L.PLANTARUM 299V AS A STARTER PROBIOTIC IN FERMENTED EGG WHITE DRINK

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Abstract

Egg white (albumen) is the sticky, colorless part of the egg surrounded by the eggshell. It consists of many functional proteins, especially ovalbumin, ovotransferrin, ovomucoid, ovomucin, and lysozyme. Probiotics are living microorganisms that have a beneficial effect on the human intestine. Recently, supplementing food with probiotics has become an important approach to prevent the adhesion of some harmful bacteria to the intestinal mucosa and reduce the symptoms associated with lactose intolerance and milk protein allergy. Egg white drink is a functional drink that is cholesterol and fat-free, and rich source of protein. In this study egg white drink was fermented by *L.plantarum* 299v as a starter culture using two different carbohydrate sources (fructose and fructooligosaccharides) as samples, and without added sugar were serving as controls.The survivability and pH value of probiotic beverages was investigated during three weeks of cold storage.

After 24 hours of fermentation, the pH decreased to a value of 6.15 and the cell count increased to 8.2 log10 CFU/ml in the control samples.

During 3 weeks of storage, the cell count was higher than 10^8 CFU/ml in all samples with or without added sugar, which is the recommended daily dose of probiotic bacteria. Moreover, the pH decreased to 3.8 when fructose and fructooligosaccharides were added, while the control samples had the highest pH (6.1) during the storage period.

Introduction

L.plantarum 299v is a Gram-positive lactic acid bacteria and a part of phylum Firmicute. Lactobacilli are facultatively heterofermentative microorganism[1] which could be found in plant-based fermented products particularly in sauerkraut, gherkin, and sourdough.It has the ability of adhesion to the intestinal mucosa, it resists to the low pH conditions in the stomach and to the high pH of bile salt in the duodenum [2], in addition to reducing cardiovascular risk [3]. Functional food is a food item that promotes health and human wellness as well as reduces the risk of serious disease [4] [5] particularly cardiovascular complications, osteoporosis, obesity, and cancer [6]. Egg consists of various dietary ingredients which protect against chronic disorder, includinglutein, zeaxanthin, choline, vitamin D, selenium, and vitamin A [7], an extra beneficial nutrient found in eggs is protein. As eggs are one of the rich sources of dietary proteins including all of the important amino acids that have a high numerous biological value [8]. It is used as a standard to compare the other protein from several sources. Egg protein dispensed among the yolk and white (3.6 g in egg white and 2.7 g in egg yolk) in the case of a massive egg with 6.3 g protein in total [9]. Egg whites incorporate ovotransferrin which binds metal ions, ovomucin has antiviral attributes, further it reduces the cholesterol absorption: Lysozyme protects against Gram-positive harmful bacteria [9], and avidin binds to biotin and improves biotin absorption as well as the important role of egg white protein in reducing the visceral fat [10]. Egg white drink (Totu drink) is a functional Hungarian beverage that contains

hen egg white, enzyme, vinegar, salt, and water, it is free of fat and cholesterol, 100 grams of the product contains 0.1 g of carbohydrate, 5.6 g of protein and it provides 23 kcal furthermore, the dry matter reaches a value of 6%.
Our study aims to test the feasibility and the survival of *L.plantarum* 299v in fermented egg white drink as well as the pH value of the final product during 3 weeks of storage under refrigerated conditions using different carbohydrate sources (fructose and fructooligosaccharides) since samples without added sugar were served as a control.

Experimental

- Fermentation : *L. plantarum* 299v has grown in MRS (de Man Rogosa Sharp) broth before 24 hours of the fermentation, then 1% of the liquid starter was added to 100 ml of egg white drink. Different types of carbohydrates solution (fructooligosaccharides, fructose)were added separately to get a 2% sugar concentration in the final products.
- Determination of the pH: By using the pH meter Mettler Toledo InLab expert pro electrode.
- Determination the growth rate: Samples were serially diluted with a saline solution followed by plating the appropriate dilutions (Pour plate method) using MRS agar and incubated for 48-72 hours at 37°C.
- Storage: the studied samples were stored at a refrigerated condition for 3 weeks, as the growth rate and pH value were measured every week.

Results and discussion

After 24 hours of fermentation samples without added sugar had a significantly lowest cell count they reached a value of 8.23 log 10 CFU/mL compared to samples with FOS 9.41 log 10 CFU/mL and samples with fructose 9.28 log 10 CFU/m fig.1. Moreover they were not significantly different from samples in the first and second weeks of refrigerated storage $p>0.05$.
Further, samples with added fructose as a carbon source were not significantly different during the storage period, the cell count ranged (from 9.27-to 9.44) log 10 CFU/mL. Also, the cell count of samples with FOS was not significantly different until the second week of storage then they increased in the third week to 9.73±0.09 log 10 CFU/mL due to sugar metabolizing by *L.plantarum* 299v.
On the other hand, samples with fructose and FOS had no significant difference from each other in the first and second week while samples with FOS were higher in the third week. assamples without added sugar had a significantly lower cell count compared to samples with added sugar during the entire studied period.
It cannot be denied that *L. plantarum* 299v was able to maintain its bioavailability at 4°C for 3 weeks. As some researchers mentioned that the minimum required number of probiotics in fermented food products to have an advantageous health effect on the human body is 10^6 CFU/ml [11] and that was compatible with our results, the cell count was higher than 10^7 CFU/ml during storage time if carbohydrate sources were added or not. Fridge temperature had also been the main role in keeping the survivability of the fermented product, Yoon and co-workers (2006) had the same findings, they evaluate the viability of probiotic cabbage juice during cold storage. The results showed that *L. plantarum* and *L.delbruekii* could maintain their bioavailability at 4 °C for several weeks.

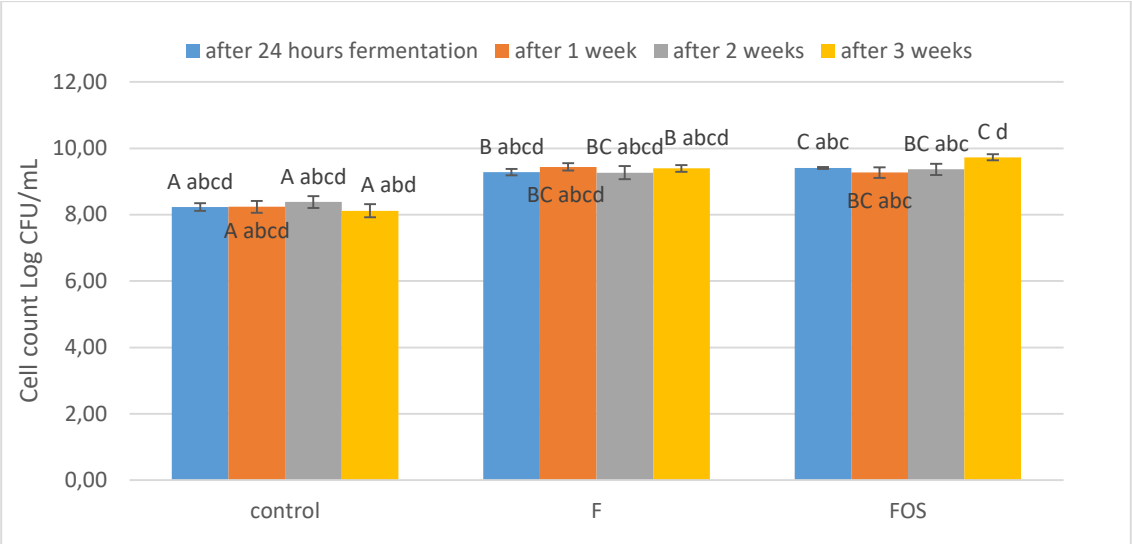

Fig (1): The survivability of *L.plantarum* 299v in fermented egg white drink during cold storage

The bigger case letters express the difference in the cell count when a different sugar type was used in the same storage period, lowercase letters indicate the difference in cell count using the same sugar type in a different storage period. control: samples without added sugar, F: samples with fructose 2%, FOS: samples with fructooligosaccharides 2%.

According to Fig.2. the pH of samples with fructose and FOS were not significantly different from each other during times, while control samples demonstrated the highest pH value compared to the others with added sugar at any period of storage, due to the low cell concentration of *L.plantarum* 299v therefore, the release of lactic acid was less.

On the other hand, the pH values after the first and second week of storage were significantly different from each other. They were both significantly lower and reached a value around pH 5.9 compared to samples after 24 hours of fermentation pH 6.15 and samples in the third week of storage pH 6.08.

The pH value of samples with fructose dropped significantly in the first week of storage, it also decrease in the second week to pH 3.69. The main reason for that may be because of the activity of *L.plantarum* 299v in the egg white drink caused by the consumption of sugar and production of short chain fatty acids and lactic acid [13] which afterward increased and reached a value of pH 3.8 Similar to fructose, the pH value of FOS samples increased significantly in the third week to pH 3.8, that's could be related to the fact that in the case of the lack of the required nutrient in the medium the microorganism starts to consume the organic acids instead thus, moreover due to the constant low pH the cells degrade.

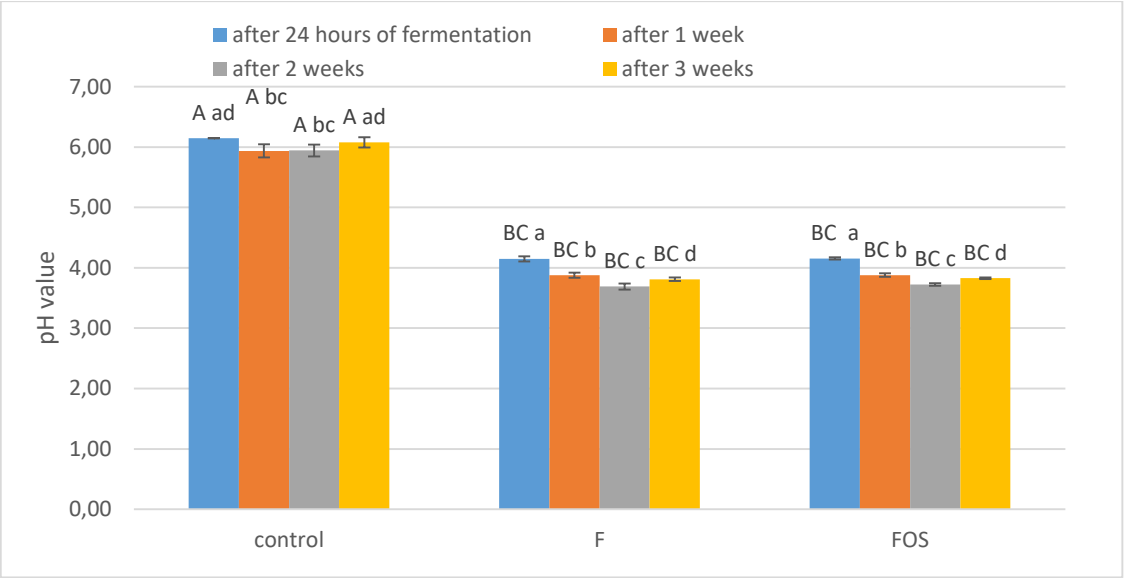

Fig (2): The pH value of fermented egg white drink during cold storage
The bigger case letters express the significant difference ($p < 0.05$) in the pH value when different sugar types were used in the same storage period, the lowercase letters indicate the significant difference ($p < 0.05$) in the pH value using the same sugar type in a different storage period. control: samples without added sugar, F: samples with fructose 2%, FOS: samples with fructooligosaccharides 2%.

Conclusion

Fermented egg white drink is a novel functional drink furthermore a rich source of probiotics and vital protein, while the fermentation extend its shelf life to more than 3 weeks under fridge temperature.

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