



Conference Article

Formulation and Evaluation of Lactose-Free Probiotic Milk Beverage with Added Dried Figs

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Abstract

*Digestive issues have become widespread due to factors such as imbalanced nutrition and the increase in fast-food consumption. The foods which are available to meet the daily probiotic consumption of individuals are limited. To overcome this nutritional need through the consumed foods, a newly formulated food product called "lactose-free milk beverage with dried fig and probiotic supplements" has been developed. It is formulated with lactose-free milk to be suitable for consumption by all individuals. Dried figs produced and exported in our country are functional ingredients not only for their nutritional values but also for their natural prebiotic effect. In addition to the prebiotic role of dried figs, *Bacillus clausii* bacteria has been used to impart probiotic properties to the product. Different ratios of dried fig puree and probiotic ratio were studied, and in vitro digestion analyses were conducted to prove the probiotic effect. Sensory evaluation of the novel functional milk beverage was also carried out. According to the preferences of a total of 60*



panelists, the product ranking results indicated that the developed formulation enriched with probiotics and dried fig was the most preferred sample. Furthermore, during the storage of the developed lactose-free milk beverage with dried fig and probiotic supplements at 4°C, color, total bacteria count, and probiotic bacteria count were determined. The research provided a new functional product designed to support individuals with digestive comfort, promote an increased probiotic population in the intestines, and address concerns related to lactose intolerance.

Keywords: Lactose-free milk, Dried fig, Probiotic, In vitro digestion, Functional food

1. Introduction

There is an observed increase in the presence of probiotic, prebiotic or vitamin-fortified, lactose-free dairy products on the market shelves. In the proposed project, considering the growing demand for lactose-free dairy products, the use of dried figs as a natural prebiotic source as well as aroma contribution, and *Bacillus clausii* as a probiotic were investigated to enhance the functional properties of the product. Turkey is rich in various types of figs due to its unique ecological conditions and is a world leader in the cultivation and trade of dried and fresh figs [1]. In the development of lactose-free probiotic milk with added dried figs, the project has benefited from the taste and aroma profile of figs while also using them as a rich source of fiber, vitamins, and minerals [2].

Probiotics can exhibit positive effects on health as well as antagonistic effects against pathogens, produce antimicrobial compounds, stabilize the intestinal microbiota, and maintain viability during storage [3]. In this context, the use of probiotics for strengthening the immune system has been recommended. It is known that traditional probiotic milks generally use species of *Lactobacillus* and *Bifidobacterium*. However, in recent years, it has been observed that other probiotic bacteria species are also being investigated. The commercial production of milk and dairy products containing probiotic bacteria of the *Bacillus* species is not widespread in Turkey. However, *Bacillus clausii* has been used in various food products abroad and in capsules sold as probiotic food supplements. *Bacillus clausii* is a part of the natural microbiota of the human intestine and is also used in cases such as diarrhea caused by antibiotics. Additionally, some studies have shown that *Bacillus clausii* strengthens the immune system and improves intestinal health [4]. The main reason for considering *Bacillus* species as probiotics is primarily due to the spores they form [5,6]. While forming spores, they have shown resistance to extreme high or low temperatures, mineral substances, hypertonic environments such as salt and sugar, and have been able to develop under these conditions [7].



The hypothesis of the study is "Lactose-free milk enriched with probiotics and dried figs is preferred by consumers in terms of sensory properties and its consumption has a positive effect on digestive system health".

2. Materials and Methods

2.1. Raw materials

Dried figs were purchased from a local market (Aydın, Turkey). Milk was purchased from a local dairy farm (Tire, İzmir, Turkey). Enterogermina® (Sanofi S.p.A., Italy) was purchased from a local pharmacy (Aydın, Turkey).

2.2. Lactose-Free Milk and Dried Fig Puree

The lactose content in the milk was reduced to 0.1% or below by breaking down lactose with β -galactosidase enzyme (10°C/5 hours, 0.07% v/v). This lactose-free milk was used in the study. Whole figs contain higher levels of compounds such as tannins, lignin, cellulose, and hemicellulose, especially derived from the skin of figs and leading to a bitter taste profile. Therefore, the preparation of fig puree was carried out by using only the flesh and seeds of dried figs (excluding the skin). Firstly, the dried figs to be used were soaked in warm water for 24 h. After soaking the figs in water, the flesh was separated, and only the inner part of the figs, excluding the skin, was used. In the next step, the obtained flesh and seeds were homogenized using a household blender to achieve a uniform mixture. This step was implemented to ensure homogeneous distribution of figs in the milk beverage. A pre-sensory evaluation of different percentage ratios (2%, 4%, 6%, and 8% w/v) of dried fig puree was conducted by 8 semi-trained panelists who were selected from students and faculty members of the Food Engineering Department at Aydın Adnan Menderes University. The ratio of dried fig puree as 6% (w/v) indicated desired taste profile of the fig-added lactose-free milk beverage.

2.3. Evaluation of Probiotic Properties

Bacillus clausii spores were revived from Enterogermina® (Sanofi S.p.A., Italy) by inoculating them into Tryptic Soy Broth (TSB) (Merck) medium. Addition of 500 μ l of *Bacillus clausii* spores into 5ml of nutrient medium resulted in 10⁹ CFU/g live probiotic microorganisms (*B. clausii* vegetative cells). Based on this, solutions containing *Bacillus clausii* spores were added to lactose-free milk with 6% (w/v) dried figs at the rates of 1%, 1.5%, and 2% (w/v). The Pasteurization process was applied to the final product in glass jars at 65°C for 30 minutes. After pasteurization, the samples were allowed to reach room



temperature, and the probiotic bacteria count was determined on day 0 according to the special method for *Bacillus clausii* [8]. After being stored for 24 hours at 4°C, the probiotic bacteria count was also determined on day 1. The samples were diluted using the standard serial dilution method. Dilutions were prepared in sterile peptone water and spread plate method was used to inoculate Tryptic Soy Agar (TSA) medium. Petri dishes were then incubated at 37°C for 2 days and the colonies were counted.

2.4. In vitro Digestion Analysis

The viability of the probiotic microorganisms was determined after digestion. In vitro digestion was performed in simulated stomach and intestinal environments [9]. For this purpose, stock solutions representing simulated gastric fluids (SGF) and simulated intestinal fluids (SIF) with appropriate pH and enzyme combinations representing the stomach and small intestine environments were prepared. All stock solutions were allowed to reach 37°C in a water bath before digestion analysis, and then the sample was mixed with a 1:10 ratio of stock solution (SGF/SIF) for each phase. For digestion in the stomach environment, 1 g of the sample was mixed with 1.6 g of pepsin, 1 g of NaCl, and 3.5 ml of HCl (36.46 g/mol), prepared with distilled water to obtain a volume of 500 mL and adjusted to pH 1.2, using 10 mL of SGF. The mixture was incubated in a water bath at 37°C and 100 rpm for 2 hours, followed by termination of digestion. For digestion in the intestinal environment, 1 g of the sample was mixed with 6.67 g of pancreatin, 4.53 g of KH₂PO₄, and 51.3 ml of NaOH (0.2N), prepared with distilled water to obtain a volume of 500 mL and adjusted to pH 6.8, using 10 mL of SIF. The mixture was incubated in a water bath at 37°C and 100 rpm for 2 hours, followed by termination of digestion. After passing through a sterile filter (0.45 µm), the solution was transferred to sterile tubes. Serial dilutions were plated on Tryptic Soy Agar medium and incubated at 37°C for 48 hours to determine the viable count of *B. clausii* bacteria passing through the simulated stomach and intestinal environments.

2.5. Sensory Analysis

Sensory analyses were conducted with a panel consisting of 60 consumers aged between 20-50 years, including faculty members, research assistants, and students from the Department of Food Engineering at Adnan Menderes University, all of whom were regular consumers of milk (at least once a week). All panelists were familiar with basic sensory evaluation techniques, and before the sensory evaluation, they participated in



description sections to familiarize themselves with the specific vocabulary used to describe dairy beverages. Approximately 40 mL samples were served in white polyethylene cups at room temperature (25°C) for each analysis. Samples were coded with three-digit random numbers, and water was used for palate cleansing between samples. Appearance, aroma, flavor, sweetness, texture, and overall liking were evaluated using a 5-point hedonic scale.

2.6. Storage

Following the new product development process, it is also important to monitor the quality changes and the ability of the product to maintain its probiotic properties during storage. During storage of the developed lactose-free fig probiotic milk beverage at 4°C, color, total viable bacteria count, and probiotic bacteria counts were determined in order to have information about the shelf-life.

3. Results

3.1. Probiotic Properties

The results showed that milk samples in which *Bacillus Clausii* spores were added and that underwent pasteurization, the viable *Bacillus Clausii* counts were 6.22 ± 0.11 log cfu/g, 7.58 ± 0.04 log cfu/g, and 7.07 ± 0.01 log cfu/g for the 1%, 1.5%, and 2% additions, respectively. The highest viability was observed in the sample with a 1.5% addition of *Bacillus Clausii* spores. For a product to be considered "probiotic," the count of probiotic microorganisms must exceed a certain threshold. The accepted minimum probiotic count is at least 10^6 (1 million) viable probiotic microorganisms per gram or milliliter by the product's expiration date. This amount is deemed the minimum level required for probiotics to have beneficial health effects [10]. *Bacillus Clausii* addition rates were found sufficient in all three samples; however, the 1.5% addition was preferred due to the highest observed proliferation.

3.2. In vitro Digestion

Based on the data obtained, the average count of *Bacillus clausii* probiotic bacteria in the stomach contents was determined to be 6.24 ± 0.07 log CFU/g. In the intestines, this value was found to be 8.35 ± 0.05 log CFU/g. When compared to the numerical values of other probiotic bacteria strains in the stomach and intestines, some significant differences emerge. For instance, the counts of *Lactobacillus* and *Bifidobacterium* strains in the stomach



contents, as noted in previous studies, are significantly lower than those of *Bacillus clausii*. Similarly, the counts of Lactobacillus and Bifidobacterium strains in the intestines are also lower than those of *Bacillus clausii* [11].

These differences indicate that different probiotic strains behave differently throughout the digestive system and that their survival abilities vary depending on the stomach and intestinal environments. For example, it can be inferred that *Bacillus clausii* has a weaker survival ability in the stomach environment but can colonize the intestinal flora more effectively. The findings suggest that the transition and survival abilities of probiotic strains throughout the digestive system should be considered when selecting and using probiotics to support digestive health.

3.3. Sensory evaluation

Sensory analysis results for 3 different milk samples presented to the panelists are shown in Table 1. Samples include Lactose-free milk with added probiotics and dried fig (MWPF), reference sample as Lactose-free milk(R), and Lactose-free milk with added dried fig (MWF).

Table1. Sensory analysis results for lactose-free probiotic milk with and without dried figs compared with the standard milk sample.

| Product code | Appearance | Aroma | Taste | Sweetness | Texture | Overall Liking |
|--------------|-------------------------|---------------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| MWPF | 4,00 ^b ±0,9 | 4,27 ^a ±0,84 | 4,10 ^{a,b} ±0,9 | 4,17 ^a ±0,88 | 4,21 ^a ±0,87 | 4,23 ^a ±0,72 |
| R | 4,46 ^a ±0,68 | 3,77 ^b ±0,95 | 3,90 ^b ±1,06 | 3,67 ^b ±1,0 | 4,19 ^a ±0,73 | 3,92 ^a ±0,85 |
| MWF | 3,92 ^b ±0,9 | 4,02 ^{a,b} ±0,98 | 4,33 ^a ±0,78 | 4,31 ^a ±0,69 | 4,08 ^a ±0,79 | 4,19 ^a ±0,7 |

According to the statistical results, the average values for appearance are as follows: 4.46^a ± 0.68 for the reference sample, 3.92^b ± 0.9 for the milk sample with added dried fig, and 4.00^b ± 0.9 for the probiotic milk sample with added dried fig. No statistically significant difference (p>0.05) was found between the appearances of the newly formulated samples. However, as expected, they differ from the reference milk sample in appearance (p<0.05). For aroma, the average values are as follows: 3.77^b ± 0.95 for the reference sample, 4.02^{a,b} ± 0.98 for the milk sample with added dried fig, and 4.27^a ± 0.84 for the probiotic milk sample with added dried fig. There was an improvement in aroma of the newly formulated samples (p<0.05). When evaluating taste, the average values are as follows: 4.33^a ± 0.78 for the milk sample with added dried fig, 3.90^b ± 1.06 for the reference sample, and 4.10^{a,b} ± 0.9 for the probiotic milk sample with added dried fig. The milk sample with added dried fig received the highest score, indicating it was found to be tastier than the



probiotic milk sample with added dried fig, although the difference was not statistically significant ($p > 0.05$). For sweetness, the newly formulated samples were rated $4.17^a \pm 0.88$ and $4.31^a \pm 0.69$, with no statistical difference observed between them ($p > 0.05$). However, the reference sample was rated $3.67^b \pm 1.0$, which was significantly different ($p < 0.05$) from the newly formulated samples. Regarding texture, no statistical difference ($p > 0.05$) was found between the reference sample and the newly formulated milk samples.

Lastly, according to the statistical results, the overall preference values are as follows: $3.92^a \pm 0.85$ for the reference sample, $4.19^a \pm 0.7$ for the milk sample with added dried fig, and $4.23^a \pm 0.72$ for the probiotic milk sample with added dried fig. No statistical difference was found among the three milk samples ($p > 0.05$). However, the probiotic milk sample with added dried fig received the highest score, making it the most preferred milk sample.

3.4. Storage results

During the storage period, total aerobic bacteria count (TABC < 30 colonies) were detected below 1 log cfu/g on days 1, 3, 5 and 10. This indicates that there was no microbial contamination during the production process. However, an increase in the number of microorganisms was observed at the end of the 14th day. While the TABC of the samples was determined as 1.45 ± 0.03 log cfu/g at day 14, this value reached 3.67 ± 0.06 log cfu/g at day 21. In addition, it was observed that phase separation started in the samples on day 21; this situation, supported by the decrease in the number of probiotic bacteria, indicates that the shelf life of the product has been completed.

According to the results obtained from storage analyses, a high initial *Bacillus clausii* bacteria count was detected. On the first day, the probiotic bacteria count was found to be 7.34 ± 0.35 log cfu/g. In the analysis carried out on the third day, the probiotic bacteria count was measured as 7.33 ± 0.07 log cfu/g, and on the fifth day, it was measured as 7.29 ± 0.05 log cfu/g. The bacteria peak value was 7.40 ± 0.02 log cfu/g on the 10th day, and then the counts decreased to 7.00 ± 0.01 log cfu/g. On the 21st day, the probiotic bacteria count was found below 6 log cfu/g.

Considerable changes were observed in the L^* values representing the lightness value of the product in the color parameters. While an increase in the L^* value was observed at the end of the 5th day, a decrease was observed on the 10th and 14th days. a^* values indicated a tendency of the product towards a reddish hue. The increases on the 5th and 10th days suggest that the pigments present in dried figs influenced the color tone of the



product. The generally consistent nature of b^* values suggested that the yellowish color components of the product remained consistent throughout the storage process.

4. Discussion and Conclusion

This study successfully developed a lactose-free milk beverage enriched with dried fig and *Bacillus clausii* probiotics. Throughout the production process, from pasteurization to the addition of probiotics and in vitro digestion, the beverage maintained its nutritional integrity. During storage at 4°C, the beverage exhibited stable color, and viable probiotic counts, ensuring its quality. The resulting product is beneficial for individuals with lactose intolerance and increasing probiotic counts in the intestines. This innovative beverage offers a functional dairy alternative with probiotic benefits. This project has completed a significant R&D effort for a functional dairy product that has not yet been commercially produced and aimed to demonstrate the feasibility of new research ideas. The successful completion of this study has made a significant contribution to the practical application of knowledge and skills acquired through food engineering education.

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