BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Impact of Elaeagnus angustifolia flour added to bio-yogurt on probiotic survival and monitoring of in vitro acid tolerance in synthetic gastric fluid

Alper Güngören[1]*, Ayşegül Demircioğlu[2], Yasin Akkemik[1], Ahmet Güner[3]

[1]Kastamonu University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kastamonu - Turkey
[2]Uludağ University, Institute of Health Sciences, Department of Food Hygiene and Technology, Bursa - Turkey
[3]Selçuk University, Faculty of Veterinary Science, Department of Food Hygiene and Technology, Konya - Turkey

***Corresponding Author:** Alper Güngören, Kastamonu University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Kuzeykent Neighborhood, Orgeneral Atilla Ateş Paşa Street, No: 19, ZIP CODE: 37150, Kastamonu - Turkey, e-mail: agungoren@kastamonu.edu.tr

Cite as: Güngören, A., Demircioğlu, A., Akkemik, Y., & Güner, A. (2023). Impact of elaeagnus angustifolia flour added to bio-yogurt on probiotic survival and monitoring of in vitro acid tolerance in synthetic gastric fluid. *Brazilian Journal of Food Technology*, *26*, e2023070. https://doi.org/10.1590/1981-6723.07023

Abstract

The present study aimed to investigate the effect of oleaster flour on *Lactobacillus acidophilus, L. delbrueckii* subsp. *bulgaricus,* and *Bifidobacterium animalis* subsp. *lactis* in probiotic yogurt during its storage period and whether oleaster flour has a protective effect against gastric fluid for these probiotic bacteria. For that purpose, the effect of oleaster flour at different doses (1%, 2%, and 3% w/v) on the titratable acidity, pH, and microbiological properties was investigated throughout cold storage. In addition, on the first day of storage, *in vitro* tolerance of probiotics in pH adjusted to (pH 2.0-ph 4.0) simulated gastric fluid was investigated for 1, 60, 120, and 180 min. Yogurt with a higher dosage (2%-3%) of oleaster flour had a higher pH and lower titratable acidity. Moreover, the addition of 3% oleaster flour showed a preservative effect on *L. acidophilus, L. delbrueckii* subsp. *bulgaricus* and *B. animalis* subsp. *lactis* during storage. On the first day of storage in pH 4.0 for synthetic gastric fluid, *in vitro* acid tolerance of all probiotics showed stability for 180 minutes. Also, at pH 2.0 SGF, *B. animalis* subsp. *lactis* was below the detectable limit in the control and 1% of groups. However, the 2% and 3% groups showed nearly 3 log cfu/g viability at the end of 180 min. These positive effects were related to the buffering effect of the oleaster peel. Thus, these results could prove that oleaster flour can be used for the production of bio-yogurt.

Keywords: Bio-yogurt; Probiotic survive; Gastric tolerance; Oleaster; *Bifidobacterium animalis* subsp. *lactis*; *Lactobacillus acidophilus*.

Highlights

- Oleaster peel may have a buffering effect on milk acidity
- The acid tolerance of probiotics may increase when oleaster fruit is added to the yogurt
- To improve quality, bio-yogurt can be fortified with 2% and 3% of oleaster flour

1 Introduction

The functional food industry needs to discover new areas due to increasing consumer demands for public health. As conscious consumers know, dairy-based functional products improve the function of the immune system and reduce cancer risks (Pandey et al., 2016; Sarkar, 2019; Yildiz & Ozcan, 2019). Particularly, yogurt is a long-time known and consumed milk product thus comprising fermented milk with standard symbiotic starter cultures. In general, yogurt can be separated into two groups: standard culture yogurt and bio-yogurt (probiotic yogurt). Standard culture contains two species of bacteria named *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These strains may not play a well role as probiotics in the host (McFarland, 2015; McKinley, 2005; Sarkar, 2019). Meanwhile, bio-yogurt, or probiotic yogurt, is supported with probiotic strains such as *Bifidobacterium animalis* subsp. *lactis*, *L. acidophilus*, *L. brevis,* etc. (Meybodi et al., 2020). Probiotics are simply defined as live microorganisms that provide health benefits to users when consumed in sufficient numbers (Hill et al., 2014). Therefore, probiotic products need to contain an adequate number of viable probiotic cultures from at least 6 to 9 log CFU per serving at that time of consumption to certify their health effects (Kaur et al., 2022). When bio-yogurt is consumed, probiotic bacteria must first survive the passage through the upper gastrointestinal tract and then linked to provide beneficial effects to the host. It's known that the low pH of gastric juice and the antimicrobial effect of gastric pepsin act as hurdles against the transportation of bacteria into the intestinal tract. Generally, the pH of the stomach varies between 2.5 and 3.5. However, it may range from 1.5 to 6.0 in relation to food intake depending on some factors such as diet (excessive protein intake), anti-acid drug use, age, etc. (Abuhelwa et al., 2017; Huang & Adams, 2004). Foods are the predominant delivery system for probiotic bacteria throughout the gastrointestinal tract. Even if fermented milk is a relatively good protector for ingested bacteria under gastrointestinal tract conditions, some research has shown that so many factors, such as storage conditions, acidity, and oxidative stress, affect the survival of these bacteria. Consequentially, these factors decrease the viability of probiotic bacteria in a short time (Champagne et al., 2018; Thomas, 2016).

Elaeagnus angustifolia L.*,* often known as oleaster, Russian olive, or silver berry, is a member of the *Elaeagnacea* (Araliaceae) family and has more than 90 species. It is mostly grown in parts of Asia, Europe, and North America. The oleaster (*E. angustifolia* L.) is defined as a relatively small, reddish brown, elliptical fruit (Hamidpour et al., 2016). Also, these fruits have high nutritional value. Approximately, oleaster contains 27.1% of glucose, 22.3% of fructose, and 12% of protein (Akbolat et al., 2008; Sahan et al., 2015). Previously, the oleaster flour (OF) effect on set-type yogurt physicochemical, textural, and microstructural characteristics was researched by Öztürk et al. (2018). According to Öztürk et al. (2018), yogurt enrichment with OF accelerated the fermentation time, reduced syneresis, enhanced cohesiveness and viscosity index, and improved antioxidant activity. Moreover, researchers didn't observe any statistical difference between the control and 2% of oleaster samples with regards to flavor or general acceptability in sensory properties. Besides all these positive effects, may oleaster also positively affect the probiotic cultures in bio-yogurt? To the best of our knowledge, there is limited information in the literature about the effect of oleaster flour on probiotic microorganisms. Thus, current research has two aims. The first one was to investigate the effect of oleaster flour on *L. acidophilus, L. delbrueckii* subsp. *bulgaricus,* and *Bifidobacterium animalis* subsp. *lactis* in probiotic yogurt during its storage period. The second aimed to investigate whether oleaster flour has a protective effect against gastric fluid for these probiotic bacteria.

2 Materials and methods

The oleaster fruits were purchased from a local market. The OF was prepared as described by Öztürk et al. (2018). The unpeeled oleaster fruits were pitted and incubated for 12 h at 30 °C. Afterward, the oleasters were milled with an ultra-centrifugal mill (ZM 200, Retsch, Germany). Commercial UHT sterile whole milk (3% of fat, 3% of protein, and 4.5% of carbohydrates) and commercial freeze-dried

yogurt culture (VİVO, Food and Dairy Product Industry and Trade Co. Ltd.) in a direct vat set containing *S. thermophilus, L. acidophilus, L. delbrueckii* subsp. *bulgaricus, and B. animalis* subsp. *lactis* (at least 9 log cfu/g) were purchased from a local market.

2.1 Yogurt production and groups

The standardized whole milk without any treatment was used as a control for experimental yogurt production. In the present study, OF was added to milk at a concentration of 1.0%, 2.0%, and 3.0% (w/v). For each group, heat treatment was used at 90 ± 2 °C for 10 minutes and then cooled to 42 ± 2 °C. The milk was then inoculated with freeze-dried yogurt cultures in the amount recommended by the manufacturer (1 vial/5L) and thoroughly mixed with a sterile spoon. The groups were poured into the sterilized cups (200 ml) and incubated at 42 ± 2 °C. When the pH level hit around 4.6, fermentation was stopped, and samples were closed with a lid and cooled to 4 ± 2 °C in the refrigerator (Figure 1).

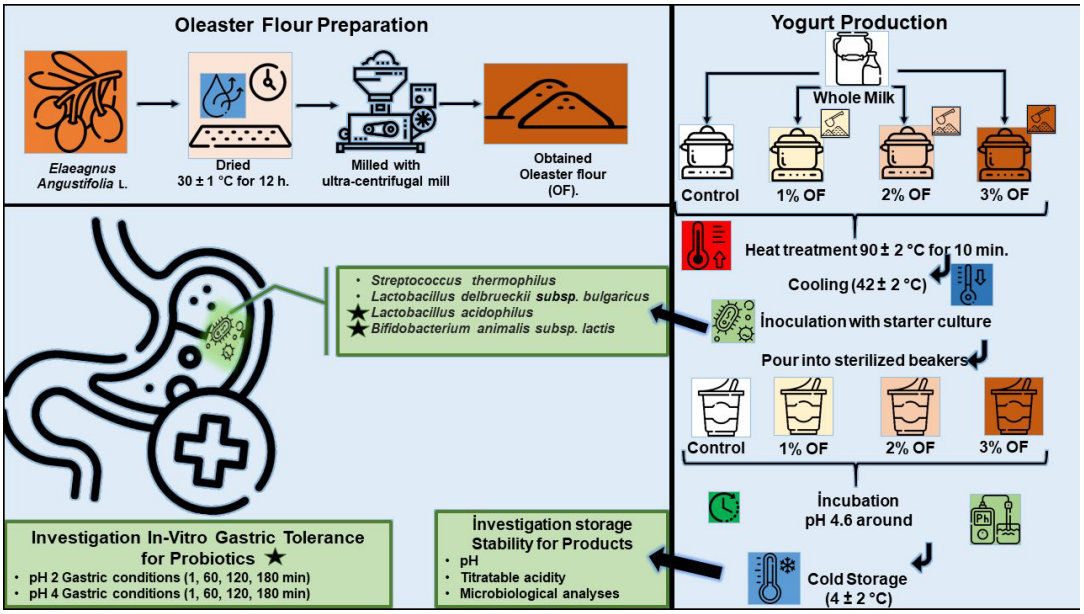

Figure 1. Schematic illustration of the preparation of oleaster flour, preparation of yogurt with oleaster flour, and analyses.

2.2 pH and Titratable acidity analyses

Titratable acidity was determined using the titration method (Tyl & Sadler, 2017), and pH values were measured by using a digital pH meter (Hanna Instruments, HI-4221, USA).

2.3 Microbiological analyses

All microbiological analyses were performed using the pour plate method described in the ISO standard (International Organization for Standardization, 2003). *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *bulgaricus* were enumerated using pH-modified MRS agar base (Himedia, Mumbai, India) (pH 5.2, anaerobic, 43 ± 1 °C, 72 h) (Tharmaraj & Shah, 2003). For the enumeration of *S. thermophilus*, M17 agar base (Himedia, Mumbai, India) (aerobic, 35 ± 1 °C, 48 h) was used (Corry et al., 2003). For enumeration of *B. animalis* subsp. *lactis*, modified MRS (mMRS) agar was used as previously described by Allgeyer et al. (2010). To prepare mMRS, 5 mL of a 10% L-cysteine hydrochloride solution (LCH, Merck, Darmstadt, Germany), 10 mL of an 11% lithium chloride (LiCl) solution (Merck, Darmstadt, Germany), and 5 mL of a

0.01% dicloxacillin antibiotic solution (Merck, Darmstadt, Germany) were added per liter of MRS agar base. The plates were incubated under anaerobic conditions at 35±1 °C for 72 hours. Microbiological analyses were measured after 1, 7, 14, 21, and 28th days of storage at 4 ± 2 °C.

2.4 Monitoring of in vitro gastric tolerance analysis

Synthetic gastric fluid (SGF) was prepared to determine *in vitro* gastric tolerance to probiotic bacteria in formulated yogurt. Briefly, Pepsin (1:10 000, ICN) (Merck, Darmstadt, Germany) was suspended to the ultimate concentration of 3 g/L in sterile NaCl (0.05%). Then, a prepared solution was filtered and sterilized (0.22-µm SFCA syringe filter). SGF was prepared considering the pH changes in the stomach, which were brought to two different pH values. The solution of pH was adjusted to 2.0 and 4.0 with concentrated HCl (Huang &Adams, 2004). On the first day of storage, one gram of yogurt from each group was transferred into sterile falcon tubes containing 9 mL of gastric juice (adjusted pH 2 and pH 4) at 37 °C. The mixtures were homogenized by using a vortex for 10 s and incubated at 37 °C. Afterward, 1 mL of aliquots was removed from mixtures after 1, 60, 120, and 180 min for determine gastric juice tolerance. Samples were serially diluted with 0.1% peptone water, and then *Lactobacillus* and *B. animalis* subsp. *lactis* counts were determined as previously described.

2.5 Statistical analyses

All data analyses were performed using a computer program (SPSS software version 21). Changes in bacterial count, gastric tolerance analysis, pH, and titratable acidity were analyzed using the one-way Analysis of Variance (ANOVA) method to detect significant differences. Samples were analyzed in triplicate. All data were expressed as mean ± standard deviation of all replicates. The means of the results were compared using Duncan's test, with a 95% confidence interval.

3 Results and discussion

3.1 Titratable acidity and pH

It is known that during the fermentation of yogurt at 42 °C, the microorganisms produce acid and lower the pH, which continues throughout cold storage (Yue et al., 2022). The results of pH and titratable acidity (TA) are demonstrated in Figure 2.

The pH and acidity values of the yogurt samples on the first day were different than expected. This can be attributed to the starter culture used or the long time the samples come to 4 ± 2 °C storage temperature. By not cooling the samples quickly as well as performing a rapid increase in the number of lactobacilli can lower the pH and increase the acidity (Yue et al., 2022). Microbiological observations in Table 1. agree with this argument.

During cold storage, pH decreased, and titratable acidity increased. Except for the control group, there was no difference between the experimental groups in terms of titratable acidity after the 7th day of storage ($p > 0.05$), which is consistent with our results (Casarotti & Penna, 2015; Öztürk et al., 2018). It was observed that the group with 3% OF had a higher pH than the control group on the first day of storage. This high pH value persisted until the end of storage except the 21st day. The decrease in pH was more pronounced in the control group than in the samples supplemented with OF throughout the storage time ($p < 0.05$). This situation can be attributed to the presence of some buffering compounds in oleaster fruit peel (Casarotti & Penna, 2015; Öztürk et al., 2018). According to Öztürk et al. (2018), peeled OF showed lower pH and higher acidity than unpeeled OF. Different parts of the fruit contain different proportions of organic acid and soluble sugar, which directly impact pH and titratable acidity (Sarvarian et al., 2022). It may be possible that the same phenomenon could be seen in this research.

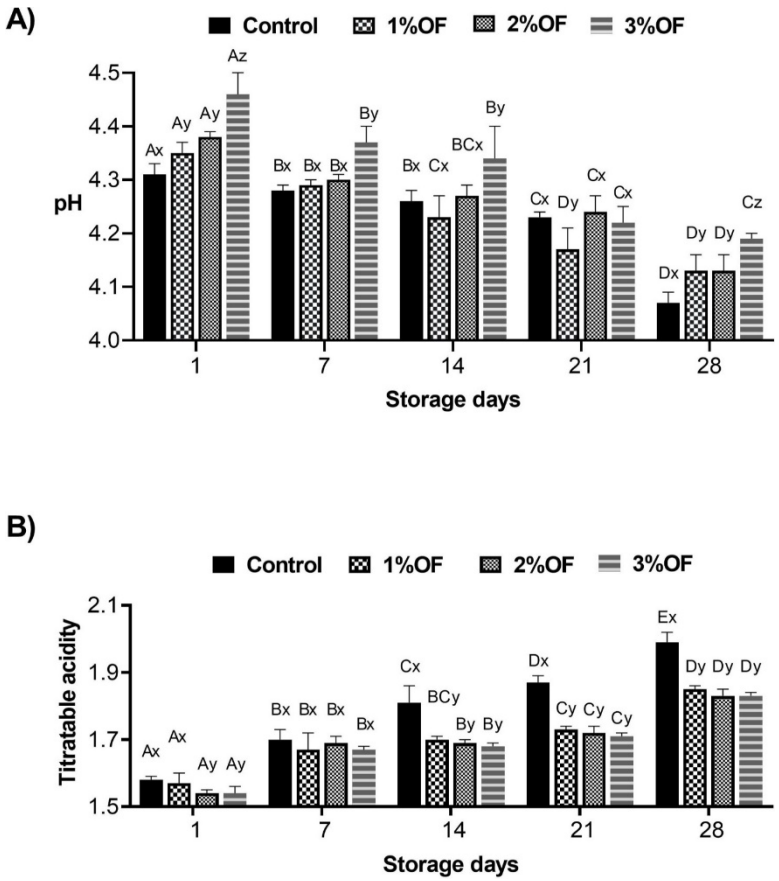

Figure 2. Changes in pH (A) values and titratable acidity (B) of yogurt during storage at 4 ± 2 °C for 28 days. Results are representing mean ± standard deviation (n: 3), with statistical differences $p < 0.05$. A-D: The means with different superscripts among the sampling days are significantly different, xyz: The means with different superscripts among the groups. Control (C) sample without oleaster flour, 1%OF, 2%OF, 3%OF: 1%, 2%, 3% (w/v) oleaster flour added in milk, respectively.

Table 1. The number of *Streptococcus thermophilus*, *Lactobacillus acidophilus, L. delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium animalis* subsp. *lactis* in yogurt samples during cold storage.

Bacteria	Yogurt samples	Storage days				
		1	7	14	21	28
S. thermophilus (log cfu/g)	Control	8.69 ± 0.25^{ax}	8.64 ± 0.34^{ax}	8.63 ± 0.34^{ax}	8.59 ± 0.29^{ax}	8.49 ± 0.49^{ax}
	1% OF	8.70 ± 0.29^{ax}	8.67 ± 0.27^{ax}	8.65 ± 0.25^{ax}	8.57 ± 0.18^{ax}	8.52 ± 0.22^{ax}
	2% OF	8.72 ± 0.23^{ax}	8.67 ± 0.18^{ax}	8.62 ± 0.16^{ax}	8.62 ± 0.30^{ax}	8.54 ± 0.25^{ax}
	3% OF	8.68 ± 0.32^{ax}	8.62 ± 0.27^{ax}	8.61 ± 0.22^{ax}	8.59 ± 0.25^{ax}	8.52 ± 0.29^{ax}
L. acidophilus, and *L. delbrueckii* subsp. *bulgaricus* (log cfu/g)	Control	8.83 ± 0.26^{ax}	8.74 ± 0.28^{ax}	8.67 ± 0.23^{ax}	8.15 ± 0.23^{bx}	7.81 ± 0.22^{bx}
	1% OF	8.80 ± 0.19^{ax}	8.72 ± 0.16^{ax}	8.68 ± 0.19^{ax}	8.17 ± 0.26^{bx}	7.85 ± 0.17^{bx}
	2% OF	8.77 ± 0.26^{ax}	8.74 ± 0.25^{ax}	8.67 ± 0.25^{ax}	8.20 ± 0.28^{bx}	7.91 ± 0.15^{bx}
	3% OF	8.69 ± 0.23^{ax}	8.66 ± 0.21^{ax}	8.64 ± 0.19^{ax}	8.28 ± 0.25^{ax}	8.31 ± 0.26^{ay}
B. animalis subsp. *lactis* (log cfu/g)	Control	6.93 ± 0.20^{ax}	6.64 ± 0.49^{ax}	6.60 ± 0.14^{ax}	5.72 ± 0.20^{by}	5.27 ± 0.27^{bx}
	1% OF	6.79 ± 0.24^{ax}	6.73 ± 0.24^{ax}	6.60 ± 0.31^{ax}	6.03 ± 0.32^{bxy}	5.33 ± 0.25^{cx}
	2% OF	6.73 ± 0.24^{ax}	6.61 ± 0.31^{ax}	6.56 ± 0.34^{ax}	5.97 ± 0.28^{bxy}	5.38 ± 0.40^{cx}
	3% OF	6.67 ± 0.16^{ax}	6.61 ± 0.21^{ax}	6.60 ± 0.29^{ax}	6.49 ± 0.31^{ax}	6.26 ± 0.11^{ay}

OF: Oleaster Flour, Results are expressed as the mean ± standard deviation (n = 3). $^{a\text{-}c}$: The mean values with different letters in the same line are significantly different ($p < 0.05$); ^{xy}: The mean values with different letters in the same column are significantly different ($p < 0.05$).

3.2 Survival of *S. thermophilus, L. acidophilus, L. delbrueckii* subsp. *bulgaricus* and *B. animalis* subsp. *lactis* during cold storage

Variations in the numbers of fermentation bacteria in bio-yogurt (*S. thermophilus, L. acidophilus, L. delbrueckii* subsp. *bulgaricus*, and *B. animalis* subsp. *lactis*) during storage are shown in Table 1. During storage, some losses in viability were observed in the *S. thermophilus* population; these small changes had no microbiological significance, as the changes were less than 0.5 log cfu/g ($p > 0.05$). This slight decrease observed over the storage period was also observed in other studies (Bedani et al., 2013; Casarotti & Penna, 2015; Zare et al., 2011; Zahid et al., 2022). As known, most lactobacilli are microaerophilic, while bifidobacteria are anaerobic and very sensitive to dissolved oxygen levels. Protecting the number of *S. thermophilus* in the last days of storage provides a small advantage over other anaerobic bacteria, as it is able to scavenge reactive oxygen species and prevent lipid peroxidation by reducing oxygen levels (Zahid et al., 2022; Michael et al., 2015).

The initial number of *L. acidophilus and L. delbrueckii* subsp. *bulgaricus* ranged from 8.69 to 8.83 log cfu/g and was similar to the number of streptococci in fresh yogurt. At the end of storage, the viability of lactobacilli was reduced and ranged from 7.85 to 8.31 log cfu/g. At the same time, the viability of lactobacilli was significantly higher in the samples enriched with 3% OF compared with the control group ($p < 0.05$). It is noteworthy to know which compounds in the OF have this protective benefit for lactobacilli. Predictably, lactobacilli's survival might have been caused by the high content of dietary fiber, fructose, and glucose in oleaster (Akbolat et al., 2008; Öztürk et al., 2018; Sahan et al., 2015). According to the current study's results, it may be noted that lactobacilli use compounds found in oleaster as an additional growth source. The lactobacilli data are consistent with other studies on bio yogurt fortified with various fruits such as lentils, apples, bananas, or grapes (Casarotti & Penna, 2015; Kycia et al., 2020; Zare et al., 2011; Zahid et al., 2022).

On the first day, relatively few *B. animalis* subsp. *lactis* were present in all samples. This may be a result of low pH levels, a short fermentation time, a high amount of milk fat, and dissolved oxygen (Kurtuldu & Ozcan, 2018; Meybodi et al., 2020; Ozcan et al., 2017; Thomas, 2016). When the results for *B. animalis* subsp. *lactis* were analyzed, it was found that the duration of storage and the addition of OF significantly affected the viability of the bacteria. The initial number detected varied from 6.67 to 6.93 log cfu/g. Interestingly, this initial number was higher in the non-supplemented group associated with the lactobacilli. This phenomenon can be attributed to the antimicrobial compounds such as organic acids and phenolic acids found in the oleaster (Meybodi et al., 2020). After 28 days of cold storage, the viability of *B. animalis* subsp. *lactis* varied between 5.27 and 6.26 log cfu/g. In the control group, there was a loss of about 1.5 log cfu/g of *B. animalis* subsp. *lactis* during storage. Several studies have found similar results (Bedani et al., 2013; Kycia et al., 2020; Michael et al., 2015).

The results showed that yogurt fortified with OF increased the survival of *B. animalis* subsp. *lactis* during 28 days of cold storage. In general, lactobacilli are able to grow and survive in fermented products with a pH ranging from 3.7 to 4.3 (Nami et al., 2023). On the other hand, bifidobacteria are known to have too low tolerance to acidity (Kycia et al., 2020; Meybodi et al., 2020). Öztürk et al. (2018) found that total soluble solids content increased with increasing concentration of oleaster in yogurt formulation. As the soluble solids content increases, the oxygen damage of bifidobacteria decreases. This could explain the slightly higher *B. animalis* subsp. *lactis* count in the group with 3% OF.

3.3 Assessment of *In Vitro* Gastric Tolerance for *B. animalis* subsp. *lactis, L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*

Pepsin's antibacterial properties and the low pH of the stomach are known to efficiently prevent the passage of microorganisms into the intestinal system. One of the main standards used to choose probiotic strains to ensure their ability to survive is an assessment of gastric tolerance. Generally, probiotic strains

have been evaluated mostly using *in vitro* methods, such as carefully monitored incubations in simulated gastric juice (SGJ) (Gaucher et al., 2019; Sanz, 2007). The effect of different pH-adjusted in SGJ on the survival of *L. acidophilus, L. delbrueckii* subsp. *bulgaricus* and *Bifidobacterium animalis* subsp. *lactis* is presented in Figures 3 and 4, respectively.

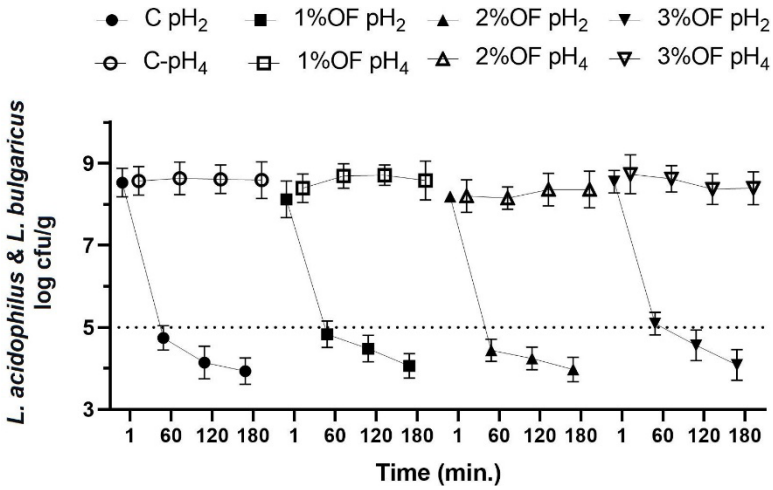

Figure 3. The survival of *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus* of yogurt samples during 180 min in simulated gastric fluid at pH 2.0 and 4.0 on the first day of cold storage.

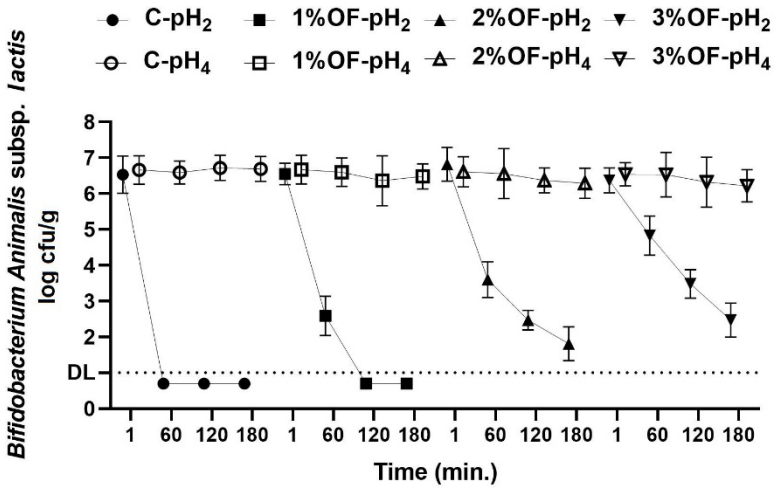

Figure 4. The survival of *Bifidobacterium animalis* subsp. *lactis* of yogurt samples during 180 min in simulated gastric fluid at pH 2.0 and 4.0 on the first day of cold storage.

Probiotic strains showed a progressive decrease at pH 2.0 in viability for 180 minutes. The survival of *L. acidophilus, L. delbrueckii* subsp. *bulgaricus* viability was not different between groups neither at pH 2.0 nor 4.0 ($p > 0.05$). At pH 4.0, the numbers of bacteria in these groups remained the same, but at pH 2.0, there were approximately 4 log cfu/g losses in all groups. The decrease in lactobacillus viability continued over time in all groups. The groups with doses of 2% and 3% OF after 60 minutes were statistically different from the control and 1% OF groups ($p < 0.05$). After 180 minutes, there was no statistically significant change between the groups ($p > 0.05$). Generally speaking, bifidobacteria strains proved to be significantly less acid resistant than lactobacilli (Balthazar et al., 2022). In the current research, our results did not find any difference regarding this subject. However, in exceptional cases, differences in acid stress tolerance can result from the use of different bacterial strains and exposure to different extrinsic or intrinsic factors.

Another study, in disagreement with the present study, showed that in plain and fruity yogurt in synthetic gastric juice adjusted to pH 2.0, *L. acidophilus* counts decreased by about 1.0 and 4.0 log cfu/g after 60 min (Ranadheera et al., 2012). Studies point out that the viability of probiotics in fruit yogurts is limited due to increased acidity (Casarotti & Penna, 2015; Ranadheera et al., 2012; Zare et al., 2011). However, in this study, there was no difference between the yogurts with OF added and the control group ($p > 0.05$). The presence of oleaster peel may have regulated acidity and then promoted lactobacilli viability.

When the SGF had a pH of 2.0, *B. animalis* subsp. *lactis* viability showed a dramatic decrease at pH 2.0 in the control group. After 60 minutes, it was below the detection limit. The group with the addition of 1% OF was also below the detection limit in the analysis after 120 minutes ($p < 0.05$). As the oleaster addition increased, *B. animalis* subsp. *lactis* viability increased, and all minute differences were statistically significant in treated groups ($p < 0.05$). The researchers likewise investigated the survival of *B. animalis* subsp. *lactis* and *L. acidophilus* in plain and fruity yogurt made from goat's milk in synthetic gastric juice adjusted to pH 2.0 and pH 4.0 (Ranadheera et al., 2012). According to researchers, in plain and fruit yogurt, *B. animalis* subsp. *lactis* has been found below the detection limit at pH 2.0 after 30 and 60 min, respectively. Current research results for *B. animalis* subsp. *lactis* is consistent with Ranadheera et al. (2012).

Matsumoto et al. (2004) found that strains from *B. longum*, *B. adolescentis*, and *B. pseudocatenulatum* dramatically reduced after an hour of incubation at a pH 3.0 solution. However, the *B. animalis* strain survived the exposure to pH 3.0–5.0 for 3 h. Moreover, another study showed the viability of *L. acidophilus* and *B. animalis* subsp. *lactis* that remained mostly unaffected by simulated gastric transit at pH 3.0 and pH 4.0 in plain and fruit yogurt (Ranadheera et al., 2012). Similarly, in this study, when the SGF mixture was adjusted to PH 4.0, *B. animalis* subsp. *lactis* survived in all groups for 180 minutes ($p > 0.05$).

4 Conclusion

Although plain and fruit yogurts have a similar shelf life, their physicochemical and microbiological properties are different. Especially tiny changes in acidity can have harmful consequences for probiotics. It is known that fruit acid increases acidity in fruit yogurts and negatively affects some probiotic viability. However, in this study, the presence of some substances from oleaster peel slightly buffered the progression of acidity.

The results of the present investigation showed that even though the survival of *B. animalis* subsp. *lactis,* *L. acidophilus,* and *L. delbrueckii* subsp. *bulgaricus* is affected by pH 2.0, these strains may survive well at pH 4.0 in stomach conditions. Also, the amount of surviving probiotic bacteria was significantly higher as the amount of oleaster added to the yogurt increased in gastric fluid conditions adjusted to pH 2.0. So, these results showed that oleaster flour can be applied to produce bio-yogurt.

References

Abuhelwa, A. Y., Williams, D. B., Upton, R. N., & Foster, D. J. R. (2017). Food, gastrointestinal pH, and models of oral drug absorption. *European Journal of Pharmaceutics and Biopharmaceutics*, *112*, 234-248. PMid:27914234. http://dx.doi.org/10.1016/j.ejpb.2016.11.034

Akbolat, D., Ertekin, C., Menges, H. O., Guzel, E., & Ekinci, K. (2008). Physical and nutritional properties of oleaster (Elaeagnus angustifolia L.) growing in Turkey. *Asian Journal of Chemistry*, *20*(3), 2358.

Allgeyer, L. C., Miller, M. J., & Lee, S. Y. (2010). Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics. *Journal of Dairy Science*, *93*(10), 4471-4479. PMid:20854980.

Balthazar, C. F., Guimarães, J. F., Coutinho, N. M., Pimentel, T. C., Ranadheera, C. S., Santillo, A., Albenzio, M., Cruz, A. G., & Sant'Ana, A. S. (2022). The future of functional food: emerging technologies application on prebiotics, probiotics and postbiotics. *Comprehensive Reviews in Food Science and Food Safety*, *21*(3), 2560-2586. PMid:35470949.

Bedani, R., Rossi, E. A., & Saad, S. M. I. (2013). Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. *Food Microbiology*, *34*(2), 382-389. PMid:23541206. http://dx.doi.org/10.1016/j.fm.2013.01.012

Casarotti, S. N., & Penna, A. L. B. (2015). Acidification profile, probiotic in vitro gastrointestinal tolerance, and viability in fermented milk with fruit flours. *International Dairy Journal*, *41*, 1-6. http://dx.doi.org/10.1016/j.idairyj.2014.08.021

Champagne, C. P., Gomes da Cruz, A., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, *22*, 160-166. http://dx.doi.org/10.1016/j.cofs.2018.04.008

Corry, J. E. L., Curtis, G. D. W., & Baird, R. M. (2003). M17 agar. In J. E. L. Corry, G. D. W. Curtis & R. M. Baird (Eds.), *Handbook of culture media for food microbiology*. Burlington: Elsevier.

Gaucher, F., Bonnassie, S., Rabah, H., Marchand, P., Blanc, P., Jeantet, R., & Jan, G. (2019). Review: adaptation of beneficial propionibacteria, lactobacilli, and bifidobacteria improves tolerance toward technological and digestive stresses. *Frontiers in Microbiology*, *10*, 841. PMid:31068918.

Hamidpour, R., Hamidpour, S., Hamidpour, M., Shahlari, M., Sohraby, M., Shahlari, N., & Hamidpour, R. (2016). Russian olive (Elaeagnus angustifolia L.): from a variety of traditional medicinal applications to its novel roles as active antioxidant, anti-inflammatory, anti-mutagenic, and analgesic agent. *Journal of Traditional and Complementary Medicine*, *7*(1), 24-29. PMid:28053884. http://dx.doi.org/10.1016/j.jtcme.2015.09.004

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: the international scientific association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews. Gastroenterology & Hepatology*, *11*(8), 506-514. PMid:24912386. http://dx.doi.org/10.1038/nrgastro.2014.66

Huang, Y., & Adams, M. C. (2004). In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. *International Journal of Food Microbiology*, *91*(3), 253-260. PMid:14984773. http://dx.doi.org/10.1016/j.ijfoodmicro.2003.07.001

International Organization for Standardization – ISO. (2003). *ISO 7889: Yogurt - Enumeration of characteristic microorganisms - Colony-count technique at 37C*. Geneva: ISO.

Kaur, H., Kaur, G., & Ali, S. A. (2022). Dairy-based probiotic-fermented functional foods: an update on their health-promoting properties. *Fermentation (Basel, Switzerland)*, *8*(9), 425. http://dx.doi.org/10.3390/fermentation8090425

Kurtuldu, O., & Ozcan, T. (2018). Effect of β-glucan on the properties of probiotic set yoghurt with Bifidobacterium animalis subsp. lactis strain Bb-12. *International Journal of Dairy Technology*, *88*(S1), 34-41. http://dx.doi.org/10.1111/1471-0307.12414

Kycia, K., Chlebowska-Śmigiel, A., Szydłowska, A., Sokół, E., Ziarno, M., & Gniewosz, M. (2020). Pullulan as a potential enhancer of Lactobacillus and Bifidobacterium viability in synbiotic low fat yoghurt and its sensory quality. *LWT*, *28*, 109414. http://dx.doi.org/10.1016/j.lwt.2020.109414

Matsumoto, M., Ohishi, H., & Benno, Y. (2004). H+-ATPase activity in Bifidobacterium with special reference to acid tolerance. *International Journal of Food Microbiology*, *93*(1), 109-113. PMid:15135587. http://dx.doi.org/10.1016/j.ijfoodmicro.2003.10.009

McFarland, L. V. (2015). From yaks to yogurt: the history, development, and current use of probiotics. *Clinical Infectious Diseases*, *60*(Suppl.2), S85-S90. PMid:25922406.

McKinley, M. C. (2005). The nutrition and health benefits of yogurt. *International Journal of Dairy Technology*, *58*(1), 1-12. http://dx.doi.org/10.1111/j.1471-0307.2005.00180.x

Meybodi, N. M., Mortazavian, A. M., Arab, M., & Nematollahi, A. (2020). Probiotic viability in yoghurt: A review of influential factors. *International Dairy Journal*, *109*, 104793. http://dx.doi.org/10.1016/j.idairyj.2020.104793

Michael, M., Phebus, R. K., & Schmidt, K. A. (2015). Plant extract enhances the viability of lactobacillus delbrueckii subsp. Bulgaricus and lactobacillus acidophilus in probiotic nonfat yogurt. *Food Science & Nutrition*, *3*(1), 48-55. PMid:25650127. http://dx.doi.org/10.1002/fsn3.189

Nami, Y., Kiani, A., Elieh-Ali-Komi, D., Jafari, M., & Haghshenas, B. (2023). Impacts of alginate–basil seed mucilage–prebiotic microencapsulation on the survival rate of the potential probiotic Leuconostoc mesenteroides ABRIINW.N18 in yogurt. *International Journal of Dairy Technology*, *76*(1), 138-148. http://dx.doi.org/10.1111/1471-0307.12909

Ozcan, T., Yilmaz-Ersan, L., Akpinar-Bayizit, A., & Delikanli, B. (2017). Antioxidant properties of probiotic fermented milk supplemented with chestnut flour (Castanea sativa Mill). *Journal of Food Processing and Preservation*, *41*(5), e13156. http://dx.doi.org/10.1111/jfpp.13156

Öztürk, H. İ., Aydın, S., Sözeri, D., Demirci, T., Sert, D., & Akın, N. (2018). Fortification of set-type yoghurts with Elaeagnus angustifolia L. flours: effects on physicochemical, textural, and microstructural characteristics. *LWT*, *90*, 620-626. http://dx.doi.org/10.1016/j.lwt.2018.01.012

Pandey, A., Sanromán, M. Á., Du, G., Soccol, C. R., & Dussap, C. G. (2016). *Current developments in biotechnology and bioengineering: Food and beverages industry*. Amsterdam: Elsevier.

Ranadheera, C. S., Evans, C. A., Adams, M. C., & Baines, S. K. (2012). In vitro analysis of gastrointestinal tolerance and intestinal cell adhesion of probiotics in goat's milk ice cream and yogurt. *Food Research International*, *49*(2), 619-625. http://dx.doi.org/10.1016/j.foodres.2012.09.007

Sahan, Y., Gocmen, D., Cansev, A., Celik, G., Aydin, E., Dundar, A. N., Dulger, D., Kaplan, H. B., Kilci, A., & Gucer, S. (2015). Chemical and techno-functional properties of flours from peeled and unpeeled oleaster (Elaeagnus angustifolia L.). *Journal of Applied Botany and Food Quality*, *71*, 157-166.

Sanz, Y. (2007). Ecological and functional implications of the acid-adaptation ability of Bifidobacterium: A way of selecting improved probiotic strains. *International Dairy Journal*, *17*(11), 1284-1289. http://dx.doi.org/10.1016/j.idairyj.2007.01.016

Sarkar, S. (2019). Potentiality of probiotic yoghurt as a functional food – a review. *Nutrition & Food Science*, *49*(2), 182-202. http://dx.doi.org/10.1108/NFS-05-2018-0139

Sarvarian, M., Jafarpour, A., Awuchi, C. G., Adeleye, A. O., & Okpala, C. O. R. (2022). Changes in physicochemical, free radical activity, total phenolic and sensory properties of orange (Citrus sinensis L.) juice fortified with different oleaster (Elaeagnus angustifolia L.) extracts. *Molecules (Basel, Switzerland)*, *27*(5), 1530. PMid:35268631.

Tharmaraj, N., & Shah, N. P. (2003). Selective enumeration of Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, bifidobacteria, Lactobacillus casei, Lactobacillus rhamnosus, and propionibacteria. *Journal of Dairy Science*, *86*(7), 2288-2296. PMid:12906045. http://dx.doi.org/10.3168/jds.S0022-0302(03)73821-1

Thomas, L. V. (2016). Probiotics– the journey continues. *International Journal of Dairy Technology*, *69*(4), 469-480. http://dx.doi.org/10.1111/1471-0307.12354

Tyl, C., & Sadler, G. D. (2017). pH and Titratable Acidity. In: S. S. Nielsen (Ed.), *Food analysis* (pp. 389-406). Cham: Springer.

Yildiz, E., & Ozcan, T. (2019). Functional and textural properties of vegetable-fibre enriched yoghurt. *International Journal of Dairy Technology*, *72*(2), 199-207. http://dx.doi.org/10.1111/1471-0307.12566

Yue, Y., Wang, S., Lv, X., Wang, C., Xu, B., Ping, L., Guo, J., Li, X., Evivie, S. E., Liu, F., Li, B., & Huo, G. (2022). Analysis of the complete genome sequence of Lactobacillus delbrueckii ssp. Bulgaricus with post-acidification capacity and its influence on yogurt in storage. *Journal of Dairy Science*, *105*(2), 1058-1071. PMid:34802736.

Zahid, H. F., Ranadheera, C. S., Fang, Z., & Ajlouni, S. (2022). Functional and healthy yogurts fortified with probiotics and fruit peel powders. *Fermentation (Basel, Switzerland)*, *8*(9), 9. http://dx.doi.org/10.3390/fermentation8090469

Zare, F., Boye, J. I., Orsat, V., Champagne, C., & Simpson, B. K. (2011). Microbial, physical and sensory properties of yogurt supplemented with lentil flour. *Food Research International*, *44*(8), 2482-2488. http://dx.doi.org/10.1016/j.foodres.2011.01.002

Funding: None.

Received: June 05, 2023; **Accepted:** Sept. 11, 2023

Associate Editor: Maria Gabriela Bello Koblitz.