

## EFFECT OF DIFFERENT TREATMENTS OF FERMENTED PROBIOTIC MILLET DRIED MIX ON CHEMICAL COMPONENTS

Comment [a1]: Effect of different treatments on Chemical

### Abstract

Millet is gaining importance nowadays due to its high nutritional and therapeutic benefits. People are more focusing on functional food to derive their health benefits. Fermented probiotic millet dried mix was developed by fermentation of germinated finger millet flour and Reconstituted Skim Milk (RSM) with *Lactobacillus acidophilus*. Maximum viability of  $9.52 \log_{10}$  cfu/g was showed after 30 h fermentation at 10% inoculum level of *L. acidophilus* and 1:1.5 ratio of millet to RSM. After freeze drying of fermented probiotic millet mix with 2% Skim Milk Powder (SMP) as a cryoprotective agent, viability was retained to  $9.50 \log_{10}$  cfu/g. After freeze drying, the fermented probiotic finger millet dried mix had 4.76% moisture, 11.94% crude protein, 6.50% crude fiber, 4.74% total ash, 1.90% alcoholic acidity and 1.73 mol/kg phytate.

Comment [a2]: Not a fit here

**Keywords:** Finger millet, *L. acidophilus*, RSM, freeze drying

### Introduction

Global production of millet was 30,945 thousand MT. With 40% of the world's millet production, India was the largest millet producer with 12,500 thousand MT (USDA, 2023). Millets are a rich source of calcium (10-348 mg/100g), iron (2.2-17.7 mg/100g), zinc (32.7-60.6 mg/100g) and phosphorus (200-339 mg/100g), vitamin such as thiamine (0.15-0.60 mg/100g), niacin (0.09-1.11 mg/100g) and riboflavin (0.28-1.65 mg/100g), that makes them almost a perfect energy food (Kumar *et al.*, 2018). In addition to their potential to decrease cholesterol, millets also have additional health benefits including antioxidative, anti-ulcerative, hypoglycemic and anti-inflammatory qualities (Sharma *et al.*, 2021).

The finger millet grains have the highest concentrations of phosphorus (130.0-283.0 mg/100g) and calcium (162.0-358.0 mg/100g). About 44.7% of essential amino acids such as lysine, isoleucine, leucine, phenylalanine, methionine, cysteine and tryptophan are found in finger millet grains. Additionally, the important fatty acids palmitic and linolenic acid that are necessary for the growth of the brain and neural tissue are present in finger millet grains (Ramashia *et al.*, 2019). Antinutrient levels can be decreased by using processing techniques such as decortication, heating, soaking, germination and fermentation as they restrict the bioavailability of minerals (Samtiya *et al.*, 2021).

Functional foods are defined as "those foods and food components that offer a health benefit above and beyond basic nutrition," and in particular, "a food that resembles, or may be a conventional food that is consumed as part of a regular diet and is demonstrated to have physiological benefits and reduce the risk of chronic diseases above and beyond basic nutritional functions" (Coda *et al.*, 2017).

Probiotics are the good example of functional food. Probiotics are "live microorganisms that when administered in sufficient amounts, confer a health benefit on the host" (WHO,

2014). Probiotic foods made from cereal can be a significant global source of cheap calories and other nutrients. They are useful for the production of functional meals because they contain a variety of phytochemicals including phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols (Sharma *et al.*, 2014). Cereal grains offer typical benefits of whole cereal together with probiotic and prebiotic benefits. Additionally, fermentation has been shown to boost nutrients including methionine, lysine and tryptophan in a variety of cereal grains. A dietary mixture based on cereal that was fermented with a probiotic strain increased the amount of B complex vitamins, specifically thiamine and niacin (Budhware *et al.*, 2020).

The main goal of the current study was to ferment finger millet with *L. acidophilus* probiotic culture, enhancing the nutritional and functional benefits of the product thereby resulting in the development of a functional product with millets and probiotics benefits.

## **MATERIALS AND METHODS**

The finger millet was obtained from reputed local market. Skim milk powder (Nandini brand) was sourced from local market, Bengaluru, Karnataka, India.

### **Cultures**

Probiotic *Lactobacillus acidophilus* maintained in the department of Dairy Microbiology, Dairy Science College, Hebbal, Bengaluru was used for the product. The working cultures were maintained in sterile skim milk and sub cultured at weekly intervals.

### **Preparation of millet**

Finger millet was soaked for 24 h in sterile potable water at a ratio of 1:3 (millet: water) and then germinated for an additional 48 h at 30°C. Following a 24 h drying in an open incubator set at 45°C, the germinated millet was then ground and sieved in a sterile environment. The resulting germinated millet flour was autoclaved for 15 min at 121°C.

### **Growth study of *L. acidophilus* in germinated finger millet flour**

Autoclaved germinated finger millet flour was mixed with 9% sterile RSM at various ratios of 1:0.5, 1:1 and 1:1.5 to prepare germinated millet paste. Probiotic milk culture *L. acidophilus* was incorporated at inoculum levels of 5 and 10% into germinated millet paste and incubated at 37°C for 72 h under anaerobic condition. Throughout the 72 h fermentation period, the viability of *L. acidophilus* was assessed for every 6 h. Viability was determined by using standard procedure given by Harrigan (1998). The period of fermentation at which maximum viability was seen with corresponding inoculum level and ratio of germinated millet flour and RSM were optimized.

### **Drying of optimized fermented probiotic finger millet mix**

The optimized fermented probiotic finger millet mix was dried using air drying and freeze-drying techniques to lower the moisture content, extend the shelf life and to preserve the maximum viable count.

### **Air drying of optimized fermented probiotic finger millet mix**

The fermented probiotic millet mix was air dried for 24 h at 20, 25 and 30°C in a sterile BOD incubator. Moisture content (FSSAI 03.005:2022), viable count, spores, coliforms, yeast and mold counts (Harrigan, 1998) were assessed before and after air drying.

### **Freeze drying of optimized fermented probiotic finger millet mix**

Addition of skim milk powder at the rate of 2% was added to the optimized fermented probiotic millet mix as a cryoprotective agent. It was then freeze dried at -40°C for 8 h at 0.01 mm Hg vacuum. Moisture content (FSSAI 03.005:2022), viable count, spores, coliforms and yeast and mold counts (Harrigan, 1998) were analysed before and after freeze drying with and without addition of SMP. The drying condition with highest probiotic viability was optimized.

### **Effect of different treatments of fermented probiotic finger millet dried mix on chemical components**

Optimized samples were taken after soaking, germination, autoclaving, fermentation and drying in order to ascertain the change in chemical constituent during the various steps followed in the manufacturing of fermented probiotic millet dried mix and were analysed for moisture (FSSAI 03.005:2022), crude protein (FSSAI 03.016:2022), crude fiber (AOAC 978.10), total ash (FSSAI 03.010:2022), alcoholic acidity (FSSAI 03.014:2022) and phytate (Sharma *et al.*, 2016).

## **RESULTS AND DISCUSSION**

### **Growth study of *L. acidophilus* in germinated finger millet flour**

After 30 h of fermentation, the finger millet paste that was prepared using autoclaved finger millet flour and RSM ratio of 1:0.5, 1:1 and 1:1.5 with 5% *L. acidophilus* inoculum exhibited maximum viability of 7.78, 9.25 and 9.30 log<sub>10</sub> cfu/g. Similarly, addition of 10% *L. acidophilus* inoculum to finger millet paste exhibited maximum viability of 7.93, 9.28 and 9.52 log<sub>10</sub> cfu/g after 30 h of fermentation (Table 1). In order to acclimatize the culture to the milk environment and to stimulate their growth, skim milk was added. The growth of the culture increased during the first 30 h of fermentation after which it steadily decreased with increased fermentation time. This might be due to the inhibitory effect of excess lactic acid produced during fermentation on the growth of the culture. There were both significant and non-significant differences between the various hours of fermentation. Maximum viability of 9.52 log<sub>10</sub> cfu/g was observed after 30 h of fermentation period at 10% inoculum level of *L. acidophilus* and 1:1.5 millet to RSM ratio. Hence it was optimized.

On par with the above study, Devagaopalan *et al.* (2018) investigated *L. acidophilus* growth during fermentation of ragi koozh for a period of 24 h. The viability had increased from  $8 \log_{10} \text{cfu/ml}$  and further declined to  $6.5 \log_{10} \text{cfu/ml}$  after 15 and 24 h of fermentation. Increased acidity and decreased nutrition availability might be the cause for the drop in viability. Similarly, Akshaykumar *et al.* (2018) conducted growth study of *L. acidophilus* on supplemented black gram dal. Viable count increased from  $7.80$  to  $9.57 \log_{10} \text{cfu/g}$  and further decreased to  $6.50 \log_{10} \text{cfu/g}$  after 24 and 48 h of fermentation.

**Table 1: Growth study of *L. acidophilus* in germinated finger millet flour**

Fermentation period (h)	Ratio of autoclaved germinated flour and RSM					
	1:0.5		1:1		1:1.5	
	<i>L. acidophilus</i> (%)					
	5	10	5	10	5	10
	Log <sub>10</sub> cfu/g					
0	6.03 <sup>e</sup>	6.27 <sup>fg</sup>	6.03 <sup>f</sup>	6.27 <sup>f</sup>	6.03 <sup>h</sup>	6.27 <sup>f</sup>
6	6.48 <sup>de</sup>	6.62 <sup>def</sup>	6.72 <sup>e</sup>	6.75 <sup>h</sup>	6.78 <sup>g</sup>	7.28 <sup>e</sup>
12	7.06 <sup>bc</sup>	7.11 <sup>bcd</sup>	7.44 <sup>d</sup>	7.64 <sup>f</sup>	7.66 <sup>ef</sup>	8.00 <sup>d</sup>
18	7.36 <sup>ab</sup>	7.44 <sup>abc</sup>	8.23 <sup>c</sup>	8.24 <sup>cd</sup>	8.29 <sup>c</sup>	8.68 <sup>bc</sup>
24	7.47 <sup>ab</sup>	7.64 <sup>ab</sup>	8.70 <sup>b</sup>	8.76 <sup>b</sup>	8.79 <sup>b</sup>	9.02 <sup>b</sup>
30	7.78 <sup>a</sup>	7.93 <sup>a</sup>	9.25 <sup>a</sup>	9.28 <sup>a</sup>	9.30 <sup>a</sup>	9.52 <sup>a</sup>
36	7.46 <sup>ab</sup>	7.57 <sup>abc</sup>	8.42 <sup>bc</sup>	8.67 <sup>bc</sup>	8.84 <sup>b</sup>	9.01 <sup>b</sup>
42	7.30 <sup>ab</sup>	7.40 <sup>abc</sup>	8.37 <sup>bc</sup>	8.40 <sup>bcd</sup>	8.48 <sup>bc</sup>	8.75 <sup>bc</sup>
48	7.05 <sup>bc</sup>	7.18 <sup>bcd</sup>	8.16 <sup>c</sup>	8.18 <sup>de</sup>	8.22 <sup>cd</sup>	8.42 <sup>cd</sup>
54	6.73 <sup>cd</sup>	6.96 <sup>cde</sup>	7.69 <sup>d</sup>	7.78 <sup>ef</sup>	7.90 <sup>de</sup>	8.12 <sup>d</sup>
60	6.15 <sup>e</sup>	6.38 <sup>efg</sup>	7.40 <sup>d</sup>	7.43 <sup>fg</sup>	7.49 <sup>f</sup>	7.94 <sup>d</sup>
66	5.48 <sup>f</sup>	5.82 <sup>gh</sup>	6.99 <sup>e</sup>	7.05 <sup>gh</sup>	7.05 <sup>g</sup>	7.30 <sup>e</sup>
72	5.08 <sup>f</sup>	5.25 <sup>h</sup>	5.82 <sup>f</sup>	5.86 <sup>f</sup>	5.89 <sup>h</sup>	6.43 <sup>f</sup>
<b>CD (P=.05)</b>	<b>0.30</b>	<b>0.35</b>	<b>0.07</b>	<b>0.25</b>	<b>0.22</b>	<b>0.29</b>

Note:

- RSM: Reconstituted Skim Milk

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#### Air drying of optimized fermented probiotic finger millet mix

Viability of *L. acidophilus* had decreased from 9.52 to 8.50, 7.90 and 7.50  $\log_{10} \text{cfu/g}$  after air drying at 20, 25 and 30°C (Table 2). The presence of spores, yeast and mold may be due to exposure of product to open air. Due to the decreased viability and presence of spores and yeast and mold, this method was not opted for further studies. A statistically significant ( $P=.05$ ) differences in viability were noted throughout drying regimes with the exception of drying between 25 and 30°C.

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On par to the above study, Akshaykumar *et al.* (2019) reported decreased viability from 9.45 to 8.94, 8.71 and 8.04  $\log_{10} \text{cfu/g}$  after air drying of *L. acidophilus* incorporated black gram

dhal at 20, 25 and 30°C. Similarly, Ramachandra *et al.* (2009) air dried *L. acidophilus* 111 incorporated rice husk for 20 h. After air drying at 15, 20, 25 and 30°C, the viable count had declined from 10.80 to 8.92, 9.00, 8.87 and 8.40 log<sub>10</sub>cfu/g respectively.

**Table 2: Air drying of optimized fermented probiotic finger millet mix**

Treatments	Moisture (%)	Viability	Spores	Coliforms	Yeast and Mold
Before drying	61.90 <sup>a</sup>	9.52 <sup>a</sup>	0.00 <sup>c</sup>	Nil	0.00 <sup>b</sup>
<b>After air drying (°C)</b>					
20	7.00 <sup>b</sup>	8.50 <sup>b</sup>	2.70 <sup>b</sup>	Nil	1.30 <sup>a</sup>
25	6.80 <sup>b</sup>	7.90 <sup>c</sup>	3.07 <sup>a</sup>		1.48 <sup>a</sup>
30	5.74 <sup>c</sup>	7.50 <sup>c</sup>	3.30 <sup>a</sup>		1.60 <sup>a</sup>
<b>CD (P=.05)</b>	<b>0.00</b>	<b>0.39</b>	<b>0.33</b>		<b>0.33</b>

**Freeze drying of optimized fermented probiotic finger millet mix**

The viability of *L. acidophilus* in fermented probiotic finger millet mix had declined from 9.52 to 9.02 log<sub>10</sub> cfu/g after freeze drying. Before freeze drying, 2% skim milk powder was added as a cryoprotective agent, which assisted to retain viable cells of 9.50 log<sub>10</sub> cfu/g even after freeze drying (Table 3). As a cryoprotective agent, skim milk powder has created a viscous coating on the cell surface, preventing the formation of ice crystals. Freeze drying proved ideal for drying probiotic mix since it produced higher viable count and hence it was optimized. There was no statistically significant difference noted between freeze drying with addition of SMP and before drying, although there was a statistically significant (P=.05) difference identified between samples dried without SMP and samples before drying.

Similarly, Kanimozhi *et al.* (2023) reported that the survival rate of freeze dried *Lactobacillus* sp. using 5% skim milk powder as a cryoprotective agent was 93.98%. On contrary, Akshaykumar *et al.* (2019) reported the decline in viability of freeze-dried *L. acidophilus* incorporated black gram dhal from 9.50 to 7.47 log<sub>10</sub>cfu/g.

**Table 3: Freeze drying of optimized fermented probiotic finger millet mix**

Treatments	Moisture (%)	Viability	Spores	Coliforms	Yeast and Mold
Before drying	61.90 <sup>a</sup>	9.52 <sup>a</sup>		Nil	
<b>After freeze drying (8h)</b>					
Without SMP	4.76 <sup>b</sup>	9.02 <sup>b</sup>		Nil	
With 2% SMP	4.76 <sup>b</sup>	9.50 <sup>a</sup>			
<b>CD (P=.05)</b>	<b>0</b>	<b>0.41</b>			

### **Effect of different treatments of fermented probiotic finger millet dried mix on chemical components**

The optimized fermented probiotic millet mix had 4.76% moisture, 11.94% crude protein, 6.50% crude fiber, 4.74% total ash, 1.90% alcoholic acidity and 1.73 mol/kg of phytate (Table 4). There was a statistically significant ( $P=0.05$ ) difference in moisture levels except for 48 h germinated millet and autoclaved sample and there was statistically significant difference in phytate content among the treatments.

### **Soaking of finger millet**

After soaking for 24 h, the moisture content of finger millet flour increased from 13.50 to 34.47% and this could be due to the absorption of moisture from the soaking media. The phytate level decreased from 5.32 to 4.06 mol/kg as a result of phytic acid breakdown by phytase and leaching into the soaking medium. Enzymatic activity caused the alcoholic acidity to rise from 0.17 to 0.19%. Because of the drop in phytate level, ash increased from 2.31 to 2.50%. Protein increased from 7.23 to 7.28% and crude fiber from 4.00 to 4.12% and this increase might be due to the decline in phytate content.

On contrary, Abioye *et al.* (2022) investigated the influence of soaking circumstances on the proximate composition of finger millet flour. After 24 h of soaking at 30°C, the moisture content increased from 7.08 to 8.89%, the protein content dropped from 9.47 to 5.78%, the ash content grew from 1.10 to 1.94%, the crude fiber content reduced from 2.36 to 1.34% and the phytate content decreased from 0.75 to 0.44 mg/100g.

### **Germination of finger millet**

The breakdown of complex organic molecules like lipids, phytin and protein into simpler substances may be the cause for the increase in alcoholic acidity from 0.17 to 0.28%. The total ash increased from 2.31 to 2.91%, which could be attributed to the reduction of metal-binding water-

soluble secondary metabolites or the conversion of phytate to inositol by phytase, which would have liberated the orthophosphate and released the minerals into the millet seeds that had germinated. The synthesis of proteins, the breakdown of antinutritional substances and the synthesis of enzymes by germinated grains may have contributed to the rise in crude protein from 7.23 to 7.91%. Phytate dropped from 5.32 to 3.34 mol/kg, and the phytase enzyme may have been activated to cause this phytate to degrade and leak out. The amount of crude fiber increased from 4.00 to 4.80%, and the production of cellulose and hemicellulose may have contributed to this rise.

On par with the above study, Sharma *et al.* (2023) investigated the physicochemical parameters of finger millet after soaking and germinating for 24 and 48 h. To create finger millet flour, germinated seeds were dried at 40°C for 24 h, then ground and sieved. The moisture content increased from 8.47 to 8.65% while the protein content increased from 7.36 to 8.51%, fiber increased from 3.75 to 4.55%, ash content from 2.35 to 2.84%, drop in fat from 2.05 to 1.31% and the amount of phytate dropped from 15.98 to 9.77 mg/g were observed.

### **Autoclaving of germinated finger millet flour**

Following germination, these seeds were ground, sieved to create germinated flour, then autoclaved for 15 min at 121°C. Moisture increased from 8.00 to 8.33%; moisture at sterilization could be the cause of the

is. Crude protein decreased from 7.91 to 7.54%, phytate from 3.34 to 2.71 mol/kg, total ash from 2.91 to 2.70%, alcoholic acidity from 0.28 to 0.20% and crude fiber from 4.80 to 4.30%.

The separation of the bran layer during milling could be the cause of this drop. The impact of germination and fermentation on the nutritional makeup of food blends including pearl millet was investigated by Arora *et al.* (2009). After 15 min at 121°C in the autoclave, the moisture content of the germinated pearl millet declined from 28.41 to 28.39%, the crude protein from 13.89 to 13.78, the ash from 2.93 to 2.90%, the titratable acidity rose from 2.15 to 2.22 g lactic acid/100 mL and the crude fiber declined from 0.79 to 0.70%.

### **Fermentation of germinated finger millet flour**

The addition of reconstituted skim milk caused the moisture content to increase from 8.33 to 61.90% after fermentation. Alcoholic acidity increased from 0.28 to 1.62% as a result of probiotic organism fermentation, which causes glucose to be converted into lactic acid. Total ash increased from 2.70 to 3.06% due to reduction of anti-nutrients. The possible reason for the rise in crude protein from 7.54 to 7.70% during fermentation is the solubilization of insoluble proteins. Phytate's hydrolysis caused it to drop from 2.71 to 1.32 mol/kg. Crude fiber declined from 4.30 to 4.21%, possibly as a result of lactic acid bacteria using the fiber as a carbon source during the fermentation process and enzymatically breaking down the fiber's components.

On par with the above study, Jan *et al.* (2022) explored the impact of fermentation treatment on the nutritional makeup of finger millet. Fermentation was carried out with *L. brevis* for 12, 24 and 36 h. After 12, 24 and 36 h, the moisture content increased from 9.7 to 10.97, 11.40 and 11.85%. Fiber also declined from 5.67 to 4.48, 3.68 and 3.49%. Ash levels increased from 3.10 to 3.2, 3.53 and 3.63%. Protein increased from 9.10 to 10.28, 10.44 and 13.13% from 9.10 to these values. The phytic acid decreased from 629 to 250.49, 230.88 and 207.94 mg/100g.

### **Freeze drying of fermented probiotic millet mix**

Due to the concentration of the mix caused by the elimination of moisture after freeze drying, the moisture content decreased from 61.90 to 4.76% and the alcoholic acidity increased from 1.62 to 1.90%. Drying caused increase in total ash of 3.06 to 4.74%, protein from 7.70 to 11.94%, crude fiber from 4.21 to 6.50% and phytate from 1.32 to 1.73 mol/kg. This increase in nutrients could be due to the result of the concentration process during freeze drying. On contrary to the above study, Kabeer *et al.* (2023) assessed the impact of freeze drying on the nutritional qualities of powdered finger millet porridge. Moisture level decreased from 15.63 to 9.5%, ash from 4.53 to 4.486, fiber from 3.61 to 3.59%, fat from 1.589 to 1.545% and protein content from 7.72 to 7.60%. Water loss from the interior surface to the surrounding could be the cause of this loss.

**Table 4: Effect of different treatments of fermented probiotic finger millet dried mix on chemical components**

Treatments	Moisture	Crude protein	Crude fiber	Total ash	Alcoholic acidity	Phytate (mol/kg)
	(%)					
Finger millet flour	13.50 <sup>c</sup>	7.23 <sup>c</sup>	4.00 <sup>c</sup>	2.31 <sup>c</sup>	0.17 <sup>b</sup>	5.32 <sup>a</sup>
After soaking (24h)	34.47 <sup>b</sup>	7.28 <sup>c</sup>	4.12 <sup>c</sup>	2.50 <sup>c</sup>	0.19 <sup>b</sup>	4.06 <sup>b</sup>
48 h Germinated finger millet after drying	8.00 <sup>d</sup>	7.91 <sup>b</sup>	4.80 <sup>b</sup>	2.91 <sup>b</sup>	0.28 <sup>b</sup>	3.34 <sup>c</sup>
After autoclaving (121 <sup>o</sup> C/15 mins)	8.33 <sup>d</sup>	7.54 <sup>bc</sup>	4.30 <sup>bc</sup>	2.70 <sup>bc</sup>	0.20 <sup>b</sup>	2.71 <sup>d</sup>
After fermentation (30h)	61.90 <sup>a</sup>	7.70 <sup>b</sup>	4.21 <sup>c</sup>	3.06 <sup>b</sup>	1.62 <sup>a</sup>	1.32 <sup>e</sup>
After freeze drying (8 h)	4.76 <sup>e</sup>	11.94 <sup>a</sup>	6.50 <sup>a</sup>	4.74 <sup>a</sup>	1.90 <sup>a</sup>	1.73 <sup>f</sup>
<b>CD(P=.05)</b>	<b>0.00</b>	<b>0.36</b>	<b>0.36</b>	<b>0.36</b>	<b>0.29</b>	<b>0.36</b>

### Conclusion

Fermented probiotic millet dried mix was developed by fermentation of germinated finger millet flour and Reconstituted Skim Milk with *L. acidophilus*. Maximum viability of  $9.52 \log_{10} \text{cfu/g}$  was seen after 30 h fermentation period at 10% inoculum level of *L. acidophilus* and 1:1.5 millet to RSM ratio. Air drying resulted in decreased viability and indicated the presence of spores and yeast and mold in the product. Prior to drying, the viable count of *L. acidophilus* was  $9.52 \log_{10} \text{cfu/g}$ , however after freeze drying fermented probiotic millet mix with 2% SMP as cryoprotective agent it was retained to  $9.50 \log_{10} \text{cfu/g}$ . After freeze drying, the fermented probiotic finger millet dried mix had 4.76% moisture, 11.94% crude protein, 6.50% crude fiber, 4.74% total ash, 1.90% alcoholic acidity and 1.73 mol/kg phytate.

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