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# Sporicidal Treatments to Produce Germinated Finger Millet

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The current investigation was to ascertain the most efficient sporicidal treatment for germinated finger millet in order to inactivate the spores that could interfere with the fermentation process by acting as a competing microflora for starter culture during the manufacturing of probiotic millet food.

**Study Design:** By applying different dry and wet sporicidal treatments to the finger millet, the study attempted to inactivate the spores and create germinated finger millet. Following each treatment, the number of aerobic spores was enumerated by the pour plate technique.

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**Place and Duration of Study**: Department of Dairy Microbiology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India between June 2023 and April 2024.
**Methodology:** Both wet and dry sporicidal treatments were used. It comprised of blanching, autoclaving, hot air oven treatment and the combined treatment of hot air oven and autoclaving.
**Results:** Aerobic spore count were reduced from 3.86 to 1.00 $\log_{10}$ cfu/g after autoclaving finger millet for 30 min at 121℃. Considering that the autoclaving procedure did not encourage germination, sporicidal and fungicidal treatments were followed for 48 h germinated finger millet. After autoclaving germinated finger millet flour, the initial counts of spores and fungus which were 4.98 and 3.56 $\log_{10}$ cfu/g were completely eliminated. Statistically significant ($P$=.05) difference was found between the treatment sample and the control.
**Conclusion:** Autoclaving the germinated finger millet flour at 121℃ for 15 min resulted in a complete reduction of spores and fungus count. Hence, this treatment was followed during the preparation of probiotic millet food.

*Keywords: Finger millet; spores; germination; fungi.*

## 1. INTRODUCTION

Finger millet (*Eleusine coracana*) is one of the major millet belonging to the Poaceae family. With 11.33 lakh tons of production, Karnataka was India's largest producer of finger millet [1]. Finger millet is known as ragi in Kannada, kelvaragu in Tamil, mandua in Hindi and ragulu in Telugu [2]. It is rich in calcium, phosphorus and essential amino acids such as lysine, isoleucine, leucine, phenylalanine, methionine, cysteine and tryptophan. Additionally, the important fatty acids such as palmitic and linolenic acid that are necessary for the growth of the brain and neural tissue are present in finger millet grains [3]. It offers several health advantages including antibacterial, anti-carcinogenic, antioxidant, lowering blood cholesterol, strengthening bones, regulating blood pressure, enhancing children's haemoglobin status and having the ability to cure wounds [4].

Finger millet has found numerous applications in the food industry. They are employed in the making of extruded products, fermented foods, weaning foods and baked products [5].

Starch makes up the majority of the carbohydrates in finger millet with 62.13 g per 100 g [6]. *Bacillus* spp. are amylolytic in nature. They produce α-amylases, which are endo-acting amylases that break down the internal α-(1-4) glycosidic linkages in starch polymers to yield glucose or maltose molecules [7].

Spore-forming bacteria are ubiquitous in nature. Bacteria that generate spores are crucial for food deterioration and foodborne illnesses. Endospores that are dormant are resilient to a variety of environmental stressors such as radiation, heat, salt, acidity, oxygen and/or water deprivation and a lack of nutrients. However, spores have the ability to detect changes in their immediate environment such as the availability of nutrients. Foods that are high in nutrients may induce the germination process of spores, after that spores can resume exponential cell division by reverting to their vegetative cell state. Food deterioration may result from spore germination in a finished food product, vegetative cell proliferation and may be even initiate sporulation. When it comes to foodborne pathogens, foodborne disease can result from consuming foods containing pathogenic species spores that have the potential to germinate and grow in the gut or from consuming foods in which the spores have already started to grow and proliferate. In the second scenario, a foodborne illness could be caused by consuming toxins found in the food (food poisoning) or by consuming the pathogen's vegetative cells which would then create toxins in the gut (foodborne infection) and cause diarrhoea [8].

The target of inactivation in many food processing methods is bacterial endospores, particularly those of the Bacillus species. Bacillus genus are gram-positive, rod shaped bacteria that are naturally found in soil and vegetation, which includes species like *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. pumilus* and *B. thuringiensis*. These microorganisms are among the most significant human pathogens or those that cause quality damage. In particular, *B. subtilis*, *B. stearothermophilus* and *B. amyloliquefaciens* are the primary bacteria that cause processed food deterioration, while *B. cereus* and *B. anthrax* are representative harmful spore forming bacteria.

Thus, it's critical to eliminate Bacillus spores linked to pathogenicity and degradation in order to sterilize processed foods [9].

Steam sterilization is another name for autoclaving since microorganisms are killed using pressured steam. Compared to other sterilization techniques like dry heat, wet heat sterilization is much more effective. To reach the steam's temperature of 121.1°C and pressure of 15 psi, air must be evacuated during this operation. For effective sterilization, materials must be kept for 15 to 20 min. Any form of microbe, including spores will not survive the autoclaving process [10].

The term "fermented foods" refers to "foods or beverages produced through controlled microbial growth and the conversion of food components through enzymatic action" [11].

In this study, an attempt was made to determine effective sporicidal treatments to produce germinated finger millet which is free from spore formers. Because spores are amylolytic in nature, they may interfere by degrading starch present in millet during fermentation process and thereby results in undesirable outcome. This endeavour to destroy spores in finger millet is intended to make it handy for the manufacturing of fermented probiotic millet food and to obtain desired end products by fermentation of millet by probiotic organisms.

## 2. MATERIALS AND METHODS

### 2.1 Finger Millet

Finger millet was obtained from a reputed local market, Bengaluru, Karnataka, India.

### 2.2 Various Sporicidal Treatments given to the Selected Millet

To get rid of all the spores, the millet was subjected to many sporicidal treatments. Both wet and dry treatments were applied to the millet. Millet was subjected to dry sporicidal treatment by being exposed for 1 h inside a hot air oven set at 100℃. Moist sporicidal treatments included blanching the millet for 30 s at 98℃, autoclaving it for 15 min at 121℃; autoclaving it at 121℃ for 30 min; and giving it a combination treatment that involved heating the millet for 1 h at 100℃ in a hot air oven and then autoclaving it for 15 min at 121℃. After each treatment, aerobic spores were enumerated [12].

#### 2.2.1 Enumeration of aerobic spores

Using a sterile mortar and pestle, 11.0 g of millet were triturated using sterile phosphate buffer. To create a 1:10 dilution, it was then added to 99.0 ml of sterile phosphate buffer. After being heated to 80℃ for 10 min 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 plate that had been labeled. Molten sterile 2% nutrient agar maintained at 45℃ was poured into marked petri dishes and allowed to solidify. By inverting the plates, all of the poured plates were incubated at 37℃ for 24 to 48 h. The number of nutrient agar colonies was counted after the incubation time. The average count of the countable plate which ranged from 30 to 300 was reported as $\log_{10}$ cfu/g [13].

### 2.3 Germination of Finger Millet

The millet was submerged in clean potable water at a ratio of 1:3 (millet: water) for 24 h at 30°C, the surplus water was drained off. The soaked millets were wrapped in a sterile muslin cloth and placed in an incubator set at 30°C for 48 h to facilitate germination. After germination, millets were dried in an open incubator for 24 h at 45°C and crushed and sieved to obtain germinated finger millet flour [14].

### 2.4 Different Sporicidal and Fungicidal Treatments for Germinated Finger Millet

Following germination, the spores and fungus count were enumerated. At various stages after germination, treatments of autoclaving at 121℃ for 15 min were administered to ascertain the point at which the number of spores and fungi would decrease. Autoclaving was done after the milling and sieving of germinated millet in an open and sterile environment as well as after germination. The spores and fungus count following each treatment were enumerated.

#### 2.4.1 Enumeration of yeast and mold

About 99.0 ml of sterile phosphate buffer was mixed with 11.0 g 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 plate. Sterile malt extract agar kept at 45°C was poured into designated petri plate 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 [13].

### 2.5 Statistical Analysis

R software (version 4.1.2) was used to analyse the data and perform statistical computations. For every treatment, three replications (n=3) of the corresponding variable data were gathered. The data were analysed using one way ANOVA and in cases where the F value is significant, the critical difference was calculated with 95% confidence level (*P*=.05) to determine the presence of significant differences, which were then displayed in the tables with superscripts. The formula for critical difference (CD) is

$$CD = \frac{\sqrt{2 \times MSS\ (E)}}{R}\ t\alpha \quad @\ 0.05$$

Where, MSS (E) = Mean Sum of Squares of the error

R = number of replications

tα = table t value at the α level of significance

## 3. RESULTS AND DISCUSSION

### 3.1 Various Sporicidal Treatments given to the Finger Millet

After being treated at 100°C for 1 h in a hot air oven, the aerobic spore count decreased from 3.86 to 2.54 $\log_{10}$ cfu/g. The aerobic spore count of finger millet lowered from 3.86 to 2.18 $\log_{10}$ cfu/g after blanching it for 30 s at 98°C and decreased from 3.86 to 1.70 $\log_{10}$ cfu/g after autoclaving finger millet for 15 min at 121°C. After subjecting them in the hot air oven at 100℃ for 1 h and autoclaving them for 15 min at 121°C, the aerobic spore count decreased from 3.86 to 1.78 $\log_{10}$ cfu/g. In the same way, autoclaving for 30 min at 121°C decreased the aerobic spore count from 3.86 to 1.00 $\log_{10}$ cfu/g (Fig. 1 and Plate 1). When compared to various dry and wet treatments, autoclaving finger millet at 121°C for 30 min was found to be more effective in killing spores where a maximum reduction of 2 log was observed. All of the treatments showed statistically significant (*P*=.05) differences with the exception of autoclaving at 121°C for 15 min and autoclaving at 121°C for 30 min following hot air treatment at 100°C which did not exhibit any significant differences. The aerobic spore formers could have been killed by the moist heat used in autoclave sterilization and due to more time contact.

On par with the above study, the impact of several dry and moist sporicidal treatments on black gram dhal was investigated. The aerobic spore with an initial count of 3.47 $\log_{10}$ cfu/g was completely destroyed when dhal was exposed to a hot air oven at 100°C for 1 h followed by sterilization at 121°C for 30 min. It's probable that hot air sensitized the spores and autoclaving later assisted in destroying the remaining spores or vegetative cells. Hence this method was used for preparation of solid state fermented black gram dhal [12].

### 3.2 Different Sporicidal and Fungicidal Treatments for Optimized Germinated Finger Millet

Sporicidal treatments before germination prevented the millets from sprouting. Hence, sporicidal treatments were followed after germination.

The initial aerobic spore count of 4.98 and the fungal count of 3.56 $\log_{10}$ cfu/g were observed in germinated finger millet. After 15 min of autoclave sterilization at 121°C, the number of spores and fungus in the germinated finger millet decreased from 4.98 to 1.60 and from 3.56 to 0.00 $\log_{10}$ cfu/g. Spore and fungal counts of the germinated finger millet flour which was made by grinding and sieving autoclaved germinated finger millet were 1.00 and 2.26 $\log_{10}$ cfu/g. After being ground and sieved in an open environment, germinated finger millet was autoclaved for 15 min at 121°C. The results demonstrated reduction in the number of spores and fungi from 4.98 to 1.03 and 3.56 to 1.00 $\log_{10}$ cfu/g. Likewise, autoclaving germinated finger millet that had been ground and sieved in a sterile atmosphere for 15 min at 121°C resulted in a reduction of spore and fungi counts from 4.98 to 0.00 and 3.56 to 0.00 $\log_{10}$ cfu/g respectively (Table 1).

With the exception of germinated finger millet flour made from autoclaved germinated finger millet and autoclaved germinated finger millet flour that was ground and sieved in an open environment, statistically significant (*P*=.05) differences were seen for the spores count across all treatments. Moist heat sterilization might have killed spores and fungus present in

the germinated finger millet either by destruction of nucleic acid or cell membrane by creating pores and leaking of cell constituents.

The literature regarding sporicidal and fungicidal treatments of germinated millets was very scanty.

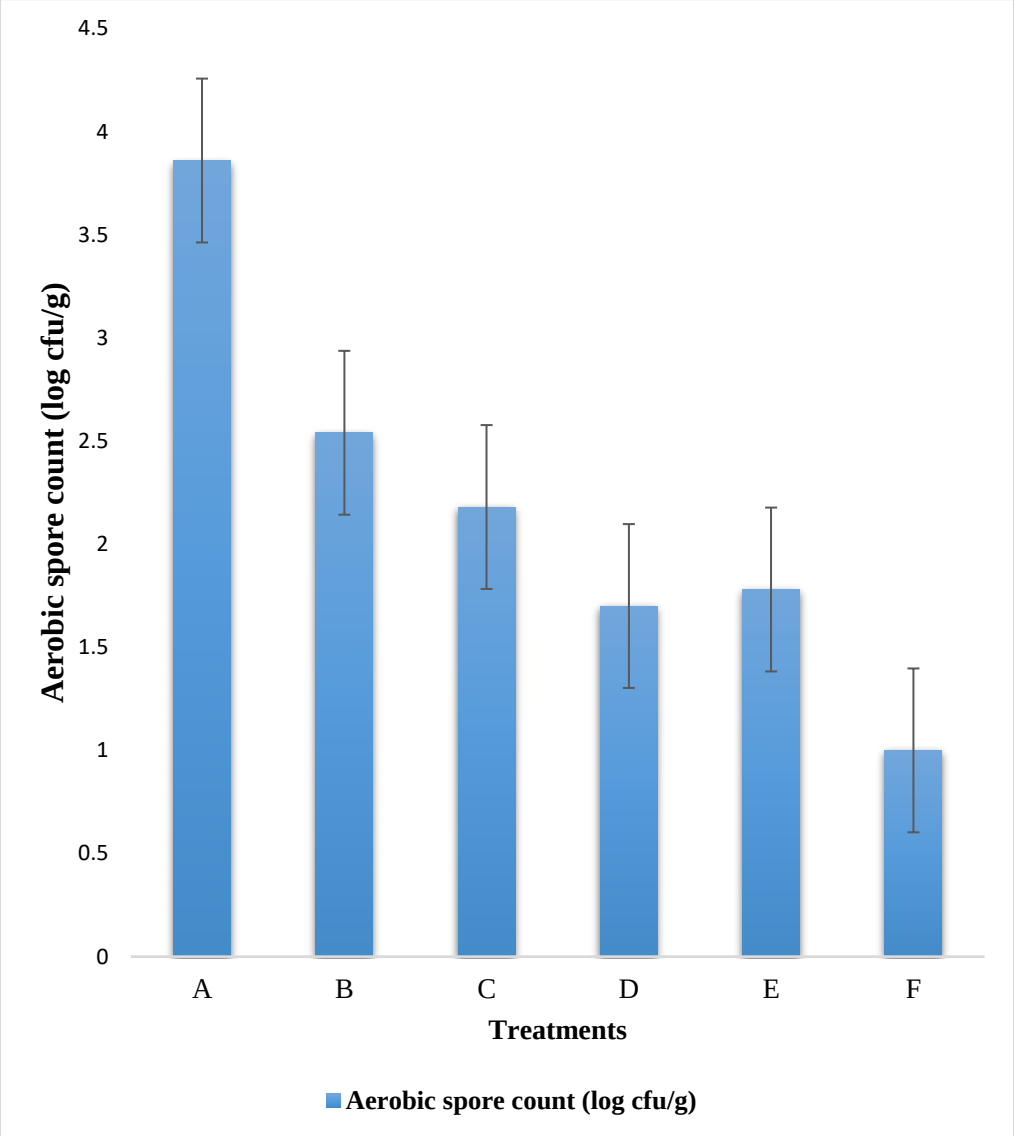


**Fig. 1. Various sporicidal treatments given to the finger millet**

*Note: A: Finger millet (control); B: Hot air oven (100℃/1 h); C: Blanching (98℃/30 s); D: Autoclaving (121℃/15 min); E: Hot air oven (100℃/1 h) and Autoclaving (121℃/30 min); F: Autoclaving (121℃/15 min)*

**Table 1. Different sporicidal and fungicidal treatments for optimized germinated finger millet**

| Treatments | Spores count | Yeast and mold count $log_{10}$ cfu/g |
|---|---|---|
| Control | 4.98[a] | 3.56[a] |
| Autoclaved millet | 1.60[b] | 0.00[d] |
| Flour from autoclaved millet | 1.00[c] | 2.26[b] |
| Autoclaved millet flour (open environment) | 1.03[c] | 1.00[c] |
| Autoclaved millet flour (sterile environment) | 0.00[d] | 0.00[d] |
| **CD (*P*=.05)** | **0.33** | **0.29** |

- *CD= Critical Difference, all the values are average of three trials; same superscripts indicate non-significance while different superscript indicates significant difference at P=.05*

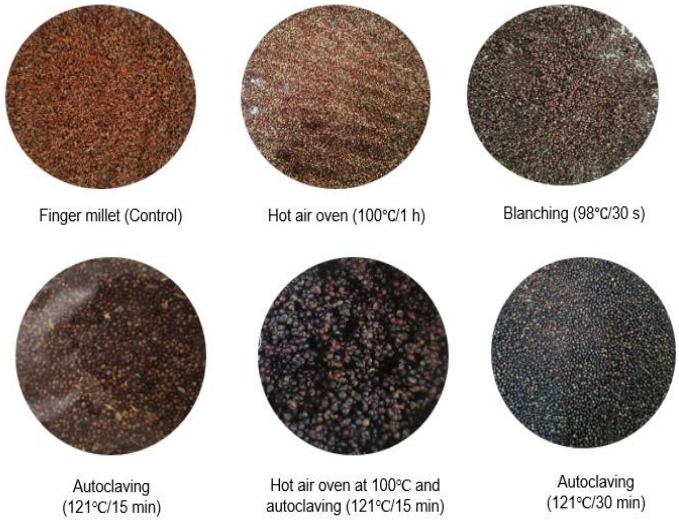


**Plate 1. Various sporicidal treatments given to the finger millet**

## 4. CONCLUSION

Among various sporicidal treatments given to finger millet, autoclaving at 121℃ for 30 min resulted in a maximum reduction of spores from 3.86 to 1.00 $\log_{10}$ cfu/g. Finger millet subjected to various sporicidal treatments did not undergo the sprouting process. Hence, sporicidal treatment was followed after 24 h soaking and 48 h germination. Autoclaving germinated finger millet flour that had been ground and sieved in a sterile atmosphere for 15 min at 121°C resulted in a complete reduction of spores and yeast and mold. Therefore, throughout the millet fermentation process, only the added probiotic bacteria uses the substrate and produces desired end products by using the germinated finger millet flour which is devoid of spores.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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