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A *BIFIDOBACTERIUM* FERMENTED EGG WHITE DRINK WITH DIFFERENT CARBOHYDRATE SOURCES

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Abstract: Probiotics have been shown to benefit human health, however, they are usually found in dairy products, which cannot be consumed by people with lactose intolerance or milk protein allergies. Egg white drink is a good alternative and might be a suitable carrier for probiotics. In this context, the usability of egg white drink for the fermentation by *Bifidobacterium longum* Bb46 and *Bifidobacterium longum* DSM 16603 and the effect of adding different carbohydrate sources (glucose, fructose and saccharose) was investigated. After 24 hours of fermentation, changes in pH, total cell count and protein profile were also examined. A reduction in pH was observed particularly when carbohydrate sources were added to egg white drink compared to control samples. Generally, the total cell count was greater than 8.3 $\log_{10}$CFU/mL, and the cell count of *B. longum* DSM 16603 was considerably higher than *B. longum* Bb46 in egg white drink supplemented with glucose and saccharose. Following SDS protein profile analysis of all studied samples, ovalbumin, ovoflavoprotein, and ovomucoid were detected, although their associated bands were weaker when carbohydrates were added. To sum up, *B. longum* DSM 16603 can be applied for the production of fermented probiotic egg white drink in the presence of fructose, which might make the drink suitable for diabetics.

Keywords: egg white, probiotics, fermentation, protein, bifidobacteria

1. Introduction

Nowadays, consumers have an increased demand for functional foods that have health-promoting effects (Aguiar et al. 2019). Three different types of food ingredients can be used for designing these types of food: living microorganisms (probiotics), non-digestible carbohydrates, and bioactive metabolites (Do Espírito Santo et al. 2011). Probiotics were defined as "live microorganisms that confer health benefits on the host when administered in adequate amounts" (Jha et al. 2020). In the last few years, *Bifidobacterium* genera have attracted research and commercial interest as a probiotic bacteria (Pokusaeva et al. 2011, Prasanna et al. 2014). Moreover, *Bifidobacterium* has been proven that predominantly colonize the human gut and have beneficial effects on boosting the immunity system and relieving symptoms of lactose intolerance (Yakoob & Pradeep 2019). Bifidobacteria have varying capacities to metabolize carbohydrates and transform them into many by-

products mainly acetate along with lactate, ethanol, and formate (Pokusaeva et al. 2011). Most traditional probiotic products are milk-based however, these products cannot be consumed by groups of people who suffer from lactose intolerance or who are allergic to milk proteins. For these consumers, egg white is considered a good alternative source to milk in the production of probiotic products. As a valuable source of functional protein and high digestibility, eggs provide many essential nutrients to humans (Belitz et al. 2004). *Bifidobacterium* has mainly been used in the fermentation of dairy products (Prasanna et al. 2014), or fruit juices (Sheehan et al. 2007), however, there is a lack of information on the fermentation of egg white.

In this research, the possibility of developing an egg white drink was investigated by assessing the suitability of egg white for the cultivation of *Bifidobacterium* genera.

2. Materials and methods

2.1. Materials

Egg white drink were obtained from Caprivous LTD (Szigetcsép, Hungary), and they were refrigerated at 10± 1 °C until use. *Bifidobacterium longum* DSM 16603 and *Bifidobacterium longum* Bb46 were brought from Probiotical and Christian Hansen, respectively.

2.2. Methods

2.2.1. Production of fermented egg white

100 ml of egg white drink was mixed with a 2% carbohydrate solution (fructose, glucose, and saccharose) separately and 1 % of *Bifidobacterium* inoculum cultivated in TPY (Trypticase-phytone-yeast extract) broth before 24 hours of the fermentation at 37 °C in anaerobic condition. The cultured egg white drink was incubated at 37°C for 24 hours in anaerobic Jar. Three replications were made for each one. After fermentation, the viable cell count, pH value, and protein profile were determined.

2.2.2. Determine the total cell count and pH

The cell count was determined by the pour plate method using TPY agar. Plates were incubated for 48-72 hours at 37 °C in anaerobic conditions in Jar. The pH was determined using a Mettler Toledo InLab expert pro electrode pH meter.

2.2.3. Determination of protein profile

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) according to the method of Laemmli (1970) with stacking gel 4% (pH 6.8) and resolving gel 15% (pH 8.8) was applied to determine the protein profile of the samples.

2.2.4. Statistical analysis

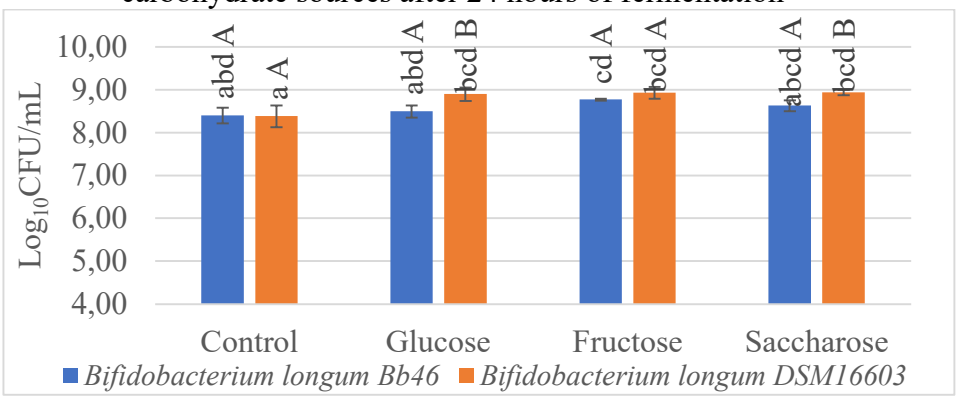
All statistical procedures were conducted using the software IBM SPSS27 (Armonk 2020). Data were analyzed by multivariate analysis of variance (MANOVA) and mean comparisons by post hoc test at $p<0.05$.

3. Results

3.1. The viability of *Bifidobacterium* strains in egg white drink

Considerably, the cell counts in *Figure 1,* increased by about 2 $\log_{10}$ in all studied samples over the initial value which was 6.88 $\log_{10}$CFU/mL in the case of *B. longum* DSM 16603, and 5.97 $\log_{10}$CFU/mL regarding *B. longum* Bb46, as a consequence of the consumption of the nitrogen and carbohydrate in the medium. Additionally, the cell concentration was not significantly different between *B. longum* DSM 16603 and *B. longum* Bb46 in the control samples and samples with fructose, 8,8-8,9 $\log_{10}$CFU/mL. On the other hand, the total cell counts of *B. longum* DSM 16603 in the fermented egg white with glucose and saccharose was considerably higher than *B. longum* Bb46. Although the cell count of *B. longum* DSM 16603 was not significantly different when glucose, fructose or saccharose were added reaching a value of 8.9 $\log_{10}$CFU/mL, it was completely higher than the control samples 8.38 $\log_{10}$CFU/mL.

Figure 1: The viability of *Bifidobacteria* in egg white drink with different carbohydrate sources after 24 hours of fermentation



Source: Author's own editing based on his own research

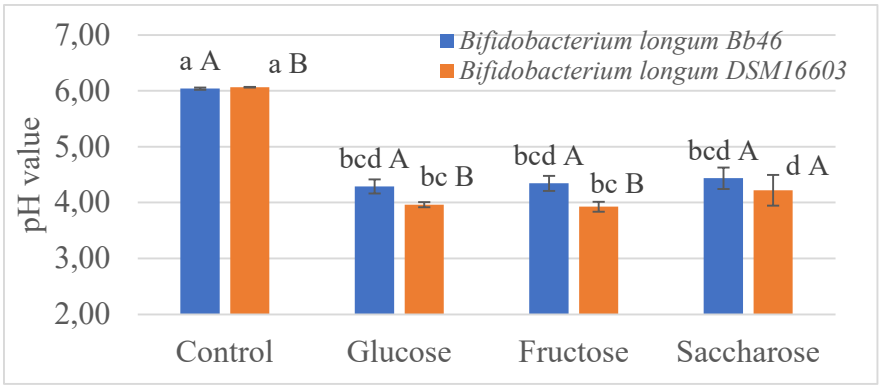
Higher case letters indicate the difference between *Bifidobacterium* strains when the same carbohydrate sources was added, lower case letters represent the differences when different carbohydrate sources were added using the same strain.

3.2. Study the pH value of egg white drink

In agreement with *Figure 2*, the pH dropped after 24 hours compared to the initial pH value which was pH 6.24. Furthermore, the pH value of fermented samples by *B. longum* Bb46 was generally higher than fermented samples by *B. longum* DSM 16603 when carbohydrate sources were added. On the other hand, there was no considerable difference in the pH value of fermented egg white samples with *B.*

longum Bb46 when different carbohydrate sources were added. In contrast, adding saccharose solution recorded a higher level of pH value (pH 4.2) in comparison with samples with glucose (pH 3.96), and fructose (pH 3.93) in fermented samples by *B. longum* DSM 16603.

Figure 2: The pH value of fermented egg white drink with different carbohydrate sources after 24 hours of fermentation



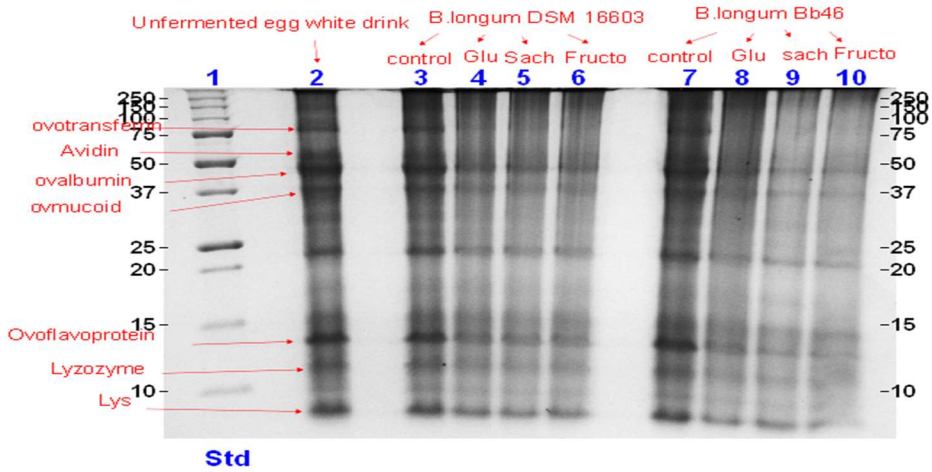
Source: Author's own editing based on his own research

Higher case letters indicate the difference between *Bifidobacterium* strains when the same carbohydrate source was added, lower case letters represent the differences when carbohydrates different sources were added using the same strain.

3.4. Study the protein profile of fermented egg white drink

As presented on *Figure 3*, five major proteins, ovalbumin, ovomucoid, ovoflavoprotein, ovotransferrin, and lysozyme were detected on the gel. A slight reduction in the intensity of ovomucoid and ovalbumin was observed in control samples compared to fresh unfermented samples due to the fermentation process.

Figure 3: The protein profile of fermented egg white drink with different carbohydrate sources after 24 hours of fermentation



Source: Author's own editing based on his own research

1:standard, 2:fresh unfermented egg white drink, 3:control: fermented egg white drink by *B.longum* DSM16603 without carbohydrate sources, 4: fermented egg white drink by *B.longum* DSM16603 with glucose, 5: fermented egg white drink by *B.longum* DSM16603 with saccharose, 6: fermented egg white drink by *B.longum* DSM16603 with fructose, 7:control: fermented egg white drink by *B.longum* Bb46 without carbohydrate sources, 8: fermented egg white drink by *B.longum* Bb46 with glucose, 9: fermented egg white drink by *B.longum* Bb46 with saccharose, 10: fermented egg white drink by *B.longum* Bb46 with fructose.

After 24 hours of fermentation, samples with mono or disaccharide presented a considerable reduction in the quantity of ovalbumin, ovomucoid, ovoflavoprotein and lysozyme, however, the avidin disappeared. Also, lysine was detected at a greater intensity in both fresh and control samples than in carbohydrate-added samples these could be attributed to the increase in cell count concentration that induced by adding carbohydrate sources, which consequently resulted in consuming more protein and peptides by *Bifidobacteria*.

Moreover, when carbohydrate sources were added, samples with *B. longum* DSM 16603 had stronger intensity bands of ovalbumin, ovoflavoprotein, and lysozyme compared to fermented samples by *B.longum* Bb46. Although ovotransferrin bands were still visible on the gel in fermented samples by *B. longum* DSM 16603, however, they disappeared in fermented samples by *B.longum* Bb46. Since *Bifidobacteria* can consume many types of carbohydrates, there was no noteworthy difference between bands relating to monosaccharides and others in samples with disaccharides.

4. Discussion

Based on *Figure 1*, the viable cell count in all studied samples was higher than 10^8 CFU/mL, which is in agreement with (Lee & Salminen 1995), who found that 10^6-10^7 viable CFU/g is needed to provide an adequate daily dosage of 10^6-10^9 viable bacteria. Furthermore, when fructose was added to egg white, the growth of *B. longum* Bb46 improved, however, it was not much different from samples with saccharose, as the total cell count reached 9 $\log_{10}$CFU/mL, since *Bifidobacterium* can metabolize ribose, galactose, fructose, glucose, and sucrose, and this ability varies significantly from strain to strain (Pokusaeva et al. 2011). Supporting this, a similar observation was made by Chou & Hou (2000), in their study of soy milk fermentation they reported an increase in *B. longum* B6 growth when isomaltooligosaccharide, glucose, lactose, or galactose was added to the medium. Also, Farnworth et al. (2007) found that fructose was the most utilized sugar by *Bifidobacterium* while glucose, raffinose, and stachyose were used much less. In contrast, our results showed higher *Bifidobacterium* cell concentrations compared to the results of Kamaly (1997) who cultured *Bifidobacterium* in soy milk with a total cell count of 7.7 $\log_{10}$CFU/mL. *Figure 2*, depicts a reduction in pH due to production of lactic acid, propionic acid, butyric acid, and acetic acid by “Bifidus pathways” from carbohydrate presence in the medium (Havas et al. 2015). In addition, after adding carbohydrates, pH levels between 4.35 and 3.98 were found, which was close to results from Farnworth et al. (2007). During their study, different *Bifidobacterium*

strains were grown in soy beverages and cow milk, and the pH values ranged from pH 4-4.3 in the final product. In *Figure* 3, ovalbumin intensity decreased after fermentation. Since it is a glycoprotein and the carbohydrate is covalently linked to it (Harvey et al. 2000), *Bifidobacteria* may rely on these proteins as nitrogen and carbon sources in the absence of carbohydrates in order to meet their metabolic demands. In conclusion, *B. longum* DSM 16603 can be used for the production of a fermented probiotic egg white drink in the presence of fructose, which could make the drink suitable for diabetics and lactose intolerants.

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