***Original Research Article***

# Formulation of foxtail millet probiotic powder enriched with high protein and fibre



## ABSTRACT

**Aims:** The study aimed to formulate a foxtail millet probiotic powder enriched with high protein and fibre.
**Study design:**This was an experimental, laboratory-based study.
**Place and Duration of Study:**The study was conducted at the Department of Dairy Microbiology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India, between January 2024 and October 2024.
**Methodology:**Foxtail millet was sterilized by autoclaving at 121°C for 15 minutes, while chia seeds and whey protein concentrate (WPC) were sterilized via autoclaving and UV treatment, respectively. Microbiological analysis for contaminants, including aerobic spores, yeasts, and molds, was performed using the pour plate method. The incorporation levels of probiotic cultures into the millet flour were optimized based on their viability. The resulting millet-probiotic powder was enriched with protein (from WPC) and fiber (from chia flour) to meet the Recommended Dietary Allowance (RDA) for these nutrients using trial and error method.The protein and fiber contents were quantified using the micro Kjeldahl method and crude fiber estimation, respectively. Statistical analyses were conducted using ANOVA, with a critical difference (CD) at a 5% significance level employed to assess the significance of differences between treatments.
**Results:**Sterilization methods significantly reduced microbial counts in foxtail millet flour, chia flour, and whey protein concentrate (WPC). Autoclaving at 121°C for 15 minutes completely sterilized millet and chia flours, while UV-C treatment effectively sterilized WPC, reducing microbial contamination to undetectable levels. The mixed culture of *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12, and *Streptococcus thermophilus* demonstrated the highest viable counts across various inoculation levels. A 5% inoculation rate was selected for further studies due to its optimal balance of viability and resource efficiency.Whey protein concentrate (WPC) with 80.04% protein content and chia flour with 24.6% fibre were incorporated into the probiotic millet dry mix, enhancing both protein and fibre content to meet Recommended Dietary Allowances (RDA) for adults.A formulation of 74.5g foxtail millet, 68g chia flour, and 7.5g WPC per 150g was optimized, yielding 27.05g of protein (50.09% of the RDA) and 20.39g of fibre (50.99% of the RDA), resulting in a nutritionally dense and functional product.
**Conclusion:**The sterilization methods successfully reduced microbial contamination in foxtail millet flour, chia flour, and whey protein concentrate, ensuring a safe base for probiotic inclusion. The optimized formulation, combining foxtail millet, chia flour, and whey protein concentrate, resulted in a nutritionally dense dry mix, providing 50.09% of the

Recommended Dietary Allowance for protein and 50.99% for fiber. This approach demonstrates the potential for developing a functional product that enhances both nutritional value and probiotic viability.

*Keywords: Foxtail millet, Probiotics, Whey Protein Concentrate, Chia flour, Functional product, Protein enrichment, Fibre enrichment*

## 1. INTRODUCTION

Millets have gained recognition as nutrient-dense ancient grains rich in protein, fiber, and essential micronutrients. Additionally, millet contains phytochemicals like polyphenols and lignans, which offer antioxidant and anti-inflammatory properties. Its non-glutinous nature also makes it suitable for individuals with gluten intolerance or celiac disease (DayakarRao *et al*., 2017).Recent studies have shown that foxtail millet has the highest protein and crude fiber content when compared with other millets (Santhosh *et al*., 2024a).

Probiotics, defined as live microorganisms that provide health benefits when consumed in adequate amounts, have garnered significant attention for their potential to positively impact gut health and metabolic processes. They play a pivotal role in modulating gut microbiota, influencing energy metabolism, appetite regulation, and inflammation reduction (Mahadzir *et al.,* 2017). Research indicates that probiotics enhance glucose regulation, lipid metabolism, and reduce systemic inflammation, particularly in cases of obesity and metabolic syndrome (Sanchez *et al.,* 2017). Such improvements in gut microbiota and metabolic health underscore the value of incorporating probiotics into functional food formulations.

Dietary protein is another critical component in weight management, as high-protein diets have been linked to increased satiety, reduced calorie intake, and enhanced energy expenditure. Protein also promotes lean muscle mass retention, which is crucial in combating obesity. Moreover, higher protein intake has shown positive effects on cardiometabolic health, making it an essential nutrient in obesity prevention (Drummen*et al.,* 2018).

Similarly, dietary fiber is indispensable for metabolic health and weight management. Fiber enhances satiety, lowers calorie intake, and supports gastrointestinal function due to its resistance to digestion. It also aids in maintaining healthy blood glucose levels, reducing cholesterol concentrations, and promoting the growth of beneficial gut bacteria, contributing significantly to metabolic health (Solah *et al.,* 2017).

Given the synergistic benefits of probiotics, protein, fiber, and millets, this study focuses on the formulation and evaluation of a millet-based probiotic powder enriched with high protein and fiber. This innovative product aims to address the nutritional needs of individuals while contributing to weight management and overall metabolic health. By leveraging the combined health benefits of these components, the study seeks to provide an effective dietary strategy for preventing obesity and improving well-being in diverse populations.

## 2. MATERIAL AND METHODS

### 2.1 Materials

Foxtail Millet (*Setaria italica)*, whey protein concentrate, chia seeds, probiotic cultures of *Lactobacillus acidophilus* LA-5and *Bifidobacterium bifidum* BB-12 and a mixed probiotic culture containing *Lactobacillus acidophilus* LA-5*, Bifidobacterium bifidum* BB-12 and *Streptococcus thermophilus.*

## 2.2Preparation of sterilized foxtail millet flour

Foxtail millet (*Setaria italica*) was soaked in potable water at a millet-to-water ratio of 1:2. The soaking process was conducted at 30°C for 24 hours to enhance hydration and prepare the grains for subsequent germination (Santhosh *et al.*, 2024b). After soaking, the water was drained, and the millet was tied in sterile muslin cloth for germination. The germination process was carried out at 30°C for 48 hours under controlled environmental conditions in an incubator.

Post-germination, the millet was dried at 30°C until a final moisture content of 5–7% was achieved. The drying process was carefully monitored to ensure uniform moisture reduction while preserving the nutritional integrity of the grains. The dried millet was then milled into fine flour and sieved using a 150-micron mesh to ensure uniform particle size. The sieved millet flour was transferred to a conical flask and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure (Santhosh *et al.,* 2024c).

## 2.3Preparation of sterilized chia flour

Chia seeds were ground into a fine powder, transferred to a conical flask, and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure.

## 2.4Preparation of sterilized whey protein concentrate

The whey protein concentrate (WPC) procured from the market was sterilized using ultraviolet (UV) light. The WPC was exposed to a UV lamp with an intensity of 100 $\mu Wcm^{-2}$ for a duration of 60 minutes, resulting in a UV-C dose of $3.6 \times 10^6$ $Jm^{-2}$

## 2.5 Microbiological testing for the presence of contaminants

Millet flour, WPC and chia flour was subjected for testing for the presence of contaminants like spores and yeast and mold using pour plate method, both before and after sterilization.

### 2.5.1 Enumeration of aerobic spores

Using a sterile mortar and pestle, 11.0g of the sample was triturated using sterile phosphate buffer. To create a 1:10 dilution, it was then added to 99.0ml of sterile phosphate buffer. After being heated to 80℃ for 10 minutes in a water bath, the first dilution was cooled to less than 10℃. Then the needed dilutions were prepared serially by the first dilution.

Serially diluted samples were then transferred to sterile petri plates that had been labelled. Molten sterile 2% nutrient agar (NA) maintained at 45℃ was poured into marked petri dishes and allowed to solidify. By inverting the plates, all the poured plates were incubated at 37℃ for 24 to 48h. The number of nutrient agar colonies was counted after the incubation time. The average count of the countable plates which ranged from 30 to 300 were reported as $\log_{10}$ CFU/g(Harrigan, 1998).

### 2.5.2 Enumeration of yeast and mold

About 99.0ml of sterile phosphate buffer was mixed with 11.0g of the weighed samples to prepare the first dilution. The first dilution was then used to prepare the subsequent dilutions in a sequential manner.

Samples that had been serially diluted were thereafter transferred to labelled sterile petri dishes. Sterile malt extract agar (MEA) kept at 45°C was poured into designated petri plates and were allowed to solidify. All of the poured plates were incubated at 30°C for 3-5 days by inverting the plates. Following the incubation period, the number of malt extract agar colonies was counted. The average of countable plate which varied from 30 to 300, was expressed as $\log_{10}$ CFU/g (Harrigan, 1998).

## 2.6Selection of probiotic culture

Probiotic cultures of *Lactobacillus acidophilus* LA-5*, Bifidobacterium bifidum* BB-12 and combination culture of *Lactobacillus acidophilus* LA-5*, Bifidobacterium bifidum* BB-12 and *Streptococcus thermophilus* in freeze dried format 5, 7.5 and 10% (in terms of millet flour) were added to the optimized sterilized millet flour. The optimum level of culture addition was determined based on the viability of the probiotic culture in the millet flour.

### 2.6.1Viability of probiotic organisms

Probiotic cultures at concentrations of 5%, 7.5%, and 10% were incorporated into 15 g of sterilized millet flour and mixed thoroughly. The viability of the probiotic cultures in the millet flour was assessed using the pour plate technique.

To prepare the first dilution, 11.0 g of the probiotic culture-incorporated millet flour sample was weighed and added to 99.0 mL of sterile phosphate buffer. Serial dilutions up to $10^{-}$□, $10^{-}$□, and $10^{-}$□ were prepared from the initial dilution. These dilutions were transferred to marked sterile petri plates. Approximately 10–15 mL of sterile de Man, Rogosa, and Sharpe (MRS) agar, maintained at 45°C, was poured into the plates, which were then allowed to solidify.

Once solidified, the plates were inverted and placed in an anaerobic candle jar for incubation at 37°C for 72 hours. After the incubation period, colonies on the MRS agar were counted. The average count from plates with colony ranges of 30 to 300 was reported as $\log_{10}$ CFU/g. Sterilized millet flour without added probiotic cultures was used as the control(Harrigan, 1998).

## 2.7Testing of Protein and Fibre sources

### 2.7.1 Estimation of Protein content

Whey protein concentrate was tested for its protein content using the micro Kjeldahl technique. A Kjeldahl tube was filled with 0.5g of thoroughly mixed sample, 0.1ml of 5% copper sulphate solution, 1.5g of potassium sulphate and five to ten boiling aids. Next, 15ml of concentrated sulphuric acid were introduced via the tube's wall. After a gentle mixing process, the contents were digested until they were clear and devoid of any leftover substance. Then, the digest was let to cool to 25±2℃. After adding 100ml of distilled water, it was placed in an auto distillation unit and allowedto distill with 50% sodium hydroxide. The ammonia that was released was then trapped in 25ml of boric acid. 25ml of distillate were titrated with 0.02N hydrochloric acid until the colour turned pink. Then, same procedure was followed for blank by substituting the sample with 1ml of water and 0.17g of sucrose. The total nitrogen and crude protein percentages were then calculated using the formula provided below (FSSAI 03.016:2022).

$$W_n = \frac{1.4007 \times (V_S - V_B) \times N}{W}$$

$$\text{Crude protein (\%)} = W_n \times 6.25$$

Where, $W_n$ = Nitrogen content of sample, expressed as % by mass
$V_S$ = Volume in ml of the standard HCl used for sample
$V_B$ = Volume in ml of the standard HCl used for blank test
N = Normality of standard HCl
W = Mass of test portion in g

### 2.7.2 Estimation of Crude Fibrecontent

Chia flour was tested for its crude fibre content using the following method. A conical flask containing 2g of sample and 200ml of 0.128M sulphuric acid solution was boiled for 30 minutes on a hot plate. The boiled solution was filtered through the funnel, which was lined with cotton cloth. To remove acid residues completely, the filtrate was carefully rinsed with hot water. The sample procedure was repeated by adding 200ml of 0.313M sodium hydroxide solution to the filtrate. Subsequently, the filtrate was gathered into a crucible that had been cleaned and dried. It was then placed on a hot plate to remove any remaining water and it was heated to 130°C for 2h. It was then cooled in a desiccator and weighed ($W_1$). The sample was then placed in a muffle furnace at 550℃ for 2h, cooled in a desiccator for 20 minutes and weighed ($W_2$) (AOAC 978.10).

$$\text{Crude fibre \%} = \frac{W_1 - W_2}{Ws} \times 100$$

Where, $W_S$ = Weight in g of sample
$W_1$ = Weight in g of crucible with fibre
$W_2$ = Weight in g of crucible with ash

## 2.8Formulation of millet probiotic powder enriched with high protein and fibre

The millet flour supplemented with probiotics was further enriched with protein (whey protein concentrate) and fiber (chia flour) to meet the Recommended Dietary Allowance (RDA) values. The enrichment process was conducted using a trial-and-error approach to achieve the desired nutrient levels. The protein and crude fibre content was determined for each combination.

The Recommended Dietary Allowance (RDA) represents the average daily intake level sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a specific gender and life stage group (Institute of Medicine, 1998). For adults, the RDA for protein to prevent deficiency is 0.83 g/kg body weight/day, equating to approximately 54 g/day for men and 45.7 g/day for women.Additionally, the Indian Council of Medical Research (ICMR) recommends a daily intake of at least 40 g of dietary fiber for adults, based on a 2000 kcal diet (ICMR-NIN, 2020).

## 2.9Statistical Analysis

The data was analysed using R software [R. version 4.1.2 copyright] for statistical computing. Data on the response variables was collected for three replications of the trails and the ANOVA tables was prepared to analyse the data. The critical difference was

calculated (*P=.05*), where the F value was significant, and used to identify whether significant differences existed and indicated in the table using superscripts.

$$\text{Critical difference (CD)} = \frac{\sqrt{2 \times MSS(E)} \times t\alpha}{r}$$

Where, MSS (E) = Mean Sum of squares of the error
r = number of replications
tα = table t value of the α level of significance

## 3. RESULTS AND DISCUSSION

### 3.1 Microbiological quality of ingredients

The microbiological analysis of foxtail millet flour, chia flour, and whey protein concentrate (WPC) before and after sterilization highlights the effectiveness of different sterilization methods in eliminating microbial contaminants as shown in Table 1.

Before sterilization, foxtail millet flour exhibited an aerobic spore count of 4.21 $\log_{10}$ CFU/g and a yeast and mold count of 4.48 $\log_{10}$ CFU/g, indicating a high level of microbial contamination. Autoclaving the flour at 121°C for 15 minutes at 15 psi resulted in the complete elimination of both aerobic spores and yeast and mold counts. These findings align with the results of Jayashree *et al.* (2024), who reported the complete eradication of spores and fungal contamination in germinated finger millet flour subjected to similar autoclaving conditions.

The high efficacy of autoclaving can be attributed to the application of moist heat, which damages microbial cell membranes and denatures nucleic acids and proteins, rendering the cells non-viable (Huesca-Espitia *et al.,* 2016). For instance, Huesca-Espitia *et al.* demonstrated that autoclaving at 121.1°C effectively inactivated heat-resistant *Geobacillus stearothermophilus* spores by denaturing critical enzymes such as α-glucosidase.

Chia flour showed an initial aerobic spore count of 4.56 $\log_{10}$ CFU/g and a yeast and mold count of 3.24 $\log_{10}$ CFU/gbefore sterilization. Similar to foxtail millet flour, autoclaving at 121°C for 15 minutes completely eliminated these contaminants. The results reaffirm the superiority of moist heat sterilization in achieving microbial safety.

WPC initially showed an aerobic spore count of 2.38 $\log_{10}$ CFU/g, while no yeast and mold were detected. Post-sterilization using UV-C rays, both aerobic spore and yeast and mold counts were reduced to undetectable levels. This method's efficacy is consistent with findings by Yu *et al.* (2024), who demonstrated that unsterilized goat milk powder exposed to a UV-C intensity of 100 μWcm$^{-2}$ for 15 and 35 minutes achieved reductions in total bacterial count from an initial 5.21 $\log_{10}$ CFU/g to 3.77 $\log_{10}$ CFU/g(low dose) and 4.16 $\log_{10}$ CFU/g(high dose), respectively. The reduction in microbial counts by UV-C treatment can be attributed to its ability to cause DNA damage, leading to microbial inactivation.

Dikkala*et al.* (2018) observed significant fungal count reductions in foxtail millet grains when subjected to heat treatment at 150–170°C for 1.5 minutes; however, complete sterilization was not achieved. This underscores the advantage of autoclaving as a superior sterilization method, particularly for achieving complete microbial elimination in food ingredients.

The results highlight the effectiveness of autoclaving at 121°C for 15 minutes at 15 psi in completely sterilizing foxtail millet flour and chia flour, while UV-C rays were equally effective

in sterilizing WPC. These findings emphasize the importance of selecting appropriate sterilization methods to ensure microbial safety in food products.

**Table 1. Impact of Sterilization on Microbial Load in Key Ingredients**

| Ingredient | Treatment | Aerobic Spore Count | Yeast and Mold Count |
|---|---|---|---|
| | | log10 CFU/g | |
| Foxtail millet flour | Before sterilization* | 4.21 | 4.48 |
| | After sterilization* | Nil | Nil |
| Chia flour | Before sterilization* | 4.56 | 3.24 |
| | After sterilization* | Nil | Nil |
| Whey Protein Concentrate | Before sterilization** | 2.38 | Nil |
| | After sterilization** | Nil | Nil |

Note: CD = All the values are average of three trials (n=3) and * indicates that sterilization was done by autoclaving at 121ºC for 15 minutes at 15 psi pressure and ** indicates that sterilization was done using UV-C lamp.

## 3.2Selection of probiotic culture

The total viable count of microbial cultures inoculated into foxtail millet flour at various inoculation levels were assessed to select the most effective probiotic combination. The results, presented in Table 2, demonstrate significant differences in viable counts among different probiotic cultures and inoculation rates.

*Lactobacillus acidophilus* LA-5 culture showed a progressive increase in total viable count with increasing inoculation rates. At a 5% inoculation rate, the viable count was 10.72 viable $\log_{10}$ CFU/g, increasing the inoculation rate to 7.5% raised the viable count to 11.15 $\log_{10}$ CFU/g, showing a statistically significant increase. The highest count for this culture was observed at a 10% inoculation rate, with a viable count of 11.36 $\log_{10}$ CFU/g, demonstrating further significant enhancement. This trend indicates that higher inoculation rates lead to increased viable counts.

*Bifidobacterium bifidum* BB-12 culture also exhibited an increase in total viable count with rising inoculation rates. At 5% inoculation, the viable count was 10.72 $\log_{10}$ CFU/g. Increasing the rate to 7.5% resulted in a significant increase to 11.41 $\log_{10}$ CFU/g, and at 10%, the viable count reached 11.46 $\log_{10}$ CFU/g. These results indicate that *Bifidobacterium bifidum* BB-12culturealso benefits from higher inoculation rates, with statistically significant increases in viable count at each increment.

A combination of *Lactobacillus acidophilus* LA-5, *Bifidobacteriumbifidum* BB-12, and *Streptococcus thermophilus*, displayed the highest total viable counts across all inoculation rates. At a 5% inoculation rate, this mixed culture achieved a viable count of 11.04 $\log_{10}$ CFU/g, which was significantly higher than the 5% rates of the other two individual cultures. Increasing the inoculation rate to 7.5% further enhanced the viable count to 11.48 $\log_{10}$ CFU/g, and at 10%, the mixed culture achieved the highest viable count of11.51 $\log_{10}$ CFU/g, significantly outperforming the individual cultures.

The critical difference (CD) at the 5% significance level was 0.019, confirming that the differences in viable counts among the different probiotic cultures and inoculation rates were statistically significant. Based on the results, the combination culture consistently showed the highest total viable counts across all inoculation rates, with the maximum count recorded at 10%. However, considering practicality and economic efficiency, a 5% inoculation rate was selected for further studies. This selection balances high probiotic viability with resource optimization, ensuring a substantial probiotic effect without excessive culture requirements.

The results of this study demonstrate that inoculating foxtail millet flour with different probiotic cultures significantly increases total viable counts, exceeding the minimum threshold of one billion CFU, as recommended by the International Scientific Association of Probiotics and Prebiotics (Hill *et al.*, 2014). Both *Lactobacillus acidophilus* LA-5 and *Bifidobacteriumbifidum* BB-12 showed a marked rise in viable counts as the inoculation rate increased, underscoring the role of higher inoculation rates in boosting probiotic viability.

The mixed culture of *Lactobacillus acidophilus* LA-5, *Bifidobacteriumbifidum* BB-12 *Streptococcus thermophilus* consistently exhibited the highest performance across all inoculation levels, with a peak viable count of 11.51 $\log_{10}$ CFU/g at a 10% inoculation rate. This outperformed the individual cultures, with *Lactobacillus acidophilus* LA-5 reaching a maximum of 11.36 $\log_{10}$CFU/g and *Bifidobacteriumbifidum* BB-12 reaching 11.46 $\log_{10}$ CFU/g. Importantly, the increased viability observed in the mixed culture can be attributed to differences in the actual CFU/g between single-strain and multi-strain cultures. Although the weight of inoculum added was the same for all cultures, the total number of CFU/g varied, leading to higher viable counts in the mixed culture. This finding aligns with Bujna *et al.* (2018), who reported that mixed cultures of *Bifidobacterium bifidum* BB-12 and *Lactobacillus casei* 01 achieved higher viable counts compared to individual strains.

While the 10% inoculation rate yielded the highest viable counts, the 5% inoculation rate for the mixed culture was found to be the most practical, balancing probiotic viability with resource efficiency.

**Table 2. Selection of Probiotics based on Total Viable Count**

| Microbial Culture | Inoculation rate (% millet flour) | Total Viable count ($\log_{10}$ CFU/g) |
|---|---|---|
| | 5 | $10.72^{g}$ |
| *Lactobacillus acidophilus* LA-5 | 7.5 | $11.15^{e}$ |
| | 10 | $11.36^{d}$ |
| | 5 | $10.72^{g}$ |
| *Bifidobacterium bifidum* BB- | | |

| | | |
|---|---|---|
| 12 | 7.5 | 11.41[c] |
| | 10 | 11.46[b] |
| Combination culture of *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12 and *Streptococcus thermophilus* | 5 | 11.04[f] |
| | 7.5 | 11.48[b] |
| | 10 | 11.51[a] |
| CD (*P=.05*) | | 0.019 |

Note: CD = Critical Difference and all the values are average of three trials (n=3) and same superscript indicate non-significance while different, indicate statistically significant difference at *P=.05*

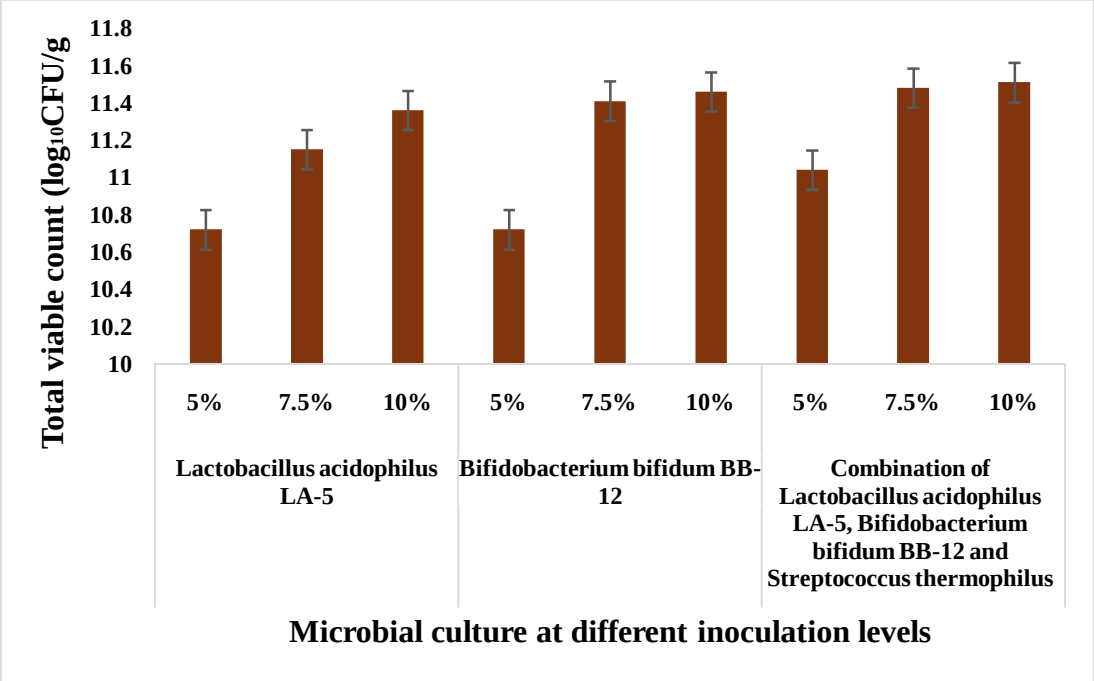


**Fig. 1. Total viable count of probiotic cultures at different inoculation levels**

### 3.3 Protein and Fibre sources

Whey protein concentrate, with a protein content of 80.04%, was added to the probiotic millet dry mix to boost its protein levels, ensuring they meet the Recommended Dietary Allowance (RDA) for adults. Chia seed powder, containing 24.6% crude fibre, was

incorporated as a fibre source to enhance the mix's fibre content in accordance with RDA requirements.

The current study incorporated whey protein concentrate (WPC-80) and chia seed powder into a probiotic millet dry mix to enhance its protein and fibre content, aligning with the Recommended Dietary Allowance (RDA) for adults. Whey protein concentrate (WPC-80) is known for its high protein content, and the present study used a WPC with a protein content of 80.04%, consistent with the findings of Zebrowska-Gamdzyk*et al.* (2018), who reported a protein content of 78.2% for WPC-80. In another study, Kim (2015) documented a slightly higher protein content of 82.60% for WPC-80, indicating some variability depending on the source and processing methods.

Chia seeds were incorporated as a source of fibre in the mix. The crude fibre content of chia seeds used in this study was 24.6%, slightly higher than the 21.12% reported by Sebo (2017) and lower than the 31.03% reported by Ibrahim *et al*. (2019). This variation could be due to differences in cultivation conditions, seed processing, or measurement methods. Despite these variations, chia seeds are widely recognized for their high fibre content and health benefits, particularly their ability to improve gut health, as noted by Sebo (2017).

Overall, the addition of WPC-80 and chia seeds contributed significantly to the nutritional quality of the probiotic millet dry mix, enhancing both its protein and fibre content to meet the nutritional requirements for adults.

### 3.3 Optimization of the protein and fibre composition of the probiotic milletdry mix

A probiotic powder blend was developed using foxtail millet as the base ingredient, chia flour as a fibre source, and whey protein concentrate (WPC) as a protein source. Various formulations were tested to evaluate their protein and fibre content, along with their contributions toward the Recommended Dietary Allowance (RDA) and presented in Table 3. The final composition selected from the analysis was the blend with 74.5g of foxtail millet, 68g of chia flour, and 7.5g of WPC, yielding a total powder quantity of 150g. This formulation provided 27.05g of protein, meeting 50.09% of the RDA for protein. The fibre content was 20.39g, which contributed to 50.99% of the RDA for dietary fibre.

This combination was chosen based on its balanced nutritional profile, effectively utilizing foxtail millet's carbohydrate and protein contributions, chia flour's high fibre content, and WPC's superior protein quality. The formulation met over half of the daily protein and fibre requirements, making it a nutritionally dense and functional food product. The balance of ingredients optimized the blend for enhanced health benefits, including high protein and fibre, making it suitable for those seeking to meet significant portions of their daily nutritional needs through a single product.

The formulation of a probiotic powder blend incorporating foxtail millet, chia flour, and whey protein concentrate (WPC-80) represents a strategic approach to developing a nutrient-dense product with an emphasis on protein and fibre. The composition of 74.5g of foxtail millet, 68g of chia flour, and 7.5g of WPC per 150g delivers 27.05g of protein and 20.39g of fibre, covering 50.09% of the Recommended Dietary Allowance (RDA) for protein and 50.99% for fibre. This balance demonstrates the blend's potential as a functional food product, particularly for those seeking to meet higher daily nutritional requirements.

Foxtail millet, chosen for its well-documented nutritional value, provides the base for this blend. Prior research, such as Yadagouda and Ravindra (2022), highlighted the substantial protein content (19.53g/100g) of foxtail millet-based composites. In comparison, the current blend achieves evengreater nutritional benefits with the inclusion of chia flour and WPC, which enhance fibre and protein content, respectively. For example, Geetha *et al.* (2021) developed a millet-based health mix that offered 19.13g of protein and 5.96g of fibre per 100g, showing that the current blend's higher nutritional content makes it better suited for meeting more demanding dietary goals.

The addition of WPC improves the overall protein quality by combining plant-based proteins from foxtail millet with animal-derived proteins from WPC. This combination offers a more balanced amino acid profile, beneficial for muscle health and overall nutritional adequacy. Additionally, the high fibre content provided by chia flour supports digestive health and satiety, further positioning the blend as a functional food with multiple health benefits.

Moreover, there is significant potential to enhance the probiotic functionality of this blend. The inclusion of probiotic strains, such as *Lacticaseibacillusrhamnosus*GG and *Lactobacillus acidophilus* LA-5, as demonstrated by Geetha *et al.* (2021) and Yadagouda and Ravindra (2022), would expand its health benefits, especially by supporting gut health. Incorporating these strains would elevate the product's value, making it a multifunctional health mix.

In conclusion, this probiotic powder blend offers a highly nutritious product capable of addressing significant portions of daily protein and fibre needs. By leveraging the complementary strengths of foxtail millet, chia flour, WPC and probiotic cultures (combination cultures of *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12 and *Streptococcus thermophilus*), the formulation outperforms existing millet-based products in terms of nutritional profile. Furthermore, by adding probiotic strains, the blend could be refined into a comprehensive functional food, catering to a wide range of health-conscious consumers.

The RDA for protein to prevent deficiency is 0.83g/kg of body weight per day, which translates to approximately 54g/day for men and 45.7g/day for women. Additionally, the Indian Council of Medical Research (ICMR) recommends a daily intake of at least 40g of dietary fibre for adults, based on a 2000 kcal diet (ICMR-NIN, 2020). This blend's formulation, therefore, meets a substantial portion of these requirements, further emphasizing its nutritional efficacy.

**Table 3. Nutritional composition and RDA contribution of different Probiotic Millet Dry Mix Formulations**

| Foxtail Millet | Chia flour | WPC | Total powder quantity | Total protein | Total fibre | Protein | Fibre |
|---|---|---|---|---|---|---|---|
| | | | (g) | | | Per cent of RDA met | |
| 50 | 25 | 25 | 100 | 30.45 | 8.55 | 56.39 | 21.37 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | 50 | 25 | | 31.07 | 13.61 | 57.55 | 34.03 |
| | 25 | 50 | | 47.17 | 7.38 | 87.36 | 18.46 |
| | 50 | 30 | | 34.42 | 13.38 | 63.74 | 33.45 |
| 20 | 30 | 50 | | 47.30 | 8.40 | 87.59 | 21.0 |
| | 60 | 20 | | 28.58 | 15.87 | 52.92 | 39.67 |
| | 50 | 40 | | 41.11 | 12.91 | 76.13 | 32.28 |
| 10 | 60 | 30 | | 34.67 | 15.40 | 64.20 | 38.51 |
| | 70 | 20 | | 28.23 | 17.89 | 52.27 | 44.73 |
| 40 | 40 | 20 | | 27.48 | 11.82 | 50.89 | 29.55 |
| 15 | 68.5 | 16.5 | | 26.53 | 17.75 | 49.14 | 44.38 |
| 13 | 70 | 17 | | 26.92 | 18.03 | 49.85 | 45.08 |
| 20.5 | 61.5 | 18 | | 27.29 | 16.26 | 50.54 | 40.66 |
| | 61 | 18.5 | | 27.61 | 16.14 | 51.13 | 40.35 |
| 19 | 61 | 20 | | 28.61 | 16.07 | 52.99 | 40.18 |
| 56 | 78 | 16 | | 33.08 | 22.02 | 61.27 | 55.06 |
| 60 | 76 | 14 | 150 | 31.68 | 21.71 | 58.66 | 54.28 |
| 75 | 65 | 10 | | 28.62 | 19.67 | 53.00 | 49.18 |
| **74.5** | **68** | **7.5** | **150** | **27.05** | **20.39** | **50.09** | **50.99** |

## 4. CONCLUSION

The present study successfully formulated a foxtail millet-based probiotic powder blend enriched with high protein and fiber. By incorporating chia flour and whey protein concentrate, the optimized formulation provided 27.05 g of protein and 20.16 g of crude fiber per 150 g, meeting approximately 50% of the Recommended Dietary Allowance (RDA) for both protein and fibre. The blend also demonstrated potential as a functional food, combining the nutritional benefits of millet, chia, and whey protein with the health-promoting effects of probiotics.

Compared with conventional millet-based formulations, this novel blend offers a multifunctional approach to improving health by addressing protein and fiber deficiencies while providing probiotics that can enhance gut health. These findings establish a strong foundation for the commercial development of millet-based functional foods and highlight the importance of combining traditional ingredients with contemporary dietary innovations.

Future research should focus on consumer acceptance, large-scale production feasibility, and the exploration of additional bioactive components to further enhance the health benefits of such functional formulations.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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## ABBREVIATIONS

**AOAC:** American Association of Agricultural Chemists
**FSSAI:** Food Safety and Standards Authority of India
**ICMR:** Indian Council of Medical Research
**MEA:** Malt Extract Agar
**MRS:** de Man Rogosa Sharpe Agar
**NA:** Nutrient Agar
**NIN:** National Institute of Nutrition
**RDA:** Recommended Dietary Allowances