Comparative evaluation of air, freeze and fluidised bed drying for Solid State Fermented (SSF) lactic cultures

Akshaykumar, Malashree L and Ramachandra B

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Abstract: The present investigation was intended to compare the optimized Solid State fermented (SSF) lactic culture by using various drying preservation methods such as air drying, freeze drying and fluid bed drying. The SSF fermented cultures were dried with the moisture reduction from initial moisture of 96% to 81, 72 and 54% using air drying at 20°C, 25°C and 30°C temperatures respectively. The drying was done openly, hence, led to aerobic spore contamination in SSF lactic cultures, thus the method of air drying of SSF lactic cultures became limited. Further, freeze drying was carried out to dry SSF cultures on black gram dhal in which viable counts were reduced from 8.59 to 7.15 log_{10} cfu/g, 9.08 to 7.23 log_{10} cfu/g and 9.50 to 7.47 log_{10} cfu/g in dahi, yoghurt and acidophilus cultures respectively. Freeze drying on an average reduced the viable lactic accounts by 1.7 log counts mean while drying of 11 g of wet SSF lactic culture took 8 h to dry with moisture content of 11 per cent. Fluidized bed drier was found as suitable method to dry SSF lactic cultures at ambient temperature (25±1°C) for period of 1, 1.5 and 2 h. Out of the drying period of 1.5 h was found suitable to retain maximum viability with a satisfactory moisture content compared to 1 h and 2 h of drying. The viable counts of SSF lactic cultures maintained after fluid bed drying for 1.5 h were 8.33, 8.85 and 9.34 log_{10} cfu/g. Among the air drying, freeze drying and fluid bed drying, the best method seemed to be the fluid bed drier to dry SSF lactic cultures on supplemented black gram dhal with respect to higher viability and less moisture retainment compared to other two.

Keywords: Air drying, Black gram dhal, Freeze drying and Fluid bed drying, Solid State Fermentation (SSF)

Introduction

Milk is considered as universal food, as it has most of the nutrients required for a human being as well as a good medium for the growth of microorganisms. Fermentation of milk preserves its nutrients for long time and fermentation is made possible by inoculating the heat treated milk with lactic cultures. Fermented milk products have their own importance in the human diet by extending potential therapeutic benefits to the consumers. People have the concept of using fermented milk products as they are safe for consumption due to fermentation end products of the lactic cultures.

Fermentation is the process of transformation of simple raw materials into a range of value added products by utilizing the phenomenon of growth of microorganisms or through their activities on various substrates. It is one of the oldest technology used for the food preservation.

Based on the substrate, fermentation may be submerged fermentation (SmF) in liquid media or Solid State Fermentation (SSF) on solid substrates like nutritive (dhal, rice) or inert substrates (paddy husk). SSF technique has been widely used in preparation of fermented foods, enzymes, organic acids, polysaccharides, biomass of lactic acid bacteria, colours and flavours that involve the controlled growth of microorganisms on solid substrates in the absence of free moisture, so as to obtain large number of viable cells in concentrated form (Bhargav et al. 2008)

Biomass production of lactic acid bacteria is important to serve as active inoculums to produce fermented milk products. Lactic acid bacteria can be grown in milk or whey as liquid media or may be on solid substrate like dhal or bran. Edible substrates such as dhals are used as solid substrates to grow the cells to maximum level up to 10^{10} cells per gram due to more surface area for growth.
In conventional liquid culture, the cell growth is limited maximum up to 10^8 cells per millilitre, due to acidic pool during the growth of cells in the presence of free moisture (Koyani and Rajput, 2015).

SSF lactic cultures require preservation in order to keep them for longer without affecting their viability. This can be done by reducing moisture by various ways of drying. The objective of this study was to find the suitable dhals as SSF medium for the growth of the lactic cultures and the use of different methods to preserve the SSF grown cultures.

Materials and Methods

Lactic acid bacterial cultures

The dahi culture consisting of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *diacetylactis*, yoghurt culture that included *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactobacillus acidophilus* as probiotic culture, which had been maintained in sterile Yeast glucose chalk litmus milk (YGCLM) in the department of Dairy Microbiology, Dairy science college, KVAFSU, Hebbal, Bengaluru-24 were used in this study.

Collection and Screening of various dhals for aerobic spore counts

Dhal of 8 various types, commonly available in local market of Bengaluru such as raw bengal gram dhal, raw and roasted bengal gram dhal, black gram dhal, green gram dhal, hyacinth dhal (avaraebele), masoor dhal, red gram dhal, soya bean dhal were purchased, cleaned to remove stones and unwanted plant materials and stored in a self-sealing polythene pouches. To determine the extent of spores present in dhal, they were subjected to aerobic spore counts by plating method as per Harrigan (1998).

Various sporicidal treatment given to black gram dhal to use as solid substrate

Various treatments like dry heat treatment such as dry frying for 5 min, exposure to microwave for 1 min and exposure to 100°C for 1 h in hot air oven and wet heat treatments like hydration of dhal for 30 min, 12 h and 24 h, 0.01% and 0.05% treatment with hydrogen peroxide and tyndallization (steaming for 3 successive days) were given to dhal to reduce aerobic spore count and after treatment analysed the treated dhal for aerobic spore count as mentioned in Harrigan (1998).

Supplementation of treated black gram dhal for the growth of lactic cultures

Best sporicidal treatment to completely destroy the aerobic spore was selected and then supplemented with skim milk powder, ash guard juice, carrot juice and tomato juice.

Preparation of ash guard juice, carrot juice and tomato juice

Ash guard, carrot and tomato were obtained freshly from local market. The edible portions were obtained, washed with potable water, grated, steamed for 15 min and mashed in clean, dry, mixer. The obtained puree was filtered through muslin cloth. After filtration the juices of ash guard, carrot and tomato were collected separately in a sterile conical flask.

Final supplementation to black gram dhal

The aerobic spore free black gram dhal was supplemented with each of SMP, ash guard juice, carrot juice and tomato juice at 0.5, 1, 1.5 and 2% level. The moisture maintained was 1:0.8 level, including volume of water in juices.

Growth study of SSF cultures on supplemented black gram dhal

The maximum growth period required for good biomass of lactic culture on supplemented black gram dhal was determined at optimum growth temperature for 48 h. At every 6 h interval, aseptically drawn samples of SSF dahi, yoghurt and acidophilus cultures were subjected for viability determination (Fig 1).

Determining the viability of lactic culture grown on supplemented black gram dhal

SSF cultures of dahi, yoghurt and acidophilus drawn at different growth periods were aseptically transferred to sterile pestle and mortar, triturated with sterile 99 ml of phosphate buffer separately, required dilutions were prepared and plated using yeast glucose agar for total lactic count. The plates were incubated at 30°C for dahi culture and while in case of yoghurt and acidophilus cultures at 37°C. The viable lactic counts were expressed as log_{10} cfu/g.

Preservation of SSF lactic culture

In order to reduce the moisture content and increase shelf life, various drying methods such as air drying, freeze drying and fluid bed drying were followed for the SSF lactic cultures such as dahi, yoghurt and acidophilus grown on black gram dhal.

Air Drying of SSF lactic cultures

The SSF lactic cultures were dried at 20°C, 25°C and 30°C for a period of 20 h in BOD incubator set to that temperatures. Before and after drying, moisture content and viability of lactic cultures on fermented black gram dhal were determined.

Drying of SSF lactic cultures using Freeze drier

The SSF cultures were dried using freeze dryer at temperature of -42°C with vacuum of 0.01 mm mercury for a period of 8 h. The freeze dried SSF lactic cultures were subjected to determined moisture content and viable counts.
Drying of SSF lactic cultures using fluid bed drier

SSF fermented lactic cultures were transferred to the sterile fluid bed drier and dried at ambient temperature (25±1°C) for periods of 1, 1.5 and 2 h and the samples were aseptically drawn to determine the moisture and viable lactic counts.

Preparation of powder of dried SSF lactic cultures

The best dried SSF lactic cultures grown on supplemented black gram dhal, based on less retained moisture and higher viable count were made into powder using sterile dry mixer. Powdered SSF lactic cultures were transferred to labelled self-sealing polythene pouches (Fig 2)

Statistical Analysis

The data was analyzed using R software [R. version 3.1.3 (2015-3-09), copyright © 2015, R foundation] for statistical computing both one way and two way Completely Randomed Design (CRD) which is the most appropriate for the study. Data on the respective variables were collected for three replication for each of these treatments. ANOVA tables were prepared to analyse the data and where the F value is significant the critical difference was calculated and used to identify where significant differences existed and was indicated in the table use superscripts.

Results and Discussion

Air drying of SSF lactic cultures

The SSF cultures were dried at different temperature like 20°C, 25°C and 30°C for 20 h in BOD incubator. After drying SSF cultures, the viable counts for dahi, yoghurt and acidophilus milk were 8.02, 8.12 and 8.94 log_{10} cfu/g, 7.93 log_{10} cfu/g, 8.05 log_{10} cfu/g and 8.71 log_{10} cfu/g and 7.46, 7.56 and 8.04 log_{10} cfu/g at 20°C, 25°C and 30°C respectively. Moisture which was initially 96% reduced to 81, 72 and 54 per cent at 20°C, 25°C and 30°C respectively.

Best air drying was done at 20°C for 20 h, as viable lactic count maintained was good compared to 25°C and 30°C because, as the temperature of drying increased, simultaneously reduction in viable count of dahi, yoghurt and acidophilus milk were noticed. The decrease of viable counts in SSF dahi, yoghurt and acidophilus milk cultures were statistically non-significant with respect to various temperatures of air drying except in case of drying of SSF culture of acidophilus at 30°C. Moisture reduction in SSF lactic cultures was highest as air temperature in incubator increased to 30°C, but higher moisture retained at 20°C accounting for 81% reduction in viable counts of lactic cultures may be due to increased drying temperature at 25°C as well as at 30°C. Apart from reduction in viable lactic count, aerial contamination, especially aerobic spores and yeast occurred. More the moisture in dried SSF lactic cultures, they may also grow producing lactic acid affecting the viability of cells over a period storage (Table 1).

In compatible to the present study, Prabha et al. (1999) dried the SSF Bifidobacterium longum on black gram dhal from 20°C to 40°C for 20 h using BOD incubator. It was found that at 20°C drying, the viable count retained was 9.31 log_{10} cfu/g and further, increase in drying temperatures reduced viable count minimally accounting for 0.2 log count reduction.

The yoghurt culture grown on supplemented paddy husk medium when air died at 20°C for 20 h reduced the viability by 1 log count (Ramachandra et al. 1999). As per the study conducted by Deepa (2011), dried dahi SSF culture grown on supplemented black gram dhal and the counts retained 9.75 log_{10} cfu/g at 20°C for 18 h.

### Table 1 Air drying of SSF lactic cultures on supplemented black gram dhal

<table>
<thead>
<tr>
<th>Temperature of drying (°C)</th>
<th>Before drying</th>
<th>After drying</th>
<th>Before drying</th>
<th>After drying</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Moisture content (% dry matter basis)</td>
<td>Dahi culture</td>
<td>Yoghurt culture</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td>20°C</td>
<td>81</td>
<td>8.02</td>
<td>8.12</td>
<td>8.94</td>
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<tr>
<td>25°C</td>
<td>96</td>
<td>8.56</td>
<td>8.89</td>
<td>9.45</td>
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<tr>
<td>30°C</td>
<td>54</td>
<td>7.46</td>
<td>7.56</td>
<td>8.04</td>
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<tr>
<td>CD (p = 0.05)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.57</td>
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The results were average of three trials (n = 3) with common superscript is non-significance while different superscripts indicate significant difference at (p ≤ 0.05)
As the air drying done in an opened condition, showed spore contamination. Hence, an alternate freeze dryer was carried out to dry SSF cultures on black gram dhal.

The initial moisture of SSF lactic cultures was reduced from 96% to 11% in case of dahi, yoghurt and acidophilus SSF culture when subjected for freeze drying during 8h of drying. The viable count which was initially 8.59, 9.08 and 9.50 log_{10} cfu/g reduced to 7.15, 7.23 and 7.47 log_{10} cfu/g in dahi, yoghurt and acidophilus cultures respectively. All the three lactic cultures behaved in the same manner with reduction of viability by nearly 2 log count. Statistically significant difference did not occur among viability of SSF lactic cultures in freeze dried cultures. As time taken for drying was more, and reduction in viability of lactic cultures were high. On the contrary, Vanisri (1995) reported that reduction in viable count of freeze drying of SSF yoghurt culture was very minimal that is 0.20 log count i.e., from 9.60 log_{10} cfu/g to 9.39 log_{10} cfu/g respectively (Table 2).

To overcome the problems of freeze drying an attempt was made to dry these cultures using a sterile Fluid bed drier at temperature of (25±1°C) for various hours. The initial moisture of 96% was...
reduced to 22. 9.40 and 8.10% at 1, 1.5 and 2 h of drying, respectively. The viable counts of SSF of dahi, yoghurt and acidophilus cultures were 8.33, 8.85 and 9.34 log$_{10}$ cfu/g after fluid bed drying at 1.5 h respectively. The drying in fluid bed drier for 1.5 h at 25°C was found to be optimum as the viable count reduction was very minimum accounting for 0.3 log reductions. Drying the SSF lactic cultures in fluid bed drier for 1 and 2 h affected the viability of cultures more accounting on an average of 0.9 and 1.3 log$_{10}$ cfu/g and also moisture retained at 1 h of drying was also found high. The viable count of dahi, yoghurt and acidophilus with respect to hours of drying did not show any significance but viable counts were found to be maximum at 1.5 h hence, used 1.5 h drying period in further studies.

Among the air drying, freeze drying and fluid bed drying, the best method seemed to be the fluid bed drier to dry SSF lactic cultures on supplemented black gram dhal with respect to higher viability and less moisture retention compared to other two methods (Table 3) Soponronnarit et al. (2001) dried the soya beans by using fluid bed drier at 140°C for 5 min, initially soya beans had moisture content of 14.90% reduced to 10.40%.

**Conclusions**

In order to preserve the viability of SSF lactic cultures, these cultures were dried by various drying methods such as air drying, freeze drying and fluid bed drying. The air dried lactic cultures showed higher reduction in viable lactic count, aerial contamination, especially aerobic spores and yeast occurred, thus the method of air drying of SSF lactic cultures became limited. In case of freeze drying viability of dahi, yoghurt and acidophilus was around 1.80 log count, reduction was more in acidophilus followed by yoghurt and dahi. Moisture retained was at 11% after freeze drying and took 8 h for freeze drying hence, not very economical and feasible. Fluid bed drying for 1.5 h at 25°C, increased the shelf life of SSF lactic cultures supplemented with black gram dhal by retaining maximum viable counts of 8.12 to 9.34 log10 cfu/g and a satisfactory moisture of 9.4%.

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**References**

Deepa BG (2011) Production of direct vat set dahi cultures. M. Tech thesis submitted to KVAFSU, Bidar, India