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Storage Stability and Performance of Fluid Bed Dried Solid State Fermented (SSF) Lactic Cultures

Akshaykumar [a++*], Manjunatha H [a#], Devaraju R [b++], Ramachandra B [c†], Basavabharati [a++], Madhusudan NM [a++] and Shashi Kumar CS [a++]

[a] Department of Dairy Microbiology, KVAFSU-Bidar, Dairy Science College, Mahagaon Cross, Kalaburagi, India.
[b] Department of Dairy Engineering, KVAFSU-Bidar, Dairy Science College, Mahagaon Cross, Kalaburagi, India.
[c] Department of Dairy Microbiology, KVAFSU-Bidar, Dairy Science College, Hebbal, Bangalore, India.

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: In addition to attempting to evaluate the performance of SSF cultures in heated milk, the current study aimed to investigate the impact of refrigeration temperature storage on the fluid bed dried powder of Solid State Fermented (SSF) lactic cultures, such as dahi, yoghurt, and probiotic cultures.

[++] Assistant Professor;
[#] Dean & Professor;
[†] Professor;
**Corresponding author: Email: akshaysagar200@gmail.com;*

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Duration and Place of study: The study was carried out in single season at Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU, Bidar), Dairy Science College, Hebbal, Bangalore, Karnataka, India

Study design and Methodology: aerobic spore survival, the raw black gram dhal had the lowest spore count—1.52 log10cfu/g. The spores were killed by sterilizing the black gram dhal at 1210C for 30 minutes and then exposing it to a hot air oven at 1000C for one hour. Black gram dhal containing 1:0 was used to obtain the maximum viable starter counts of dahi (8.41 log10cfu/g), yoghurt (8.98 log10cfu/g), and probiotics (9.47 log10cfu/g) cultures during fermentation. Ash gourd, carrot, tomato, and skim milk powder liquids were added to the 8:1 moisture ratio at 1% level as growth-promoting agents. The fermented SSF cultures were dried on a fluid bed at room temperature (25^0C) for 1.5 hours. After fluid drying, the SSF culture viability of the probiotic, yoghurt, and dahi cultures dropped from (9.01 to 8.85, 8.65 to 8.33) and 9.65 to 9.34 log10cfu/g, respectively. 9.4% of the SSF cultures' moisture content was observed. Following inoculation of the heat-treated milk with these dried powders of SSF cultures of dahi, yoghurt, and probiotic cultures at 1,0.5, and 3%, the cultures took 5:00, 3:30, and 9:30 hours to establish the heat-treated milk with acidity of 0.68, 0.71, and 0.59% lactic acid, respectively.

Results: The dried SSF lactic cultures were viable for 40 days at 7±10C, or 6 log10cfu/g, when stored at that temperature. The absence of contaminants such as coliforms, aerobic spores, yeast, and molds in the stored fluid-dried SSF lactic cultures demonstrated the good hygienic practices used during the biomass production. The curdling period increased with the number of storage days at refrigeration temperatures in relation to the performance of stored SSF lactic cultures because of the decrease in viable lactic counts.

Conclusion: The minimum viable counts of probiotic, yoghurt, and dahi cultures on the 40th day of storage at refrigerator temperature were 6.45, 6.85, and 6.44 log10 cfu/g, respectively. Furthermore, none of the preserved samples had any coliforms, aerobic spores, yeast, or mould in them.

Keywords: Probiotic cultures; solid state fermented lactic cultures; black gram dhal; supplements; fluid bed drying; Dahi; yogurt; refrigeration temperature.

1. INTRODUCTION

One of the most affordable and traditional ways to produce and preserve food is through fermentation. Furthermore, fermentation offers a natural means of lowering the volume of material to be carried, eliminating unwanted components, improving the food's nutritional content and appearance, lowering the cooking energy needed, and producing a safer product [1]. Fermented foods and beverages are goods that have been allowed to undergo desired biochemical changes by the action of microbes or enzymes. These products are made using the fermentative activities. The microflora that naturally exists on the substrate may be the microorganisms causing the fermentation, or they may be added as starter cultures [2]. Food, dairy, and alcoholic drinks are all produced in large part by microorganisms. Furthermore, a number of microbial fermentation byproducts are added to food as supplements and additives (antioxidants, flavours, colourants, preservatives, sweeteners, etc). Since natural food and additives created from microbes are more attractive than those made synthetically by chemical processes, there is a lot of interest in their development and application [3].

Solid-state fermentation, or SSF, is characterised as a fermentation process that uses a solid matrix and is conducted without or very near the presence of free water; the substrate must, however, have sufficient moisture to maintain the microorganism's development and metabolism. The solid matrix may serve as a source of nutrients or it may just be a medium on which the right nutrients are impregnated to promote the growth of the microorganisms. SSF's potential is found in placing the cultivated microbe in close proximity to the substrate and attaining the maximum concentration of substrate possible for fermentation [4]. It is necessary to cultivate lactic cultures on solid substrates with the right nutrients. Reducing moisture content without severely impacting the viability of the cells requires the employment of suitable preservation procedures. Air drying, freeze drying, and fluid bed drying are a few of the methods that are employed. Of air drying, freeze drying, and drying by bed, the fluid bed drier seems to be the most successful way for drying SSF lactic

cultures on supplemented black gram dhal since it offers higher viability and lower moisture retention than the other two. This study aims to cultivate lactic cultures on appropriate edible substrate with appropriate supplements to sustain SSF lactic cultures for an extended duration. The SSF lactic cultures' dried powder is thereafter kept at refrigerator temperature to preserve its viability. This suggests that SSF culture needs low-temperature storage to preserve viable cells, which makes it an excellent inoculum for fermented milk products.

2. MATERIALS AND METHODS

2.1 Lactic Acid Bacterial Cultures

In this study, cultures of probiotic from the Dairy Science College, KVAFSU, Hebbal, Bengaluru-24 department of Dairy Microbiology were used. These cultures included *L. lactis* ssp. lactis and *L. lactis* ssp. lactis bv. diacetylactis, as well as *S. thermophilus* and *L. delbrueckii* ssp. bulgaricus and *L. acidophilus* were used as probiotics.

Gathering and examining different dhals to measure aerobic spore counts: Eight different dhal varieties, which are widely available in Bengaluru's local market, were bought, cleaned to remove debris and unwanted plant materials, later stored in polythene pouches with self-sealing. The variants were black gram dhal, green gram dhal, hyacinth dhal, masoor dhal, red gram dhal, raw bengal gram dhal and soya bean dhal. To find out how many spores were in the dhal, they were subjected to aerobic spore counts using the plating method as per Harrigan [5].

Sporicidal treatment for black gram dhal to use as solid substrate: In order to lower the aerobic spore count, Dhal was exposed to a range of treatments, such as wet heat treatments such as hydration for 30 minutes, 12 hours, and 24 hours, 0.01% and 0.05% treatment with hydrogen peroxide, and tyndallization (steaming for three consecutive days). According to Harrigan [5], an aerobic spore count was performed on the treated dhal.

Addition of treated black gram dhal to promote the development of lactic cultures: The optimal sporicidal therapy was determined, and as supplements, skim milk powder, ash guard juice, carrot juice, and tomato juice were administered to totally eliminate the aerobic spore.

Preparation of ash guard, carrot and tomato juice: We bought fresh tomatoes, carrots, and ash guard at the neighbourhood market. The edible components were removed and cleansed in potable water before being grated and steam-cooked for fifteen minutes before being mashed in a dry, clean mixer. The resulting puree was filtered via muslin cloth. After filtering, the juices from the ash guard, carrot, and tomato were separated and collected in a sterile conical flask [6].

Final supplementation to blackgram dhal: At the 0.5, 1, 1.5, and 2% levels, SMP, Ash Guard juice, carrot juice, and tomato juice were added to the aerobic spore-free black gram dhal. Juice volume included, the moisture content was kept at 1:0.8.

SSF cultures' growth investigation on supplemented blackgram dhal: The maximal growth period required for good biomass of lactic culture on supplemented blackgram dhal was found to be 48 hours at the appropriate growing temperature. Every six hours, the viability of aseptically collected samples of SSF yoghurt, dahi, and acidophilus cultures was assessed.

Assessing the viability of lactic culture cultivated on blackgram dhal supplementation: Probiotic SSF cultures, yoghurt, and dahi were acquired at different phases of development. After that, the cultures were aseptically moved to a sterile pestle and mortar and each of them was given a single trituration using 99 millilitres of sterile phosphate buffer. After making the required dilutions, the yeast glucose agar was plated in order to calculate the total lactic count. The yoghurt and probiotic cultures were incubated at 37°C, while the dahi culture was kept at 30°C in the plates. The viable lactic counts were represented in log10cfu/g.

SSF lactic culture drying with a fluid bed drier: SSF fermented lactic cultures were dried for 1, 1.5, and 2 hours at room temperature (25 ± 1^0 C) after being moved to a sterile fluid bed drier. Aseptic sampling was used to determine the viable lactic counts and moisture content.

Powder preparation of dried SSF lactic cultures: Using a sterile dry mixer, the finest dried SSF lactic cultures cultivated on supplemented black gram dhal were turned into powder based on less residual moisture and a

higher viable count. Labeled self-sealing polythene packets were filled with powdered SSF lactic cultures.

Impact of keeping SSF lactic cultures refrigerated (7±1^{0}C) on their viability and their function in sterile milk: The dried SSF powder of lactic culture packaged in self-sealing (polythene) bags and at refrigerated temperature (7±10C) to assess its viability. The viability of the SSF culture was assessed by YGA once per five days of storage at refrigerator temperature. At the same time, the culture was injected with heat-treated milk and allowed to incubate. For the set milk, the titratable acidity and curdling time were calculated. The quantity of aerobic spore, coliforms, yeast, and moulds was counted in order to further assess the safety of the conserved SSF cultures.

2.2 Statistical Analysis

R statistical tool of version 3.1.3 was used to evaluate the data and perform statistical computations for both one-way and two-way ANOVA in CRD, which is suitable method for this kind of research. For every one of these treatments, response variable data were gathered for three replications. The data were analyzed using ANOVA tables, and in cases where the F value was significant, the crucial difference was calculated. This allowed for the identification of significant differences, which were then displayed in the table using superscripts. The crucial difference (formula is CD):

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

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

The viability of fluid bed powdered SSF dahi culture stored at a temperature of (7±1^0 C) degrees Celsius for storage and their performance in heated milk.

Dahi SSF viable count culture started decreasing slowly in refrigerated condition from 8.55 to 6.44 $\log_{10}$ cfu/g on 40th storage day. The viable count reduction was more or less 1.00 up to 20th day of storage, and later storages reduced still up to 2.00 log counts. Colifroms, when stored at 7^{0}C for 40 days aerobic spores, yeast and molds were absent in SSF dahi culture. Dahi SSF culture when inoculated into heated milk, at 1% level, curdled between (5:00 and 9:30 h) at incubation of 30^{0}C with acidity ranging from (0.68 to 0.72%) LA. As the viable count in SSF dahi culture started to reduce the curdling time of milk automatically increased. The storage study of dried SSF dahi culture was concluded till a good inoculums level was observed i.e., around 6 log count. Significant difference was noticed in viable count of SSF dahi culture stored at 7^{0}C from 25th day of storage (Table 1, Fig. 1). Viable count of dahi SSF culture in refrigerated condition declined gradually in comparison to ambient temperature; this reduction was 1 log until the 20th day of storage, at which point it was 2.00 log counts. The curdling time of milk automatically increased from 5 hours on the 40th day of storage with inoculums of approximately 6.44 log count to 9:30 hours when the viable count in SSF dahi culture began to decrease.

It appears that hygienic practices were followed during the propagation, inoculation, and preparation of fermented milk products using SSF lactic cultures because the dried powder of SSF dahi cultures did not contain any coliforms, aerobic spores, yeast, or mould until the end of the storage period at both room temperature and chilled temperatures. The viability of SSF mixed dahi culture on supplemented black gram dhal decreased by 2 log at room temperature (24^{0}C), and by 4.50 log on the 60th day. However, the drop in viable log count was slower when SSF culture was held at refrigeration temperature (7^{0}C), accounting for 2 at day 49 of storage and 3.50 at day 60 of storage [7].

The viability of fluid bed powdered SSF yoghurt culture at refrigeration temperature (7±1^{0}C) and their performance in heated milk.

Yoghurt SSF culture viable counts decreased by 1 log on the 20th day of storage (from 9.07 to 8.03 log), then by the 35th day (6.94 log) to 2 log, and lastly by 2.2 log counts at the end of 40 days of storage (6.85 log). During the storage period, no signs of yeast, moulds, aerobic spores, or coliforms were found. Using a stored SSF yoghurt culture, short set yoghurt was produced in 3:30–8:00 hours, containing 0.71–0.80 percent lactic acid during the first 40 days of storage at 70°C. At low temperature storage, a statistically significant variation was seen in the viable count of SSF yoghurt culture starting on day 25. (Table 2, Fig. 1).

Table 1. Impact of refrigerated temperature (7±1^{0}C) on viability of SSF dahi cultures and their performance in heated milk

Days of storage	(Viable counts in dried powder of SSF culture log_{10} cfu/g)	Performance of dried powder of SSF culture in milk	
		(Time for Curdling in hrs.)	(Titratable acidity - %LA)
0th	8.55^a	5:00	0.68
5th	8.40^b	5:30	0.69
10th	8.25^c	6:00	0.72
15th	7.79^d	6:45	0.72
20th	7.50^e	7:00	0.72
25th	7.12^f	7:30	0.72
30th	6.71^g	8:30	0.72
35th	6.53^h	9:00	0.72
40th	6.44^i	9:30	0.72
CD($p \leq 0.05$)	**0.08**		

- *Average of three trials(n = 3).*
- *Same letters in super script indicates non-significance while different letters have significant difference ($p \leq 0.05$).*
- *Stored dried powder SSF culture showed nil counts of coliform, aerobic spore count and yeast and mold counts throughout storage period.*
- *Stored SSF powder of dahi culture was inoculated at 1% and performance was checked at incubation of 30^{0}C.*

Table 2. Impact of storage of SSF yoghurt culture on viability at refrigerated temperature (7±1^{0}C) and their performance in heated milk

Days of storage	(Viable counts in dried powder of SSF culture log_{10} cfu/g)	Performance of dried powder of SSF culture in milk	
		(Time for Curdling in hrs.)	(Titratable acidity - %LA)
0th	9.07^a	3:30	0.71
5th	8.89^b	3:45	0.75
10th	8.52^c	4:00	0.79
15th	8.23^d	4:30	0.79
20th	8.03^e	5:30	0.79
25th	7.71^f	6:15	0.79
30th	7.33^g	7:00	0.80
35th	6.40^h	7:30	0.80
40th	6.85^i	8:00	0.80
CD($p \leq 0.05$)	**0.18**		

- *Average of three trials(n = 3).*
- *Same letters in super script indicates non-significance while different letters have significant difference ($p \leq 0.05$).*
- *Stored dried powder SSF culture showed nil counts of coliform, aerobic spore count and yeast and mold counts throughout storage period.*
- *Stored SSF powder of dahi culture was inoculated at 1% and performance was checked at incubation of 42^{0}C.*

They exhibited a longer curdling period when the inoculum was freeze-dried SSF yoghurt culture. The freeze-dried SSF yoghurt culture on black gramme, maintained at 300C, was found to have dropped by 3.00 log viable count after 42 days of preservation. It took 24 hours to curdle with 0.50% acidity when the viable count dropped to 6.00 log after 42 days of storage at 420C, as opposed to 18 hours when fresh SSF culture was inoculated at 1% level (9.39 log based cfu/g) [8]. The viable count of the SSF culture dropped to 2 log after being inoculated at 1% level into sterile skim milk, and it curdled with an acidity of 0.9% LA after 24 hours [8].

Storage stability of culture (fluid bed powdered SSF probiotic *Lactobacillus acidophilus*) on

Table 3. Impact of storage of SSF *L. acidophilus* on viability at refrigerated temperature (7±1^{0}C) and their performance in heated milk

Days of storage	(Viable counts in dried powder of SSF culture log$_{10}$ cfu/g)	Performance of dried powder of SSF culture in milk	
		(Time for Curdling in hrs.)	(Curdling time in hrs.)
0th	9.38^a	9:30	0.59
5th	9.27^a	9:45	0.60
10th	9.01^a	10:15	0.63
15th	8.78^b	11:00	0.63
20th	8.31^c	11:45	0.64
25th	7.89^d	12:00	0.64
30th	7.43^e	12:15	0.64
35th	6.94^f	13:00	0.65
40th	6.45^g	13:50	0.65
CD(p ≤ 0.05)	**0.22**		

- *Average of three trials(n = 3).*
- *Same letters in super script indicates non-significance while different letters have significant difference (p ≤ 0.05).*
- *Stored dried powder SSF culture showed nil counts of coliform, aerobic spore count and yeast and mold counts throughout storage period.*
- *Stored SSF powder of dahi culture was inoculated at 1% and performance was checked at incubation of 37^{0}C.*

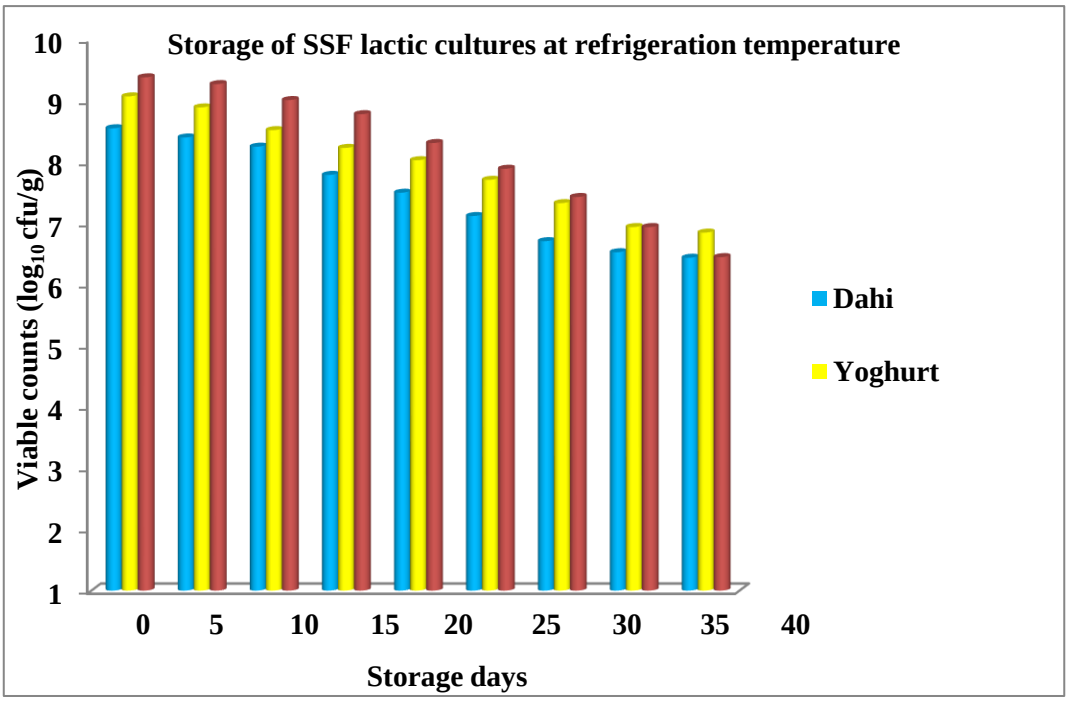

Fig. 1. Impact of storage of fluid bed dried powdered SSF lactic cultures on viability at refrigerated temperature

viability at refrigerated temperature (7±1^{0}C) and their performance in heated milk.

On the 40th day of storage, the viable counts of the acidophilus SSF culture, which had a log count of 9.38 under refrigeration, dropped to 6.45 log. The viable count drop was approximately 1 log until the 20th day of storage, and it further reduced to 3 log counts in further storages. No aerobic spores, yeast, moulds, or coliforms were

found in any of the preserved SSF cultures. When 3% of an acidophilus SSF culture was added to warm milk, it began to curdle between 9:30 and 13:50 hours at 370C, with an acidity range of 0.59 to 0.65% LA. From the 30th day of storage, a significant variation in the viable count of SSF acidophilus culture held at 70C was observed. (Table 3, Fig. 1).

The freeze-dried SSF acidophilus culture on black gramme dhal kept at room temperature at the end of the 42nd day of storage may have had a 2.50 log drop in viable count at the end of the longer curdling duration of 24 hours, despite the initial viable count of 7.00 and an acidity of 0.44 percent LA. In a related study, the Lactobacillus acidophilus 111 SSF culture was dried on rice husk and stored at room temperature (300C) in heat-sealed polyethylene bags [8]. The initial viable count of 8.81 log10 cfu/g gradually decreased to 6.14 log10 cfu/g after 30 days of storage. The cultivated material was curdling for 34 hours as opposed to a fresh SSF culture that was cultured for 26 hours. When added to Ceralac weaning food, even after six months of storage at ambient temperature, the air-dried fermented black gram dhal, which had a viable count of 7.00 of *Bifidobacterium longum* PF1, remained quite rich in viable counts, accounting for 6.70 log [9-14].

4. CONCLUSIONS

Use of dry powdered SSF lactic culture to examine the viability of probiotic cultures, chilled yoghurt, and chilled dahi was studied. Using a fluid bed, the biomass generated by SSF lactic cultures on supplemented black gram dhal was aseptically dried and kept at a temperature of 7 ± 1^0C till the 40th day of storage was up. The minimum viable counts of probiotic, yoghurt, and dahi cultures at refrigerator temperature on the 40th day of storage were 6.45, 6.85, and 6.44 log10 cfu/g, respectively. Furthermore, none of the preserved samples had any coliforms, aerobic spores, yeast, or mould in them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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