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Optimization of selected starter cultures combinations for *idli* batter fermentation

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Abstract

Four bacterial strains viz., *Lactobacillus casei* NCDC 299, *Lactobacillus rhamnosus* MTCC 5462, *Pediococcus cerevisiae* NCIM 217 and *Leuconostoc mesenteroides* 029 and two yeast strains viz. *Candida versatilis* NCIM 3431 and *Saccharomyces cerevisiae* inoculum was prepared using sterilized paneer whey. Starters in different combinations were inoculated for controlled fermentation of *idli* batter. *Idli* batter fermented without the addition of cultures was also prepared as a control. The fermented batter samples were evaluated for batter volume rise (%), pH and acidity while *idli* prepared from the fermented batter was evaluated for sensory parameters by a trained panel of experts. Highly acceptable quality *idli* were prepared from batter fermented under controlled conditions at 30 °C for 14 h using combination of *Lactobacillus rhamnosus* MTCC 5462 (0.5%), *Leuconostoc mesenteroides* 029 (0.5%), *Candida versatilis* NCIM 3431 (0.5%) and *Saccharomyces cerevisiae* (0.5%) compared to *idli* prepared from naturally fermented *idli* batter.

Keywords: *Idli* batter, whey, starters, acidity, sensory

Introduction

India is traditionally rich in fermented foods. In the Indian sub-continent, fermented foods using local food crops and other biological resources are very common. But the nature of the products and the base material varies from region to region (Sekar and Mariappan, 2007) [1]. Fermented foods such as *idli* and dahi were described as early as 700 BC. At present, there are hundreds of fermented foods with different base materials and preparation methodology. Each fermented food is associated with a unique group of micro-biota, which increases the level of proteins, vitamins, essential amino acids and fatty acids in the food product. However, fermented foods are still produced traditionally by spontaneous fermentation and only limited knowledge has been obtained regarding the micro-biota of these products (Jeyaram *et al.*, 2009) [2].

There are various problems associated with indigenous fermentation. They are uncontrolled and often unhygienic, labor-intensive, seen as primitive by some people, are normally not integrated into the economic mainstream, have limited export potential, and in some cases, the impact on nutritive value and safety is questionable (Singhal, 2005) [3].

Cereal-based fermented foods are considered as staple diets in their respective regions. Most of the foods such as *idli*, dosa, dhokla, koozhu, nan, parotta, ambali, pazhaiya soru are consumed on a daily basis by the local population. Mostly they are made at the household level and have short shelf-life (Satish kumar *et al.*, 2013) [4].

Idli is a traditional fermented food of India based on cereal and legume combination. *Idli* is a white, fermented acid (leavened), soft, spongy textured product and steamed cake of rice (*Oryza mungo*) and dehulled black gram dhal (*Phaseolus mungo*). It is widely popular and consumed in entire South India. Recently, *idli* is also becoming popular throughout India (Sridevi *et al.*, 2010) [5]. *Idli* is generally prepared by natural fermentation at household. *Idli* is very nutritious and enjoyed by all age people in India. A starter culture, a microbial preparation of a large number of cells of at least one microorganism, need to be added to raw material for desired fermentation. The bacteria identified as a part of the microflora for *idli* batter fermentation include *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Lb. fermentum*, *Lb. lactis*, *Lb. brevis*, *Streptococcus faecalis* and *Pediococcus cerevisiae*, which are essential for leavening of batter and acid production and yeasts such as *Geotrichum candidum*, *Torulopsis holmii*, *T. candida*, *Trichosporon lullulans*, *Candida fragilola*, *C. kefyfyr*, *C. tropicalis*, *Hansenula anomala* and *Rhodotorula graminis*, are responsible for pH reduction

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and may increase the thiamine and riboflavin content (Iyer and Ananthanarayan, 2008^[6], Sridevi *et al.*, 2010)^[5].

Due to its popularity, nutritional profile and urbanization, lack of time to prepare *idli* batter at home people want nutritious traditional products with the uniform quality available throughout the year. Hence, *idli* batter preparation using selected starter culture combinations for controlled fermentation could be the best way for making this traditional product with desired characteristics and uniform quality available throughout the year. With this basic objective, a series of experiments were conducted to evaluate the effect of selected starter culture's combinations on quality of *idli* batter and *idli*.

Materials and Methods

Materials

The raw materials, i.e., IR20 variety parboiled rice (*Oryza sativa*), dehulled black gram (*Phaseolus mungo*) splits and salt (Brand -Tata) were procured from the local market. During the entire study, Borosil brand of glass-ware and analytical grade chemicals were used. Glass wares and other materials were sterilized by standard procedures whenever required.

Starter cultures and their maintenance

The cultures used in the present study, viz., *Lactobacillus rhamnosus* MTCC 5462, *Leuconostoc mesenteroides* 029 and *Saccharomyces cerevisiae* were obtained from the culture collection of Dairy Microbiology Department, SMC College of Dairy Science, AAU, Anand (Gujarat). *Lactobacillus casei* NCDC 299 was procured from Dairy Microbiology Division, National Dairy Research Institute, Karnal. *Pediococcus cerevisiae* NCIM 217 and *Candida versatilis* NCIM 3431 were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. The cultures were maintained at 4 °C on MRS, M17 & PDA agar (Hi-media labs, Mumbai, India) slants according to their growth medium and sub-cultured at 15 days interval.

Sterilized paneer whey was utilized as a medium for the propagation of selected cultures. The pure culture was aseptically transferred @1% into the sterilized paneer whey and incubated at an optimum growth temperature of cultures. Pure starter culture was propagated three times and the activated culture was used as inoculum for the *idli* batter fermentation.

Preparation of Idli Batter

Idli batter was prepared from the mixture of milled rice (*Oryza sativa*) and dehulled black gram (*Phaseolus mungo*) dhal in 3:1 ratio. The raw materials after weighing were dipped for 1 min in boiling water and then soaked in sterilized water for 4 h. These ingredients were ground to a fine paste under hygienic conditions. Six different starter cultures were used to prepare different *idli* batter samples using the following combinations: A) *Lactobacillus casei* NCDC 299 (0.5%) + *Leuconostoc mesenteroides* 029(0.5%) + *Candida versatilis* NCIM 3431 (0.5%) + *Saccharomyces cerevisiae* (0.5%), B) *Lactobacillus rhamnosus* MTCC 5462 (0.5%) + *Leuconostoc mesenteroides* 029(0.5%) + *Candida versatilis* NCIM 3431 (0.5%) + *Saccharomyces cerevisiae* (0.5%) and C) *Pediococcus cerevisiae* NCIM 217 (0.5%) + *Leuconostoc mesenteroides* 029(0.5%) + *Candida versatilis* NCIM 3431 (0.5%) + *Saccharomyces cerevisiae* (0.5%). These combinations of starter cultures were added to the *idli* batter at 2% inoculum (1% LAB and 1% yeast). The batter was

fermented at 30 °C for 14 h. The control sample of *idli* batter was prepared using the traditional method (without steaming the ingredients and without boiling the water used for soaking and grinding and without any added culture natural fermentation). The batter was analyzed for the rise in batter volume (%), pH, acidity (% lactic acid) and the steamed prepared *idli* was evaluated for sensory parameters.

Quality evaluation of idli batter

The rise in batter volume (%)

The *idli* batter was poured into 100 ml sterilized measuring cylinder, up to 30 ml mark, covered with aluminum foil and was kept at 30 °C for 14 h and observed for the rise in batter volume during fermentation. The increase was measured in 'ml' (Sridevi *et al.*, 2010)^[5].

pH

The pH of the *idli* batter was determined using a microprocessor-based digital pH meter (pH Tester 30, Elico LI 610, Singapore) as per the procedure described by Ranganna, 1986^[8].

Acidity (%LA)

To determine titratable acidity, 10g of fermented batter was taken in a 100ml conical flask to which 20ml of distilled water was added. After adding 3-4 drops of phenolphthalein, the contents were mixed well and titrated against 0.1N NaOH to an endpoint of pale pink color and expressed as % lactic acid produced (AOAC, 1984)^[9].

Quality evaluation of idli

Sensory analysis

The *Idli* prepared from the batter was evaluated based on a 9-points hedonic scale for appearance, flavor, body and texture, color and appearance and overall acceptability. The sensory evaluation was carried out by an expert panel of 10 trained judges.

Statistical Analysis

All experiments were conducted with three replications and the data were subjected to statistical analysis using Completely Randomized Design as per the methods described by Steel and Torrie (1980)^[10] and using analysis of variance (ANOVA) by Microsoft Excel Program (Version 7.0). Differences were identified as significant or non-significant based on mean squares and F-test for significance at 5% level of each treatment.

Results and Discussions

Lactic acid bacteria are essential for leavening of batter and acid production while yeasts are responsible for pH reduction and increase the thiamin and riboflavin (Jama and Varadaraj, 1999)^[11]. Yeasts also produce gas that results in the rise of batter volume. Symbiotic relationship of LAB and yeasts exists in fermentation that leads to the development of desirable product and hence starter culture combinations of lactic acid bacteria and yeasts were selected for evaluating their suitability for controlled batter fermentation and its effect on the quality of *idli*. Each isolate was activated using sterilized paneer whey as a growth medium and was used as inoculums for fermentation. Starter culture combinations as per the material and methods were utilized as inoculums for batter fermentation. Batter fermented at 30 °C for 14 h and the fermented batter was analyzed for the rise in batter volume (%), pH and acidity (%LA) and *idli* prepared from the

fermented batter was tested for sensory evaluation by a trained sensory panel.

Table 1: Influence of cultures combination on pH, acidity (% LA) and % rise in volume of *Idli* Batter

Type	pH	Acidity (% lactic acid)	% batter volume rise
Control	4.76 ± 0.12	0.46 ± 0.02	67.33 ± 6.43
A	4.81 ± 0.16	0.46 ± 0.03	46.00 ± 5.29
B	4.68 ± 0.13	0.45 ± 0.03	51.80 ± 9.06
C	4.83 ± 0.11	0.43 ± 0.01	43.33 ± 3.06
C.D. Value	0.25	0.04	11.94

Each observation is a mean ± SD of three replicate experiment (n =3)

A): *Lactobacillus casei* NCDC 299 (0.5%) + *Leuconostoc mesenteroides* 029(0.5%) + *Candida versatilis* NCIM 3431 (0.5%) + *Saccharomyces cerevisiae* (0.5%), B): *Lactobacillus rhamnosus* MTCC 5462 (0.5%) + *Leuconostoc mesenteroides* 029(0.5%) + *Candida versatilis* NCIM 3431 (0.5%) + *Saccharomyces cerevisiae* (0.5%) C): *Pediococcus cerevisiae* NCIM 217 (0.5%) + *Leuconostoc mesenteroides* 029(0.5%) + *Candida versatilis* NCIM 3431 (0.5%) + *Saccharomyces cerevisiae* (0.5%), Control: Without cultures addition

The effect of cultures on *Idli* batter for pH, acidity and % rise in batter volume is shown in Table 1. The pH of batter samples ranged from 4.68 to 4.83, acidity ranged from 0.43 to 0.46 while the rise in *idli* batter volume (%) ranged from 46 to 67.33. The batter fermented with the addition of the culture did not show a significant effect on pH and acid production in

the batter during fermentation compared to the control batter ($P < 0.05$). The rise in batter volume was observed highest (67.33%) in naturally fermented batter compared to the batter fermented with the combinations of selected starter cultures. The rise in batter volume (%) observed was 51.8, 46.0 and 43.33 for B, A and C batter samples respectively while in control it was 67.33. The effect of culture combinations on % batter volume rise was significant (Table 1). The control samples showed the highest batter volume during the study and this may be due to the natural microflora of raw ingredients and tap water used in control may contribute the uncontrolled fermentation leading to more gas production and rise in batter volume.

The batter after 14 h of fermentation was used for the preparation of *idli* and the freshly prepared *idli* was subjected for sensory evaluation by a sensory panel. The sensory score of the *idli* is present in Table 2. The flavor score ranged from 7.49 to 7.7, body and texture score ranged from 7.58 to 7.90, color and appearance score ranged from 7.2 to 7.56 while overall acceptability of *idli* ranged from 7.42 to 7.74. Significant effect on addition of culture was observed on overall acceptability. The overall acceptability of *idli* was in the order of B>Control>A>C. *Idli* prepared from batter fermented with starter culture combination *Lactobacillus rhamnosus* MTCC 5462 (0.5%), *Leuconostoc mesenteroides* 029(0.5%), *Candida versatilis* NCIM 3431 (0.5%) and *Saccharomyces cerevisiae* (0.5%) scored highest overall acceptability among all the samples.

Table 2: Sensory evaluation of *idli* on 9 points hedonic scale

Sample	Flavor	Body and texture	Colour and appearance	Overall acceptability
Control	7.63 ± 0.05	7.73 ± 0.03	7.46 ± 0.05	7.60 ± 0.04
A	7.58 ± 0.02	7.76 ± 0.04	7.43 ± 0.06	7.59 ± 0.03
B	7.70 ± 0.10	7.90 ± 0.20	7.56 ± 0.16	7.74 ± 0.18
C	7.49 ± 0.09	7.58 ± 0.03	7.20 ± 0.01	7.42 ± 0.04
C.D. Value	0.14	0.20	0.17	0.17

Each observation is a mean ± SD of three replicate experiment (n =3)

Table 3: Effect of starter cultures on the hardness of *idli* (Sample size 7 cm diameter)

Samples	Hardness (Load, g)
Control	240.85 ± 16.99
A	123.16 ± 4.54
B	147.45 ± 6.30
C	131.68 ± 3.32
CD Value	17.87

Each observation is a mean ± SD of three replicate experiment (n =3)

The hardness of *idli* was measured using the texture analyzer and the results are presented in Table 3. The control *idli* had the highest hardness of 240.85 g while sample A, B, and C had the hardness values of 123.16, 147.45 and 131.68 g respectively. The effect of culture combinations on the hardness of *idli* was significant. The *idli* prepared from the batter fermented with the selected starter cultures combinations were soft compared to the *idli* prepared from a naturally fermented batter.

Conclusion

The present work was undertaken to study the effect of bacterial and yeast starters cultures in different combinations for controlled fermentation of *idli* batter. Starter culture combinations of *Lactobacillus rhamnosus* MTCC 5462,

Leuconostoc mesenteroides 029, *Candida versatilis* NCIM 3431 and *Saccharomyces cerevisiae* each @ 0.5% v/v in batter fermentation for 14 h at 30 °C produced highly acceptable sensory quality *idli*.

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