Bio Controlling of Yoghurt Using *Bifidobacterium Bifidum* and *Lactis*

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Abstract

The antimicrobial activity of *Bifidobacterium bifidum* and *lactis* against some pathogenic bacteria such as *Staphylococcus aureus, E. coli and Salmonella typhimurium* was investigated during the manufacture and preservation of four types of yoghurt; Traditional yoghurt (TY) (*Starter culture*), Bifidobifidium yoghurt (BY) (*Starter culture + Bifidobacterium bifidum*), Bifidolactis yoghurt (LY) (*Starter culture + Bifidobacterium lactis*) and Mixed yoghurt (MY) (*Starter culture + Bifidobacterium bifidum+ lactis*). Yoghurt samples were inoculated with 10^4 cfu/g *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* and preserved for 14 days at 4±1^{o}C. The inoculated bacterial count and the titratable acidity were determined in the prepared yoghurt samples at zero, 3rd, 5th, 7th and 14th day of storage. The obtained results revealed elevation in the titratable acidity and noticeable inhibition in the count of the inoculated bacteria during the storage period of prepared yoghurt samples at 4±1^{o}C.

Keywords: Biocontrol, bio preservation**,** probiotics, Bifidobacterium spp., Staphylococcus aureus, E. coli, Salmonella typhimurium, yoghurt.

Introduction

A variety of fermented milk products are made around the world, among which yoghurt is the most popular fermented dairy product especially in Egypt ***(El Kholy et al., 2014).*** Yoghurt is defined as a coagulated dairy product that results from the fermentation of milk lactose to lactic acid by the action of lactic acid starter culture of both *Streptococcus thermophilus* and *Lactobacillus bulgaricus* ***(Oskar et al., 2004).*** Although yoghurt is usually considered to be safe, due to its acidic nature, but it may be involved in food borne illness due to process failure and contaminated raw materials. Some

microorganisms like *Staphylococcus aureus, E. coli* and *Listeria monocytogenes* were the most common foodborne pathogens able to survive in fermented dairy products ***(Karagozlu et al., 2007)***. The probiotic bacteria possess antagonistic activity against some foodborne microorganisms such as *Staphylococcus aureus, E. coli,* and *Salmonella typhimurium,* and this activity may be due to the production of inhibitory compounds like bacteriocins, hydrogen peroxide or organic acids as well as competitive adhesion to epithelium in the intestine ***(Karagozlu et al., 2007).*** Staphylococcal food illness is one of the most common food borne disease worldwide caused by *Staphylococcus aureus* bacteria. It`s intoxication, resulted from ingestion of food containing one or more preformed Staphylococcal enterotoxins ***(Cliver and Riemann, 2002).*** *E. coli* Since its discovery in 1982 has been recognized as a serious foodborne pathogen that causes hemorrhagic colitis, a disease marked by extreme abdominal pain and bloody diarrhea ***(Kasimoglu and Akgun, 2004).*** Salmonellosis represents a very important foodborne disease that keep a serious risk to human general wellbeing caused by *Salmonella typhimurium* ***(EFSA, 2010)***.

This study was aimed to investigate the ability of *Bifidobacterium bifidum* and *Bifidobacterium lactis* to antagonism the viability of *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* in prepared yoghurt samples during their storage at 4±1°C.

Materials and methods

1. Bacterial strains:

1.1. Yoghurt cultures: Yoghurt cultures (obtained from Chr. Hansen Lab., Copenhagen, Denmark) include: - Traditional yoghurt starter cultures (FD-DVS, YC-X11) include *Streptococcus thermophiles* and *Lactobacillus delbrueckiisub spp. bulgaricus* (1:1).

1.2. Bifidus yoghurt starter culture include *Bifidobacterium* strain culture (*Bifidobacterium lactis* DSM10140 and *Bifidobacterium bifidum* DSM10140). All Starter cultures were prepared according to ***Hull and Robert (1984).***

1.3. Pathogenic strains:

The pathogenic bacteria used were *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* reference strains obtained from animal health research institute, Dokki, Giza, Egypt. All strains were prepared according to ***Bachrouri et al. (2002).***

2. Preparation of Traditional & Bifidus yoghurt samples: according to ***Nighswonger et al. (1996)*****:** Four groups of yoghurt were manufactured (TY, BY, LY and MY) then each group was subdivided into three batches, each

batch was inoculated with the pathogenic bacteria (10^4 CFU/ml).

3. Determination of titratable acidity: according to ***A.O.A.C. (2000).***

4. Microbiological examination: Counting *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* according to ***A.P.H.A. (2001)*** at zero, 3rd, 5th, 7th, and 14th day of cold storage (4±1°C).

5. Statistical Analysis *(SPSS, 2011)*: The data was analyzed using a mixed method of SPSS software (Release 20, IBM CO). Means were separated by Fisher's least significant difference test, and significance was tested at $\alpha = 0.05$.

RESULTS

Table (1): *The mean Staphylococcus aureus count and its reduction % in the treated yoghurt samples*

Types of yoghurt	Traditional yoghurt (TY)		Bifidobifidium yoghurt (BY)		Bifidolactis yoghurt (LY)		Mixed yoghurt (MY)	
Storage period	Mean ±SE	R%	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%
zero time	$6.7 \times 10^4 \pm 1.5 \times 10^{4\,e}$	0.0	$2.9 \times 10^4 \pm 6 \times 10^{3\,d}$	0.0	$2.2 \times 10^4 \pm 4.8 \times 10^{3\,d}$	0.0	$1.2 \times 10^4 \pm 3 \times 10^{3\,c}$	0.0
3 day	$8.9 \times 10^2 \pm 2 \times 10^{2\,d}$	98.6%	$6.2 \times 10^2 \pm 1 \times 10^{2\,c}$	97.8%	$5.8 \times 10^2 \pm 3.9 \times 10^{c}$	97.3%	$1.3 \times 10^2 \pm 2 \times 10^{b}$	98.9%
5 day	$2.3 \times 10^2 \pm 3.8 \times 10^{c}$	99.6%	$1.1 \times 10^2 \pm 2 \times 10^{b}$	99.6%	$1.6 \times 10^2 \pm 3.6 \times 10^{b}$	99.2%	N.D. [a]	100%
7 day	$7.4 \times 10 \pm 2 \times 10^{b}$	99.8%	N.D. [a]	100%	N.D. [a]	100%	N.D.	100%
14 day	N.D. [a]	100%	N.D.	100%	N.D.	100%	N.D.	100%

*Treatments with different letters show significant difference (*P*-value <0.05)

Mean: average of triplicate. **S E:** standard error of mean. **N. D.:** not detected. **R%:** Reduction % from initial count**.**

TY: Yoghurt made with traditional yoghurt culture (*L. bulgaricus* and *S. thermophilus*)

BY: Yoghurt made with traditional yoghurt culture + *B. bifidum*

LY: Yoghurt made with traditional yoghurt culture + *B. lactis*

MY: Yoghurt made with traditional yoghurt culture + *B. bifidum*+ *B. lactis*

Table (2): *The mean E. coli count and its reduction % in treated yoghurt samples*

Types of yoghurt	Traditional yoghurt (TY)		Bifidobifidium yoghurt (BY)		Bifidolactis yoghurt (LY)		Mixed yoghurt (MY)	
Storage period	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%
zero time	$4.6 \times 10^4 \pm 1 \times 10^{4\,e}$	0.0	$4.1 \times 10^4 \pm 8 \times 10^{3\,e}$	0.0	$2.9 \times 10^4 \pm 6 \times 10^{3\,e}$	0.0	$2.3 \times 10^4 \pm 7 \times 10^{3\,d}$	0.0
3 day	$7 \times 10^3 \pm 1 \times 10^{3\,c}$	84.8%	$5.7 \times 10^3 \pm 1 \times 10^{3\,d}$	86.2%	$4.7 \times 10^3 \pm 9 \times 10^{2\,d}$	84%	$3.6 \times 10^2 \pm 1 \times 10^{2\,c}$	98.3%
5 day	$7.5 \times 10^2 \pm 2 \times 10^{2\,d}$	98.3%	$4.2 \times 10^2 \pm 1 \times 10^{2\,c}$	98.9%	$2.6 \times 10^2 \pm 6.5 \times 10^{c}$	99%	$9.9 \times 10 \pm 2 \times 10^{b}$	99.5%
7 day	$2.7 \times 10^2 \pm 4 \times 10^{b}$	99.4%	$5.8 \times 10 \pm 1 \times 10^{b}$	99.8%	$4.9 \times 10 \pm 1 \times 10^{b}$	99.8%	N.D.[a]	100%
14 day	$2.2 \times 10 \pm 0.9 \times 10^{a}$	99.9%	N.D. [a]	100%	N.D. [a]	100%	N.D.	100%

Table (3): *The mean Salmonella typhimurium count and its reduction % in treated yoghurt samples*

Types of yoghurt	Traditional yoghurt (TY)		Bifidobifidium yoghurt (BY)		Bifidolactis yoghurt (LY)		Mixed yoghurt (MY)	
Storage period	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%	Mean ± SE	R%
zero time	$4.9 \times 10^4 \pm 1 \times 10^{4\,e}$	0.0	$2.7 \times 10^4 \pm 5 \times 10^{3\,d}$	0.0	$2.5 \times 10^4 \pm 4.8 \times 10^{3\,e}$	0.0	$1.3 \times 10^4 \pm 3 \times 10^{3\,d}$	0.0
3 day	$2.9 \times 10^3 \pm 4.5 \times 10^{2\,d}$	94%	$4 \times 10^3 \pm 1 \times 10^{3\,e}$	85%	$2.1 \times 10^3 \pm 5 \times 10^{2\,d}$	91.4%	$4.6 \times 10^2 \pm 1 \times 10^{2\,c}$	96.4%
5 day	$3.9 \times 10^2 \pm 9 \times 10^{c}$	99%	$2.9 \times 10^2 \pm 8 \times 10^{c}$	98.9%	$1.4 \times 10^2 \pm 2.6 \times 10^{c}$	99.4%	$5.8 \times 10 \pm 1 \times 10^{b}$	99.5%
7 day	$1 \times 10^2 \pm 2 \times 10^{b}$	99.7 %	$2.6 \times 10 \pm 1 \times 10^{b}$	99.9%	$1.5 \times 10 \pm 0.5 \times 10^{b}$	99.9%	N.D. [a]	100%
14 day	$1 \times 10 \pm 0.4 \times 10^{a}$	99.8 %	N.D. [a]	100%	N.D. [a]	100%	N.D.	100%

Table (4): *Titratable acidity throughout the storage period of the treated yoghurt samples expressed as acidity%:*

Storage period	*Staphylococcus aureus*				*E. coli*				*Salmonella typhimurium*			
	TY	BY	LY	MY	TY	BY	LY	MY	TY	BY	LY	MY
zero time	0.82	0.83	0.72	0.72	0.63	0.63	0.72	0.72	0.64	0.67	0.71	0.72
3 day	0.81	0.85	0.85	0.85	0.63	0.63	0.67	0.74	0.67	0.70	0.72	0.76
5 day	0.85	0.85	0.99	0.87	0.67	0.72	0.68	0.75	0.67	0.72	0.74	0.74
7 day	0.87	0.87	0.99	0.94	0.72	0.72	0.72	0.76	0.67	0.76	0.76	0.76
14 day	0.87	0.88	0.99	0.95	0.73	0.73	0.73	0.76	0.68	0.76	0.77	0.78

Discussion

The results demonstrated in Table (1) showed that the *Staphylococcus aureus* bacterial counts in the prepared samples were 6.7 x 10^4 ± 1.5 x 10^4, 2.9 x 10^4 ± 6 x 10^4, 2.2 x10^4 ± 4.8 x10^3 and 1.2 x10^4 ± 3 x10^3 CFU/g in TY, BY, LY and MY samples, respectively at the zero day of cold storage at 4^0C. *Staphylococcus aureus* was undetectable in TY on the 14th day of storage. On the other hand**,** *Staphylococcus aureus* was not detected in BY and LY samples from the 7th day of storage, but was not detected in MY samples from the 5th day of storage. Different results were obtained by ***Abdel-Aziz (2011)*** and ***Marwa (2011)*** who reported that *Staphylococcus aureus* was not detected in BY on the 5th day of storage followed by TY sample in the 7th day of storage. Longer time (16 days in TY and 12 days in BY) reported by ***Oscar et al. (2007).*** On the contrary, *Staphylococcus aureus* disappeared in TY after 24 hrs followed by BY after 48 hours ***Yesillik et al. (2011)***.

E. coli count in TY, BY, LY and MY expressed at Table (2) was 4.6 x 10^4 ± 1 x 10^4, 4.1 x 10^4 ± 8 x10^3, 2.9 x 10^4 ± 6 x10^3 and 2.3 x 10^4 ± 7 x10^3 CFU/g at zero time, respectively. It was not detected for MY on the 7th day of storage. While by the 14th day of storage *E. coli* was 2.2 x10 ± 0.9 x10 in TY and it was undetectable in BY and LY. Similar results were reported by ***Amer et al. (2010)*** and ***Marwa (2011).*** Furthermore, ***Abdel-Aziz (2011)*** found that the initial count of *E. coli* completely disappeared at 11th day of storage in BY, while in TY samples *E. coli* was not detected in the day 14th.Meanwhile, ***Kasimoglu and Akgun (2004)*** reported that the elimination of *E. coli* was after 48 hrs in traditional yoghurt. Many studies have found that *Bifidobacterium spp.* showed strong inhibitory activity against *E. coli* and *Staphylococcus aureus* in yoghurt ***(Muhammad et al., 2015).***

Salmonella typhimurium count at zero time was 4.9 x 10^4 ± 1 x 10^4, 2.7 x 10^4 ± 5 x 10^3, 2.5 x 10^4 ± 4.8 x 10^3 and 1.3 x 10^4 ± 3 x 10^3 cfu/g in TY, BY, LY and MY respectively. While it was not detected for MY on the 7th day of storage. By the 14th day of storage *Salmonella typhimurium* count was 1x10 ± 0.4 x 10 in TY and was not detected in BY and LY as showed in Table (3). *Salmonella typhimurium* can survive for up to 5 days at 4 ° C in both TY and BY when the yoghurt pH is < 4.5, but up to 10 days if pH is 4.5 or higher ***Hal-Haddad (2003)***. ***Nassib et al., (2006)*** found that *Salmonella typhimurium* survived longer in BY than TY, and this was due to the development of lower pH by traditional starters.

In Table (4) all yoghurt samples showed increase in titratable acidity during the storage and the yoghurt fortified with probiotic strains should higher increase in the acidity than the traditional yoghurt and that may be one of the antimicrobial mechanisms the probiotic could use against the pathogenic microorganisms. The previous results showed that the more the acidity produced, the more decline in pathogenic bacterial count. These results agreed with those obtained by ***Abdel-Aziz (2011)*** and ***El-Kholy et al., (2014).***

Clinical studies have shown that certain probiotics can effectively reduce infection. Possible mechanisms of probiotic action include prevention of adsorption, intracellular internalization of pathogens, production of metabolites and substances that directly affect pathogens, and interaction with cells **(**immune regulation) in building protection ***(Power et al., 2014).***

Conclusion

This study demonstrated the capability of *B. bifidum* and *B. lactis* to inhibit the growth of *Staphylococcus aureus, E. coli* and *Salmonella typhimurium* during the storage of yoghurt and the possibility of using them for bio preservation of yoghurt. As the presence of pathogenic bacteria as *Staphylococcus aureus* considered as a public health hazard, thus the hygienic standard needs to be strengthened during manufacture and preservation to ensure production of safe yoghurt. Furthermore, It is recommended to promote consuming dairy products fortified with probiotics for their health benefits and saving the product against pathogenic microorganisms.

References

Abdel-Aziz, M. M. ***(2011):*** The role of Bifidobacterium in improvement of physical, chemical and microbial quality of fermented dairy products. Ph.D. Thesis. Faculty of Vet. Med. Benha Univ.

Amer, A.; Aiad, A. and Abd-Allah, M. ***(2010):*** Effect of yoghurt processing and ice-cream

manufacture on viability of some food- borne bacteria. Assiut Vet. Med. J., 56(127):108-119.

A.O.A.C. ***(2000):*** Dairy products in: Official methods of analysis. Association of Official and Analytical Chemists, Inc., Gaithersburg, USA.

A.P.H.A. (American Public Health Association) ***(2001)*****:** Compendium of methods for the microbiological examination of foods. 4th Ed. Amer. Public Health Asso., Washington, D.C., U.S.A.

Bachrouri, M; Quinto, E. J. and Mora, M. T. ***(2002)*****:** Survival of *Escherichia coli* O157:H7 during storage of yogurt at different temperature. J. of Food Sci., 67 (5) 1899-1903.

Cliver, D.O. and Riemann, H.P. ***(2002):*** Food- borne Diseases (2nd ed.), California, Academic Press.

EFSA (European Food Safety Authority) ***(2010):*** The Community Summary Report on Trends and Sources of Zoonosis, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2008. EFSA J., 8 (1): 1496.

El Kholy, A. M.; El-Shinawy, S. H.; Meshref, A. M. S. and Korany, A. M. ***(2014):*** Screening of antagonistic activity of probiotic bacteria against some food-borne pathogens. J. of Applied and Enviro. Microbiol., 2 (2):53-60.

Hal-Haddad, K. S. ***(2003):*** Survival of *Salmonellae* in bio-yoghurt. Int. J. Dairy Technol. 56 (4), 199-202.

Hull, R. R. and Robert, A. V. ***(1984):*** Differential enumeration of *Lactobacillus acidophilus* in yogurt. J. Aust. Dairy Technol., 3: 160-164.

Karagozlu, N.; Karagozlu, C. and Ergonul, B. ***(2007):*** Survival characteristics of *E. coli O157:H7*, *S. typhimurium* and *S. aureus* during kefir fermentation. Czech J. Food Sci., 25: 202–207.

Kasımog˘lu, A. and Akgùn, S. ***(2004):*** Survival of *E. coli* O157:H7 in the processing and post-processing stages of acidophilus yogurt. Inter. J. of Food Sci. and Tech., 39 (5): 563-569.

Marwa, A. Meawad ***(2011):*** Effect of bacteriocins produced by some lactic acid bacteria on the quality of yoghurt. MD Thesis, Benha University.

Muhammad, Z.; Muhammad, A.; Muhammad, A; Ghulam, M.; Aqeela, Y. and Hafiz M. A. H. ***(2015):*** Antimicrobial activity of bacteriocins isolated from Lactic Acid Bacteria against resistant pathogenic strains. Int. J. of Nut. and Food Sci., 4 (3): 326-331

Nassib, T. A.; El-Din, M. Z. and El-Sharoud, W. M. ***(2006):*** Effect of thermophilic lactic acid bacteria on the viability of *Salmonella* serovar *typhimurium* PT8 during milk fermentation and preparation of buffalo's yogurt. Int. J. of Dairy Tech., 59 (1): 29-34.

Nighswonger, B. D.; Brashears, M. M. and Gilliland, S. E. ***(1996):*** Viability of *Lactobacillus acidophilus* and *Lactobacillus casei* in fermented milk products during refrigerated storage. J. of Dairy Sci., 79: 212–219.

Oscar, C.; Caroline, P.; Caroline, C.; Laura, V. and Maria, L. ***(2007):*** Evaluation of the effect of *Lactobacillus rhamnosus* probiotic culture added to yogurt over *Staph. aureus, E. coli O157:H7, L. monocytogenes* and *Salmonella enteritidis* populations. Arch Latinoam Nutr, 57 (1): 51-5.

Oskar, A.; Simin, N. and Robert, M. R. ***(2004):*** Yogurt and gut function Review, Am J Clin. Nutr, 80, 245-56.

Power, S. E.; Toole, P. W. O.; Stanton, C.; Ross, R. P. and Fitzgerald, G. F. ***(2014):*** Intestinal microbiota, diet and health, British J. of Nutrition, 111 (3): pp. 387–402.

SPSS ***(2011):*** Statistical Package for Social, Science 20.0.0 SPSS Inc., Chicago, IL., USA.

Yesillik, S.; Yildirim, N.; Dikici, A.; Yildiz, A. and Yesillik, S. ***(2011):*** Antibacterial effect of some fermented commercial and homemade dairy products and 0.9% lactic acid against selected foodborne pathogens. Asian J. of Animal and Veterinary Advances; 6 (2): 189-195.

الملخص العربي

تم في هذه الدراسة تصنيع الزبادي بأربع انواع النوع الاول الزبادي العادي (TY) تحتوي علي بادئا الزبادي فقط والنوع الثاني (BY) وهو زبادي مضاف اليه بادئا الزبادي + البيفيدو بيفيديم و النوع الثالث (LY) وهو زبادي مضاف اليه بادئا الزبادي + بكتريا البيفيدو لاكتس والنوع الرابع (MY) وهو زبادي مضاف اليه بادئا الزبادي + بكتريا البيفيدو بيفيدم + بكتريا البيفيدو لاكتس. وتم اضافة عدد معلوم من البكتريا الممرضة من المكور العنقودي الذهبي وبكتريا الايشيريشيا كولاي وبكتريا السالمونيلا تيفيميوريم. ثم تم فحص انواع الزبادي عند تكوين الخثرة وعند اليوم الثالث والخامس والسابع والرابع عشر لقياس نسبة الحموضة وقياس العدد الكلي لبكتيريا المكور العنقودي الذهبي وبكتريا الايشيريشيا كولاي وبكتريا السالمونيلا تيفيميوريم وقد اظهرت النتائج ارتفاعا في نسبة الحموضة وتناقصا في عدد البكتريا المضافة علي مدار فترة التخزين.