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EFFECT OF SELECTED LACTIC ACID BACTERIA AND YEAST STARTER CULTURE COMBINATIONS ON QUALITY OF *IDLI* BATTER AND *IDLI*

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ABSTRACT
Starter culture combinations of lactobacilli culture (@ 0.5%) (*Lactobacillus fermentum* AI_2, *Lactobacillus casei* NCDC 299, *Lactobacillus rhamnosus* MTCC 5462 and *Lactobacillus helveticus* MTCC 5463), other LAB culture (@0.5%) (*Streptococcus thermophilus* MTCC 5460, *Pediococcus cereviceae* NCIM 2171 and *Leuconostoc mesentroides* 029) with a yeast culture (@ 0.5%) (*Candida versatilis* NCIM 3431 and *Saccharomyces cereviceae) as* inoculums was evaluated. *Idli* batter fermented naturally without starter culture was also prepared as control. The cultures were first activated in pre-sterilized paneer whey by three times transfer and the vigorous culture was used as inoculums for controlled batter fermentation and afterward, idli was prepared. Fermented batter was evaluated for rise in batter volume (%), pH and acidity and from this fermented batter idli was prepared and evaluated for sensory parameters. Effect of combinations of starter culture on *idli* batter were found statistically significant ($P<0.05$) on batter volume rise (%), pH and acidity. Among 24 combinations most promising three combinations found were T24, T18 and T22 and were further evaluated along with control and found significant effect on the lactobacilli and yeast count (log cfu/g) ($P<0.05$). The effect of culture combination was significant ($P<0.05$) on flavor, body and texture and overall acceptability of idli while non significant on colour and appearance ($P > 0.05$). The order of preference with respect to overall acceptability score was T22 > T24 > T18 > C.

KEY WORDS: Lactic acid bacteria, yeast, starter culture, *Idli*, *Idli* batter.

INTRODUCTION

Traditionally India is 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). Fermented foods such as *idli* and dahi were described as early as 700BC. 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). 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).

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). *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. fementum, Lb. lactis, Lb. brevis, Streptococcus faecalis* and *Pediococus 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. kefyr, C. tropicalis, Hansenula anomala* and *Rhodotorula graminis*, are responsible for pH reduction and may increase the thiamine and riboflavin content(Iyer and Ananthanarayan, 2008, Sridevi *et al*., 2010).

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-wares 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 fermentum* AI_2, *Lactobacillus rhamnosus* MTCC 5462, *Lactobacillus helveticus* MTCC 5463, *Leuconostoc mesentroides* 029, *Streptococcus thermophilus* MTCC 5460 and *Saccharomyces cereviceae* obtained from 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 cereviceae* NCIM 217 and *Candida versatilis* NCIM 3431 were procured from National Collection of Industrial Micro-organisms (NCIM), National Chemical Laboratory, Pune. The cultures were maintained at 4°C on MRS, M17 & PDA agar (Himedia labs, Mumbai, India) slants according to their growth medium and sub-cultured at 15 day intervals.

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 (@ 0.5% (v/v)) for the 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 to remove surface microflora and then soaked in sterilized water for 4 h. These ingredients were ground to a fine paste under hygienic conditions. The activated cultures were taken (@ 0.5 % each from 1, 2 and 3) into different combinations as inoculums for controlled batter fermentation. (1) Lactobacilli bacteria (@ 0.5%): *(Lactobacillus fermentum* AI_2, *Lactobacillus casei* NCDC 299, *Lactobacillus rhamnosus* MTCC 5462, *Lactobacillus helveticus* MTCC 5463) (2) Other LAB (@ 0.5%): *(Streptococcus thermophilus* MTCC 5460, *Pediococcus cereviceae* NCIM 2171, *Leuconostoc mesentroides* 029) and (3) Yeast cultures (@ 0.5%): *(Candida versatilis NCIM* 3431, *Saccharomyces cereviceae)*. The possible combinations of starter culture were selected by taking each one from lactobacilli culture @0.5 % *(Lactobacillus fermentum* AI_2, *Lactobacillus casei* NCDC 299, *Lactobacillus rhamnosus* MTCC 5462, *Lactobacillus helveticus* MTCC 5463), Other LAB (@ 0.5%): *(Streptococcus thermophilus* MTCC 5460, *Pediococcus cereviceae* NCIM 2171, *Leuconostoc mesentroides* 029) and Yeast cultures (@ 0.5%): (*Candida versatilis* NCIM 3431, *Saccharomyces cereviceae)* with total @1.5% of inoculums. Twenty fourn combinations are shown in table 1. The batter was fermented at 30^{o}C for 14h. 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.

TABLE 1: Coding of selected starter cultures combination used as inoculums for batter fermentation

Treatment	Starter culture combinations
C	Without Starter culture (Natural Fermentation)
T1	*Lactobacillus casei* NCDC299+*Pediococcus cereviceae* NCIM 2171+ *Candida versatilis* NCIM 3431
T2	*Lactobacillus casei* NCDC 299 + *Pediococcus cereviceae* NCIM 2171+ *Saccharomyces cereviceae*
T3	*Lactobacillus casei* NCDC 299 + *Leuconostoc mesentroides* 029 + *Candida versatilis* NCIM 3431
T4	*Lactobacillus casei* NCDC 299 + *Leuconostoc mesentroides* 029 + *Saccharomyces cereviceae*
T5	*Lactobacillus rhamnosus* MTCC 5462 + *Pediococcus cereviceae* NCIM 2171+ *Candida versatilis* NCIM 3431
T6	*Lactobacillus rhamnosus* MTCC 5462 + *Pediococcus cereviceae* NCIM 2171+ *Saccharomyces cereviceae*
T7	*Lactobacillus rhamnosus* MTCC 5462 + *Leuconostoc mesentroides* 029 + *Candida versatilis* NCIM 3431
T8	*Lactobacillus rhamnosus* MTCC 5462 + *Leuconostoc mesentroides* 029 + *Saccharomyces cereviceae*
T9	*Lactobacillus rhamnosus* MTCC 5462 + *Streptococcus thermophilus* MTCC 5460 + *Candida versatilis* NCIM 3431
T10	*Lactobacillus rhamnosus* MTCC 5462 + *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae*
T11	*Lactobacillus helveticus* MTCC 5467 + *Streptococcus thermophilus* MTCC 5460 + *Candida versatilis* NCIM 3431
T12	*Lactobacillus helveticus* MTCC 5463 + *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae*
T13	*Lactobacillus helveticus* MTCC 5463 + *Pediococcus cereviceae* NCIM 2171+ *Candida versatilis* NCIM 3431
T14	*Lactobacillus helveticus* MTCC 5463 + *Pediococcus cereviceae* NCIM 2171+ *Saccharomyces cereviceae*
T15	*Lactobacillus helveticus* MTCC 5463+ *Leuconostoc mesentroides* 029 + *Candida versatilis* NCIM 3431
T16	*Lactobacillus helveticus* MTCC 5463 + *Leuconostoc mesentroides* 029 + *Saccharomyces cereviceae*
T17	*Lactobacillus fermentum* AI_2+ *Streptococcus thermophilus* MTCC 5460 + *Candida versatilis* NCIM 3431
T18	*Lactobacillus fermentum* AI_2+ *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae*
T19	*Lactobacillus fermentum* AI_2+ *Pediococcus cereviceae* NCIM 2171+ *Candida versatilis* NCIM 3431
T20	*Lactobacillus fermentum* AI_2+ *Pediococcus cereviceae* NCIM 2171+ *Saccharomyces cereviceae*
T21	*Lactobacillus fermentum* AI_2+ *Leuconostoc mesentroides* 029 + *Candida versatilis* NCIM 3431
T22	*Lactobacillus fermentum* AI_2+ *Leuconostoc mesentroides* 029 + *Saccharomyces cereviceae*
T23	*Lactobacillus casei* NCDC 299 + *Streptococcus thermophilus* MTCC 5460 + *Candida versatilis* NCIM 3431
T24	*Lactobacillus casei* NCDC 299 + *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae*

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 ºC for 14 h and observed for the rise in batter volume during fermentation. The increase was measured in 'ml' (Sridevi *et al.*, 2010).

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.

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).

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.

Selection of best three combinations

Based on the above analysis data the best three starter culture combinations giving the maximum raise in batter volume and sensory score of the product were selected as optimized product and further subjected for physico-chemical, microbial and sensory analysis of the product.

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) 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).

TABLE 2: Influence of cultures combination on pH, acidity (% LA) and % rise in volume of *Idli* Batter

Treatments	pH	Acidity (% lactic acid)	Batter volume rise, %
C	5.08±0.00	0.45±0.00	96.87±2.22
T1	4.26±0.03	0.48±0.00	22.64±2.15
T2	4.54±0.03	0.47±0.01	39.08±1.47
T3	4.21±0.05	0.50±0.01	14.63±2.39
T4	4.55±0.07	0.47±0.00	35.08±1.08
T5	4.31±0.01	0.49±0.00	11.31±0.65
T6	4.58±0.00	0.47±0.01	36.67±2.91
T7	4.29±0.01	0.49±0.00	25.34±3.53
T8	4.41±0.01	0.47±0.00	49.80±10.20
T9	4.62±0.01	0.48±0.00	92.39±25.64
T10	4.61±0.01	0.48±0.00	85.08±23.92
T11	4.61±0.00	0.48±0.00	85.68±22.29
T12	4.62±0.01	0.48±0.00	83.29±23.65
T13	4.61±0.01	0.48±0.00	76.69±20.35
T14	4.61±0.01	0.48±0.00	84.57±22.29
T15	4.61±0.00	0.48±0.00	53.36±12.77
T16	4.65±0.02	0.47±0.01	61.96±18.24
T17	5.12±0.00	0.48±0.00	95.37±0.63
T18	5.09±0.00	0.48±0.00	103.97±2.31
T19	5.12±0.00	0.49±0.00	96.71±0.64
T20	5.08±0.00	0.48±0.00	99.38±1.72
T21	5.12±0.00	0.48±0.00	86.29±0.98
T22	5.09±0.00	0.48±0.00	99.41±1.69
T23	5.11±0.00	0.48±0.00	90.08±1.09
T24	5.09±0.00	0.48±0.00	103.99±1.17
CD (0.05)	0.06	0.01	35.19

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

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.

The effect of cultures on *Idli* batter for pH, acidity and % rise in batter volume is shown in Table 2. The pH of batter samples ranged from 4.21 to 5.12, acidity ranged from

0.45 to 0.50 while the rise in *idli* batter volume (%) ranged from 11.31 to 103.99. The batter fermented with the addition of the culture showed a significant effect on pH, acid production and percentage batter volume rise during fermentation compared to control batter (P<0.05). The rise in batter volume was observed highest (103.99%) in T24 while in control it was 96.87. The effect of culture combinations on % batter volume rise was significant(P<0.05). The overall acceptability score of the *idli* prepared by the starter culture combination of *Lactobacillus casei* NCDC 299 + *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae (*T24), *Lactobacillus fermentum* AI_2+ *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae* (T18) and *Lactobacillus fermentum* AI_2 + *Leuconostoc mesentroides* 029 + *Saccharomyces cereviceae* (T22) was 8.38, 8.28 and 8.27 respectively. These three starter culture combinations have the higher acceptability compared to the control (7.96). Based on overall acceptability score of products and batter volume rise (%), these best three combinations of starter culture were selected for further study.

TABLE 3: Effect of selected starter cultures on sensory attributes i.e., flavour, body and texture, colour and appearance and overall acceptability (9 point hedonic scale) of Idli

Treatment	Flavour	Body and Texture	Colour and Appearance	Overall Acceptability
C	7.87±0.29	7.71±0.17	8.3±0.11	7.96±0.18
T1	7.31±0.24	7.21±0.31	7.86±0.25	7.46±0.20
T2	7.96±0.09	8.25±0.16	8.10±0.14	8.10±0.08
T3	7.63±0.21	7.37±0.28	7.63±0.26	7.54±0.09
T4	8.01±0.13	8.29±0.17	8.14±0.15	8.15±0.08
T5	7.65±0.22	7.07±0.25	7.50±0.30	7.40±0.17
T6	8.21±0.11	8.35±0.17	8.17±0.15	8.24±0.06
T7	7.37±0.25	7.07±0.24	7.66±0.21	7.37±0.17
T8	8.17±0.14	8.17±0.15	7.55±0.31	7.97±0.21
T9	7.96±0.16	7.95±0.18	8.27±0.11	8.06±0.10
T10	8.11±0.16	7.87±0.22	8.23±0.14	8.07±0.10
T11	7.87±0.19	7.83±0.20	8.23±0.11	7.98±0.13
T12	7.91±0.15	7.83±0.22	8.19±0.12	7.98±0.10
T13	8.09±0.17	7.74±0.24	8.22±0.13	8.02±0.14
T14	8.09±0.17	7.93±0.18	8.19±0.13	8.07±0.08
T15	7.97±0.13	7.83±0.20	8.02±0.10	7.94±0.06
T16	8.17±0.18	7.87±0.21	7.98±0.11	8.00±0.09
T17	7.94±0.16	8.19±0.13	8.25±0.10	8.13±0.10
T18	8.27±0.12	8.29±0.23	8.30±0.11	8.28±0.01
T19	7.93±0.17	8.09±0.15	8.20±0.11	8.07±0.08
T20	8.19±0.09	8.37±0.15	8.23±0.10	8.23±0.06
T21	7.97±0.18	7.95±0.19	8.28±0.10	8.06±0.11
T22	8.13±0.18	8.31±0.12	8.26±0.11	8.27±0.05
T23	8.21±0.11	8.17±0.12	8.24±0.10	8.21±0.02
T24	8.45±0.13	8.42±0.13	8.27±0.10	8.38±0.05
CD (0.05)	0.48	0.54	0.45	0.32

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

Effect of best three combinations of selected starter cultures on physico-chemical, microbial quality of idli batter and sensory characteristics of *idlis*

Percentage batters volume rise varied from 83.33 to 96 (Table 3). Percentage volume rise was found highest 96 for sample T24 while 90 for sample T18. Sample T24 and control had 83.33 percentage volume rise. The effect of culture combinations was significant (P<0.05) on percentage batter volume rise. Effect of culture combinations was found non-significant ((P>0.05) on pH while significant effect was found on acidity of fermented batter.

TABLE 4: Effect of three best combinations of cultures on volume rise, acidity and pH of batter

Samples	Rise in batter volume (%)	Acidity (%LA)	pH
C	83.33^{c}±2.04	0.46^{b}±0.3	5.08^{a}±0.01
T18	90.00^{b}±1.15	0.48^{a}±0.1	5.09^{a}±0.00
T22	83.33^{c}±1.76	0.48^{a}±0.2	5.10^{a}±0.01
T24	96.00^{a}±1.15	0.48^{a}±0.1	5.00^{a}±0.00

Data are presented as Means ± SEM (n=3)

The rate of lactic acid production in the batter is dependent on the availability of sugars for the utilization of the microorganisms, with the consequent decrease in pH of the batter. Lower of the pH or increase in acidity has been reported to be a common feature in idli batter during fermentation by Venkatasubbaiah et al. (1984). According to Steinkraus et al. (1967) idlis prepared from batters with a pH in the range of 4.1 -5.3 had satisfactory flavour when steamed. Acidification of the batter by microorganisms is one of the most important changes taking place in batter

during fermentation. Nagarathnama and Siddappa (1965) have observed that the texture, taste and flavor of idli depend largely on the acidity developed during fermentation of batter. The consistency of batter was also free flowing at this stage.

Microbial analysis of Idli batters

Batter fermented using best three starter culture combinations and control was analyzed for lactobacilli count, yeast and mold count and coliform count.

TABLE 5: Lactobacilli count (log_{10} cfu g^{-1}) of batters fermented using selected cultures

Treatments	Lactobacilli count (log_{10} cfu g^{-1}) (Initial)	Lactobacilli count (log_{10} cfu g^{-1}) (Final)	Increase in Lactobacilli count
C	2.86±0.04	6.53±0.02	3.67^{a}
T18	5.73±0.00	6.51±0.02	0.78^{c}
T22	5.71±0.01	6.53±0.02	0.82^{b}
T24	5.72±0.01	6.55±0.02	0.83^{b}

Data are presented as Means ± SEM (n=3)

Initially at 0 hour of fermentation lactobacilli count were 2.86 for control while for T18, T22 and T24 it was 5.73, 5.71 and 5.72 respectively. Compared to control, all the three combinations of starter culture added batter has higher initial lactobacilli count. This may be due to addition of pure culture inoculum containing very high population of lactobacilli. After 14 hours increase in lactobacilli count was 3.86, 0.78, 0.82 and 083 for Control, T18, T22 and T24 respectively. The change was higher in control while in treated it was very less this may be due to the initial adoption of inoculum to batter. The increase in lactobacilli count was significantly ($P<0.05$) affected by the culture combinations. Sridevi *et al.*, (2010) had observed upto 8.79 cfu/g in batter prepared using lactic acid bacteria and yeast as starter culture.

TABLE 6: Yeast and mold count (log_{10} cfu g^{-1}) of batter

Treatments	Yeast count (log_{10} cfu g^{-1}) (Initial)	Yeast count (log_{10} cfu g^{-1}) (Final)	Increase in Yeast count
C	3.61±0.01	7.75±0.02	4.13^{c}
T18	3.1±0.01	7.61±0.01	4.51^{b}
T22	3.06±0.01	7.64±0.00	4.58^{a}
T24	3.09±0.02	7.65±0.00	4.56^{ab}

Initially at 0 hour, yeast count of batters was 3.61, 3.1, 3.06 and 3.09 for Control, T18, T22 and T24 respectively. While after 14 hours of fermentation count were 7.75, 7.61, 7.64 and 7.65 for Control, T18, T22 and T24 respectively. Increase in yeast count was significantly ($P<0.05$) different for each others. In culture added products e.g. T18, T22 and T24 count of yeast at 0 hour and 14 hours of fermentation were almost similar. This is because; *Saccharomyces cereviceae* yeast culture was added in all treated batter at same rate from the same inoculum medium for the definite incubation time. Compared to control, all three combination of starter culture added samples had higher increase in yeast count. This can be correlated with rise in batter volume (%) in samples. The microbial load indicated high yeast count related to high rise in the batter volume this might be responsible for soft texture to prepared *idli.* Similar results were observed by Sridevi *et al.*, (2010).

Coliforms were absent in all the samples suggesting that production was carried out under good hygienic condition.

Sensory evaluation of Idli

The effect of culture combination was significant ($P < 0.05$) on flavor, body and texture and overall acceptability while non-significantly ($P > 0.05$) on colour and appearance. Compared to control (Natural fermentation), products prepared using combination of starter culture scored higher for flavor, body and texture and overall acceptability.

TABLE 7: Effect of selected culture combinations on sensory parameters of idli

Treatments	Flavour	Body & Texture	Color & Appearance	Overall Acceptability
C	7.33^{b}±0.16	7.67^{b}±0.13	8.2^{a}±0.11	7.74^{b}±0.25
T18	8.33^{a}±0.11	8.2^{a}±0.11	8.2^{a}±0.11	8.25^{a}±0.05
T22	8.37^{a}±0.10	8.3^{a}±0.11	8.2^{a}±0.11	8.29^{a}±0.05
T24	8.3^{a}±0.11	8.3^{a}±0.11	8.2^{a}±0.11	8.27^{a}±0.03

Data are presented as Means ± SEM (n=3)

The overall acceptability of idli varied from 8.25 to 8.29 while for control it was 7.74. Idli prepared from batter fermented using *Lactobacillus fermentum* AI_2+ *Leuconostoc mesentroides* 029+ *Saccharomyces cereviceae* (T22) scored the maximum (8.29); while idli prepared using *Lactobacillus casei* NCDC 299 + *Streptococcus thermophilus* MTCC 5460+ *Saccharomyces cereviceae* (T24) scored 8.27; and *Lactobacillus fermentum* AI_2+ *Streptococcus thermophilus* MTCC 5460 + *Saccharomyces cereviceae (*T18) scored 8.25. T22 was found highest scored 8.37 for flavor. This might be due to production of flavor compounds produced by *Leuconostoc mesenteroides* present. In fermented milk products *Leuconostoc mesenteroides* are known to produce pleasant

flavor. The order of preference with respect to overall acceptability score was T22 > T24 > T18 > C while for flavor the order was T22 > T18 > T24 > C. The overall acceptability of *idli* prepared were found to be acceptable (>7.0) among the panelists.

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 fermentum* $AI_{2,}$ *Leuconostoc mesentroides* 029 and *Saccharomyces cereviceae each* @ 0.5 % v/v as inoculum in batter fermentation for 14 h at 30 ºC produced highly acceptable sensory quality *idli*. These culture combinations can be utilized for the controlled batter fermentation to obtain uniform quality *Idli.*

REFERENCES

Agrawal, R., Rati, E.R., Vijayendra, S.V.N., Varadaraj, M.C., Prasad, M.S. Krishna Nand. Flavour Profile of Idli Batter Prepared from Defined Microbial Starter Cultures.World Journal of Microbiology and Bio-technology; 2000; 16(7)687-690.

Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. Washington, DC, USA, 1984.

Iyer, B.K. and Ananthanarayan, L. Effect of α-amylase Addition on Fermentation of Idli—a Popular South Indian Cereal- legume-based Snack Food. LWT Food Science and Technology; 2008; 41(6) 1053-1059.

Jama Y.H. and Varadaraj M.C. Antibacterial effect of plantaricin LP84 on food borne pathogenic bacteria occurring as contaminants during idli batter fermentation. World Journal of Microbiology and Biotechnology. 1999; 15:27–32.

Jeyaram K., Singh A., Romi W., Devi A.R., Singh W.M., Dayanithi H. Traditional fermented foods of Manipur. Indian Journal of Traditional Knowledge. 2009; 8:115 121.

Ranganna S. Handbook of analysis and quality control of fruits and vegetable products. 1986. New Delhi: Tata McGraw-Hill publications.

Satish Kumar, R. Kanmani, P. Yuvaraj, N. Paari, K.A. Pattukumar V. & Arul V. Traditional Indian fermented foods: a rich source of lactic acid bacteria, International Journal of Food Sciences and Nutrition, 2013;64(4) 415-428, DOI: 10.3109/09637486.2012.746288

Sekar and Mariappan. Usage of traditional fermented products by Indian rural folks and IPR. Indian journal of traditional knowledge. 2007; 6(1):111-120.

Singhal, R.S. Cereal–based fermented foods of South East Asia. In: 2nd International Conference on Fermented Foods, Health Status, and Social Well being, organized by SASNET-FF at Anand, December 17-18, 2005, pp.128-135.

Sridevi, J. Prakash Halami, M. Vijayendra, S.V.N. Selection of starter cultures for idli batter fermentation and their effect on quality of idlis. Journal of Food Science and Technology, 2010; 47(5) 557-563.

Steel, R.G.D. and Torrie, J.H. Principles and Procedures of Statistics, Second edition, New York: McGraw Hill, 1980.