

Comparative analysis on the Polyphenol Concentration of Conventional over Probiotic based Pearl Millet Foods by Gas Chromatography Mass Spectroscopy

Priyanka.B¹, K. Sangeetha²

¹Research scholar, Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. Pincode: 602105.

²Project guide, Corresponding Author, Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India. Pincode: 602105.

Abstract

Aim: To estimate the polyphenol concentration of Probiotic fermented Pearl millet by Gas Chromatography Mass Spectroscopy. **Materials and Methods:** The polyphenol concentration was estimated in the Probiotic treated pearl millet and in the non treated Pearl millet (Uninoculated) using GCMS method. Pearl millet was allowed to ferment for 24 hrs after the addition of probiotics (*Bacillus mesentericus* and *Lactobacillus sporogenes*) and fermented for 18 hours. ClinCalc.com and the prior web were used to calculate the sample. The alpha error level was set at 0.05 percent, the confidence interval was set at 95 percent, the G power was set at 80 percent, and the enrollment ratio was set at 1. (N=18). Using IBM SPSS V 28.0 software, T tests were performed between the samples. **Results:** The polyphenol concentration was estimated to be 514 mg/100g for the probiotic treated innovative Pearl millet and 654 mg/100g for Conventional Pearl millet and found to be statistically insignificant. **Conclusion:** The reduction in polyphenol in the treated samples represents the increase in the concentration of other important minerals for absorption during intake when compared to the untreated samples.

Keywords: Innovative Pearl Millet, Probiotics, GCMS Milk, Curd, *Bacillus mesentericus*, *Lactobacillus*, *Bacillus mesentericus*.

DOI: 10.47750/pnr.2022.13.S03.025

INTRODUCTION

Pearl millet (Bajra and Bulrush Pearl) is a cereal grown yearly of 26 million hectare in Africa and the Indian subcontinent (Ranasalva and Visvanathan 2014). Pearl millet is a vital, stable and an important source of iron and zinc for sufficing the micronutrient deficiencies at very low cost. (Sharma and Kapoor 1996). Polyphenols provide bioactive pathways to lower free radical-induced oxidative stress and are likely to have a role in the prevention of aging and oxidative stress-related disorders such as cancer, cardiovascular disease, and neurological disease (Nambiar et al. 2012).

Literature was searched in accordance to the title in platforms including google scholar, science direct, and pubmed. An average of 95 papers were found in google scholar. However the bioavailability and bioaccessibility of the minerals get reduced due to the presence of inhibitory factors like phytic acid and polyphenols (Arora et al. 2003). Fermentation and Soaking has been found to decrease the level of polyphenols in the pearl millets to 15% (Krishnan and Meera 2018). The other factors like sprouting, pressure cooking, Blanching also reduce the polyphenol concentration (Mishra and Sabikhi 2020); (Hithamani and Srinivasan 2014). The addition of curd in the pearl millet has increased the digestibility and palatability (Elyas et al. 2002). Decreasing the polyphenol concentration increases the bioavailability of iron and Zinc. Hence in this study the two different probiotics *Lactobacillus sporogenes* and *Bacillus mesentericus* were used to ferment the pearl millet based food and their effect on the reduction of polyphenols. The positive outcome of the study resulted in the development of probiotic based Pearl millet food with the estimation of polyphenol concentration. (Turpin, Humblot, and Guyot 2011). There have been publications on the effects of several probiotics on Pearl millet fermentation and polyphenol availability, but no data on the effects of *Lactobacillus sporogenes* and *Bacillus*

mesentericus coupled on polyphenol availability has been reported yet. (Martín-Cabrejas 2019). Our team has extensive knowledge and research experience that has translate into high quality publications (Bhansali et al. 2021; Jayanth et al. 2021; Sudhakar, Ravel, and Perumal 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 innovative Pearl millets and the polyphenol availability exists however the combined use of the two Probiotics *Lactobacillus sporogenes* and *Bacillus mesentericus* and their polyphenol availability has not yet been reported. The aim of the research is to estimate the polyphenol concentration between Pearl millet treated with probiotic strains like *Lactobacillus sporogenes* and *Bacillus mesentericus* by Gas Chromatography Mass Spectroscopy method.

MATERIALS AND METHODS

The research was done at the Department of Biotechnology Laboratory in Saveetha School of Engineering, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai. The total number of groupings used was two: Probiotic based innovative Pearl millet and Conventional Pearl millet. The sample size was 18 for this research. 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 (Ilango and Antony 2021; Ganguly, Sabikhi, and Singh 2021; Semwal, Kamani, and Meera 2021).

About 20g of powdered pearl millet was mixed with 100 ml of fresh milk and steam sterilized at 121 degree C for 20 minutes to make the unique probiotic-based pearl millet composition. To that pure culture of *Lactobacillus sporogenes* and *Bacillus mesentericus* isolated on MRS agar was inoculated into the produced pearl millet mixture, with an uninoculated microorganisms standard pearl millet preparation as control. (Arora, Jood, and Khetarpaul 2011).

The Polyphenol concentration was estimated for both the samples by the Gas Chromatography Mass Spectrometry method by standard procedures (Khan et al. 2017).

Statistical analysis

Statistical analysis was performed by IBM SPSS 28 version software. There were no independent variables. The dependent variables are Probiotic based Pearl millet and Conventional Pearl millet. Standard deviation and standard mean errors were performed using IBM SPSS version 28 software (Khan et al. 2017)).

Results

The polyphenol concentration was estimated by the GCMS method. The concentration was estimated to be 514 mg/100g for Probiotic Pearl millet and 654 mg/100g for Conventional Pearl millet. A significant reduction in the polyphenol concentration in the fermented samples was observed in comparison with the untreated sample. The polyphenol concentration values of Probiotic based pearl millet and Conventional pearl millet are summarized in Table 1, Fig 1 and Fig. 2.

Table 2 represents the paired T test analysis of two samples by IBM SPSS V 28.0 software. The results were shown 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. Polyphenol concentrations were observed in the Probiotic based Pearl millet samples in our study. Our results were inconsistent with the polyphenol concentration of the pearl millet analyzed in their region showing higher concentration

Discussion

In this study the polyphenol concentration was estimated between the two samples Probiotic based Pearl millet and Conventional Pearl millet by GCMS method. The concentration of polyphenol in the Probiotic based Pearl millet samples in our investigation showed a considerable decrease. The polyphenol concentration of pearl millet examined in their location showed higher concentrations, contradicting our findings (Ilango and Antony 2021; Ganguly, Sabikhi, and Singh 2021).

The polyphenols in pearl millet occur in the range of 307–714 mg/100 g (Abdelrahman et al. 2005). In our examination, after 24 hrs of fermentation the polyphenol concentration decreased from 654 mg/100g to 514 mg/100g which is highly inconsistent with our results. After 2 hours of fermentation in other studies had shown a subsequent significant reductions at 4, 6, 8, 10, 12, and 14 hours from 304 mg/100g to 122mg/100g (Hag et al. 2002). Also the temperature has influenced the polyphenol content and a significant drop occurs when the

fermentation duration is increased (Dhankher and Chauhan 1987). Pearl millet fermented with *Lactobacilli* lost substantially more polyphenols than pearl millet fermented with yeasts (P<0.05); pearl millet fermented with *L.fermentum* had the least quantity of polyphenols of all the types of fermentations investigated. (Khetarpaul and Chauhan 1989). Polyphenols are also thought to be important in boosting health by lowering the risk of metabolic syndrome and the difficulties that come with type 2 diabetes. (Hassan, Sebola, and Mabelebele 2020). The sampling time and the other parameters in our study were set at 37°C for 24 hours of fermentation, which is a constraint. Further standardization of fermentation temperature and time of incubation may further decrease the polyphenol concentration and the increased bioavailability of micronutrients could result in the development of innovative probiotic-based pearl millet diets for the micronutrient deficient population.(Ilango and Antony 2021).

Conclusion

After 24 hrs of fermentation the polyphenol concentration decreased from 654 mg/100g to 514 mg/100g which is highly inconsistent with our results .Significant decrease in the polyphenol concentration in the fermented Pearl based millets were observed and hence further standardization of temperature,pH,and inoculum size could further decrease the polyphenol concentration. Hence the novel approach on the use of probiotics *Lactobacillus sporogenes* and *Bacillus mesentericus* in the innovative 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.

Acknowledgement:

The authors would like to express their gratitude towards Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (formerly known as Saveetha University), for providing the necessary infrastructure to carry out this work successfully.

Funding

We thank the following organizations for providing financial support that enabled us to complete the study.

1. SAS Foods.
2. Saveetha University
3. Saveetha Institute of Medical and Technical Sciences
4. Saveetha School of Engineering
- 5.

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Figures And Tables

Table 1. Polyphenol concentration of Probiotic treated Pearl millet against Conventional Pearl millet.

Temperature	Probiotic based Pearl millet in mg	Conventional Pearl millet in mg
28	424	467
31	432	458
34	445	489
37	514	654
40	412	523
43	528	564
46	488	501
49	467	555
52	453	498

Table 2. Polyphenol 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
POLYPHENOL CONCENTRATION	PROBIOTIC BASED PEARL MILLET	9	462.5556	40.26820	13.42273
	PEARL MILLET	9	523.2222	60.71610	20.23870

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 significance	Two sided significance	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference

										Lower	Upper
O D	Equal variances assumed	.784	.389	-2.498	16	.012	.024	-60.66667	24.28528	-112.14915	-9.18418
	Equal variances not assumed			-2.498	13.897	.013	.026	-60.66667	24.28528	-112.78969	-8.54364



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.

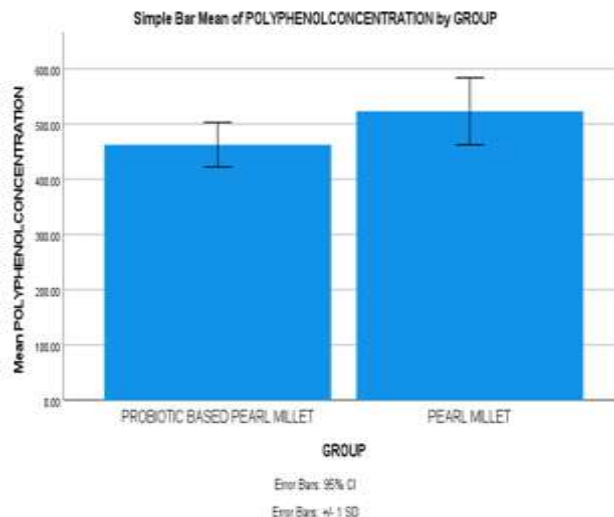


Fig 3. Bar graph representing the Polyphenol concentration with Probiotic based pearl millet against conventional pearl millet ($P \leq 0.001$). X-axis represents the probiotic based pearl millet and pearl millet Y-axis indicating the mean polyphenol concentration. $SD \pm 1$.