Open Journal of Applied Sciences, 2019, 9, 564-572
http://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Survival of *Staphylococcus aureus* in Bio-Yoghurt

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How to cite this paper: Soliman, N.S.M. and Ahmed, L.I. (2019) Survival of *Staphylococcus aureus* in Bio-Yoghurt. *Open Journal of Applied Sciences*, **9**, 564-572.
https://doi.org/10.4236/ojapps.2019.97044

Received: June 4, 2019
Accepted: July 19, 2019
Published: July 22, 2019

Abstract

Introduction: Due to the increasing demand for natural probiotic products, yogurt is the most popular type of fermented dairy products. **Aim:** Yoghurt produced using probiotic bacteria and its effect on *Staphylococcus aureus* as a pathogenic microorganism is discussed as compared with traditional yoghurt. **Material and method:** The influence of yoghurt using starter culture on *S. aureus* during manufacture and storage for three brands of Lab. made yoghurt (A, B and C) was judged as excellent concerning the total acceptability. **Result:** The inoculated *S. aureus* decreases in number by the end of the storage period. In addition, the best effect of inhibition was observed in brand (B) using yoghurt starter with *Lactobacillus acidophilus*, followed by brand (C) using yoghurt starter *L. acidophilus* together with *Bifidobacterium bifidum* and the least effect was belonging to brand (A) using yoghurt starter only. This inhibitory effect may be attributed either to the probiotic bacteria itself or to the antibacterial substances secreted by them. **Conclusion:** The authors recommended the consumption of bio-yoghurt as it is not only palatable but also, safe due to improved hygienic quality.

Keywords

Yoghurt, Probiotics, *Lactobacillus acidophilus, Bifidobacterium bifidum*

1. Introduction

Milk fermentation is initiated by the growth of lactic acid bacteria, thus increasing the acidity and inhibiting the growth of most other objectionable organisms. Therefore, fermented milk is delicious and palatable [1] [2].

The most popular type of fermented milk in Egypt is yogurt. Its nutritive value is attributed to the fat content, sugar and casein. In GIT, fermented milk assists the absorption of calcium and phosphorus, besides it inhibits the bacterial flora of intestine which may lead to constipation, autointoxication or colitis. Therefore, it is recommended for sick and convalescent people [3].

Dairy foods containing viable probiotic bacteria represent one of the functional foods, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora [4]. In addition to improving gut health, probiotics may play a beneficial role in several medical conditions, including lactose intolerance, cancer, allergies, hepatic disease, *Helicobacter pylori* infections, urinary tract infections, hyperlipidemia and assimilation of cholesterol [5].

Enterotoxigenic *S. aureus* strains in dairy products possess a potential health hazard to consumers, and their presence is used as a part of hazard analysis and risk assessment of milk and milk products [6]. Outbreaks of food poisoning by *S. aureus* are often associated with unsanitary handling of food at an inappropriate temperature for a prolonged period of time [7]. Milk and dairy products constitute 1% - 9% (mean 4.8%) of all *S. aureus* outbreaks in Europe [8]. Last reported outbreak caused by *S. aureus* was recorded by CDC in USA in 2011 and it was linked to cheese.

The quality and safety of yoghurt depend on quality of milk, starter culture used, efficiency of processing and the hygienic practice applied in the dairy plant. This study aims to improve hygienic quality of yoghurt by using probiotic strains as starter cultures for bio-yoghurt production and determine its acceptability for the consumer and its effect on *S. aureus* as one of the most common pathogenic microorganisms isolated from yoghurt. In recent years, bio-yoghurt has been designed to include live strains of *L. acidophilus* and species of *Bifidobacterium* with usually yoghurt starter cultures. The number of viable cells, namely the "therapeutic minimum" needs to be consumed regularly for transfer of the "probiotic" effect to consumers. Consumption should be more than 100 g per day of bio-yoghurt containing more than 10^6 cfu/gm. Probiotic bacteria [9]. The selection of the appropriate probiotic bacteria and strains is an important factor affecting its functionality. The interactions among the probiotic strains selected to manufacture of dairy product should be taken in consideration to select the best combination(s) and to optimize their technological performance in the process and their survival in the products during storage [10] [11]. The addition of probiotic sources such as inulin has also been shown to improve the stability and sensory characterization of fermented milk products [12].

2. Material and Methods

2.1. Used Milk

Buffalo's milk was obtained from the Department of dairy production, Faculty of Agriculture, Cairo University.

2.2. Yoghurt Starter Culture

Lyophilized fermented and probiotic cultures for direct vat set (DVS) used in this study were kindly provided by Chr. Hansen Laboratories, Copenhagen, Denmark. The used cultures were classified as:

Y.C-280: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*.

La-5: *Lactobacillus acidophilus.*

Bb-12: *Bifidobacterium bifidum* (Bb-12).

All cultures were prepared according to the manufacturer's description.

2.3. Reference *Staphylococcus aureus* Strain Used

Reference strain of *Staphylococcus aureus* (25923) S.A., was used. The organism was kindly obtained from ATCC (American type culture collection). The organism was inoculated in Brain Heart Infusion Broth for 18 hours to yield a final concentration of 7.3 log cfu/ml as determined by spreading technique on Baired Barker agar medium.

2.4. Preparation and Inoculation of Yoghurt

Three brands of Yoghurt (A, B & C) were prepared in the laboratory using the procedure described by [13].

For each brand of yoghurt, one liter of the raw milk was heated to 80˚C for 30 minutes, and then cooled to 40˚C - 44˚C. The exact amount of starter was added according to the brand of yoghurt (**Table 1**), followed by stirring. The inoculated milk was divided into two parts. The first part was inoculated with fresh *S. aureus* (25923) S.A. culture, while the second part was left as a control. The inoculated milk was divided into sterile cups and incubated at 45˚C for 4 - 5 hours. After complete fermentation, the cups were transferred to the refrigerator to be stored at 4˚C.

2.5. Organoleptic Examination

Organoleptic examination of the control part of each brand was applied after one day storage at 4˚C [5].

2.6. Determination of pH Value

It was applied according to [14], using a digital pH meter. Three readings were recorded and the average was calculated.

2.7. Viability of Inoculated *S. aureus*

During yoghurt manufacture, samples were taken at zero time inoculation and every an hour of the incubation period to determine pH and *S. aureus* count

Table 1. Brands of laboratory prepared yoghurt in relation to the used starter culture.

Brand	Used starter culture			Ratio of starter cultures
	Yc-280	La-5	Bb-12	
A	+	−	−	
B	+	+	−	1:1
C	+	+	+	1:1:1

according to [15]. Samples were also taken from each brand every 24 hours for 7 days of storage.

The trial was carried out on triplicates and the average results were calculated and recorded. All results were statistically analyzed using Excel program.

3. Results and Discussion

Many factors can affect the growth and survival of food-borne microbial pathogens in food. Investigation of the effects of environmental conditions on the growth and survival of food-borne pathogens are crucial to control and limit their potential risks [7]. Predictive modeling has been applied in food microbiology to describe the growth behavior of specific pathogens. [16] has divided the microbial growth models into kinetic models and probability models. Kinetic and probability models may be closely related. Application of microbial growth models to predict the growth behavior of specific microorganisms in yoghurt has been widely studied in recent years [17] [18].

The hygienic quality practices are necessary to be applied in the processing, handling and transferring of the product to avoid the contamination of food with *S. aureus* and eventual production of its enterotoxins [19]. Many researchers reported that yoghurt could be contaminated by *S. aureus* and survived fermentation during storage period till 8 - 10 days. In this period, toxins could be formed and persist after inhibition of the organism by low pH. Also, by using probiotic starter culture the population of *S. aureus* could be lowered to non-detectable level within short time of storage [20].

Data depicted in **Table 2** reveal that among the three brands of control negative produced yoghurt (A, B and C) all of them gained very good grade in container and closure, excellent in appearance, flavour and body & texture, while in acidity only the brand (C) gained the grade excellent and the other two brands were very good. On the other hand all the three brands were judged as excellent concerning the overall acceptability.

Data presented in **Figures 1-4** demonstrate the behavior of pH and survival of *S. aureus* during the fermentation period at 42˚C for 5 hours and storage period at 7˚C for 7 days for brand (A), the pH fall from 6.5 to 4.7 during fermentation, while slow decrease was observed during the 7 days storage from 4.8 to 4.4. Regarding to the inoculated *S. aureus* starting from zero time (log cfu/g. 7.3) during fermentation no visible decrease was observed only at 5 hours. By the end of storage period (7th day) the *S. aureus* count decreased to (5.8 log), showing drop as (1.5 log).

Inspection to the results tabulated in **Figures 1-4** illustrates the behavior of pH and survival of *S. aureus* during the manufacture and storage of Brand B yoghurt. pH decreased from 6.4 to 4.7 during manufacture and dropped from 4.8 to 4.4 during the 7 days storage ay 7˚C, which simulate the behavior of pH in brand (A). On observing *S. aureus* count starting from zero time to the end of fermentation period, there is no difference (7.3 log), but in the storage we found

Table 2. Grading of control negative yoghurt samples based on their organoleptic examination (average of triplicates).

Judged item	Brand (A)	Brand (B)	Brand (C)
	Grade	Grade	Grade
Container & Closure	Very Good	Very Good	Very Good
Appearance	Excellent	Excellent	Excellent
Flavour	Excellent	Excellent	Excellent
Body & Texture	Excellent	Excellent	Excellent
Acidity	Very Good	Very Good	Excellent
Overall Acceptability	Excellent	Excellent	Excellent

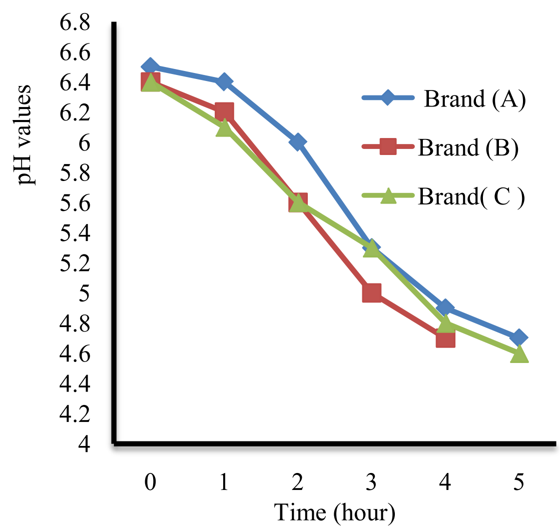

Figure 1. Changes in pH during manufacture of 3 brands yoghurt (average of triplicates).

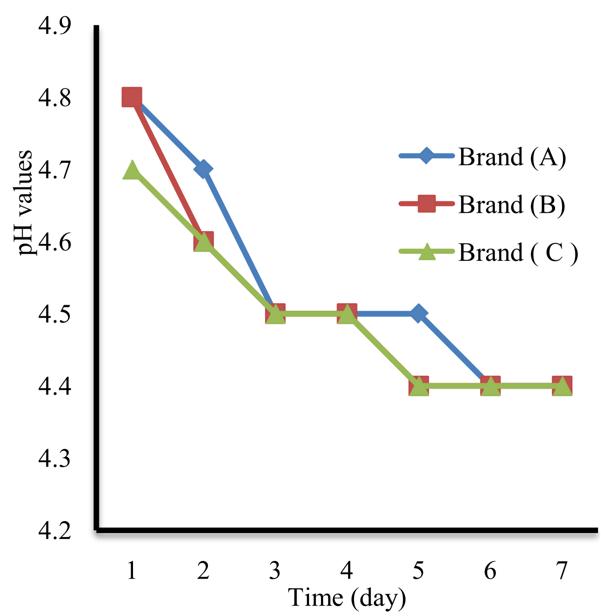

Figure 2. Changes in pH during storage of 3 brands yoghurt (average of triplicates).

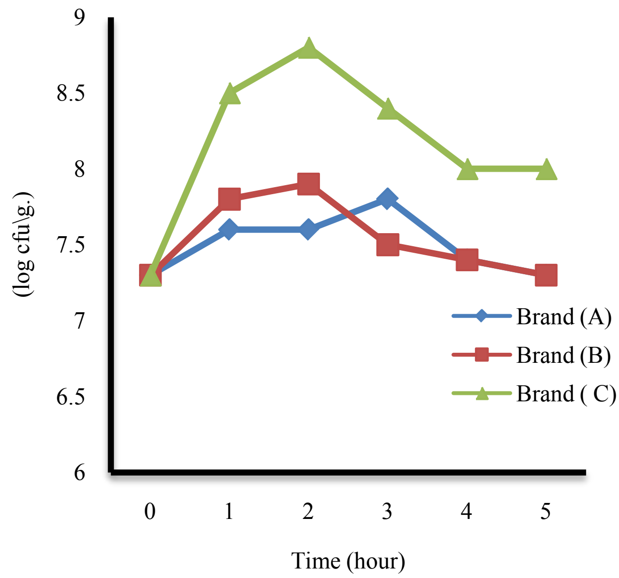

Figure 3. Changes in viable *S. aureus* counts (log cfu/g.) in 3 brands yoghurt during manufacture (average of triplicates).

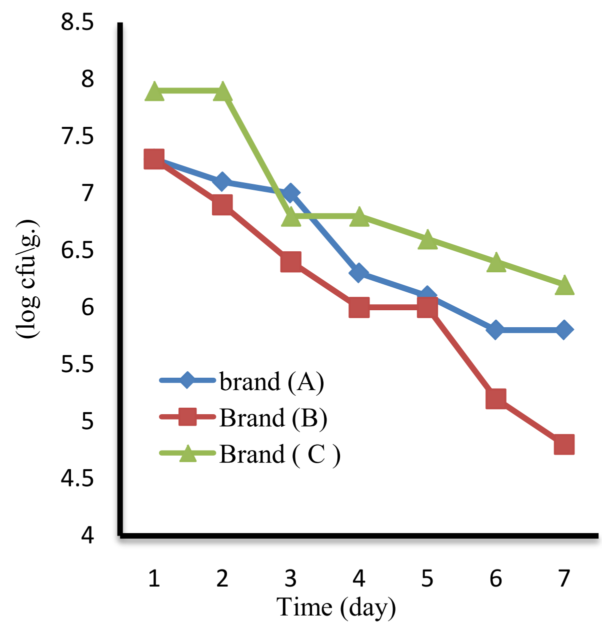

Figure 4. Changes in viable *S. aureus* count (log cfu/g.) in 3 brands yoghurt during storage (average of triplicates)

that the count decreased to (4.8 log) at the end of storage period (7th day), showing drop as (2.5 log).

Also, data existing in **Figures 1-4** illustrated the behavior of pH and survival of *S. aureus* during the manufacture and storage of Brand C yoghurt. pH fall from 6.4 to 4.6 in fermentation stage, and decreased to 4.4 at the end of the storage period. For *S. aureus* count, there was an observed increase in count from (7.3 log) to (8 log) during fermentation, while there was drop in count to (6.2 log) at the end of storage period (7th day), with drop as (1.8 log).

From the obtained data we can conclude that the inoculated *S. aureus* decreased in number by the end of the storage period, as well as, the best bactericidal effect was observed in brand (B) using yoghurt starter with *L. acidophilus*, followed by brand (C) using yoghurt starter with L. acidophilus together with *Bifidobacterium bifidum* and the least effect was belonging to brand (A) using the traditional yoghurt starter only. This inhibitory effect may be attributed either to the probiotic bacteria themselves or to the antibacterial substances secreted by them.

However, it is a well-known fact that the behavior of selected *S. aureus* strains in test yoghurts differs considerably under the same conditions. It follows from the results of our experiment that during the fermentation of yoghurt the concentration of *S. aureus* remains practically unchanged, which is reduced during refrigerated storage by the yoghurt culture inhibitory substances. The results show that the yoghurt culture reduces the counts of *S. aureus* added to milk by 1 - 2 log during the cold storage of yoghurt.

In the case of a high contamination of milk with *S. aureus,* the antibacterial effect of yoghurt culture is insufficient to avoid the risk of food poisoning. Therefore, the desirable inhibitory effect of yoghurt culture on some pathogenic micro-organisms must not lead to a lowering of hygiene standards during the production, storage and distribution of yoghurt [21]. Further studies about these functional traits of different probiotics in other dairy products are needed.

4. Conclusion

Due to the increasing demand for safe and functional foods, fermented dairy products especially yogurt are increasing consumption which is increasing by time. Bio-yoghurt is not only palatable for the consumer but it also improves the final product hygienic quality and decreases the risk of pathogenic microorganisms presence.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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