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Comparative studies of *Listeria monocytogenes* survival in bifidus and traditional yoghurt during production and storage

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A B S T R A C T

Antagonistic activity of *Bifidobacterium bifidum* against *Listeria monocytogenes* during the production and storage of Traditional yoghurt (TY) (*Streptococcus thermophiles*+ *Lactobacillus bulgaricus*) and Bifidus yoghurt (BY) samples (*Streptococcus thermophiles*+ *Lactobacillus bulgaricus*+ *Bifidobacterium bifidum*). Traditional & Bifidus yoghurt samples were inoculated with 10^6 cfu/g *L. monocytogenes* and stored for 21 days at 4±1ºC. Samples were taken at zero time (fresh samples) then after 3, 5, 7, 10, 14 and 21 days of storage for titratable acidity measurement and bacterial count of the culture starters and *L. monocytogenes* counts. The results revealed that the titratable acidity of TY & BY was increased during the 21 days of storage, while the population of *L. monocytogenes* became non-detectable level in 10^{th} days of storage in BY and still detected in TY till the day 14th of storage and became 2.20± 0.31 $\log_{10}$ cfu/g. The count of all starter cultures (*S. thermophiles*+ *Lb. bulgaricus*+ *B. bifidum*) in both yoghurts increased during the fermentation and cold storage and remained stable with values >6 $\log_{10}$ cfu/g throughout the storage period at 4±1°C.

Keywords: probiotics, *Bifidobacteria*, *L. monocytogenes*, yoghurt, bifidus yoghurt.

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1- INTRODUCTION

During the last century, many reports were published, with variable results, regarding the benefits to health of consuming yoghurt. More recent studies focused on varies health benefits of the consumption of fermented milks with probiotic microorganisms (Kehagias et al., 2006; Ouwehand et al., 2002). The probiotics to be incorporated into milk or any other food must fulfill several criteria related to safety, health benefits, and processing parameters (Mattila-Sandholm et al., 2002). "Probiotics" are defined as" live microorganisms which when administered in adequate amounts (between 10^6-10^7 cfu/g) confer a health benefit on the host" (FAO/WHO, 2010). Therefore, viability and activity of the probiotic bacteria in yoghurt are of important consideration. Among the most often used, probiotic bacteria are species of the genus *Bifidobacterium* (Kehagias et al., 2006). *Bifidobacteria* constitute a major part of human intestinal microflora and play an important role in maintaining good health. The potential health benefits ascribed to *bifidobacteria* include inhibition of bacterial pathogens, reduction of serum cholesterol levels and colon cancer risks, stimulation of immune response, improvement of lactose tolerance, calcium absorption and vitamin synthesis. In addition to assistance of digestion, prevention of radiotherapy associated diarrhea, and control of intestinal gram-negative bacteria (Palacios et al., 2008; Wu et al., 2011). At present, *bifidobacteria* are increasingly in corporate into fermented dairy products. *Bifidobacteria* grow slowly in milk and the usual practice is to add yoghurt starter bacteria to enhance the fermentation process for making probiotic yoghurt. Several factors have been claimed to affect the viability of probiotics in yoghurt, including acidity, hydrogen peroxide, oxygen content, concentration of lactic acid and acetic acids, temperature of storage etc., during manufacture and storage of yoghurt (Kehagias et al., 2006). *L. monocytogenes* is Gram-positive foodborne pathogen, can cause illnesses extending from those with mild flu-like symptoms or gastroenteritis to more serious, potentially fatal conditions such as bacteremia and meningitis and in pregnancy can cause preterm delivery, fetal loss, neonatal infection, or infant death (Liu, 2006; McLauchlin et al., 2004).

Between1998 and 2008 in the USA, at least 25% of reported outbreaks of listeriosis were of dairy origin (Allerberger and Wagner, 2010).

This study is aimed to compare and evaluate the effect of bifidus and traditional yoghurt samples on survival of *L. monocytogenes* during production and during storage at 4±1ºC.

2- MATERIALS AND METHODS

2.1. Yoghurt cultures

Yoghurt cultures (were obtained from Chr. Hansen Lab., Copenhagen, Denmark). Traditional yoghurt starter culture (FD-DVS, YC-X11) contains *Streptococcus thermophiles & Lactobacillus delbrueckiisub spp. bulgaricus* (1:1). Bifidus yoghurt starter culture contains Bifidobacterium bifidum Bb-12+ *Streptococcus thermophiles+ Lactobacillus bulgaricus.* All Starter cultures were prepared according to Hull and Robert (1984).

2-2. L. monocytogenes

L. monocytogenes NCTC 13372/ ATCC® 7644 was obtained from TCS Bioscience Ltd, United Kingdom and activated at Food Hygiene department- Animal Health Research Institute- Dokki, Giza, Egypt. It was prepared according to Bachrouri et al. (2002).

2.3. Preparation of Traditional & Bifidus yoghurt samples: according to Nighswonger et al. (1996).

2.4. Chemical examination:

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

2.5. Microbiological examination:

Yoghurt samples were taken at Zero time, 3, 5, 7, 10, 14 and 21 days of cold storage (4±1ºC), thoroughly mixed aseptically immediately after opening of yoghurt cup, and from each prepared samples, 10 folds' serial dilutions were prepared according to APHA (2001) for the count of the following: *S. thermophiles, Lb. bulgaricus* according to Kailasapathy et al. (2008) and *B. bifidum* according to Tharmaraj and Shah (2003). *L. monocytogenes* counts: according to A.P.H.A. (American Public Health Association) (2001).

2.6- Statistical analysis: Data were analyzed by using SPSS (2000)

3- RESULTS

The research was carried out to compare and evaluate the effect of traditional and bifidus yoghurt samples on survival of *L. monocytogenes* during production and storage at 4±1ºC and analyzed for T.A% and total counts of starter cultures and *L. monocytogenes.* The titratable acidity of both TY & BY was increased and reached 0.69, 0.71, 0.78, 0.80 and 1.25 then spoiled by visualized mould growth in TY, while in BY reached 0.67, 0.69, 0.75, 0.78, 0.82, 0.93 and 1.17 at zero time, 3, 5, 7, 10, 14, and 21 days of storage, respectively (Table 1). The viability of the inoculated *L. monocytogenes* reached 7.00, 6.20, 5.80, 5.00, 3.13 and 2.20 $\log_{10}$ cfu/g in TY, while in BY reached 6.60, 5.40, 3.33 and 1.47 at zero time, 3, 5 and 7 days of storage, respectively and became non-detectable level in 10th days of storage (Table 2). The changes in the viable counts of *S. thermophilus, Lb. bulgaricus and B. bifidum* in Bifidus yoghurt samples during production and storage (Fig. 1), The count of all starter cultures (*S. thermophiles + Lb. bulgaricus + B.bifidum*) in bifidus yoghurt increased during the fermentation and cold storage, *S. thermophiles*, it was 7.25, 7.35, 8.30, 8.50, 7.50, 7.40 and 6.60 $\log_{10}$cfu/g, while *Lb. bulgaricus* 7.35, 7.65, 8.40, 8.25, 7.60, 7.55 and 6.35 $\log_{10}$cfu/g and *B. bifidum* became 7.50, 8.10, 8.80, 8.60, 7.60, 7,40 and 6.60 $\log_{10}$cfu/g at zero time, 3, 5, 7, 10, 14, and 21 days of storage, respectively.

Table (1): The mean values of titratable acidity (T.A %) in prepared yoghurt samples during refrigerated storage (mean ± SD)

Samples Storage days	TY	BY
Zero	0.69±0.02	0.67±0.02
3	0.71±0.02	0.69±0.01
5	0.78±0.03	0.75±0.03
7	0.80±0.02	0.78±0.02
10	0.85±0.02	0.82±0.03
14	1.25±0.13	0.93±0.03
21	S	1.16±0.03

TY: Traditional yoghurt BY: Bifidus yoghurt. S: Spoilage of sample by visualized mould growth

Table (2): Viability of *L. monocytogenes* in the TY&BY during their refrigerated storage (mean $\log_{10}$ cfu/g ± SD)

Yoghurt sample	Days of storage						
	0	3	5	7	10	14	21
TY	7.00±0.26	6.20±0.36	5.80±0.17	5.00±0.36	3.13±0.31	2.20±0.30	S
BY	6.60±0.10	5.40±0.03	3.33±0.15	1.47±0.25	<1		

TY: Traditional yoghurt BY: Bifidus yoghurt S: Spoilage of sample by visualized mould growth

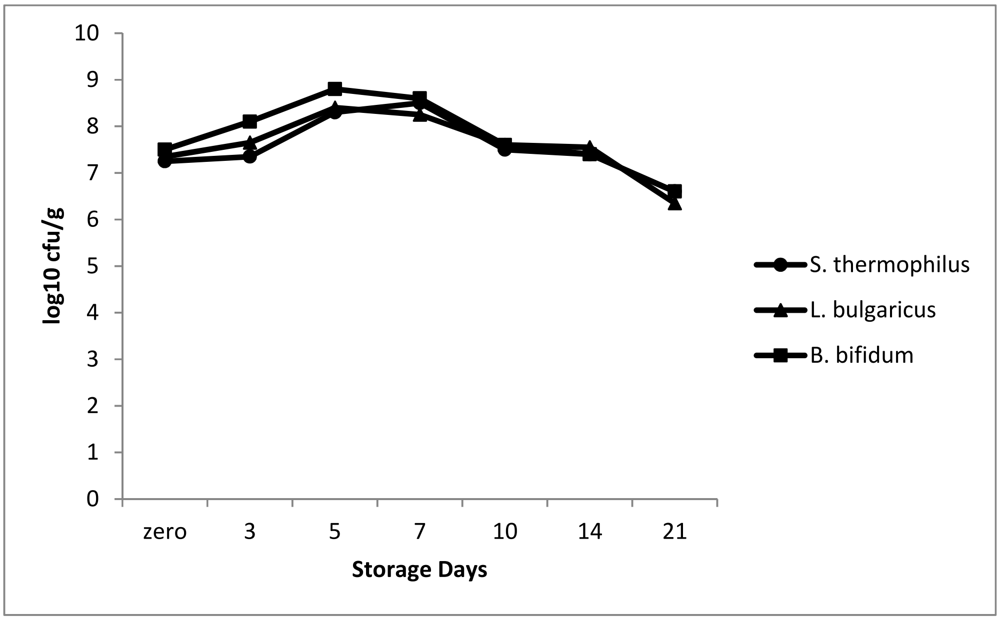

Fig. (1): The mean counts of *S. thermophilus, Lb. bulgaricus* and *B.bifidum* counts during production and storage of traditional and bifidus yoghurt.

4- DISCUSSION

Titratable acidity (TA) of yoghurt is 0.70-0.80%, the acid production in yoghurt depends on the growing of microorganisms and their ability for fermentation of the lactose in milk. Yoghurt is manufactured by addition of *S. thermophilus and Lb. bulgaricus.* These organisms are active even at refrigerated temperatures and still can produce small amounts of lactic acid by fermentation of lactose resulting in noticeable TA increase (Shah et al., 1995). Table (1) represented the mean titratable acidity in TY & BY, which increased over time from initial 0.69 and 0.67% at termination of fermentation (4h) to 1.25 and 0.93 % by 14 days of storage at 4±1ºC for TY and BY, respectively. The mean value of titratable acidity of all samples increased during storage period, but TY samples showed significantly higher values ($P<0.05$) than BY samples.

The higher titratable acidity in TY samples than those in BY samples may be due to the amount of initial inoculum (3%) of yoghurt starter culture (*S. thermophilus* and *Lb. bulgaricus*) compared to (1.5%) in BY, in addition to the slow rate of bifidobacteria in the production of acidity (Dave and Shah, 1997). These results agreed with those obtained by Hussein and Kebary (1999) and Abdel-Aziz-Mona (2011) who found that bifidus yoghurt had lower acidity compared to traditional yoghurt. The acidification in yoghurt depends on the growing of microorganisms in the selection of starter culture and probiotic bacteria and their ability for lactic acid formation (Rahnama-Fatemeh et al., 2013). LAB is active even at refrigerated temperature and still can produce small amounts of lactic acid by fermentation of lactose resulting in noticeable TA increase (Shah et al., 1995).

Data presented in Table (2) showed the viability of the inoculated *L. monocytogenes* in the prepared yoghurt samples during production and cold storage (4±1ºC). The count of *L. monocytogenes* showed decreased in all yoghurt samples and its count became 3.3 and 5.00 $\log_{10}$ cfu/g after 5 days of storage. Elimination of *L. monocytogenes* was observed on the 10th day in BY samples, while still detected in TY till the day 14th of storage and became 2.20± 0.31 $\log_{10}$ cfu/g. Similar results was obtained by Azizkhani and Toorya (2016) and Toorya (2016) who found that *Bifidobacterium* Bb12 showed inhibitory effect against *L. monocytogenes* in vitro, and (Rayes-Amnah, 2012) found that LAB inhibited the growth of *L. monocytogenes* in vitro.

From the above mentioned results which showed that bifidobacteria had an inhibitory effect against *L. monocytogenes* microorganism more than that of the yoghurt starter culture (*S. thermophilus* and *Lb. bulgaricus*), that may be due to the antimicrobial agents produced by *Bifidobacteria* such as organic acids; mainly acetic acid and lactic acids (Bruno and Shah, 2002) and bacteriocins (Lengkey (Lengkey and Adriani, 2009).

The viability of probiotic bacteria in yoghurt must kept sufficiently high to ensure that consumers receive health benefits. These benefits include the prevention of diarrhea, balancing of intestinal microflora, stimulation of the immune system, antitumor properties and alleviation of lactose tolerance (El-Kholy et al., 2014; Guktepe, 2006). In order to produce these benefits. It is important to note that the number of lactic acid bacteria present in different systems of yoghurt was constant over time, as it kept in a range of 6 to 8 $\log_{10}$ cfu/g, which is the need to exercise bactericidal action as well as being the recommended number by FAO/WHO (2010) as the amount of bacteria needed for exercising beneficial effects on the body (Dave and Shah, 1997; Gueimonde et al., 2004). The changes in the viable counts of *S. thermophilus, Lb. bulgaricus* and *B. bifidum* in yoghurt during production and storage are given in Fig. (2), it is clear that the $\log_{10}$ cfu/g of all yoghurt samples slightly increased till the 5th and the 7th days and then decreased slowly to the end of the storage period and reached 6.60, 6.35 and 6.60 $\log_{10}$ cfu/g. Over 21 days' storage at 4°C there was some decline in the numbers of bifidobacteria but the counts remained stable with values around 6-8 $\log_{10}$ cfu/g. Similar observations were reported by Abdel-Aziz-Mona (2011) Abd El-Gawad et al. (2014); El-Kholy et al. (2014); Mani-López et al. (2014); Meg et al. (2013); Ranasinghe and Perera (2016); Rayes-Amnah (2012) and Azizkhani and Toorya (2016)

Donkor et al. (2007) concluded that the ability of probiotic to survive in yoghurt was strain dependent, in addition *Bifidobacteria* could survive in yoghurt at sufficient levels (>10^6 cfu/g) for up to 28 days. Variation in the probiotic viability data among different authors may probably be attributed to strain variation, acid accumulation, interaction with starter cultures and storage condition.

5- CONCLUSION

The results demonstrated the capability of *B. bifidum* to inhibit the growth of *L. monocytogenes*, in vitro. Survival of *S. thermophilus, Lb. bulgaricus* and *B. bifidum* in yoghurt were satisfactory as they remained viable at levels > 10^7 cfu/ g after 21 days of storage at 4±1°C, which indicate that the yoghurt would be a suitable vehicle for probiotic bacteria. Bifidobacteria has been shown to possess inhibitory activity toward the growth of *L. monocytogenes* during the fermentation and storage of yoghurt, as the presence of pathogenic bacteria as *Staph. aureus* pose a risk for public health. Therefore, the hygienic standard needs to be strengthened during manufacture and storage to ensure production of safe, high quality yoghurt.

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