

Comparative Analysis on the Calcium Concentration of Conventional over Probiotic Based Pearl Millet Foods by Atomic Absorption Spectrophotometric Analysis.

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Abstract

Aim: To estimate the Calcium concentration of Probiotic based fermented innovative Pearl millet by Atomic Absorption Spectrophotometric analysis. **Materials and Methods:** Probiotics Lactobacillus and Bacillus mesentericus were used and compared with the uninoculated Pearl millet for Calcium estimation by atomic absorption spectroscopy after 18 hrs. The sample was calculated by using clincalc.com. For alpha error- threshold at 0.05%, 95% confidence interval, G power at 80% and enrollment ratio 1 (N=18). T tests were performed between the samples using IBM SPSS V 28.0 software. **Results:** Calcium concentration was estimated to be 112.5 mg/100g for probiotic pearl millet and 187.8 mg/100g for Conventional pearl millet. Comparison of the results by statistical T test analysis showed it as statistically significant. **Conclusion:** A moderate increase in the concentration of Calcium was observed in the Conventional pearl millet composition which can be further standardized for use as supplements to treat malnutrition.

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

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INTRODUCTION

Pearl millets (*Pennisetum glaucum*) are widely cultivated millet species and principal sources of minerals like calcium, zinc, iron, and vitamins (Ranasalva and Visvanathan 2014). The crop pearl millet is farmed over 26 million hectares in Africa and the Indian subcontinent each year. The presence of anti-nutritional substances (phytate, tannins, and polyphenols) that reduce mineral availability and poor storage quality due to greater lipase activity are the main factors that limit its use (Amara and Shibl 2015)(Dudhate et al., n.d.)(Amara and Shibl 2015). The Calcium concentration is generally higher in pearl millet than any other minerals and is 40 mg/100g. The Calcium content in millets ranges from 14.98 ± 1.5 to 344.45 ± 2.62 mg/100 g with a mean value of 110.6 mg/100 g. (Dias-Martins et al. 2018). Fermented Ca is also known to be beneficial to those with diabetes, especially type 1 diabetics, who have lower bone mineral density than healthy people (Mirza et al. 2014).

Literature was searched in accordance to the title in platforms including google scholar, science direct, and pubmed. An average of 65 papers were found in google scholar. However the bioavailability is decreased due to the presence of anti-nutrient factors like phytic acids and polyphenols. Fermentation process using different microorganisms has been reported to reduce the anti-nutrient factors like phytic acids and polyphenols whilst increasing the bioavailability of the various minerals and vitamins (Dudhate et al., n.d.). The literature sources reports the effect of the 1 gm of fiti sachet containing the probiotics Lactobacillus rhamnosus and Streptococcus thermophilus on the changes in the chemical composition by reducing the moisture, ash, fiber, fat and protein content and also their effect on the different carbon sources (Dias-Martins et al. 2018); (Achi and Asamudo 2019); (Dias-Martins et al. 2018). The primary activities of fermented calcium in food include providing rigidity and structure, moderating vascular and muscle contractions and dilations, and transmitting nerve signals. (Puranik et al. 2017). Hence the study was undertaken to determine the effect of the aforementioned probiotic treatment on the calcium concentration of the pearl millet (Turpin, Humblot, and Guyot 2011).

Our team has extensive knowledge and research experience that has translate into high quality publications (Bhansali et al. 2021; Jayanth et al. 2021; Sudhakar et al. 2021; Sathiyamoorthi et al. 2021; Deepanraj et al. 2021; Raju et al. 2021; Arun Prakash et al. 2020; Kamath et al. 2020; Shanmugam et al. 2021; Rajasekaran et al. 2020; Adhinarayanan et al. 2020; Rajesh et al. 2020; Aurtherson et al. 2021). The reports on the different probiotics use on the fermentation of Pearl millets and the calcium availability exists however the combined use of the two Probiotics Lactobacillus sporogenes and Bacillus mesentericus and their calcium availability has not yet been reported (Ganguly, Sabikhi, and Singh 2021).The reports on the different probiotics use on the fermentation of Pearl millets and the calcium availability exists however the combined use of the two Probiotics Lactobacillus sporogenes and Bacillus mesentericus and their calcium availability has not yet been reported (Ganguly, Sabikhi, and Singh 2021). The aim of this project is to estimate the calcium concentration of the Probiotic based innovative pearl millet using Lactobacillus sporogenes and Bacillus mesentericus by atomic absorption spectrophotometry analysis.

Material and Methods

The research work was carried out at the Microbiology Lab at Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai. Total number of groupings was two (Probiotic based Pearl millet and Conventional Pearl millet). The sample size is 18. Pre-test power the sample size was calculated by clincalc.com. alpha threshold at 0.05, confidence interval at 95%, g power at 80% and enrolment ratio at 1 (Ganguly, Sabikhi, and Singh 2021; Semwal, Kamani, and Meera 2021).

To prepare the novel probiotic based pearl millet composition, 20 g of powdered pearl millet was mixed with 100 ml of milk and steam sterilized at 121 °C for 20 min. To that, the pure culture of Lactobacillus and Bacillus mesentericus isolated in MRS agar was inoculated to the prepared pearl millet mixture and the uninoculated conventional preparation of pearl millet without the microorganisms was used as control for comparison. Lactobacillus sporogenes was isolated from the curd following the serial dilution method in MRSA and Bacillus mesentericus was isolated from Bifilac sachet (Stefano et al. 2017).

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Statistical analysis

Statistical analysis was performed by IBM SPSS version 28 software. The dependent variables are two, Probiotic based Pearl millet and Conventional Pearl millet and there were no independent variables. Paired T test; standard deviation in standard of mean errors was performed using IBM SPSS version 28.0 (Ganguly, Sabikhi, and Singh 2021; Semwal, Kamani, and Meera 2021).

Results

Calcium concentration was estimated by the Atomic Absorption Spectrophotometry. The Calcium concentration was estimated to be 112.2 mg/100g for Probiotic pearl millet and 187.8 mg/100g for Conventional pearl millet. The calcium concentration values of Probiotic based pearl millet and Conventional pearl millet and its different values were summarized in Table 1, Fig. 1 and Fig. 2.

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 significant.

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

Discussion

In this study Calcium concentration was estimated by performing Atomic Absorption Spectrophotometry method for the Probiotic based Pearl millet and Conventional Pearl millet. The Calcium concentration was estimated to be 112.2 mg/100g for Probiotic based Pearl millet and 187.8mg/100g for Conventional pearl millet. There was a moderate decrease in the concentration of Calcium observed in the Probiotic based Pearl millet samples in our study. Our results were inconsistent with the calcium concentration of the pearl millet analyzed in their region showing higher concentration.

The Probiotic based pearl millet composition used in this study is a novel preparation in which the milk was used as an additional substrate and the microorganisms used were *Lactobacillus sporogenes* and *Bacillus mesentericus*. Also the complete sterilization was carried out to understand clearly the influence of these microorganisms on the concentration with and without fermentation. Accordingly our calcium concentration in our tested samples ranged from 112.2 to 187.8 mg/gm. This was very near to the calcium content estimated in MP-7792 V (Tomar et al. 2021); (Kunchala et al. 2017); (Tomar et al. 2021). Unlike in the other findings, a two fold increase in the concentration of the calcium was also reported as 295.58 mg/gm in the fermented samples (Adebiyi et al. 2017). In the germinated pearl millets, significant decrease in the calcium content has been reported from to 41.3 mg/gm in comparison to our results. (Suma and Urooj 2014)

The limitation in our study is that the sampling time and conditions was standard and performed at 37 c for 24 hrs of fermentation. Hence the different treatment temperature, fermentation time and inoculum size can be used and extended in future studies for further optimisation. The use of lactic acid bacteria reduces the phytic acid which makes the availability of calcium in higher concentration and the fermentation process could increase the digestion of food (Arora, Jood, and Khetarpaul 2011). Hence further standardization of the fermentation hours and time could help in the development of novel probiotic based innovative pearl millet foods for the calcium deficient individuals (Ilango and Antony 2021).

Conclusion

Fermentation and processing of the Pearl millet could increase the concentration of the available nutrients. The calcium concentration estimated in our studies ranged from 112.2 mg/100g to 187.8 mg/100g which was highly significant and hence further optimisation could help in the development of probiotic based pearl millet foods. Hence the novel approach on the use of probiotics *Lactobacillus sporogenes* and *Bacillus mesentericus* in the pearl millet composition as a probiotic and their role in fermentation needs to be explored further.

DECLARATION

Conflict of interest

No conflict of interest in these manuscripts.

Authors contribution

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

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FIGURES AND TABLES

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

Probiotic based pearl millet in mg	Conventional pearl millet in mg
112.2	187.8
110.1	183.5
109.2	180.4
108.5	178.9
109.1	174.6
111.4	169.3
112.3	165.2
107.1	155.5
105.5	148.3

Table 2. Calcium concentration of Probiotic based pearl millet and Conventional pearl millet

Group Statistics					
GROUP	N	Mean	Std. Deviation	Std. Error Mean	

CALCIUM CONCENTRATION	PROBIOTIC BASED PEARL MILLET	9	108.5000	2.72397	.90799
	PEARL MILLET	9	171.5000	13.19110	4.39703

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							
		F	Sig.	t	df	One sided	Two sided	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
										Lower	Upper
Calcium concentration	Equal variances assumed	13.015	.002	-14.023	16	<.001	<.001	000	4.48980	-72.51796	53.48204
	Equal variances not assumed			-14.023	8.681	<.001	<.001	-63.00000	4.48980	-73.21381	52.78619



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 hrs.

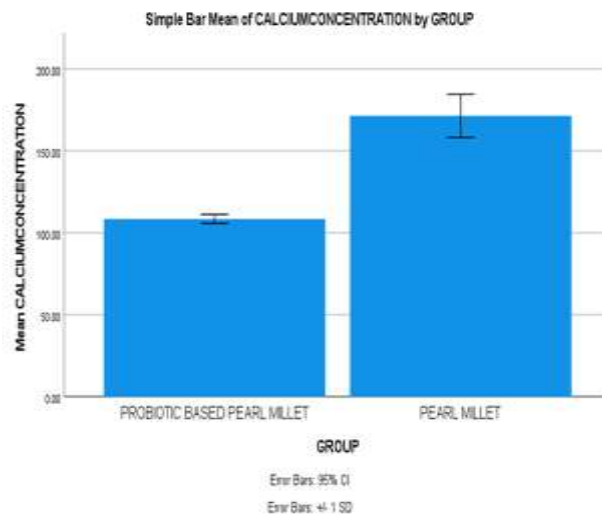


Fig. 3. Probiotic based Pearl millet shows significant decrease when compared to the Conventional Pearl millet ($P < 0.001$). X-axis represents the probiotic based pearl millet and pearl millet Y-axis indicating the mean calcium concentration. $SD \pm 1$.