Original Article

Effect of Adding Lactoferrin on Some Foodborne Pathogens in Yogurt

Mai Fetouh[1*], Hend Elbarbary[2], Ekbal Ibrahim[1], Ahmed Maarouf[1]

1. *Animal Health Research Institute-Benha Laboratory, Benha, Egypt.*
2. *Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Benha, Egypt.*

Use your device to scan and read the article online

How to Cite This Article Fetouh, M., Elbarbary, H., Ibrahim, E., & Maarouf, A. (2023). Effect of Adding Lactoferrin on Some Foodborne Pathogens in Yogurt. *Iranian Journal of Veterinary Medicine, 17*(3):189-198. http://dx.doi.org/10.32598/ijvm.17.3.1005313

doi: http://dx.doi.org/10.32598/ijvm.17.3.1005313

Article info:

Received: 25 Sep 2022

Accepted: 03 Dec 2022

Publish: 01 July 2023

ABSTRACT

Background: Lactoferrin is a natural biological active cationic protein that can be used as a yogurt additive to inhibit the growth of foodborne pathogens.

Objectives: The present study evaluated the antimicrobial effects of lactoferrin against *Bacillus cereus*, *Enterococcus faecalis*, and *Candida albicans* inoculated in laboratory prepared yogurt at refrigerator temperature.

Methods: The pre-warmed skimmed milk was inoculated by commercial starter and then divided into 3 parts, each inoculated by different inoculum and a certain concentration of lactoferrin (0.5% and 1.5%) and incubated at 42°C for 4 h till curd formation. Tenfold serial dilutions were performed for each group and refrigerated at4±1°C for up to 14 days to be examined every day.

Results: The obtained results showed that the treated yogurt samples with lactoferrin had significant reductions in *B. cereus*, *Ent. faecalis*, and *C. albicans* counts than untreated samples (control positive). Generally, yogurt samples treated with 1.5% lactoferrin showed the highest reduction percentages on *B. cereus* and *C. albicans* than 0.5% lactoferrin. In addition, *B. cereus* showed more susceptibility to lactoferrin than *Ent. faecalis*; the maximum reduction of the inoculated *B. cereus* was observed on the sixth day of the incubation to be 99.99%. Maximum reduction of the tested *C. albicans* was observed after the seventh day.

Conclusion: The application of lactoferrin showed a potentially significant antimicrobial effect against *B. cereus*, *Ent. faecalis*, and *C. albicans* in refrigerating conditions, so lactoferrin is recommended to be used in yogurt production for safe product manufacturing.

Keywords: Yogurt, Lactoferrin, *Bacillus cereus*, *Enterococcus faecalis*, *Candida albicans*

*** Corresponding Author:**
Mai Fetouh
Address: *Animal Health Research Institute – Benha Laboratory, Benha, Egypt.*
Phone: *+20 (11) 20553158*
E-mail: *maiabdallahphd@gmail.com*

1. Introduction

Yogurt is a fermented dairy product popular among people in Egypt and worldwide. It is a rich source of minerals such as calcium, proteins, fats, and useful microorganisms such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Alighazi et al., 2020). In recent years, scientists have tried to increase the organoleptic and health properties of yogurt using different methods (Fernandez & Marette, 2017).

Its production and consumption are growing continuously due to its health benefits, let alone its high nutritive value (El Kholy et al., 2014). Also, fortifying yogurt with lactoferrin can add more health benefits (Tomita et al., 2009; Tsukahara et al., 2020). However, yogurt is highly vulnerable to bacterial contamination, and hence it is easily perishable (Girma et al., 2014).

Factors affecting the hygienic quality of raw milk, especially mastitis, is an important disease that affects dairy herds and products around the world, which has mainly been caused by different pathogens, including *Staphylococcus aureus* (*S. aureus*). This pathogen tends to form biofilm and may be antibiotic resistance (Foroutan et al., 2022). The microbial quality of yogurt represents the quality of raw milk. Due to unsanitary conditions, there is a possibility of microbial contamination, which may have serious effects on the consumer's health (Qajarbeygi et al., 2017).

Using natural antimicrobial compounds such as lactoferrin in food has gained much attention from consumers and the food industry. This interest is primarily due to two major factors. First, the misuse and mishandling of antibiotics have resulted in the dramatic rise of a group of microorganisms, including foodborne pathogens, that are not only antibiotic-resistant but also more tolerant to several food processing and preservation methods. In addition, increasing consumers' awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products in foods. This condition has prompted the food industry to look for alternative preservatives that can enhance the safety and quality of foods. Compounds derived from natural sources, such as lactoferrin (LF), can be used for food safety due to their antimicrobial properties against a broad range of foodborne pathogens (Tajkarimi et al., 2010; Gyawali & Ibrahim, 2014; Niaz et al., 2019).

Lactoferrin is a glycoprotein that can bind and transfer iron; it is found in many secretions in the body as saliva, serum, and tears, and is highly present in milk and colostrum. Lactic fermentation of foods, such as yogurt, increases the availability of iron (Yen et al., 2011; Lisko et al., 2017).

Lactoferrin is known to be a multifunctional or multitasking protein. It has antimicrobial, antioxidant, anticancer, anti-inflammatory, and immune modulators, and it represents an excellent natural alternative substance that reduces the use of chemical preservatives (Ochoa & Cleary, 2009; Legrand, 2016). So, the present study was planned to determine the antimicrobial effects of LF on the viability of some pathogenic microbes, *Bacillus cereus*, *Enterococcus faecalis*, and *Candida albicans* strains in yogurt.

2. Materials and Methods

Bovine lactoferrin (LF) was purchased from Hygint pharmaceuticals, Alexandria, Egypt. It was prepared using sterile distilled water to obtain LF solution at concentrations of 0.5% and 1.5% (Ombarak et al., 2019). LF is the ideal compound of choice due to its stability and supply of high iron bioavailability, and it does not affect the sensory properties or nutritional value of yogurt (El-Kholy et al., 2011).

Activation of yogurt starter cultures

Lyophilized mixed starter cultures containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) were obtained from the Cairo MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University. Lyophilized mixed starter cultures were added to sterile 11% reconstituted skimmed milk powder and incubated at 37°C for 24 h (Tavakoli et al., 2019).

Inoculum preparation

Pure cultures of *B. cereus* and *E. faecalis* were activated on TSB (tryptic soya broth) at 37°C/24 h. The organisms were activated for 3 successive sub-cultures till obtaining the concentration of 10^6 CFU/mL (Hassan et al., 2011), and *C. albicans* was activated on modified Sabouraud dextrose broth at 25°C±1°C for 2-3 days till obtaining the concentration of 15×10^3 CFU/mL (Laref & Guesses, 2013).

Preparations of yoghurt

The skim milk was heated to 85°C for 30 min and immediately cooled to 45°C, and then inoculated with the activated starter cultures (Corrieu & Be'al., 2016), followed by the addition of the examined pathogen and LF. Samples were grouped as follows:

B. cereus groups

G1: Yogurt made with 2% yogurt starter cultures (control negative).

G2: Yogurt+10^6 CFU/mL *B. cereus* (control positive).

G3: Yogurt+10^6 CFU/mL *B. cereus*+0.5% LF.

G4: Yogurt+10^6 CFU/mL *B. cereus*+1.5% LF.

E. faecalis Groups

G1: Yogurt made with 2% yogurt starter cultures (control negative).

G2: Yogurt+10^6 CFU/mL *E. faecalis* (control positive).

G3: Yogurt+10^6 CFU/mL *E. faecalis*+0.5% LF.

G4: Yogurt+10^6 CFU/mL *E. faecalis*+1.5% LF.

C. albicans Groups

G1: Yogurt made with 2% yogurt starter cultures (control negative).

G2: Yogurt+10^3 CFU/mL *C. albicans* (control positive).

G3: Yogurt+10^3 CFU/mL *C. albicans*+0.5% LF.

G4: Yogurt+10^3 CFU/mL *C. albicans*+1.5% LF.

All samples were packed into sterile polyethylene cups, labeled, and incubated at 44°C till curd formation, then stored at 4°C for 14 days and examined every day to monitor *B. cereus*, *E. faecalis*, and *C. albicans* counts (FDA, 2001; Domig et al., 2003 & ISO, 2008, respectively). Tests were performed in triplicate.

Microbiological examinations

Preparation of samples

It was performed according to ISO (2017).

Counting of inoculated pathogens

B. cereus counting was performed on *B. cereus* agar (FDA, 2001), while *E. faecalis* counting was performed on KF Streptococcus agar (Domig et al., 2003), and *C. albicans* counting on modified Sabouraud dextrose agar (ISO, 2008).

Statistical analysis

The experiment for studying the effect of different lactoferrin concentrations on some isolates was conducted in three repetitions. Data were coded, then entered and analyzed using the SPSS software, version 26-2018 (SPSS; IBM Corp, NY, USA) for Microsoft Windows 10.

3. Results

The antibacterial activity of Lactoferrin on the viability of *B. cereus* in yogurt

The antibacterial activity of LF on the viability of experimentally inoculated pathogenic *B. cereus* strains in yogurt is shown in Table 1. It was revealed that treated groups (0.5% and 1.5% LF) showed lower *B. cereus* counts than the control group, where the mean *B. cereus* counts in examined yogurt samples after the addition of 0.5% LF were lowered from $4.2\times10^6\pm0.06\times10^6$ on day 0 to $1.2\times10^2\pm0.03\times10^2$ on day 14 of storage with reduction percentage from 67.56% to 99.99% on the first and fourteen days, respectively. In the case of using 1.5% LF, the mean counts were lowered from $4.2\times10^6\pm0.06\times10^6$ on day 0 to $1.2\times10^2\pm0.12\times10^2$ on day 9 of inoculation with a reduction percentage from 90.81% to 99.99% on the first and ninth days, respectively, but not detected after that (<10^2 CFU/g) with 100% reduction percentage after the ninth day, comparing to *B. cereus* counts in the control samples which were increased from $4.2\times10^6\pm0.06\times10^6$ on day 0 to $9.6\times10^9\pm0.05\times10^9$ on day 9 of storage. In addition, all results showed a significant ($P<0.05$) decrease (growth inhibition) in *B. cereus* in the yogurt samples treated with 1.5% LF and 0.5% LF during refrigerated storage when compared with non-treated samples.

The antibacterial activity of LF on the viability of *E. faecalis* strain

Table 2 presents the antibacterial activity of LF on the viability of experimentally inoculated pathogenic *E. faecalis* strains in yogurt samples. The treated groups showed lower *E. faecalis* counts than the control groups, where the mean *E. faecalis* counts in examined yogurt samples after the addition of 0.5% LF decreased from

Table 1. Antibacterial activity of lactoferrin on viability of *Bacillus cereus* inoculated into yoghurt during refrigeration storage (4°C)

Storage Time (Day)	Control Group Negative	Control Group Positive	0.5% Lactoferrin Group		1.5% Lactoferrin Group	
		Mean±SEM	Mean±SEM	R%	Mean±SEM	R%
0		$4.2x10^{6}\pm0.06x10^{6}$	$4.2x10^{6}\pm0.06x10^{6}$	--	$4.2x10^{6}\pm0.06x10^{6}$	--
1		$7.4x10^{6}\pm0.01x10^{6a}$	$2.4x10^{6}\pm0.06x10^{6ab}$	67.56	$6.8x10^{5}\pm0.06x10^{5b}$	90.81
2		$9.8x10^{6}\pm0.06x10^{6a}$	$3.7x10^{5}\pm0.03x10^{5ab}$	96.2	$1.1x10^{5}\pm0.03x10^{5b}$	98. 8
3		$1.1x10^{7}\pm0.01x10^{7a}$	$3.1x10^{5}\pm0.03x10^{5b}$	97.18	$7.2x10^{4}\pm0.06x10^{4c}$	99.3
4		$2.1x10^{7}\pm0.07x10^{7a}$	$9.0x10^{4}\pm0.03x10^{4b}$	99.57	$2.8x10^{4}\pm0.08x10^{4c}$	99.86
5		$3.7x10^{7}\pm0.02x10^{7a}$	$5.5x10^{4}\pm0.03x10^{4b}$	99.85	$8.5x10^{3}\pm0.01x10^{3c}$	99.97
6		$5.1x10^{8}\pm0.04x10^{8a}$	$3.4x10^{4}\pm0.03x10^{4b}$	99.99	$4.3x10^{3}\pm0.002x10^{3c}$	99.99
7	ND	$9.4x10^{8}\pm0.03x10^{8a}$	$1.2x10^{4}\pm0.03x10^{4b}$	99.99	$9.6x10^{2}\pm0.008x10^{2c}$	99.99
8		$1.9x10^{9}\pm0.06x10^{9}$	$7.7x10^{3}\pm0.06x10^{3}$	99.99	$5.4x10^{2}\pm0.005x10^{1}$	99.99
9		$9.6x10^{9}\pm0.05x10^{9}$	$4.2x10^{3}\pm0.03x10^{3}$	99.99	$1.2x10^{2}\pm0.10x10^{2}$	99.99
10		S	$1.3x10^{3}\pm0.05x10^{3}$		$<10^{2}$	
11		S	$9.3x10^{2}\pm0.03x10^{2}$		$<10^{2}$	
12		S	$8.9x10^{2}\pm0.05x10^{2}$		$<10^{2}$	
13		S	$9.1x10^{2}\pm0.01x10^{2}$		$<10^{2}$	
14		S	$1.2x10^{2}\pm0.03x10^{2}$		$<10^{2}$	

[a, b, c, ab]Mean values within the same row with different superscript letters are statistically different at P≤0.05.

Abbreviatioins: S: Spoiled sample; SEM: Standard error of means; R%: Reduction % of *B. cereus* count.

$5.9\times10^{6}\pm0.03\times10^{6}$ on day 0 to $1.9\times10^{5}\pm0.03\times10^{5}$ on day 14 of storage with reduction percentage from 23.3% to 99.9% on the first and ninth days, respectively. In the case of using 1.5% LF, the mean counts decreased from $5.9\times10^{6}\pm0.03\times10^{6}$ on day 0 to $1.3\times10^{4}\pm0.03\times10^{4}$ on day 14 of storage with a reduction percentage from 27.4% to 99.9% on first and ninth days, respectively, comparing to *E. faecalis* counts in the control samples which it increased from $5.9\times10^{6}\pm0.03\times10^{6}$ on day 0 to $9.1\times10^{9}\pm0.03\times10^{9}$ on day 9 of storage. Moreover, all results showed a significant (P<0.05) decrease (growth inhibition) of *E. faecalis* in the yogurt samples treated with 0.5% and 1.5% LF during cold storage when compared with non-treated samples.

The antifungal activity of LF on the viability of *C. albicans* in yogurt

The antifungal activity of LF on the viability of experimentally inoculated pathogenic *C. albicans* strains in yogurt is shown in Table 3. The treated groups showed lower *C. albicans* counts than the control group, where the mean *C. albicans* counts in examined yogurt samples after the addition of 0.5% LF decreased from $15\times10^{3}\pm0.03\times10^{3}$ on day 0 to $4.0\times10^{2}\pm0.003\times10^{2}$ on day 11 of storage with reduction percentage from 4.76% to 99.99% at the first and eleventh days, respectively, but not detected after that ($<10^{2}$) with 100% reduction percentage. In the case of using 1.5% LF, the mean counts decreased from $15\times10^{3}\pm0.03\times10^{3}$ on day 0 to $1.0\times10^{2}\pm0.01$ on day 9 of inoculation, with a reduction percentage from 23.81% to 99.9% on the first and ninth days, respectively, but not detected after that ($<10^{2}$) with 100% reduction percentage, comparing to *C. albicans* counts in the control samples which increased from $15\times10^{3}\pm0.03\times10^{3}$ on day 0 to $91\times10^{5}\pm0.06\times10^{5}$ on day 10 of inoculation, but not detected after that as the samples were spoiled. Moreover, all results showed a significant (P<0.05) decrease (growth inhibition) of *C. albicans* in the yogurt samples treated with 0.5% and 1.5% LF during cold storage when compared with non-treated samples.

Table 2. Antibacterial activity of lactoferrin on viability of *E. faecalis* inoculated into yogurt during their refrigeration storage (4°C)

Storage Time (Day)	Control Group Negative	Control Group Positive	0.5% Lactoferrin Group		1.5% Lactoferrin Group	
		Mean±SE	Mean±SE	R%	Mean±SE	R%
0		$5.9x10^{6}±0.03x10^{6}$	$5.9x10^{6}±0.03x10^{6}$	--	$5.9x10^{6}±0.03x10^{6}$	--
1		$7.3x10^{6}±0.1x10^{6a}$	$5.6x10^{6}±0.06x10^{6a}$	23.3	$5.3x10^{6}±0.03x10^{6a}$	27.4
2		$9.5x10^{6}±0.06x10^{6a}$	$5.3x10^{6}±0.03x10^{6ab}$	44.2	$4.5x10^{6}±0.06x10^{6b}$	52.6
3		$2.6x10^{7}±0.06x10^{7a}$	$5.0x10^{6}±0.03x10^{6b}$	80.7	$4.0x10^{6}±0.03x10^{6b}$	84.6
4		$5.4x10^{7}±0.03x10^{7a}$	$4.6x10^{6}±0.06x10^{6b}$	91.4	$3.5x10^{6}±0.05x10^{6b}$	93.5
5		$8.2x10^{7}±0.03x10^{7a}$	$4.1x10^{6}±0.03x10^{6b}$	95.0	$9.1x10^{5}±0.03x10^{5c}$	98.9
6		$3.1x10^{8}±0.06x10^{8a}$	$3.2x10^{6}±0.03x10^{6b}$	98.9	$7.2x10^{5}±0.06x10^{5c}$	99.9
7	ND	$8.1x10^{8}±0.03x10^{8a}$	$2.1x10^{6}±0.03x10^{6b}$	99.7	$4.7x10^{5}±0.03x10^{5c}$	99.9
8		$5.4x10^{9}±0.03x10^{9a}$	$9.0x10^{5}±0.03x10^{5b}$	99.8	$2.4x10^{5}±0.05x10^{5c}$	99.9
9		$9.1x10^{9}±0.03x10^{9a}$	$7.2x10^{5}±0.03x10^{5b}$	99.9	$8.3x10^{4}±0.01x10^{4c}$	99.9
10		S	$5.7x10^{5}±0.06x10^{5}$		$5.6x10^{4}±0.06x10^{4}$	
11		S	$4.5x10^{5}±0.03x10^{5}$		$3.5x10^{4}±0.06x10^{4}$	
12		S	$3.0x10^{5}±0.03x10^{5}$		$3.1x10^{4}±0.03x10^{4}$	
13		S	$2.1x10^{5}±0.03x10^{5}$		$2.6x10^{4}±0.03x10^{4}$	
14		S	$1.9x10^{5}±0.03x10^{5}$		$1.3x10^{4}±0.03x10^{4}$	

[a, b, c, ab]Mean values within the same row with different superscript letters are statistically different at P≤0.05.

Abbreviatioins: S: Spoiled sample; SEM: Standard error of means; R%: Reduction % of *E. faecalis* count.

4. Discussion

Lactoferrin is a natural component used as a food additive to inhibit the growth of pathogenic microorganisms (Ombarak et al., 2019). It is a protein that occurs naturally in milk and nowadays is increasingly supplemented in foods for its multiple functions and its antimicrobial effects on many bacteria and yeast (Zorina et al., 2018 & Niaz et al., 2019). Lactoferrin is essential to produce a supplemented yogurt that could alleviate symptoms caused by some gastrointestinal problems (Bruni et al., 2016; Zarzosa-Moreno et al., 2020).

Concerning the antibacterial effect of LF on *B. cereus* counts in yogurt samples, the results showed that the control samples had the highest counts of *B. cereus* at cold storage compared to other treatments, and also, yogurt samples treated with 1.5% LF had the highest reduction percentage of *B. cereus* counts than 0.5% LF (Table 1). These results agree with those of (Karam-Allah, 2022), who recorded that lactoferrin has a strong inhibitory effect against *B. cereus* by using LF at a concentration of 100 mg/g in stirred yogurt. In addition, Ombarak et al., (2019) recorded that 4% LF inhibited *B. cereus* strains and decreased their counts in experimentally inoculated Kareish cheese.

The mechanism of LF action is the direct interaction between the positively charged protein regions with anionic molecules present on the surface of some microorganisms, resulting in increased membrane permeability that leads to bacterial and fungal damage (Haversen et al., 2010).

Lactoferrin has antimicrobial activities for many pathogenic microbes, including Enteropathogenic *E. coli*, *E. faecalis*, *B. cereus*, and *C. albicans* (Farnaud & Evans, 2003; Pan et al., 2007).

Lactoferrin's antibacterial activity on Gram-positive bacteria is attributed to its binding to iron, leading to the inhibition of bacterial growth via restriction of the avail-

Table 3. Antifungal activity of lactoferrin on viability of *C. albicans* inoculated into yogurt during their refrigeration storage (4°C)

Storage Time (Day)	Control Group Negative	Control Group Positive	0.5% Lactoferrin Group		1.5% Lactoferrin Group	
		Mean±SEM	Mean±SEM	R%	Mean±SEM	R%
0		$15x10^3±0.03x10^3$	$15x10^3±0.03x10^3$	--	$15x10^3±0.03x10^3$	--
1		$21x10^3±0.1x10^{3a}$	$20x10^3±0.05x10^{3a}$	4.76	$16x10^3±0.03x10^{3a}$	23.81
2		$34x10^3±0.05x10^{3a}$	$16x10^3±0.05x10^{3b}$	52.9	$86x10^2±0.06x10^{2c}$	74.7
3		$71x10^3±0.05x10^{3a}$	$10x10^3±0.06x10^{3b}$	85.91	$51x10^2±0.03x10^{2c}$	92.8
4		$82x10^3±0.03x10^{3a}$	$82x10^2±0.005x10^{2b}$	90.0	$35x10^2±0.03x10^{2c}$	95.73
5		$27x10^4±0.03x10^{4a}$	$65x10^2±0.006x10^{2b}$	97.59	$12x10^2±0.006x10^{2c}$	99.55
6		$61x10^4±0.05x10^{4a}$	$42x10^2±0.03x10^{2b}$	99.31	$8.0x10^2±0.003x10^c$	99.86
7	ND	$88x10^4±0.05x10^{4a}$	$21x10^2±0.003x10^{2b}$	99.76	$7.0x10^2±0.003x10^c$	99.92
8		$37x10^5±0.05x10^{5a}$	$9.0x10^2±0.003x10^b$	99.97	$5.0x10^2±0.1^b$	99.99
9		$55x10^5±0.05x10^{5a}$	$7.0x10^2±0.003x10^b$	99.99	$1.0x10^2±0.01^c$	99.99
10		$91x10^5±0.06x10^{5a}$	$6.0x10^2±0.006x10^b$	99.99	$<10^2$	100
11		S	$4.0x10^2±0.003x10$		$<10^2$	
12		S	$<10^2$		$<10^2$	
13		S	$<10^2$		$<10^2$	
14		S	$<10^2$		$<10^2$	

[a, b, c]Mean values within the same row with different superscript letters are statistically different at P≤0.05.

Abbreviatioins: S: Spoiled sample; SEM: Standard error of means; R%: Reduction % of *C. albicans* count.

ability of iron as a nutrient for bacteria, and to its effects on lipoteichoic and teichoic acids, which leads to depolarization and disruption of bacterial membranes than on the cytoplasmic contents (Brandenburg et al., 2001; Orsi, 2004; Liu et al., 2011).

Moreover, lactoferrin can counter different important mechanisms evolved by microbial pathogens to infect and invade the host, such as adherence, colonization, invasion, and production of biofilms, and also cause mitochondrial and caspase-dependent regulated cell death and apoptosis-like in pathogenic yeasts (Yen et al., 2011; Sharbfi et al., 2016; Zarzosa-Moreno et al., 2020).

Meanwhile, for *E. faecalis*, the recorded results showed a slight decrease of *E. faecalis* in the yogurt samples treated with 1.5% and 0.5% LF, which were counted till the end of refrigerating storage days. However, they were much lower than non-treated samples at cold storage (Table 2). Bellamy et al., (1992) reported that bovine lactoferrin had limited effects on *E. faecalis* at 150 µg concentration, and Zorina et al. (2018) reported that *E. faecalis* showed resistance when using LF at a concentration of 206.3±51.1 pg and advise to use high-concentration of bovine LF.

Moreover, regarding the antifungal effect of lactoferrin on *C. albicans* counts in yogurt samples, the obtained results showed that the treated groups had much lower counts (growth inhibition) compared with the control ones. Also, yogurt samples treated with 1.5% lactoferrin had the highest reduction percentage of *C. albicans* counts than 0.5% lactoferrin (Table 3). *C. albicans* was found to be highly susceptible to inhibition and inactivation by concentration within the 18 to 150 µg/mL range (Bellamy et al., 1993); lactoferrin concentration (20 µg/mL) caused a rapid loss of viability of *C. albicans* isolates (Samaranayake et al., 2001). The concentration of lactoferrin was required for the suppression of *C. albicans* (11.3±1.5 and 43.8±9.5 pg/mL) (Zorina et al.,

2018) and also conceded the previous reports of (Gonzalez Chavezt et al., 2009; Bruni et al., 2016) who found that lactoferrin had candidacidal activity and disrupted the important virulence mechanisms in *C. albicans* through sequestering Fe^{3+} ions, thinning and inhibiting hyphal development, altering the permeability of the cell surface, significantly preventing biofilms and decreasing the internal thiol levels with 20% in *C. albicans*, resulting in the death of cells.

According to the present study, we concluded that lactoferrin had highly antibacterial against *B. cereus* than *E. faecalis* which showed more resistance to it, so we need a higher concentration. Lactoferrin has antifungal effects on *C. albicans*, and naturally-occurring antimicrobials such as lactoferrin for yogurt preservation are gaining great attention due to consumers' trends and help to reduce the addition of chemical preservatives.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

We would like to acknowledge the Animal Health Research Institute for the scientific support of this research.

References

Alighazi, N., Noori, N., Gandomi, H., & Basti, A. A. (2020). Effect of ziziphora clinopodioides essential oil stress on viability of Lactobacillus acidophilus and bifidobacterium bifidum microencapsulated with alginate -chitosan and physiochemical and sensory properties of probiotic yoghurt. *Iranian Journal of Veterinary Medicine, 15*(2), 234-253. [DOI:10.22059/IJVM.2020.303329.1005092]

Bellamy, W., Takase, M., Wakabayashi, H., Kawase, K., & Tomita, M. (1992). Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. *The Journal of Applied Bacteriology, 73*(6), 472–479. [DOI:10.1111/j.1365-2672.1992.tb05007.x] [PMID]

Bellamy, W., Wakabayashi, H., Takase, M., Kawase, K., Shimamura, S., & Tomita, M. (1993). Killing of candida albicans by lactoferricin b, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. *Medical Microbiology and Immunology, 182*(2), 97–105. [DOI:10.1007/BF00189377] [PMID]

Brandenburg, K., Jürgens, G., Müller, M., Fukuoka, S., & Koch, M. H. (2001). Biophysical characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. *Biological Chemistry, 382*(8), 1215–1225. [DOI:10.1515/BC.2001.152] [PMID]

Bruni, N., Capucchio, M. T., Biasibetti, E., Pessione, E., Cirrincione, S., & Giraudo, L., et al. (2016). Antimicrobial activity of lactoferrin-related peptides and applications in human and veterinary medicine. *Molecules, 21*(6), 752. [DOI:10.3390/molecules21060752] [PMID] [PMCID]

Corrieu, G., & Béal, C. (2016). Yoghurt: The product and its manufacture. In: B. Caballero, P. Finglas, & F. Toldrá, (Eds.), *The Encyclopedia of Food and Health* (pp. 617-624). Oxford: Academic Press. [DOI:10.1016/B978-0-12-384947-2.00766-2]

Domig, K. J., Mayer, H. K., & Kneifel, W. (2003). Methods used for the isolation, enumeration, characterisation and identification of Enterococcus spp. 2. Pheno- and genotypic criteria. *International Journal of Food Microbiology, 88*(2-3), 165–188. [DOI:10.1016/S0168-1605(03)00178-8] [PMID]

El-Kholy, A. M., Osman, M. M., Gouda, A., & Ghareeb, W. A. (2011). Fortification of yoghurt with iron. *World Journal of Dairy & Food Sciences, 6*(2), 159-165. [Link]

El-Kholy, A. M., El-Shinawy, S. H., Meshref, A. M. S., & Korny, A. M. (2014). Screening of antagonistic activity of probiotic bacteria against some foodborne pathogens. *Journal of Applied and Environmental Microbiology, 2*(2), 53-60. [DOI:10.12691/jaem-2-2-4]

Farnaud, S., & Evans, R. W. (2003). Lactoferrin--a multifunctional protein with antimicrobial properties. *Molecular Immunology, 40*(7), 395–405. [DOI:10.1016/S0161-5890(03)00152-4] [PMID]

Food and Drug Adminstration. (2020). Bacillus cereus. In S.M. Tallent, A. Knolhoff, E. J. Rhodehamel, S. M. Harmon, & R. W. Bennett (Eds.), *BAM bacteriological analytical manual*. Maryland: Food and Drug Adminstration. [Link]

Fernandes, K. E., & Carter, D. A. (2017). The antifungal activity of lactoferrin and its derived peptides: Mechanisms of action and synergy with drugs against fungal pathogens. *Frontiers in Microbiology, 8*, 2. [DOI:10.3389/fmicb.2017.00002]

Fernandez, M. A., & Marette, A. (2017). Potential health benefits of combining yogurt and fruits based on their probiotic and prebiotic properties. *Advances in Nutrition, 8*(1), 155S–164S. [DOI:10.3945/an.115.011114] [PMID] [PMCID]

Foroutan, S., Eslampour, M. A., Emaneini, M., Jabalameli, F., Akbari, G. (2022). Characterization of biofilm formation ability, virulence factors and antibiotic resistance pattern of Staphylococcus aureus isolates from subclinical bovine mastitis. *Iranian Journal of Veterinary Medicine, 16*(2), 144-154. [DOI:10.22059/IJVM.2021.323994.1005174]

Ghajarbeygi, P., Palizban, M., Mahmoudi, R., Sadeghi Niaraki, A., & Soltani, A. (2016). Hygienic quality of traditional and industrial yoghurt produced in Qazvin province of Iran. *Archives of Hygiene Sciences. 6*, 39-43. [DOI:10.29252/ArchHygSci.6.1.39]

Girma, K., Tilahun, Z., & Haimanot, D. (2014). Review on milk safety with emphasis on its public health. *World Journal Dairy Food Science, 9*(2), 166-183. [Link]

González-Chávez, S. A., Arévalo-Gallegos, S., & Rascón-Cruz, Q. (2009). Lactoferrin: Structure, function and applications. *International Journal of Antimicrobial Agents, 33*(4), 301.e1–301.e3018. [DOI:10.1016/j.ijantimicag.2008.07.020] [PMID]

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food Control, 46*, 412-429. [DOI:10.1016/j.foodcont.2014.05.047]

Hao, L., Shan, Q., Wei, J., Ma, F., & Sun, P. (2019). Lactoferrin: Major physiological functions and applications. *Current Protein and Peptide Science, 20*(2),139-144. [DOI:10.2174/1389203719666180514150921] [PMID]

Hassan, P.A. H., Peh, K., & Liong, M. (2011). Antimicrobial activity of metabolites of various strains of Lactobacillus acidophilus. Proceeding of the International Conference on Advanced Science. *Engineering and Information Technology, 1*(6), 694-697. [DOI:10.18517/ijaseit.1.6.139]

Haversen, L., Kondori, N., Baltzer, L., Hanson, L. A., Dolphin, G. T., & Duner, K., et al. (2010). Structure-microbicidal activity relationship of synthetic fragments derived from the antibacterial alpha-helix of human lactoferrin. *Antimicrobial Agents Chemotherapy, 54*(1), 418-425. [DOI:10.1128/AAC.00908-09] [PMID] [PMCID]

ISO. (2017). *6887-1 Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – part 1: General rules for the preparation of the initial suspension and decimal dilutions (ISO 6887-1: 2017).* Geneva: International Organization for Standardization. [Link]

ISO (21527-1). (2008) Microbiology of food and animal feeding stuff- Horizontal method for enumeration of yeasts and mould. Part 1: Colony count technique in products with water activity greater than 0.95. Retrieved from: [Link]

Karam-Allah, A. A., Abo-Zaid, E. M., Refae, M. M., Shaaban, H. A. G., Saad, S. A., & Hassanin, A. M. (2022). Functional stirred yoghurt fortified with buffalo, bovine, mix colostrum and lactoferrin, effect of lactoferrin on pathogenic bacteria and amino acids of buffalo, bovine colostrum and lactoferrin. *Egyptian Journal of Chemistry, 65*(7), 583-594. [DOI:10.21608/EJCHEM.2021.106920.4907]

Laref, N., & Guessas, B. (2013). Antifungal activity of newly isolates of Lactic acid bacteria. *Innovative Romanian Food Biotechnology, 13*, 80-88. [Link]

Legrand, D. (2016). Overview of lactoferrin as a natural immune modulator. *Journal Pediatric, 173*, S10-S15. [DOI:10.1016/j.jpeds.2016.02.071] [PMID]

Lisko, D. J., Johnston, G. P., & Johnston, C. G. (2017). Effects of dietary yogurt on the healthy human gastrointestinal (GI) Microbiome. *Microorganisms, 5*(1), 6. [DOI:10.3390/microorganisms5010006] [PMID] [PMCID]

Liu, Y., Han, F., Xie, Y., & Wang, Y. (2011). Comparative antimicrobial activity and mechanism of action of bovine lactoferricin-derived synthetic peptides. *Biometals : An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine, 24*(6), 1069–1078. [DOI:10.1007/s10534-011-9465-y] [PMID]

Niaz, B., Saeed, F., Ahmed, A., Imran, M., Maan, A. A., & Khan, M. K. I., et al. (2019). Lactoferrin (LF): A natural antimicrobial protein. *International Journal of Food Properties, 22*(1), 1626-1641. [DOI:10.1080/10942912.2019.1666137]

Nwamaka, N. T., & Chike, A. E. (2010). Bacteria population of some commercially prepared yoghurt sold in Enugu State, Eastern Nigeria. *African Journal of Microbiology Research, 4*(10), 984-988. [Link]

Ochoa, T. J., & Cleary, T. G. (2009). Effect of lactoferrin on enteric pathogens. *Biochimie, 91*(1), 30–34. [DOI:10.1016/j.biochi.2008.04.006] [PMID] [PMCID]

Ombarak, R. A, Saad, M. A., & Elbagory, A. M. (2019). Biopreservative effect of lactoferrin against foodborne pathogens inoculated in Egyptian soft cheese "kariesh cheese". *Alexandria Journal Veterinary Science., 63*(2), 97-103. [DOI:10.5455/ajvs.76015]

Orsi N. (2004). The antimicrobial activity of lactoferrin: Current status and perspectives. *Biometals : An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine, 17*(3), 189–196. [DOI:10.1023/B:BIOM.0000027691.86757.e2] [PMID]

Pan, Y., Rowney, M., Guo, P., & Hobman, P. (2007). Biological properties of lactoferrin: An overview. *Australian Journal of Dairy Technology, 62*(1), 31-42. [Link]

Qajarbeygi, P., Palizban, M., Mahmoudi, R., Sadeghi Niaraki, A., & Soltani, A. (2017). Hygienic quality of traditional and industrial yoghurt produced in Qazvin province of Iran. *Archives of Hygiene Sciences, 6*(1), 39-43. [Link]

Samaranayake, Y. H., Samaranayake, L. P., Pow, E. H. N., Beena, V. T., & Yeung, K. W. S. (2001). Antifungal effects of lysozyme and lactoferrin against genetically similar, sequential Candida albicans isolates from a human immunodeficiency virus-infected Southern Chinese Cohart. *Journal of Clinical Microbiology, 39*(9), 3296-3302. [DOI:10.1128/JCM.39.9.3296-3302.2001] [PMID] [PMCID]

Sharbafi, R., Moradian, F., & Rafeie, A. R. (2016). [Evaluation of antimicrobial activity of lactoferrin against P. Aeruginosa and E. coli growth (Persian)]. *Journal Babol University Medical Sciences, 18*(7),19-23. [DOI:10.22088/jbums.18.7.19]

SPSS, I. (2018). *Corp Ibm SPSS statistics for windows, version 26.0.* Armonk, NY: IBM Corp, Released. [Link]

Tajkarimi, M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control, 21*(9), 1199-1218. [DOI:10.1016/j.foodcont.2010.02.003]

Tavakoli, M., Habibi Najafi, M. B., & Mohebbi, M. (2019). Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt. *Heliyon, 5*(2), e01204. [DOI:10.1016/j.heliyon.2019.e01204] [PMID] [PMCID]

Tomita, M., Wakabayashi, H., Shin, K., Yamauchi, K., Yaeshima, T., & Iwatsuki, K. (2009). Twenty-five years of research on bovine lactoferrin applications. *Biochimie, 91*(1), 52–57. [DOI:10.1016/j.biochi.2008.05.021] [PMID]

Tsukahara, T., Fujimori, A., Misawa, Y., Oda, H., Yamauchi, K., & Abe, F., et al. (2020). The preventive effect of lactoferrin-containing yogurt on gastroenteritis in nursery school children-intervention study for 15 weeks. *International Journal of Environmental Research and Public Health, 17*(7), 2534. [DOI:10.3390/ijerph17072534] [PMID] [PMCID]

Yen, C. C., Shen, C. J., Hsu, W. H., Chang, Y. H., Lini, H. T., & Chen, H. L., et al. (2011). Lactoferrin: An iron-binding antimicrobial protein against Escherichia coli infection. *Biometals, 24*, 585-594. [DOI:10.1007/s10534-011-9423-8] [PMID]

Zarzosa-Moreno, D., Avalos-Gómez, C., Ramírez-Texcalco, L. S., Torres-López, E., Ramírez-Mondragón, R., & Hernández-Ramírez, J. O., et al. (2020). Lactoferrin and its derived peptides: An alternative for combating virulence mechanisms developed by pathogens. *Molecules, 25*(24), 5763. [DOI:10.3390/molecules25245763] [PMID] [PMCID]

Zorina, V. N., Vorobeva, O. N., Zorin, N. A. (2018). Antimicrobial activity of the human and bovine lactoferrin against Gram- positive bacteria and C. albicans. *Journal Microbiology, Epidemiology and Immunobiology, 95*(2), 54-58. [DOI:10.36233/0372-9311-2018-2-54-58]

This Page Intentionally Left Blank