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

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1. INTRODUCTION AND OBJECTIVES

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

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

It's important to recognize that including prebiotics in probiotic products can greatly improve the probiotic ability to survive, increase the absorption of calcium and magnesium, and stimulate the production of short-chain fatty acids that are extremely beneficial for the body. However, these beneficial microorganisms are typically found in dairy products, which are only consumed by certain segments of the population. Additionally, individuals with special diets may need to exclude these beneficial products from their diet and seek alternative options.

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

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

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

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

Objectives

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

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

The specific objectives were the following:

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

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Egg white drink (ToTu drink)

Caprivous LTD (Budapest, Hungary) provided fresh egg-white drink (ToTu) bottles, which were kept cooled at 4±1°C until usage. Egg white drink is made by separating egg yolk, then the egg white is heated to about 80 °C, and the pH is adjusted to around 6.5. After enzymatic treatment, the dry matter was adjusted to 6.

2.1.2 Carbohydrate sources

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

2.1.3 Fruit juices

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

2.1.4 Bacterial strain

Bacterial strains include 16 *Lactobacillus* strains such as *Lactiplantibacillus plantarum* 299v, *Lacticaseibacillus casei* 01, *Lactobacillus acidophilus* 150 and *Lactobacillus acidophilus* La-5 (Chr. Hansen). *Lactobacillus helveticus* R-52, *Lacticaseibacillus rhamnosus Rosell*-11, *Levilactobacillus brevis* HA-112, *Limosilactobacillus fermentum* HA-179**,** *Lactobacillus helveticus Lafti* RL10**,** *Limosilactobacillus reuteri* HA-188**,** *Lacticaseibacillus rhamnosus* HA-111**,** and *Ligilactobacillus salivarius* HA-118 (Lallemand Health Solutions), *Ligilactobacillus salivarius* CRL 1328, *Lactobacillus crispatus* LCRO1, *Lacticaseibacillus rhamnosus* GG ATCC53103, and *Limosilactobacillus fermentum* LF08 (Probiotical S.p.A.). As well as *Bifidobacterium longum* DSM 16603 (Probiotical), *Bifidobacterium longum* Bb46, *Bifidobacterium lactis* Bb12 (Chr. Hansen). *Bifidobacterium bifidum* Rosell-71, *Bifidobacterium lactis* Lafti^{R}B94, and *Bifidobacterium longum* Rosell-175 (Lallemand Health Solutions). They were activated by adding them to the MRS liquid media in the case of *Lactobacillus* strains and TPY liquid media in the case of *Bifidobacterium* and incubating them for 24 hours at a temperature of 37 °C and then used to inoculate the egg white drinks. As the initial cell counts of the probiotic strains used were approximately 10^9 CFU/mL.

2.1.5 Media

MRS (De Man, Rogosa, Sharpe) Agar and **TPY (**Tryptone Peptone Yeast Extract**) Agar** were applied for selective isolation of *Lactobacillus* and *Bifidobacterium* respectively.

Tryptic Soy Agar (TSA) was prepared to determine the antagonistic activity of fermented egg white drink products.

2.1.6 Saline solution

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

2.2 Methods

2.2.1 Fermentation of egg white drink fortified with different carbohydrate sources

Egg white drink was mixed with different types of carbohydrate solution 2% (w/v) then, they were incubated with a 1% (v/v) *Lactobacillus* and *Bifidobacterium* strains that were grown in MRS medium before 24 hours of starting the fermentation at 37° C for 24 hours under aerobic conditions in the case of *Lactobacillus* strains and anaerobic conditions in the case of bifidobacteria. Control samples were also prepared without carbohydrate addition. The applied carbohydrate sources including monosaccharides (glucose), disaccharides (sucrose and fructose), and polysaccharides (fructo-oligosaccharides, galacto-oligosaccharides, and xylo-oligosaccharides) solution 2% (w/v). The evaluation was based on the total cell counts, pH, and rheological characteristics after 24 hours of fermentation.

2.2.2 Fermentation of egg white drink fortified with different fruit juices

Egg white (EW) drink and fruit juices - peach (PE), pineapple (PI), and strawberry (ST) - were mixed separately in 3:1 ratio (v/vThe monoculture fermentations were initiated with 1% (v/v) of inoculum using *L. casei* 01 and *L. salivarius* CRL 1328. The incubation temperature was 37°C for 24 hours under aerobic conditions.

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

L. casei 01, and *L. salivarius* CRL 1328 lyophilizate were grown separately in MRS broth before 24 hours of the fermentation, then they were mixed separately with egg white drink, and pineapple/strawberry juice in 4:1, 4:2, and 4:3 (v/v) ratios egg white drink to pineapple/strawberry juice. Samples without fruit juice were served as control samples. The incubation temperature was 37 °C for 16 hours.

2.2.4 Fermentation of egg white drink by a mixed culture

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

2.2.5 Determination of the pH

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

2.2.6 Determination of the cell count

Tenfold serial dilution of fermented egg white drink with a 0.85% (w/v) NaCl saline solution followed by a pour plate method was used to determine the cell count. The plates were incubated for 72 hours at a constant 37°C, or until colonies were clearly visible. The average colony count has been multiplied by the reciprocal of the sample volume to calculate the visible colonies on the plates accounting for the proper dilution factor. The incubation was carried out aerobically for *Lactobacillus* and anaerobically for *Bifidobacterium* .

2.2.7 Determination of specific growth rate and generation time

The specific growth rate (μ) of the cell populations during the exponential phase was measured graphically, as it represents the slope of the growth curve at various time points of fermentation (Perni et al., 2005). The generation time (t_g) was measured using equation (1)

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

Where t_g is the generation time (hour) andμ is the specific growth rate (1/hour).

2.2.8 Determination of volumetric productivity

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

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

2.2.9 Antagonistic activity

Antagonistic effects of the fermented product were determined by well-diffusion agar assay (Hossain et al., 2022). The pathogenic strain included *Escherichia coli* 8739, *Escherichia coli* 0157: H7, *Enterobacter cloacae*, *Listeria innocua*, and *Enterococcus faecalis*.

2.2.10 Viscosity measurement

The viscosity of each egg white drink sample was measured by MCR 92 rheometer (Anton Paar, France), in rotational mode equipped with a concentric cylinder. 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. Herschel—Bulkley model was successfully fitted to describe the flow curve of the samples based on Abbasnezhad and coworkers (2015) publication.

$$\tau=\tau_0+K\dot{\gamma}^n \quad (3)$$

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

2.2.11 Total protein content determination

The protein measurement was performed based on the Bradford method (Bradford,1976). For the calibration curve, a tenfold serial dilution was prepared from bovine serum albumin (BSA) with a concentration of 10 mg/mL.To determine the protein content, 10 μl of the appropriate dilution of the samples was pipetted onto a microplate and reacted with 200 µl of Bradford reagent. After 1 minute, the absorbance of the samples was measured at 595 nm.

2.2.12 Protein profile

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) was performed to determine the protein profile of egg white drink and fermented egg white drink samples according to protein size (mass). Gels were cast in the laboratory using unpolymerized monomer and buffer components, the stacking gel was 4% pH=6.8, 15% resolving gel pH=8.8. For better results, the protein samples on the same gel were loaded with different protein standards. The protein samples were heated for 1 min at 100 °C in the presence of SDS and β-mercaptoethanol, and bands were stained with Coomassie Brilliant Blue (R- 250) before loading (Laemmli, 1970). After the electrophoresis process was completed (parameters), the gels were washed with 20% trichloroacetic acid for 20 minutes. They were then rinsed in 850 ml of distilled water, 50 ml of acetic acid, and 100 ml of ethanol for 30 minutes. Next, a mixture of 0.2g Coomassie, 50 ml distilled water, 10 ml acetone, and 50 ml ethanol was used to colour the gel for 30 minutes. The gels were then left overnight in a 10% acetic acid solution using a vortex mixer. To analyse the gels, a digital picture was captured using the Biorad ChemDoc XRS imaging system (USA) and Quantity One software. Finally, the gels were analysed using Image Lab version 5.1 software.

2.2.13 Total phenolic content determination

Total phenolic content was determined by the Folin–Ciocalteu method. 1 mL of fermented egg white drink was centrifuged at 3000 rpm for 2 min in Eppendorf tube, then 10 µL of the supernatant was added to 1250 µl from a mix of tenfold diluted Folin-Cioalteau reagent and 240 µL methanol:water (4:1) solvent. Afterward, the mixture was homogenized and allowed to react for 1 min, then 1 mL of 0.7 M Na_2CO_3 was added, vortexed, and was allowed to stand for 5 min at 50 °C in a water bath before measurement. The absorbance was measured at λ = 765 nm. Gallic acid was utilized as a standard to create a calibration curve.

2.2.14 Titratable acidity determination

The samples were titrated to a pH of 8.2 using 0.1N NaOH. The following formula was used to calculate titratable acidity:

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

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

2.2.15 Colour measurements

The colour of the samples was measured with a Konica-Minolta CR-400 chromameter (Konica Minolta Sensing Inc., Osaka, Japan) at room temperature.

2.2.16 Sensory evaluation

One day prior to evaluation, the fermented samples were refrigerated at 4°C. Ten participants conducted the sensory analysis in a laboratory for sensory analysis. Samples were served in plastic cups that were coded with three-digit random numbers that were written in a random order. During the testing process, people were given water to drink and bread to eat to clean their mouths. Under bright light, each test was done on a separate table. The tests were conducted using Addinsoft's XLSTAT Sensory Solution (Pro module) software (Kókai et al., 2003).

2.2.17 Determine the shelf life of the products

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

2.2.18 Statistical methods

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

3. RESULTS AND DISSCUSION

3.1 Screening of different *Lactobacillus* and *Bifidobacterium* for the fermentation of egg white drink

Probiotic bacteria was able to propagate in egg white drink with and without glucose 2% (w/v) since after 24 hours of fermentation, samples fermented by *Lactobacillus* strains without added sugar, the pH ranged from 4.86 to 5.61, while in the presence of carbohydrate, it ranged from pH 3.54 to 4.51. When fermenting with Bifidobacteria, the pH without glucose ranged from 5.52 to 5.75 , and with glucose, it ranged from 4.08 to 4.60.

After strain selection, the egg white drink was fermented with *L. helveticus* R52, *L. acidophilus* 150, *L. rhamnosus* Rosell 11, *L. casei* 01, *L. salivarius* CRL 1328, and *L. plantarum* 299 v 1% (v/v) as a monoculture fermentation. After 24 hours of fermentation in the presence of glucose 2% all the studied probiotics could propagate in egg white drink, as *L. plantarum* 299v and *L. casei* 01 increased by 2 logs while 1 Log in the case of *L. rhamnosus* Rosell 11 and *L. helveticus* R52. In addition, the greatest pH level was achieved in samples with *L. helveticus* R52, contrary to *L. plantarum* 299v and *L. casei* 01 samples which had the lowest value. Also, *Bifidobacterium* strain thrived in egg white drink with glucose 2% (w/v) since *B. longum* Bb46 growth was not considerably different compared to *B. longum* DSM 16603. Studying the specific growth rate h^{-1} and generation time (h) showed that *L. plantarum* 299v exhibited the fastest growth with a specific growth rate value of 0.14 h^{-1} and the shortest generation time at 4.95 h. *L. helveticus* R-52 had the slowest growth and the longest generation time. *B. longum* Bb46 demonstrated quicker propagation in the glucose-enriched egg white drink compared to *B. longum* DSM 16603. In general *Bifidobacteria* grew better than *Lactobacilli.*

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

L. plantarum 299v, *L. acidophilus* 150, and *L. casei* 01 were selected to be studied for their preference for different carbohydrate sources, including monosaccharides (glucose), disaccharides (sucrose and fructose), and polysaccharides (fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and xylo-oligosaccharides (XOS)) solution 2% (w/v). The evaluation was based on the total cell counts, pH, and rheological characteristics after 24 hours of fermentation. Egg white completed with fructose, FOS, GOS, or XOS reacted differently when a different strain was used. It was observed that *L. acidophilus* 150 showed the highest preference for glucose, fructose, and saccharose, while *L. casei* 01 preferred glucose and fructose, on the other hand, *L. plantarum* 299v favoured FOS. Nevertheless, no discernible difference was observed between the three strains when xylo-oligosaccharides (XOS) were utilized in egg white fermentation.

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

The study found that K values of *L. acidophilus* 150 samples were not significantly different in control samples and samples with FOS (0.004 Pa.s^n). However, adding GOS resulted in a higher K value compared to samples with other carbohydrate sources. Likewise, the K value of the samples containing XOS and *L. casei* 01 was greater, reaching 0.009 Pa.s^n. However, out of all the samples examined, those that had fructose or FOS addition together with *L. casei* 01 had the lowest K values. Additionally, samples with *L. plantarum* 299v and glucose addition had the highest K value compared to fermented samples with other sugar sources.

The flow behaviour index of the examined materials was more than one, indicating dilatant shear thickening, except in samples containing XOS, where it was 0.98, indicating shear thinning or pseudoplastic behaviour. Shear thinning is a time-independent flow in which the apparent viscosity decreases as the shear rate rises. Additionally, samples of *L. casei* 01 with fructose and FOS added exhibit strong dilatant properties. In the case of control samples and samples with GOS, the flow behaviour index of *L. casei* 01 and *L. acidophilus* 150 did not differ from each other. Meanwhile adding glucose, sucrose, GOS, and XOS to fermented samples by *L. plantarum* 299v presented a higher n value compared to other strains when the same sugar type was tested.

3.3 The effect of storage on the fermented egg white drink

Fermented egg white drink by *L. plantarum* 299v and *L. casei* 01 utilizing fructose and FOS (fructo-oligosaccharides) as carbohydrate sources were monitored during cold storage.

It was found that after 24-hour fermentation process and three weeks of refrigeration, the samples without a carbohydrate source had a significantly lower cell counts compared to the samples that contained one.

The total cell counts of samples with *L. plantarum* 299v and carbohydrate sources were higher than 9 Log_{10} CFU/mL over the storage period. Control samples were decreased to 8.12±0.19 Log_{10} CFU/mL in the third week. Samples with fructose had consistent cell counts ranging. Also, samples containing FOS had relatively stable cell counts until the second week of storage, when they greatly increased. Compared to the fermented samples with fructose the samples with FOS showed no significant difference in the cell count in the first two weeks.

In addition, the survivability of *L. casei* 01 was extremely stable the probiotics count was higher than 8.4 Log_{10} CFU/mL throughout storage time. Correspondingly, 3 weeks of cold storage did not affect the growth of probiotics, further, the total cell numbers of *L. casei* 01 were not greatly different when using fructose or FOS. However, it was better to extend the storage for a few months to specify the ideal time of storage.

The control samples had the highest pH value compared to the others with sugar addition at any period of storage. Additionally, the pH levels of samples containing fructose and FOS and *L. plantarum* 299v did not differ significantly from each other over time. In the third week of storage, the pH value of fermented samples with fructose or FOS increased and reached pH 3.8 which could be attributed to the fact that when microorganisms lack the required nutrients in the medium, they start consuming organic acids instead.

In the case of *L. casei* 01 was used as a starter culture, the pH values of samples with FOS were greatly different as they decreased over time. In contrast, fructose samples during the first and second weeks were not considerably different from each other as they dropped substantially in the third week of storage to pH 3.62 which may have been caused by the proliferation of microorganisms. Thus, the higher acidity of the probiotic egg white drink can affect positively the microbiological stability of the product consequently extending its shelf life from two days to over 3 weeks.

Studying the rheological parameters revealed that the yield stress values were higher than 0 in all studied samples and storage periods. In the term of *L. plantarum* 299v, control samples had the lowest yield stress and were closer to a 0 when compared to samples with carbohydrate sources, and they remained stable over refrigeration for up to 3 weeks. FOS samples had higher yield stress than fructose samples then it decreased in the third week. The consistency coefficient (K) values for control and FOS samples remained constant throughout the study period. While, fructose samples showed an increase in the third week, reaching 0.0033 Pa. s^n compared to 0.019 for FOS samples and 0.038 for control samples.

In the case of *L. casei* 01, the yield stress of samples with FOS, did not significantly differ throughout storage time, but they were quite lower values compared to fermented samples after 24 hours of fermentation. The consistency coefficient of egg white drink increased significantly after the fermentation from 0.0008 to 0.004 Pa.s^n in control samples, and to 0.003 Pa.s^n in samples with FOS since K values varied from 0.0008 to 0.004 Pa.s^n. On the other hand, samples containing fructose did not vary significantly from fresh samples throughout the time. Examining the flow behaviour index in all studied samples, revealed shear thickening or dilutant properties, as indicated by n values exceeding 1 over the storage period.

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

Egg white drink and fruit juices - peach, pineapple, and strawberry - were mixed separately in 3:1 ratio (v/v). The combination of egg white drink and fruit juice provided the free amino acids and peptides required by lactic acid bacteria, particularly the proteins found in egg whites, as well as the vitamins and fermentable carbohydrates found in fruit juices. After 4 hours of fermentation, *L. salivarius* acclimated faster than *L.casei* 01 to the egg white drink containing EW:PE and EW:PI or EW:ST samples. However, *L. casei* 01 exhibited a considerably greater total cell count than *L. salivarius* CRL 1328 after 8 hours and up to 24 hours, except for EW:ST samples, there was no significant difference between *L. casei* 01 and *L. salivarius* CRL 1328 growth at the 4 and 8-hour intervals.It was also observed that extending the fermentation time over 24 hours played a significant role in promoting the growth of *L. casei* 01 when peach juice was added. However, the growth rate did not differ significantly when pineapple and strawberry were added separately, even after 16 and 24 hours of fermentation. the cell counts in EW: PE samples increased significantly, reaching 9.06 Log_{10} CFU/mL after 24 hours. Similarly, the cell counts in EW: PI and EW: ST reached 8.97 and 8.83 Log_{10} CFU/mL respectively, with no significant differences among them. Additionally, the fermentable sugar in fruit juices affects *Lactobacillus* cell viability, which contributes to the significant growth of the bacterial strain viability.

It's worth noting that after 8 hours of fermentation, all samples increased by approximately 3 Logs over the initial cell count, which is in line with the daily recommended therapeutic dose of probiotics.

Furthermore, lactic acid was produced faster by *L. casei* 01 than by *L. salivarius* CRL 1328 since *L. salivarius* CRL 1328 samples had significantly higher pH values compared to *L .casei* 01 samples after 24 hours of fermentation, as the pH level of EW:PE, EW:PI, and EW: ST reached (pH 3.77, pH 3,80, pH 3.86) respectively in samples with *L. casei* 01 and (pH 3.83, pH 4.08, pH 3.95) respectively in *L. salivarius* CRL 1328 samples.

SDS PAGE was performed to determine the protein profile of fermented samples. Five significant protein bands were identified on the fermented egg white drink mixed with different fruit juices, including ovo-transferrin at 81.2-89.8 kDa, ovalbumin at 44.4-47.6 kDa, ovomucoid at 35.3-36.6 kDa, ovo-flavoprotein at 12.4-13.1 kDa, and lysozyme at 14.4 kDa. In accordance with a densitometric study the density of some protein bands reduced due to the probiotics' proteolytic activity, which led the protein to degrade into amino acids and peptides in order to proliferate. For instance, ovalbumin bands were clearly apparent on the gel, although the strength was greater in fresh egg white drink samples than in others. Peach juice, when used in egg white drink fermentation with *L. salivarius* CRL 1328, led to significantly stronger density bands of ovalbumin, ovo-flavoprotein, ovomucoid, and ovo-transferrin in egg whites compared to pineapple and peach juices. However, due to the strong proteolytic system that *L. casei* 01 possesses, the bands related to ovalbumin were fainter in the case of *L. casei* 01 compared to those of samples with *L. salivarius* CRL 1328 when the same fruit juice was added. Although lysozyme was not considerably different in fermented samples, they were higher in the fresh egg white drink which could be due to the addition the juices in a 3:1 ratio egg white drink: fruit juices.

Egg white with strawberry juice samples were the darkest and most red, indicating the best overall acceptability when fermented by *L. salivarius* CRL 1328. Additionally, samples with a 3:1 EW to pineapple juice ratio exhibited pseudoplastic behaviour after 24 hours of fermentation.

The cold storage over 8 weeks demonstrated that samples containing *L. casei* 01 with strawberry or pineapple juice stayed steady, with their proliferation remaining greater than 8.44 Log_{10}CFU/mL. In contrast, *L. salivarius* decreased to 4.62 Log_{10}CFU/mL after the fourth week of storage.

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

To find the most suitable ratio of After 16 hours of fermentation, *L. casei* 01 and *L. salivarius* CRL 1328 could grow in egg white drink with or without adding juice since the initial cell count of *L. salivarius* CRL 1328 increased considerably from 5.02 Log_{10} CFU/mL to not less than 7.7 Log_{10} CFU/mL, while the initial cell count of *L. casei* 01 was intensified from 7.7 to 8.16 Log_{10} CFU/mL.

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

A total cell count of 9.17 Log_{10} CFU/mL was achieved in the 4:3 EW: PI and *L. casei* 01 combination, while the ratios of 4:1 and 4:2 EW: PI did not considerably differ in the total cell count. Both samples reached 8.6 Log_{10} CFU/mL but differed significantly from control samples. To summarize, pineapple juice helps enhance the proliferation of tested probiotic bacteria.

The pH of fresh unfermented egg white drink was not significantly different, compared to the pH values of control samples after 16 hours of fermentation. As the fermentation time might be insufficient to cultivate the probiotic strains in control samples since probiotic strains require carbohydrate sources to grow and release organic acids, which lower the pH level of the medium. However, a significant difference was observed when PI was added to *L. salivarius* CRL 1328 and *L.casei* 01 samples reaching a pH value of 4 compared to control samples (pH 6.41), considering that different ratios of the juice did not significantly differ in pH when *L. salivarius* CRL 1328 was used as a starter strain.

Although the total phenolic content (TPC) of control samples did not differ significantly from unfermented samples, fermented beverages containing pineapple juice, had a slightly higher level of the phenolic content of 0.1 mg GAE /mL versus 0.09 mg GAE /mL in unfermented samples. In the 4EW:1PI, and 4EW:3PI combinations, there was no significant difference in TPC between *L. casei* 01 and *L. salivarius* CRL. However, fermented 4EW:2 PI combination by *L. salivarius* CRL 1328 samples were special with different characteristics as they showed higher TPC than *L. casei* 01 samples. Fermented egg whites with *L. salivarius* CRL 1328 had a significantly greater phenolic content in ratios 4:2 and 4:3 EW: PI compared to the control and 4:1 samples. In the case of *L. casei* 01, the higher juice ratio did not affect the phenolic content as much as in the 4:1 sample.

The yield stress ($\tau 0$) of fermented samples were greater than 0 at the same time it was significantly higher in the fermented samples with *L. casei* 01 compared to the fresh egg white drink. In the case of samples with *L. salivarius* CRL 1328, the control samples were not significantly different from the fresh egg white drink. However, adding pineapple juice to egg white drink fermentation increased the stress yield, as the 4EW:2PI combination required the highest power to move their structure. Contrastingly, *L. salivarius CRL* 1328 samples had a higher $\tau 0$ compared to *L. casei* 01 samples thus, they needed a higher force to move the network structure, while control samples were not significantly different in τ_0 from unfermented fresh egg white drink.

The initial consistency coefficient (K) decreased remarkably after the fermentation, adding juice also reduced the K value significantly compared to control samples, in the case of *L. salivarius* CRL 1328, 4EW:2PI samples were not considerably different from 4EW:1PI, or 4EW:3PI blends. However, 4EW:3PI samples had a significantly higher K value (0.000003) compared to 4EW:1PI (0.000001), on the other hand, using *L. casei* 01 indicated different results, where 4EW:2PI samples recorded the lowest K value (0.00007). Moreover, the consistency coefficient of *L. salivarius* CRL 1328 samples was significantly lower than *L. casei* 01 when pineapple juice was added. Further, the flow behaviour index (n) indicated that all samples had a dilatant or shear thickening behaviour as n values were greater than 1, which is increased after the fermentation in the case of *L. casei* 01, the highest value was detected in 4EW:2PI.

The colour parameter determination showed that as the pineapple juice ratio was raised, b* increased while L* and a* dropped.

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

Adding strawberry juice to egg white drink improves the bacterial growth process greatly. It was observed that there was no noticeable shift in *L. casei* 01 cell growth when different strawberry juice was added after 8 hours of fermentation time. After 24 hours of fermentation, the samples with the 4EW:2ST formula had the greatest population of cells (9.61 Log_{10} CFU/mL). Furthermore,

At the same time, the populations of *L. salivarius* CRL 1328 in the control and 4EW:1ST samples were notably the lowest, as they were not significantly different from each other. In contrast, the total cell count in the 4EW:2ST and 4EW:3ST samples were considerably the highest, reaching 8.6 Log_{10} CFU/mL. Comparatively speaking, *L. casei* 01 had better growth than *L. salivarius* CRL 1328 after 24 hours of fermentation, regardless of whether strawberry juice was added or not.

The decrease in pH correlates with an increased strawberry juice ratio, suggesting a consequent enhancement in lactic acid production. The pH level of fermented beverage with *L. casei* 01 was not significantly different from those with *L. salivarius* CRL 1328. In general samples with strawberry juice recorded a lower pH value compared to control samples as a result of increasing the production of organic acid due to the presence of carbohydrates in the strawberry juice.

3.7 Study the effect of mixed culture in the fermentation of egg white drink with and without strawberry juice

Egg white beverages fermented with *L. casei* 01 and *L. salivarius* CRL 1328 as a mixed culture were investigated.

The pH of samples with mixed culture fermentation was higher than the samples fermented with *L. salivarius* CRL 1328 alone. The total cell counts in the mixed culture samples were higher, recording a value of 7.94, compared to 7.78 in the samples with *L. salivarius* CRL 1328, but there was no significant difference compared to the samples with *L. casei* 01.

The total phenolic compounds expressed as gallic acid mg/mL increased significantly from 0.08 to 0.2 mg/mL in the fermented samples. Moreover, there was no significant difference in the phenolic content if monoculture or mixed starter culture was utilized. On top of that, adding strawberry juice in a 1:3 ratio to egg white drink slightly enhanced the total phenolic content as the final product had higher total phenolic content in comparison to samples without further addition, possibly belonging to the anthocyanin group, which is the dominant phenolic group in strawberry.

All fermented beverages have antimicrobial activity against *E. coli* 8739, especially when EW is mixed with strawberry, regardless of the strain and whether it was a mixed or monoculture fermentation. As a result of low pH as well as fermentation by-products which present an antimicrobial activity. In addition, fresh EW drink samples also showed antagonistic activity. Also, fermented EW: ST samples by *L. casei* 01 or *L. salivarius* CRL 1328 individually showed a larger clear zone than others, which indicates an enhanced antimicrobial effect against *E. coli* 0157: H7. Interestingly, all samples have antimicrobial activity against *E. cloacae* but the clarity zone size varies from sample to sample since it is smaller in fermented samples by a monoculture fermentation and samples without strawberry puree but expanded when strawberry juice was incorporated. *Listeria innocua* and *Enterococcus faecalis* showed similar effects when strawberry juice was added, as adding it enhanced antagonistic activity.

What is more, when the studied strains were growing in MRS broth, they exhibited antagonistic activity against the tested pathogen, both when mixed or used separately. However, the diameter of the clear zone increased when strains were mixed, while the results were similar when the test was conducted on *E. coli* 0157:H7.

4. CONCLUSION AND RECOMMENDATIONS

Egg white drink is high in functional proteins and is also free of cholesterol and fat, making it an excellent replacement for those who want to avoid dairy drinks. It can also serve as a vehicle for probiotics. The characteristics of fermented beverages are influenced by the starter culture and fermentation conditions.

Screening different probiotic lactic acid bacteria and bifidobacteria for the fermentation of egg white, reaveled that *L. casei* 01 and *L. plantarum* 299v, recorded the highest cell concentration and lowest pH levels. In addition, *Bifidobacterium longum* Bb46 had less generation time compared to *Bifidobacterium longum* DSM 16603. To investigate the influence of various carbohydrate sources, including monosaccharides (glucose and fructose), disaccharides (sucrose), and oligosaccharides (fructo-oligosaccharides, galacto-oligosaccharides, and xylo-oligosaccharides), *L. acidophilus* 150, *L. plantarum* 299v, and *L. casei* 01 were selected as the most promising. The results demonstrated that *L. casei* 01 had the greatest growth in samples with fructose, fructo-oligosaccharides, and galacto-oligosaccharides. Also, the cell counts of *L. plantarum* 299v was considerably higher in samples with fructo-oligosaccharides.

Fermented egg white drink samples with monosaccharides (such as glucose or fructose) or oligosaccharides (such as fructo-oligosaccharides or galacto-oligosaccharides) and *L. plantarum* 299v had the highest yield stress. Another strain, *L. casei* 01, required more energy to start flowing its structure when sucrose and xylo-oligosaccharides were added to the egg white drink. The consistency coefficient (K) of both control and egg white samples with fructose fermented by *L. plantarum* 299v indicated a higher viscosity compared to samples with *L. acidophilus* 150 and *L. casei* 01. The flow behaviour index of the fermented samples represented a shear thickening behaviour. Studying microbial stability of *L. casei* 01 and *L. plantarum* 299v for three weeks of cold storage, utilizing fructose and fructo-oligosaccharides as distinct sources of carbohydrates showed stable cell populations over three weeks, with higher counts for *L. plantarum* 299v in FOS samples compared to fructose samples. The stress yield of control samples fermented by *L. plantarum* 299v did not change with storage time, however, it reduced in samples containing carbohydrate sources. Moreover, the stress yield of *L. casei* 01 control samples declined in the third week of storage and remained constant for fructose and FOS samples. K value didn't change when carbohydrate was added to the fermentation of the EW drink. Also, a shear thickening behaviour was noticed over time.

Fruit juices incorporation into the fermentation process of egg whites enhances the overall nutritional value and supports the growth of probiotics. After 24 hours of fermentation, there was no significant difference in total cell counts when different fruit juices (peach, pineapple, and strawberry) were added. Although, the growth of *L. casei* 01 was notably higher compared to *L. salivarius* CRL 1328, *L. salivarius* CRL 1328 samples displayed consistently higher pH levels compared to *L. casei* 01 samples. The protein profile of fermented EW drink mixed with fruit juices did not change much in terms of the protein concentration, and the bands remained recognizable on the gel. Based on the results of sensory evaluation, *L. salivarius* CRL 1328 samples with strawberry juice obtained the highest overall sensory assessment score. The stress yield of fermented samples containing pineapple juice and *L. casei* 01 was considerably higher than that of samples containing other fruit juices. The flow behaviour index showed that adding pineapple juice to EW fermentation consequently impacts its flow behaviour as they had pseudoplastic characteristics while they exhibited a shear thickening behaviour once peach or strawberry juice was added. Also, the K value of samples with *L. salivarius* CRL 1328 did not differ when various fruit juices were added while in the case of *L. casei* 01 pineapple samples were the highest. Examining the viability over the course of eight weeks under cold storage revealed that, the cell count of *L. casei* 01 in the fourth week, was approximately three logs higher than that of *L. salivarius* CRL 1328.

Incorporating varying percentages of pineapple juice (100%) into egg white drink resulted in a significant rise in the *L. casei* 01 population and a decrease in the pH level of the 4:3 EW:PI samples. Nevertheless, increasing the ratio of pineapple juice from 4:1 to 4:3 in the EW:PI mixture did not lead to a significant impact on the total cell counts of *L. salivarius* CRL 1328 or the pH level.

Increasing the percentage of pineapple juice in egg white drink caused a rise in the stress yield of 4:1 and 4:2 EW:PI samples. The consistency coefficient of *L. salivarius* CRL 1328 samples was significantly lower than *L. casei* 01. Fermented egg white drink samples combined with pineapple juice 100% in various ratios showed a dilutant behaviour. The determination of colour parameters indicated that increasing the incorporation ratio of pineapple juice increased the a* and b* values and decreased the L* value.

The study of the effect of different ratios of strawberry puree on the fermentation of egg white drink by *L. casei* 01 and *L. salivarius* CRL 1328 found that adding strawberry puree to the drink enhanced the growth of the bacteria with the highest cell populations observed in the 4:2 EW:ST and 4:3 EW: ST samples after 24 hours of fermentation by *L. casei* 01. In the case of *L. salivarius* CRL 1328, the highest growth was observed in 4:1 EW:ST samples after 16 hours of fermentation. Furthermore, increasing the strawberry juice ratio led to an increase in the L*, b*, and a* values.

During the fermentation process of a mixture of egg white drink to strawberry puree in a ratio 3:1 using a mixed culture of *L. casei* 01 and *L. salivarius* CRl 1328, the lactic acid content was higher in samples fermented with the mixed culture compared to those fermented with *L. casei* 01 as a monoculture fermentation. In addition, samples that were fermented with mixed culture without strawberry puree addition recorded the lowest percentage of lactic acid. Samples of mixed and monoculture-fermented EW drinks with and without ST puree were found to possess antimicrobial activity since a greater inhibition zone was observed in samples containing ST puree against selected pathogens.

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

Summarising, fermentation is a good way to produce probiotic egg white-based beverages with health-promoting effects.

Overall, this research demonstrates the feasibility of producing probiotic beverages using egg white beverage with various carbohydrates and fruit juices. The choice of probiotic strain and processing parameters can affect the viability, functionality, and sensory properties of the final product. This work paves the way for the development of a novel, nutritious, and shelf-stable probiotic beverage derived from egg white, suitable for lactose-intolerant consumers and those seeking a protein-rich functional drink.

5. NEW SCIENTIFIC RESULTS

1. It was stated, that a variety of microorganisms were able to multiply in egg white drink after 24 hours including *Lactobacillus helveticus* R52, *L.acidophilus* 150, *L.rhamnosus Rosell* 11, *L.casei* 01, *L.salivarius* CRL 1328, *L.plantarum* 299v and *Bifidobacterium longum* Bb46, however, 2% of glucose addition is also beneficial to reach Log_{10} CFU/mL >7. The greatest cell counts and lowest pH were observed in *L.casei* 01 and *L.plantarum* 299v after a 24-hour fermentation period.
2. It can be concluded, that the application of different carbohydrate sources (glucose, fructose, sucrose, fructo-oligosacchrides, galacto-oligosaccharides, and xylo-oligosaccharides) to the fermentation of EW drink by *L. acidophilus* 150, *L. plantarum* 299v, and *L. casei* 01 showed differences in carbohydrate preference. *L. casei* 01 had better growth when fructose, fructo-oligosacchrides, and galacto-oligosaccharides was added to the egg white drink. *L. plantarum* 299v preferred fructo-oligosacchrides for growth and its pH level was not affected by mono- or disaccharides. However, *L. casei* 01 had the lowest pH with polysaccharides.
3. The viscosity of a fermented egg white beverage is greatly influenced by the strains used and the type of carbohydrate source. The ToTu drink samples exhibited shear thickening behaviour when fermented with *L. plantarum* 299v, *L. casei* 01, or *L. acidophilus* 150 in the presence of these carbohydrates.
4. During 3-week cold storage at 4°C, the viable cell count of *L. plantarum* 299v and *L. casei* 01 in a fermented egg white drink with fructose and fructo-oligosacchrides in 2% concentration was greater than 10^7 CFU/mL.
5. The shelf life of the ToTu drink was extended from two days to at least three weeks by the fermentation process with *L. plantarum* 299v and *L. casei* 01, while maintaining its shear thickening behaviour.
6. Fermentation of egg white combining peach, pineapple, or strawberry juice (25% fruit) in a ratio of 1:3 by *L.salivarius* CRL 1328 or *L.casei* 01 boosts nutritional content and promotes probiotic development. The highest total cell count was obtained by *L.casei* 01 among all fruit juices, surpassing *L.salivarius* CRL 1328. *L.casei* 01 grew to its maximum in 16 hours with pineapple or strawberry juice but required 24 hours to reach the greatest cell count with strawberry. *L.salivarius* CRL 1328 cell count did not change significantly after 8 hours of fermentation, indicating that an 8-hour fermentation may be effective.
7. It was found, that the flow behaviour index at 20° C showed pseudoplastic attributes when pineapple juice 25% in a 3:1 was added to egg white fermented by *L.salivarius* CRL1328 or *L.casei* 01; nevertheless, when strawberry and peach juices (25%) were used, they maintained shear thickening properties.
8. It was observed, that the addition of different ratios of 100% pineapple or strawberry juice (4:1, 4:2, and 4:3 EW: PI, 4:2 and 4:3 EW: ST) to the fermentation of egg white by *L. casei* 01 and *L. salivarius* CRL 1328, respectively resulted in an increase in their growth after 24 hours.
9. The total phenolic content of egg white: pineapple in the ratio 4:2 and 4:3 fermented by *L. salivarius* CRL 1328 can be considerably increased by 18.18% compared to control samples.

6. LIST OF PUBLICATIONS AND CONFERENCES

Journal article publications

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

Conference full paper publications

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

Conference abstract publications

1. **Mourad R.,** Csehi B., & Bujna, E. (2021). The effect of adding different sugar types on the properties of fermented egg milk product. *International Conference on Agricultural and Food Chain Safety Development*, Budapest, Hungary.
2. **Mourad R.**, Csehi B., & Bujna, E. (2021). Fermentation of egg white milk by probiotic bacteria. *The 4th International Conference on Biosystems and Food Engineering*, E425, pp.1, http://www.biosysfoodeng.hu/2021/USB/pdf/E425.pdf
3. **Mourad R**., Csehi B., Németh, Cs., & Bujna, E. (2021). The effect of adding different sugar types on the properties of fermented egg milk product, *Proceedings of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting*, pp.90,
4. **Mourad R.**, Csehi B., & Bujna, E. (2022). Studying the shelf-life of a probiotic fermented egg white milk product, *4th International Conference on Food Science and Technology*, pp. 21, http://www.foodconf.hu/files/BOA2022.pdf
5. **Mourad R**., Csehi B., & Bujna, E. (2022). Probiotic fermentation of enzyme-treated egg white drink mixed with pineapple juice. *9th Asia-Pacific Probiotics Symposium*.
6. **Mourad R**., Aljanabi I., Csehi, B., & Bujna E. (2023). Development of a non-dairy Probiotics drink from egg white-based product mixed with different fruit juices. *5th International Conference on Biosystems and Food Engineering*, E556, pp.1. http://biosysfoodeng.hu/USB/pdf/E556.pdf
7. **Mourad R**., Aljanabi I., Csehi B., & Bujna, E. (2023). Egg white fortification with probiotics and peach juice. *A Magyar Táplálkozástudományi Társaság XLVI*. pp.30, https://www.doki.net/tarsasag/taplalkozas/upload/taplalkozas/document/dec.20_honlapra_2023._oktober_20nyomda_szept._25_4judit_ri_ix_6_vandorgyules_gyor_program_nyomda_elott_final_09_20_logokkal.pdf?web_id=A5C42F4E392CAA2
8. **Mourad R**., Csehi B., & Bujna, E. (2023). Egg white-based product with probiotics and pineapple juice, *Proceedings of János Lippay – Imre Ormos – Károly Vas (LOV) Scientific Meeting*.