ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 6, pp. 17752-17756, June, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

AN ATTEMPT TO REDUCE THE FERMENTATION TIME FOR MAKING IDLIS FASTER

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DOI: http://dx.doi.org/10.24327/ijrsr.2017.0806.0416

ARTICLE INFO

Article History:

Received 18th March, 2017
Received in revised form 10th April, 2017
Accepted 06th May, 2017
Published online 28th June, 2017

Key Words:

Organo-leptic, Idli batter, fermentation, indigenous flora

ABSTRACT

Fermentation of idli batter requires 20-24 hours including soaking and leavening time. Present study aimed to isolate the microorganisms responsible for the fermentation, from freshly ground idli batter samples (four South Indian samples and four Maharashtra sample) and thereby use them in different combinations and concentrations to reduce the fermentation time. Microorganisms isolated from South samples were *Leuconostocsps., Lactococcussps., Lactobacillus sps.* and that from Maharashtra samples were *Micrococcus sps., Leuconostocsps., Lactobacillus sps.* Addition of these isolated microorganisms in different concentrations and combinations to the freshly ground idli batter showed that combination 1 for South sample and combination 3 for Maharashtra sample were significantly effective in yielding good quality idlis as determined from the organoleptic properties like appearance, colour, texture, flavour and taste with fermentation period of 2 hours. Thus fermentation time was reduced from 8-10 hours to 2 hours by addition of indigenous.

INTRODUCTION

Idli, a traditional food of South India is an example of an important group of fermented foods that depend on the indigenous flora of the ingredients to bring about fermentation and is very widely consumed throughout India and also becoming popular in other countries (Steinkraus, 1995). Apart from its unique texture properties, idli makes an important contribution to the diet as a source of protein, calories and vitamins, especially B-complex vitamins, compared to the raw unfermented ingredients (Reddy *et al*., 1982). From nutritional and health status point, idli appears to be an ideal human food for all ages and all times.

Idli is a white, fermented (acid leavened), soft, spongy texture product or steamed cake of rice (*Oryzasativum*) and gram dhal (*Phaseolusmungo*). Two significant changes occurring in idli fermentation are leavening and acidification of the batter (Jama and Varadaraj 1999). Starter culture 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 great importance. In this way, they enhance shelf life & microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product (Leroy and Vuyst 2004). Traditionally, rice and black gram in various proportions are soaked and ground adding water in mortar and pestle to yield a batter with the desired consistency. Black gram, the leguminous component of idli batter, serves not only as effective substrate but also provides the maximum number of micro-organism for fermentation (Ghosh and Chattopadhyay 2010).

The quality of idli varies widely in taste, flavor and texture due the variety of rice and dal used for the preparation, which directly points out towards the microflora of the variety of the rice and dal (Balasubramanian and Viswanathan 2007; Soni and Sandhu 1989). Idli preparation in the conventional manner takes at least 20-24 hours. Reduction in the fermentation time of the idli batter is of great commercial significance for large-scale as well as household idli production and can be potentially achieved by addition of enzymes or enzymes producing microorganisms. The available instant idli pre-mixes do provide the desired textural characteristic but lack the typical fermented aroma. On other hand, idli prepared in different households do not have consistent quality (Nisha *et al*., 2005). Fermented foods with scientifically developed cultures in appropriate concentration can aid the commercialization of these products.

Thus, the present study aimed to determine the effect of different microorganisms on idli batter fermentation and in turn on the quality of the idlis. Furthermore, we made an attempt to

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reduce the fermentation time by adding indigenous microorganisms isolated from the idlli batter, in different concentrations and combinations to the freshly ground idli batter.

MATERIALS AND METHODS

Collection of samples

Freshly ground Idli batter sample was collected from four Maharashtra restaurants and four South Indian restaurant as it has been observed that there is a significant difference in quality of idlis from these two areas.

Determination of Physico-Chemical properties

The physic-chemical properties namely pH, titrable acidity and batter volume were determined for all the samples at 8hrs, 18-20hrs, and 36hrs after obtaining the freshly ground idli batter.

pH

The idlibatter sample was diluted 1:1 in 10 ml of sterile distilled water and pH was determined by using a pH meter.

Titrable acidity

Titrable acidity was determined in terms of per cent of acid in the sample (Nielsen, 1994). Briefly, batter sample was diluted 1:1 in 10 ml of sterile distilled water and was titrated against 0.1 N NaOH.

Batter volume

Each batter sample was poured into sterile 100 ml measuring cylinder up to 50 ml mark, covered with aluminium foil and was incubated at 30^0C for different time points. Rise in batter volume due to CO_2 production after fermentation was determined using the markings on measuring cylinder.

Determination of Organoleptic properties

Organoleptic properties of idli samples were determined by 20 different volunteers. The point scores of these volunteers were measured on the basis of samples tested for their organoleptic properties namely appearance, colour, texture, flavour, taste and the percentage score for each parameter was calculated.

Identification of Microorganisms responsible for idli batter fermentation

Serial-dilution agar plating method was used to enumerate the total number of microorganisms present in idli samples. All the idli batter samples were serially diluted up to 10^6. These dilutions were spread on nutrient agar media and then incubated at 37^0C. Identification and characterization of bacteria was done using a series of cultural, morphological and biochemical tests given by Bergy's manual of determinative.

Additional Inoculum preparation

The bacteria isolated from idli batter samples were enriched in 50 ml Nutrient Broth, incubated overnight on shaker and their cfu/ml was determined. These bacteria were then added, in different concentrations and different combinations (Table 1a and 1b) to 50 ml freshly ground idli batter samples (South sample 2 and Maharashtra sample 5) and incubated for 2 hours at room temperature.

Table 1a Combinations of isolated organisms added to South Sample

Microorganisms	cfu/ml	Comb 1 (µl)	Comb 2 (µl)	Comb 3 (µl)	Comb 4 (µl)
Leuconostoc spp.	7×10^6	1000	400	350	600
Lactococcus spp.	7×10^4	350	350	1000	600
Lactobacillus spp.	8×10^4	400	1000	400	550

Table 1b Combinations of isolated organisms added to Maharashtra Sample

Microorganisms	cfu/ml	Comb 1 (µl)	Comb 2 (µl)	Comb 3 (µl)
Leuconostoc spp.	7×10^6	1000	2000	4000
Lactococcus spp.	7×10^4	2000	4000	8000
Lactobacillus	8×10^4	1500	3000	6000

RESULTS

Physio-chemical properties of idli batter samples

pH of the batter

pH of the batter decreased with an increase in the hours of fermentation. South sample 2 showed the lowest pH of 4.1 after 36 hrs whereas, Maharashtra Sample 5, showed the lowest pH value 4.5 (Table 2a & 2b).

Titrable acidity

In titrable acidity parameter, South Sample 2 showed highest acidity value of 17.5 ml whereas; Maharashtra Sample 5 showed highest acidity value of 15.6 ml (Table 2a & 2b). Thus, titrable acidity increased with increase in hours of fermentation.

Batter volume

Batter volume of South Sample2rose to a highest of 82 ml whereas; Maharashtra Sample 5 rose to a maximum volume of 78 ml (Table 2a & 2b). The rise in batter volume was found to be proportional to the rise in the hours of the fermentation. Thus, indirectly pointing out the generation of CO_2, which is the major reason for the softness of the idlis.

Table 2a Physio-Chemical Properties for South Samples

	Time (hours)	S1 (South)	S2 (South)	S6 (South)	S7 (South)
Ph	8	4.69	4.54	6.8	9.8
	18-20	4.5	4.3	5.6	7.2
	36	4.3	4.1	4.5	5.1
Acidity (ml)	8	13.8	13.6	12.8	10.0
	18-20	14.5	14.0	13.3	11.8
	36	17.1	17.5	15.4	12.7
Volume (ml)	8	50	50	50	50
	18-20	72	68	71	75
	36	80	82	80	78

Table 2b Physio-Chemical Properties for Maharashtra Samples

	Time (hours)	S5 (Maharashtra)	S8 (Maharashtra)	S3 (Maharashtra)	S4 (Maharashtra)
pH	8	7.2	8.3	5.8	5.6
	18-20	6.4	7.0	5.4	5.2
	36	4.5	5.0	4.6	5.0
Acidity (ml)	8	13.3	10.0	9.7	9.8
	18-20	14.8	13.1	11.3	10.5
	36	15.6	13.7	12.0	10.7
Volume (ml)	8	50	50	50	50
	18-20	58	65	52	62
	36	78	76	55	76

Determination of Organoleptic Properties

The percentage calculated for all the criteria to evaluate the quality of idlis for all eight samples showed that there was a wide variation in the qualities of idlis prepared from South and Maharashtra batter sample (Table 3a & 3b). South sample 2 and Maharashtra sample 5 showed highest percentage in terms of all parameters and thus were found to be acceptable in terms of appearance, colour, texture, flavour and taste.

Table 3a Percentage of Appearance, Colour, Texture, Flavours, Taste for South Samples

Samples	Appearance			Colour		Texture			Flavours			Taste		
	V.G	G	S	W	C	3+	2+	1+	V.G	G	S	V.G	G	S
S1	35	45	15	0	100	25	60	15	20	60	4	35	60	5
S2	35	55	10	95	5	60	40	0	40	60	0	35	65	0
S6	15	70	15	70	30	40	50	10	30	70	0	25	65	10
S7	15	55	30	30	70	45	45	10	25	50	25	30	5	45

Note: Total number of volunteers = 20
V.G – Very Good, G- Good, S –Satisfied, W – White, C – Creamy

Table 3b Percentage of Appearance, colour, Texture, Flavours, Taste for Maharashtra Samples

Samples	Appearance			Colour		Texture			Flavours			Taste		
	V.G	G	S	W	C	3+	2+	1+	V.G	G	S	V.G	G	S
S4	20	40	40	25	75	20	45	35	25	50	25	35	25	40
S3	25	45	30	85	15	25	35	40	15	55	30	25	55	20
S5	40	60	0	95	5	50	30	10	45	40	5	45	50	5
S8	35	60	5	95	5	35	40	25	30	40	30	35	45	20

Note: Total number of volunteers = 20
V.G – Very Good, G- Good, S -Satisfied

Isolation, identification and characterization of the microorganisms isolated from different Idli batter samples

Based on the cultural, morphological and biochemical characters, the organisms isolated from south samples were *Lactococcuspiscicum, Leuconostocmesenteroides, Lactobacillus delbruckii* and that isolated from Maharashtra samples were *Leuconostoccitreum, Lactobacillus fermentum, Lactococcuspiscicum* and *Micrococcus roseus* (Table 4). Since the molecular identification of these isolates was not performed, we would mention only the genus of the isolates throughout the article.

Table 4 Total Plate count in idli batter samples

South sample	TPC (CFU/ml)	Bacteria
Sample 1	77×10^5	*Lactococcus spp.*
Sample 2	33×10^4	*Leuconostoc spp.* *Lactococcus spp.* *Lactobacillus spp.*
Sample 3	60×10^5	*Lactobacillus spp.*
Sample 4	28×10^3	*Lactobacillus spp.*
Maharashtra sample	TPC (CFU/ml)	Bacteria
Sample 5	37×10^4	*Leuconostoc spp.* *Lactobacillus spp.*
Sample 6	28×10^3	*Lactococcus spp.*
Sample 7	25×10^4	*Lactobacillus spp.*
Sample 8	40×10^4	*Lactobacillus spp.* *Leuconostoc spp.* *Micrococcus spp.*

Effect of additional inoculum to the idli batter

By using the microorganisms isolated from the idli batter samples which scored highest in terms of their organoleptic properties (Table 5), in different concentrations and combinations, an attempt to reduce the time of idli batter fermentation was made. After incubation of two hours with the additional inoculum, the batter volume was increased significantly for all combinations, but the highest rise was seen in Comb1 for South sample and in Comb3 for Maharashtra sample (Table 6a & 6b). This rise was almost similar to the rise in original batter volume after 36 hours. Thus the additional inoculum fastened the process of fermentation and indeed the formation of CO_2 which led to the rise in the volume.

Table 5 Parameters of two idli batter samples which scored highest in terms of their organoleptic properties

Name of the Sample	pH	Acidity	volume	TPC(cfu/ml)	Microorganisms isolated
South sample 2	4.1	17.5	82	33×10^4	*Leuconostoc spp.* *Lactococcus spp.* *Lactobacillus spp.*
Maharashtra sample 5	4.5	15.6	78	37×10^4	*Leuconostoc spp.* *Lactobacillus spp.*

Table 6a Effect of additional inoculum inidli batter (South Sample) on volume

Combinations	Incubation Period	Initial volume (ml)	Final volume (ml)
1	2	50	75
2	2	50	68
3	2	50	73
4	2	50	71

Table 6b Effect of additional inoculum in idli batter (Maharashtra Samples) on volume

Combinations	Incubation Period	Initial volume (ml)	Final volume (ml)
1	2	50	63
2	2	50	68
3	1	50	71

Comparison of Organoleptic properties

The idils prepared from Comb1 for South sample and Comb3 of Maharashtra sample, were found more acceptable in terms of appearance, colour, texture, flavour and taste and increase in volume within the fermentation period of 2 hours (Table 7a & 7b).

Table 7a Organoleptic evaluation of idlis produced from batter with additional inoculum (South Sample)

Combinations	Appearance			Colour		Texture			Flavours			Taste		
	V.G	G	S	W	C	3+	2+	1+	V.G	G	S	V.G	G	S
Comb1	60	30	10	80	20	65	25	10	50	30	20	55	35	10
Comb2	40	35	25	65	35	45	30	25	35	35	30	35	35	30
Comb3	30	35	35	50	50	45	35	20	30	35	35	35	35	30
Comb4	35	30	35	55	45	40	35	25	25	30	45	35	40	25

Note: Total number of volunteers = 20
V.G – Very Good, G- Good, S -Satisfied

Table 7b Organoleptic evaluation of idlis produced from batter with additional inoculum (Maharashtra Sample)

Combinations	Appearance			Colour		Texture			Flavours			Taste		
	V.G	G	S	W	C	3+	2+	1+	V.G	G	S	V.G	G	S
Comb1	30	35	35	50	50	45	35	20	30	35	35	35	35	30
Comb2	30	30	40	45	55	40	30	30	35	35	30	35	30	35
Comb3	55	25	20	70	30	50	35	15	55	35	10	55	35	10

Note: Total number of volunteers = 20
V.G – Very Good, G- Good, S -Satisfied

DISCUSSION

Comparison of South sample & Maharashtra sample showed that South sample possess low pH, high acidity, more rise in volume with increase in hours of fermentation. All these properties are mainly due to the role played by Lactic Acid Bacteria (LAB). Most of the organisms isolated from different idli batter are *Leuconostocmesenteroides* and *Streptococcus faecalis*as the dominant microorganisms. The other organisms are *Saccharomyces cerivisiae*, *Debaromyceshansenii, Hansenulaanomala*, *Torulopsis candida, Trichosporonbeigelii*and *Pediococcuscerivisiae*. And some others isolated *Lactobacillus fermentum*, *Torulopsis*sp., *Candida* sp. and *Trichosporonpullulans* (Purushothaman *et al*., 1993; Agrawal *et al*., 2000). In consistent with these findings, we also isolated *Leuconostoc sp., Lactococcus sp., Lactobacillus sp.* from South samples and *Micrococcus sp., Leuconostoc sp., Lactobacillus sp.* from Maharashtra samples.
The organoleptic evaluation of all the eight samples showed that there was wide variation in samples from South and Maharashtra. The reason could be different concentrations of the indigenous fermenting bacteria in the respective batter. Heterofermentative *Leuconostoc*is known to be responsible for rise of batter and its aroma, while the homofermentative *Lactococcus* provide flavour (Mukherjee *et al*., 1965). Thus, in the present study, South Sample 2 containing *Leuconostocspp., Lactococcusspp., Lactobacillus sp.* (TPC 33 X 10^4) & Maharashtra Sample5 containing *Leuconostocspp. and Lactobacillus sp.* (TPC 37 X 10^4) were found to be acceptable in terms of appearance, colour, texture, flavour and taste and hence these samples were considered ideal for inoculum preparation for time reduction experiments.

Increase in acidity level with increase in time was observed which justify the decrease in the pH values of the batter. This may be, mainly associated with the development of lactic acid which lowers the pH and produces carbon dioxide, which results in leavening of the batter. The reason for increase in batter volume can be attributed to the microbial growth and secretion of enzymes, which catalyse the hydrolysis of carbohydrates, lipids, proteins, anti-nutritional and toxic factors (Rolle, 1998). Some reported the addition of sour butter milk or commercial yeast pellets to the idli batter enhanced the leavening (Sridevi *et al*., 2010). With this idea in the background, we attempted to rise up the batter using the native bacteria, in additional concentration. That is, instead of allowing the native bacteria to grow and increase in number over a time span of 8-10 hours, we increased the number by adding extra inoculum of the native bacteria allowing them to speed up the fermentation process.

In the present study, addition of isolated microorganisms in different concentrations and combinations showed that combination1 for South sample and combination3 for Maharashtra sample were more effective in yielding good quality idlis as determined from the organoleptic properties. These samples were found significantly acceptable in terms of appearance, colour, texture, flavour and taste within the fermentation period of only 2 hours. The volume of the batter raised for these samples, after a period of 2 hours of incubation, was maximum and significant. Almost same rise in volume was observed after 36 hours when no extra inoculum was added. A similar study explored the possibility of expediting the idlibatter fermentation process by adding an exogenous source of α- amylase enzyme (5, 15 and 25 U per 100 g batter of amylase) to the idli batter (Iyer and Ananthanarayan 2008). Different parameters were monitored and sensory attributes were also studied and compared with that of the control set. The fermentation time was reduced from a conventional 14 h to 8 h and the sensory attributes of the final product were also successfully maintained.

CONCLUSION

Reduction in the fermentation time of the idli batter is of great commercial significance for large scale idli production and can be potentially achieved by addition of isolated microorganisms. Attempt of reducing time of fermentation by adding extra amount of known and intended microorganisms to the freshly ground idli batter was successful to an extent but further detail studies are needed to commercialize the idea.

Acknowledgement

Authors are thankful to Dr. D. Y. Patil Arts, Commerce & Science College, Pimpri, Pune, for providing facilities available at their laboratory.

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How to cite this article:

Panicker, SG., Zate, A and Patil, M.2017, An Attempt To Reduce The Fermentation Time For Making Idlis Faster. *Int J Recent Sci Res.* 8(6), pp. 17752-17756. DOI: http://dx.doi.org/10.24327/ijrsr.2017.0806.0416

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