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Enumeration of Microflora from Ingredients and *Idli* Batter

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ABSTRACT

Background: Microorganisms are responsible for characteristic changes in the fermented foods. They result in the sequential changes during fermentation of food, resulting in souring, leavening and changes in flavour, body and texture. The present study was aimed to enumerate the natural microflora present in raw ingredients used as well in fresh and fermented idli batter.

Methods: Study was conducted during the period 2019-2020 to develop solid state fermented cultures for whey based *idli* batter at Department of Dairy Microbiology, Dairy Science College, Bangalore. The raw ingredients used for *idli* preparation were sourced from local market of Bangalore. Lactic counts such as leuconostoc, enterococci, pediococci and lactobacilli, as well as yeast counts were enumerated using different selective media by pour plate method.

Result: The leuconostoc count of rice rava, black gram dhal and fresh *idli* batter on sucrose agar ranged from 2.0 to 3.78 $\log_{10}$ cfu/g; Lactobacilli count on MRS agar varied from 2.0 to 4.11 $\log_{10}$ cfu/g. Yeast count on potato dextrose agar ranged from 1.6 to 2.3 $\log_{10}$ cfu/g. Lactic counts (includes leuconostoc, lactobacillus, pediococci, enterococci) in fresh *idli* batter ranged from 3.63 $\log_{10}$ cfu/g to 4.77 $\log_{10}$ cfu/g, whereas in fermented *idli* batter varied from 2.56 $\log_{10}$ cfu/g to 8.74 $\log_{10}$ cfu/g. The yeast counts in fermented batter ranged from 9.23 to 9.56 $\log_{10}$ cfu/g.

Key words: Enumeration, Fermentation, *Idli* batter, Lactic counts, Microflora, Viable counts.

INTRODUCTION

Fermented foods and beverages are defined as “Foods made through desired microbial growth and enzymatic conversions of food components”. Fermentation involves the microbial growth and enzymatic activity on food components, which is deliberate and controlled to generate the desirable attributes (Marco *et al*., 2021). A range of indigenous fermented foods are prepared from cereal grains. Most popular indigenous fermented foods from cereals in India are *idli, dosa, aapam, dhokla, kinema, ambali, gundruk, sinki, enduripitha, jelebi, puttu* and many more *Idli*, *dosa* and dhokla are rice-based fermented foods.

Among the fermented foods of India *idli* is a fermented, steamed product with a soft and spongy texture is a highly popular and widely consumed breakfast in India. Traditionally, the product is made from naturally fermented batter prepared from a mixture of polished rice (*Oryza sativa*) and dehulled black gram dhal (*Phaseolus mungo*), with varying proportions. Washed polished rice and black gram, dhal were soaked separately in potable water overnight and ground. Slurries of rice and black gram dhal were mixed to form a thick batter. The batter was kept overnight at room temperature for fermentation. Then, the fermented batter was poured into a concave shaped *idli* pan for steaming, finally yielding the savoury spongy cake, *idli* (Das *et al*., 2012).

Predominant microorganisms responsible for souring and leavening of *idli* batter were found to be *Leuconostoc mesenteroides*. In the later stages of fermentation, growth of *Streptococcus faecalis* (presently known as *Enterococcus faecalis*) and later, *Pediococcus cerevisiae* becomes significant. The fermentation of *idli* demonstrates a leavening action caused by the activity of the heterofermentative lactic acid bacterium, *Leu. mesenteroides* (Mukherjee *et al*., 1965).

Soni and Sandhu (1989) enumerated lactic acid bacteria in the fermented batter and found them ranging from 10^6-10^9/g that included *Leuconostoc mesenteroides, Enterococcus faecalis, Lactobacillus fermentum* and *Pediococcus cerevisiae* essential for leavening of batter and acid production in *idli*. It was found that the prevalence (68% of samples) of yeasts such as *Saccharomyces cerevisiae, Geotrichum candidum, Debaryomyces hansenii*, *Rhodotorula graminis* and *Hansenula anomala* were responsible for pH reduction, might increase thiamine and riboflavin content.

The main objective of the current study was to enumerate the microflora from raw ingredients, paneer whey, idli batter before and after fermentation and to isolate them and use as solid-state fermented cultures for batter fermentation.

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MATERIALS AND METHODS

The raw ingredients, rice rava, black gram dhal, and common salt, were purchased from local market of Bangalore. The same brand rice rava and black gram dhal were used throughout the study. The filtered water and *paneer* whey were used for soaking of ingredients. Paneer whey was collected from Students' Experimental Dairy Plant, Dairy Science College, Bangalore.

Preparation of *idli* batter

Ingredients, rice (*Oryza sativa*) rava and black gram dhal (urd dhal or black lentils), (*Phaseolus mungo*) were taken at 2:1 ratio (w/w) basis, cleaned and washed thoroughly 3-4 times with filtered water. Rice rava was soaked in one time of its dry weight filtered water for 1 h and black gram dhal in 1.5 times its dry weight filtered water for 4 h. After completion of soaking black gram dhal was ground in electrical mixer to coarse, pasty consistency. It was mixed with soaked rice rava and stirred well. The water used for soaking was not discarded and completely incorporated during grinding. Common salt was added to the fresh batter at 0.80% w/w basis of total ingredients. Mixed well and kept for fermentation at ambient temperature (27°C) for 12 h or based visual observation of rise in batter volume.

Preparation of paneer whey based *idli* batter

Paneer whey was incorporated during soaking stage of ingredients by replacing water with whey at 70% whey and 30% water. Soaked black gram dhal was ground and mixed well with soaked rice rava.The mixed fresh batter was subjected to fermentation at ambient temperature of 27°C for 12 h. The microflora of fresh as well as fermented batter was enumerated.

Enumeration of microbial load

Ingredients were washed thoroughly using potable water. Individually washed ingredients, rice rava and black gram dhal, *paneer* whey, fresh batter and fermented batter were serially diluted using phosphate buffer and plated for lactic counts such as leuconostoc, enterococci, pediococci and lactobacilli using ready to use sucrose agar, bile esculin agar, acetate agar and Lactobacillus MRS agar (HiMedia) respectively. Plates were incubated at 30°C/48 h for leuconostoc, enterococci, pediococci and at 37°C/48 h for lactobacilliin a anaerobic jar. Yeastswere enumerated using potato dextrose agar (PDA) and incubated at 25°C /3 days (Harrigan, 1998). The results obtained in the present study, are the average of three trials. The data was analyzed using Least Significant Difference (LSD) test of R Studio software [R.version 4.03. (2020-12-18), R Studio version 1.3.1093].

RESULTS AND DISCUSSION

The microflora of rice rava, black gram, paneer whey, fresh idli batter and fermented idli batter was enumerated by pour plate method using different selective media for lactic acid bacteria (LAB) and yeast.

It was found that leuconostoc count on sucrose agar ranged from 2.0 to 3.78 $\log_{10}$ cfu/g, enterococci were not detected in bile esculin agar while lactobacilli count varied from 2.0 to 4.11 $\log_{10}$ cfu/g on MRS agar and yeasts on PDA ranged from 1.6 to 2.3 $\log_{10}$ cfu/g. Fresh *idli* batter showed highest leuconostoc count of 3.78 $\log_{10}$ cfu/g, black gram dhal showed lowest leuconostocs count of 2.0 $\log_{10}$ cfu/g. Similarly, lactobacilli count was found to be highest (4.11 $\log_{10}$ cfu/g) in fresh *idli* batter and lowest (2.0 $\log_{10}$ cfu/g) in black gram dhal. Yeast count was lowest in fresh *idli* batter accounting for 1.60 $\log_{10}$ cfu/g and highest in both rice rava and black gram dhal of 2.30 $\log_{10}$ cfu/g (Table 1). There was a significant difference in leuconostoc and lactobacilli counts in different ingredients but no significant difference was observed in yeast count. No significance difference occurred in the microflora of rice rava, but black gram dhal and fresh batter showed the significant difference (P =.05).

As the pH of all these media varied from 6.4 to 6.6, the spreader colonies (aerobic bacterial spore formers) were noticed in plates, affected the colony counts. Hence, the pH of media for bacterial count was reduced to 5.5 by addition of 10% sterile lactic acid, which might be selective for fermentative microflora. Further the viable counts of ingredients *viz.* washed rice rava, black gram and fresh *paneer* whey were enumerated with low pH media. No colonies were noticed in case of rice rava and black gram, which might be attributed to removal of microflora by washing as well by inhibition of aerobic spore forming bacteria by lowering pH of media to 5.5. Fresh *paneer* whey showed viable counts ranging from 1.0 $\log_{10}$ cfu/ml to 2.42 $\log_{10}$ cfu/ml MRS agar (Table 2). There was no significant difference in the microbial counts of rice rava, black gram dhal (P=.05), but in comparison with *paneer* whey the difference was significant in the viable counts of leuconostoc, lactobacilli and pediococci (P=.05).

On the contrary, Baruzzi *et al*. (2002) found viable counts of both bacilli and cocci obtained using MRS and M17 Agar as 5.39 and 6.08$\log_{10}$ cfu/ml, respectively in 24 h fermented whey having pH 3.80 during Scamorza Altamurana cheese, a naturally fermented Italian cheese processing and ripening.

According to Steinkraus *et al*. (1967) initial bacterial counts in rice-black gram mixture for *idli* ranged from 10^3- 10^5 (3-5 log) cfu/g. Soni *et al*. (1986) studied the microbiology of *dosa* fermentation, in which they found that total bacterial count on nutrient agar in raw rice ranged from 10^5 to 10^6/ g while black gram dhal showed 10^6 cfu/g, whereas yeast count on malt extract agar for rice and black gram dhal was 10^3 cfu/g. Similar trend was noticed in the present study with respect to bacterial and yeast counts.

Leuconostoc count on sucrose agar in control batter (100% water) was 3.63 $\log_{10}$ cfu/g and 4.58 $\log_{10}$ cfu/g in whey-based batter with 70:30 whey: water combination. There was significant difference noticed in these counts (P=.05). No viable counts observed on bile esculin agar, MRS agar as well as acetate agar for control batter, but in case of

whey-based batter lactobacilli count on MRS agar was 4.47 log_{10}cfu/g and pediococci count on acetate agar was 4.77 log_{10} cfu/g (Table 3). Significant difference was noticed in the counts of lactobacilli and pediococci of both the types of batters (*P*=.05).

Further, the viable counts were enumerated post-fermentation, in control as well as whey-based batter. The leuconostoc count on sucrose agar in control (100% water) was 8.38 log_{10}cfu/g and 8.63 log_{10}cfu/g in whey-based batter. No significant difference was found in leuconostoc count of both control and whey-based batter (P=.05). Counts of enterococci on bile esculin agar for control were 1.02 log_{10} cfu/g and 2.56 log_{10}cfu/g in whey-based batter. Counts of lactobacilli on MRS agar was 5.42 log_{10}cfu/g in control batter

Table 1: Enumeration of microflora of raw ingredients used in *idli* batter preparation.

Raw ingredients	Type of media used for microflora			
	Sucrose agar (Leuconostoc)	Bile esculin agar (Enterococci)	Lactobacillus MRS agar (Lactobacilli)	Potato dextrose agar (Yeasts)
	Viable Count (log_{10} cfu/g)			
Rice rava	3.30[a]	0	3.48[a]	2.30[a]
Black gram dhal	2.00[b]	0	2.00[b]	2.30[a]
Fresh *idli* batter	3.78[c]	0	4.11[c]	1.60[b]
CD (*P*=.05)	0.17	-	0.20	0.05

- All values were average of three trials.
- CD - Critical difference.
- Plates poured with sucrose agar, bile esculin agar and potato dextrose agar were incubated at 30°C while Lactobacillus MRS agar at 37°C for 48 h.
- Same superscripts in the column indicate non-significance while different superscripts indicate significant difference.

Table 2: Enumeration of microflora of raw ingredients used in *idli* batter preparation with modification in pH of media.

Raw ingredients	Type of the media used for microflora				
	Sucrose agar (Lactobacilli)	Bile esculin agar (Leuconostoc)	Lactobacillus MRS Agar (Enterococci)	Acetate agar count (Pediococci)	Potato dextrose agar (Yeast)
	Viable count (log_{10} cfu/g or ml)				
Rice rava	0.00[b]	0	0.00[a]	0.00[b]	0
Black gram dhal	0.00[b]	0	0.00[a]	0.00[b]	0
Paneer whey	1.20[a]	0	2.42[a]	1.00[a]	0
CD (*P*=.05)	0.31	-	0.16	0.31	-

- All values were average of three trials.
- CD -Critical difference.
- pH of the media was adjusted to 5.5 before pouring with sterile 10 % lactic acid.
- Plates poured with sucrose agar, bile esculin agar, acetate agar and potato dextrose agar were incubated anaerobically in candle jar at 30°C while MRS agar at 37°C for 48 h.
- Same superscripts in the column indicate non-significance while different superscripts indicate significance difference.

Table 3: Enumeration of microflora of optimized *idli* batter before fermentation

Idli batter	Type of media used for microflora			
	Sucrose agar (Leuconostoc)	Bile esculin agar (Enterococci)	Lactobacillus MRS agar (Lactobacilli)	Acetate agar count (Pediococci)
	Viable count (log_{10} cfu/g)			
Batter with 100% water (Control)	3.63[a]	0	0.00[a]	0.00[a]
Batter with 70% *paneer* whey	4.58[b]	0	4.47[b]	4.77[b]
CD (*P*=.05)	0.45	-	0.31	0.38

- All values were average of three trials.
- CD - Critical difference.
- pH of the media was adjusted to 5.5 before pouring.
- Plates poured with sucrose agar, bile esculin agar and acetate agar were incubated anaerobically in candle jar at 30°C while MRS agar at 37°C for 48 h.
- Same superscripts in the column indicate non-significance while different superscripts indicate significant difference.

and 8.74 log_{10} cfu/g in whey-based batter. Count of pediococci on acetate agar was 4.70 log_{10}cfu/g in control batter and 8.63 log_{10}cfu/g in whey-based batter (Table 4). Significant difference was noticed in enterococci, lactobacilli and pediococci counts of both the types of batters (*P*=.05). Distribution of leuconostoc ranged from 30 to 43%, pediococci from 24 to 30%, lactobacilli from 28 to 31% and enterococci from 5 to 9% in control and whey-based batter (Fig 1).

Table 4: Enumeration of microflora of optimized *idli* batter after fermentation.

Idli batter	Type of media used for microflora			
	Sucrose agar (Leuconostoc)	Bile esculin agar (Enterococci)	Lactobacillus MRS agar (Lactobacilli)	Acetate agar count (Pediococci)
	Viable count (log_{10} cfu/g)			
Batter with 100% water (Control)	8.38[a]	1.02[a]	5.42[a]	4.70[a]
Batter with 70% *paneer* whey	8.63[a]	2.56[b]	8.74[b]	8.63[b]
CD (*P*=.05)	0.31	0.21	0.44	0.32

- All values were average of three trials.
- CD - Critical difference.
- *Idli* batter fermented at 30°C for 12 h.
- pH of the media was adjusted to 5.5 before pouring.
- Plates poured with sucrose agar, bile Esculin agar and acetate agar were incubated anaerobically in candle jar at 30°C while MRS agar at 37°C for 48 h.
- Same superscripts in the column indicate non-significance while different superscripts indicate significant difference.

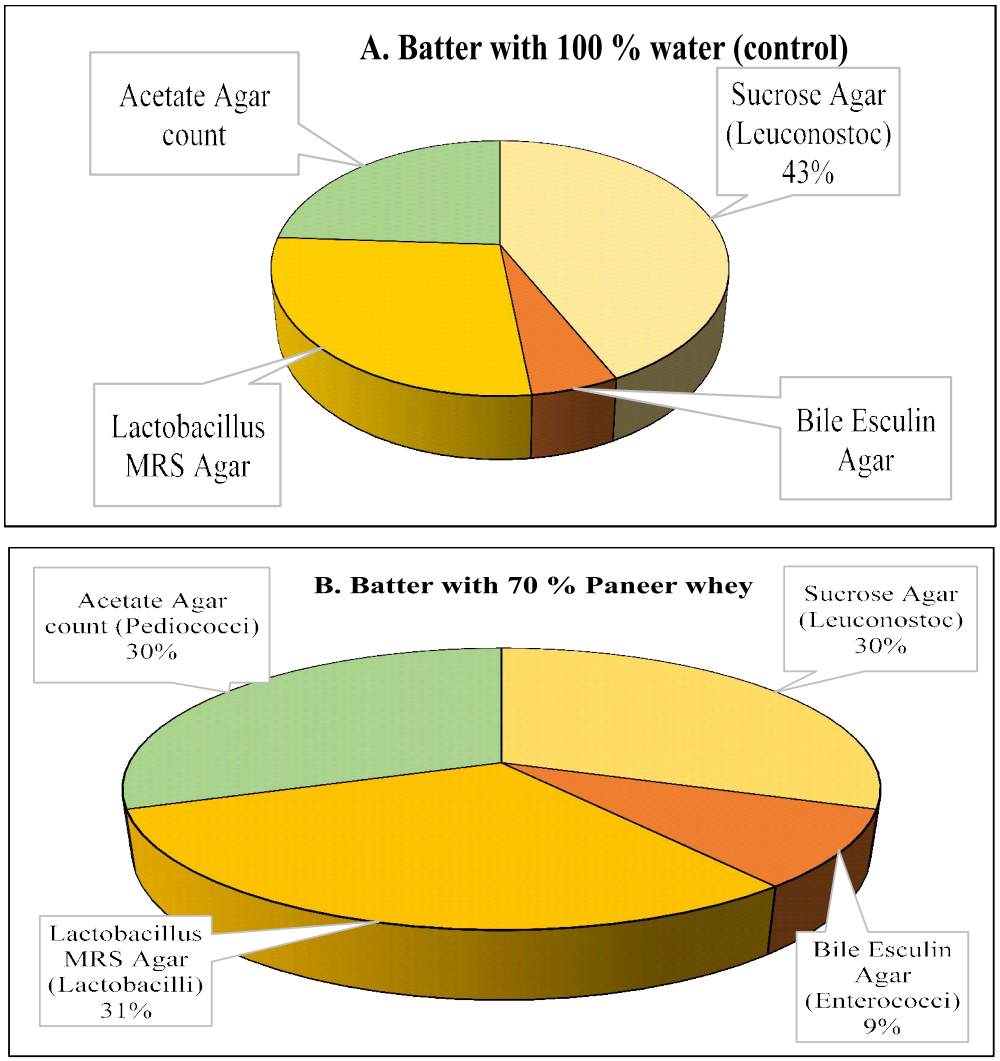

Fig 1: Microflora of optimised *idli* batter after fermentation.

Sridevi *et al*. (2010) studied on addition of selected lactic cultures and yeast to fresh idli batters and evaluated their effect on idli quality. The viable counts were enumerated for the batters stored at 10°C and 30°C for 6 days. The lactic counts ranged from 8.6 to 10.01 $\log_{10}$ cfu/g and yeast count from 8.7 to 10.1 $\log_{10}$ cfu/g.

On contrary Saravanan *et al*. (2015) studied diversity of microflora isolated from traditionally fermented idli batter, where the initial counts of lactic acid bacteria and aerobic microflora were in the range of 7.5 - 8.2 log cfu/g and later the counts increased to 8.6-8.8 log cfu/g after 3 h of fermentation and 8.3 to 9.2 log cfu/g after 9 h of fermentation. But microbial load decreased slowly after 12 to 15 h of fermentation.

Panicker *et al*. (2017), reduced fermentation time from 8-10 h to 2 h by addition of isolated bacteria *Leuconostoc* spp., *Lactococcus* spp. and *Lactobacillus* spp., at different inoculation rates to 50 ml fresh ground batters and incubated at room temperature for 2h. Total viable counts of these cultures in fermented *idli* batter ranged from 3.45 $\log_{10}$cfu/g to 6.88 $\log_{10}$cfu/g on MRS agar.

CONCLUSION

Microbial load of ingredients used for idli preparation plays an important role in batter fermentation. The leuconostoc count in rice rava, black gram dhal and fresh *idli* batter on sucrose agar ranged from 2.0to 3.78 $\log_{10}$ cfu/g, lactobacilli count on MRS agar varied from 2.0to 4.11 $\log_{10}$ cfu/g. Yeast count on potato dextrose agar ranged from 1.6 to 2.3 $\log_{10}$ cfu/g. Lactic counts in control *idli* batter increased from 3.63 $\log_{10}$ cfu/g before fermentation to 8.38 $\log_{10}$ cfu/g after fermentation, whereas the *idli* batter with incorporated paneer whey the lactic counts increased from 4.47 $\log_{10}$ cfu/g before fermentation to 8.74 $\log_{10}$ cfu/g after fermentation at 30°C for 12h.

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