Selection of starter cultures for *idli* batter fermentation and their effect on the quality of the *idlis*

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Abstract:

The effect of starter cultures on the quality of *idli* batter fermentation was studied. *Idli* batter samples were prepared using the lactic starter cultures like *Pediococcus pentasaceous* (Pp), *Enterococcus faecium* MTCC 5153 (Ef), *Ent. faecium* IB2 (Ef-IB2), individually, along with a yeast culture, *Candida versatilis* (Cv). The *idli* batter prepared using the Ef and Ef-IB2 cultures gave better results, when evaluated for the raise in batter volume (80 ml), level of CO_2 production (23.8%), titratable acidity (2.36-3.46 % lactic acid) and pH (4.3-4.4). Storage stability of the batter made with selected starter cultures was determined by analyzing the *idlis* prepared using the batter stored for one and five days for texture, nutrient composition and sensory quality. Slight variations in the results were seen among the *idlis* of different combination of cultures, whereas these results are better than that of the idlis made using natural fermented idli batter. Sensory profile of the idlis prepared using starter cultures had high score (3.9-4.4) when compared to the control (3.6) for overall acceptability.

Keywords: *Idli*, Microbial profile, Nutrition, Starter culture, Storage stability, Texture

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Introduction

The production of fermented foods is one of the oldest food processing technologies known to man. The diversity of the population of India has given rise to a large number of traditional fermented foods which have been extensively reviewed (Soni and Sandhu 1990, Achaya 1994). Cereals and legume based foods are a major source of economical dietary energy and nutrients, worldwide. The use of desirable microorganisms, particularly those of lactic acid bacteria (LAB), yeasts and fungi have been well documented (Steinkraus 1995). Among several of the Indian traditional foods, *idli*, a fermented steamed product with a soft and spongy texture is highly popular and very widely consumed throughout India and is also becoming popular in other countries. From the nutritional and health status point, *idli* appears to be an ideal human food for all ages and at all times. *Idli* is a white, fermented (acid leavened), soft, spongy texture product and steamed cake of rice (*Oryza sativum*) and dehulled black gram dhal (*Phaseolus mungo*), widely popular and consumed is the entire South India. *Idli* fermentation has been the subject of many research investigations, covering aspects such as methods of preparation, microbiology and nutritive value (Desikachar et al 1960, Mukerjee et al 1965, Steinkraus et al 1967, Ramakrishnan 1979, Venkatasubbaiah et al 1984, Thyagaraja et al 1992, Agarwal et al 2000, Rati et al 2003, Rati et al 2006). Two significant changes occurring in *idli* fermentation are leavening and acidification of the batter (Jama and Varadaraj 1999).

A starter culture can be defined as a microbial preparation of large number of cells of at least one microorganism to be added to a raw material to ferment food by accelerating and steering the fermentation process. They cause rapid acidification of the raw material through the production of organic acid, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product (Leroy and De Vuyst 2004). The bacteria identified as a part of the microflora for *idli* batter fermentation included *Leuconostoc mesenteroides, Lactobacillus delbruckii, Lb. fermentum, Lb. lactis, Lb. brevis, Streptococus faecalis* and *Pediococus cerivisiae,* which are essential for leavening of the batter and for acid production in *idli* and

yeasts such as *Geotrichum candidum, Torulopsis lolmii, T. candida, Trichosporon pullulans, Candida, C. fragilola, C. kefyr, C. tropicalis, Hansenula anomala* and *Rhodotorula graminis*, which are responsible for pH reduction and may increase the thiamin and riboflavin content (Agrawal et al 2000, Charan and Kadam 1989, Mukherjee et al 1965, Purushothamen et al 1993, Reddy et al 1981, Soni and Sandhu 1990, Steinkraus 1995, Thyagaraja et al 1992, Venkatasubbaiah et al 1984). In a direction towards reducing the fermentation time of *idli* batter and increasing the shelf life of fermented batter, an improved process for the preparation of shelf-stable *idli* batter was made available (Varadaraj et al 1999). The purpose and scope of the present study was to develop a suitable combination of starter cultures for *idli* batter fermentation and to evaluate the quality of the batter and *idli*s for nutritional, textural and sensory quality.

Materials and methods

Media: For cultivation and maintenance of the lactic acid bacteria, *Lactobacillus* deMan Rogosa sharpe agar (MRS agar) and for yeasts Sabroud Dextrose (SD) Agar (Hi-media labs, India) were used. The lactic cultures were incubated at 37°C for 16 h for the cells to reach late exponential phase in static condition and yeast culture was grown at 30° C for 48 h.

Preparation of idli *batter*: *Idli* batter was prepared from the mixture of milled rice (*Oryza sativa*) and dehulled black gram dhal (*Phaseolus mungo*) in 4:1 ratio. The ingredients (rice and dhal) were processed using good manufacturing process (Agrawal et al, 2000). Soaking was done in potable water for 6-8 h. These ingredients were ground to a fine paste under hygienic conditions (Varadaraj et al 1999) and pH of the fresh *idli* batter was checked. Four different starter cultures were used to prepare different *idli* batter samples using the following combinations: i) *Pedicoccus pentasaceus* + *Candida versatilis*, ii) *Enterococcus faecium* MTCC 5153 + *Candida versatilis* and iii) *Enterococcus faecium* IB2 + *Candida versatilis* (these combinations of starter cultures were added to the *idli* batter sample at 1% inoculum (0.5% LAB and 0.5% yeast)). *Idli* batter prepared using the traditional method (without steaming the ingredients and without boiling the water used for soaking and grinding) without any externally added culture (natural fermentation) was used as a control. For the analysis of texture, nutrient composition and sensory

profile of *idli* samples, the batter samples prepared individually using 3 different combinations of cultures and naturally fermented batter were packed in PET-LDPE laminated pouches and stored at 30 °C.

Analysis of idli *batter*

Raise in Batter volume: The *idli* batter prepared using 3 different culture combinations and the batter without any added culture was poured into 500 ml measuring cylinders, individually, up to 100 ml mark, covered with aluminum foil and were kept at 30 ºC for 24 h and observed for raise in batter volume. The raise in CO_2 production can be correlated to the increase in the batter volume. The four *idli* batter samples were freshly prepared and packed in three different packaging materials. The amount of CO_2 produced was analyzed and compared.

Analysis of the batter: The pH of the *idli* batter samples was checked, individually using a microprocessor based digital pH meter (Henna Instruments, Singapore). The CO_2 produced during fermentation of *idli* batter packed in various types of packing material (PET laminated LDPE pouches, air tight HDPE tins and aluminum foil laminated LDPE pouches) was analyzed using PBI Dansen analyzer. The microbial load of the batter stored at 30 ºC and 10 °C was checked for 0-7 days. The batter samples were serially diluted with 0.8% saline individually and appropriate dilutions were pour plated individually using MRS agar (for lactics) and spread plated on SD agar (for yeasts) plates. The plates were incubated at 37 °C for 24 h to 48 h. The colonies were counted using colony counter. The microbial load was estimated until the product became deteriorated (with visual observation of yeast and molds growth).

Evaluation of idli*s prepared using selected starter culture*: The *idlis* made from the *idli* better samples prepared using selected combinations of starter cultures and stored for 1 and 5 days were evaluated for textural, sensory and nutritional parameters. The hardness of the *idlis* was tested using Instron texture analyzer (Lloyds Instron texturomenter LRSK, UK) through the sheer force measurement. The shear force values in Neuton (N) recorded under the operating conditions of 100 kg load all, 100 mm/min plunger speed and shear plunger. The sensory evaluation of *idlis* prepared by the selected combinations of starter cultures was carried out

organoleptically. The *idlis* were evaluated by 50 untrained judges in respect of color, flavor, appearance, taste and overall acceptability, using 5 point Hedonic scale rating (Watts et al 1989). The average mean of the scores was calculated. Nutrient analysis of the *idlis* prepared was carried out to compare the nutrients present in it. For estimation of moisture content a method described by Bemal et al (2004) was used. *Idlis* (10 g) prepared using different batter were dried to a constant weight in a hot air oven at 100°C and the moisture content was estimated using the formula:

$$\text{moisture (\%)} = \frac{W1-W2}{W} \times 100$$

Where, W was weight of sample, W1 was weight of sample + moisture dish and W2 was weight of dried sample + moisture dish. To determine the ash content (Bemal et al 2004), 5g of batter samples were placed in pre-dried crucibles, individually and kept in a muffle furnace at 550 °C until they were ashed and it gets the clean and white in appearance. After cooling, the ash content was calculated using the formula as indicated above, where, W was weight of the sample (g), W1 was weight of the empty of crucible (g) and W2 was weight of the crucible with ash (g). Thiamine content was estimated after extracting it from the batter in acidic condition. It was oxidized to thiachrome by alkaline potassium ferrycyanide and the intense blue colour fluorescence exhibited by thiochrome was measured fluorometrically as described by Bemal et al (2004). The carbohydrate content was determined by calorimetric method using as described by Ranganna (1977). The energy or calorific value of *idlis* was determined by oxidizing a known weight of *idlis* in Bomb calorimeter and the heat produced was measured and expressed in terms of kilo calories (Kcal). Nitrogen content was measures using microkjeldahl method (Bemal et al 2004). Fat soluble material of *idlis* was extracted with ether from an oven dried sample using soxhlet extraction method (Bemal et al 2004). The iron content of *idlis* was determined by converting the iron to ferric form using oxidizing agent (hydrogen peroxide) and treated with potassium thiocyanate to form the red ferric thiocyanate, which was measured colorimetrically at 480 nm.

Statistics: The data was subjected to statistical analysis by Duncan's multiple range test (DMRT) (Duncan 1955).

Results & Discussion

In this paper we report preparation of *idli* batter using the selected lactic cultures along with yeast culture. Both the *Enterococcus* cultures used in this study are found to produce anti-listerial bacteriocin (Sangeetha et al 2007). The *idli* batter samples prepared using three different starter combinations, along with batter sample prepared by natural fermentation were analyzed for 5 days from the day of preparation. The raise in batter volume, titratable acidity and pH for all the samples were analyzed. The results are given in Table-1. From the results, it can be seen that the pH of the samples range between 4.50 and 4.30. The combination of Ef and yeast showed high acidity compared to other combinations of starter cultures. Titratable acidity of the samples differed from 3.46 to 2.30%. At the end of fifth day of observation, the combination of starter cultures namely Ef and yeast and also Ef-IB2 and yeast gave better raise in volume up to 80% suggesting that it was producing acid (due to lactics) and gas production (mainly by yeast) and with a titratable acidity of 2.30%. Further the samples were checked for the rate of CO_2 production. The increase in volume is due to gas production by the yeast during fermentation, which is a measure of their metabolic activity.

Quantitative analysis of CO_2 production: The amount of CO_2 produced by different combination of cultures is indicated in Table-2. From the results, it can be predicted that the rate of CO_2 production was found to be higher in the samples packed with aluminium foil –LDPE laminate pouches compared to other two types of packing materials. It might be due to the non-permeability of aluminium foil to gas. The highest amount of CO_2 production in natural fermentation was found to be 45.6% in laminated LDPE pouches. Among all the three combinations of the cultures used, the combination of Ef and yeast resulted in higher CO_2 production in all the types of packing material and the highest of 32.2% was noticed in the pouches prepared with laminated LDPE. It might be concluded that the combination of starter cultures of Ef-IB2+Cv and Ef+Cv were able to produce the high rate of CO_2 due to the metabolic activity of yeast and its compatibility with added lactic culture. The rate of CO_2 production due

to microbial fermentation can directly be correlated to the raise in batter volume. This is due to the presence of large number of yeast present in batter that produces CO_2.

Texture analysis: The fresh *idli* samples prepared individually using 3 different combinations of cultures and naturally fermented batter were analyzed for their texture using Instron Texture analyzer. It was done to examine the softness of the *idlis*. The results were expressed in Newton units. Analysis was performed on the *idlis* prepared using one day and five day old batter (Table -3). The results revealed that the hardness of the *idlis* increased with increase in storage period and it was high on 5 day old *idli* batter sample when compared to one day old *idli* batter. The idlis prepared using naturally fermented batter required high unit of Newton to break. The combinations of Ef-IB2 & yeast and Ef & yeast required less unit of Newton. Hence, the *idlis* prepared from these combinations were found to be quite soft compared to *idlis* prepared from the combination of Pp+ yeast culture. The combination of Ef and yeast was found to be the best in terms of softness of the *idlis*. The hardness of *idlis* was increased due to the increase in acidity. This reveals that the texture of the *idli* was affected by the change in acidity. The *idlis* were further analyzed for the nutrient content present in the one day and 5 day old *idli* batter samples.

Nutrient analysis of idlis: Proteins, essential amino acids/ peptides, essential fatty acids, vitamins and minerals provide nutritional requirements to satisfy the metabolic needs of the consumer (Steinkraus 1997). The nutrient analysis of *idli* samples prepared by different combinations was performed in order to found the nutrient content present in the *idlis* fermented using different starter cultures. The moisture content, proximate values, iron and thiamine were analyzed. The observations of the above are presented in Table-4. The observations of the first and the fifth day samples showed variations in the nutrient content. The nutrient content was found to be rich in first day sample compared to fifth day sample. In the first day sample, Pp+Cv combination gave better result for protein and fat, the carbohydrate content higher in Ef-IB2 +Cv. The natural fermented idlis had high contents of carbohydrate, protein and iron. In the 5 day old sample, the more carbohydrate content in *idlis* prepared using Pp+Cv, high fat content in Ef-IB2+Cv and high energy in the *idlis* prepared using EF+Cv combination were noticed. The

thiamine was not detected in any combination of *idli*. It may be due to the polished rice used for the batter preparation. From the results, much difference was not seen among the *idlis* prepared with these combinations of cultures and the *idlis* prepared using the batter fermented naturally. One day and 5 day old *idli* batter samples were further analyzed for the organoleptic properties. Fermentation of *idli* batter has a significant effect on the increase of vitamins B, C and essential amino acids and in the reduction of anti nutrients (Phytate-50% hydrolyzed), enzyme inhibitors and flatus sugars (Steinkraus 1983). Several researchers reported that methionine increases from 10.6 to 60.0% during fermentation (Steinkraus et al 1967, Balasubramanian and Vishmanathan 2004). The presence of yeast seems to favour formation of folates (Kariluoto et al 2004) and thiamine (Ternes and Freund 1998). Formation of acidity can both increase levels of bioactive compounds (such as total amount of phenolic compounds) or decrease levels of some other compounds, such as thiamine, ferulic acid, dehydrodimers, tocopherols and tocotrienols (Liukkonen et al 2003, Boskov Hansen et al 2002, Ternes and Freund 1998).

Microbial profile: The microbial load of the *idli* batter prepared using selected combinations of starter cultures was determined starting from day one to day 7 of storage at 30 °C and 10 °C and the results are provided in Table-5. Increase in the viable count was observed till day 4 and day 6 in the samples maintained at 30 °C and at 10 °C conditions, respectively. After that, the samples kept at 30 °C were observed to be spoiled, whereas the samples stored at 10 °C the decrease in the viable count was observed in all the combination of starter cultures. In the study of day one samples, the microbial load indicated high yeast count related to high CO_2 production, less lactic count, which gave soft texture to *idlis* as the acidity was less. In the day 5 sample, low yeast count was observed compared to lactic count, produced high acidity and gave the hardness to the final product. This confirms the necessity of the cold storage, to extend the shelf life of the batter beyond one day. Decrease in viable count beyond 4 days of storage might be due to increased acidic conditions due to the fermentation of the ingredients by the lactic cultures.

Sensory profile: The food product prepared should be acceptable by consumers. It should meet the demands of the consumers. The sensory evaluation of the *idlis* made using the batter

fermented by different combinations of starter culture and stored for one and five days was carried out by 50 untrained panel members. The *idlis* prepared by using natural fermented batter were used as control. The results are presented in Table-6. The results indicated that the overall acceptability was decreased with increase in storage period of the *idli* batter. In the first day, the Pp+Cv combination of *idli* had high score for appearance, flavour and taste. However, the overall acceptability and the colour scores were good for Ef-IB2+Cv combination. On the fifth day, the appearance, colour, flavour scores were good for Pp+Cv. However, overall acceptability was good for the *idlis* prepared using Ef+Cv cultures. Over all from the sensory evaluation, the *idlis* made out of the Ef+Cv combination showed good result than compared to the *idlis* prepared using the combination of Ef-IB2 +Cv after 5 days storage.

Conclusions

Although slight variations in the results were seen among the *idlis* of different combination of cultures, these results are better than that of the *idlis* made using naturally fermented *idli* batter. At the same time, the starter cultures are found to produce anti-listerial bacteriocins. This will help to extend the storage period of the batter, besides giving probiotic benefits to the consumers.

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Table 1. Effect of starter culture in increasing the batter volume, titratable acidity and pH during *idli* batter fermentation.

Storage	Combination of starter cultures*											
Period	PP +Cv			Ef-IB2+Cv			Ef +Cv			NF		
(day)	RBV	TA	pH	RBV	TA	pH	RBV	TA	pH	RBV	TA	pH
0	100	ND	6.73	100	ND	6.73	100	ND	6.73	100	ND	6.73
1	105±2.3^{a}	0.67±0.1^{a}	4.73±0.2^{a}	115±2.1^{b}	0.90±0.1^{a}	4.78±0.1^{a}	115± 1.8^{b}	1.17±0.1^{a}	4.73±0.1^{a}	30±3.5^{c}	0.96± 0.1^{a}	4.78±0.2^{a}
2	105 ±3.1^{a}	1.73±0.1^{b}	4.62±0.3^{a}	135± 3.2^{b}	1.44±0.1^{b}	4.77±0.2^{a}	135±4.1^{b}	1.66±0.1^{a}	4.70±0.1^{a}	140± 3.1^{c}	1.64±0.1^{b}	4.70±0.1^{a}
3	105±4.8^{a}	2.11± 0.2^{c}	4.58±0.3^{a}	155± 3.5^{d}	2.04±0.2^{c}	4.59±0.1^{a}	155±5.5^{c}	2.31±0.2^{a}	4.51±0.2^{a}	140±2.5^{b}	2.16± 0.2^{c}	4.59±0.3^{a}
4	105±3.7^{a}	2.67±0.1^{d}	4.56±0.2^{a}	180±4.0^{c}	2.67±0.3^{e}	4.53±0.3^{a}	180±6.1^{c}	2.94±0.3^{a}	4.42±0.3^{a}	140±3.2^{b}	2.90±0.1^{d}	4.53±0.2^{a}
5	105±2.5^{a}	3.30±.0.2^{e}	4.42±0.4^{a}	180±3.8^{c}	2.36±0.1^{d}	4.44±0.2^{a}	180±5.8^{c}	3.46±0.1^{a}	4.34±0.2^{a}	140±2.8^{b}	3.46±0.2^{e}	4.46±0.1^{a}

* PP – *Pediococcus pentosaceous*; Ef- *Enterocuccus faecium* MTCC 5153; Ef-IB2 –*Ent. faecium* IB_2; Cv- *Candida versatilis;*

NF- Natural fermentation; RBV- Raise in batter volume; TA- Titratable acidity (% lactic acid); ND- Not determined;

Results are average of 3 independent experiments done in duplicate with Standard deviation. Mean scores, in a row within the treatment, without a common letters are significantly different ($P < 0.05$) by DMRT.

Table 2. Rate of carbon dioxide production by different starter cultures during *idli* batter fermentation

Fermentation period (days) / Type of packaging material	Amount of CO_2 produced (%) / Combinations of starter culture*			
	Pp+Cv	Ef-IB2+Cv	Ef + Cv	NF
PET laminated LDPE pouches				
1	14.6±1.5^{b}	15.3±1.4^{b}	16.2±1.5^{b}	12.0±1.4^{a}
2	16.2±1.6^{b}	16.2±1.3^{b}	17.3±1.4^{b}	12.0±1.5^{a}
3	17.1± 1.5^{a}	17.7±1.4^{a}	18.7±1.5^{a}	21.3±1.4^{b}
4	19.3±1.6^{a}	19.9±1.5^{a}	20.2±1.5^{a}	21.3±1.6^{a}
5	20.3± 1.5^{a}	22.2±1.4^{b}	23.8±1.6^{b}	23.0±1.5^{b}
Aluminum Foil laminated LDPE pouches				
1	16.4±1.1^{a}	21.6±1.1^{b}	21.9±1.2^{b}	23.0±1.4^{b}
2	22.7±1.2^{a}	26.4±1.2^{b}	26.9±1.4^{b}	27.3±1.3^{b}
3	25.3±1.2^{a}	30.0±1.4^{b}	29.0±1.5^{b}	35.0±1.4^{c}
4	27.8±1.5^{a}	30.9±1.3^{b}	31.1±1.5^{b}	41.4±1.4^{c}
5	29.3±1.4^{a}	30.2±1.2^{a}	32.2±1.4^{b}	45.6±2.1^{c}
HDPE Plastic ware with air tight lid				
1	12.2±1.1^{b}	9.3±1.0^{a}	9.6±1.1^{a}	12.3±1.1^{b}
2	13.7±1.0^{b}	11.7±1.2^{a}	12.2±1.1^{a}	13.9±1.2^{b}
3	15.9±1.2^{b}	13.9±1.1^{a}	15.8±1.1^{b}	15.2±1.4^{b}
4	16.3±1.1^{ab}	15.4±1.1^{b}	17.3±1.2^{b}	16.8±1.5^{ab}
5	17.0±1.3^{a}	17.2±1.1^{a}	20.8±1.4^{b}	17.9±1.4^{a}

* as given in Table-1

Results are average of 3 independent experiments done in duplicate with standard deviation. Mean scores, in a row within the treatment, without a common letters are significantly different ($P < 0.05$) by DMRT.

Table 3. Textural Analysis of *idlis* prepared using different starter cultures

Fermentation period (Days)	Combination of starter cultures*			
	P_P +Cv	Ef- IB2+Cv	Ef+Cv	NF
1	6.42 ± 1.1^{a_s}	5.30 ± 0.9^{a}	5.71 ± 0.5^{a}	9.54 ± 1.1^{b}
5	8.54 ± 1.2^{b}	8.98 ± 1.1^{b}	8.24 ± 0.9^{b}	ND

^{S}-Newton, ND - Not determined

* as given in Table-1

Results are average of 3 independent experiments done in duplicate with standard deviation. Mean scores, in a column within the treatment, without a common letters are significantly different ($P < 0.05$) by DMRT.

Table 4. Nutrient analysis of *idlis* prepared using selected culture

Parameter	Combination of starter cultures*						
	Day 1				Day 5		
	PP +Cv	Ef-IB2+Cv	EF+Cv	NF	PP +Cv	Ef-IB2+Cv	Ef+Cv
Moisture (g %)	51.92±1.4[a]	50.94± 2.8[a]	50.21±2.6[a]	50.51±1.1	50.24± 3.1[a]	47.97±2.8[a]	48.96±3.6[a]
Energy (Kcal)	42.80±2.5[b]	43.40±2.7[b]	43.10±3.2 [b]	43.20±1.0	37.20±2.4[a]	37.70±1.9[a]	38.10±3.1[a]
Carbohydrate (g %)	8.10±1.1[a]	7.70±1.4[a]	7.90±0.8[a]	8.20± 0.9	7.40± 0.9 [a]	6.30±0.8[a]	6.70±1.5[a]
Protein (g %)	3.32±0.6[a]	3.23±0.6[a]	3.23±0.1[a]	3.32±0.2	2.97±0.4[a]	2.88±0.3[a]	2.97±1.3[a]
Fat (g %)	0.15±0.1[a]	0.14±0.1[a]	0.14±0.1[a]	0.14±0.1	0.12± 0.2[a]	0.13±0.1[a]	0.13±1.4[a]
Iron (mg)	1.90±0.1[b]	2.85±1.2[b]	2.85±0.1[b]	3.80±0.3	ND	0.95±0.1[a]	0.95±0.1[a]
Ash content (g %)	36.66±2.2[a]	37.85±2.3[a]	38.35±2.1[a]	38.19±2.8[a]	35.44± 2.1[a]	36.37±2.5[a]	37.42±2.4[a]

* as given in Table-1; ND-Not detected;

Results are average of 3 independent experiments done in duplicate with standard deviation.

Mean scores, in a row within the treatment, without a common letters are significantly different ($P < 0.05$) by DMRT.

Table 5. Microbial profile of *idli* batter at different storage temperatures

Storage Period (days)	Microbial count (Log10 cfu/g)											
	PP +Cv*				Ef-IB2+Cv				Ef+Cv			
	10 °C		30 °C		10 °C		30 ° C		10 °C		30 °C	
	Lactics	Yeasts	Lactics	Yeasts	Lactics	Yeasts	Lactics	Yeasts	Lactics	Yeasts	Lactics	Yeasts
0	-	-	7.29[a]	7.33[a]	-	-	7.06[a]	7.34[a]	-	-	7.07[a]	7.40[a]
1	8.56±1.1[a]	8.74±1.2[a]	9.20±1.4[b]	9.25±1.4[b]	8.56±1.2[a]	8.68±1.2[a]	9.12±1.3[b]	9.24±1.2[b]	8.33±0.9[a]	8.64±0.6[a]	9.10±0.1[b]	9.40±1.3[b]
2	8.66±0.9[a]	8.84±1.3[a]	9.35± 1.5[b]	9.29±1.2[b]	8.79±1.1[a]	8.82±1.0[a]	9.32±0.8[b]	9.34±1.1[b]	8.58±0.8[a]	8.82±0.5[a]	9.39±1.5[b]	9.40±1.2[b]
3	8.79±0.8[a]	9.00±1.5[a]	9.54±1.4[b]	9.42±1.3[b]	9.00±1.4[a]	9.09±1.1[a]	9.51±0.9[b]	9.48±1.2[b]	8.63±0.6[a]	8.93±0.9[a]	9.56±1.3[b]	9.49±1.5[b]
4	ND	ND	10.07±1.6[c]	10.01±1.4[c]	ND	ND	10.07±1.2[c]	10.12±1.6[c]	ND	ND	9.93±1.6[b]	9.93±1.7[b]
5	ND	ND	9.22±1.0[b]	8.95±1.1[b]	ND	ND	9.22±1.1[b]	9.06±1.1[b]	ND	ND	9.26±1.0[b]	8.79±1.1[b]
6	9.09±1.2[a]	9.06±1.4[a]	ND	ND	9.04±1.2[a]	9.11±1.1[a]	ND	ND	8.99±1.0[a]	9.05±1.0[a]	ND	ND
7	8.92±1.1[a]	8.99±1.1[a]	ND	ND	9.01±1.3[a]	9.08±1.0[a]	ND	ND	8.89± 0.9[a]	8.97±0.6[a]	ND	ND

* as given in Table-1; ND- Not Determined; Results are average of 3 independent experiments done in duplicate with standard deviation.

Mean scores, in a row within the treatment, without a common letters are significantly different ($P < 0.05$) by DMRT.

Table 6. Sensory profile of freshly prepared *idlis* prepared using selected combinations of starter cultures

Parameter	Sensory score / Combination of starter cultures							
	P_P +Cv*		Ef-IB2+Cv		Ef+Cv		NF	
	1**	5	1	5	1	5	1	5
Appearance	3.7 ± 0.1^{a}	3.5 ± 0.1^{a}	3.7 ± 0.2^{a}	3.1 ± 0.2^{a}	3.1 ± 0.1^{a}	3.2 ± 0.1^{a}	3.5 ± 0.1^{a}	ND
Colour	3.5 ± 0.1^{a}	2.8 ± 0.2^{a}	4.0 ± 0.4^{b}	2.6 ± 0.1^{a}	3.7 ± 0.4^{a}	2.5 ± 0.1^{a}	3.6 ± 0.2^{a}	ND
Flavour	4.5 ± 0.3^{c}	3.0 ± 0.1^{b}	4.4 ± 0.4^{c}	2.8 ± 0.3^{a}	4.1 ± 0.5^{b}	2.8 ± 0.3^{a}	3.8 ± 0.2^{a}	ND
Taste	4.3 ± 0.2^{a}	2.3 ± 0.2^{a}	4.0 ± 0.3^{a}	2.2 ± 0.2^{a}	4.2 ± 0.4^{a}	2.4 ± 0.2^{a}	3.9 ± 0.2^{a}	ND
Overall acceptability	3.9 ± 0.3^{b}	2.3 ± 0.2^{a}	4.4 ± 0.3^{d}	2.3 ± 0.1^{a}	4.2 ± 0.5^{c}	2.4 ± 0.2^{b}	3.6 ± 0.2^{a}	ND

* As given in Table-1; ** Storage period of batter (in days) at 30°C

Mean scores, in a row within the treatment, without a common letters are significantly different ($P < 0.05$) by DMRT. (n- 50)