Impact of partial replacement of rice with other selected cereals on *idli* batter fermentation and *idli* characteristics

Monika Rani¹ · Dhanashree Amame¹ · Laxmi Ananthanarayan¹

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Abstract *Idli* is a naturally fermented food, prepared using dehulled black gram *dal* and parboiled rice. In the first phase of the study, replacement of rice with some underutilized cereals like amaranth and finger millet was carried out at different proportions (25%, 50%, 75% and 100%, w/w) followed by sensory evaluation of the prepared *idlis*. *Idlis* prepared with 25% (w/w) replacement of rice with finger millet (25F) were more acceptable than *idlis* prepared with 25% (w/w) replacement of rice with amaranth (25A). In the second phase of the study, 50% replacement of rice was carried out with cereals in combinations (25% each, w/w) such as amaranth + finger millet (AF), amaranth + sorghum (AS), amaranth + pearl millet (AP), finger millet + sorghum (FS), finger millet + pearl millet (FP) and sorghum + pearl millet (SP), of which FS and FP batter variants were accepted by the panellists and taken ahead for further analysis. Control, 25F, FS and FP batter variants were subjected to various biochemical analysis up to 20 h of fermentation. Replacement of rice in these batter variants led to comparable changes with an increase in the levels of microbial counts, amylase, protease and phytase activities, titratable acidity, reducing sugars, soluble proteins and antioxidant activity during fermentation. A greater reduction in trypsin inhibitor activity (TIA) was also observed. *Idlis* prepared with replacement of rice by other cereals (25F, FS and FP) resulted in improved textural values and bulk

density as compared to control *idlis*, though the colour was affected.

Keywords *Idli* · Rice · Partial replacement · Millets

Abbreviations

25A	25% amaranth
AF	25% amaranth + 25% finger millet
AS	25% amaranth + 25% sorghum
AP	25% amaranth + 25% pearl millet
25F	25% finger millet
FS	25% finger millet + 25% sorghum
FP	25% finger millet + 25% pearl millet
SP	25% sorghum + 25% pearl millet

Introduction

Idli is a traditional cereal-legume based fermented steamed product, consumed as a breakfast food in many parts of India (Agrawal et al. 2001). It has a spongy texture, attractive appearance, soft mouthfeel and is easy to digest, hence making it a preferred choice of food. It is traditionally prepared by soaking parboiled rice (*Oryza sativa*) and dehulled black gram *dal* (*Phaseolus mungo*), usually taken in 3:1 (w/w) proportion followed by grinding. Salt (0.9%) is added and the mixture is allowed to ferment overnight at room temperature (Balasubramanian and Viswanathan 2007). Post fermentation, the batter is dispensed in *idli* moulds as small cakes and steamed.

Replacing rice and black gram *dal* completely or partially with other cereals and legumes respectively can be explored to increase the nutritional value of *idli* and can also provide scope for incorporation of some underutilized cereals. Cereals are important sources of proteins, vitamins,

✉ Laxmi Ananthanarayan
laxmi.ananth.iyer@gmail.com;
l.ananthanarayan@ictmumbai.edu.in

¹ Food Engineering and Technology Department, Institute of Chemical Technology, Nathalal Parekh Marg, Matunga, Mumbai, Maharashtra 400019, India

carbohydrates, minerals and dietary fibre (Blandino et al. 2003). In addition, they possess bioactive compounds such as flavonoids, tocopherols (Gani et al. 2012) that confer health benefits. Rice is a staple cereal of India and is consumed in ample amounts. Various other cereals such as amaranth, finger millet, sorghum and pearl millet are high in nutritional quality but their consumption is decreasing due to easy availability of rice and wheat through public distribution systems (Ratnavathi and Patil 2013). Replacement of rice in *idli* batter with other cereals will encourage the consumption of these underutilized cereals which are consumed in considerably lower amounts than rice. Use of finger millet (*Eleusine coracana*) improves the mineral content (Shobana et al. 2013). The protein (lysine) content, fiber, fat and ash are considerably higher in amaranth grain as compared to other cereal grains (Singh et al. 2014). Sorghum (*Sorghum bicolor*) occupies an important place in asian diet as it is a source of proteins, minerals, carbohydrates and also enhances dietary fibre content (Nazni and Shalini 2010a). Pearl millet (*Pennisetum glaucum*) serves as an integral source of energy for the poor sections in the region of its cultivation (Nazni and Shalini 2010b). Several authors have made attempts to replace rice in *idli* batter partially or completely with other cereals such as mixed millets (Krishnamoorthy et al. 2013), kodo millet (Neelam et al. 2013), sorghum (Nazni and Shalini 2010a) and pearl millet (Nazni and Shalini 2010b). Although, replacement of rice in *idli* batter with these cereals has been attempted, the biochemical aspects of fermentation in *idli* batter on replacement of rice with other cereals have not been studied.

The present work focuses on replacement of rice in *idli* batter partially with amaranth, finger millet, sorghum, and pearl millet and analyses the effect on batter fermentation and *idli* characteristics. The batter properties studied include microbial counts, various enzyme activities, trypsin inhibitor activity, physicochemical and biochemical properties. Colour, texture, bulk density and sensory properties were analyzed to assess the quality of *idlis* obtained from partial substitution of rice with other cereals.

Materials and methods

Materials

Raw materials

Parboiled rice, dehulled black gram *dal*, amaranth, finger millet, sorghum, pearl millet, and table salt (Tata, India) were purchased from local market of Mumbai.

Chemicals and reagents

MRS agar, MRS broth, potato dextrose broth, agar powder, standards such as glucose, maltose, bovine serum albumin (BSA-fraction V) and tyrosine were purchased from Himedia (India). N-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). $3 \times$ crystallized trypsin and Bradford reagent were procured from SRL (India) and Bio-Rad (USA), respectively. All chemicals used were of analytical grade.

Methods

Preparation of *idli* batter and *idli*

Parboiled rice and dehulled black gram *dal* were taken in the ratio of 3:1 (w/w), soaked separately in water for 4 h and the water was drained. Rice was ground coarsely and black gram *dal* was ground to a fine paste with addition of 150 mL of water, followed by mixing to form batter with addition of 0.9% salt. Optimally fermented *idli* batter (12 h, 30 ± 2 °C) was poured into greased *idli* moulds and steamed for 15 min.

Screening of cereals for replacement of rice

Screening of cereals was done by sensory evaluation of *idlis* prepared from 12 h fermented batters with replacement of parboiled rice (all replacements on w/w basis of rice) individually with 25%, 50%, 75% and 100% amaranth and finger millet. Further, six combinations of cereals studied for 50% replacement of rice on w/w basis were 25% amaranth + 25% finger millet (AF), 25% amaranth + 25% sorghum (AS), 25% amaranth + 25% pearl millet (AP), 25% finger millet + 25% sorghum (FS), 25% finger millet + 25% pearl millet (FP) and 25% sorghum + 25% pearl millet (SP).

Sensory evaluation

The *idlis* were evaluated using 9-point hedonic scale (ranging from 1 = dislike extremely to 9 = like extremely) for colour, aroma, taste, texture and overall acceptability. A panel of 20 semi-trained members was chosen to carry out the study.

Evaluation of microbial counts in *idli* batter

LAB count in *idli* batter One gram *idli* batter was suspended in 10 mL sterile saline (0.9%) and mixed thoroughly on a vortex mixer. Appropriate dilutions were prepared in sterile saline and spread on MRS agar plates

and were kept at 37 °C for 48 h. Colony forming units were counted. For counting, only those dilutions were considered in which the colony count fell between 30 and 300.

Yeast count in idli batter One gram *idli* batter was suspended in 10 mL sterile saline (0.9%) and mixed thoroughly on a vortex mixer. Appropriate dilutions were prepared in sterile saline and spread on PDA plates and kept at 28 °C for 48 h and colony forming units were counted. For counting, only those dilutions were considered in which the colony count fell between 30 and 300.

Evaluation of amylase, protease and phytase activities in idli batter

Five grams *idli* batter was suspended in 10 mL 0.01 M phosphate buffer, pH 6.9 and mixed thoroughly on a vortex mixer, followed by centrifugation (J2-MC; Beckman Coulter, Brea, CA, USA.) at 7840 *g* for 15 min at 4 °C. The estimation of amylase activity in the supernatant was done by measuring absorbance at 540 nm using maltose as standard as followed by Udupa et al. (1989).

Five grams *idli* batter was suspended in 10 mL 50 mM phosphate buffer, pH 7.5 and mixed thoroughly on a vortex mixer, followed by centrifugation at 7840 × *g* for 15 min at 4 °C. Protease activity was estimated by measuring absorbance at 280 nm, using tyrosine as standard (Soni et al. 1986).

Five grams *idli* batter was suspended in 10 mL 0.1 M acetate buffer, pH 5 and mixed thoroughly on a vortex mixer, followed by centrifugation at 7840 *g* for 15 min at 4 °C. The estimation of phytase activity in the supernatant was done by measuring blue colour at 700 nm (Lata et al. 2013).

Evaluation of trypsin inhibitor activity (TIA) in idli batter

Trypsin inhibitor was extracted from *idli* batters as described by Senanayake et al. (2013). The sample (extracted inhibitor) was diluted 20 times with distilled water and used for estimation. Trypsin inhibitor activity was estimated by incubating trypsin with BAPNA (substrate) in presence of the inhibitor extracted from *idli* batters. The absorbance was measured at 410 nm and TIA was calculated using the formula:

$$TIA = 2.632 * D * \frac{A1}{S}$$

where 2.632 = constant based on the weight of pure trypsin inhibited per mL, D = Dilution factor, A1 = Change in absorbance due to trypsin inhibition per 1 mL of diluted sample, S = sample weight in grams.

Evaluation of physicochemical parameters of idli batter

The physicochemical properties of *idli* batter included titratable acidity, pH and batter volume. These were measured after every 2 h till 20 h during fermentation for control, 25F, FS and FP.

Measurement of titratable acidity Measurement of titratable acidity was done according to the procedure described by Balasubramanian and Viswanathan (2007). Reading from the burette was recorded and percent titratable acidity was calculated based on the formula:

$$\% \text{ Lactic acid} = \text{mL of 0.1 NaOH} * \text{Normality of NaOH} * 0.09 * 100 / \text{sample weight}$$

Measurement of pH The pH of *idli* batter was measured directly after the specified time using a digital pH meter (Eutech Instruments, Singapore).

Measurement of batter volume Thirty millilitres of freshly prepared batter was dispensed in 100 mL measuring cylinder and was kept undisturbed at 30 °C. The rise in batter volume was recorded in 'mL' after every 2 h.

Determination of reducing sugars and soluble proteins in idli batter

The reducing sugars were determined by DNSA method. Five grams *idli* batter was suspended in 10 mL 0.2 M phosphate buffer, pH 6 and mixed thoroughly on a vortex mixer, followed by centrifugation at 7840 *g* for 15 min at 4 °C. The estimation of reducing sugars in the supernatant was done by measuring colour change at 540 nm, using glucose as standard (Iyer and Ananthanarayan 2008).

The soluble proteins were estimated by Bradford method (Bradford 1976). One gram *idli* batter was suspended in 10 mL 0.1 M Tris buffer, pH 9 and mixed thoroughly on a vortex mixer, followed by centrifugation (J2-MC; Beckman Coulter, Brea, CA, USA.) at 7840 *g* for 15 min at 4 °C. 10 µL of the diluted extract (supernatant diluted 1:10 in 0.1 M Tris buffer, pH 9) was added in ELISA plate with 200 µL of Bradford reagent and change in colour was recorded at 595 nm. Bovine serum albumin (BSA) was used as a standard.

Antioxidant value of idli with cereal replacement

Idli batter was dried in an oven (Labline) at 60 °C for 24 h and ground to powder. Further, the antioxidant activity was estimated by DPPH assay, using ascorbic acid standard curve as followed by Das et al. (2013). The absorbance was

taken after 30 min at 517 nm. The percent inhibition activity was calculated as:

$$\frac{A_0 - (A - A_e)}{A_0} * 100$$

where A_0 = DPPH· without sample, A = Sample plus DPPH·, A_e = Sample without DPPH·.

The antioxidant activity was further reported as equivalent of ascorbic acid.

Evaluation of idli characteristics prepared with partial replacement of rice with other cereals

The quality of *idlis* prepared with partial replacement of parboiled rice (w/w) i.e. 25F, FS and FP was analyzed by measuring bulk density, texture and colour of the *idli* samples.

Bulk density The bulk density (g/cm^3) of the *idli* samples was measured by seed displacement method as followed by Iyer and Ananthanarayan (2008).

Texture The texture of *idli* was analyzed in terms of hardness, by compression test using a Texture Analyzer (TA.TX2i; Stable Micro Systems, Surrey, UK) with 2 mm stainless steel cylinder probe P/36R and 50 kg load cell. The analysis was done using the software texture Expert Exceed. The force was measured in terms of compression (g). The test speed was 1 mm/s and the probe was allowed to compress 5 mm into the sample.

Colour The colour was evaluated using Hunter Lab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA), using the software Easy Match. 'L' value (an indicator of whiteness), 'a' value (an indicator of redness) and

'b' value (an indicator of yellowness) were noted and compared for all the samples.

Statistical analysis

One-way ANOVA method using IBM SPSS statistics software (version 20) was used for statistical analysis. All experiments were carried out in triplicates and results are denoted as mean $\pm$ SD. Values of $p > 0.05$ were considered to be statistically significant.

Results and discussion

Screening of cereals for replacement of rice

The present study was undertaken to achieve replacement of rice in *idli* formulation with other cereals, to encourage the consumption of various underutilized cereals in the diets of Indian population. Initial part of work included screening of cereals like amaranth and finger millet wherein rice was replaced with 25%, 50%, 75% and 100% (w/w) these two cereals.

It can be observed from the sensory scores as shown in Table 1 that 25A *idli* scored less on aroma, taste and overall acceptability when compared with control and as the percentage of replacement with amaranth was increased, all sensory attributes decreased significantly. However, 25A *idli* showed no significant difference in texture scores as compared to control *idlis*. Shevkani and Singh (2014) have reported that incorporation of amaranth improves viscoelasticity of the batter and hence, more air is retained resulting in improved springiness. Based on

Table 1 Sensory evaluation of *idlis* prepared by replacement of rice with amaranth and finger millet in different proportions

Idli variants*	Sensory attributes				Overall acceptability
	Colour	Aroma	Taste	Texture	
Control	8.02 $\pm$ 0.69 ^a	7.4 $\pm$ 0.73 ^a	7.25 $\pm$ 0.57 ^b	7.25 $\pm$ 0.61 ^a	7.45 $\pm$ 0.66 ^a
25% amaranth	7.35 $\pm$ 0.24 ^a	6.62 $\pm$ 0.13 ^c	6.37 $\pm$ 0.33 ^d	7.20 $\pm$ 0.19 ^a	7.15 $\pm$ 0.12 ^b
50% amaranth	7.17 $\pm$ 0.12 ^b	6.22 $\pm$ 0.12 ^d	4.45 $\pm$ 0.25 ^f	6.52 $\pm$ 0.26 ^b	6.25 $\pm$ 0.23 ^d
75% amaranth	7.12 $\pm$ 0.13 ^b	5.70 $\pm$ 0.34 ^f	3.4 $\pm$ 0.29 ^g	6.25 $\pm$ 0.26 ^d	5.62 $\pm$ 0.13 ^c
100% amaranth	7.01 $\pm$ 0.20 ^b	5.40 $\pm$ 0.26 ^g	2.62 $\pm$ 0.46 ⁱ	6.20 $\pm$ 0.25 ^e	5.27 $\pm$ 0.29 ^f
25% finger millet	7.5 $\pm$ 0.80 ^a	7.07 $\pm$ 0.63 ^a	7.9 $\pm$ 0.52 ^a	7.17 $\pm$ 0.10 ^a	7.55 $\pm$ 0.57 ^a
50% finger millet	6.52 $\pm$ 0.58 ^c	6.7 $\pm$ 0.52 ^b	6.62 $\pm$ 0.70 ^c	6.45 $\pm$ 0.58 ^c	6.82 $\pm$ 0.56 ^c
75% finger millet	5.05 $\pm$ 0.45 ^d	5.9 $\pm$ 0.53 ^c	5.52 $\pm$ 0.27 ^e	5.25 $\pm$ 0.64 ^f	5.62 $\pm$ 0.22 ^c
100% finger millet	3.97 $\pm$ 0.37 ^e	4.35 $\pm$ 0.65 ^h	3.25 $\pm$ 0.69 ^h	2.97 $\pm$ 0.63 ^g	3.8 $\pm$ 0.67 ^g

(i) Values reported are mean values of scores with SD, obtained from n = 20 panellists

(ii) Values followed by the different superscript in each column are significantly different at $p > 0.05$

(iii) *Idli variants include those with partial or complete replacement of parboiled rice (ranging from 25 to 100%) for the rice: black gram *dal* ratio of 3:1

overall acceptability, 25A was considered for further studies. 25F showed no significant difference in any of the sensory attributes and was well accepted by the panellists with scores between 7.07 and 7.55, indicating that the sensory attributes of the product were comparable with the control. However, *idli* variants with rice having higher replacements by finger millet (50%, 75% and 100%) demonstrated lower acceptability by the panellists. Therefore, 25F was taken ahead for further studies.

In the next phase of the study, rice was replaced with other cereals taken in combination of two cereals (25% each, w/w) for preparation of *idli* batters followed by sensory evaluation of prepared *idlis*. This was done in order to determine if higher replacement of rice was possible. *Idli* variants used for the study were AF, AS, AP, FS, FP and SP, prepared as mentioned in “Screening of cereals for replacement of rice” section.

On comparison of the sensory scores presented in Table 2, it can be observed that FS and FP *idli* variants scored comparable if not better than 25A and 25F (Table 1). AF *idli* variant scored very low in terms of colour, therefore it was not considered for further analysis. The taste and texture scores of AS, AP and SP *idli* variants were much lower which significantly decreased the acceptability of *idlis*. Hence, based on the overall acceptability, FS and FP *idli* variants were chosen for further studies.

Four *idli* batter variants i.e. control, 25F, FS and FP were evaluated for various characteristics such as microbial counts, enzyme activities, trypsin inhibitor activity, physicochemical and biomolecule parameters, in order to study the effect of replacement of rice (w/w) with finger millet, sorghum and pearl millet.

Effect of cereal replacement on microbial growth in *idli* batters

The predominance of LAB during fermentation in different batters is visible in Fig. 1a. The LAB and yeast count (Fig. 1a, b) significantly increased during the fermentation process. Batters having rice replaced with cereals showed a higher count as compared to control, probably due to the contribution of microorganisms responsible for fermentation from added cereals (Blandino et al. 2003).

Effect of cereal replacement on amylase, protease and phytase activities in *idli* batter

Amylases act on starch and hydrolyse it into reducing sugars while proteases act on proteins and cleave them into peptides. During the process of fermentation, a significant increase in amylase activity was evident in case of control, 25F, FS and FP as observed in Fig. 2a. Similarly, a significant increase in protease activity was observed during the fermentation in control, 25F, FS and FP (Fig. 2b). The increase in activity of these two enzymes can be attributed to the presence of LAB, in the initial stages of fermentation and yeast, which is predominant at latter stages of fermentation (Fig. 1b). A positive contribution of yeasts towards higher activity of amylases and proteases has been reported by Soni (1991). Unlike amylase and protease activity, a different trend was observed in case of phytase activity. From Fig. 2c, it can be seen that the phytase activity of rice replaced batter variants showed higher phytase activity when compared with control till 12th h of fermentation, after which, the activity declined in case of 25F, FS, and FP as compared to control. An increased phytase activity in case of batters having rice replaced with other cereals could be due to a higher LAB count as seen in Fig. 1a. As can be observed from Fig. 2c, even at 0th h of

Table 2 Sensory evaluation of *idlis* prepared by replacement of rice with different combinations of cereals

<i>Idli</i> variants*	Sensory attributes				Overall acceptability
	Colour	Aroma	Taste	Texture	
Control	8.02 ± 0.69 ^a	7.4 ± 0.73 ^a	7.25 ± 0.57 ^b	7.25 ± 0.61 ^b	7.45 ± 0.66 ^a
25% amaranth and 25% finger millet	3.8 ± 0.34 ^c	6.45 ± 0.25 ^c	5.8 ± 0.30 ^c	6.3 ± 0.32 ^c	6.42 ± 0.37 ^b
25% amaranth and 25% sorghum	7.41 ± 0.52 ^{ab}	6.75 ± 0.11 ^b	3.98 ± 0.43 ^d	2.93 ± 0.15 ^e	5.64 ± 0.11 ^c
25% amaranth and 25% pearl millet	7.39 ± 0.28 ^b	6.58 ± 0.32 ^c	4.28 ± 0.43 ^d	5.92 ± 0.22 ^c	5.99 ± 0.18 ^c
25% finger millet and 25% sorghum	7.65 ± 0.71 ^a	7.27 ± 0.55 ^a	8.21 ± 0.12 ^a	7.37 ± 0.10 ^b	7.75 ± 0.17 ^a
25% finger millet and 25% pearl millet	7.69 ± 0.68 ^a	7.25 ± 0.55 ^a	7.98 ± 0.35 ^b	7.51 ± 0.15 ^a	7.68 ± 0.21 ^a
25% sorghum and 25% pearl millet	7.58 ± 0.32 ^a	5.61 ± 0.14 ^d	2.71 ± 0.13 ^e	3.52 ± 0.17 ^d	4.98 ± 0.17 ^d

(i) Values reported are mean values of scores with SD, obtained from n = 20 panellists

(ii) Values followed by the different superscript in each column are significantly different at $p > 0.05$

(iii) **Idli* variants include those with 50% replacement of parboiled rice with combination of two cereals for the rice: black gram *dal* ratio of 3:1

Fig. 1 Change in **a** growth of LAB and **b** growth of yeast, due to rice replacement in *idli* batter with 25% (w/w) finger millet (25F), 25% (w/w) each of finger millet-sorghum (FS) and 25% (w/w) each of finger millet -pearl millet (FP). *The different alphabets at top of the bar graphs indicate significant difference between *idli* batter variants while similar alphabets indicate no significant difference

fermentation, the phytase activity of batter variants containing a combination of cereals was higher as compared to control. This can be due to the presence of plant phytases contributed by the combination of cereals. Further, an increasing trend in phytase activity was observed due to the contribution of phytases from both plant microbial sources. Anastasio et al. (2010) have reported the contribution of LAB towards degradation of phytate during fermentation.

Effect of cereal replacement on trypsin inhibitor activity (TIA) in *idli* batter

Although trypsin inhibitors are majorly present in legumes (Bora 2014), their presence in cereal grains is well documented (Reddy and Pierson 1994; Sosulski et al. 1988). From Fig. 2d, it was observed that at 0th h, 25F, FS, and FP showed higher TIA as compared to the control batter. As the fermentation process progressed, a significant decline in TIA was seen with greater reduction in FP as compared to control, 25F and FS batter variants. The decline in TIA

could possibly be due to the increased protease activity, as evident from Fig. 2b, thereby leading to the hydrolysis of trypsin inhibitor in *idli* batter variants. Shrivastava and Ananthanarayan (2014) observed a decline in TIA in traditional *idli* batter on fermentation. The decline in TIA plays an important role in maintaining the nutritional content as trypsin inhibitors hinder the availability of minerals and vitamins (Porres et al. 2003).

Effect of cereal replacement on physicochemical parameters of *idli* batter

Idli batter variants, 25F, FS and FP and control batter were subjected to studies on physicochemical characteristics such as titratable acidity, pH and changes in batter volume. Physicochemical parameters were studied in order to understand if the fermentation was progressing in all the batter variant samples under study.

Effect of cereal replacement on titratable acidity and pH

The titratable acidity increased markedly that resulted in a decline in pH during fermentation in control, 25F, FS, and FP batter variants, as can be seen from Fig. 3a, b, showing that lactic acid fermentation has taken place in all batter variants. It was observed that 25F, FS and FP batter variants had significantly higher acidity as compared to control as evident from Fig. 3a that correlates to a higher reduction in pH (Fig. 3b). An increase in acidity can be correlated to the presence of LAB during the fermentation process, as is evident from Fig. 1a.

During fermentation, the metabolic activity of LAB present in *idli* batter leads to increase in acidity and decrease in pH of the batter. Although, the microflora responsible for carrying out fermentation is majorly contributed by black gram *dal* used in preparation of *idli* batter (Krauss et al. 1993), cereals such as finger millet (Dharmi and Devi 2017), sorghum (Tinay et al. 1979) and pearl millet (Songré-Ouattara et al. 2008) have been reported to show capability for fermentation. Antony and Chandra (1997) observed a reduction in pH during finger millet fermentation. Au and Fields (1981) and Khetarpaul and Chauhan (1989) have reported a significant increase in titratable acidity during the course of fermentation in sorghum and pearl millet, respectively. Elkhailifa et al. (2004) depicted increase in titratable acidity and a corresponding decline in pH during the fermentation of sorghum, which is related to the presence of LAB during initial phases.

Effect of cereal replacement on batter volume

From Fig. 3c, it is observed that during initial hours of fermentation, there was no change in batter volume but as

Fig. 2 Change in **a** amylase, **b** protease, **c** phytase and **d** trypsin inhibitor activity, due to rice replacement in *idli* batter with 25% (w/w) finger millet (25F), 25% (w/w) each of finger millet -sorghum (FS), 25% (w/w) each of finger millet -pearl millet (FP). *The

different alphabets at top of the bar graphs indicate significant difference between *idli* batter variants while similar alphabets indicate no significant difference

the fermentation progressed, the increase became evident in all the batter variants. At the 12th h of fermentation, 112% increase in batter volume was observed in case of 25F, 134% in case of FS, and 121% in FP as compared to 63% in control. The increase in batter volume (leavening) is related to the activity of yeasts. Yeast is predominantly present in the latter phases of fermentation process. Yeast utilizes sugars and releases carbon dioxide, which gets entrapped in the network of foam produced by globulin, present in *urad dal*. Arabinogalactan, a mucilaginous layer in *urad dal* prevents disruption of foam network and retains the carbon dioxide, hence resulting in increase in batter volume (Reddy et al. 1982). All the rice replaced batter variants showed a higher increase in batter volume than the control batter, which could be correlated to higher yeast counts observed in these batter variants (Fig. 1b). Further, constituents present in the cereals used for replacement of rice in these batter variants could potentially contribute towards stabilizing the foam.

Effect of cereal replacement on reducing sugars and soluble proteins in *idli* batter

The cereals used in our study for replacement of rice in *idli* batter are good sources of starch and proteins (Ratnavathi and Patil 2013; Saleh et al. 2013). Reducing sugars are produced as a result of action of amylases on starch while proteins are solubilized by the action of proteases. A gradual and significant increase in reducing sugar and soluble protein content was observed in all the batter variants during the 20 h of fermentation. From Fig. 3d, e, it is seen that 25F, FS and FP showed higher values for reducing sugars and soluble proteins than control batter. The reducing sugar and soluble protein content increased gradually up to 16th h of fermentation. A considerably higher amount of reducing sugars and soluble proteins was observed in rice replaced batter variants than control, probably due to a higher amylase and protease activity in these batter samples (Fig. 2a, b), thereby leading to hydrolysis of starch and proteins.

Fig. 3 Change in **a** titratable acidity, **b** pH, **c** batter volume, **d** reducing sugar and **e** soluble protein content and **f** antioxidant activity, due to rice replacement in *idli* batter with 25% (w/w) finger millet (25F), 25% (w/w) each of finger millet -sorghum (FS) and 25%

(w/w) each of finger millet -pearl millet (FP). *The different alphabets at top of the bar graphs indicate significant difference between *idli* batter variants while similar alphabets indicate no significant difference

Effect of cereal replacement on antioxidant activity

Whole grain cereals and their products being recognized as sources of antioxidant substances, are recommended for healthy diets. Millets and sorghum have been reported to have high antioxidant properties (Ragaee et al. 2006). Due to replacement of rice with combination of 25% (w/w) each of finger millet + sorghum and finger millet + pearl millet, the antioxidant activity of *idli* batter variants increased (Fig. 3f).

It was observed that after 12 h of fermentation, the antioxidant activity increased significantly as compared to 0th h in all the four cases. The increase in antioxidant activity in case of replacement of rice with cereals may be

due to the presence of antioxidant compounds such as polyphenols, flavonoids in finger millet (Sreeramulu et al. 2009), pearl millet and sorghum (Ragaee et al. 2006). However, the increase in antioxidant activity in rice replaced batters after 12 h fermentation was not much higher, whereas in case of control batter, the antioxidant activity increased significantly in fermented (12 h) batter as compared to unfermented (0 h) batter. Hur et al. (2014) have also reviewed the positive role of fermentation and lactic acid bacteria in production of antioxidant compounds.

Table 3 Effect of partial replacement of rice with other cereal(s) on bulk density, texture and colour of the *idlis*

<i>Idli</i> variants*	Bulk density (g/cm ³)	Texture (load, g)	colour		
			L value	a value	b value
Control	0.61 ± 0.05 ^a	167.96 ± 5.40 ^a	70.47 ± 0.015 ^a	0.20 ± 0.010 ^d	11 ± 0.043 ^a
25% finger millet	0.59 ± 0.02 ^b	163.12 ± 3.17 ^b	40.24 ± 0.030 ^d	4.04 ± 0.032 ^a	7.02 ± 0.020 ^d
25% finger millet and 25% sorghum	0.54 ± 0.07 ^d	155.45 ± 4.07 ^d	42.68 ± 0.015 ^b	3.26 ± 0.032 ^c	8.17 ± 0.030 ^b
25% finger millet and 25% pearl millet	0.57 ± 0.03 ^c	160.15 ± 3.23 ^c	41.16 ± 0.015 ^c	3.55 ± 0.025 ^b	7.65 ± 0.050 ^c

(i) Values reported are mean values of triplicate scores with SD

(ii) Values followed by the different superscript in each column are significantly different at $p > 0.05$

(iii) **Idli* variants include those with partial replacement of parboiled rice with other cereal(s) having high sensory acceptability for the rice: black gram *dal* ratio of 3:1

Effect of replacement of rice on *idlis* prepared using different combinations of cereals

The data presented in Table 3 indicates that the *idlis* prepared with replacement of rice had lower bulk density and softer texture as compared to control. The lower bulk density values can be attributed to higher batter volumes (Fig. 3c) resulting in softer *idlis*. The colour values showed significant difference in *idli* variants when compared to control *idli*. ‘L’ indicates whiteness of the product, which was highest for control and lowest for 25F. The low ‘L’ values for 25F, FS, and FP are due to the brown colour contributed by the finger millet, which can also be related to higher ‘a’ value (redness). The ‘b’ value represents yellowness of the product. It was observed that due to replacement of rice with finger millet, sorghum and pearl millet, the yellowness decreased as compared to control *idli*.

Conclusion

The importance of cereals in our diet is very well appreciated from a nutritional point of view. In the present study, the partial replacement of rice in *idli* batter preparation with other cereals has contributed to increased microbial activity, enhanced enzyme activities, decline in trypsin inhibitor along with improved physicochemical and biochemical parameters during fermentation. The partial replacement of rice (50%, w/w) with other cereals led to improved overall acceptability of *idli* while colour was the most affected parameter due to rice replacement by other cereals in *idli* preparation. A positive impact on texture was observed. The work clearly demonstrates the potential to use rice replacement with other cereals in *idli* as a way to increase the consumption of other nutritious though underutilized cereals.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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