Atomic absorption spectrophotometric study to compare the zinc content of conventional and probiotic Pearl millet foods

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ABSTRACT

Aim: To estimate the amount of zinc concentration in Probiotic treated innovative pearl millet by the atomic absorption spectrophotometric analysis. **Materials and Methods:** The probiotic strains *Lactobacillus sporogenes* and *Bacillus mesentericus* were inoculated to the pearl millet composition and allowed to ferment for 18 hrs. Zinc Concentration was estimated for both the fermented and non fermented pearl millet composition. The samples were then calculated by clincalc.com and prior web. The alpha error threshold was set at 0.05%, the confidence interval was set at 95%, enrollment ratio at 1 and g power was set at 80%. Using IBM SPSS version 28.0 software. T tests were performed between the samples (N=18). **Results:** Zinc concentration was found to be 0.7mg/100g for Probiotic treated innovative pearl millet and 1.2 mg/100g for Conventional pearl millet and was found to be statistically insignificant. **Conclusion:** A decline in the zinc concentration in the fermented sample was observed when compared to the untreated samples.

Keywords: Innovative Pearl Millet, Probiotics, Atomic Absorption Spectrophotometry, Milk, *Lactobacillus sporogenes, Bacillus mesentericus.*

INTRODUCTION

Pearl millet, an important source of zinc, is a low-cost approach for addressing micronutrient deficiencies in millet-consuming areas and might be used to make nutrient-dense dishes. (Krishnan and Meera 2018)**.** Zinc deficiency is a major public health concern, especially in underdeveloped nations where diets consist primarily of bland cereal staples. These cereal grains are essential for newborns and young children since complementing porridges are made from various fermented cereals. Insulin synthesis, maturation, secretion, and signaling are all performed by the insulin-producing pancreatic -cell, which has some of the highest zinc concentrations in the body. [Citation error].

Literature was searched in accordance to the title in platforms including google scholar, science direct, and pubmed. An average of 81 papers were found in google scholar. Children are typically given supplemental porridge 2-3 times per day (Gabaza et al. 2018). About 3 billion suffer from Micronutrient deficiencies annually and among that zinc is required daily for about 8mg /day. [Citation error]. In the pearl millet, zinc bioaccessibility was shown to be high in fractions with minimal phytic acid and insoluble fiber. (Krishnan and Meera

2018). Natural fermentation with yeasts, bacteria, and fungi is used to make cereal grains. Several of them can be served as a side dish, breakfast, light lunch, or main dish. The microbiology of a number of regularly consumed cereal-based fermented foods is complicated and unclear. Probiotic bacteria are well-known for their health-promoting properties. [Citation error]. Fermentation has been demonstrated to increase the availability of micronutrients by decreasing the polyphenol contents. Hence the positive outcome of this study could help develop the products to balance the micronutrient deficiency [Citation error]. Also the use of probiotics could increase the digestibility and palatability which could be highly beneficial for the children and Geriatric population. Because zinc is a cofactor for numerous proteins involved in immune regulation, it is necessary for optimal immune system development and function. There have been reports on the effects of several probiotics on Pearl millet fermentation and zinc availability, but there has yet to be a report on the combined usage of *Lactobacillus sporogenes* and *Bacillus mesentericus* and their zinc availability. As a result, the research was carried out to identify the effects of the probiotic therapy on the zinc concentration of pearl millet. [Citation error].Previously our team has a rich experience in working on various research projects across multiple disciplines(Madhesh et al. 2021; Bishir et al. 2020);(Vimalraj et al. 2020; Sivasamy, Venugopal, and Mosquera 2020)(Madhesh et al. 2021; Bishir et al. 2020)

The reports on the different probiotics used on the fermentation of Pearl millets and the zinc availability exists however the combined use of the two Probiotics *Lactobacillus sporogenes* and *Bacillus mesentericus*and their zinc availability has not yet been reported. The study was performed to evaluate the zinc concentration of probiotic-based innovative Pearl millet utilizing *Lactobacillus sporogenes* and *Bacillus mesentericus* by AAS method.

MATERIALS AND METHODS

The study was conducted at Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, at the Department of Microbiology Laboratory. Probiotic treated Pearl millet and Conventional Pearl millet make up the total number of categories. This study had a sample size of 18. Clincalc.com was used to determine the sample size before the test. The alpha criterion was set at 0.05, the confidence interval was set at 95%, the g power was set at 80%, and the enrolment ratio was set at 1 ([Citation error].

To prepare the unique probiotic-based pearl millet composition in a 500ml beaker, 20g of powdered pearl millet was mixed with 100 ml of fresh milk and steam sterilized at 121°C for 20 minutes. To that probiotic based pearl millet mixture, pure culture of *Lactobacillus sporogenes* and *Bacillus mesentericus* isolated on MRS agar was inoculated and the uninoculated standard pearl millet preparation without microorganisms was used as comparative control [Citation error].

The zinc concentration was estimated by atomic absorption spectrophotometric

analysis by standard procedures (Adebiyi et al. 2017).

STATISTICAL ANALYSIS

IBM SPSS 28 version software was used for statistical analysis. There were no independent variables in this experiment. Pearl millet with probiotics and conventional pearl millet without probiotics are the dependent variables. IBM SPSS version 28 software was used to execute a paired T test and calculate the standard deviation in standard of mean errors ([Citation error].

RESULTS

The atomic absorption spectrophotometry method was used to calculate the zinc content. For Probiotic Pearl millet, the concentration was estimated to be 0.7 mg/100g, while for Conventional Pearl millet, it was calculated to be 1.2 mg/100g. Table 1, Fig. 1 and Fig. 2 describe the zinc concentration values of Probiotic based pearl millet and Conventional pearl millet, as well as their various values.

Table 2 represents the paired T test analysis between the Probiotic based Pearl millet and conventional Pearl millet food composition . It was found to be statistically insignificant and are represented in Fig. 3.

Table 3 results represent the mean±standard error variation between the Probiotic based Pearl millet and Conventional Pearl millet. Zinc observed in the Probiotic based Pearl millet samples in our study. Our results were inconsistent with the Zinc concentration of the pearl millet analyzed in their region showing higher concentration.

DISCUSSION

The zinc concentration in Probiotic based Pearl millet and Conventional Pearl millet was determined using the Atomic Absorption Spectrophotometry method in this study. For probiotic-based Pearl millet, the zinc content was estimated to be 0.7 mg/100 g, while for conventional pearl millet, it was assessed to be 1.2 mg/100g. The content of zinc in the Probiotic based Pearl millet samples showed a sharp decline when compared to the conventional pearl millet.

At 0 hours, the zinc extractability was 44%, increasing to 89 percent after 96 hours of fermentation at 50°C. Fermentation at other temperatures was less successful in enhancing zinc extractability throughout this time period. After 24 hours of fermentation at 30°C, 40°C, and 50°C, the amount of the growth was greatest. This meant that fermenting for longer than 24 hours at 30°C, 40°C, or 50°C did not increase zinc extractability, which is similar to our research that shows gradual increase in zinc content after 18 hrs of fermentation. (Mahajan and Chauhan 1988). At a temperature of 40°C, pH 5, and a period of 38.86 hours, the maximum concentration of zinc was found to be 12mg/100g. At 30° C, pH 3, and a time length of 4 hours, the lowest zinc concentration of 4.5mg/100g was attained, when the temperature of fermentation was increased the zinc content in the pearl millet was also increased. (Srivastava et al., n.d.).

The sampling time and the other parameters in our study were set at 37°C for 24 hours of fermentation, which is a constraint. The sample period and

conditions in our investigation was standard and the fermentation was completed after around 18 hours. As a result, future studies can use and expand the different treatment temperatures, fermentation periods and inoculum quantities to better future optimization [Citation error]. Phytic acid is reduced when lactic acid bacteria are used, allowing for a higher concentration of zinc to be available. As a result, standardizing fermentation hours and minutes may benefit in the development of novel probiotic-based pearl millet diets for persons who are iron deficient. (Arora, Jood, and Khetarpaul 2011). Thus the observations from our study reveals the requirement of significant optimisation procedures to obtain the increased concentration of Zinc.

CONCLUSION

The concentration of accessible zinc minerals could be increased by the fermentation process . Our tests found zinc concentrations ranging from 0.7mg/100g to 1.2 mg/100g and so further optimization could aid in the creation of probiotic-based innovative pearl millet diets to supplement the micronutrient deficiencies.

DECLARATION

Conflict of interest

No conflict of interest in these manuscripts.

Authors contribution

Author BP was involved in data collection, data analysis, manuscript writing, Author SS was involved in conceptualization, data validation, and critical review of manuscript.

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FIGURES AND TABLES

Table 1. Zinc concentration values of Probiotic based pearl millet and Conventional pearl millet and its different values.

Probiotic based Pearl millet in mg/100g	Conventional Pearl millet in mg/100g
0.7	1.2
0.5	1.1
0.4	1.8
0.33	1.6

0.3	1.4
0.28	1.3
1.25	1.1
1.15	2.3
1.9	2.8

Table 2. Zinc concentration values of Probiotic based pearl millet and Conventional pearl millet and its different values were summarized.

Group Statistics					
	GROUP	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
ZINC CONCENTRATION	PROBIOTIC BASED PEARL MILLET	9	.6456	.55018	.18339
	PEARL MILLET	9	1.6222	.58689	.19563

Table 3. Paired T test analysis between the Probiotic based Pearl millet and conventional Pearl millet food composition.

		Levene's Test for Equality of Variances		**t-test for Equality of Means**							
										95% Confidence Interval of the Difference	
		F	**Sig.**	**t**	**df**	**One sided significance**	**Two sided significance**	**Mean Difference**	**Std. Error Difference**	**Lower**	**Upper**
OD	**Equal variances assumed**	.092	.766	-3.642	16	.001	.002	-.97667	.26815	-1.54512	-.40821
	Equal variances not assumed			-3.642	34	.001	.002	-.97667	.26815	-1.54531	802

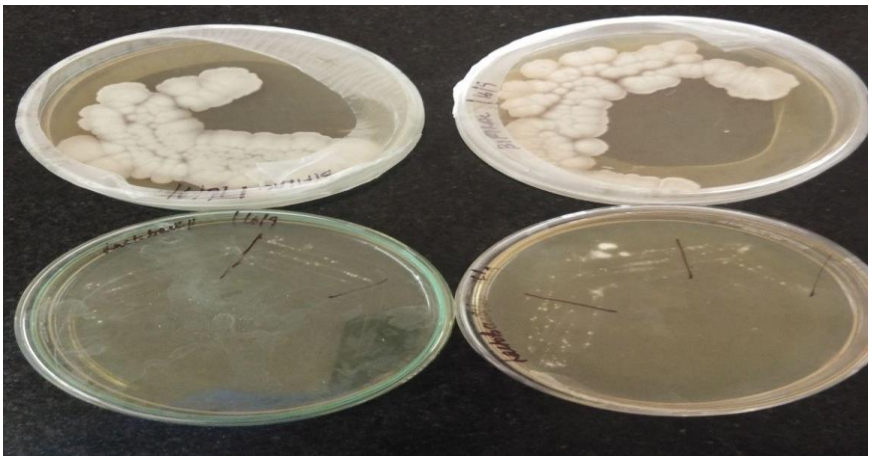

Fig. 1. Bifidobacterium and Lactobacillus cultured from curd and bifilac sachet as Probiotic strains

Fig. 2. Beakers containing fermented pearl millet and milk. The fermentation time is 18 hr.

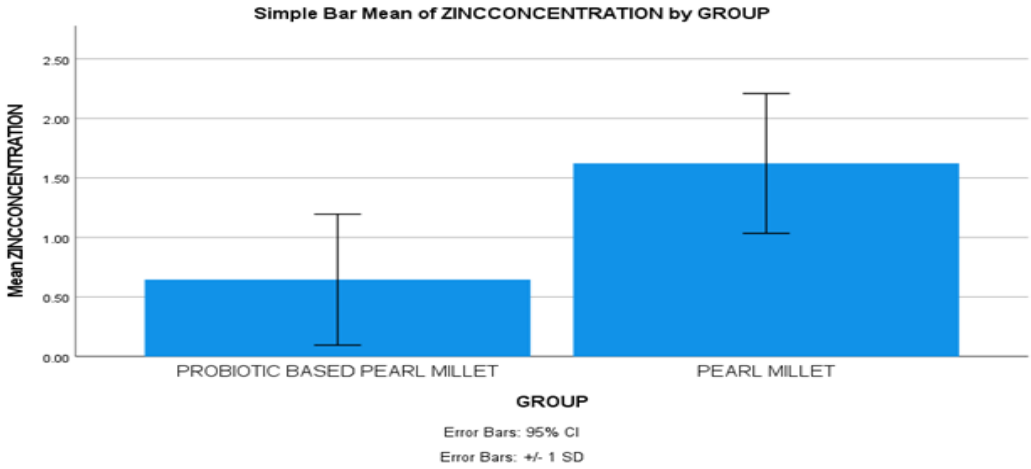

Fig. 3. Probiotic based Pearl millet shows significant decrease when compared to the Conventional Pearl millet (P<=0.001). X-axis represents the probiotic based pearl millet and pearl millet Y-axis indicating the mean Zinc concentration. SD+/- 1.