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ORIGINAL ARTICLE

Bio-fortification and shelf-life extension of *idli* batter using curry leaves (*Murraya koenigii*)

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Abstract Among several traditional foods of India, *idli* is one of the most popular and commonly consumed steamed products. A new method of adding *Murraya koenigii* (curry leaves) to *idli* batter as a vehicle for fortification and extension of shelf-life has been developed. Dried curry leaves powder was incorporated with other ingredients like rice and dehusked black gram in different proportions to optimize the most palatable formulation. Rate of fermentation and microbial changes in the batter; nutritional qualities, texture and sensory properties of the prepared product were assessed. Incorporation of curry leaves powder (5 %) in *idli* batter increased the shelf-life and also increased the flavour, texture and appearance of the *idli*. The calcium content of the prepared *idli* was 10 times more than that of the control *idli*, while dietary fiber content increased by 18.6 %. Anti-microbial activity of the curry leaves in *idli* batter extended the shelf-life from 2 to 5 days when stored at 30 °C.

Keywords *Idli* · Shelf-life · Fortification · Curry leaves · Calcium

Introduction

Idli is one of the popular foods consumed throughout India and is also becoming popular in other countries. In the pursuit of shelf-life extension of ready to cook *idli* batter, microbial population dynamics of fermentation, time and temperature, batter characteristics and end products of fermentation needs to be understood.

Curry leaves plant is native to India and Srilanka which is available at very low cost. Curry leaves are sweet smelling leaves of small tree, *Murraya koenigii* (Linn.) Spreng. of *Rutaceae* family. The leaves are slightly bitter and highly aromatic. It is a perennial leafy vegetable and primarily used in Indian cooking to provide flavor for vegetables, pickles, soups, as well as meat. They add to smell and taste of food in addition to food value. They are rich in vitamins A (carotene 21,000 µg, β-carotene 7110 µg), B, C, E and minerals such as calcium 830 mg, phosphorus 57 mg and iron 0.93 mg per 100 g of fresh leaves (Gopalan 1998).

It helps in digestion, is good for eyesight, may help to prevent the growth of cataract and is a good remedy for heart burn, nausea and vomiting. It has been reported to have antioxidant, antimicrobial, anti-diabetic and anti-dysenteric activities (Yankuzo et al. 2011). It is also known to have anti-inflammatory, anticancer, antinociceptive, antihelminthic, anticholinesterase and antiamnesic activities (Tembhurne and Sakarkar 2011).

The bioactive components include caryophyllene, cadinene, cadinol, sabinene, pinene, phellandrene, terpinene, lauric acid, palmitic acid, carbazole alkaloids, bornyl acetate and humulene. Carbazole alkaloids have antiplatelet activity, vasorelaxing effects and anticarcinogenic effects in dimethylhydrazine (DMH) treated rats (Khanum et al. 2000). Marked reduction of systolic and diastolic blood pressure was observed among 20 hypertensive patients whose diet was supplemented with curry leaves chutney containing 5 g of curry leaves powder for 60 days and this has been attributed to the presence of bioactive compounds in curry leaves. They are used in very small

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quantities and because of their slightly hard texture they are generally discarded from the dish while eating. Hence, the nutrition potential of curry leaves remains underutilized. One way to ensure greater consumption of curry leaves is to use them in dried form (Paul et al. 2013).

The products made include tea fortified with dried curry leaves powder, ready to drink beverage containing extract of curry leaves with lemon juice and sugar in tetra packs, antacid mix made from curry leaves powder, cumin powder and salt which can be an alternative for other antacids available in market and spiced paste made from curry leaves.

Although its potential to inhibit microbial growth has been documented, the property has not been exploited so far in food systems. This research has been carried out to overcome short shelf-life and enhance the nutrient content of commercially available *idli* batters.

Materials and methods

Procurement of raw materials

Rice (CR1009 variety) and black gram dhal (urad dhal) (Vamban 6 variety) were bought from the local market and stored at room temperature in appropriate containers until used. Fresh curry leaves (Cenkampu variety) purchased from the market were air-dried (30 ± 2 °C) for 6 h and ground to fine powder and stored at room temperature in air tight container until used. Commercial *idli* batter was purchased from the local market and used for the comparison studies. The standard preservatives sodium benzoate and sodium metabisulfite were purchased from Himedia, Mumbai.

Control Idli batter preparation (CB)

Rice (CR1009) and black gram in the ratio 3:1 (w/w) were washed separately with tap water to remove the dust and soaked in twice the amount of drinking water for 4 h at room temperature (25 °C). *Idli* batter was prepared by grinding soaked rice (coarse batter) and dhal (smooth batter) in a grinder with addition of water. Salt (2 % w/w) was added and the batter was mixed well with hand and allowed to ferment in an incubator at 30 °C for 24 h. To study the physico-chemical properties during fermentation, samples of fermented batter were drawn at 4 h intervals and subjected to analyses.

Curry leaves idli batter preparation (CLB)

Idli batter fortified with curry leaves was prepared by mixing dry curry leaves powder (5 % w/w) to the 12 h fermented *idli* batter. The fermentation was carried out for 24 h and samples drawn at 4 h intervals for analyses as in the control batter.

Batter characteristics

Moisture

The moisture content was determined by moisture analyzer (Sartorius, MA 35). A minimum of two grams of the batter sample was used and the value was recorded as percentage moisture.

pH and titratable acidity (TA)

Sample of 10 g batter was mixed with 100 mL of distilled water and vortexed for 2 min and the pH determined 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.

Bulk density

Idli batter (100 mL) was measured in a measuring cylinder and its weight determined in an analytical balance (BL220H, Shimadzu, Japan) to determine the bulk density (ratio of mass by volume) of the sample and expressed as kg m^{-3}.

Viscosity measurement

Batter (100 mL) was taken for all viscosity measurements. The viscosity of the fermented batter was measured using Brookfield Viscometer model DV-E with disc spindle (S-62, S-18) speeds of 20, 50 and 100 rpm.

Texture

The parameters like firmness, consistency, cohesiveness, index of viscosity of the *idli* batters were measured using a TAXT2 Texture Analyzer (Stable Micro system, USA) equipped with the back extrusion probe.

Color

The color of the *idli* batter was measured using color measurement device (Ultra Scan VIS, Hunter Associates Laboratory, Reston, Virginia, USA). The L* denotes the whiteness level. The scale for a* varies from green (negative) to red (positive) and the scale for b* corresponds to a yellow-blue scale on which yellow is positive.

Particle morphology analysis by scanning electron microscope (SEM)

Idli and *idli* batter were oven-dried at 55 °C and crushed using mortar and pestle. Using 1 mm sieve coarse and fine particles were separated. Fine particles were taken for SEM analysis. The surface of the samples were examined with a scanning electron microscope (Hitachi S-2400) at 30 kV accelerating voltage. The surface morphology of particles at different fermentation time was observed.

Microbiological changes

The *idli* batter samples were subjected to microbiological analysis and the following tests were done.

Total bacterial count

Idli batter sample of 10 g was diluted in 90 mL of 0.85 % sterile saline. It was then serially diluted and spread plated on plate count agar. The plates were incubated at 37 °C for 48 h and the colonies were counted and expressed as $CFUg^{-1}$ of the batter.

Lactic acid bacteria (LAB) count

Idli batter sample of 10 g was diluted in 90 mL of 0.85 % sterile saline. It was serially diluted and spread plated on Man, Rogosa, and Sharpe (MRS) agar. The plates were incubated at 37 °C for 48 h and the colonies were counted and expressed as $CFUg^{-1}$ of the batter.

Yeast and mold count

Idli batter sample of 10 g was diluted in 90 mL of 0.85 % sterile saline, serially diluted and spread plated on Sabouraud Dextrose Agar (SDA) containing chloramphenicol 0.050 g/L). The plates were incubated at 25 °C for 3–5 days and the colonies were counted and expressed as $CFUg^{-1}$ of the batter.

Characteristics of curry leaf powder

Preparation of extract

Five grams of curry leaves powder was mixed with 20 mL methanol in closed opaque glass bottles and left for 24 h at 40 °C. The supernatant was separated by centrifugation at 3500 rpm for 10 min followed by addition of 20 mL chloroform to the dry residue and kept for incubation as mentioned above. The extraction procedure was repeated with hexane. Each of the solvent extracts were evaporated separately in dry bath at 55 °C and the final residue were dissolved in double distilled water, pooled and stored at 4 °C until analysis.

Antimicrobial activity

The antibacterial activity of curry leaf extract was determined using agar well diffusion method. Nutrient agar was inoculated with microorganisms by spread plate method. The microorganisms used were given in Table 4. The bacterial strains were obtained from Microbial Type Culture Collection (MTCC) and gene bank at Institute of Microbial Technology, Chandigarh, India. The yeast strains were isolated from idli batter. Wells were punched in agar using punch borer (4 mm) and loaded with the extract (25–100 µL). The plates were incubated at 37 °C for 24 h and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition (Holt et al. 1994).

Phytochemicals in curry leaves and curry leaves idli by GC–MS

Presence of active components in the solvent extracts of curry leaves prepared with methanol was detected by GC–MS (Gas Chromatography-Mass Spectroscopy). The GC–MS analysis of the samples was performed on an Aglient 6890 N Series gas chromatograph coupled with mass spectroscopy. The samples were injected into a DB 5 ms capillary column (30 m length × 250 mm internal diameter × 25 µm film thickness). The carrier gas was helium at a flow rate 1.0 mL min^{-1}, linear velocity 37 cm s^{-1}. The initial column temperature was 80 °C, then increased linearly at 10 up to 150-5 up to 280 °C and held for 5 min. The total run time was 34 min. The temperature of the injection port was 280 °C and interface temperature was 28 °C. The injection volume was 2 µL. The mass spectra were recorded in Electron Ionization (EI) mode at 2006 eV. Compound identification was accomplished by comparing the retention times with those of authentic compounds and fragmentation pattern, as well as with mass spectra in the NIST spectral library stored in the computer software (version MSD CHEM STATION, G1701EAE02.00.493) of the GC–MS. The quantification of the compounds was derived from the percentage area.

Detection of functional groups by FTIR

Fourier transform infrared spectrophotometer (FTIR) CARY 630 (Agilent Technologies Pvt. Ltd. Germany) was used for the analysis. The measurement was based on the Universal Diamond ATR with spectral range exactly between 4000 and 650 cm^{-1} and spectral resolution 4 cm^{-1}. The scan no. is 8. High sensitivity Deuterated L-

Alanine Tri-Glycine Sulfate (DLATGS) detector was used. The samples tested were 3–5 mg of dry solid powder of the pooled extract and 5–10 μL of liquid in the case of hexane extract.

Preparation of control idli (CI) and curry leaves idli (CLI)

The 12th h fermented *idli* batter of both control and curry leaves were cooked by steaming for 15 min in *idli* moulds and the *idlies* were studied for the following characteristics.

Idli characteristics

Color

Color of *idli* is an important quality factor for consumer acceptance. Among L*, b* and a* parameters, lightness is considered more important as color attribute for *idli*. The surface color of *idli* samples were made as in the case of batter.

Texture analysis of idli

The hardness/firmness of the *idli* (based on the gas pockets entrapped in the *idli*) were measured using a TAXT2 Texture Analyzer (Stable Micro system) equipped with the AACC 36 mm cylindrical probe (P/36R). Firmness is defined as the force (in grams, kilograms or Newtons) required for penetrating the product. The maximum force was recorded as the hardness of the *idli*.

Internal structure of idli by ink print test

A single *idli* was cut across evenly into two equal halves. An ink print of the cross-section was made to study the internal structure of the pores in the product (Nazni and Shalini 2010).

Nutrient composition

The proximate composition was determined according to the Association of Official and Analytical Chemists (AOAC 2005) methods. Dietary fiber, calcium and iron content was analysed by AOAC 991.43 and AOAC 935.13 methods respectively (AOAC 2003).

Sensory analysis

The sensory characteristics of *idli* made from batter fermented for 12 h were determined using a sensory panel consisting of 20 people including both genders in age groups 18–45 years. Samples of identical size, shape, and quantity were prepared and served in plain, identical, white trays. The panelists had no prior information about the test products. They were asked to evaluate and compare the *idli* samples with control *idli* which was prepared from commercial *idli* batter. The attributes of color, appearance, texture, mouth feel, taste, flavor and overall acceptability were evaluated by the panelists using the 5-point hedonic scale. The scale of values was given with descriptive terms. The order of presentation was balanced and randomized to eliminate contrast effect and positional bias. The samples were presented under controlled laboratory conditions illuminated with white incandescent lights.

Storage studies of batter

Fresh ground *idli* batter (200 g each of control and fortified, in 5 batches) were packed in metallized polyethylene pouches, sealed and stored at 4 and 30 °C for 5 days. One pack of each batter was used every day for all the analysis mentioned in batter characteristics.

Sensory analysis of idli from stored batter

Idli was prepared every day from both the control and curry leaves batter stored at 30 and 4 °C and subjected to sensory analysis as described above.

Statistical analysis

All analytical tests were carried out in triplicates and expressed as mean ± standard deviation. ANNOVA was used to determine the statistical significance. p value was calculated from ANNOVA using Microsoft office excel programe (version 2010).

Results and discussion

pH and titratable acidity

The results relating to change in pH and titratable acidity of *idli* batter during fermentation are presented in Fig. 1a, b. The increasing acidity of the batter due to the production of lactic acid by lactic acid bacteria was clearly evident. The pH of commercial batter (4.32 ± 0.02) was similar to the 16 h fermented sample.

Addition of curry leaves to the 12 h batter reduced the rate of fermentation as there was not much change in the pH values ($p < 0.05$). Also, inhibition of fermentation leading to slow change in pH was observed on the addition of curry leaf powder at the start of fermentation (0 h batter). The anti-microbial activity of curry leaves (Table 4) may be responsible for the reduced fermentation rate due to

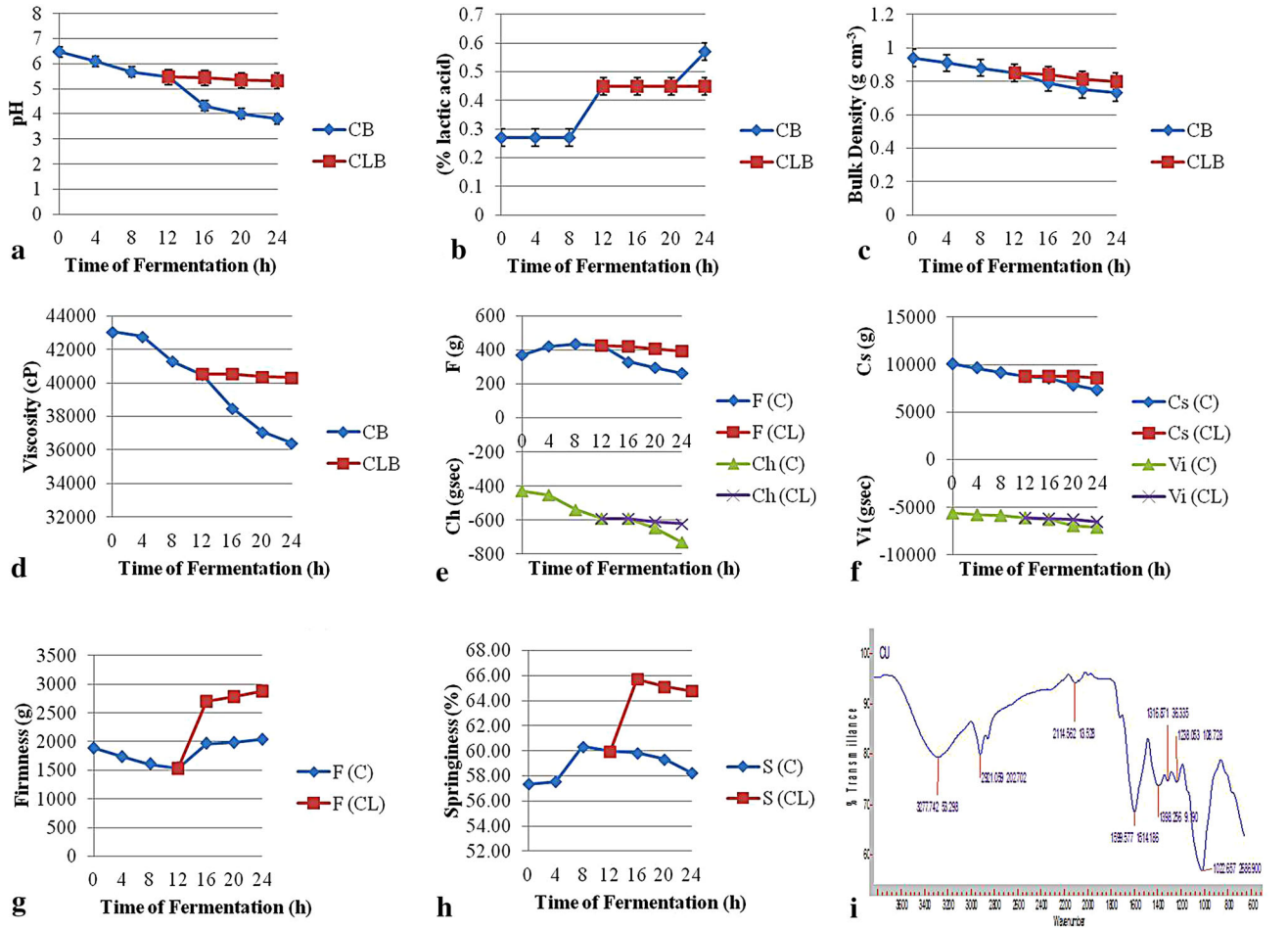

Fig. 1 Physical properties of the control and curry leaves idli batter. **a** pH of control and curry leaves idli batter. **b** Titratable acidity of control and curry leaves idli batter. **c** Bulk density of control and curry leaves idli batter. **d** Viscosity of control and curry leaves idli batter. **e** Textural characteristics (firmness and cohesiveness) of control and curry leaves idli batter. **f** Textural characteristics (consistency and index of viscosity) of control and curry leaves idli batter. **g** Textural characteristics (firmness) of control and curry leaves idli. **h** Textural characteristics (springiness) of control and curry leaves idli. **i** FTIR analysis of curry leaves powder

suppression of bacterial growth. In case of curry leaves batter the pH 4.32 ± 0.02 (control batter, 16 h) was not obtained even after 24 h of fermentation.

Balasubramanian and Viswanathan (2007) who studied *idli* batter from polished parboiled rice and decorticated black gram blend in a ratio of 2:1, 3:1 and 4:1 (v/v) reported pH and titratable acidity in the range of 5.9–4.1 and 0.443–0.910 %, respectively. Annan et al. (2005) reported that the pH of the control batter and soybean batter was almost the same but the acidity was found to be significantly higher in the fortified batter. This is due to the buffering effect caused by the higher content of soluble proteins, amino acids and also free fatty acids of the beans. Likewise, Rekha and Vijayalakshmi (2011) observed the increase in acidity within 2 h of natural fermentation in control batter and after 6 h of natural fermentation in okara fortified batter resulting in accelerated fermentation. Earlier, Soni and Sandhu (1991) also reported the role of lactic acid bacteria in reducing the pH up to 4.4–4.5 and increasing the acid content of batter as the fermentation progressed, providing a favourable environment for the prominent growth of yeast.

Bulk density and viscosity

Bulk density of batter gradually decreased with increase in fermentation time as seen from Fig. 1c which may be due to the CO_2 production resulting from the metabolic activity of hetero-fermentative lactic acid bacteria and yeast (Thyagaraja et al. 1992). The volume of batter doubled after 12 h of fermentation. Bulk density of 12 h fermented batter (0.85 ± 0.2 g cm^{-3}) was slightly higher than the commercial batter (0.93 ± 0.01 g cm^{-3}). Addition of curry leaves had slight changes in the bulk density of the

batter (0.8 ± 0.01 g cm^{-3}) when compared to the control (0.73 ± 0.02 g cm^{-3}) during 24 h of fermentation.

These findings are in accordance with the data reported by Rekha and Vijayalakshmi (2011) where at the end of 10 h natural fermentation at 30 °C there was a 20 % increase in volume in the unfortified *idli* batter and a 55 % increase in volume in okara fortified batter. Ghosh and Chattopadhyay (2011) reported that increase in blend ratio significantly increased the batter density. Balasubramanian and Viswanathan (2007) reported similar bulk density between 0.94 and 0.59 g cm^{-3} in *idli* batter with different ratios of rice and black gram [2:1, 3:1 and 4:1 (v/v)]. The blend ratios didn't affect the bulk density whereas it decreased with increase in fermentation time.

As in the case of bulk density, viscosity of the curry leaves fortified batter showed a slow decrease when compared to the unfortified batter (Fig. 1d). The steep decrease in the viscosity of the control batter was due to the CO_2 production by microbial action during fermentation which was inhibited/reduced in the case of curry leaves batter during the 24 h fermentation period.

Texture analysis

Texture of batter in terms of firmness (F) showed little change till 8th h of fermentation after which it decreased significantly, while consistency (Cs) decreased steadily during 24 h of fermentation (Fig. 1e, f). Both firmness and consistency were least in the commercial batter. This may be due to the variation in ingredients and processing method, as well as the chemical leavening agents added. In contrast, cohesiveness (Ch) and index of viscosity (Vi) decreased with fermentation time and the commercial samples showed the highest values. Firmness of control *idli* batter was 33.4 % lesser than the curry leaves *idli* batter. The changes in texture among the samples during the fermentation may be due to the production of acids and CO_2 by the microorganisms as well as particle size variation of the ingredients.

Colour characteristics

The control and commercial batter showed similar colour characteristics while curry leaves batter showed variation in L*, a*, and b* values due to the addition of curry leaf powder. The lightness value decreased from 86.5 to 51.61 indicating a darker coloured batter with slight greenish tinge (−0.49, 7.20 to +0.05, 15.11) as shown from the colour values given in Table 1. Similar colour changes were also observed for the *idli* with a greenish product prepared from curry leaves batter rather than the white coloured product prepared from conventional *idli* batter.

Table 1 Colour characteristics of idli batters and idli

	L*	a*	b*
Control batter	83.82 ± 0.02	−0.34 ± 0.01	9.24 ± 0.06
Commercial batter	85.12 ± 0.4	−0.43 ± 0.08	8.62 ± 0.08
Curry leaves batter	51.61 ± 0.01	0.05 ± 0.11	15.11 ± 0.3
Control idli	85.91 ± 0.04	−0.20 ± 0.15	11.36 ± 0.07
Curry leaves idli	40.48 ± 0.03	1.98 ± 0.31	14.25 ± 0.09

Microbial analysis

Rate of fermentation

The fermentation is dominated by lactic acid bacteria (LAB) whose count increased steadily from 4 h and reached a maximum at 12 h. The total bacterial count (TBC) which was higher initially, increased till 8 h of fermentation and then decreased significantly (Fig. 2a). The decrease in non-LAB counts may be due to the antagonistic action of LAB producing lactic acid, hydrogen peroxide and bacteriocins as well as decrease in pH resulting in unfavourable growth of non-LAB. Although moulds were detected in the unfermented batter up to 4 h of fermentation, they were not detectable subsequently with the progress in fermentation. Yeasts however increased and reached a maximum at 16 h and remained unchanged till 24 h, contributing to the leavening of the batter. Addition of curry leaf powder to the 12 h fermented batter resulted in lesser growth of bacteria and yeast was completely inhibited. Rekha and Vijayalakshmi (2011) reported that in okra fortified *idli* batter there was a gradual increase in mesophilic bacterial and LAB count with fermentation time as well as yeast and mould count. Thus the bacterial counts were higher in okra fortified batter than control batter. It has been previously reported that ingredients in foods such as black gram affect the microbial population as they provide a source of nutrients for the growth of microorganisms during fermentation (Ghosh and Chattopadhyay 2011). The steady increase in lactic acid bacteria found in sourdough was enhanced by the presence of amino acids (Gobetti et al. 1994). Reduction in fermentation time of *idli* batter from 14 to 8 h was achieved by addition of an exogenous source of α-amylase enzyme by Iyer and Ananthanarayan (2008).

Storage studies

Storage of *idli* batters was done at two temperatures (30 and 4 °C) (Fig. 2b, c). TBC decreased steadily with increasing storage time both at 30 and 4 °C, although the counts were higher at lower temperature. Similarly, the

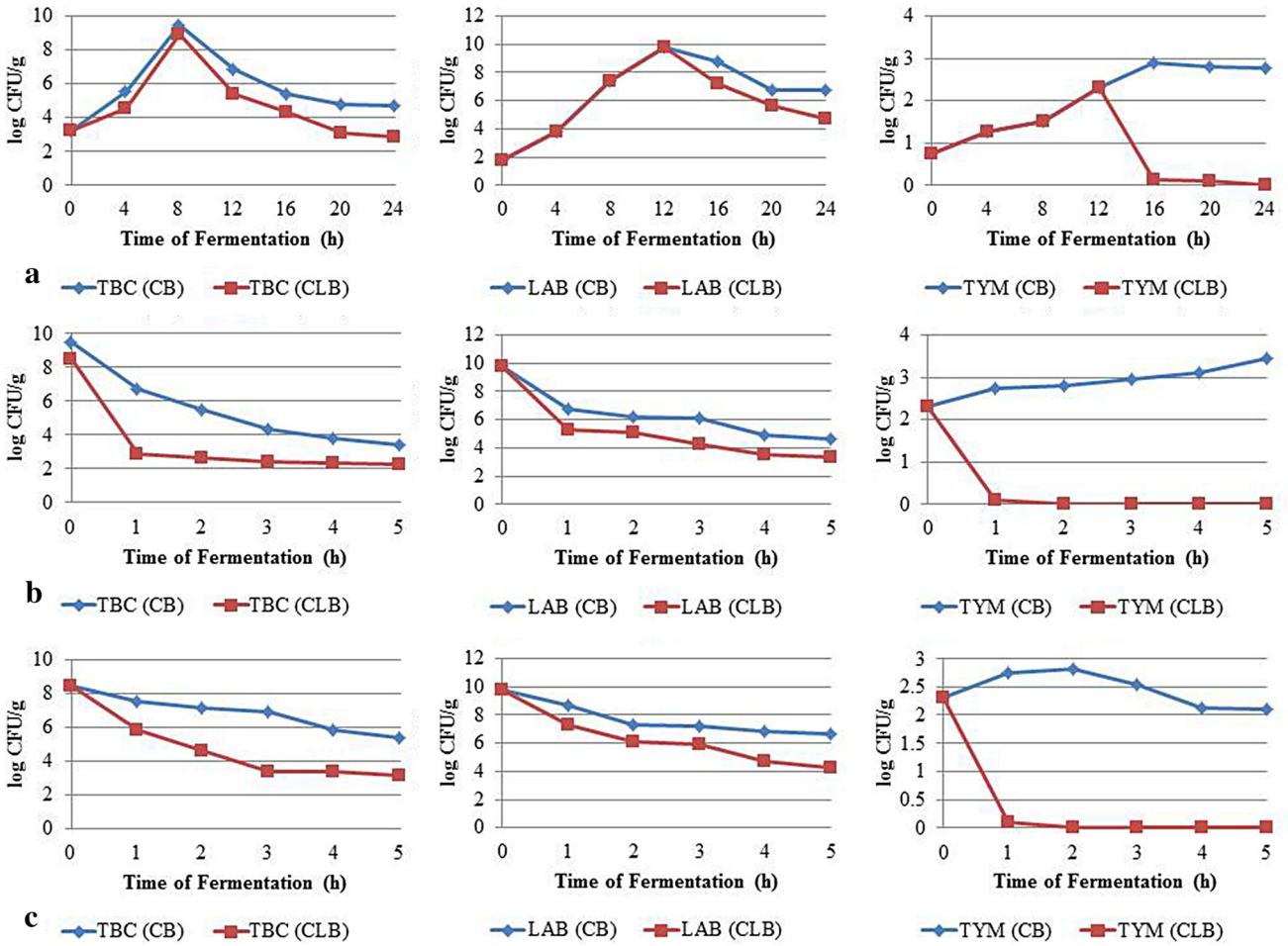

Fig. 2 **a** Microbial changes during 24 h of fermentation of control and curry leaves idli batter. **b** Microbial changes during storage of control and curry leaves idli batter at 30 °C for 5 days. **c** Microbial changes during storage of control and curry leaves idli batter at 4 °C for 5 days

LAB count also showed a decrease but at a slower rate with better retention at 4 °C than 30 °C. Curry leaf powder in batter stored at 30 °C suppressed TBC significantly from the first day itself and counts remained steady till 5th day. When stored at 4 °C, lower suppression was seen. In the case of curry leaves batter LAB suppression was less significant and the counts were higher in batter stored at 4 °C. Similar observations of low yeast and mould count have been documented by Sridevi et al. (2010) on *idli* batter stored for 5 days at 30 and 10 °C when compared to LAB count.

Yeast counts showed a different response on storage compared to LAB. There was a steady increase in yeast at room temperature in the control batter whereas the counts remained steady at 4 °C. In contrast yeasts were completely suppressed at both temperatures in the curry leaves batter from day 1. The slower growth of microbes in curry leaves batter could be due to the presence of antimicrobial compounds which apparently affected yeasts more than bacteria.

Nutritional analysis

The proximate composition of the prepared *idlies* from both the batters (control and curry leaves batter) are given in Table 2. The crude protein and fat content were slightly

Table 2 Composition of idli prepared with and without curry leaves

	Control idli	Curry leaves idli
Moisture (%)	66 ± 0.3	64 ± 0.06
Carbohydrate (%)	17.13 ± 0.1	18.46 ± 0.3
Crude protein (%)	11.89 ± 0.08	12.25 ± 0.4
Fat (%)	0.19 ± 0.03	0.54 ± 0.01
Ash (%)	0.21 ± 0.09	3.76 ± 0.3
Total dietary fiber (g/100 g)	22.23 ± 0.1	26.36 ± 0.07
Soluble fiber (g/100 g)	7.53 ± 0.05	14.15 ± 0.02
Insoluble fiber (g/100 g)	14.7 ± 0.4	12.22 ± 0.08
Calcium (mg/100 g)	26.5 ± 0.07	228.42 ± 0.15
Iron (mg/100 g)	6.12 ± 0.2	36.8 ± 0.13

Table 3 Sensory scores of idli prepared from batters with and without curry leaves during fermentation and storage

	Appearance	Color	Texture	Flavor	Mouthfeel	Aftertaste	Overall Acceptability
Control idli 12 h	8.44 ± 0.07	7.1 ± 0.06	6.98 ± 0.11	7.86 ± 0.1	7.92 ± 0.3	7.75 ± 0.12	7.68
Curry leaves idli 12 h	9.37 ± 0.06	8.76 ± 0.07	8.5 ± 0.18	9.4 ± 0.2	7.8 ± 0.4	7.62 ± 0.24	8.58
Control idli 16 h	7.28 ± 0.03	6.55 ± 0.22	6.64 ± 0.11	6.93 ± 0.02	7.7 ± 0.34	7.42 ± 0.4	7.17
Curry leaves idli 16 h	9.12 ± 0.07	8.33 ± 0.21	8.12 ± 0.03	8.41 ± 0.06	7.6 ± 0.02	7.53 ± 0.01	8.19
Control idli 20 h	6.57 ± 0.07	6.24 ± 0.31	6.14 ± 0.06	6.22 ± 0.21	6.51 ± 0.4	6.32 ± 0.08	6.33
Curry leaves idli 20 h	9.11 ± 0.09	8.21 ± 0.3	8.11 ± 0.07	8.21 ± 0.02	7.42 ± 0.3	8.14 ± 0.02	8.2
Control idli 24 h	6.43 ± 0.08	5.17 ± 0.03	4.22 ± 0.09	5.28 ± 0.13	5.47 ± 0.2	5.41 ± 0.06	5.33
Curry leaves idli 24 h	8.3 ± 0.05	7.21 ± 0.04	7.42 ± 0.2	8.31 ± 0.12	7.46 ± 0.22	7.39 ± 0.03	7.68
30 °C							
Control idli day 1	8.42 ± 0.03	8.31 ± 0.01	7.93 ± 0.13	7.68 ± 0.21	6.59 ± 0.03	6.97 ± 0.2	7.65
Curry leaves idli day 1	9.12 ± 0.07	9.36 ± 0.13	8.33 ± 0.14	8.75 ± 0.1	8.43 ± 0.04	7.87 ± 0.02	8.64
Control idli day 2	8.34 ± 0.06	7.68 ± 0.01	6.52 ± 0.01	8.1 ± 0.07	6.61 ± 0.5	7.7 ± 0.08	7.5
Curry leaves idli day 2	9.31 ± 0.3	9.06 ± 0.07	8.2 ± 0.11	7.8 ± 0.06	8.6 ± 0.01	8.02 ± 0.09	8.5
Control idli day 3	7.17 ± 0.03	7.26 ± 0.06	5.62 ± 0.07	6.06 ± 0.05	5.72 ± 0.2	6.14 ± 0.1	6.33
Curry leaves idli day 3	8.1 ± 0.04	8.33 ± 0.08	7.6 ± 0.06	7.72 ± 0.14	7.12 ± 0.03	8.13 ± 0.2	7.83
Control idli day 4	6.32 ± 0.06	7.25 ± 0.09	4.5 ± 0.3	5.22 ± 0.01	4.63 ± 0.05	5.32 ± 0.4	5.54
Curry leaves idli day 4	8.08 ± 0.1	7.83 ± 0.03	8.12 ± 0.2	7.63 ± 0.02	8.24 ± 0.4	7.1 ± 0.04	7.83
Control idli day 5	5.84 ± 0.03	6.27 ± 0.16	3.83 ± 0.07	3.2 ± 0.12	3.7 ± 0.2	3.16 ± 0.03	4.33
Curry leaves idli day 5	8.02 ± 0.12	7.62 ± 0.13	7.21 ± 0.03	7.43 ± 0.08	8.2 ± 0.01	7.6 ± 0.02	7.68
4 °C							
Control idli day 1	8.2 ± 0.06	7.81 ± 0.3	8.01 ± 0.02	7.84 ± 0.12	7.1 ± 0.02	8.03 ± 0.13	7.83
Curry leaves idli day 1	9.12 ± 0.05	9.37 ± 0.2	8.14 ± 0.01	9.1 ± 0.05	8.1 ± 0.12	8.19 ± 0.2	8.67
Control idli day 2	8 ± 0.02	8.14 ± 0.3	7.12 ± 0.05	8.12 ± 0.03	7.22 ± 0.01	6.4 ± 0.21	7.5
Curry leaves idli day 2	9.3 ± 0.04	9.1 ± 0.09	8.4 ± 0.04	8.32 ± 0.01	8.1 ± 0.01	8 ± 0.3	8.5
Control idli day 3	7 ± 0.02	7 ± 0.17	6.01 ± 0.01	6.01 ± 0.3	6 ± 0.03	6 ± 0.06	6.33
Curry leaves idli day 3	8.1 ± 0.06	7.8 ± 0.01	7.84 ± 0.02	8.2 ± 0.01	6.62 ± 0.01	8.42 ± 0.02	7.83
Control idli day 4	6.14 ± 0.03	7.14 ± 0.08	5.11 ± 0.07	6.22 ± 0.1	5.54 ± 0.1	6.1 ± 0.18	6
Curry leaves idli day 4	8.22 ± 0.02	8.13 ± 0.18	7.8 ± 0.02	7.54 ± 0.19	8.3 ± 0.14	7 ± 0.02	7.83
Control idli day 5	6.4 ± 0.01	6.7 ± 0.02	5.12 ± 0.4	4.01 ± 0.04	4.56 ± 0.1	5.2 ± 0.06	5.33
Curry leaves idli day 5	8.1 ± 0.05	8 ± 0.01	7.36 ± 0.3	7.43 ± 0.02	7.5 ± 0.01	7.64 ± 0.1	7.67

higher in curry leaves *idli* with little change in the moisture. Marked increase in soluble dietary fiber (87.9 %) occurred in the curry leaves *idli* while, insoluble fiber decreased marginally (16.9 %). Overall, the total dietary fiber was 18.58 % higher in curry leaves *idli*. Significant increase in calcium content (tenfold) occurred in the fortified *idli*.

Texture of idli

Texture of *idli* in terms of firmness was slightly higher for curry leaves *idli* than the control *idli* but within the softness range (Fig. 1g, h). The springiness (S) of the control *idli* increased till 8 h of fermentation and showed a slight decrease till 24 h, whereas springiness of the *idli* increased with addition of curry leaves during 24 h of fermentation. The hardness of traditional *idli* decreased with okra substitution during a 10 h fermentation period as observed by Rekha and Vijayalakshmi (2011). The sponginess and fluffy texture of the *idli* is based on the yeast growth in naturally fermented batters. The texture changes may also be due to the ionic changes in the protein network induced by the decrease in pH during fermentation. The disruption of protein would reduce the firmness and springiness of the *idli*.

Sensory analysis

The sensory evaluation data confirmed the preference for the *idli* prepared with the addition of curry leaves at all fermentation time periods (12, 16, 20 and 24 h). However, *idli* from 12 h fermented batter was preferred most in terms of appearance, colour, texture, mouthfeel, flavour, aftertaste and overall acceptability (Table 3). Although the

acceptability remained similar for the 12 and 16 h fermented batters, significant decrease in scores ($p < 0.05$) were evident for *idlies* from 20 and 24 h fermentation particularly in the control batter which turned sour leading to lower scores for flavour and after-taste. Further texture scores also declined due to loss of gas retention and porosity which correlated with the increased firmness and little change in the springiness. The addition of curry leaves proved advantageous in suppressing the sour taste and maintaining the structure and texture of the *idli*. The instrumental analysis of texture also showed increase of springiness.

Both temperature and time of storage of *idli* batter influenced the sensory qualities of the *idli*. Curry leaves incorporated *idli* received better sensory scores compared to the control *idli* from the stored batters. The sensory attributes dropped in the control batter after two days at 30 °C while it was better retained for 5 days in the presence of curry leaves. Storage at refrigerated temperature (4 °C) apparently preserved the quality of the batters resulting in higher acceptability than at 30 °C. Except appearance and colour all other sensory attributes declined in the control *idli* while it is maintained through the 5 days storage of the batter in the curry leaves *idli* both at 30 and 4 °C.

Therefore, addition of curry leaves to the *idli* batter not only enhances its sensory attributes, but also aids in maintaining these attributes in the products even after storage of the batter for 5 days.

Anti-bacterial activity of curry leaves

Table 4 represents the zone of inhibition (mm) for the antibacterial activity of curry leaves extract as compared to the common preservatives used. Curry leaves effectively inhibited the two yeast strains compared to the bacterial pathogens tested. However, it was not effective against *Enterobacter faecalis* and *Salmonella paratyphi*-A. It was interesting to note that that the curry leaves extract was more effective in inhibiting both bacteria and yeast compared to the chemical preservatives. The antibacterial activity of curry leaves has contributed to the suppression of bacteria and yeast in the fermenting batter.

GC–MS analysis

Total of 10 and 23 compounds identified from pooled extract of curry leaves and curry leaves idli respectively. Main compounds identified were: 1-methyl-pyrrolidine-2-carboxylic acid (35.16 %), 2-methyl-1H-phenanthro 3,4-d-imidazol-10-ol (34.99 %), pyrrolidin-1-acetic acid (11.84 %) and caryophyllene (8.58 %); all other compounds were present in significantly lower amounts from 0.91 to 2.45 % and 0.02 to 2.76 % respectively for curry leaves and curry leaves idli (Tables 5, 6). Carbazole alkaloids have previously been isolated from different parts (leaves, stem bark and seed) of curry leaves. Alkaloids isolated from curry leaves such as mahanine, pyrayafoline-D and murrayafoline-I act as chemotherapeutic agent (Ito et al. 2006) as well as prevents damage of pancreatic cells apart from oxidative stress relief (Arulselvan and Subramanian 2007).

FTIR, SEM and ink-print analysis

Fourier transform infrared spectroscopy (FTIR) analysis of curry leaves powder confirmed the presence of alcohol, phenol, alkanes, aldehyde, aromatic compounds, secondary alcohol, aromatic amines and halogen compounds (Fig. 1i).

Table 4 Antimicrobial activity of curry leaves extract

	Zone of inhibition (mm)		
	Curry leaves	Sodium benzoate	Sodium metabisulfate
Enterococcus faecalis (MTCC 112)	ND	6.00 ± 0.2	6.00 ± 0.12
Escherichia coli (MTCC 728)	16.00 ± 0.01	9.00 ± 0.01	9.00 ± 0.09
Klebsiella pneumoniae (MTCC 2653)	11.00 ± 0.03	ND	ND
Pitchia kudriavzevii (URCS7, BankIt No. 1824798)	25.00 ± 0.08	ND	ND
Proteus vulgaris (MTCC 426)	13.00 ± 0.05	ND	ND
Saccharomyces boulardii (URCS1, BankIt No. 1824798)	24.00 ± 0.02	ND	ND
Salmonella paratyphi-A (MTCC 734)	ND	ND	ND
Salmonella paratyphi-B (MTCC 724)	16.00 ± 0.09	ND	ND
Salmonella typhi (MTCC 754)	14.00 ± 0.1	6.00 ± 0.07	6.00 ± 0.07
Salmonella typhimurium (MTCC 736)	10.00 ± 0.02	5.00 ± 0.04	5.00 ± 0.08
Shigella dysenteriae (MTCC 1437)	17.00 ± 0.04	7.00 ± 0.05	7.00 ± 0.3
Shigella flexneri (MTCC 1457)	17.00 ± 0.06	6.00 ± 0.09	6.00 ± 0.02
Staphylococcus aureus (MTCC 1377)	17.00 ± 0.2	11.00 ± 0.05	11.00 ± 0.1

Table 5 GC–MS analysis of curry leaves extract

	RT	Area (%)
1-Methyl-pyrrolidine-2-carboxylic acid	4.973	35.16
Pyrrolidin-1-acetic acid	5.287	11.84
Caryophyllene	8.105	8.58
.alpha.-Caryophyllene	8.632	1.7
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2.alpha.,4a.alpha.,8a.beta.)]-	9.241	2.31
Caryophyllene oxide	10.66	0.91
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5.alpha.)]-	14.948	1.04
Phytol	19.967	2.45
2-Ethylacridine	28.655	1.02
2-Methyl-1H-phenanthro[3,4-d)imidazol-10-ol	32.355	34.99

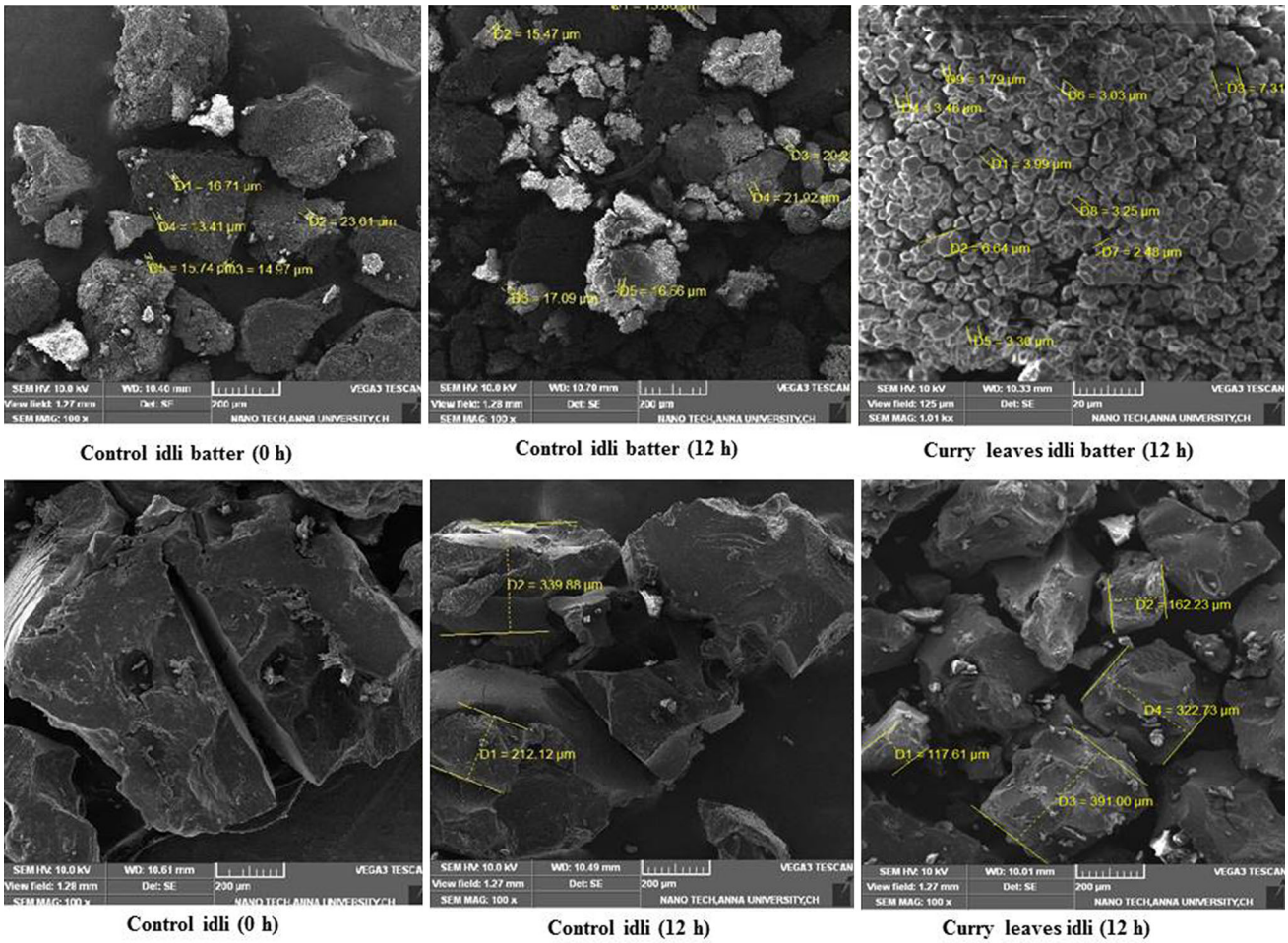

Fig. 3 SEM analysis of control and curry leaves idli batter and idli

The particle size variation as the fermentation proceeds due to the microbial activity was evident from the scanning electron microscopy (SEM) of the batters and *idli* (Fig. 3). In case of control batter after 12 h of fermentation, the larger particles were broken down into smaller particles (size reduction of 34 %) by the enzymatic activity of the fermenting microbes. Smaller particles were found in the *idli* prepared from control batter. Larger particles were

Table 6 GC–MS analysis of curry leaves idli

	RT	Area (%)
1-Methyl-pyrrolidine-2-carboxylic acid	4.973	35.16
Pyrrolidin-1-acetic acid	5.287	11.84
Hexane,2-nitro-	7.458	0.35
Caryophyllene	8.105	8.58
.alpha.-Caryophyllene	8.632	1.7
Oxalic acid, cyclohexyl octyl este	8.633	1.01
Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-,[2R-(2.alpha.,4a.alpha.,8a.beta.)]-	9.241	2.31
2-Pentanone	10.2	0.02
Caryophyllene oxide	10.66	0.91
2-Hexanone	11.4	0.32
Ethanone	13.8	0.6
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-,[1R-(1.alpha.,2.beta.,5.alpha.)]-	14.948	1.04
Phytol	19.967	2.45
Propanal	21.2	1.22
3-Methylbutanal	22.8	2.1
9-Decenal	23	2.76
Decanoic acid	26.89	0.11
2-Ethylacridine	28.655	1.02
Octadecanoic acid	29.07	0.45
Hexadecanoic acid	31.9	0.61
Undecanoic acid	32.1	0.41
2-Methyl-1H-phenanthro[3,4-d)imidazol-10-ol	32.355	34.99
3-Propoxyamphetamine	34.93	0.13

observed in the prepared *idli* added with curry leaf powder that indicated the reduction in fermentation rate of the batter.

A special test called ink print test was done to record the appearance of *idli* by photography of ink prints (Nazni and Shalini 2010). These prints show the number of pores per square centimeter in graph sheets which is an indication of the softness of *idlies*. Higher number of pores was noted in the control idli as the fermentation time increased when compared to the control idli from the unfermented batter. Curry leaves idli had less number of pores indicating the reduction in the microbial activity during fermentation. However, the texture of the idli was found to be within the softness range. From this the difference in texture resulting from the distribution of air pockets in the *idli* samples was evident.

Conclusions

The incorporation of curry leaves powder (5 %) in the *idli* batter increased the shelf-life at room temperature (30 °C) up to 5 days with retention of quality. The sensory qualities of the fortified *idli* were preferred by panelists. Nutritional enhancement of dietary fiber and calcium content is an added benefit. Results obtained suggest that curry leaves is a potential raw material for making a novel *idli* product.

Compliance with ethical standards

Conflict of interest None.

References

Annan NT, Plahar WA, Poll L, Jakobsen M (2005) Effect of soybean fortification on Ghanaian fermented maize dough aroma. Int J Food Sci Nutr 56:315–326

AOAC (2003) AOAC official method 991.43 total, soluble, and insoluble dietary fiber in foods. In: Horwithz W (ed) Official methods of analysis of the AOAC international, 17th edn. AOAC International, Gaithersburg

AOAC (2005) Official methods of analysis of AOAC International. AOAC International, Gaithersburg

Arulselvan P, Subramanian SP (2007) Beneficial effects of *Murraya koenigii* leaves on antioxidant defence system and ultra-structural changes of pancreatic β-cells in experimental diabetes in rats. Chem Biol Interact 165:155–164

Balasubramanian S, Viswanathan R (2007) Properties of idli batter during its fermentation time. J Food Process Preserv 31:32–40

Ghosh D, Chattopadhyay P (2011) Preparation of idli batter, its properties and nutritional improvement during fermentation. J Food Sci Technol 48:610–615

Gobetti M, Corsetti A, Rossi J (1994) The sourdough microflora: interactions between lactic acid bacteria and yeasts: metabolism of amino acids. World J Microbiol Biotechnol 10:275–279

Gopalan C (1998) Micronutrient malnutrition in SAARC—the need for a food-based approach. NFI Bull 19:1–4

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative microbiology. Williams and Wilkins, Maryland

Ito C, Itoigawa M, Nakao K, Murata T, Tsuboi M, Kaneda N, Furukawa H (2006) Induction of apoptosis by carbazole alkaloids isolated from *Murraya koenigii*. Phytomedicine 13:359–365

Iyer BK, Ananthanarayan L (2008) Effect of α-amylase addition on fermentation of idli—a popular south Indian cereal—Legume-based snack food. LWT Food Sci Technol 41:1053–1059

Khanum F, Anilakumar KR, Krishna KS, Viswanathan KR, Santhanam K (2000) Anticarcinogenic effects of curry leaves in dimethylhydrazine-treated rats. Plant Foods Hum Nutr 55:347–355

Nazni P, Shalini S (2010) Standardization and quality evaluation of idli prepared from pearl millet (*Pennisetum glaucum*). Int J Curr Res 5:84–87

Paul SK, Goyal S, Yadav P, Yadav S, Parashari D (2013) Phytochemistry and pharmacological aspects of *Murraya koenigii* Linn. Res J Pharm Technol 6:695–697

Rekha CR, Vijayalakshmi G (2011) Accelerated fermentation of 'idli' batter using soy residue okara. J Food Sci Technol 48:329–334

Soni SK, Sandhu DK (1991) Role of yeast domination in Indian idli batter fermentation. World J Microbiol Biotechnol 7:505–507

Sridevi J, Halami PM, Vijayendra SVN (2010) Selection of starter cultures for idli batter fermentation and their effect on quality of idlis. J Food Sci Technol 47:557–563

Tembhurne SV, Sakarkar DM (2011) Effects of *Murraya koenigii* leaf extract on impaired gastrointestinal motility in streptozotocin-induced diabetic rats. J Integr Med 9:913–919

Thyagaraja N, Otani H, Hosono A (1992) Studies on microbiological changes during the fermentation of Idly. Lebensm-Wiss Technol 25:77–79

Yankuzo H, Ahmed QU, Santosa RI, Akter SFU, Talib NA (2011) Beneficial effect of the leaves of *Murraya koenigii* (Linn.) Spreng (Rutaceae) on diabetes-induced renal damage in vivo. J Ethnopharmacol 135:88–94