

Investigating the shelf-life of probiotics fermented egg white-based beverage using prebiotics

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ABSTRACT

With growing attention to health and lifestyle changes, functional foods have become crucial and in demand. These foods are a rich source of probiotics and prebiotics, but most probiotic products are dairy-based, making them inappropriate for people with lactose intolerance or milk protein allergies. Nevertheless, egg white offers a viable substitute and is considered one of the best sources of functional proteins. As an alternative food matrix, they come highly recommended for those who are hypersensitive to dairy products or who follow a high-protein diet, such as athletes. In this context, egg-white drink with different carbohydrate sources, including monosaccharide (fructose) and oligosaccharide (fructooligosaccharide), was fermented by *Lactocaseibacillus casei* 01. After 24 h of fermentation, the total cell count was higher than $8 \log_{10}$ CFU mL⁻¹ thus, the egg white drink was suitable for *L. casei* 01 to grow. Additionally, the survival of *L. casei* 01, the pH value, and the rheological properties of fermented beverages within three weeks of refrigerated storage were also investigated. Throughout the storage period, the control samples exhibited considerably lower cell count and higher pH values compared to the samples with carbohydrate sources, also, samples containing the same carbohydrate source showed no noticeable changes. Viscosity measurements of the studied samples showed a shear thickening behaviour during the time.

KEYWORDS

Fructooligosaccharides, fermented egg white, survivability, rheology

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INTRODUCTION

Functional foods including probiotics and prebiotics have been found to have positive effects on health (Stanton et al., 2005; Ganesh, 2006). Probiotics, such as *Lactocaseibacillus casei* 01, have been known to offer various health benefits and are commonly used in a range of products, especially dairy and non-dairy items, meat, bakery, and fruits (Pimentel et al., 2021). Prebiotics are dietary carbohydrates that help selectively stimulate the growth of probiotics in the colon, increase resistance to invading pathogens, reduce the possibility of colon cancer, and improve calcium and iron bioavailability (Nilegaonkar et al., 2010). In addition to plant-based materials, egg white-based drink is an excellent substitute for dairy products for lactose intolerance and milk protein allergies and might be a suitable food matrix for probiotics as well as prebiotics. Egg whites include 88% water, 11% protein, 0.2% fat, and 0.8% ash. There are four major proteins found in egg whites: ovalbumin, conalbumin, ovomucoid, and lysozyme (Campbell et al., 2003). Furthermore, egg whites have several beneficial properties that involve emulsifying, foaming, and gelling therefore are extensively utilized in food processing (Nagy et al., 2021). Fructooligosaccharides (FOS) have been proven to be advantageous carbon sources for the growth of probiotics. However, there is a noticeable scarcity of information regarding the effect of prebiotics on egg white drink fermentation. Hence, more research is required to entirely comprehend the possible advantages of this combination. Despite the relevance of rheological properties in food production, monitoring, sensory assessment, and structural investigations (Hayta et al., 2001), insufficient knowledge exists related to the rheology of fermented egg white drinks. Our research aims to investigate the growth of *Lactocaseibacillus casei* 01 in egg-white drinks using two types of carbohydrates, FOS, and fructose separately. During three weeks of storage in refrigerated conditions, the final products were tested for changes in some parameters (survivability of *L. casei* 01, pH value, and viscosity).

MATERIALS AND METHODS

Materials

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%. 100 g of the product contains 0.1 g of carbohydrates, 5.6 g of protein, and 6% dry matter and provides a total of 23 kcal. Fresh egg-white drinks were obtained from Capriovus LTD (Szigetcsép, Hungary), and they were refrigerated at $(4 \pm 1 \text{ }^\circ\text{C})$ until use. *L. casei* 01 was brought from Christian Hansen company.

Egg white fermentation

Fresh egg white beverage was mixed with 1% (V/V) of cultivated *L. casei* 01 in MRS (de Man Rogosa Sharp) broth that was incubated prior to 24 h of starting the manufacturing process. Then, different forms of carbohydrate solutions 20% (W/V) (fructooligosaccharide, fructose) were inserted into the mixture individually to obtain a 2% (W/V) sugar concentration. Samples without sugar addition were served as a control. The incubation temperature was 37 °C for 24 h.



Determination of *L. casei* 01 survival and the pH level of egg white drink after the fermentation and during the cold storage

Following sequential dilutions in a saline solution, samples were plated on MRS agar and incubated at 37 °C for 48–72 h. The pH was measured using a Mettler Toledo InLab expert pro electrode pH meter.

Viscosity measurement. Anton Paar's RheoCompass software (version 1.21.852) was used to manage the MCR 92 rheometer (Anton Paar, France), which was operating in the rotational mode. In order to investigate the flow behaviour of the samples at $T = 20$ °C, the shear rate was raised from 10 to 500/min. The yield stress, consistency, and flow behaviour index were evaluated by the Herschel-Bulkley model (1) (Abbasnezhad et al., 2015).

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

where τ – shear stress (Pa); τ_0 – yield stress (Pa); $\dot{\gamma}$ – shear rate (1/s), K – consistency coefficient ($\text{Pa}\cdot\text{s}^n$), n – flow behaviour index. The rheological characteristics of the samples were precisely characterized by the model. The fitted models' determination coefficient values (R^2) were greater than 0.95.

Statistical analysis. All determinations were made in triplicates, and data were analyzed by multivariate analysis of variance (MANOVA) and mean comparisons by post hoc test (A Tukey HSD test was used in all cases, except for pH levels of fructose samples, for which Games Howell was used because homogeneity was violated). All statistical procedures were conducted using the software IBM SPSS27 (Armonk, NY, 2020).

RESULTS AND DISCUSSION

Survivability of *L. casei* 01 in fermented egg white drink during cold storage

A 24-h egg white fermentation with FOS and fructose was carried out with *L. casei* 01 before storage. Following Fig. 1, a fermentation of *L. casei* 01 resulted in the initial cell concentration increasing from 6.7 to 8.98 \log_{10} CFU mL^{-1} in samples with FOS, and 9.04 \log_{10} CFU mL^{-1} in samples with fructose, while in control samples it was significantly lower cell concentration reached a value of 8.04 \log_{10} CFU mL^{-1} . These phenomena could be explained by the high requirements of nutrients that *Lactobacillus* needs to propagate. In our case, although *L. casei* 01 was able to grow in egg white drink without carbohydrate sources by using the carbohydrate bound to protein in the glycoprotein present in egg white drink, however, its growth was better when carbohydrate sources were added. Additionally, fructose can be used by a wide range of microorganisms because it is a simple sugar and easily fermentable. After 3 weeks, there was no noticeable distinction in cell count between the egg white samples containing fructose and FOS, on the other hand, the survivability was extremely stable as Daneshi et al. (2012) indicated that cold storage maintains the cell populations of *L. casei* 01. Moreover, the probiotics count was higher than 8.4 \log_{10} CFU mL^{-1} throughout storage time and it was in agreement with the results of Nighswonger et al. (1996) as the viability of *L. casei* GG in cultivated buttermilk and yogurt was stable upon 28 days of refrigeration. These results also agreed with Łopusiewicz et al. (2019) who



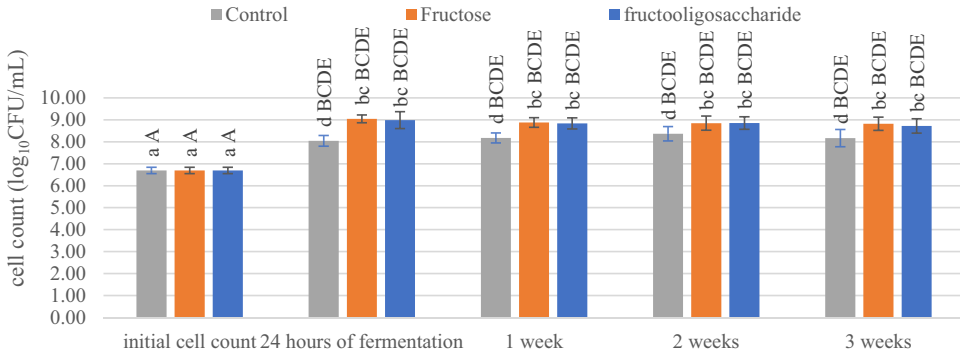


Fig. 1. The viability of *L. casei* 01 in fermented egg white drink with different carbohydrates throughout cold storage. Lowercase letters indicate the difference between sugar types in the same storage period, and uppercase letters indicate the difference during the time using the same sugar

studied the survivability of *Lactobacillus* along 21 days of storage at 6 °C in fermented flaxseed cake, which showed that the bacterial counts were maintained higher than 10⁷ CFU mL⁻¹. Correspondingly, 3 weeks of cold storage did not affect the growth of probiotics, additionally, the survivability of *L. casei* 01 was not greatly different when using fructose or fructooligosaccharide. However, it was better to extend the storage for a few months to specify the ideal time of storage.

pH value changes of fermented egg white beverage during cold storage

In compliance with Fig. 2, the initial pH value of the egg white drink (6.80) dropped significantly after 24 h of fermentation reaching a level of pH 5.91 in control samples since it was higher compared to samples with carbohydrate sources, because of the higher growth in samples with carbohydrates that consequently lead to increased production of organic acids. Meantime, control samples were not considerably different in the first and third weeks, the pH value was around 5.8 in both. Moreover, the pH values of samples with FOS were greatly different as they decreased over time, after 2 weeks reduced to pH 3.8, and dropped on the 21 days of

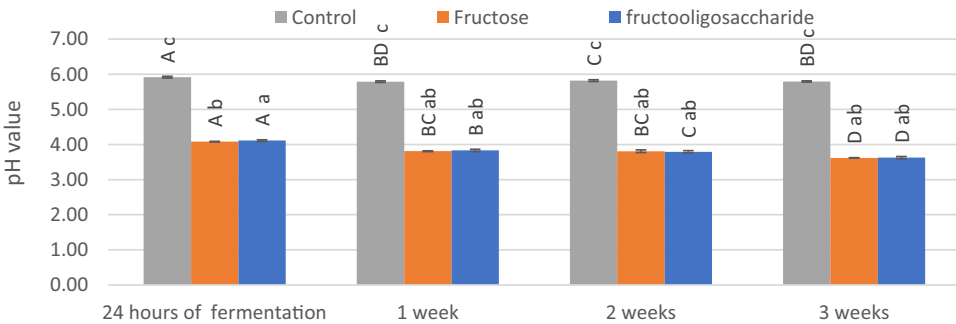


Fig. 2. The pH value of fermented egg white beverages with different carbohydrate types during cold storage. Lowercase letters indicate the difference between sugar types in the same storage period, and uppercase letters indicate the difference during the time using the same sugar



fermentation. Yeo and Liong (2010) reported an increase in lactic acid production when fermented soy drink by *Lactocaseibacillus casei* ATCC 393 was supplemented with FOS. In contrast, fructose samples during the first and second weeks were not considerably different from each other as they dropped substantially in the third week of storage to pH 3.62 which may have been caused by the proliferation of microorganisms. After the third week of storage, there were no considerable changes in pH between the fermented samples with fructose and FOS. Thus, the higher acidity of the probiotic egg white drink can affect positively the microbiological stability of the product consequently extending its shelf life from two days to over 3 weeks.

The changes in viscosity of fermented egg white drink during the time

In accordance with the yield stress (Fig. 3a) of the control samples slightly raised after 24 h of the fermentation in comparison with fresh samples thus, they required a higher force to move their structure before starting the flow. Later on, it significantly decreased in the second and third week of cold storage in the case of control samples. After the first week, fructose samples showed a dramatic drop in yield stress, followed by a slight increase without any significant difference from fresh samples. As compared to control samples in the same storage period, samples with fructose required less force to remove. In the case of samples with FOS the yield stress did not significantly differ throughout storage time, but they were quite lower values compared to fermented samples after 24 h of fermentation.

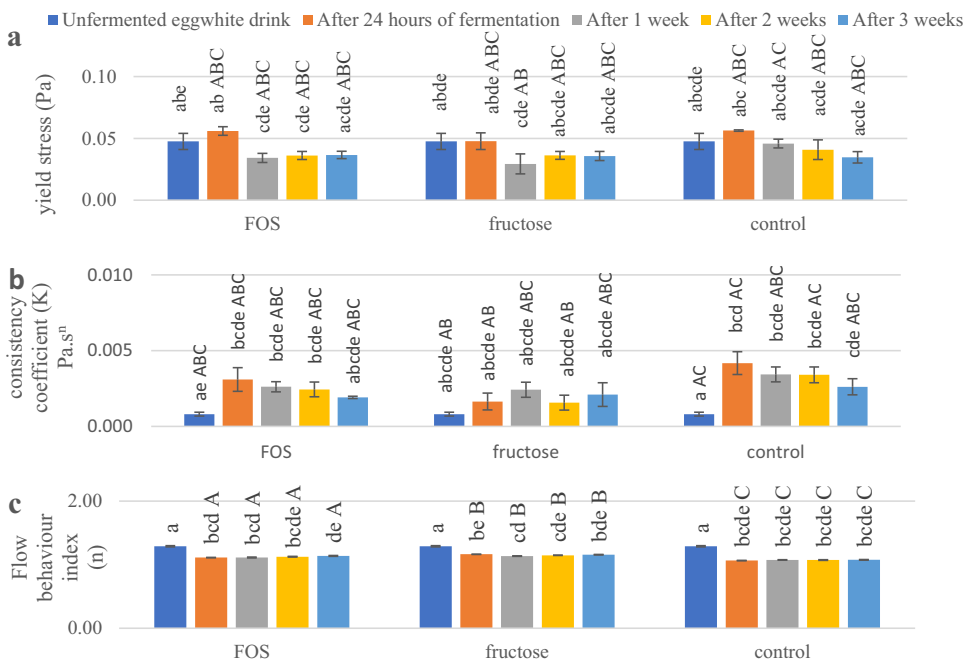


Fig. 3. The rheological parameters for fermented egg white drink during cold storage. (a) yield stress, (b) consistency coefficient, (c) flow behaviour index. Lowercase letters indicate the difference between sugar types in the same storage period, and uppercase letters indicate the difference during the time using the same sugar



Undeniably the consistency coefficient (K) of egg white drink (Fig. 3b) increased significantly after the fermentation from 0.0008 to 0.004 Pa.sⁿ in control samples, and to 0.003 Pa.sⁿ in samples with FOS since K values varied from 0.0008 to 0.004 Pa.sⁿ. That means fermentation raised the strength of the structure against breakdown by increasing water retention and protein aggregation. On the other hand, samples containing fructose did not vary remarkably from fresh samples throughout the time. After 3 weeks of storage, the K values were not remarkably different in samples involving fructose and FOS, since they were both equivalent to fresh samples. These results were the same as the results of Kumbár et al. (2015) since an increase in K value was found during the cold storage of liquid egg white drink in the first three weeks. Studying the flow behaviour index (Fig. 3c) expressed a non-Newtonian fluid that has shear thickening behaviour, additionally, fresh samples had the significantly highest n value. The reduction in n value and the increase in K could be attributed to the dissociation of the protein network due to the shearing (Aboulfazli et al., 2015). Our findings differ from those of Kumbár et al. (2015) who reported that fresh egg white exhibited shear thinning behaviour. This discrepancy may be attributed to the enzymatic treatment used during egg white drink processing. Additionally, Varga-Tóth et al. (2023) observed a pseudoplastic behaviour in egg white drink enriched with mixed berries and bovine collagen peptides, which contradicts our results. However, it should be noted that they conducted their viscosity measurements at a different temperature (15 °C), which is known to affect viscosity.

CONCLUSION

Lactocaseibacillus casei 01 was able to propagate in egg white drink, and the probiotic count was greater than 8.03 log₁₀ CFU mL⁻¹ over the course of 3 weeks of refrigeration at 4 °C. Furthermore, the inclusion of carbohydrate sources, particularly FOS, is highly recommended due to its positive influence on probiotic growth. After 24 h of fermentation, the dilatant properties of fermented egg white drink declined with a shear-thickening behaviour, which may provide insight into the selection and design of industrial production equipment.

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