentation of enzyme-treated egg white drink mixed with pineapple juice. In *9th Asia-Pacific Probiotics Symposium*.

7. **Mourad, R.,** Aljanabi, I., Csehi, B., & Bujna, E. (2023). Development of a non-dairy probiotic drink from egg white-based product mixed with different fruit juices. In *5th International Conference on Biosystems and Food Engineering* (E556, p. 1). http://biosysfoodeng.hu/USB/pdf/E556.pdf
8. **Mourad, R.,** Aljanabi, I., Csehi, B., & Bujna, E. (2023). Egg white fortification with probiotics and peach juice. In *A Magyar Táplálkozástudományi Társaság XLVI* (p. 30). https://www.doki.net/tarsasag/taplalkozas/upload/taplalkozas/document/dec.20_honlapra_2023._oktober_20nyomda_szept._25_4judit_ri_ix_6_vandorgyules_gyor_program_nyomda_elott_final_09_20_logokkal.pdf?web_id=A5C42F4E392CAA2
9. **Mourad, R.,** Csehi, B., & Bujna, E. (2023). Egg white-based product with probiotics and pineapple juice. In *Proceedings of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting*.

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