



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2018; 7(7): 267-270

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www.thepharmajournal.com

Received: 12-05-2018

Accepted: 15-06-2018

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## Effects of zinc on the acidification pattern of fermented milk by yoghurt starter culture

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### Abstract

As an initial investigation of using different  $Zn^{2+}$  concentration to evaluate acidification, the fermentation time, pH and the viable counts of bacteria of fermented milk during fermentation were studied. Fermented milk with 6.0 and 7.0 ppm of  $Zn^{2+}$  presents the shortest fermentation time (<4 h) compared with other samples (more than 4 h) except that of the control and sample with 1 ppm of  $Zn^{2+}$  (5 h). Among all fermented milk samples, the counts of *S. thermophiles* and *L. bulgaricus* were significantly highest in the addition of 7.0 ppm of  $Zn^{2+}$ . Zinc at the concentration of 1.0 ppm had no significant difference compared to the control sample in fermentation time and viable count of *S. thermophilus* and *L. bulgaricus*. It seems clear that the prevention of zinc deficiency among young children remains the best policy, not only on moral ground, but also on economic ones. These results of present study will contribute a great deal of work to find an adequate way to prevent zinc deficiency via fermented milk.

**Keywords:** Fermented milk, zinc, acidification, yoghurt starter

### Introduction

#### Review of literature

Zinc is an essential mineral for many basic physiological functions and cellular metabolism in human body. Its deficiency causes many disorders and approximately two billion people in the world are estimated to be at risk of inadequate zinc intake (Song et al 2009) [1]. Zinc is also readily available as dietary supplements. But, zinc fortification of foods is a potentially more useful strategy for the control of zinc deficiencies, especially for risk groups such as elderly, vegetarians, lactating women and children (Walingo 2009) [2].

Dairy products are good sources for fortification strategies, not only due to worldwide consumption by all groups at risk of deficiency, but also because of the high nutritional value, the buffer effect in the digestion and absorption processes, and the positive effects on growth, cognition, and morbidity (Joon et al., 2017, Kaur et al., 2017, Joon et al., 2018, Chawla et al., 2018) [3-6]. Several works has been done on lactic acid bacteria (Singroha et al., 2017, Mishra et al., 2011, Mishra et al., 2012, Mishra et al., 2016, Mishra et al., 2018), [7-11]. Zinc is widely distributed in some foods such as red meat, turkey, oyster etc. But, milk and most of milk products are low in zinc. Dairy products only provide about 19-31% of total zinc intake in western countries (Hunt and Nielsen 2009) [12] without any fortification. The fortification of such kind of milk and milk-based products with zinc will improve their nutritional status as functional foods. Zinc sulfate and zinc gluconate are authorized as zinc sources for specific nutritional purposes in foods and food supplements by European Regulation (EC) N. 1925/2006.

To date, there are very few studies reported on the fortification of milk and milk products with zinc salts and the responses of lactic acid bacteria to milk fortification with zinc salts. Therefore, the primary aim of the study is to evaluate effect of zinc sulphate fortification in milk on yoghurt starter culture to determine their acidification activities.

### Materials and Methods

#### Materials

Fresh whole milk was obtained in clean plastic containers from Experimental Dairy Plant, College of Dairy Science and Technology, GADVASU (Ludhiana) and taken to Dairy Microbiology Department, College of Dairy Science and Technology for standardization of milk to 3.0 % fat and 13% SNF.

Skim powder milk (Amul brand, Gujarat, India) was obtained from local market. Yoghurt starter cultures NCDC 144 (*L. bulgaricus* and *S. thermophilus*) were procured from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal, India. De Man Rogosa and Sharpe (MRS) broth and M-17 broth (HiMedia, India) were used for propagation of the *L. bulgaricus* and *S. thermophiles*, respectively. All chemicals and media used in this study were of analytical grade. Cells were removed by centrifugation and maintained in 10 ml of sterile 12% reconstituted skim milk supplemented with 2% glucose and 1% yeast extract. These cell suspensions were stored at 4 °C until they were added to milk. All cultures including working cultures were propagated successively three times prior to use. The purity of all the bacterial cultures was always ascertained by Gram staining prior to use for any experiment.

**Preparation of standardized milk added with Zn<sup>2+</sup>**

The whole milk was standardized to 3.0 % fat and 13% SNF and divided in 8 parts of 1000 ml each. Then Zn<sup>2+</sup> (ZnSO<sub>4</sub>·7H<sub>2</sub>O) were added into the milk at the final concentration of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ppm, respectively. In Control sample no Zn<sup>2+</sup> was added. All the milk samples were heat treated at 90 °C for 10 min.

**Fermentation characters of fermented milk**

Each sample (1000 mL in total) with different added concentrations of Zn<sup>2+</sup> (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ppm, respectively) was inoculated with yoghurt starter culture NCDC 144 (3%, w/v). The sample without zinc was used as a control. All samples were fermented at 42 °C for 6 h. A portion of fermented milk was taken out for pH and viable counts analysis at 0, 1, 2, 3, 4, 5 and 6 h of incubation, respectively. The fermentation time was recorded when pH dropped to 4.70, and the other portion of fermented milks were transferred to an ice bath immediately to stop the fermentation.

**Microbiological Analysis**

Enumeration of the *L. bulgaricus* and *S. thermophilus* were carried out at the end of fermentation. 10 g of yoghurt samples were diluted in 90 mL of sterile 0.01% (w/v) peptone water and mixed properly. Aliquots (0.1 mL) of the serial dilutions were spread in duplicate on MRS and M17 agar (HiMedia, India). MRS plates were incubated at 42 °C in jars with the Anaerogen system (HiMedia, India) for 48-72 h.

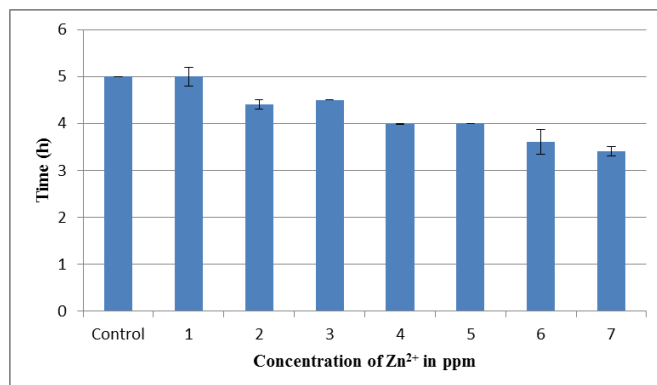
**Statistical Analysis**

Duncan’s multiple range tests were employed to determine the significance of the difference within treatments for each analysis. Total of 3 replicates were performed and the mean values were calculated. Values were considered significantly different when P < 0.05. All statistical analyses were performed using the SPSS13.0 statistics program.

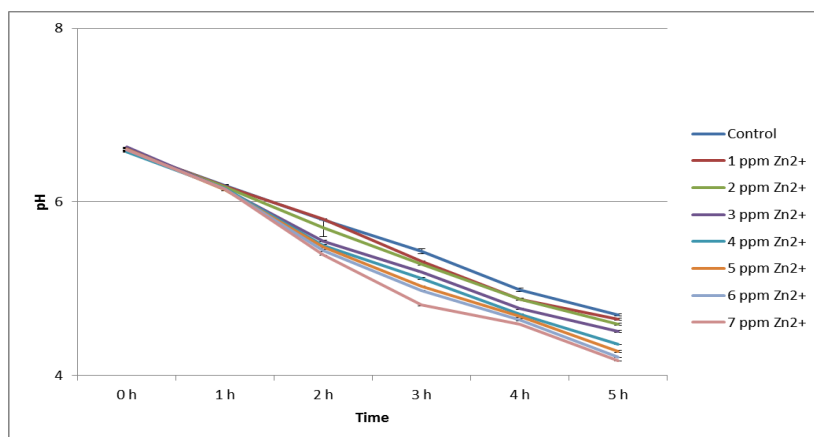
**Results and Discussion**

**Fermentation time and pH changes of fermented milk**

The changes of fermented time and pH during fermentation were influenced by the concentration of Zn<sup>2+</sup> (Figure 1 and 2). From the figure it is evident that as the concentration of zinc salt is increasing fermentation time is decreasing. When 7.0 ppm zinc ion concentration was used it took only 3.4 h to bring the pH of the milk to 4.6 in comparison to control sample (without zinc) where it took almost 5 h to bring that pH to the same level. Similarly from figure 2 it is evident that as the concentration of zinc salt is increasing pH is decreasing accordingly. After 5 h incubation, pH in control sample has gone down to 4.7 only but in case of milk with 7.0 ppm zinc ion concentration the pH went down to 4.1 in the same period. The results presented in this work indicated that a higher concentration of Zn<sup>2+</sup> resulted in a smaller fermentation time. Furthermore, the fermented milk with 6 and 7 ppm of Zn<sup>2+</sup> presents the shortest fermentation time (<4 h) compared with other samples (more than 4 h) except that of the control and sample with 1 ppm of Zn<sup>2+</sup> (5 h). The results of the present study indicate that the addition of 1.0 ppm of Zn<sup>2+</sup> to milk had no significant effect on the fermentation time.



**Fig 1:** The fermentation time changes of fermented milk with various concentrations of Zn<sup>2+</sup>. Values are mean values (n = 3). Control means no Zn<sup>2+</sup> was added.

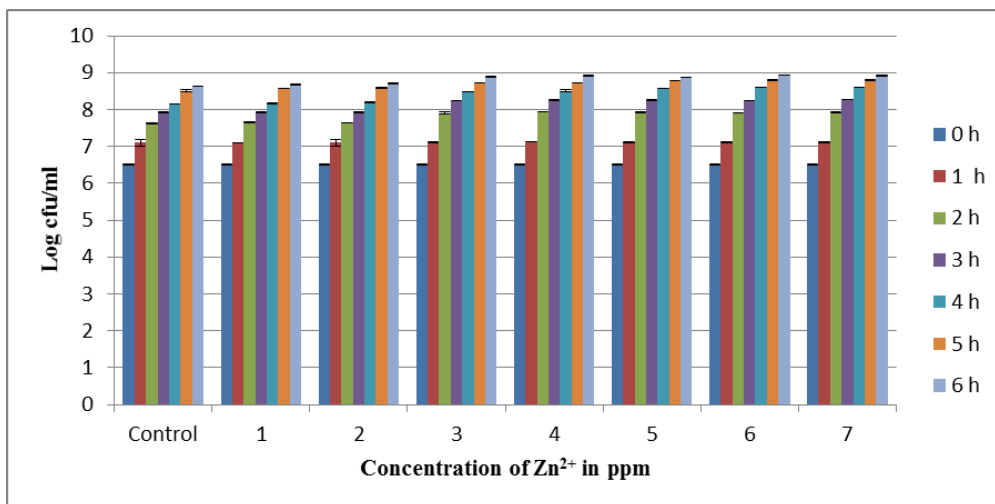


**Fig 2:** The pH changes of fermented milk during fermentation in the presence of various concentrations of Zn<sup>2+</sup>. Values are mean values (n = 3). Control means no Zn<sup>2+</sup> was added.

**Viable counts of *S. thermophilus* during fermentation**

The changes of viable counts of *S. thermophilus* showed similar trends in the pH during fermentation (Figure 3). The growth of *S. thermophilus* increased slowly with the increase in Zn<sup>2+</sup> concentration (Figure 3). For fermented samples with added 0 (control), 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ppm of Zn<sup>2+</sup>, the viable counts of *S. thermophilus* were increased from the initial 3.16 × 10<sup>6</sup> CFU/mL (the initial counts for all samples were the same) to 4.26 × 10<sup>8</sup>, 4.78 × 10<sup>8</sup>, 5.01 × 10<sup>8</sup>, 6.30 × 10<sup>8</sup>, 8.31 × 10<sup>8</sup>, 7.55 × 10<sup>8</sup>, 8.51 × 10<sup>8</sup> and 8.12 × 10<sup>8</sup>

CFU/mL, respectively, after incubation at 42 °C for 6 h. Analysis of variance (ANOVA) showed that when the concentration of Zn<sup>2+</sup> was above 2 ppm, there were significant changes in viable counts after incubation at 42 °C for 6 h (*P* > 0.05). Figure 3 also showed that Zn<sup>2+</sup> at the concentration of 1.0 ppm had no significant difference compared to the control sample in viable count of *S. thermophilus* (*P* > 0.05). Among all fermented milk samples, the counts of *S. thermophilus* were significantly highest in the addition of 7.0 ppm of Zn<sup>2+</sup>.

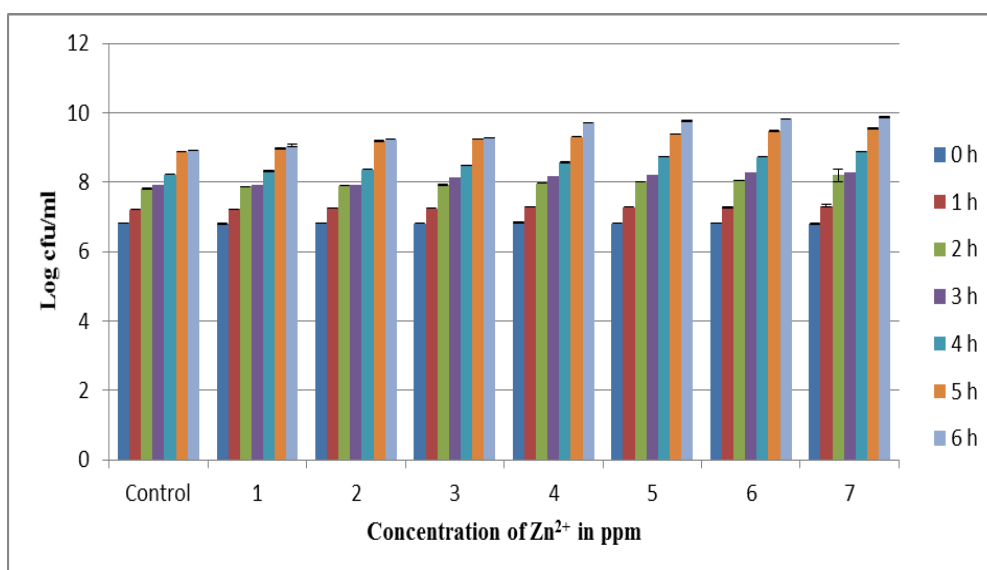


**Fig 3:** The viable count changes of *S. thermophilus* at 42 °C for 6 h by addition different concentration of Zn<sup>2+</sup>. Values are mean values (n = 3). Control means no Zn<sup>2+</sup> was added.

**Viable counts of *L. bulgaricus* during fermentation**

The changes of viable counts of *L. bulgaricus* showed similar trends in the pH during fermentation (Figure 3). The growth of *L. bulgaricus* increased slowly with the increasing of Zn<sup>2+</sup> concentration (Figure 3). For fermented samples with added 0 (control), 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ppm of Zn<sup>2+</sup>, the viable counts of *L. bulgaricus* were increased from the initial 6.45 × 10<sup>6</sup> CFU/mL (the initial counts for all samples were the same) to 8.31 × 10<sup>8</sup>, 1.12 × 10<sup>9</sup>, 1.69 × 10<sup>9</sup>, 1.90 × 10<sup>9</sup>, 5.12 × 10<sup>9</sup>, 5.88 × 10<sup>9</sup>, 6.76 × 10<sup>9</sup> and 7.41 × 10<sup>9</sup> CFU/mL, respectively, after incubation at 42 °C for 6 h. Analysis of variance (ANOVA) showed that when the concentration of

Zn<sup>2+</sup> was above 2 ppm, there were significant changes in viable counts after incubation at 42 °C for 6 h (*P* > 0.05). Boyaval (1989) [13] evaluated addition of 0.016 mM and 0.3 mM Zn<sup>++</sup> to the growth medium and its effect on *Lactobacillus delbrueckii* growth. They reported that although 0.016 mM Zn<sup>++</sup> showed a stimulative effect on the growth of LAB. Mrvic *et al.* (2009) [14] have investigated biosorption capacity of LAB species with a media consisting of 10–90 mg/L zinc and have not seen any toxic effect of zinc on LAB. Furthermore, they have seen that zinc ions enhance probiotic effect of *L. plantarum*.



**Fig 4:** The viable count changes of *L. bulgaricus* at 42 °C for 6 h by addition different concentration of Zn<sup>2+</sup>. Values are mean values (n = 3). Control means no Zn<sup>2+</sup> was added.

## Conclusions

The Zn<sup>2+</sup> addition to milk represented a positive effect to reduce the fermentation time of yoghurt. Zinc at the concentration of 1.0 ppm had no significant difference compared to the control sample in fermentation time and viable count of *S. thermophilus*. 7.0 ppm of Zn<sup>2+</sup> is within the prescribed limit under dietary requirement given by RDA. Further study focusing safety aspects of zinc ions in fermented food is in progress in our laboratory. By this study it can be concluded that application of zinc salts in fermented milk products production will result in obtaining dairy products with new functional properties and health benefits.

## Acknowledgements

Mr. Santosh Kumar Mishra is the recipient of funds from Union Grant Commission, New Delhi, India, under Major Research Project and duly acknowledges the financial assistance received.

## Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

## Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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