MSc THESIS

ISAM AL-JANABI

2023

HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOENGINEERING AND ALCOHOLIC DRINK TECHNOLOGY

EGG WHITE AND FRUIT JUICE FERMENTATION BY PROBIOTIC BACTERIA

ISAM AL-JANABI

BUDAPEST
2023

Hungarian University of Agriculture and Life Sciences

Institute of Food Science and Technology

Programme name : MSc Food Engineering

Specialization name: Product and technology development

Place of thesis preparation: Department of Bioengineering and Alcoholic Drink Technology, Institute of Food Science and Technology, MATE.

Student name: Isam Khudhyer Zain Aljanabi

Thesis title: Egg White and Fruit Juice Fermentation by Probiotic Bacteria.

Supervisors: Dr. Bujna Erika, associate professor

Reem Mourad, PhD student

Date of issuing the thesis: May 3, 2023

Dr. Quang Duc Nguyen

Head of Department

Dr. Erika Bujna, Reem Mourad

Supervisors

Dr. László Friedrich

responsible for specialization

Table of Contents

1. INTRODUCTION

In most communities, food plays a significant role in promoting human nutrition and health. The biological significance of food's constituents in sustaining human health will replace food's initial function as a source of energy and development, and the market for food consumption and production will be directed toward functional foods. A product that improves health and offers essential nourishment is known as functional food (Zandi et al,. 2016). Growing consumer interest in maintaining a healthful lifestyle has fueled the efforts of researchers and food manufacturers to develop functional foods in recent years. These are products that have been fortified or enriched to provide additional health benefits beyond their fundamental nutritional functions (Francini and Sebastiani, 2013; Corbo et al., 2014), capable of promoting physiological improvement and lowering the risk of disease (Verschuren, 2002). Probiotic microorganism-containing foods are among the most important functional foods. Since probiotics have positive effects on the microbial balance of the intestine and the health of the entire body, the market for this food group is expanding (Zandi et al., 2016). The two categories of probiotic products are: Various probiotic dairy products, including yoghurts, cheese, cream, yoghurt water, butter, ice cream, infant formula, whey beverages, and dairy desserts (Mohammadi et al., 2012). and non-dairy probiotic products like cereals, sweets, various beverages like juice and non-alcoholic beer, infant food, and meat products (Sohrabvandi et al., 2010). In addition to dietary products, probiotic pharmacological products in the form of tablets or capsules are used to treat a variety of medical issues, including intestinal infections, diarrhea, vaginal infections, constipation, and lactose intolerance. There are more probiotic microorganisms in pharmaceutical products than desiccated, frozen ones (Mortazavian and Sohrabvandi, 2006). The physicochemical properties of food carriers affect the viability of probiotics during transfer in the digestive system. Components of foods containing probiotics influence resistance to gastric acid and bile, binding to the mucus of the digestive tract, and production of acid (Ranadheera et al., 2010). Proper and balanced nutrition is essential for a healthy lifestyle, but there are more and more people who cannot consume certain products because they suffer from some kind of food allergy or intolerance. One of the most common is lactose intolerance, which is a type of digestive disorder, and the point is that people suffering from intolerance cannot fully digest and break down milk sugar because their body does not produce enough of the necessary lactase enzyme. As a result, lactose is not absorbed in the small intestine but passes on to the large intestine, where it breaks down into fatty acids and hydrogen gas, which causes bloating, diarrhea and intestinal cramps. The symptoms can sometimes be more severe, depending on the amount or lack of the enzyme (Czétényi, 2014). Another significant problem is milk protein allergy, the root cause of which is unfolded or insufficiently unfolded proteins, which trigger an immunological response in the body. The problem can appear even in infancy. For people suffering from allergies, it is not always possible to decide which protein fraction causes the unpleasant symptoms, so they must follow a dairy-free diet throughout their lives. Due to the reasons listed above, the demand for functional foods is increasing throughout the world. ToTu products made from egg white can be suitable alternatives for those who cannot consume dairy products due to a medically diagnosed illness or who just want to change their lifestyle and do something to improve their health. The ToTu drink can also be included in an ovo-vegetarian or paleo diet that rejects modern processed additives. It is not milk-based but is also consumed by athletes and bodybuilders due to its high protein content. The consumption of raw egg white is not really widespread due to its taste and other physical properties. Fermented egg white with *Lactobacillus* are used to solve this problem, thus facilitating its use. Based on all of this, in my thesis I aimed to produce a fermented ToTu drink containing pre- and probiotics. I used the *L. casei* 01 and *L.salivarius* CRL1328 strain as a probiotic, which has a beneficial effect on the body, which can further increase the content value of the egg-based product. There is constant consumer demand for the expansion of the market for free products, and with the help of my research, additional milk substitutes (fermented products) that can be produced from the ToTu drink can be filled, which also have extra added value due to the probiotics.

2. THE AIM OF THE RESEARCH

Society's life is becoming more and more dependent on nutrition. Attitudes to food, age, health status, various trends, economic and financial situation, etc., change depending on the environment. All contribute to the development of consumer habits. People care more and more about what they eat, where it comes from, what it's made of, what processes have been applied to it and what internal characteristics it has. They consciously pay attention to a healthy diet and lifestyle, and the demand for healthy foods is integrated into today's thinking. They are actively looking for opportunities that help them live a healthy life. The so-called functional foods, which are proven to have a good effect on health, can be easily incorporated into a healthy lifestyle and daily diet by enriching or reducing or removing an ingredient. As a result, the demand for free foods is also increasing on the market. The ToTu drink made from egg white, which is the subject of my thesis, can also be classified here, as it is lactose, milk, fat and preservative free and has a low calorie but high protein content. The purpose of my study is to discover the properties of the probiotic-enriched ToTu drink, it will be possible to develop any fermented product made from the ToTu drink, which can be a new dairy-free alternative for people suffering from lactose intolerance or milk protein allergy. During the research, I planned to investigate the fermentability of ToTu drink using *L.casei* 01 and *L.salivarius* CRL1328 strain and the effects of adding fruit juices in terms of bacterial development. I examined the following parameters of the fermented ToTu drink after fermentation and during six weeks of refrigerated storage under 4 °C:

• pH
• viable cell number
• protein content
• viscosity
• color
• sensory evaluation
• storage stability

The purpose of the storage experiment is to be able to draw conclusions about shelf life and internal characteristics by comparing the results of the properties tested on the samples after a certain period of time. and also Improve the sensory and nutritional properties of the egg white drink by adding probiotic

3. LITERATURE OVERVIEW

3.1 LACTIC ACID BACTERIA

3.1.1 History, Origin, Overview

The first pure culture of a lactic acid bacteria (LAB) ("*Bacterium lactis*") was obtained by J. Lister in 1873, ten years after L. Pasteur explored lactic acid fermentation (between 1857 and 1863). Although humans have consumed fermented foods for more than 5000 years, starter cultures for the manufacture of cheese and sour milk were only developed around 1890. (Schlegel and Folkerts 1999; Stiles and Holzapfel 1997). Foods that spoil quickly, including milk, meat, fish, and some vegetables, can be preserved, improved in flavor, and given more nutritional value by lactic acid-producing fermentation, which has been known for thousands of years and used by many different cultures (Halász, 2021). The first monograph by Orla-Jensen appeared in 1919. A typical lactic acid bacterium cultivated under standard conditions is aerotolerant, acid-tolerant, organotrophic, and a rod or coccus that is strictly fermentative. It is devoid of cytochromes and incapable of synthesizing porphyrins. Its characteristics can vary depending on the conditions. In the presence of hemes, catalase and cytochromes may be produced, and lactic acid can be further metabolized, resulting in decreased lactic acid amounts. Cell division happens in one plane, except pediococci. Typically, the cells are immobile. They require intricate growth factors, including vitamins and amino acids. There is no clear definition of LAB conceivable (Axelsson, 2004). Most of the microorganisms employed in food fermentation and to improve the taste and texture of fermented foods are lactic acid bacteria. Their primary metabolic byproduct from glucose is lactic acid, and they also produce compounds that hinder the growth of spoilage bacteria and pathogens, such as bacteriocins, hydrogen peroxide, diacetyls, etc. (Mokoena, 2017).

Figure 1 depicting a phylogenetic tree with the evolutionary relationships between the various species of lactic acid bacteria currently known, including *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella*. Many times, milk and dairy products are linked to the bacterial groups *Lactococcus* and *Lactobacillus*, but these genera are not limited to a single habitat. The genus *Streptococcus* is the most common bacterium in milk and other dairy products, but the genus *Enterococcus, Leuconostoc,* and *Pediococcus* are also frequently found (Bjö et al., 2011).

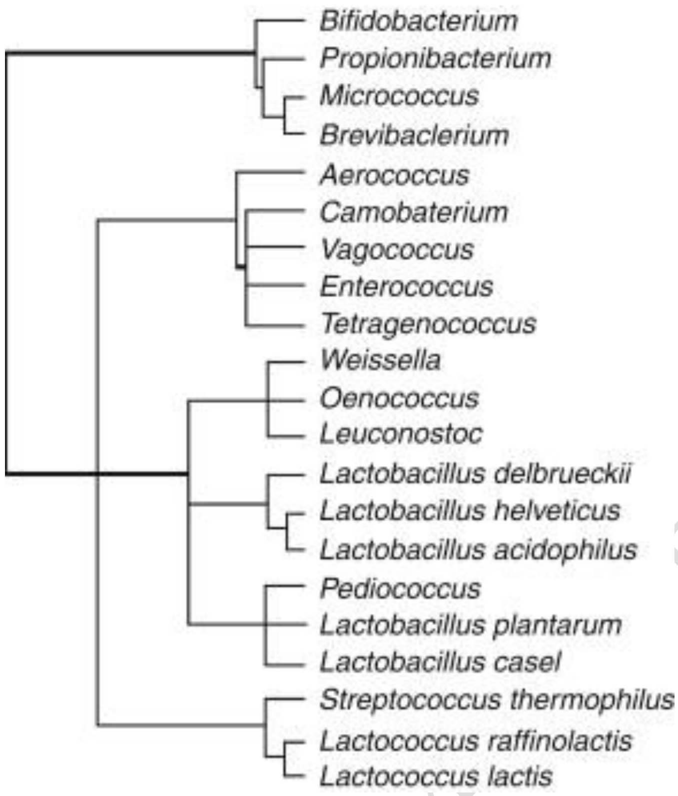

Figure 1. A phylogenetic relationships of lactic acid bacteria (Bjö et al., 2011).

3.1.2 Classification of Lactic Acid Bacteria

Orla- Jensen's (1919) paper provides the general framework for categorizing lactic acid bacteria. First, the lactic acid bacteria are classified into rods (*Lactobacillus* and *Carnobacterium*) and cocci (all other genera) based on their morphology in this genus-level classification system. Under standard conditions, the way in which glucose is fermented is the next most useful character for separating lactic acid bacterial genera (unlimited supply of glucose, growth factors such as amino acids, vitamins, and nucleic acid precursors, and limited oxygen availability). Bacteria producing lactic acid under these conditions can be classified as either (I) homofermentative or (II) heterofermentative. In contrast to the heterofermentative bacteria, which produce lactic acid and carbon dioxide, the homofermentative bacteria convert sugar almost quantitatively into lactic acid. Tolerance to sodium chloride, temperature, and media pH all had different effects on growth (Halász, 2021).

3.1.3 Lactic Acid Fermentation

Fermented foods are crucial to the protection of millions of people's health, nutrition, and social standings around the world. Sour dough bread, sorghum beer, all fermented milks, and most "pickled" (fermented) vegetables are all fermented using lactic acid bacteria, the most essential bacterium in good food fermentations.

The most important lactic acid bacteria used in food fermentations include *Lactobacillus acidophilus, L. bulgaricus, L. plantarum, L. pentoaceticus, L. brevis,* and *L. thermophilus.* Species that exclusively create lactic acid are said to be "homofermentative," while those that also produce other volatile chemicals and trace amounts of alcohol are said to be "heterofermentative" (Battcock and Azam-Ali, 1998). *Leuconostoc mesenteroides* is commonly found in pickle and sauerkraut fermentations. The fermentation of these foods into a more pleasant lactic acid form is triggered by this bacteria. Carbon dioxide and acids produced by *Leuconostoc mesenteroides* quickly lower the pH, preventing the growth of pathogens. Carbon dioxide is released in the place of oxygen, creating an anaerobic environment ideal for the development of following *Lactobacillus* species. Vegetables retain their vibrant hues and any ascorbic acid present is stabilized by the absence of oxygen (Battcock and Azam-Ali, 1998). Some fermented foods, like Swiss cheese, get their distinctive flavor and texture from members of the Gram-positive *Propionibacteriaceae* family, which also produces the holes that give the cheese its distinctive appearance. Lactic acid is metabolized by these bacteria to produce acetic and propionic acids and carbon dioxide. Dairy product fermentation also relies on the presence of a number of other bacteria, including *Leuconostoc citrovorum*, *Streptococcus lactis,* and *Brevibacterium* species. The optimum pH for growth of different types of microorganisms varies. Near-neutral pH is ideal for most bacteria. Fermented foods take use of the fact that different groups of microbes have distinct pH requirements by allowing different groups to take over as the pH of the food's environment shifts. Some bacteria can even thrive in environments with a low pH. The *Lactobacillus* and *Streptococcus* species are two examples of acid-resistant bacteria that are important in the fermentation of dairy and vegetable products (Battcock and Azam-Ali, 1998). Most lactic acid bacteria perform well between 18 and 22 °C and can handle high salt environments. Due to their salt tolerance, lactic acid fermenters are able to initiate metabolism, producing acid that further hinders the growth of undesirable organisms.

Bacteria can only thrive in water with a water activity of 0.9 or greater. Yeasts and fungi tend to thrive on foods with a lower water activity, while there are a few species that can survive water activities below this. Almost always, the fermentation of food requires the cooperation or sequential action of more than one bacterium. Bacteria of varying species, as well as other microbes like yeast and mold, have specific requirements for optimal growth. There are hardly any fermentations that only use pure cultures. Once an organism starts fermentation, it will continue to thrive until the byproducts of the fermentation kill off the organism. During this time of expansion, additional creatures mature and are prepared to take over if the current inhabitants' environment ever becomes too hostile. Bacteria typically act as the catalyst for growth, followed by yeasts and eventually molds (Battcock and Azam-Ali, 1998).

3.1.4 Lactic Acid Bacteria Metabolism

The breakdown of carbohydrates and associated substances primarily to lactic acid is the key aspect of the metabolism of lactic acid bacteria. Along with this, energy (adenosine triphosphate = ATP) is produced. The most common byproduct of fermentation is lactic acid, while variations in the end product are possible due to changes in growing conditions. The most common pathways for fermentation are as follows:

-Fermentation of disaccharides

Disaccharides can enter the cell either as free sugars or sugar phosphates, depending on the delivery mechanism. specialized hydrolases split the unbound disaccharides into monosaccharides, which subsequently enter the major routes. Phosphohydrolases catalyze the conversion of disaccharide phosphates into monosaccharide and monosaccharide phosphate, respectively. The most intriguing aspect of lactose fermentation, from the standpoint of the dairy sector, is the production of lactic acid. Following hydrolysis, lactose turns into glucose and galactose, which can enter the cell unbound or bound to a phosphate and then enter the primary pathway of lactic acid generation (Halász, 2021).

-Hexose fermentation

Hexose is the most frequently encountered substrate for lactic acid bacteria among the sugars contained in food. Since lactic acid is the byproduct of glucose fermentation under standard conditions (excess sugar and restricted oxygen availability), homofermentative lactic acid bacteria should theoretically be able to create two molecules of lactic acid from one molecule of glucose. Many lactic acid bacteria may ferment hexoses besides glucose; these sugars, after undergoing isomerization and/or phosphorylation, join the primary routes of glycolysis. The phosphorylated hexose is further broken down into triose phosphates and oxidized and dephosphorylated to produce pyruvic acid during the fermentation process. The last step in the fermentation process is turning pyruvic acid into lactic acid (Halász, 2021).

-Fermentation of Pentoses

Lactic acid bacteria can quickly and efficiently ferment pentoses. To enter the pentose-phosphate pathway, pentoses are first transformed by epimerases within the cell to either ribulose-5-phosphate or xylulose-5-phosphate. Lactic acid bacteria's nitrogen metabolism is linked to both the hydrolysis and synthesis of proteins, in addition to the fermentation of carbohydrates that constitutes the bulk of their primary metabolic pathways. For instance, cheese making relies heavily on these procedures (Halász, 2021).

3.1.5 Use of Lactic Acid Bacteria in the Dairy Industry

Fermented milk can take numerous forms. Pasteurized skim or semi-skimmed milk is used to produce cultured buttermilk, which is then fermented with lactic acid and flavoring bacteria such *Streptococcus lactis* and *Streptococcus cremoris* as mentioned in table 1. To create sour cream, lactic acid and aroma-producing bacteria are added to pasteurized cream and allowed to ripen. Yogurt can range in fat content from 1% to 5%, depending on the product. The Lactic Acid Bacteria employed in this process are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Kefir is a kind of fermented milk product that is sour and has a low alcohol content. It is produced by a bacterial and yeast fermentation process. Most cheeses go through a lactic fermentation process. Ingredients in making cottage cheese. Milk that has been heated and sterilized. Lactic streptococci and rennet are responsible for the coagulation, as well as the production of the characteristic flavor and scent (Reed, 1983).

Table 1. Lactic acid bacteria and their uses in fermentation processes (Ali, 2010).

Types of fermented products	Lactic acid bacteria
Dairy products	
Hard cheeses without eyes	*L lactis subsp. lactis, L lactis subsp. cremoris*
Cheeses with small eyes	*L lactis subsp. lactis, L lactis subsp. lactis var. diacetylactis, L lactis subsp. cremoris, Leuc.mesenteroides subsp. cremoris*
Swiss- and Italian-type cheeses	*Lb. delbrueckii subsp. lactis, Lb. helveticus, Lb. caset, Lb. delbrueckdi subsp. bulgaricus, S. thermophilus*
Butter and buttermilk	*L lactis subsp. lactis, L lactis subsp. lactis var. diacetylactis, L lactis subsp. cremoris, Leucmesenteroides subsp. cremoris*
Yoghurt	*Lb. delbrueckii subsp. bulgaricus, S. thermophilus*
Fermented, probiotic milk	*Lb. casei, Lb. acidophilus, Lb. rhamnosus, Lb. johnsonii, B. lactis, B. bifidum,*
	B. breve
Kefir	*Lb. kefir, Lb. keftranofacies, Lb. brevis*
Fermented meats	
Fermented sausage (Europe)	*Lb. sakei, Lb. curvatus*
Fermented sausage (USA)	*P. acidilactici, P. pentosaceus*
Fermented fish products	*Lb. alimentarius, C. piscicola*
Fermented vegetables	
Sauerkraut	*Leuc. mesenteroides, Lb. plantarum, P. acidilactici*
Pickles	*Leuc. mesenteroides, P. cerevisiae, Lb. brevis, Lb. plantarum*
Fermented olives	*Leuc. mesenteroides, Lb. pentosus, Lb. plantarum, P. acidilactici, P. pentosaceus, Lb. plantarum, Lb.fermentuan*
Fermented vegetables soy sauce	*T. halophilus*
Fermented cereals Sourdough	*Lb. sanfransiscensis, Lb. farciminis, Lb. fermentum, Lb. brevis, Lb. plantarum, Lb. amylovorus, Lb.reuteri, Lb. pontis, Lb. panis, Lb. alimentarius, W. cibaria*
Alcoholic beverages	
Wine (malolactic fermentation)	*0. oeni*
Rice wine	*Lb. sakei*

B: *Bifidobacterium*, C: *Carnobacterium*, L: *Lactococcus*, Lb: *Lactobacillus*, Leuc: *Leuconostoc*, O: *Oenococcus*, P:*Pediococcus*, S:*Streptococcus*, T: *Tetragenococcus*, W: *Weissella*

3.1.6 *Lactobacillus casei* Group

Formerly known as *Lactobacillus casei* 01, the organism now known as *Lacticaseibacillus casei* belongs to the largest genus in the family *Lactobacillaceae* and is a lactic acid bacteria (Pimentel, et al., 2021; Hill, et al., 2018). This bacteria is an acid-tolerant, non-spore-forming, facultative anaerobic or microaerophile. For years, scientists have argued over how to classify this group due to their inability to say *L. casei* strains apart from *L. paracasei* ones. *L. casei* subsp. *rhamnosus, L. casei* subsp. *alactosus, L. casei* subsp. *casei, L. casei* subsp. *tolerans*, and *L. casei* subsp. *pseudoplantarum* are the five recognized subspecies of this once-considered single species. Similarities in phenotype, physiology, and biochemistry were used to establish a taxonomic framework for this genus. The human reproductive and digestive systems are common habitats for this non-sporing, rod-shaped, gram-positive microorganism (Zheng, et al., 2020). Health researchers have focused a lot of attention on *L. casei* because of its adaptability to different environments. *L. casei* has a number of commercial uses, including the fermentation of dairy products and the use as a probiotic. In 1901, *Lactobacillus delbrueckii*, a crucial species in making yogurt, was first characterized. In 1935, a Japanese researcher named Shirota identified the beneficial strain of *Lactobacillus casei*. (It increases the number and efficiency of macrophages by populating the small intestine and causing the formation of antimicrobial compounds). For these reasons, *Lactobacillus casei Shirota* was one of the earliest probiotics to hit the market, with its initial sales occurring in Japan in 1955 (Yerlikaya, 2014).

3.1.7 Characteristics of *Lactobacillus casei* 01

Lactobacillus casei is a Gram-positive, nonmotile, nonspore-forming, and catalase-negative bacteria . Rods of (0.7-1.1) (2.0-4.0) micrometer, frequently with square ends, that occur individually, in pairs, or in chains are called cells. L-Lys-d-Asp peptidoglycan and polysaccharides found in the cell wall define the serological specificity (B or C) based on the amount of rhamnose or glucose-galactose present. The cell wall does not contain teichoic acids. The DNA has a G+C composition of 45–47%. Since most *L. casei* and *Lactobacillus paracasei* strains cannot be distinguished from one another using molecular methods, the taxonomy of the *L. casei* group has been a subject of much discussion. The three strains of *Lacticaseibacillus rhamnosus* are *L. casei* (type strain: ATCC 25599TM), *L. paracasei* subsp. *paracasei* (type strain: ATCC 25302TM), and *L. paracasei* subsp. *tolerans* (type strain: ATCC 25599TM), according to the currently accepted nomenclature and taxonomic division of the *L. casei* group. Despite the fact that just a few strains of the species *L. casei* have undergone extensive research, Table 2 lists some of the key behavioral characteristics that distinguish these three species (Gobbetti and Minervini et al., 2014).

Table 2. Some of the main phenotypic traits characterizing the species of the *Lactobacillus casei* group (Gobbetti , et al., 2014).

Strains	Growth at 10°C	Growth at 45°C	Resistance to 72°C for 40 seconds	Lactose	Maltose	Rhamnose	Sucrose
Lactobacillus casei	+	−	−	−	D	−	−
Lactobacillus paracasei subsp. *paracasei*	+	−	−	D	+	−	+
Lactobacillus paracasei subsp. *tolerans*	+	−	+	+	−	−	−
Lactobacillus rhamnosus	D	+	−	+	+	+	+

D, 50–90% of strains have this specification.

3.1.8 Health Benefits of *Lactobacillus casei* 01

There are investigations have been conducted *in vitro* (test tube) and/or *in vivo* (human clinical studies and mouse model). Since the influence on health varies according on the strain, it's crucial to keep an eye out for symptoms that might be unique to particular strain. When *L. casei* 01 (9 log cfu/mL) was added to ice creams, the level of hazardous ammonia was reduced while the production of beneficial microbial metabolites such propionate, acetate, lactic acid, and butyrate was boosted in an *in vitro* gut model (Pimentel, et al., 2021). In addition, it increased beneficial bacteria like *Lactobacillus* and *Bifidobacterium* while decreasing the numbers of dangerous microorganisms. (clostridia and fecal coliforms). *L. casei* 01 had a more noticeable impact than *L. acidophilus* La-5. (Chaikam et al., 2013). After two weeks of treatment with low-fat fermented goat milk containing *L. casei* 01 (6 log cfu/mL) and passion fruit by-product (1%), Casarotti et al. (2020) found that the number of *Lactobacillus* and *Bifidobacterium* genera increased while the number of *Megamonas, Prevotella*, and *Succinivibrio* genera decreased. The authors conducted their research on a SHIME system (a Simulator of Human Intestinal Microbial Ecosystem) using fecal inoculum from obese individuals. Based on their results, the *in vitro* tests suggest that *L. casei* may be involved in the modulation of intestinal microbiota through the production of beneficial microbial metabolites,propionate, acetate, lactic acid, and butyrate. *L. casei* 01 has antibacterial action, which may explain why the number of pathogens has dropped. However, the mechanisms through which this strain exerts its antibacterial action remain unclear. Pimentel and co-workers (2021) also found that *L. casei* 01 has antimicrobial effect on *Staphylococcus aureus* in vitro conditions. Lin et al., (2015) found that the acid producing *L. casei* 01 affected the development and biofilm formation of *Streptococcus mutans*. In addition, they imply that this strain has the potential to generate bacteriocins with molecular weights below 3000 Da.

Higher inhibition of angiotensin converting enzyme (ACE) activity was also seen when *L. casei* 01 was added to ice creams (6 log cfu/mL) and cheeses (8.32 log cfu/g), which was linked to the proteolytic activity of this strain and the production of bioactive peptides (Balthazar et al., 2018). In a clinical study (randomized double-blind pilot trial), the consumption of Minas Frescal cheese added with *L. casei* 01 (50 g for 28 days, 8.32 cfu/g) was shown to improve the lipid profile, blood pressure, and hematological parameters of hypertensive overweight women (n 30). These improvements included lower levels of total cholesterol (p 0.018), low-density lipoprotein-cholesterol (p 0.022), (Sperry, et al., 2018). Human clinical trials using *L. casei*-containing products showed that it improved lipid profile, blood pressure, and hematological parameters in hypertensive overweight women, and postprandial glycemia control in healthy individuals. The development of renal calculi may also be slowed, and intestinal regulation and oxidative stress may be affected, according to *in vitro* and animal model investigations. However, these cannot be proven without clinical research. Only three studies involved human participants for clinical trials (Grom et al., 2020).

3.1.9 *Lactobacillus salivarius* Group

The lactic acid bacteria known as *Lactobacillus salivarius* is a common resident of the human digestive tract and mouth. It belongs to the genus *Lactobacillus salivarius,* which contains numerous closely related bacterial species. In 1919, it was originally identified by Orla-Jensen, who had isolated it from human saliva and placed it in the genus *Lactobacillus* (Selle et al., 2019). The probiotic qualities of the *Lactobacillus salivarius* group have been shown to benefit digestive health and the immune system. The lactic acid generated by these bacteria can help to generate an acidic environment in the Small intestine, which can help to prevent the growth of pathogenic bacteria. *Lactobacillus salivarius* has been explored for both its probiotic and medicinal potential. Irritable bowel syndrome (IBS) disease, inflammatory bowel disease (IBD), and oral infections are just some of the illnesses that preliminary research suggests it may help cure or prevent (Neville, et al., 2010). Researchers are optimistic that the *Lactobacillus salivarius* group could lead to the discovery of useful novel probiotics and medicinal compounds (Holscher and Groeger., 2018). Like

Lactobacillus fermentum, *Lactobacillus acidophilus*, *Lactobacillus amylovorus* and *Lactobacillus salivarius* belongs to the family of *lactobacilli*. Some additional reading on the *Lactobacillus salivarius* group is provided in table 3 below (Talarico et al., 1988).

Table 3. Species and their source that are genetically related to *Lactobacillus salivarius* (Neville et al., 2010).

Species	Source
Lactobacillus apodemi	Feces of Japanese large wood mouse
Lactobacillus animalis	Dental plaque of primates
Lactobacillus aviarius	Chicken intestines
Lactobacillus ceti	Lungs of beaked whale
Lactobacillus equi	Horse feces
Lactobacillus hayakitensis	Horse intestines
Lactobacillus murinus	GI tract of rat and mouse
Lactobacillus saerimneri	Pig feces
Lactobacillus ruminis	Bovine rumen
Lactobacillus salivarius	Oral cavity of human
Lactobacillus agilis	Piglets, humans, pigeons, sewage
Lactobacillus aquaticus	Freshwater pond
Lactobacillus caconum	Cocoa fermentations
Lactobacillus ghanensis	Cocoa fermentations
Lactobacillus hordei	Malted barley
Lactobacillus sucicola	Sap of oak trees
Lactobacillus mali	Pressed apple juice
Lactobacillus nagelii	Fermenting grape wine
Lactobacillus oeni	Bobal grape wines
Lactobacillus satsumensis	Shochu mashes (by-product of distilledspirit production)
Lactobacillus uvarum	Fermenting grape musts
Lactobacillus vini	Fermenting grape musts
Lactobacillus acidipiscis	Fermented fish
Lactobacillus capillatus	Fermented stinky tofu brine
Lactobacillus pobuzihi	Fermented cummingcordia (pobuzihi;traditional Taiwanese food)

3.1.10 Characteristics of *Lactobacillus salivarius*

As a Gram-positive bacterium, *Lactobacillus salivarius* plays a significant role in the commensal bacterial communities of humans, pigs, and chickens, particularly in the intestinal microbiota of these animals (Gong, et al., 2007). Due to its resilience to acid and bile (Fang, et al., 2009), its ability to cling to vertebrate mucus (van Pijkeren et al., 2006), and its bile salt hydrolase activity, this lactic acid-producing bacterium is a viable probiotic possibility (Fang, et al., 2009). In addition, this bacteria safeguards hosts from pathogenic infections by several mechanisms, such as antimicrobial action against pathogens (Messaoudi, et al., 2013), decreased pathogen adherence (O'hara, et al., 2006), and impacts on host immune cells (O'Mahony, et al., 2006). Attenuation of arthritis in an interleukin 10 knockout mice (Sheil, 2004) and improvement of Crohn's disease symptoms have been seen in various *in vivo* studies of *L. salivarius'* impact on illness (Mattila-Sandholm et al., 1999). Figure 2. provides a quick overview of the species that make up the *Lactobacillus salivarius* clade and where they were initially isolated. As 2010, a total of 25 species had been assigned to the group.(Neville, et al., 2010)

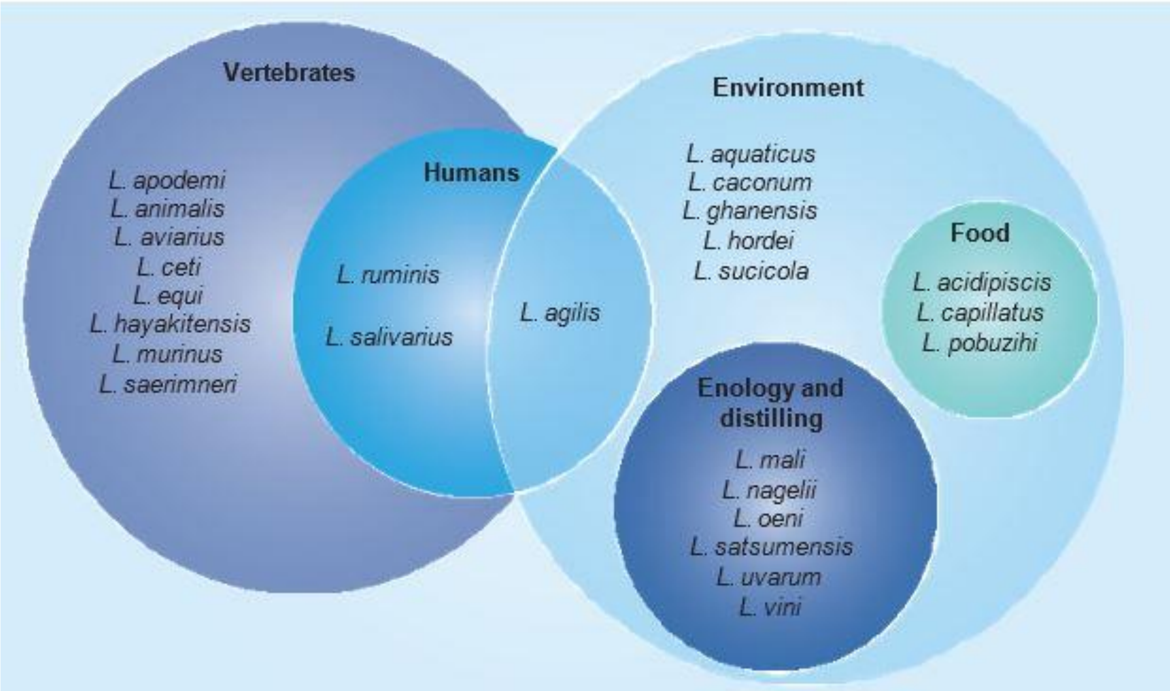

Figure 2. the species of *Lactobacillus salivarius* clade (Neville et al., 2010)

3.1.11 Metabolic Processes of *Lactobacillus salivarius*

L. salivarius is a homofermentative bacterium (Kuratsu, et al. 2010)., meaning it can only use the Embden-Meyerhof-Parnas (EMP) pathway. Glucose, ribose, adonitol, galactose, fructose, mannose, mannitol, sorbitol, and N-acetylglucosamine are only some of the monosaccharides it may ferment; other sugars include maltose, lactose, sucrose, and trehalose (all disaccharides) (Claesson et al. 2006). The human digestive system is a favorable environment for *L. salivarius* since all of these sugars are either consumed by humans or produced by them (Claesson et al. 2006). As these processes are concerned, *L. salivarius* is a facultative-anaerobe (Stern et al. 2006). Bacteriocins, or short peptides with antibacterial action, can be metabolized by *L. salivarius* (Messaoudi, et al. 2013). The presence of this trait gives *L. salivarius* its probiotic qualities in humans. For example, *L.*

salivarius'sAbp118 bacteriocin has been demonstrated to directly antagonize bacteria that cause food-borne disease in humans (Corr, et al., 2007). Some of the other bacteriocins produced by this bacteria are effective in treating acne (Deidda, et al. 2018). Antibiotics and disinfectants derived from bacteriocins produced by lactic-acid bacteria have seen increased application in recent years (Messaoudi et al. 2013).

3.1.12 Health Benefits of *Lactobacillus salivarius*

Lactobacillus salivarius is a kind of beneficial bacteria that lives naturally in the human gut and mouth. Researchers have found that it has a number of health perks, like making the immune system work better. *Lactobacillus salivarius* can make it easier for the immune system to fight off virus infections by making more cytokines. Cytokines are proteins that help control the immune system (Donatiet et al., 2015), also getting your gut healthy. *Lactobacillus salivarius* has been shown to lower inflammation in the gut, improve the function of the intestinal barrier, and ease the symptoms of gastrointestinal disorders like irritable bowel syndrome (IBS). This helps IBS patients feel less pain and gas in their stomachs (Paik et al., 2015). Helping to lower oral pathogens and improve oral health is a way to support oral health and reduce the amount of *Streptococcus mutans* in saliva, which is a bacteria that can cause tooth decay (Perera et al., 2016). To prevent allergies, the immune system's reaction to allergens needs to be changed. (Børsting et al., 2012). Getting people to lose weight by reducing inflammation and making their guts healthier. Research published in the journal Beneficial Microbes found that when overweight people took *Lactobacillus salivarius* for 12 weeks, their weight, body mass index (BMI), and waist circumference all went down significantly (Starke et al., 2015).

3.2 PROBIOTICS AND FUNCTIONAL FOOD

3.2.1 Definitions

Probiotic is a relatively recent word meaning "for life" that is used to describe bacteria that has been linked to health benefits for people and animals. Eli Metchnikoff, a Russian-born Nobel Prize winner working at the Pasteur Institute at the turn of the last century, is credited with first noting the beneficial effects of certain strains of bacteria. He stated, "The dependency of the intestinal microbes on the food makes it possible to use measures to regulate the flora in our bodies and to replace the harmful microbes by advantageous microbes" (Anukam, 2007). In recent years, the term "functional food" has emerged to characterize edibles that include nutrients that contribute to host health in ways beyond simple nutrition. Products like probiotics, which have been shown to improve health by acting on the body's microbes, fall under this category (Ziemer and Gibson, 1998). Foods containing probiotics are an example of functional foods because they go beyond the nutritional needs of the body.

3.2.2 Microorganisms with Probiotic Applications

Lactic acid bacteria strains, specifically those from the genera *Lactococcus* and *Bifidobacterium*, have any nutritional significance at all (Holzapfel et al., 2001). Because of their distinct metabolic process, the genus *Bifidobacterium* has historically been included inside this group.

3.2.3 Probiotic Qualities That Are Desirable

The therapeutic effects of a probiotic strain are contingent on the strain meeting certain criteria. Current *in vitro* tests have identified the following properties as important for oral administration: acid and bile tolerance, adhesion to mucosal and epithelial surfaces, competitive exclusion of pathogens, and pathogen prevention adhesion and colonization, antimicrobial activity against pathogenic bacteria, and bile salt hydrolase activity (Saarela et al., 2000). The final product's colony forming units per gram of product should be based on the dosage levels of probiotics shown to be beneficial in human research. If you want to earn the full probiotic effect, you need to consume somewhere between 10^8 and 10^9 CFU of probiotic microorganisms every day (Sanders, 2008), so make sure your probiotic product has at least a 10^6 CFU/mL or gram concentration. Strains should also be viable and able to multiply under commercial and industrial conditions.

Industrial difficulty aside, viability is a necessary condition for probiotic performance since it enhances mechanisms like adherence, gut permeability reduction, and immunological modulation (Kosin & Rakshit, 2006).

Certain criteria must be met before a strain can be called a probiotic culture (Daliri & Lee, 2015): be harmless to the host, cause no allergic reactions in the host, can be cultured in large quantities, can be added to food with little effort, can survive technological manipulations like heating and low-oxygen conditions in packaging, can be kept at a minimum level throughout the product's storage period, the strain has to originate in the host's digestive system in order to be deemed "autochthonous" (Daliri and Lee, 2015). Unconventional sources of microorganisms are being screened for potential probiotics isolated from a wide variety of non-intestinal sources and non-dairy fermented food products, such as traditional fermented foods, traditional fermented drinks, vegetable and fruit juices (Sornplang and Piyadeatsoontorn, 2016).

3.2.4 Probiotic Bacteria

Lactic acid bacteria, like *Lactobacillus, Lactococcus, Streptococcus, Enterococcus,* and *Bifidobacterium*, are the most often utilized probiotic bacteria. The main probiotic strains used in food are *L. acidophilus, L. casei, L. plantarum, Limosilactobacillus reuteri, L. rhamnosus, Lactobacillus paracasei, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium adolescentis, Saccharomyces bourlardii, Saccharomyces cerevisiae* and *Propionibacterium freudenreichii* (Stürmer et al., 2012). Whether or not *Lactobacillus bulgaricus* and *Streptococcus thermophilus* belong in the probiotic category is debatable because of a lack of proof of their proliferation in the digestive system. However, you can get them in abundance in fermented milk products. Exopolysaccharides (EPS) and peptides are two examples of healthful metabolites that bacteria can create (Pimentel, et al., 2019). The most common bacteria that have been shown to have probiotic effects are shown in Table 4.

Table 4. The main types of microorganisms known as probiotic cultures (Petrova & Petrov, 2017).

Lactobacillus	***Bifidobacterium***	***Saccharomyces***	***Other Microorganisms***
L. acidophilus	*B. animalis*	*S. boulardii*	*Streptococcusthermophilus*
L. brevis	*B. breve*	*S. cerevisiae*	*Streptococcus diacetylactis*
L. casei	*B. bifidum*		*Propionibacterium freundenreichi*
L. fermentum	*B. lactis*		*Pediococcus acidilactici*
L. gasseri	*B. longum*		*Enterococcus faecium*
L. paracasei	*B. infantis*		*Enterococcus durans*
L. plantarum	*B. thermophilum*		*Enterococcus faecalis*
L. reuteri	*B. essensis*		*Leuconostoc mesenteroides*
L. brevis	*B. laterosporus*		*L. mesenteroides*
L. rhamnosus			
L. bulgaricus			
Sporolactobacillus inulinus			
L. helveticus			
L. johnsonii			
L. cellobiosus			
L. curvatus			
L. crispatus			
L. amylovorus			
L. gallinarum			

3.2.5 Probiotics and Their Positive Effects on Health

Increasing research shows that consuming probiotic cultures has several health benefits, lending credence to the advantages of these microorganisms (Kechagia et al,. 2013), such as: antimutagenic, anticarcinogenic, and antidiarrheal properties, immune system stimulation, lowering serum cholesterol, improving lactose metabolism, antimicrobial activity, reducing gastrointestinal infections, and improving intestinal health are just a few of the many benefits of probiotics (Stavropoulou and Bezirtzoglou, 2020). There are two ways in which probiotics are thought to safeguard humans. One important function they provide is in the digestive system. When the digestive system is functioning properly, dangerous substances including bacteria, poisons, chemicals, and waste are filtered out and eliminated. Maintaining a steady bacterial population in the gut is important for proper motility and barrier function. Infectious diarrhea, antibiotic-associated diarrhea, intestinal transit, irritable bowel syndrome, abdominal pain and bloating, ulcerative colitis, *Helicobacter pylori* infection, nonalcoholic fatty liver disease (NAFLD), and necrotizing enterocolitis are all conditions for which probiotics have been shown to have some positive effects in clinical trials. The immune system is another area where probiotics prove useful. This position is seen by some as pivotal. The protective immune mechanisms fights off bacteria and viruses. When it fails to function properly, we are more susceptible to infections, allergic responses, and autoimmune illnesses including ulcerative colitis, Crohn's disease, and rheumatoid arthritis. The goal is to avoid these diseases by ensuring a healthy equilibrium from birth. It's never too late to get the benefits of an immune system that works well (Wu and Wu, 2012).

For adults, probiotics have been shown to decrease the incidence of upper respiratory infections and the associated antibiotic use (Guarner and Malagelada, 2003). Antibody responses to both natural infections and vaccines have been found to be improved by the consumption of some probiotics. Consumption of *L. rhamnosus* GG-fermented milk greatly increased specific antibody responses in children infected with rotavirus, as shown by the research of Kaila and colleagues (1992). Organic acids (lactic acid and acetic acid), hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins/bacteriocin-like compounds are just some of the antimicrobial chemicals that have been found to be produced by probiotic bacteria. Both lactic acid and acetic acid slow bacterial metabolism by lowering the pH of the gut contents (Mishra and Lambert, 1996). Both Gram positive and Gram negative bacteria are sensitive to the antibacterial effects of hydrogen peroxide (Mishra and Lambert, 1996, Holland et al., 1987).

Family Health Guide reports that many persons who are lactose intolerant can eat yogurt without experiencing any negative side effects. This is because the microorganisms in yogurt facilitate lactose digestion in the small intestine, preventing the sugar from becoming undigested in the large intestine. *L. bulgaricus* and *Streptococcus thermophilus*, two cultures used in yoghurt starter, also aid in lactose digestion (Harvard Medical School's, 2005).

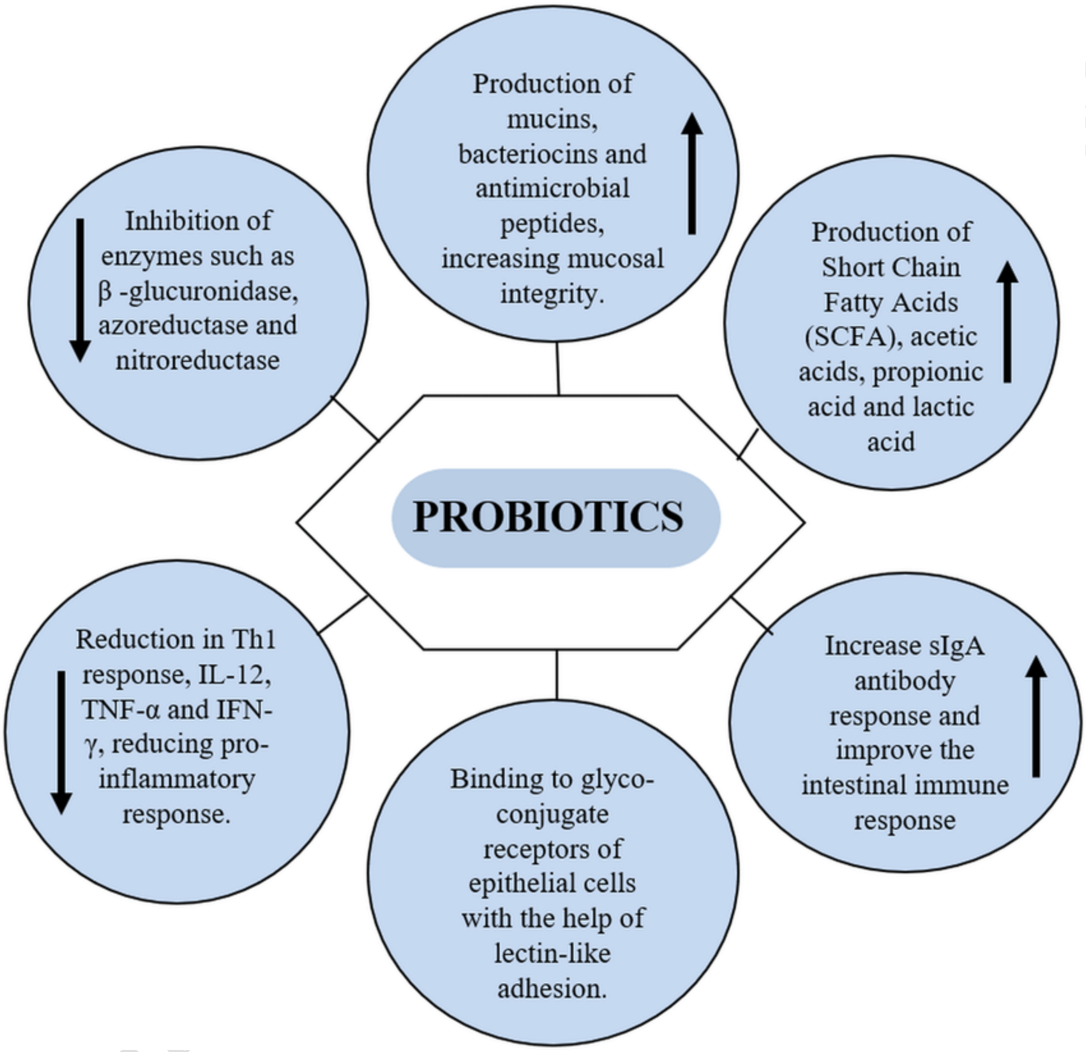

Figure 3. Proposed health effect mechanism involving probiotics (Basak and Gokhale 2021).

3.3 CHICKEN EGG

3.3.1 Overview

Eggs are one of the few items that are consumed over the world by people of all faiths and cultures (Stadelman and Cotterill, 2001). The chicken egg is one of the few naturally preserved biological specimens and is widely regarded as an excellent food choice due to its high protein, fat, vitamin, and mineral content. Eggs have been decreasing in popularity in industrialized countries over the past few decades due to their high cholesterol and fat content. Eggs have been deemed unhealthy and high in cholesterol by the medical community, hence their consumption has been discouraged, especially among the elderly. Eggs, however, are widely acknowledged for the health benefits they provide. Eggs' functional features include their ability to froth and emulsify, as well as imparting a distinctive color and flavor to a variety of foods.

The egg's three primary parts are the shell (9–12%), the white (60%) and the yolk (33–33%). found that a whole egg contains 75% water, 12% proteins, 12% lipids, 1% carbs, and 1% minerals (Kovacs-Nolan et al., 2005). White and yolk both contain proteins, but the yolk is also rich in lipids. Yolk is made up of water (50%), protein (15-17%), lipids (31-35%), and carbohydrates (1%), and is surrounded by a membrane made of vitelline. The proteins in egg yolk are divided into four groups: lipovitellins (36%), livetins (38%), phosvitin (8%), and low-density lipoproteins (17%). In addition, the yellow pigment carotinoides (1% of yolk) is responsible for this characteristic (Stadelman and Cotterill, 2001).

3.3.2 Chemical Composition of Egg White

Egg white is predominantly composed of water (88%) and protein (11%), with trace quantities of carbohydrates, ash, and lipids (1%). Ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%) are considered as the primary proteins and cystatin (0.05%), avidin (0.05%), ovomacroglobulin (0.5%), ovoflavoprotein (0.8%), ovoinhibitor (1.5%) and ovoglycoprotein (1.0%) are the minor proteins found in egg white (Kovacs, et al., 2005). These proteins have been acknowledged for their functional significance. Each protein possesses numerous functional properties, and the proteins have been isolated from egg white using a variety of techniques. Table 5 below shows the basic characteristics of egg proteins.

Table 5. Major egg white proteins and selected properties (Mine, 1995)

Protein	Protein content inegg white %	Molecular weight (kDa)	pI Isoelectric point	Denaturation temperature (°C)
Ovalbumin	54	44.5	4.5	84.0
Ovotransferrin	12	77.7	6.1	61.0
Ovomucoid	11	28.0	4.1	77.0
Ovomucin	3.5	$5.5\text{-}8.8\times10^3$	4.5-5.0	Unknown
Lysozyme	3.4	14.3	10.7	75.0

Ovalbumin

Ovalbumin comprises 54% of the total egg white proteins (Stadelman and Cotterill, 2001). Ovalbumin is the primary egg white protein synthesized in the hen's oviduct. Ovalbumin has a molecular weight of 45 kDa and 386 amino acids. In addition to the N-terminal acetyl group, ovalbumin has three additional locations of post-synthesis modification (Huntington and Stein, 2001). The N-terminal amino acid of ovalbumin is acetylated glycine, while the C-terminal amino acid is proline (Nisbet et al., 1981). Additionally, it is referred to as a glycoprotein and possesses an N-terminal carbohydrate group. Ovalbumin consists of three components, A1, A2, and A3, which, respectively, contain 2, 1, and no phosphate group. According to (Stadelman and Cotterill 2001).

Ovotransferrin

Ovotransferrin is a monomeric glycoprotein with a molecular weight of 76 kDa and 686 amino acids. It contains 15 disulfide bonds and has the same amino acid sequence as transferrin in human serum (Oe et al., 1988). Schade and Caroline (1944) first characterized ovotransferrin, which comprises 12 percent of the total egg white protein. It was formerly known as conalbumin, but its name was changed to ovotransferrin after it was discovered that it binds iron (Williams, 1968). One molecule of ovotransferrin can bind to and transport two iron molecules. Each lobe of ovotransferrin is comprised of two distinct and domains. Ovotransferrin is folded into two lobes and four domains. These two domains are connected by antiparallel strands that operate as a hinge to open and close (Huopalahti et al., 2007). There are two primary variants of ovotransferrin: apo- (iron-free) and holo (iron-bound) forms. The chemical and physical properties of these two types of ovotransferrin are notably distinct (Azari and Baugh, 1967), whereas the apo-form is monochromatic, the holo-form is salmon pink in color. The apo-form compared to the holo-form is more resistant to chemical and physical conditions. At pH >7.0, iron (Fe^{3+}) can readily bind to ovotransferrin, but at pH 4.5, it is released (Ko and Ahn, 2008). Each lobe contains two iron-binding sites within the interdomain fissure (Baker and Baker, 2004). These ligands consist of tyrosine, aspartic acid, and histidine residues. Both ovotransferrin and lactoferrin found in milk have iron-scavenging and iron-transporting properties (Abdallah and Chahine, 1999).

Lysozyme

Lysozyme is an additional essential protein present in egg white. There are naturally many forms of lysozyme, but the lysozyme found in eggs is the most soluble and stable. Lysozyme was discovered in 1946 by Alderton and co-workers. N-acetyl-muramic-hydrolase is a ubiquitous enzyme that can hydrolyze the -linkage between N-acetylneuraminic acid and N-acetylglucosamine in the bacterial cell membrane it is composed of lysine and leucine at the N- and C-termini, respectively. Lysozyme has a molecular weight of 14,400 Da and consists of a single polypeptide chain containing 129 amino acids. This protein is typically found as a monomer in nature, but occasionally as a dimer with greater thermal stability (Stadelman and Cotterill, 2001; Huopalahti et al., 2007). According to Huopalahti et al. (2007), it is a fundamental protein found in egg white that is considered to be of high quality. Lysozyme possesses four disulfide bridges, which contribute to its high thermal stability, and its isoelectric point is 10.7. It tends to conjugate to negatively charged proteins like ovomucin in egg white (Wan et al., 2006).

Ovomucin

Ovomucin is another significant egg white protein, accounting for 3.5 percent of the total egg white protein (Stadelman and Cotterill, 2001). Ovomucin is composed of soluble and insoluble components; the soluble component has a molecular weight of 8,300 Da and the insoluble component has a molecular weight between 220 and 270 kDa (Omana et al., 2010). It is one of the proteins with the highest molecular weight with a carbohydrate attached and is responsible for the gel like structure of egg white. Ovomucin is composed primarily of two classes of subunits, designated and ovomucin is heterogeneous (Hiidenhovi et al., 1999). The 1 and 2 subunits of ovomucin contain fewer carbohydrate groups than ovomucin, which is abundant with carbohydrates. Ovomucin's extremities have more coiled regions, similar to human mucin's structure. Due to the presence of long-coiled regions, a structure of random coiling is observed. Ovomucin is primarily composed of serine and threonine (Robinson and Monsey, 1975), whereas ovomucin is composed predominantly of acidic amino acids, such as aspartic acid and glutamic acid. (Omana et al., 2010). However, there was no difference between ovomucin from dense egg white and thin egg white. Previous research has demonstrated that ovomucin contains at least three varieties of carbohydrate chains, which are composed of galalctose, galactosamine, sialic acid, and sulfate in a molecular ratio of 1:1:1:1. The average amount of carbohydrates in ovomucin is 33% (Mine, 2008).

Ovomucoid

Ovomucoid is among the most glycosylated proteins found in egg white. Ovomucoid has a molecular weight of 28 kDa, but the band in SDS-PAGE appears between 30 and 40 kDa. It is well-known as a trypsin inhibitor and is regarded as the primary allergen in egg white. Each ovomucoid molecule engages one trypsin molecule, and its three-dimensional structure is held together by three disulfide bonds (Hoppe, et al. 2010). Kovacs-Nolan et al. (2000) demonstrated that peptides derived from ovomucoid with pepsin exhibit IgE-binding activity and retain their trypsin-inhibiting properties. Matsuda et al. (1985) reported that ovomucoid retained its immunoreactivity even after pepsin hydrolysis; however, the role of carbohydrates in ovomucoid immunoreactivity is unknown. Nagata and Yoshida (1984) reported that ovomucoid is effective against *Streptomyces erythraeus*. Even the protein is a trypsin inhibitor, which is a negative characteristic; it has the ability to control microorganisms. Therefore, it can be utilized as a food antimicrobial agent.

3.3.3 Nutritional values of egg white proteins

As a whole, the egg is thought to be a good source of protein and lipids, but the egg white is mostly water (88%) and protein (11%), with no lipids (Stadelman and Cotterill, 2001). Ovomucin is a highly glycosylated protein, and Omana et al. (2010) found that about 33% of ovomucin is made up of carbs. So, ovomucin can be thought of as a good form of nutrients that can give you protein and carbohydrates, which are both very important. Ovalbumin is the main protein in egg whites. It has a well-balanced mix of amino acids, so it can be used as a great source of protein in many foods. The rest of the egg white proteins are also good sources of amino acids that the body needs.

3.3.4 Potential Applications of Modified Egg White Proteins

Hydrolyzed forms of egg white proteins retain many of the beneficial qualities of the native proteins. The proteins in egg whites can be hydrolyzed by a number of different enzymes and in a range of environments. Many functional peptides with positive health effects have been produced and patented in recent decades. Enzymes like pepsin, trypsin, and chymotrypsin are used to create these biologically active peptides. Egg white has been employed as a substrate for enzymatic hydrolysis, yielding bioactive peptides by a number of studies. For example, Chiang et al. (2006) hydrolyzed egg white with thermolysin, yielding bioactive peptides that can suppress ACE activity. Peptides were created by hydrolyzing egg white with pepsin, a method also used by Miguel and Aleixandre (2006). Peptides having the pattern Tyr-Arg-Glu-Glu-Arg-Tyr-Pro-Ile-Leu were the most effective at inhibiting ACE activity, followed by Arg-Ala-Asp-His-Pro-Phe-Leu and Ile-Val-Phe. The blood pressure of rats with spontaneous hypertension was lowered when they were fed these peptides. The ACE-inhibitory action of hydrolyzed ovalbumin was demonstrated to be high (Miguel et al., 2007). Seven ACE-inhibitory peptides, were isolated from hydrolyzed ovalbumin by Fujita et al. (2000). Blood lipid levels were decreased and substantial ACE-inhibitory effects were observed in some of the peptides derived from ovalbumin (Manso et al., 2008). Peptides isolated from both egg white and ovalbumin were found to have a vasodilatory action (Hoppe, et al. 2010). According to Yu et al. (2012), of the peptides generated, ovokinin and ovokinin 2–7 showed the most notable ACE- inhibitory effects. Ovotransferrin, like ovalbumin, has been applied for its bioactive peptides. Ovotransferrin hydrolysates shown potent antibacterial action. Peptides isolated from ovotransferrin have been found to have potent antibacterial properties, as described by Ibrahim et al. (2000). However, hydrolysis of ovotransferrin eliminated its iron-binding capacity (Wu and Acero-Lopez, 2012). Strong antioxidant activity was also seen in peptides isolated from ovotransferrin. Protective benefits against oxidative stress and DNA damage in human leukocytes were shown after ovotransferrin was hydrolyzed with enzymes such protamex, alkalase, trypsin, and -chymotrypsin (Moon et al., 2013). Ovotransferrin, with the peptide sequence Lys-Val-Arg-Glu-Gly-Thr, has been shown to have potent ACE-inhibitory activity and vasodilatory activity when hydrolyzed (Wu and Acero-Lopez, 2012). Immunomodulating activity against T-cells was seen for peptides generated from ovomucoid, and macrophage-stimulating activities were observed for peptides produced from ovomucin (Kovacs-Nolan et al., 2005).

3.4 PINEAPPLE FRUIT

3.4.1 Pineapple origin, Properties

One of the world's most valuable commercial fruit crops is pineapple *Ananas comosus* (L.) Merr. Because of its delicious flavor, it is often called the "queen of fruits" (Baruwa, 2013). After the banana and citrus, pineapple is the third most widely consumed tropical fruit (Bartholomew et al., 2003). Pineapples can be eaten raw, cooked, juiced, or stored for later use. This is a seasonal fruit that spoils quickly. Sugar accounts for 14% of the total in ripe fruit, while protein-digesting bromelin and plenty of citric acid, malic acid, vitamins A and B are also present. The exact ingredients in pineapple juice change based on where it was grown, when it was harvested, and how it was processed. The sweetness and tanginess of this fruit come from the perfect harmony of sugar and acid. Thailand, the Philippines, Brazil, and China are the world's four largest producers of pineapples, accounting for about half of global output. Most of the remaining fruit comes from India, Nigeria, Kenya, Indonesia, Mexico, and Costa Rica, who are also major producers (Joy, 2010). Pickles made from green pineapple are a thing. The tender leaves and the pulp that remains after the juice has been extracted are both utilized as cattle feed. Pineapple is used in the production of several different foods, including squash, syrup, and jelly. Pineapple is also processed into vinegar, alcohol, citric acid, calcium citrate, etc. There are some medical conditions for which a pineapple-based diet is recommended (Moniruzzaman, 1988). Bromelain is a proteolytic digestive enzyme, according to the U.S. National Library of Medicine. Bromelain, when taken with meals, facilitates protein metabolism by converting proteins to amino acids (Debnath et al., 2012).

3.4.2 Nutritional Value of Pineapple

Pineapple is a fantastic tropical fruit with health advantages and a lively, tropical flavor. Pineapple is rich in several nutrients, including calcium, potassium, vitamin C, carbs, crude fiber, water, and various minerals, all of which aid in digestion and contribute to healthy weight maintenance. Pineapple is a popular fruit, and its low fat and sodium content are healthful benefits (Sabahelkhier et al., 2010). Vitamin content ranges from 10 mg to 25 mg (Rasid et al., 1987). Most studies on pineapple composition have focused on the fruit itself. Pineapple has between 81.2 and 86.2% water content, and between 13% and 19% total solids; its primary sugars are sucrose, glucose, and fructose. Up to 85% of total solids can be attributed to carbohydrates, while fiber only accounts for 2-3%. It contains a high concentration of citric acid, the most common organic acid. Ash, nitrogenous chemicals, and lipids (0.1%) make up a small percentage of the pulp. The real protein content of nitrogenous substances is between 25% and 30%. Bromelin, a protease, is responsible for the proteolytic activity of around 80% of this fraction. There are many minerals in fresh pineapple, including calcium, chlorine, potassium, phosphorus, and sodium (Dull, 1971). Vitamin C is abundant in pineapple juice thanks to the ascorbic acid it contains. Vitamin C, or ascorbic acid, goes by many different names, is an active antioxidant that aids in the body's absorption of iron and fights off bacterial and viral infections. Half of an adult's daily vitamin C requirement can be met by consuming half a cup of pineapple juice (Joy, 2010). Manganese, a trace mineral needed to bone development and the production and activation of certain enzymes, is one of many critical minerals found in pineapples. Copper, another trace mineral, can be found in pineapples. It controls blood pressure and heart rate and aids in iron absorption (Debnath, et al., 2012).

3.5 STRAWBERRY FRUIT

3.5.1 Strawberry Origin, Properties

The garden strawberry, often known as the strawberry or just strawberry (*Fragaria ananassa*), is a widely cultivated hybrid species of the genus Fragaria, also known as the strawberries and farmed for their fruit all over the world. The fruit's distinctive aroma, vivid red color, juicy texture, and sweetness are all well-liked characteristics. Both fresh and prepared delicacies including jam, juice, pies, ice cream, milkshakes, and chocolates are consumed in great amounts. A lot of products, including confectionery, soap, lip gloss, perfume, and many others, contain artificial strawberry flavorings and fragrances (Manganaris et al., 2013).

3.5.2 Nutritional Value of Strawberry

A 100-gram serving of strawberries contains more than 100% of the recommended daily intake (RDI) for vitamin C, making them a superior source of the vitamin. Additionally, they are an exceptional source of fiber, potassium, and folate. There are only 32 calories in a 100-gram portion of strawberries, making them a calorie-efficient food. They have low salt and fat content (Giampieri et al., 2015). Polyphenols, flavonoids, and anthocyanins are just few of the many bioactive components found in strawberries. Because of their antioxidant and anti-inflammatory qualities, these chemicals play a crucial role in warding off chronic diseases like cancer, cardiovascular disease, and diabetes. Strawberries contain a wide variety of bioactive chemicals, the majority of which are polyphenols such ellagic acid, quercetin, and kaempferol. Catechins, epicatechins, and proanthocyanidins are all examples of flavonoids, another class of beneficial substances present in strawberries. The anthocyanins that give strawberries their red hue also provide them anti-inflammatory and antioxidant benefits (Giampieri et al., 2015). There are a lot of benefits to eating strawberries. Consumption of strawberries has been linked in several studies to a lower chance of developing cancer, diabetes, and cardiovascular disease. Antioxidant and anti-inflammatory effects of the polyphenols and flavonoids contained in strawberries may provide protection against certain chronic diseases. Also, research indicates that eating strawberries can help with memory, digestion, and weight control (Basu, et al., 2010).

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Fruit Juices

25% pineapple fruit juice and strawberry fruit juice (Hey-Ho, Budapest, Hungary) were purchased commercially from the local market. The initial pH of the pineapple juice was adjusted to pH 6.1 and for strawberry juice was 6.5 and saved under cooling conditions at 4± 1 °C till use.

4.1.2 Egg White Drink

Fresh egg-white drink (ToTu) bottles were obtained from Caprivous LTD (Budapest, Hungary), and they were refrigerated at 4± 1 °C until use. The egg white drink is made using table salt, vinegar and protease enzyme. can also be classified here, as it is lactose, milk, fat and preservative free and has a low calorie but high protein content as mentioned in table 6. The product is reminiscent of milk, especially long-life milk, in both texture and color, and to some extent in taste.

Table (6): Nutritional Values of Egg white drink (ToTu) (Caprivous LTD, 2023)

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

4.1.3 Bacterial Strain

The *Ligilactobacillus salivarius* CRL 1328 and *Lacticaseibacillus casei* 01 strain was obtained from the bacterial collection of local laboratory (MATE, Food Science and Technology Institute, Fermentation laboratory at Department of Bioengineering and Alcoholic Drink Technology). The bacteria were activated in the fermentation laboratory by adding them to the MRS liquid media and incubating them for 24 hours at a temperature of 37 °C degrees and then used to inoculate the mixture of egg whites and fruit juice.

4.1.4 MRS media

MRS (De Man, Rogosa, Sharpe) media is a selective and differentiated culture medium used to isolate and count lactic acid bacteria, Prepare MRS agar by combining the appropriate amount of the contents as in table 7 with distilled water and autoclaving for 15 minutes at 121 °C.

Table (7). Composition of the MRS medium (De Man and Sharpe, 2003).

Components	Quantity
Glucose	20 g
Proteose peptone	10 g
Beef extract	8 g
Yeast extract	4 g
Sodium acetate	5 g
Triammonium citrate	2 g
Dipotassium hydrogen phosphate	2 g
Tween 80	1 ml
Magnesium sulphate	0.2 g
Manganese sulphate	0.05 g
Agar	15 g
Distilled water	1000 ml

4.1.5 Saline Solution

(0.85% NaCl) was prepared in the laboratory by adding 8.5 g of NaCl to 1000 Ml of distilled water and has been distributed to tubes (4.5 ml) for each tube and sterilized by autoclave at 121 °C for 15 Minutes

4.2 METHODS

4.2.1 Analysis of Bacterial Cell Count and Vitality

and also Fermented egg white was serially diluted with sterile saline (0.85% NaCl) by transferring 0.5 ml from the first tube to the second, 0.5 ml from the second tube to the third, etc., to 10^4-10^7 dilutions, and 0.1 mL of the diluted samples was poured into petri dishes for inoculation. Cooling down the MRS agar to 45 to 46 °C before pouring it into inoculated petri dishes. Let the agar to settle to room temperature and solidify, then plates should be incubated at 37°C for 72 hours, or until colonies are visible. Count the number of visible colonies on the plates with the appropriate dilution factor by multiplying the average number of colonies on the plates and dividing by the sample volume used.

4.2.2 Fermentation of Egg White Drink with Fruit Juices

The fermentation process was conducted according to the following process: *L. casei* 01, and *L.salivarius* CRL 1328 were grown separately in MRS broth before 24 hours of the fermentation. Egg white drink and fruit juices were mixed at the ratio of 3:1 (v/v) under sterile condition. The fermentation was started with 1% of inoculum and incubating at 37 °C under aerobic conditions. Two replication were made for each formula. The fermentation time was 16h-24 h,. During the fermentation process, 2 ml of fermented egg white were taken respectively 4 h, 8 h, 16h, and 24 h to determine total protein content, pH values and 0.5 ml for analysis of viable bacterial cell count. At the end of fermentation, the fermented egg white drinks are stored under cool conditions (4± 1 °C) and the reology, colour and viscosity were also determined.

4.2.3 Analysis of pH

The pH values of the samples was measured using digital pH meter (Mettler-Toledo GmbH, Switzerland) after calibration by standard solutions. During fermentation process at different times and also during shelf life experiment the pH of the samples was determined.

4.2.4 Viscosity Measurement

The viscosity of each fermented 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° 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.

$$\tau=\tau_0+K\dot{\gamma}^n \quad (1)$$

where τ—refers to shear stress (Pa); τ_0—indicates the yield stress (Pa); $\dot{\gamma}$—is the shear rate (1/s), K—refers to the consistency coefficient ($Pa \cdot s^n$), and n—is the flow behavior index (dimensionless).

4.2.5 Color Determination

The color 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),

b* value ranged from blueness (-60) to yellowness (+60) (Dimitrellou et al., 2020). Additionally, the total color difference (ΔE*) between two colors in the L*a*b* color space was calculated. This difference can be used to characterize the color change of the sample in accordance with the human perceptual threshold. The ΔE* was computed as the difference between the sample values following fermentation.

4.2.6 Protein Content Determination

The quantity and quality of the proteins of the fermented egg white drink were determined using gel electrophoresis for the samples during fermentation process as follows: 0h, 4h, 8h, and 16h. Gel electrophoresis is a common technique for separating and analyzing proteins according to their size and charge. Sodium dodecyl sulfate-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. 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) (Laemmli, 1970).

4.2.7 Sensory Evaluation

Besides the fermentation procedure, the sensory analysis of fermented beverages were 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.

4.2.8 Determine The validity Period of The product

The product validity experiment was conducted for fermented egg whites by knowing the viability of the bacteria during the storage period by analyzing the total number of living cells of the bacteria and pH values as follows:, 2 weeks, 4 weeks, and 6 weeks. Two replicates were prepared for each sample and the samples were stored in a refrigerator (4± 1 °C).

5. RESULTS

Through this study, the fermentability of the egg white drink (ToTu) was verified and the shelf life of the product by using two kind of the probiotic strains (*L.casei* 01 and *L.salivarius* CRL 1328) was determined. The egg white was completed with pineapple and strawberry juices in a ratio: of 3:1. The pH, viscosity, microbial cell number and viability, colour, sensory evaluation and change in total protein content were investigated during the fermentation process. as following:

•**Sample 1:** Pineapple juice + Egg white drink inoculated with *L.casei* 01

•**Sample 2:** Strawberry juice + Egg white drink inoculated with *L.casei* 01

•**Sample 3:** Pineapple juice + Egg white drink inoculated with *L.salivarius* CRL 1328.

•**Sample 4:** Strawberry juice + Egg white drink inoculated with *L.salivarius* CRL 1328.

5.1 FERMENTATION OF EGG WHITE DRINK

5.1.1 Changes in pH Values

After 24 hour fermentation the change of pH values in the fermented egg white drink samples with *L.casei* 01 was presented in Figure 4., where the pH of the fermented egg white drink decreased highly with the fermentation time. In more detail, for the fermented egg white drink with pineapple juice, the pH value decreased from initial pH 5.55 to 3.80 ±0.01 , during 24 h at 37°C, and in the case of strawberry the decrease was from 5.78 to 3.86 ± 0.004 thus, significant changes were observed compared with the initial pH values. During fermentation *L. casei* 01 hydrolyzes the carbohydrates that originally exist in the egg white and juice and produce lactic acid, as another researchers have reported in similar experiments regarding lactic acid fermentations of various fruit juices (Nualkaekul, 2011). It can be said that the decrease in the pH value of the pineapple juice was higher than in the case of strawberry juice at the end of fermentation because of the initial pH of pineapple juice was higher than strawberry juice.

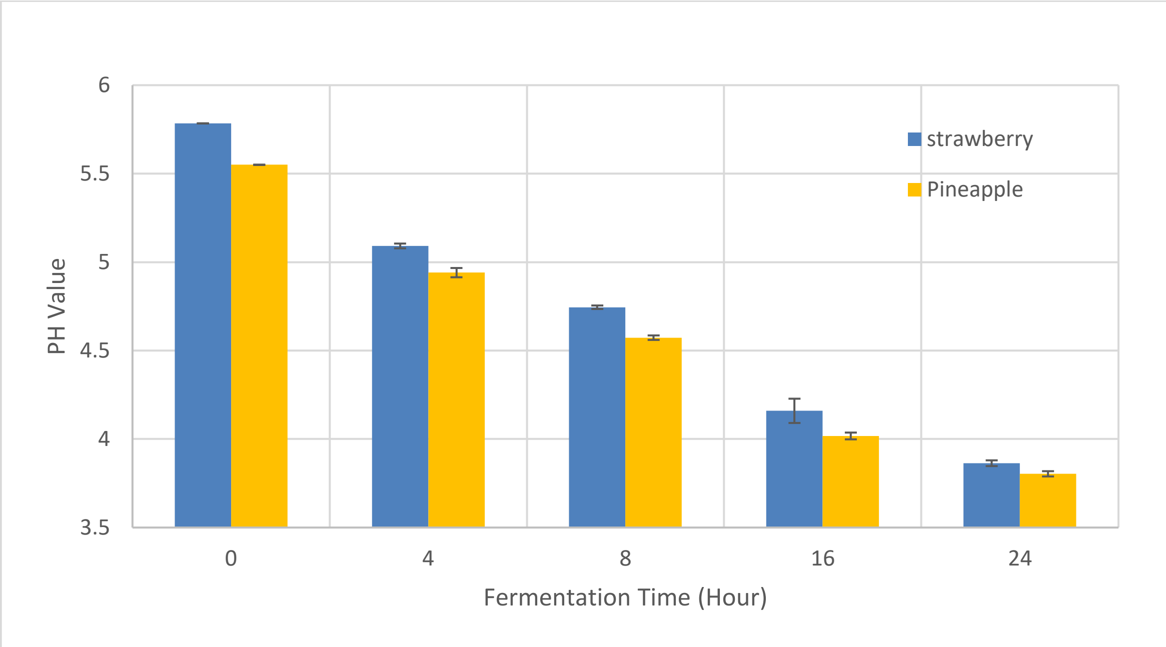

Figure 4. Acidity changing after 24 h of fermentation of two different mixtures (egg white and fruit) with *L.casei* 01

Based on the results of pH values in Figure 5 which present the fermentation of egg white drink with other probiotic strain of *Lactobacillus salivarius* CRL 1328 as starter culture. The pH value of egg white drink fermented with this strain also decreased highly during fermentation, where it showed significant change compared with the initial pH value of pineapple and strawberry juice are 5.55 to 4.04 ± 0.004 and 5.78 to 3.94 ± 0.01, respectively. . The results in Figure 4 and Figure 5 show us that there are few differences between fermentation by the two strains in the pH values at the end of fermentation. The pH value after 24h fermentation with *L.casei* 01 was lower than *L.salivarius* CRL1328. It can be concluded that *L.casei* 01 produces lactic acid with a higher concentration than *L.salivarius* CRL1328 in this condition of fermentation.

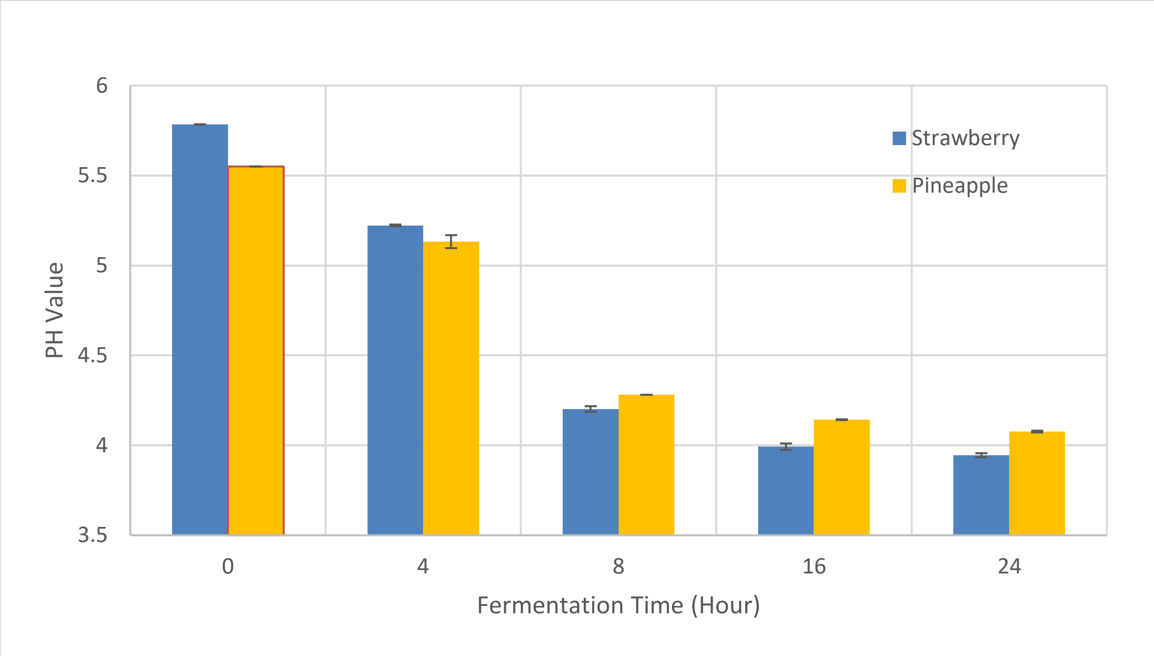

Figure 5. Acidity changing after 24 h of fermentation of two different mixtures with *L.salivarius* CRL 1328

5.1.2 Change of viable cell number

The fermentation time lasted until 24 hours, where the change in cell number and pH changes were measured during the fermentation. Concerning the viable cell number, it was observed (Figure 6), the viable cell count has shown significant growth increasing during the fermentation time for *L.casei* 01, as the initial cell count was approximately 7.38 Log10 CFU/mL and increased to 8.97± 0.13 Log10 CFU/mL at 24 h for pineapple supplemented egg white sample, while the strawberry sample recorded a similar increase in growth 8.83± 0.19 Log10 CFU/mL compared to the pineapple sample. The viable cell count of microorganisms is one of the most crucial criteria for probiotic products. According to the common consensus (Terpou et al., 2019; Mantzourani et al., 2019), probiotic products should contain a significant number of probiotic bacteria above the limit of 6 Log10 CFU/mL, for health benefits for consumers. Based on the results in Figure 7 all values of the viable cell at 24 h were within range as a probiotic product. The significant growth of the bacterial strain can be attributed to the fermentable sugar content of the fruit juices, which affects the *Lactobacillus* cell viability (Hossain et al., 2020).

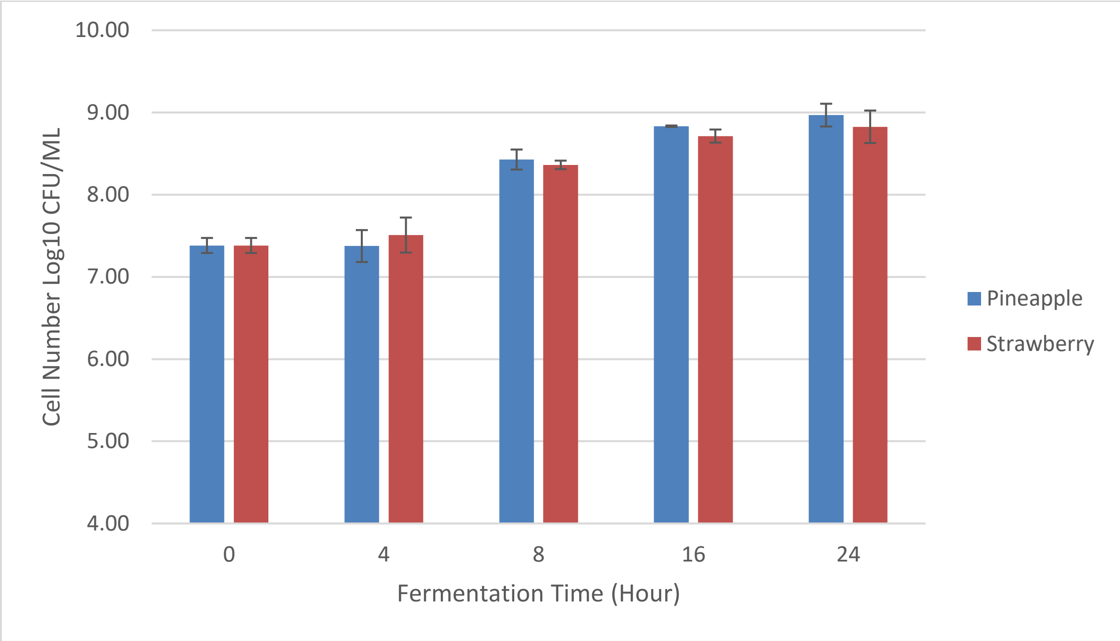

Figure 6. Viable Cell Number after 24 h of fermentation of two different mixtures with *L.casei* 01.

The results in Figure 7 the change of viable cell number during 24 h fermentation of egg white drink by *L.salivarius* CRL1328 are shown increasing in viable cell count from initial 5.43 to 8.10±0.32 Log10 CFU/mL after 24 h in the pineapple juice sample. Similar results, 8.18±0.22 Log10 CFU/mL were obtained in case of egg white with strawberry juice under the same conditions. The results in Figure 6 and Figure 7 have shown no significant difference between the activity of cell growth in the two strains during fermentation process. It was observed that the cell growth efficiency of *L.casei* 01 was 0.87 Log10 CFU/mL higher than from *L.salivarius* CRL1328 in case of fermented egg white with pineapple juice sample and 0.65 log10 CFU/ML with egg white drink with strawberry juice for the same strain.

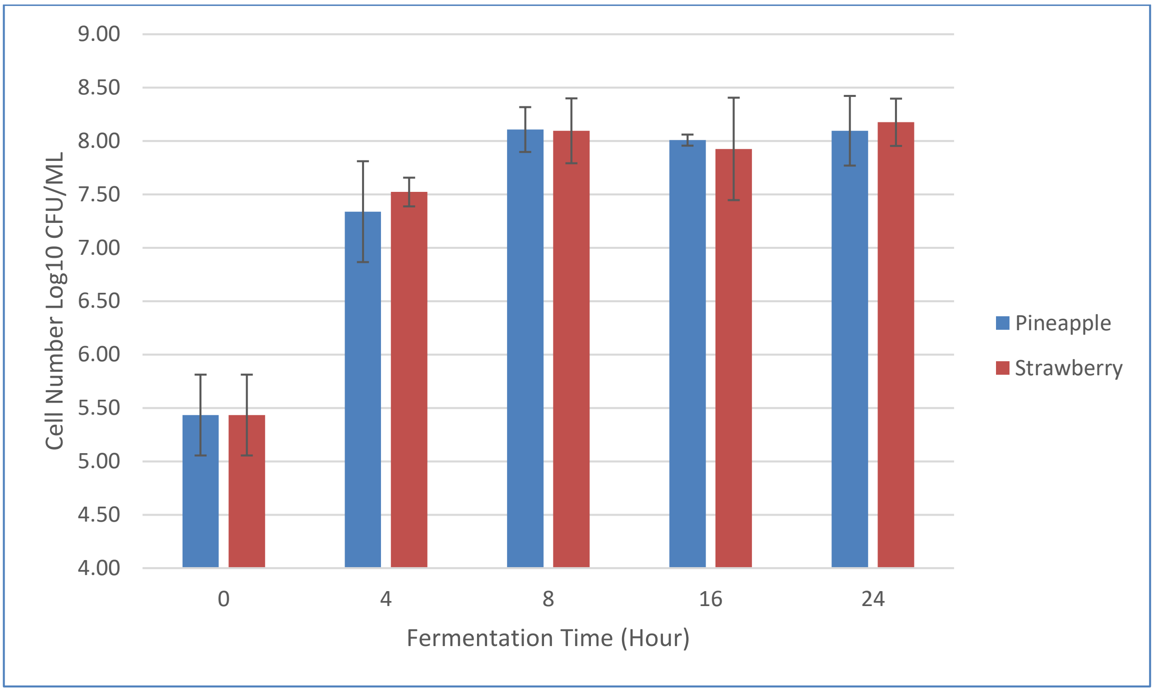

Figure 7. Viable cell number after 24 h of fermentation of two different mixtures with *L.salivarius* CRL1328.

5.1.3 Colour Evaluation

Figure (8) displays the L* values of the samples after 24 h of fermentation. Where L * value indicates the lightness ranging from blackness (0) to whiteness (100). According to the results in Figure 8, it was observed that there were significant differences between the samples of fermented egg white drink with pineapple juice and strawberry juice for both strains. Fermented egg white with strawberry juice samples were recorded in *L.casei* 01 and *L.salivarius* CRL1328 strains as 82.37±0.14 and 82.63±0.16, respectively. While the pineapple samples recorded higher values of L* for *L.casei* 01 and *L.salivarius* CRL1328 as 88.22±0.17 and 88.40±0.18, respectively. Considering for these results, it can be said that the strawberry juice samples were darker than the pineapple juice samples in both strains, due to the anthocyanin pigment that is found naturally in strawberries and is considered one of the water-soluble pigments and is responsible for the red color of strawberries as in the study of ABERS and co-workers (1979). While no significant differences were recorded in the lightness (L*values) between the two strains with the same type of juice.

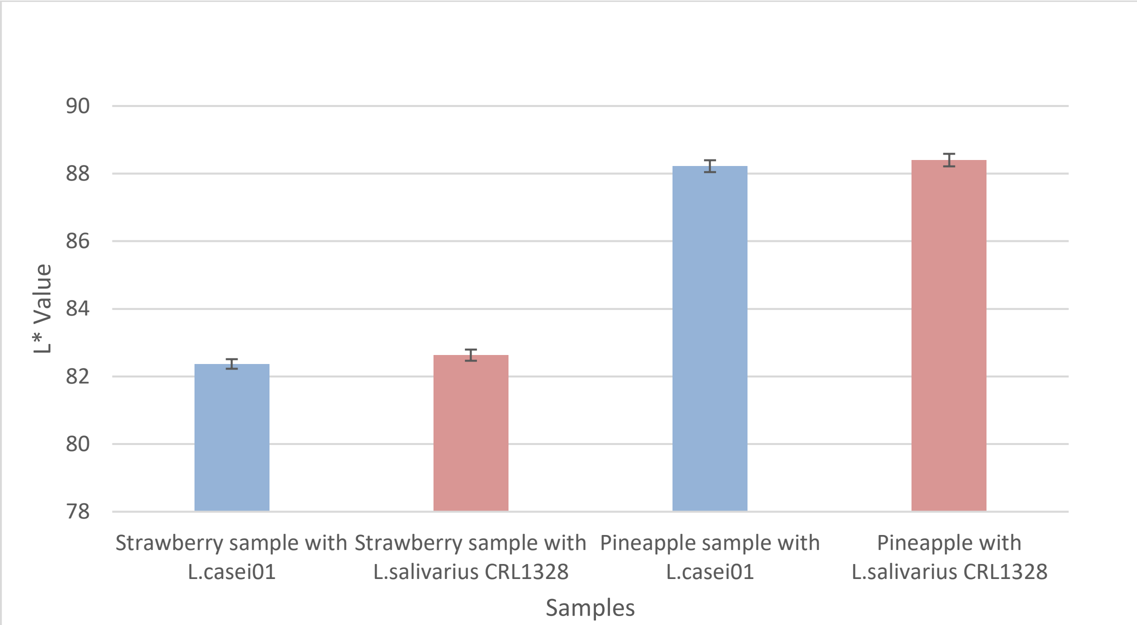

Figure 8. Color measurement L* for the fermented egg white drink with fruit juices samples after 24 h of fermentation .

Figure 9. represents a* values of fermented egg white drink after 24 h of fermentation, which ranges from (-60) greens to redness (+60), and it is evident that the egg white with strawberry juice has a higher value from the pineapple juice sample in both strains. These values are3.67± 0.15 for *L.casei* 01 and 3.34± 0.26 for *L.salivarius* CRL 1328 and these values indicate to the redness with slight differences between the values of the two strains in the fermented egg white and strawberry juice sample. As previously mentioned, due to the anthocyanin pigment in strawberry juice. In the case of the fermented egg white and pineapple juice samples, the values tended to be slightly green color with -2.48±0.09 in *L.casei* 01 and -2.17±0.11 in *L.salivarius* CRL1328 and this relates to the presence of trace amounts of sulfur of iron and sulfur molecules that exist naturally in the egg white compounds as the study mentioned (Tan, et al., 2022). The results have shown there are no significant differences between the two strains on color change.

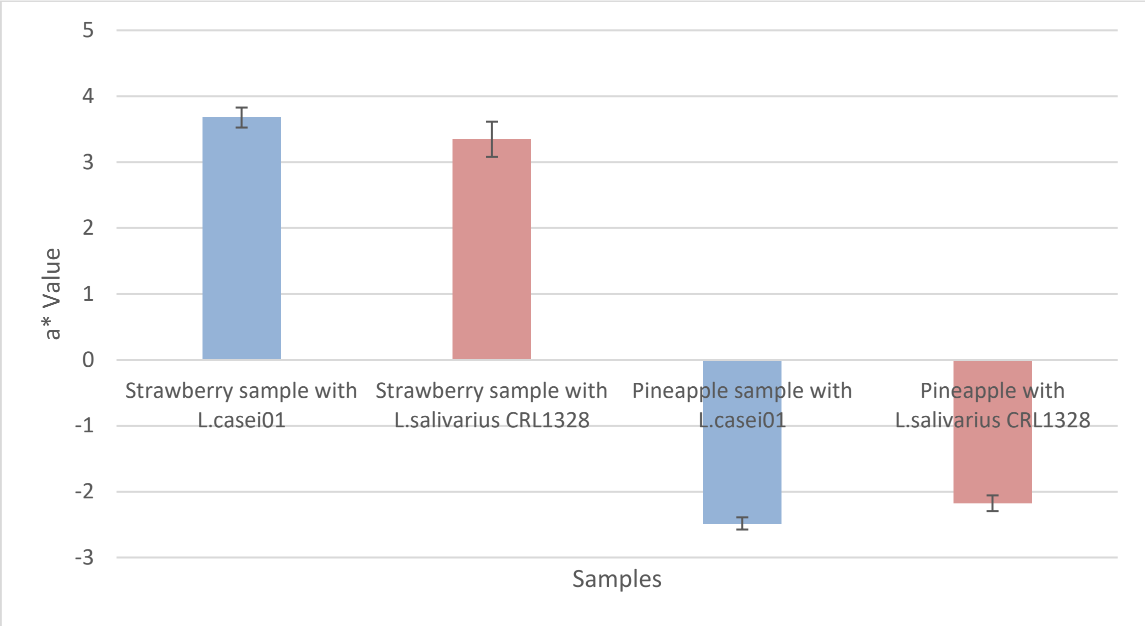

Figure 9. Color measurement a* for the fermented egg white drink with fruit juices samples after 24 h of fermentation .

The b* values of the samples were observed in Figure 10, where the range of b* value from blueness (-60) to yellowness (+60). All values in this figure appear in a yellowish color, as the values of the strawberry juice model ranged between 7.68±0.12 with *L.casei* 01 and 7.04±0.11 with a slight difference in value between the two strains. As for the b* values of the fermented egg white with pineapple juice model, it was lower than that of the strawberry juice, as it ranged between 5.67±0.17 in *L.casei* 01 and 5.37±0.10. These results indicate a large variation between samples in yellow color of the fermented egg white with the different juices. The yellow color can be attributed to the pineapple juice sample for the carotenoids, a group of natural pigments that give many fruits and vegetables their yellow, orange, and red colors. Beta-carotene is the most common carotenoid in pineapple juice, and it is responsible for its yellow color (Barretto et al., 2013). The red color of strawberries comes from a type of flavonoid called anthocyanins, while the yellow color comes from another type of flavonoid called flavonols (Jeong, et al., 2012).

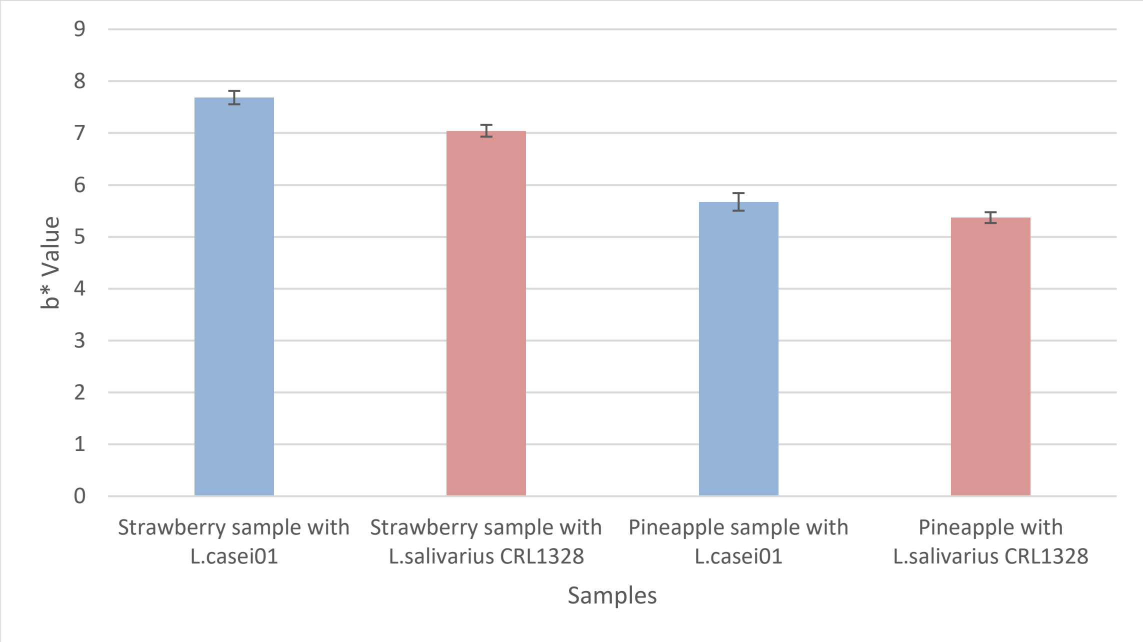

Figure 10. Color measurement b* for the fermented egg white drink with fruit juices samples after 24 h of fermentation .

5.1.4 Viscosity Measurement

The shear thinning properties of the fermented egg white drink were investigated.. The flow curves were analyzed by the Herschel—Bulkley model, and the samples were studied at temperature 20 C. From Figure 11, based on the curves between shear rates and shear stress, It can be concluded that they reflect a linear relationship between them i.e. the shear stress increases with the increasing of the shear rate and the opposite will be true. Table 8 below shows the differences between the measured samples, which represent the values of consistency coefficient K, flow index n and the yield stress Tau0.

Table 8. Values used during the rheological testing of fermented egg white samples with different juices

Sample	Yield Stress Tau0 (Pa)	Consistency coefficient K (Pas^n)	Flow index (n)
Strawberry - *L.casei* 01	0.093574	0.004742	1.033548
Strawberry – *L.salivarius* CRL1328	0.080072	0.004106	1.051019
Pineapple - *L.casei* 01	0.110600	0.007107	0.971234
Pineapple – *L.salivarius* CRL1328	0.168127	0.008251	0.953077

Where the flow behavior index shows for the analyzed samples we can observe from Figure (11) the newtonian behavior of fermented egg white drink samples at temperature 20°C was determined between the shear rate 1 to 500 (1/s) and shear stress (Pa). It can be figured out from our samples that the newtonian behavior implies that the slope of the linear relationship between shear rate and shear stress is equal to the fluid's viscosity (Jankowska, et al., 2023). Based on the flow curves in Figure 11, it can be said that after 24 hours of fermentation, the shear stress values of the pineapple juice sample with *L.salivarius* CRL1328 was 3.25 (Pa) when the shear rate is at 500 1/s. These values are higher than other samples but there are no significant differences between the tested samples. It was also observed that the share stress values were very similar between egg white with strawberry and pineapple juice samples fermented with the same strain *L.casei* 01, 0.14 and 0.17 (Pa) with shear rate 500 1/s respectively. While noticed the pineapple juices sample with both strains *L. casei* 01 and *L. salivarius* CRL1328 recorded the highest share stress values from the other samples 3.03 , 3.25 (Pa) with shear rate 500 1/s respectively.

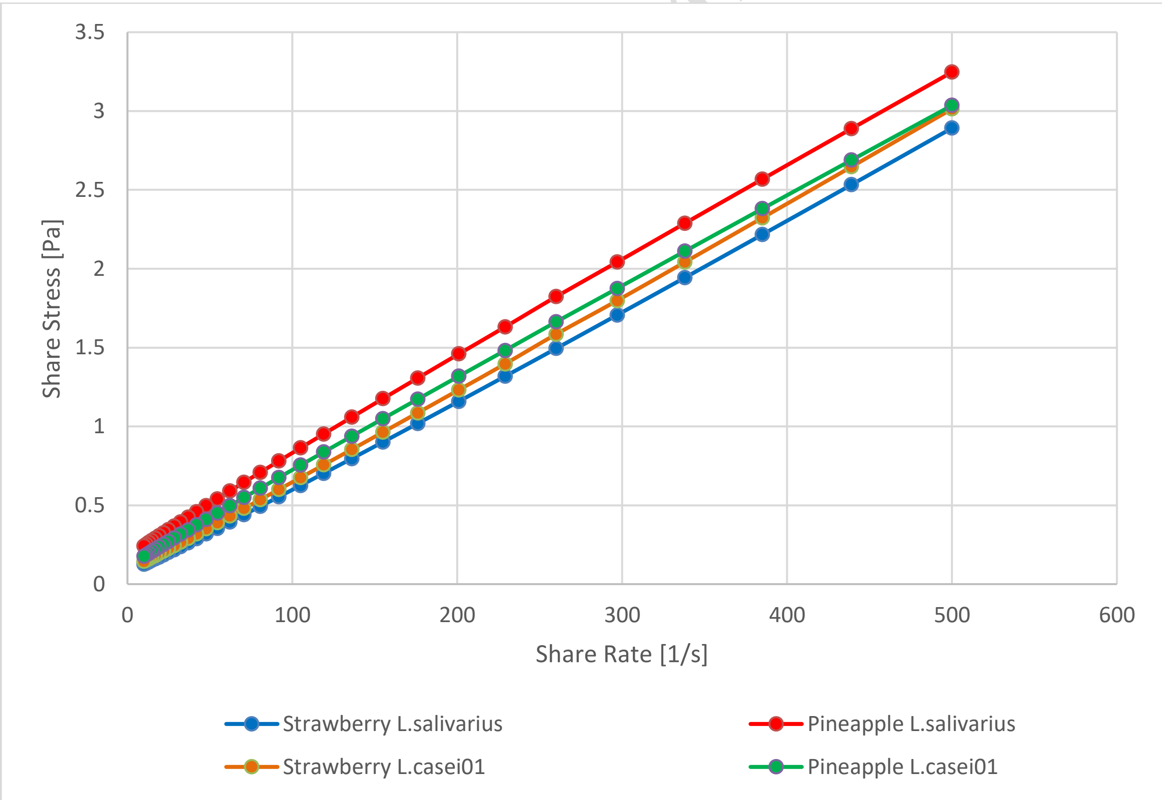

Figure 11. Flow curve of fermented egg white drink samples after 24 hours

5.1.5 Change in Protein Composition

The protein composition of fermented egg white and fruit juice samples were analyzed by SDS PAGE. According to Figure 12 and 13, a reduction in some protein intensity was observed after 16 hours of fermentation, since the probiotic bacteria had a proteolytic system which decompose the proteins into peptides that requited for its growth. Ovalbumin bands was higher intensity in fresh egg white drink samples compared to the other, since fermented samples by *L.casei* 01 with strawberry juice were considerably higher than fermented samples with other juice fruits. Ovoflavoprotein at 12.4-13.1 kDa and ovomucoid at 35.8-36.1 kDa and ovotransferrine at 89.8-82.5 kDa recorded a higher intensity bands in fermented samples by *L. salivarius* CRL 1328 with strawberry flavour rather than pineapple, further more adding pineapple juice with *L.casei* 01 resulted in a lower ovoflavoprotein than when strawberry juice were added. Although lyzozyme and lysine was not considerably different in fermented samples, they were higher in fresh egg white drink as a results of adding the juices in 3:1 ratio egg white drink:fruit juices.

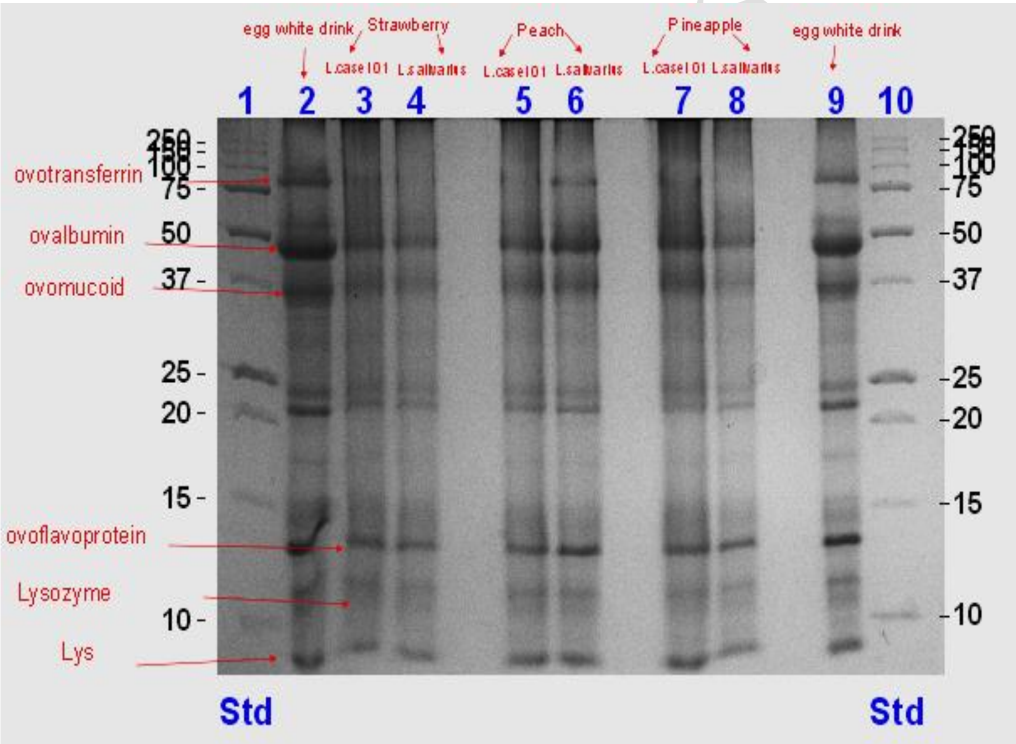

Figure 12. The protein profile of fermented egg white drink with different fruit juices

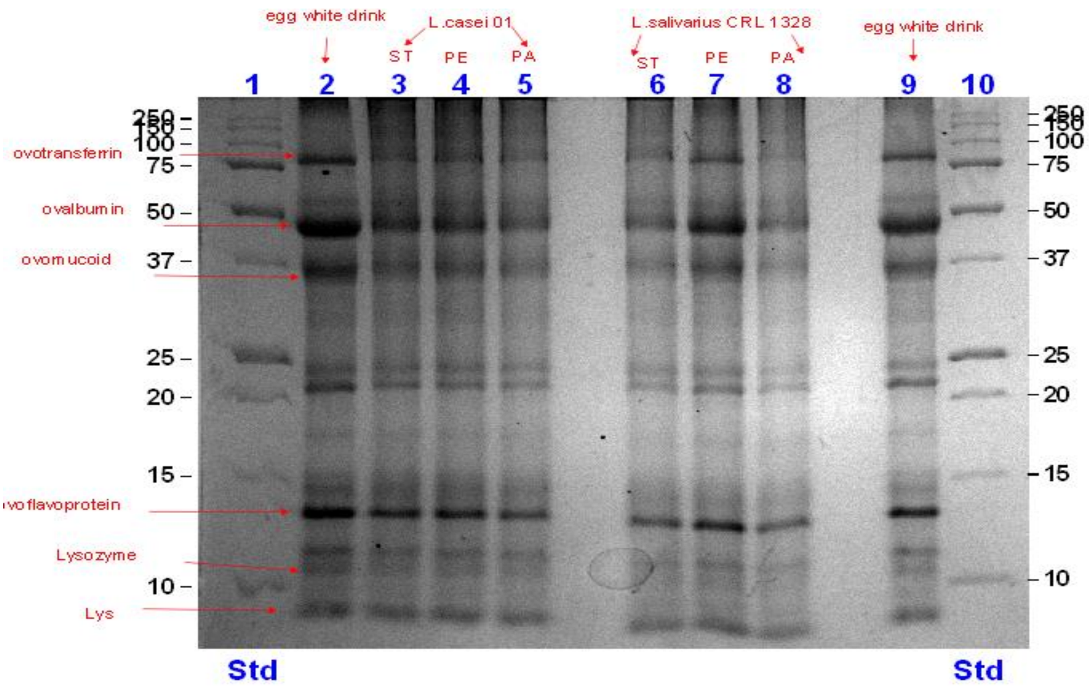

Figure 13. The protein profile of fermented egg white drink with different fruit juices (ST=strawberry juice sample , PA= Pineapple juice sample)

5.1.6 Sensory Evaluation

Four samples of fermented egg white drinks with pineapple and strawberry juices separetely using *L.casei* 01 or *L.salivarius* CRL 1328 samples were tested, and evaluated by ProfiSens. The number of attributes was 17 (color hue, viscosity, prickliness, mouthfeel, medicinal flavor, acetic taste, egg flavor, sour taste, bitter taste, sweet test fruity flavor, overall flavor intensity, pungent odor, acidic odor, sweet odor, fruity odor, and overall odor intensity), as it is shown in Figure 14. Fermented samples by *L.casei* 01 with pineapple juice were not significantly different in color hue from samples of *L.salivarius* CRL 1328 with the same juice on the other hand, it has considerably the lowest fruity odor, lowest sweet taste, and the highest sour taste which could be attributed to the high lactic acid production after the fermentation. Egg white with pineapple juice fermented by *L.salivarius* CRL 1328 had a higher fruity odor, and lower acetic taste compared to fermented egg white drink with pineapple by *L.casei* 01 without any significant difference with other samples. The egg white drink with strawberry fermented by *L.salivarius* CRL 1328 recorded the highest value of fruity odor while the parameters of fruity flavor, sweet odor, and sour taste were not considerably different in samples with fermented egg white with strawberry juice by *L. casei* 01 sample, and fermented egg white with pineapple juice by *L. salivarius* CRL1328 sample. Egg flavor and medicinal flavor recorded a low level in all studied samples, however, it was slightly higher in fermented samples by *L.casei* 01 with strawberry juice. Moreover, bitter taste had a very low score in all studied samples. The viscosity score of all the studied samples was moderate which varied from 38-46% since samples with strawberry juices record its highest. fermented egg white drink with strawberry juice by *L. casei* 01 sample had a slightly high score of mouth feel as well as a pungent flavor. To sum up, most of the assessors sorted the fermented egg white by *L.casei* 01 with pineapple as sour, fermented egg white drink with strawberry juice by *L. casei* 01 sample egg taste was pretty obvious, however, its texture was the most viscous. Additionally, fermented samples with *L.salivarius* CRL1328 and strawberry juice recorded the highest overall since the fruity smell and odor were favorable.

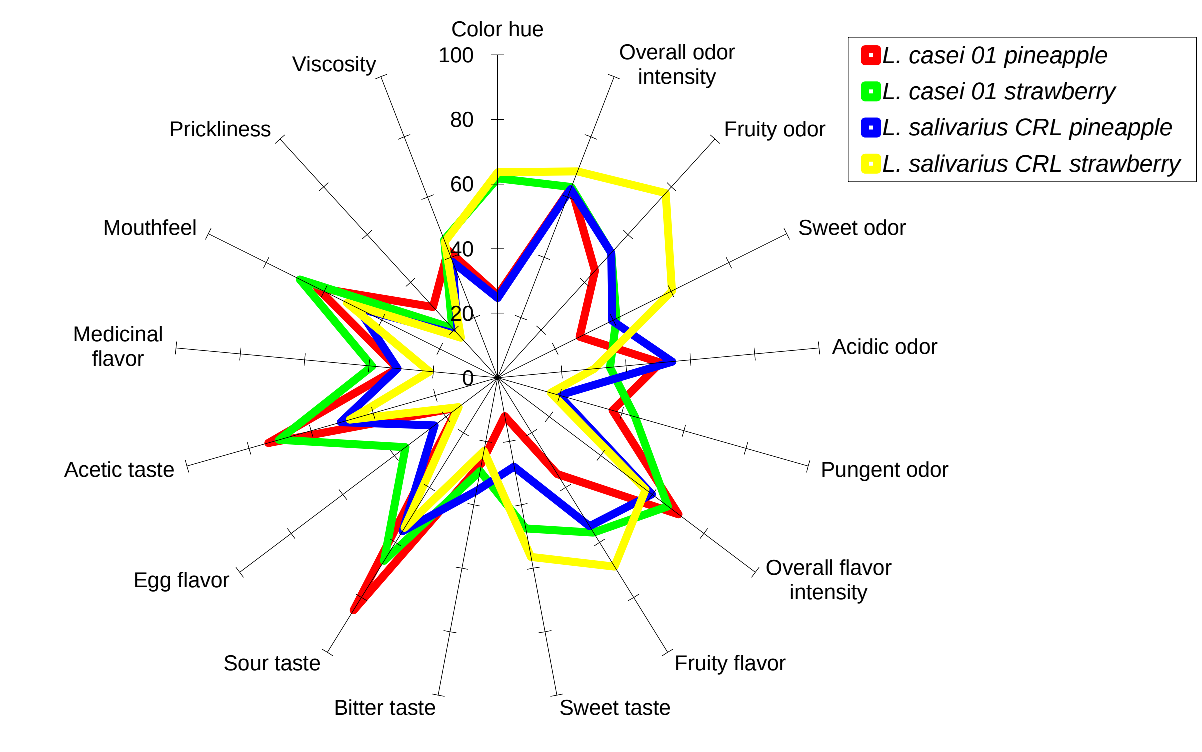

Figure 14. Quantitative sensory characteristics of probiotic fermented egg white drink samples with different fruit juice

5.2 STORAGE STABILITY

With the aim to determine the shelf-life and to develop a prediction model to estimate the shelf-life of the probiotic fermented drink, the four fermented egg white drink samples were used for storage study. After fermentation with adapted culture of *L.casei* 01 and *L.salivarius* CRL1328 at 37 °C for 16 hours, the products were stored in the refrigerator (4 °C) and were evaluated by 2 weeks, 4 weeks, and 6 weeks of fermentation.

5.2.1 Changes in pH Values

During the storage time at temperatures (4 °C), pH value of all fermented egg white drink samples showed a significant decline from 16 hours till 6 weeks (Figure 15). These values were from pH 4.40±0.006 for egg white and pineapple juice samples with *L.casei* 01 and pH 4.09±0.007 for strawberry juice samples with *L .salivarius* CRL1328 decreased to lower values 3.88±0.03 and 3.85±0.006 for pineapple juice sample with *L.casei* 01 and strawberry juice sample with *L. salivarius* CRL1328 after 6 weeks of storage. It can be seen that there are significant differences in the decrease in the acidity of samples between 16 hours of storage to 2 weeks from pH 4.40±0.006, pH 4.50±0.009 to pH 4.00±0.006, 3.98±0.007 for pineapple juice sample and strawberry juice sample with the same strain *L.casei* 01, respectively. This could be due to the high efficiency of *L.casei* 01 in producing lactic acid through the fermentation of fermentable sugar in the fruit juices sample with egg white drink (Nualkaekul, 2011). No significant change was recorded in the pH values between the strains during storage from two weeks to 6 weeks at a temperature of 4 °C, where the differences between the pH values did not exceed 0.09±0.02. It can be noticed from Figure 15 that all the pH values of *L.salivarius* CRL1328, during the storage process from 2 to 6 weeks, were always lower than *L.casei*01 values. In a different way, it can be said that both strains have recorded similar activity in producing lactic acid during the storage period. Dropping pH during storage is primarily caused by acid accumulation, as shown by the rising trend in lactic acid concentration. Since most foodborne pathogens cannot survive at pH values below 4.5, a pH value of 4.0 is sufficient to ensure the product's safety (Yaneva et al., 2021).

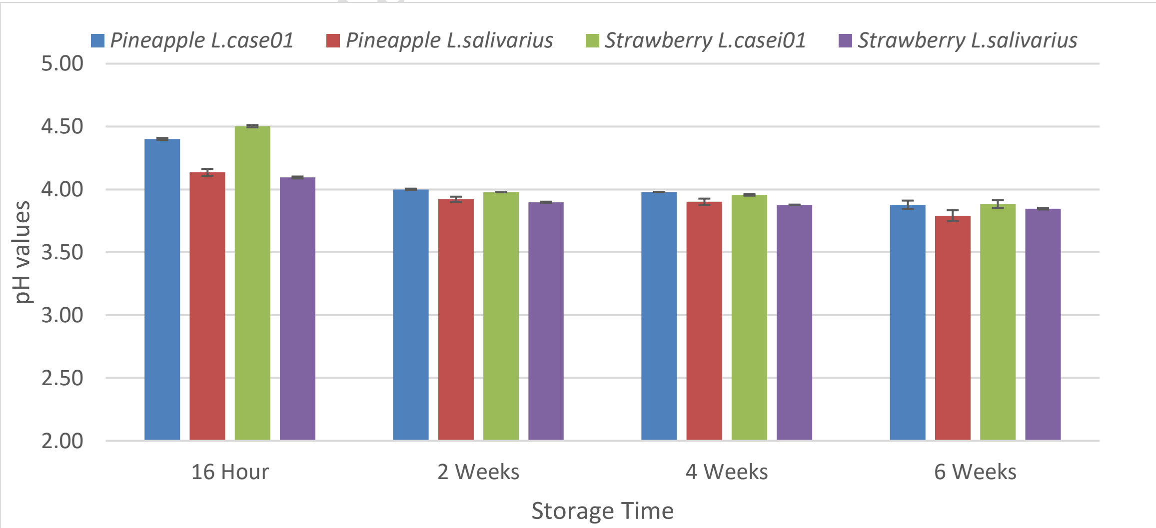

Figure 15. Acidity changing during storage of fermented egg white drink with fruit juices by lactic acid bactira for 6 weeks

5.2.2 Change of viable cell number

Changes in viable cell number in the fermented egg white drink with fruit juices during storage at 6 weeks under cooled conditions 4 C are presented in Figure 16. The population of bacteria in fermented mixtures showed different trends depending on the variety between studied strains. A slight decrease tendency can be observed from 16 hour till 6 weeks in fermented egg white with pineapple and strawberry juices with *L. casei* 01 strain 8.85±0.12, 8.60±0.07 to 8.65±0.26, 8.33±0.25 Log10 CFU/mL , respectively. While it noticed a highly significant decrease with *L. salivarius CRL1328* during storage in 4th week. Where the egg white drink with strawberry juice sample had a higher decrease than the pineapple juice sample of the same strain from 8.31±0.10 to 4.62±0.10 Log10 CFU/mL at 16 hours for pineapple juice sample when the pH value was 3.90 and it was 8.21±0.11 to 3 Log10 CFU/mL at the 4th week for strawberry juice sample when the pH value was 3.88. According for these results, it can be said that *L. salivarius* CRL1328 cannot tolerate the low acidity value or are very sensitive to acidity, while studies indicated that *Lactobacillus casei* 01 can survive in acidic environments with a pH as low as 3.0, and some strains have been shown to tolerate pH levels even lower than 3.0 (Zhang, et al.,2012). Due to the use of sugar and nutrients in the juice, the number of *L.casei* 01 increased during fermentation process. Since the nutrients such as sugar, vitamin, and amino acid in the product have not been entirely depleted after fermentation, bacteria can use them for growth during storage as the study shows (Zandi et al., 2016). From other side, for health benefits of probiotics to be evident, the minimum concentration of live probiotic bacteria at the end of the shelf life should be approximately 7 Log10 CFU/mL, according to the scientific literature (Nualkaekul & Charalampopoulos, 2011; Nualkaekul et al, 2011). During the storage period of 6 weeks, the numbers of *L.casei* 01 were in the range of 8.33 Log10 CFU/mL, therefore, it could be considered that the microbial population in the fermented drinks with fruit juices after storage in a refrigerated case could fulfill the standard of probiotic products with *L.casei* 01 about viable cells. And it is not possible with *L.salivarius* CRL1328 in such as these conditions.

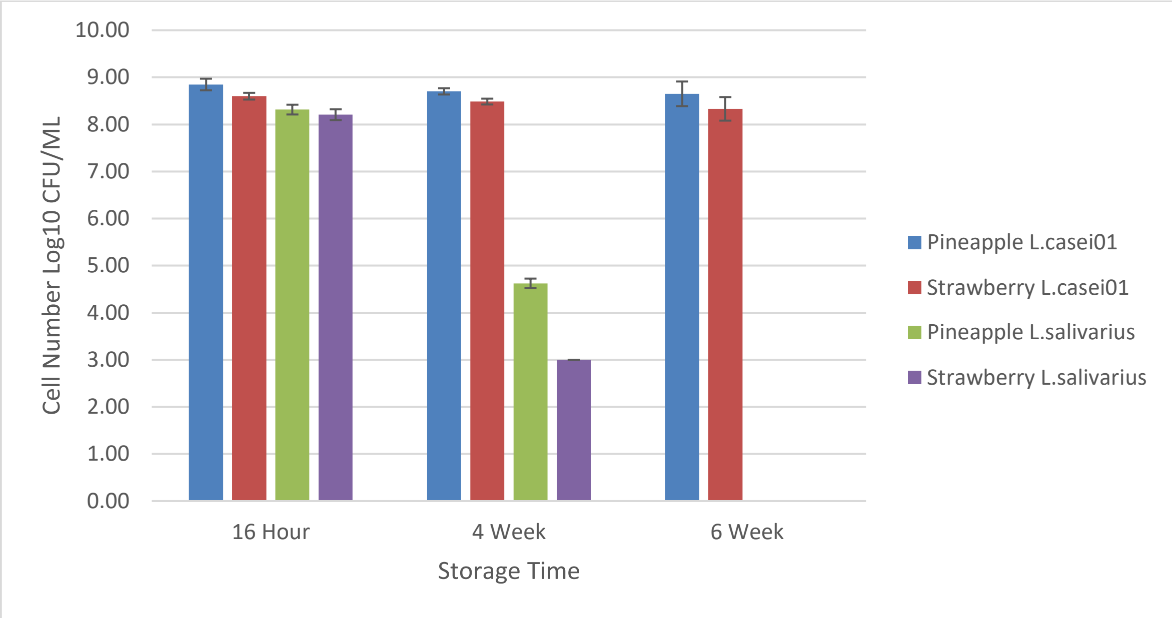

Figure 16. Viable Cell Number changing during storage of fermented egg white drink with fruit juices for 6 weeks.

6. CONCLUSIONS

Nutrition has become a significant focus in today's society, as by consuming foods with beneficial internal characteristics, we can effectively contribute to improving our health, well-being, and quality of life. The change in eating habits is also facilitated by the rapid spread of civilizational diseases, as the number of persons suffering from diabetes and various food allergies is increasing. All of these facts pose new challenges to the food industry, as consumer demand for the development and production of functional foods that, in addition to their excellent enjoyment value, have a beneficial effect on health, is increasing, and can also be consumed by people with intolerances or allergies.

The main product in my study is the ToTu drink made from egg whites which are processed by proteases enzymes. The advantages of it, that include minimal carbohydrate and salt content, highly concentrated protein content, free from fat, cholesterol and additives (stock enhancers, preservatives), and can also be consumed by people suffering from lactose intolerance and milk protein allergy. In this study, the added value of the product was increased by fermentation using two strains of the probiotic bacteria, *Lactobacillus casei* 01 and *Lactobacillus salivarius* CRL1328, which have proven beneficial properties for the human body. Moreover, two types of fruit juices (pineapple and strawberry) were used to supplement the egg white and then various characteristics, pH, number of living cells, protein content, color, viscosity, storage stability and the sensory properties of our products were examined.

The purpose of comparing the pH values is to determine to what extent the applied probiotic shows a decrease in pH vlaues based on various environmental factors. The present research indicates that a probiotic culture of *Lactobacillus casei* 01 and *Lactobacillus salivarius* CRL1328 is an appropriate starter for the development of a fermented egg white drink. Moreover, egg white and strawberry or pineapple juice are good media for the growth of probiotic bacteria without any nutrient supplement. Adding the fruit juices to the egg white drink was an effective step to increase the maximum cell number after the fermentation. The six weeks storage study shows that the starter culture *Lactobacillus casei* 01 was well tolerant to fruit juices and preserved the cell viable number in the fermented egg white to 8 Log10 CFU/mL, and decreased pH values to 3.8 which is attributed to the product's safety, and that the acids produced function as natural preservatives in the later stages, which also increases the shelf life of the product. It also showed good rheological properties for all samples represented by Newtonian behavior. Regarding to colour, the higher value of lightness L* was in the egg white with pineapple juice sample fermented by *L.salivarius*

CRL1328. While the sensory evaluation of the four fermented egg white drinks with fruit juices showed that the egg white drink with strawberry juice fermented by *Lactobacillus salivarius* CRL1328 was more preferred by the panelists. This study provided promising results for the development of new non-dairy probiotic food products. Some directions for further research can be suggested as follows:

► Investigation of the ability to ferment the egg white drink using other probiotic starter cultures or mixed cultures and investigation for its viability.

► Supplementary studies on the influence of the fermentation process on protein degradation and using other fruit juices with the starter culture

► Investigating the possibility of improving the cell viability of *L. salivarius* CRL1328 to extend the shelf life of the fermented egg white

7. SUMMARY

Modern functional food companies are focused on the development of products that offer multiple health benefits through the combination of different functional ingredients. Following this trend, the present study aimed to develop the nutritional and sensory properties of the egg white drink (ToTu) and make it into a fermented probiotic product, and discover the properties of the probiotic-enriched ToTu drink. During the research, the fermentability of ToTu drink using two strains of probiotic bacteria, *L.casei* 01 and *L.salivarius* CRL1328 was investigated and the effects of adding fruit juices (pineapple and strawberry juices) 25% fruit content in terms of bacterial growth. Where the following parameters were measured for the fermented ToTu drink with probiotic bacteria and fruit juices during and after fermentation and during six weeks of refrigerated storage at (4±1°C) by investigating the pH values using a digital pH meter (Mettler-Toledo GmbH, Switzerland), viscosity measurement by MCR 92 Anton Paar Rheometer, microbial cell number and viability which was measured by total plate count (TPC) method, color measurement with Konica-Minolta CR-400 chromameter, sensory evaluation and changes in total protein content during the fermentation process by gel electrophoresis technique. Four samples were prepared by mixing them separately with egg white drink. and pineapple or strawberry juice at the ratio of 3:1 (v/v) respectively. The fermentation was inoculated individually by 1% of *L.casei* 01 and *L.salivarius* CRL1328 .and incubated at 37 °C under aerobic conditions for 16-24 hours. The results showed that there was a significant decrease in the pH value of all the tested samples during 24 hours of fermentation from initial pH 5.55 to 3.80 ±0.01, and this is a good indicator to protect the product from microbial growth by increasing the shelf life of the product. At the same time, egg white drink with pineapple juice inoculated by *L.casei* 01 recorded the highest viable cell number growth during fermentation from 7.38 to 8.97± 0.13 Log10 CFU/mL. For the rheology test during viscosity measurement, results show the Newtonian behavior for all fermented egg white samples at a temperature of 20°C was determined between the shear rate 10 to 500 (1/s). The color measurement is important for the quality because it will affect the color of the final product. In the case of L* the highest L* values and the lightest are the pineapple with *L.salivarius* CRL 1328 followed by the pineapple juice sample with *L.casei 01*, respectively. The results also showed that there were no significant differences in the colors of the fermented samples by *L.casei* 01 or *L.salivarius* CRL1328, as a reduction in some protein intensity was observed after 16 hours of fermentation, since the probiotic bacteria had a proteolytic system which decompose the proteins into peptides that required for its growth. The egg white and

strawberry juice sample fermented by *L.casei* 01 recorded the lowest rate of protein decomposition for ovalbumin than others. Egg white and strawberry juice fermented by *L. salivarius* CRL 1328 recorded the highest value of fruity odor, fruity flavor, overall odor intensity, sweet taste and color hue, which makes it the best sample in sensory evaluation from the others. During the storage stability test, *L.casei* 01 significantly outperformed *L.salivarius* CRL1328 in terms of viable cell number for six weeks. The growth of *L.salivarius* CRL1328 decreased significantly in the fourth week during storage at 4°C, while no significant change was recorded in the pH value of all samples.

We observed that the experimental setup significantly affects the end results. The study showed promise and delivered good results in creating new functional non-dairy products, and it can be depended upon to further improve the quality of food and raise its help with sustainability.

REFERENCES

Abdallah, F. B., & Chahine, J.-M. E. H. (1999). Transferrins, the mechanism of iron release by ovotransferrin. In European Journal of Biochemistry (Vol. 263, Issue 3, pp. 912–920). Wiley. https://doi.org/10.1046/j.1432-1327.1999.00596.x

ABERS, J. E., & WROLSTAD, R. E. (1979). CAUSATIVE FACTORS OF COLOR DETERIORATION IN STRAWBERRY PRESERVES DURING PROCESSING AND STORAGE. Journal of Food Science, 44(1), 75–81. doi:10.1111/j.1365-2621.1979.tb10008.x

Alderton, G., W. H. Ward, and H. L. Fevold. 1946. Isolation of lyso- zyme from egg white. J. Biol. Chem. 157:43–58.

Ali, A .(2010). Beneficial Role of Lactic Acid Bacteria in Food Preservation and Human Health: A Review. Research Journal of Microbiology, 5: 1213-1221. URL: https://scialert.net/abstract/?doi=jm.2010.1213.1221

Anukam, K. C. (2007). Probiotics: 100 years (1907-2007) after Elie Metchnikoff's Observation Do African student's fecal microbiome compositions associate with gut health? View project Relative abundance of the micro biome and functional prediction from different body sites of an individual View project. https://www.researchgate.net/publication/228915782

Axelsson L (2004) Lactic acid bacteria: classification and physiology. In: Salminen S, von Wright A, Ouwehand AC (eds) Lactic acid bacteria microbiological and functional aspects, 3rd edn. Marcel Dekker, New York, pp 1–66

Azari, P., & Baugh, R. F. (1967). A simple and rapid procedure for preparation of large quantities of pure ovotransferrin. In Archives of Biochemistry and Biophysics (Vol. 118, Issue 1, pp. 138–144). Elsevier BV. https://doi.org/10.1016/0003-9861(67)90289-5

Baker, H. M., and E. N. Baker. 2004. Lactoferrin and iron: Struc- tural and dynamic aspects of binding and release. Biometals 17:209–216.

Balthazar, C. F., Silva, H. L., Esmerino, E. A., Rocha, R. S., Moraes, J., Carmo, M. A., & Franco, R. M. (2018). The addition of inulin and *Lactobacillus casei* 01 in sheep milk ice cream. Food Chemistry, 246, 464–472.

Barretto, L. C. de O., Moreira, J. de J. da S., Santos, J. A. B. dos, Narendra, N., & Santos, R. A. R. dos. (2013). Characterization and extraction of volatile compounds from pineapple (Ananas comosus L. Merril) processing residues. Food Science and Technology (Campinas), 33(4), 638–645. doi:10.1590/s0101-20612013000400007

Bartholomew, D.P.; Paul, R. E. and Rorbach, K.G. 2003. The pineapple 'Botany, Production and Uses', University of Hawaii Manoa Honolulu, USA. [Available: http://bookshop.cabi.org/Uploads/Books/PDF/978085995038/]

Baruwa, O.I. 2013. Profitability and constraints of pineapple production in Osun State, Negeria. 2013. Journal of Horticultural Research. 21(2):59-64. [DOI: 10.2478/johr-2013-0022].

Basak, S., & Gokhale, J. (2021). Immunity boosting nutraceuticals: Current trends and challenges. In Journal of Food Biochemistry (Vol. 46, Issue 3). Hindawi Limited. https://doi.org/10.1111/jfbc.13902

Basu, A., Rhone, M., & Lyons, T. J. (2010). Berries: emerging impact on cardiovascular health. In Nutrition Reviews (Vol. 68, Issue 3, pp. 168–177). Oxford University Press (OUP). https://doi.org/10.1111/j.1753-4887.2010.00273.x

Battcock, M., Azam-Ali, S. (1998): Fermented fruits and vegetables, a global perspective. FAO Agricultural Services Bulletin, 134, 96.

Bjö, J., Koort, J., Limsowtin, G. K. Y., Broome, M. C., & Powell, I. B. (2011). LACTIC ACID BACTERIA Taxonomy and Biodiversity (Vol. 3).

Børsting, C., Mikkelsen, M., & Morling, N. (2012). Kinship Analysis with Diallelic SNPs Experiences with the SNPforID Multiplex in an ISO17025 Accreditated Laboratory. In Transfusion Medicine and Hemotherapy (Vol. 39, Issue 3, pp. 195–201). S. Karger AG. https://doi.org/10.1159/000338957

Capriovus LTD . (2023). , Totu egg white drink product Specification https://capriovus.eu/en/totu-drink-egg-white-product/

Chiang, W.-D., Lee, M.-J., Guo, W.-S., & Tsai, T.-C. (2020). Protein hydrolysate batch production with angiotensin I-converting enzyme inhibitory activity form egg whites. In Journal of Food and Drug Analysis (Vol. 14, Issue 4). The Journal of Food and Drug Analysis (JFDA), Food and Drug Administration, Taiwan (TFDA). https://doi.org/10.38212/2224-6614.2453

Claesson, M. J., Li, Y., Leahy, S., Canchaya, C., van Pijkeren, J. P., Cerdeño-Tárraga, A. M., Parkhill, J., Flynn, S., O'Sullivan, G. C., Collins, J. K., Higgins, D., Shanahan, F., Fitzgerald, G. F., van Sinderen, D., & O'Toole, P. W. (2006). Multireplicon genome architecture of Lactobacillus salivarius. In Proceedings of the National Academy of Sciences (Vol. 103, Issue 17, pp. 6718–6723). Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.0511060103

Corbo, M.R.; Bevilacqua, A.; Petruzzi, L.; Casanova, F.P.; Sinigaglia, M. (2014). Functional beverages: The emerging side of functional foods; Commercial trends, research and health implications. Compr. Rev. Food Sci. Food Saf , 13, 1192–1206.

Corr, S. C., Li, Y., Riedel, C. U., O'Toole, P. W., Hill, C., & Gahan, C. G. M. (2007). Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. In Proceedings of the National Academy of Sciences (Vol. 104, Issue 18, pp. 7617–7621). Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.0700440104

Czétényi R. (2014). Laktózérzékenység: A tejmentes étrend nem megoldás. https://semmelweis.hu/hirek/2014/12/16/laktozerzekenyseg-a-tejmentes-etrend- nem-megoldas/

Daliri, E.B.-M., Lee, B.H. (2015). New perspectives on probiotics in health and disease. Food Sci. Human Wellness 4, 56–65. https://doi.org/10.1016/j.fshw.2015.06.002

De man, rogosa and sharpe (MRS) agar. (2003). In Handbook of Culture Media for Food Microbiology (pp. 511–513). Elsevier. https://doi.org/10.1016/s0079-6352(03)80066-8

Debnath, P.; Dey. P.; Chanda, A. and a Bhakta, T. 2012. A Survey on Pineapple and its medicinal value. Scholars Academic J. Pharm.1.

Deidda, F., Amoruso, A., Nicola, S., Graziano, T., Pane, M., & Mogna, L. (2018). New Approach in Acne Therapy. In Journal of Clinical Gastroenterology (Vol. 52, Issue Supplement 1, pp. S78–S81). Ovid Technologies (Wolters Kluwer Health). https://doi.org/10.1097/mcg.0000000000001053

Dimitrellou, D., Solomakou, N., Kokkinomagoulos, E., & Kandylis, P. (2020). Yogurts Supplemented with Juices from Grapes and Berries. In Foods (Vol. 9, Issue 9, p. 1158). MDPI AG. https://doi.org/10.3390/foods9091158

Donati, M., Cremonini, E., Di Francesco, A., Dallolio, L., Biondi, R., Muthusamy, R., & Leoni, E. (2015). Prevalence of Simkania negevensis in chlorinated water from spa swimming pools and domestic supplies. In Journal of Applied Microbiology (Vol. 118, Issue 4, pp. 1076–1082). Oxford University Press (OUP). https://doi.org/10.1111/jam.12761

Dull, G. G. 1971.The pineapple: general. In: A. C. Hulme (Ed.). The biochemistry of fruits and their products, Academic Press, New York. vol. 2: p. 303-324.

Fang, F., Li, Y., Bumann, M., Raftis, E. J., Casey, P. G., Cooney, J. C., Walsh, M. A., & O'Toole, P. W. (2009). Allelic Variation of Bile Salt Hydrolase Genes in Lactobacillus salivarius Does Not Determine Bile Resistance Levels. In Journal of Bacteriology (Vol. 191, Issue 18, pp. 5743–5757). American Society for Microbiology. https://doi.org/10.1128/jb.00506-09

Felis, G. E., & Dellaglio, F. (2007). Taxonomy of Lactobacilli and Bifidobacteria. Current issues in intestinal microbiology, 8(2), 44–61.

Food and Agriculture Organization of the United Nations & World Health Organization. (2001). Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.

Francini, A.; Sebastiani, L. (2013). Phenolic compounds in apple (Malus × domestica Borkh.): Compounds characterization and stability during postharvest and after processing. Antioxidants, 2, 181–193.

Fujita, H. (2000). Yokoyama K and Yoshikawa M, Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. J Agric Food Chem, 65, 564-569.

Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S., Bompadre, S., Quiles, J. L., Mezzetti, B., & Battino, M. (2015). Strawberry as a health promoter: an evidence based review. In Food & Function (Vol. 6, Issue 5, pp. 1386–1398). Royal Society of Chemistry (RSC). https://doi.org/10.1039/c5fo00147a

Gobbetti, M., & Minervini, F. (2014). LACTOBACILLUS | Lactobacillus casei. In Encyclopedia of Food Microbiology (pp. 432–438). Elsevier. https://doi.org/10.1016/b978-0-12-384730-0.00180-4

Gong, J., Si, W., Forster, R. J., Huang, R., Yu, H., Yin, Y., Yang, C., & Han, Y. (2007). 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. In FEMS Microbiology Ecology (Vol. 59, Issue 1, pp. 147–157). Oxford University Press (OUP). https://doi.org/10.1111/j.1574-6941.2006.00193.x

Grom, L. C., Rocha, R. S., Balthazar, C. F., Guimara˜es, J. T., Coutinho, N. M.,Barros, C. P., & Granato, D. (2020). Postprandial glycemia in healthy subjects: Which probiotic dairy food is more adequate? Journal of Dairy Science, 103, 1110–1119.

Guarner, F., & Malagelada, J. R. (2003). Gut flora in health and disease. Lancet (London, England), 361(9356), 512–519. https://doi.org/10.1016/S0140-6736(03)12489-0

Halász, A. (2021). LACTIC ACID BACTERIA. In FOOD QUALITY AND STANDARDS. -©Encyclopedia of Life Support Systems (EOLSS).

Harvard Medical School Family Health Guide "Health Benefits of Taking Probiotics." (2005)..<http://www.health.harvard.edu/fhg/updates/update0905c.shtml.

Hiidenhovi, J., Aro, H. S., & Kankare, V. (1999). Separation of Ovomucin Subunits by Gel Filtration: Enhanced Resolution of Subunits by Using a Dual-Column System. In Journal of Agricultural and Food Chemistry (Vol. 47, Issue 3, pp. 1004–1008). American Chemical Society (ACS). https://doi.org/10.1021/jf9811774

Hill, D., Sugrue, I., Tobin, C., Hill, C., Stanton, C., & Ross, R. P. (2018). The *Lactobacillus casei* Group: History and Health Related Applications. In Frontiers in Microbiology (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fmicb.2018.02107

Holland K. T. Knapp J. S. & Shoesmith J. G. (1987). Anaerobic bacteria. Blackie ; Chapman and Hall.

Holscher, H. D., & Groeger, D. (2018). The Potential Role of Probiotics in the Management of Childhood Autism Spectrum Disorders. Gastroenterology Research and Practice, 2018, 1-11. doi: 10.1155/2018/9797604

Holzapfel, W. H., Haberer, P., Geisen, R., Björkroth, J., & Schillinger, U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. The American journal of clinical nutrition, 73(2 Suppl), 365S–373S. https://doi.org/10.1093/ajcn/73.2.365s

Hoppe, Andrew. (2010). Examination of egg white proteins and effects of high pressure on select physical and functional properties.

Hossain, M. A., Hoque, M. M., Hossain, M. M., Kabir, M. H., Yasin, M., & Islam, M. A. (2020). Biochemical, Microbiological and Organoleptic Properties of Probiotic Pineapple Juice Developed by Lactic Acid Bacteria. Journal of Scientific Research, 12(4), 743–750. doi:10.3329/jsr.v12i4.46179

Huntington, J. A., & Stein, P. E. (2001). Structure and properties of ovalbumin. In Journal of Chromatography B: Biomedical Sciences and Applications (Vol. 756, Issues 1–2, pp. 189–198). Elsevier BV. https://doi.org/10.1016/s0378-4347(01)00108-6

Huopalahti, R., R. L. Fandino, M. Anton, and R. Schade, ed. 2007. Bioactive Egg Compounds. Springer, New York, NY.

Ibrahim, H. R., Sugimoto, Y., & Aoki, T. (2000). Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. In Biochimica et Biophysica Acta (BBA) - General Subjects (Vol. 1523, Issues 2–3, pp. 196–205). Elsevier BV. https://doi.org/10.1016/s0304-4165(00)00122-7

Jankowska, H., Dzido, A., & Krawczyk, P. (2023). Determination of Rheological Parameters of Non-Newtonian Fluids on an Example of Biogas Plant Substrates. In Energies (Vol. 16, Issue 3, p. 1128). MDPI AG. https://doi.org/10.3390/en16031128

Jeong, S. C., Koyyalamudi, S. R., & Pang, G. (2012). Dietary intake of Agaricus bisporus white button mushroom accelerates salivary immunoglobulin A secretion in healthy volunteers. Nutrition, 28(5), 527–531. doi:10.1016/j.nut.2011.08.005

Joy, P.P. 2010. Benefits and uses of pineapple. Pineapple Research Station, Kerala Agricultural University, Kerala, India. [http://www.kau.edu/prsvkm/Html/BenefitsofPA.htm]

Kaila, M., Isolauri, E., Soppi, E., Virtanen, E., Laine, S., & Arvilommi, H. (1992). Enhancement of the circulating antibody secreting cell response in human diarrhea by a human Lactobacillus strain. Pediatric research, 32(2), 141–144. https://doi.org/10.1203/00006450- 199208000-00002

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., & Fakiri, E. M. (2013). Health benefits of probiotics: a review. ISRN nutrition, 2013, 481651. https://doi.org/10.5402/2013/481651

Ko, K. Y., & Ahn, D. U. (2008). An Economic and Simple Purification Procedure for the Large-Scale Production of Ovotransferrin from Egg White. In Poultry Science (Vol. 87, Issue 7, pp. 1441–1450). Elsevier BV. https://doi.org/10.3382/ps.2007-00434

Kosin B, Rakshit SK.(2006). Criteria for production of probiotics. Food Technology and Biotechnology, 44(3):371–379.

Kovacs-Nolan, J. K. N., M. Phillips, and Y. Mine. 2005. Advances in the value of eggs and egg components for human health. J. Agric. Food Chem. 53:8421–8431.

Kovacs-Nolan, J., Zhang, J. W., Hayakawa, S., & Mine, Y. (2000). Immunochemical and Structural Analysis of Pepsin-Digested Egg White Ovomucoid. In Journal of Agricultural and Food Chemistry (Vol. 48, Issue 12, pp. 6261–6266). American Chemical Society (ACS). https://doi.org/10.1021/jf000358e

Kuratsu, M., Hamano, Y., & Dairi, T. (2010). Analysis of the Lactobacillus Metabolic Pathway. In Applied and Environmental Microbiology (Vol. 76, Issue 21, pp. 7299–7301). American Society for Microbiology. https://doi.org/10.1128/aem.01514-10

LAEMMLI, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature, 227(5259), 680–685. doi:10.1038/227680a0

Lin D. C. (2003). Probiotics as functional foods. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition, 18(6), 497–506. https://doi.org/10.1177/0115426503018006497

Lin, X., Chen, X., Chen, Y., Jiang, W., & Chen, H. (2015). The effect of five probiotic lactobacilli strains on the growth and biofilm formation of Streptococcus mutans. Oral Diseases, 21, e128–e134.

Manganaris, G. A., Goulas, V., Vicente, A. R., & Terry, L. A. (2013). Berry antioxidants: small fruits providing large benefits. In Journal of the Science of Food and Agriculture (Vol. 94, Issue 5, pp. 825–833). Wiley. https://doi.org/10.1002/jsfa.6432

Manso, M. A., Miguel, M., Even, J., Hernández, R., Aleixandre, A., & López-Fandiño, R. (2008). Effect of the long-term intake of an egg white hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats. In Food Chemistry (Vol. 109, Issue 2, pp. 361–367). Elsevier BV. https://doi.org/10.1016/j.foodchem.2007.12.049

Mantzourani, I.; Chondrou, P.; Bontsidis, C.; Karolidou, K.; Terpou, A.; Alexopoulos, A.; Bezirtzoglou, E.; Galanis, A.; Plessas, S. (2019). Assessment of the probiotic potential of lactic acid bacteria isolated from kefir grains:Evaluation of adhesion and antiproliferative properties in in vitro experimental systems. Ann. Microbiol, 69, 751–763.

MATSUDA, T., GU, J., TSURUTA, K., & NAKAMURA, R. (1985). Immunoreactive Glycopeptides Separated from Peptic Hydrolysate of Chicken Egg White Ovomucoid. In Journal of Food Science (Vol. 50, Issue 3, pp. 592–594). Wiley. https://doi.org/10.1111/j.1365-2621.1985.tb13751.x

Mattila-Sandholm, T., Blum, S., Collins, J. K., Crittenden, R., de Vos, W., Dunne, C., Fondén, R., Grenov, G., Isolauri, E., Kiely, B., Marteau, P., Morelli, L., Ouwehand, A., Reniero, R., Saarela, M., Salminen, S., Saxelin, M., Schiffrin, E., Shanahan, F., … von Wright, A. (1999). Probiotics: towards demonstrating efficacy. In Trends in Food Science & Technology (Vol. 10, Issue 12, pp. 393–399). Elsevier BV. https://doi.org/10.1016/s0924-2244(00)00029-7

Messaoudi, S., Manai, M., Kergourlay, G., Prévost, H., Connil, N., Chobert, J.-M., & Dousset, X. (2013). *Lactobacillus salivarius*: Bacteriocin and probiotic activity. In Food Microbiology (Vol. 36, Issue 2, pp. 296–304). Elsevier BV. https://doi.org/10.1016/j.fm.2013.05.010

Miguel, M., & Aleixandre, A. (2006). Antihypertensive Peptides Derived from Egg Proteins. In The Journal of Nutrition (Vol. 136, Issue 6, pp. 1457–1460). Elsevier BV. https://doi.org/10.1093/jn/136.6.1457

Mine, Y. (1995). Recent advances in the understanding of egg-white protein functionality. Trends in Food Science & Technology, 6(7), 225-232.

Mine, Y. 2008. Egg Bioscience and Biotechnology. John Wiley & Sons Inc., Hoboken, NJ.

Mishra, C., & Lambert, J. (1996). Production of anti-microbial substances by probiotics. Asia Pacific journal of clinical nutrition, 5(1), 20–24.

Mohammadi R, Sohrabvandi S, Mortazavian AM. (2012). The starter culture characteristics of probiotic microorganisms in fermented milks. Eng Life Sci; 12(4): 399-409.

Mokoena M. P. (2017). Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules (Basel, Switzerland), 22(8), 1255. https://doi.org/10.3390/molecules22081255

Moniruzzaman, F. M. 1988. Bangladesh Faler Chash (Fruit Cultivation in Bangladesh), 2nd Edition, Bangla Academy, Dhaka.

Moon, S. H., Lee, J. H., Lee, Y. J., Chang, K. H., Paik, J. Y., Ahn, D. U., & Paik, H. D. (2013). Screening for cytotoxic activity of ovotransferrin and its enzyme hydrolysates. In Poultry Science (Vol. 92, Issue 2, pp. 424–434). Elsevier BV. https://doi.org/10.3382/ps.2012-02680

Mortazavian A, Sohrabvandi S .(2006). Probiotics and food probiotic products,based on dairy probiotic products. First ed.Tehran: Eta Publication; p.24-34.

NAGATA, K., & YOSHIDA, N. (1984). Interaction between Trypsin-Like Enzyme from Streptomyces erythraeus and Chicken Ovomucoid. In The Journal of Biochemistry (Vol. 96, Issue 4, pp. 1041–1049). Oxford University Press (OUP).https://doi.org/10.1093/oxfordjournals.jbchem.a134921

Neville, BA PW O'Toole. (2010). Probiotic properties of *Lactobacillus salivarius* and closely related *Lactobacillus* species. In Future Microbiology (Vol. 5, Issue 5, pp. 759–774). Future Medicine Ltd. https://doi.org/10.2217/fmb.10.35

NISBET, A. D., SAUNDRY, R. H., MOIR, A. J. G., FOTHERGILL, L. A., & FOTHERGILL, J. E. (1981). The Complete Amino-Acid Sequence of Hen Ovalbumin. In European Journal of Biochemistry (Vol. 115, Issue 2, pp. 335–345). Wiley. https://doi.org/10.1111/j.1432-1033.1981.tb05243.x

Nualkaekul, S., & Charalampopoulos, D. (2011). Survival of *Lactobacillus plantarum* in model solutions and fruit juices. International journal of food microbiology, 146(2), 111–117. https://doi.org/10.1016/j.ijfoodmicro.2011.01.040

Nualkaekul, S., Salmeron, I., & Charalampopoulos, D. (2011). Investigation of the factors influencing the survival of Bifidobacterium longum in model acidic solutions and fruit juices. Food Chemistry, 129, 1037–1044.

O'Mahony, L., O'Callaghan, L., McCarthy, J., Shilling, D., Scully, P., Sibartie, S., Kavanagh, E., Kirwan, W. O., Redmond, H. P., Collins, J. K., & Shanahan, F. (2006). Differential cytokine response from dendritic cells to commensal and pathogenic bacteria in different lymphoid compartments in humans. In American Journal of

Physiology-Gastrointestinal and Liver Physiology (Vol. 290, Issue 4, pp. G839–G845). American Physiological Society. https://doi.org/10.1152/ajpgi.00112.2005

Oe, H., Doi, E., & Hirose, M. (1988). Amino-Terminal and Carboxyl-Terminal Half-Molecules of Ovotransferrin: Preparation by a Novel Procedure and Their Interactions. In The Journal of Biochemistry (Vol. 103, Issue 6, pp. 1066–1072). Oxford University Press (OUP). https://doi.org/10.1093/oxfordjournals.jbchem.a122381

Omana, D. A., Wang, J., & Wu, J. (2010). Co-extraction of egg white proteins using ion-exchange chromatography from ovomucin-removed egg whites. In Journal of Chromatography B (Vol. 878, Issue 21, pp. 1771–1776). Elsevier BV. https://doi.org/10.1016/j.jchromb.2010.04.037

Paik, W. H., Park, Y., Park, D. H., Hong, S.-M., Lee, B. U., Choi, J.-H., Lee, S. S., Seo, D.-W., Lee, S. K., & Kim, M.-H. (2015). Prospective Evaluation of New 22 Gauge Endoscopic Ultrasound Core Needle Using Capillary Sampling With Stylet Slow-Pull Technique for Intra-Abdominal Solid Masses. In Journal of Clinical Gastroenterology (Vol. 49, Issue 3, pp. 199–205). Ovid Technologies (Wolters Kluwer Health). https://doi.org/10.1097/mcg.0000000000000084

Perera, M., Al-hebshi, N. N., Speicher, D. J., Perera, I., & Johnson, N. W. (2016). Emerging role of bacteria in oral carcinogenesis: a review with special reference to perio-pathogenic bacteria. In Journal of Oral Microbiology (Vol. 8, Issue 1, p. 32762). Informa UK Limited. https://doi.org/10.3402/jom.v8.32762

Petrova, P & Petrov, K., (2017). Prebiotic–probiotic relationship: the genetic fundamentals of polysaccharides conversion by Bifidobacterium and Lactobacillus genera. In Food Bioconversion, vol. 237, p. 278. DOI:10.1016/B978-0-12-811413-1.00007-3

Pimentel, T. C., Brandão, L. R., de Oliveira, M. P., da Costa, W. K. A., & Magnani, M. (2021). Health benefits and technological effects of Lacticaseibacillus casei-01: An overview of the scientific literature. In Trends in Food Science & Technology (Vol. 114, pp. 722–737). Elsevier BV. https://doi.org/10.1016/j.tifs.2021.06.030

Pimentel.T . Klososki.S . Rosset.M . Barão.C . Marcolino.V .(2019) . FRUIT JUICES AS PROBIOTIC FOODS .Federal Institute of Paraná, Paranavaí, Brazil. https://doi.org/10.1016/B978-0-12-815851-7.00014-0

Ranadheera RDCS, Baines SK, Adams MC. (2010). Importance of food in probiotic efficacy.Food Res Int; 43: 1-7.

Rasid; Kader and Hosain. 1987. Fruits of Bangladesh, 1st ed. Rashid Publishing House, Joydevpur, Gazipur, Bangladesh. pp.232.

Reed, G. (ed.) (1983). Prescott & Dunn’s Industrial Microbiology, 4th ed., AVI publ.Co, Westport 1983. https://doi.org/10.1002/pi.4980160311

Robinson, D. S., & Monsey, J. B. (1975). The composition and proposed subunit structure of egg-white β-ovomucin. The isolation of an unreduced soluble ovomucin. In Biochemical Journal (Vol. 147, Issue 1, pp. 55–62). Portland Press Ltd. https://doi.org/10.1042/bj1470055

Saarela, M., Mogensen, G., Fondén, R., Mättö, J., & Mattila-Sandholm, T. (2000). Probiotic bacteria: safety, functional and technological properties. Journal of biotechnology, 84(3), 197–215. https://doi.org/10.1016/s0168-1656(00)00375-8

Sabahelkhier, K. M.; Hussain, A.S. and Ishag, K. E. A. 2010. Effect of maturity stage on protein fractionation, in vitro protein digestibility and anti-nutrition factors in pineapple (Ananas comosus) fruit grown in Southern Sudan. Afr. J. Food Sci.4 (8):550 -552.

Sanders M. E. (2008). Probiotics: definition, sources, selection, and uses. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 46 Suppl 2, S58–S151. https://doi.org/10.1086/523341

Schade, A. L., & Caroline, L. (1944). Raw Hen Egg White and the Role of Iron In Growth Inhibition of *Shigella dysenteriae* , *Staphylococcus aureus* , *Escherichia coli* and *Saccharomyces cerevisiae*. In Science (Vol. 100, Issue 2584, pp. 14–15). American Association for the Advancement of Science (AAAS). https://doi.org/10.1126/science.100.2584.14

Schlegel, H. G., & Folkerts, M. (1999). Geschichte der Mikrobiologie. Halle (Saale): Deutsche Akademie der Naturforscher Leopoldina.

Selle, K., Klaenhammer, T. R., & Barrangou, R. (2019). Lactobacillus genomes and their host interactions. Trends in microbiology, 27(8), 681-701.

Sheil, B. (2004). Is the mucosal route of administration essential for probiotic function? Subcutaneous administration is associated with attenuation of murine colitis and arthritis. In Gut (Vol. 53, Issue 5, pp. 694–700). BMJ. https://doi.org/10.1136/gut.2003.027789

Sohrabvandi S, Razavi SH, Mousavi SM, Mortazavian AM. (2010). Viability of probiotic bacteria in low alcohol- and non-alcoholic beer during refrigerated storage. Philipp Agric Sci, 93(1): 24-28.

Sornplang, P., & Piyadeatsoontorn, S. (2016). Probiotic isolates from unconventional sources: a review. Journal of animal science and technology, 58, 26. https://doi.org/10.1186/s40781- 016-0108-2

Sperry, M. F., Silva, H. L., Balthazar, C. F., Esmerino, E. A., Verruck, S., Prudencio, E. S., & Rocha, R. S. (2018). Probiotic Minas frescal cheese added with L. casei 01: Physicochemical and bioactivity characterization and effects on hematological/ biochemical parameters of hypertensive overweighted women – a randomized double-blind pilot trial. Journal of Functional Foods, 45, 435–443.

Stadelman, W. J., and O. J. Cotterill. 2001. Egg Science and Tech- nology. 4th ed. Avi Publ. Co., Westport, CT.

Starke, I. C., Zentek, J., & Vahjen, W. (2015). Effects of the probiotic *Enterococcus faecium* NCIMB 10415 on selected lactic acid bacteria and enterobacteria in co-culture. In Beneficial Microbes (Vol. 6, Issue 3, pp. 345–352). Wageningen Academic Publishers. https://doi.org/10.3920/bm2014.0052

Stavropoulou, E., & Bezirtzoglou, E. (2020). Probiotics in Medicine: A Long Debate. Frontiers in immunology, 11, 2192. https://doi.org/10.3389/fimmu.2020.02192

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., Pokhilenko, V. D., Levchuk, V. P., Svetoch, O. E., & Seal, B. S. (2006). Isolation of a *Lactobacillus salivarius* Strain and Purification of Its Bacteriocin, Which Is Inhibitory to Campylobacter jejuni in the Chicken Gastrointestinal System. In Antimicrobial Agents and Chemotherapy (Vol. 50, Issue 9, pp. 3111–3116). American Society for Microbiology. https://doi.org/10.1128/aac.00259-06

Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. International journal of food microbiology, 36(1), 1-29.

Stürmer, E.S., Casasola, S., Gall, M.C., Gall, M.C., (2012). A importância dos probióticos na microbiota intestinal humana. Rev. Bras. Nutr. Clin. 27, 264–272.

Talarico TL, Casas IA, Chung TC, Dobrogosz WJ. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. Antimicrobial agents and chemotherapy. 1988 Oct;32(10):1854-8. https://aac.asm.org/content/32/10/1854.short

Tan, J., Yao, Y., Wu, N., Du, H., Xu, M., Liao, M., Zhao, Y., & Tu, Y. (2022). Color, physicochemical characteristics and antioxidant activities of preserved egg white pickled at different temperatures. In LWT (Vol. 164, p. 113685). Elsevier BV. https://doi.org/10.1016/j.lwt.2022.113685

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in Food Systems: Significance and Emerging Strategies Towards Improved Viability and Delivery of Enhanced Beneficial Value. Nutrients, 11(7), 1591. https://doi.org/10.3390/nu11071591

van Pijkeren, J.-P., Canchaya, C., Ryan, K. A., Li, Y., Claesson, M. J., Sheil, B., Steidler, L., O'Mahony, L., Fitzgerald, G. F., van Sinderen, D., & O'Toole, P. W. (2006). Comparative and Functional Analysis of Sortase-Dependent Proteins in the Predicted Secretome of *Lactobacillus salivarius* UCC118. In Applied and Environmental Microbiology (Vol. 72, Issue 6, pp. 4143–4153). American Society for Microbiology. https://doi.org/10.1128/aem.03023-05

Verschuren, P. (2002). Functional Foods: Scientific and Global Perspectives. British Journal of Nutrition, 88(S2), S126-S130. doi:10.1079/BJN2002675.

Wan, Y., Lu, J., & Cui, Z. (2006). Separation of lysozyme from chicken egg white using ultrafiltration. In Separation and Purification Technology (Vol. 48, Issue 2, pp. 133–142). Elsevier BV. https://doi.org/10.1016/j.seppur.2005.07.003

Williams, J. (1968). A comparison of glycopeptides from the ovotransferrin and serum transferrin of the hen. In Biochemical Journal (Vol. 108, Issue 1, pp. 57–67). Portland Press Ltd. https://doi.org/10.1042/bj1080057

Wu, H. J., & Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. Gut microbes, 3(1), 4–14. https://doi.org/10.4161/gmic.19320

Wu, J., & Acero-Lopez, A. (2012). Ovotransferrin: Structure, bioactivities, and preparation. In Food Research International (Vol. 46, Issue 2, pp. 480–487). Elsevier BV. https://doi.org/10.1016/j.foodres.2011.07.012

Yaneva, T., Dinkova, R., Gotcheva, V., & Angelov, A. (2021). Modulation of the antioxidant activity of a functional oat beverage by enrichment with chokeberry juice. Journal of Food Processing and Preservation, 00, e16012. https://doi.org/10.1111/jfpp.16012

Yerlikaya, O. (2014). Starter cultures used in probiotic dairy product preparation and popular probiotic dairy drinks. Food Science and Technology, 34, 221–229.

Yu, Z., Liu, B., Zhao, W., Yin, Y., Liu, J., & Chen, F. (2012). Primary and secondary structure of novel ACE-inhibitory peptides from egg white protein. In Food Chemistry (Vol. 133, Issue 2, pp. 315–322). Elsevier BV. https://doi.org/10.1016/j.foodchem.2012.01.032

Zandi, M. M., Hashemiravan, mahnaz, & Berenjy, S. (2016). Production of Probiotic Fermented Mixture of Carrot, Beet and Apple Juices [JD]. Archives of Advances in Biosciences, 7(3), 17–23. https://doi.org/10.22037/jps.v7i3.11571

Zhang, J., Wu, C., Du, G., & Chen, J. (2012). Enhanced acid tolerance in *Lactobacillus casei* by adaptive evolution and compared stress response during acid stress. Biotechnology and Bioprocess Engineering, 17(2), 283–289. doi:10.1007/s12257-011-0346-6

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

Ziemer, C. J., & Gibson, G. R. (1998). An Overview of Probiotics, Prebiotics and Synbiotics in the Functional Food Concept: Perspectives and Future Strategies. In Int. Dairy Journal (Vol. 8).

AKNOWLEDGEMENT

I would like to express my deep gratitude to my supervisor **Dr. Erika Bujna** for his continuous guidance and valuable advice for me during my work to accomplish this research. Also, I would thank my second supervisor, **Mrs. Reem Mourad**, for her support during the work, and wish her all the best for her doctoral thesis.

My appreciation also goes to the professors of the Institute of Food Science and Technology (formerly the Faculty of Food Science) for their support and encouragement throughout the entire MSc. Programme.

I am thankful to the Tempus Public Foundation for providing Stipendium Hungaricum scholarship and study opportunity at Hungarian University of Agriculture and Life Sciences (formerly Szent Istvan University), in Hungary.

I express my sincere gratitude and appreciation to my classmate friends **(Anas Al Halabi , Masagus Haidir Tamimi , Valeria Romero Delgado , Andressa Bem, Yonas Tega, and Aknur Sakpan),** those with whom we have always shared good times together, for their encouragement during the study period and all hard times and sweet times, as well as my dear friends in Iraq.

I express deep and sincere gratitude to my precious family in Iraq (My father: **Dr.Khudhyer Aljanabi** and my mother: **Arabia Aljanabi**); for their blessing and inspiration. And also my brothers and sister.

Besides, to my lovely wife (**Rana Nawfel**), who has been supportive and always there for me.

Finally, I am grateful to God, for the strength and the ability to overcome all difficulties in order to accomplish this work satisfactorily.

DECLARATION

on authenticity and public assess of final essay/thesis/mater's thesis/portfolio[1]

Student's name: **_Isam Khudhyer Zain Aljanabi_**

Student's Neptun ID: _________**H3VCN3**___________

Title of the document: **Egg White and Fruit Juice Fermentation by Probiotic Bacteria.**

Year of publication: __________**2023**______________

Department: **Department of Bioengineering and Alcoholic Drink Technology**

I declare that the submitted final essay/thesis/**master's thesis**/portfolio[2] is my own, original individual creation. Any parts taken from an another author's work are clearly marked, and listed in the table of contents.

If the statements above are not true, I acknowledge that the Final examination board excludes me from participation in the final exam, and I am only allowed to take final exam if I submit another final essay/thesis/master's thesis/portfolio.

Viewing and printing my submitted work in a PDF format is permitted. However, the modification of my submitted work shall not be permitted.

I acknowledge that the rules on Intellectual Property Management of Hungarian University of Agriculture and Life Sciences shall apply to my work as an intellectulal property.

I acknowledge that the electric version of my work is uploaded to the repository sytem of the Hungarian University of Agriculture and Life Sciences.

Place and date: __**Budapest 2023**__ year ___**May**____month __**2**__day

____

Student's signature

[1]Please select the one that applies, and delete the other types.

[2]Please select the one that applies, and delete the other types.

STATEMENT ON CONSULTATION PRACTICES

As a supervisor of **Isam Khudhyer Zain Aljanabi** (**H3VCN3**), I here declare that the master's thesis has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

I recommend/don't recommend[1] the master's thesis to be defended in a final exam.

The document contains state secrets or professional secrets: yes no*[2]

Place and date: 2023. 05. 02.

Internal supervisor

[1] Please underline applicable.

[2] Please underline applicable.