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Fermented batter characteristics in relation with the sensory properties of *idli*

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Summary

Idli is one of the very popular fermented breakfast foods in the Indian subcontinent, prepared from a cereal-legume mix of rice (*Oryza sativa*) and black gram dhal (*Phaseolus mungo*). It is a natural microflora fermentation dominated by lactic acid bacteria and yeasts. Reduction in fermentation time, quality standardization and enhancement of the *idli* batter is of great commercial importance for large scale *idli* production and this can be potentially achieved though better understanding of *idli* batter fermentation. The present study documents how the dynamics of microbial growth influence the batter characteristics and *idli* qualities like firmness, cohesiveness and springiness. The results show that the lactic acid bacteria (LAB) and yeast and mould (YM) counts influence the physico-chemical properties of *idli* batter and consequently the organoleptic qualities of prepared *idli*.

Key words: *Idli, natural fermentation, LAB, YM, firmness, cohesiveness, springiness*

1. Introduction

Idli is a traditional cereal-legume based indigenous fermented food highly popular and widely consumed in India, especially in the Southern states as a snack, breakfast or dinner food and is usually served with *chutney*, *sambar*, or other accompaniments. Mixture of crushed dry spices such as *milagai podi* is also a preferred condiment. *Idli* was known in India as early as 700 CE. It has a spongy texture, attractive appearance, appetizing taste and flavor. Its easy digestibility and good nutritive value contribute to its increasing popularity in all parts of India and also in other countries (*1*). There is an increasing demand for this traditional product among Indians living abroad. Currently, the production and export of ready-to-cook *idli* mix and *idli* batter in India is a growing business with exports to various Asian, European and Gulf countries. The *idli* deserves global recognition, as special kinds of very fluffy *idli* are exported in hundreds of kilos everyday from India to Malaysian and Singaporean restaurants.

It is a good source of protein, calories and vitamins, especially B-complex vitamins, compared to the raw unfermented ingredients. Taste of *idli* depends upon the type and proportion of raw materials and the properties of batter.

Traditionally, *idli* preparation involves the following: i) rice and black gram dhal are soaked separately, ii) after draining the water, rice and black gram dhal are ground separately with occasional addition of water during the grinding process, iii) the rice and black gram dhal batters are mixed together with addition of salt, iv) the mixture is allowed to ferment overnight at room temperature, v) the fermented batter is poured in the special *idli* pans and steamed for 5-8 min) The steamed product is scooped out of pans (*2*).

Studies have been carried out replacing black gram dhal with soybean which increases vitamin B, protein, nitrogen and α-amylase level in batter during fermentation (4). Amylose content of starch present in rice plays a vital role in textural properties of *idli* (*3*). The addition of exogenous source of α-amylase enzyme (5-15U) (5, 8) and prebiotics namely, xylooligosaccharides improves the LAB growth, reduces the conventional fermentation time (14-18 h) to 6 h giving a softer texture to the *idli* (1), while maintaining the same sensory quality during fermentation, Starches are broken down making them more assimilable and pH of batter decreases from 5 up to 3 (*9,10*). With the progress of fermentation, there is increase in batter volume, acidity level, and non-protein nitrogen while the reducing sugar decreases initially and then tends to increase. Microbial source for idli fermentation can be obtained naturally from the ingredients and pre-soaking. Both LAB and yeasts are involved in fermentation.

Commercial production of *idli* batter is a time consuming process sometimes requiring 24 h to get the desired quality of *idli* and hence reduction of fermentation time is significant. However, detailed studies on *idli* batter fermentation parameters and their effects on the end product quality are scarce (2, 6, 20). Hence, this study focuses on the growth kinetics of microorganisms mediating the fermentation and textural and physico-chemical properties of *idli* batter. The correlation between microbial population dynamics, batter properties and *idli* quality are explored. This would aid in better understanding of *idli* fermentation process to get desirable product characteristics and help in the technology of large scale production.

2. Materials and methods

2.1. Materials

Rice (parboiled) and black gram dhal (dehulled) were purchased from the local market, Chennai. The microbiological media used in the study namely De Man Rogosa and Sharpe (MRS) Agar (Cat No-M369), Plate Count Agar (PCA) (Cat No-M091) and Sabouraud Dextrose Agar (SDA) (Cat No-M063) was obtained from HiMedia, Mumbai. All other chemi-

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cals like sodium chloride (NaCl), sodium hydroxide (NaOH) and chloramphenicol & reagents namely phenolphthalein and Bradford reagent used were of analytical grade and were purchased from Merck, Mumbai and HiMedia, Mumbai.

2.2. Preparation of idli batter

The raw materials rice and black gram (urad dhal) in the ratio of 4:1 (w/w) were washed thoroughly and soaked separately in twice the volume of water for an hour at room temperature (25 °C). They were ground separately in an electrical stone grinder, black gram dhal being ground to a fine paste and rice ground to a coarse consistency. Afterwards both ingredients were mixed with salt (2% w/w). The batter was then transferred to a stainless steel vessel, covered with a lid and fermented at 37 °C for 32 h. Generally natural *idli* fermentation is carried out at temperatures between 25- 40º C. In this study the fermentation was initially carried out at various temperatures and finally optimized to 37º C. A previous studies by Rajalakshmi and Vanaja (15, *11*) have also reported the same temperature condition.

2.3. Analysis of idli batter

Batter samples were taken periodically under aseptic condition every 4 h for physico-chemical and microbiological analysis. Three batches of the batters were prepared and analyzed in three consecutive weeks. All tests on the three batches were performed either in duplicates or in triplicates.

2.3.1. Microbiological analysis

The batter samples were homogenised using the high speed blender (REMI) for 60 sec under aseptic conditions. 25 g of sample was dissolved in 225 ml of the diluent (physiological saline), subsequently mixed and serially diluted in the ratio of 1:9. Since the batter is pseudo plastic (non-Newtonian) fluid in nature, the batter preparation is essential before microbiological analysis.

Samples were spread plated on PCA agar for total bacterial count and MRS agar for LAB count and incubated at 37 °C for 24- 48 h. The YM count was obtained by spread plating on SDA supplemented with chloramphenicol (1 %), incubated at room temperature (25 °C) for 3- 5 days and the counts represented as cfu/g of batter.

2.3.2. Physico-chemical analysis

The bulk density of the batter was calculated as the ratio of mass by volume and expressed as g/cm^3.

The moisture content of the batter was determined using moisture analyser (Sartorius MA 35, Germany) and expressed as percentage.

Sample of 10 g was mixed with 100 mL of distilled water and centrifuged at ***17 880 g*** for 20 min and the supernatant obtained was used to determine the pH using pH meter (Susima AP-1 plus, Chennai). An aliquot of the supernatant was also titrated against 0.1 N NaOH with phenolphthalein as indicator to determine TA expressed as percent lactic acid.

The total protein content of parboiled rice, black gram and *idli* batter (at different fermentation times namely 0 h, 12 h and 32 h) was determined by Bradford method (*12*) (Fig 1)

2.4. Idli preparation

About 40 mL of the 12th h batter was taken out at four hour intervals and dished into greased (sesame oil) *idli* pans and steamed at 100 °C for 10-15 min.

2.4.1 Physico-chemical analysis of idli

Idli prepared from the batter samples was used for chemical and sensory analysis. All tests were performed either in duplicates or in triplicates.

2.4.2. Sensory analysis of idli

The color of the *idli* was determined using color spectrophotometer (Ultrascan VIS, Hunter Lab, USA) for L*, a*, b* measurements.

The parameters like firmness, consistency, cohesiveness and index of viscosity of the batter and the firmness of the prepared *idli* were measured using the TA.XT plus Texture Analyser (Stable Microsystems, UK) with the back extrusion and 36 mm cylinder probe respectively. The set parameters were distance - 40 mm, test speed - 1.0 mm / s, data acquisition rate - 200 pps, load cell - 5 kg. The individual samples of *idli* batter were placed on the platform which attached to that of the instrument. When the probe triggers on the surface of the sample the graph begins to plot data, The individual samples of idli were placed on the platform such that they were supported by a plate and heavy duty rig was attached to the cross head of the instrument. When the probe triggers on the surface of the sample the graph begins to plot data. The distance at the trigger point is taken as the original height of the samples. The peak corresponds to force at the maximum penetration depth of 75% of the sample's original height. The subsequent decay in force is due to the relaxation of the sample over the 2 second holding period. Beyond this, the probe withdraws to a tracking force of 5 g for a defined amount of time allowing the sample to "spring back". Then the probe returns to the starting position. Firmness is defined as the force (in grams, kilograms or Newtons) required penetrating the product. The maximum force was recorded as the hardness of the idli. An average value of 3 replicates is reported. Readings were noted after calibration.

The *idli* batter and prepared *idli* was analysed by SEM at initial time period (0 h), mid-interval (12 h) and final time period (32 h). The morphology of the particles was viewed under 100X and 500X magnifications (Hitachi S-2400).

The sensory characteristics of the prepared *idli* at different fermentation times were determined using an untrained panel consisting of 10 members (5 male and 5 female, age group-25-30 yr). All members were familiar with the traditional product as *idli* is a common and frequently consumed food. The samples of uniform size and shape were coded with random numbers. The panellists were asked to score and comment on the color, appearance, texture, mouth feel, taste, flavour and overall acceptability. These eight sensory attributes were also measured on a 5-point hedonic scale with the highest score being extremely good and the lowest being poor (*13*).

2.5. Statistical analysis

Correlation analysis was performed to determine the relationship between the various textural characteristics to that of

the microbial count and the sensory properties using MS-Excel software.

3. Results and discussion

3.1. Idli batter characteristics

3.1.1. Microbiology

The growth of microbes during *idli* fermentation is evident from the microbial counts (**Fig. 2**). The total bacterial count increased sharply but steadily and attained a peak at 12 h and then started to decline steadily until 32 h. This inhibition of bacterial growth may be due to the domination of LAB and yeasts. The peak microbial growth in the three batches varied from 8- 16 h depending on the initial load at 0 h. While mould exceeded yeasts initially, only yeasts were observed after 8 h. This may be attributed to the increasing acidity and domination by LAB. Interestingly, a sharp increase in yeast which occurred at 28 h, declined rapidly at 32h.

At the start of fermentation, the lactic and non-lactic acid bacteria were found to grow together. However, subsequently the LAB dominated from 8 to 16 h. After 24 h only one species of LAB dominated as evident from colony morphology and gram reaction and is possibly a homolactic high acid producer which is acid tolerant as also observed by Thyagaraja et al (*22*).

3.1.2. Physicochemical characteristics

Idli fermentation is a primarily lactic acid fermentation evident from the increase in titratable acidity and decrease in pH (Figure 2). TA increased after 8 h from 0.27 % to 0.45 % and remained steady up to 20 h, and rose further to 0.64 % at 24 and 28 h, with a further rising at 32 h. pH dropped steadily from 5.99 to 3.69. A pH of 4.5 and acidity of 0.45 % at 12 h obtained in this study are similar to the reports of several authors although the fermentation temperature varied between 25- 37 °C (*15, 16,17*).

Bulk density decreased gradually up to 28 h after which there is little change and moisture increased steadily throughout (Table 1). Nagaraju and Manohar (*13*) also observed a steep change in density with increased volume after 4 h with no change in weight, due to gas production, largely by yeasts. The total protein content of the fermented batter increased from 438.95 g at 0 h to 547.32 g at 32 h.

Idli batter showed specific variations in texture throughout the fermentation. Among these textural parameters, consistency and cohesiveness were found to be correlated with overall acceptability of the product. A steady decrease in firmness was observed throughout the fermentation, probably due to the corresponding increase in moisture. In contrast, consistency showed considerable variation through the fermentation. Maximum consistency was evident at 12 and 16 h (**Fig. 3a**). Cohesiveness which was maximum in unfermented batter decreased sharply at 4 h and increased again up to 16 h and showed a steady decline from the 20th h. Microbial gas production, increased moisture and decreased density of batter are responsible for the variations in texture. Likewise total protein content of idli batter has increased over the fermentation period (Fig 1). Complex protein known as glyco protein present in black gram in mucilaginous form may be responsible for gas entrapment during leavening of batter (21)

3.2. Idli characteristics

3.2.1. Texture and color

The firmness of *idli* showed wide variation with fermentation time of batter. It decreased till 16 h (**Fig. 3b**), then increased steadily and at 32 h was similar to the *idli* from unfermented batter. The reduction in the firmness at 8 - 16 h may be due to the leavening action of microbes in the batter – heterolactic LAB and yeasts (*19*). Springiness of *idli* showed less change, being lower at 0 and 32 h and remaining almost similar between 4 to 28 h. This correlates with references by other authors (*14, 19, 21*). These changes in texture may be due to the collapse and loss in the gas holding capacity of the batter. Some of the lactic acid bacteria which are more prominent among the microbial population play a major role in *idli* fermentation by synthesising acid and gas resulting in leavening of batter. Likewise Yeast play important role in fermenting the existing sugars to alcohol and esters, which produce desirable flavours to *idli* (19).

There were no changes in color (L, a* and b*) values of the *idli* (Table 2).

3.2.2. Scanning Electron Microscopy (SEM)

Changes in the fermented batter were confirmed by SEM picture (**Fig. 4a**).The particles showed difference in their surfaces and increased porosity because of microbial activity. At 0 h, *idli* batter had smooth surface while at 12 h surface pores were visible due to utilization of starch during fermentation. SEM pictures (4a1,2,3,4,5,6) indicate initially a smooth surface with uniform distribution of starch granules on the surface. However further fermentation causes the starch consumption and therefore a scattered surface with clusters and porosity are visible during 12 and 32 hours. Steaming of batter resulted in larger particles and hence the12th hour idli sample has a rough surface than 0 th hour idli. The particle size in *idli* batter varied between 24.3 - 218 µm whereas in *idli* it was about 88.3 - 497 µm. *Idli* made from 12 h batter showed particles with a rough texture (**Fig. 4b**). At 32 h both the batter and *idli* became more amorphous, irregular and lost their integrity. *Idli* particles are larger than idli batter. Because the gelatinization (recrystallisation) process of starch during cooking helps to attain native structure of starch. Particle size of the *idli* batter ranged up to 2000 µm for different types of batter *with variable proportion during natural* fermentation for 23 hours, it was found that 80% of the particles after fermentation are found to below 782 µm, like wise particle size for all types batter based on fermentation time ranged from 500 to 600 µm (12). The unfermented idli batter at 1.0 kx showing starch granules but 18th hour fermented *idli* batter at 30°C showed less starch granules as well as leavening of the batter observed at at 500X magnification (7).

3.3. Sensory properties

Desirable appearance, taste, texture, mouth feel was obtained at the fermentation times between 8 - 16 h when batter had pH 4.5 with acidity of 0.45 % lactic acid. *Idli* from 12

h batter scored maximum value of 3.3 out of 5.0. The overall acceptability of the *idli* decreased as the fermentation time increased. The sensory property of *idli* in terms of overall acceptability as a function of microbial profile (**Fig. 3c**), showed that maximum LAB count corresponded to the highest score. Similarly, the overall acceptability as a function of texture corresponded with consistency and cohesiveness of batter and firmness of *idli*. Thus, LAB plays a major role and along with yeasts contributes to the texture and overall sensory quality of the *idli* (**Fig. 3c**). Lee (*9*) also showed that optimum taste during fermentation was attained when the pH and acidity reached approximately 4.0 - 4.5 and 0.5 % - 0.6 % respectively supporting our observations here.

3.4. Correlation analysis

Correlation analysis (Table 3) revealed a positive and negative correlation between LAB count and texture parameters of both batter and *idli*. Further, the texture parameters were positively correlated with overall acceptability of *idli* showing the importance of LAB growth in influencing textural parameters of batter and *idli* and hence its major role in the quality of the product.

4. Conclusions

This study conclusively shows that in natural *idli* fermentation LAB and yeasts play a major role and affect the physicochemical and the textural properties of the batter, thereby enhancing the textural and organoleptic properties of the steamed product.

The traditional method of natural fermentation for a period of 12 - 16 h at 37 °C to an end point of 4.5 yields *idli* with required textural and sensory properties.

The overall desired sensory properties of *idli* can be obtained with moderate levels of acidity and gas formed by LAB and yeasts during fermentation thus contributing to the final quality of the product.

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6. Figures and captions

Figure 1. Total protein estimation

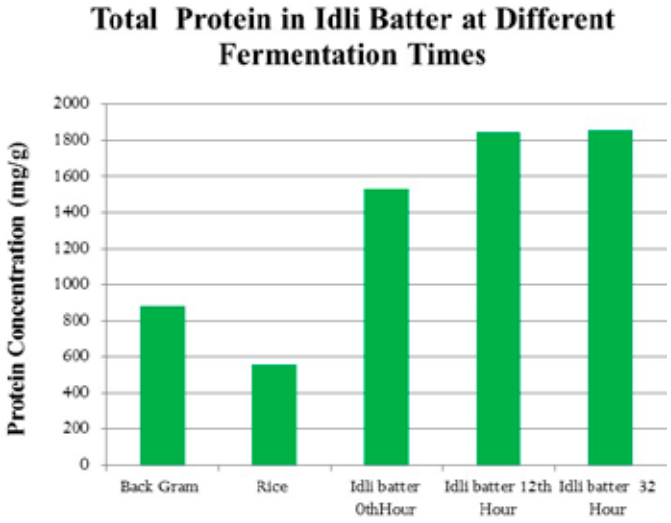


Figure 1. Total protein content in black gram, parboiled rice and idli batter (at different time of fermentation).

Figure 2. Changes in microbial profile, pH and titratable acidity during *idli* fermentation

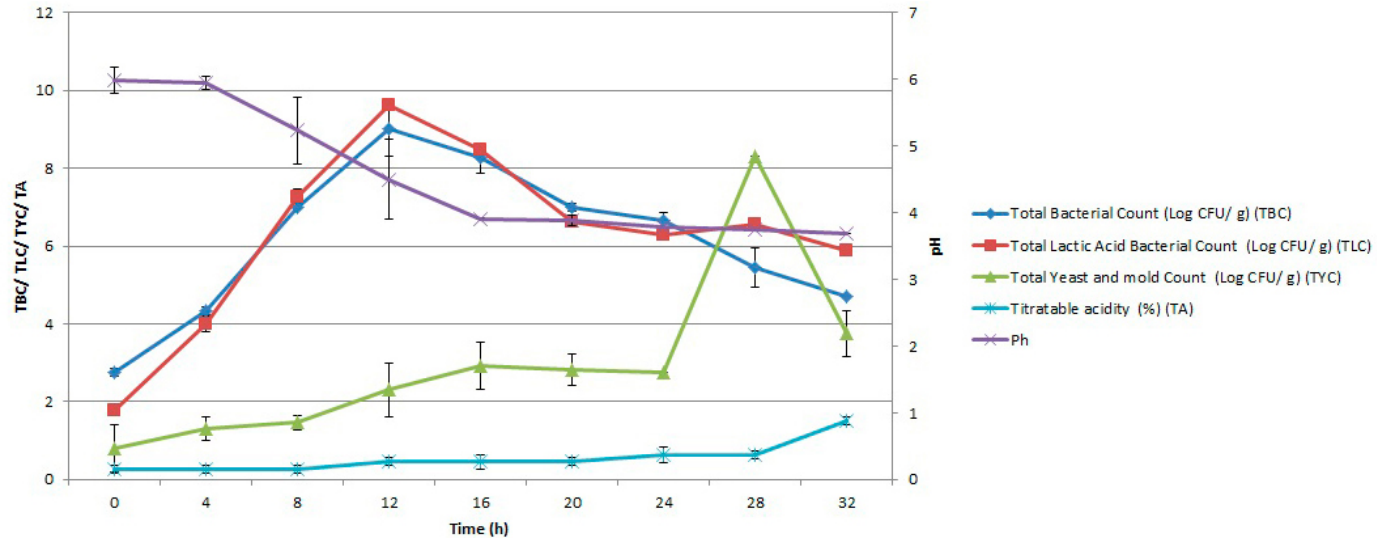


Figure 2 illustrates the changes occurring during idli batter fermentation from 0-32 h, at 4 h intervals. Mean± SD values of three independent estimations of total bacterial count, lactic acid bacteria count and yeast and mould count, pH and titratable acidity are represented.

Figure 3a. Textural qualities of *the idli* batter *Vs overall acceptability*

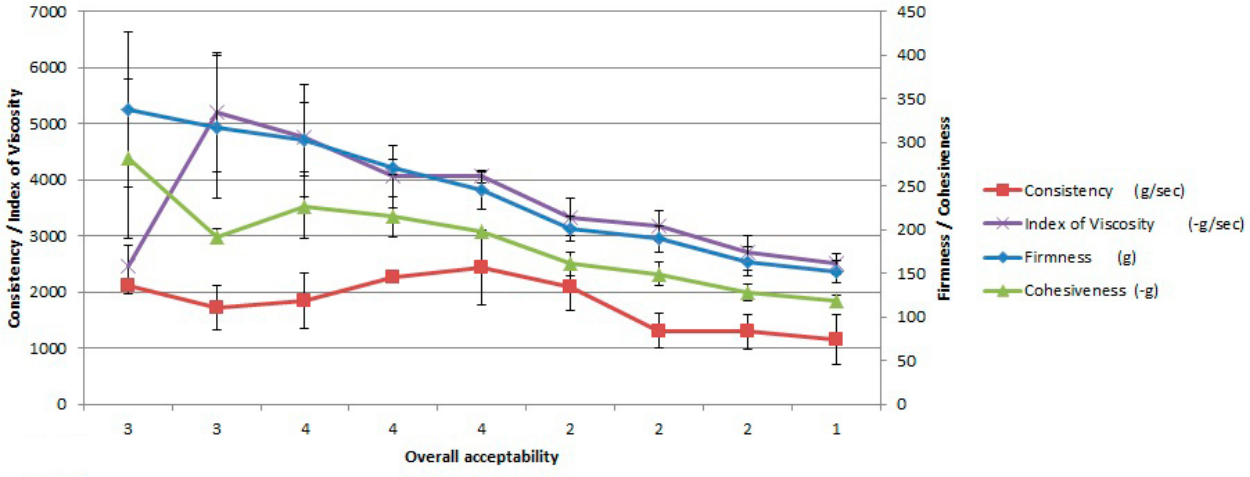


Figure 3b. Textural properties of *idli* Vs overall acceptability

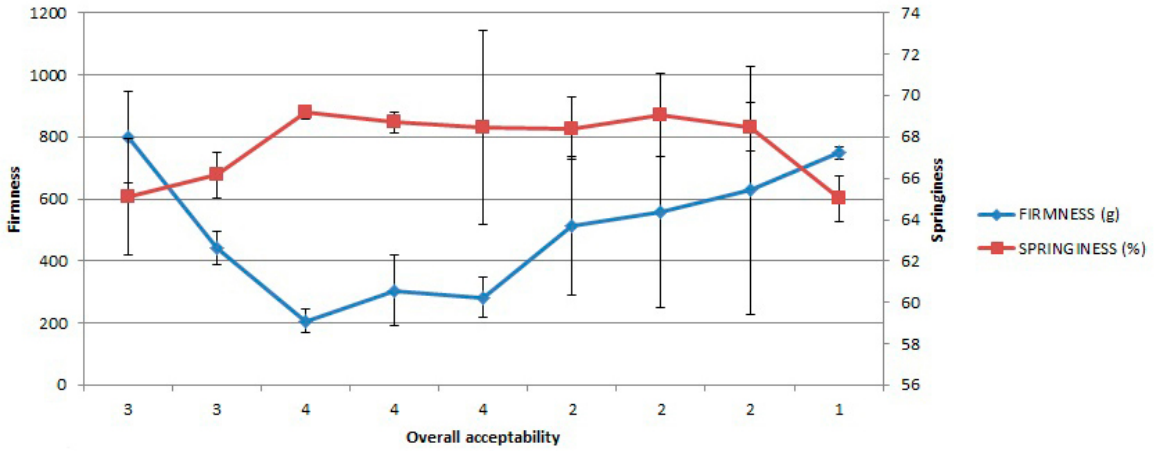
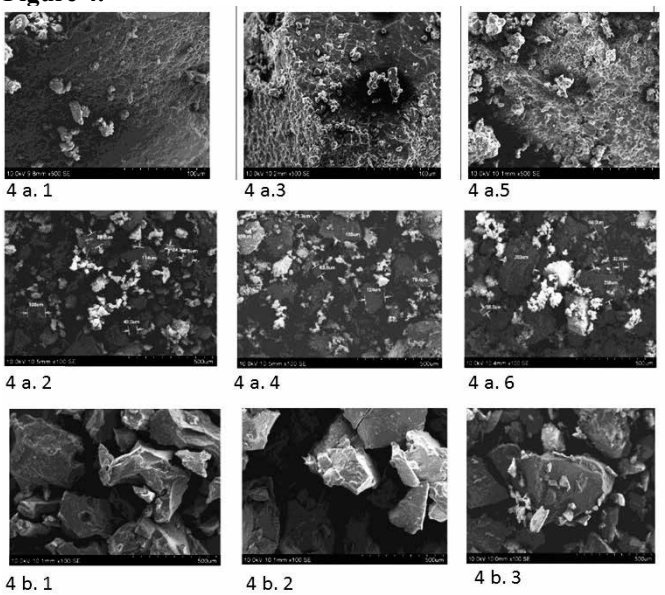


Figure 3a. *Shows the overall acceptability of* idli *with reference to a) consistency, b) index of viscosity, c) firmness & d) cohesiveness of* idli *batter*

Figure 3b. *Shows the overall acceptability of* idli *with reference to its firmness and springiness.*

The X axes of fig 3a & 3b represent the overall acceptability based on eight sensory quality attributes measured using 5 point hedonic scale with three independent estimations.

Figure 4.



Figures 4a *shows the SEM pictures of* idli *batter particles at 100 X and 500 X magnification*

4 a. 1. *At 0th hour idli batter has a smooth surface (500 X).*

4 a. 2. *0th hour idli batter (100 X).*

4 a. 3. *12th hour idli batter sample shows a porous surface with some particles development (500X)*

4 a. 4. *12th hour idli batter sample (100 X).*

4 a. 5. *32nd hour fermented idli batter shows a coalesced particles surface with porousity (500 X).*

4 a. 5. *32nd hour fermented idli (100 X).*

Figures 4b *shows the SEM pictures of idli at 100 X magnifications*

4 b. 1. *Idli from 0th hour batter shows smooth surfaced particle.*

4 b. 2. *Idli from 12 hour fermented batter shows rough surface particles*

4 b. 3. *Idli from 32 hour fermented batter shows break down of particles*

Table 1. *Bulk density and moisture Vs time of fermentation of* idli *batter*

Time (h)	Bulk density (g/ cm^3)	Moisture (%)
0	0.94 ± 0.01	60.74 ± 0.50
4	0.85 ± 0.10	61.25 ± 0.30
8	0.79 ± 0.20	62.22 ± 0.60
12	0.74 ± 0.20	61.95 ± 0.10
16	0.70 ± 0.10	62.13 ± 0.20
20	0.66 ± 0.10	61.85 ± 0.50
24	0.54 ± 0.01	62.37 ± 0.40
28	0.54 ± 0.02	61.54 ± 0.20
32	0.53 ± 0.00	64.57 ± 0.10

* *Values are mean ± SD of three batches in duplicates (six independent estimations)*

Table 2. *Texture and color Vs time of fermentation of* idli *batter*

Time (h)	Texture of *idli*		Colour of *idli*		
	Firmness	Springiness	L*	a*	b*
	(g)	(%)			
0	798.9 ± 148.0	65.12 ± 2.8	80. 08 ± 0.64	0.50 ±0.04	13.71 ± 0.46
4	442.5 ± 53.5	66.16 ± 1.1	77.69 ± 0.50	0.61 ± 0.01	12.93 ± 0.97
8	207.1 ± 39.3	69.18 ± 0.3	75.84 ± 0.20	0.52 ± 0.08	13.12 ± 1.05
12	305.0 ± 115.0	68.70 ± 0.5	68.13 ± 1.70	0.40 ± 0.01	11.54 ± 1.10
16	283.1 ± 65.6	68.46 ± 4.7	66.17 ± 1.42	0.37 ± 0.01	10.57 ± 0.15
20	513.6 ± 225.0	68.42 ± 1.5	71.76 ± 1.02	0.37 ± 0.04	11.00 ± 0.66
24	558.0 ± 305.8	69.06 ± 2.0	71.24± 0.20	0.18 ± 0.01	11.53 ± 0.03
28	627.6 ± 400.0	68.49 ± 1.2	74.15 ± 1.07	0.54 ± 0.04	11.53 ± 0.03
32	748.5 ± 18.80	65.04 ± 1.1	71.93 ± 1.12	0.54 ± 0.04	10.39 ± 1.69

* *Values are mean ± SD of three batches in duplicates (six independent estimations)*

Table 3. *Correlation analysis between LAB count and textural parameters of batter and* idli

S.No	Correlation between	r value
1	pH and bulk density	0.93
2	pH and firmness (batter)	0.93
3	pH and cohesiveness (batter)	0.79
4	pH and titratable acidity	-0.62
5	Bulk density and titratable acidity	-0.72
6	LAB and firmness (*idli*)	0.7
7	LAB and springiness (*idli*)	0.74
8	LAB and pH	-0.61
9	Firmness (batter) and cohesiveness (batter)	0.92
10	Firmness (batter) and pH	0.93
11	Firmness (batter) and bulk density	0.97
12	Firmness (batter) and titratable acidity	-0.76
13	Consistency (batter) and bulk density	0.62
14	Cohesiveness (batter) and bulk density	0.92
15	Overall acceptability and bulk density	0.7
16	Overall acceptability and firmness (batter)	0.61
17	Overall acceptability and consistency (batter)	0.9
18	Overall acceptability and cohesiveness (batter)	0.71
19	Overall acceptability and titratable acidity	-0.71