Investigation of biogenic amines content in fermented *idli* batter during storage

Baburaj Regubalan¹ · Laxmi Ananthanarayan¹

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Abstract *Idli* is a cereal-pulse based fermented food. This study profiles the qualitative and quantitative analysis of biogenic amines formed in the fermented *idli* batter prepared using varying proportions of rice to black gram *dal* at 1:1, 2:1, 3:1 and 4:1 (w/w) ratios and stored at 30 and 4 °C for 7 days. Histamine, tyramine, putrescine, cadaverine, spermidine, and spermine were investigated for the first time in the *idli* batter using HPLC technique. Putrescine and cadaverine were the predominant biogenic amines found in both prepared and market samples. Histamine and spermine were not detected in all batter samples. Increasing the proportion of rice in the *idli* batter resulted in the decrease in the total biogenic amines formed in the fermented batter with batter having more black gram dal (1:1) showing the maximum formation of total biogenic amines. *Idli* is a safe, easily digestible food to consume as the highest total biogenic amines quantified (366.87 µg/g) in 1:1 variant batter was below the harmful limit (1000 µg/g).

Keywords Biogenic amines · *Idli* batter · Histamine · Putrescine · Cadaverine · Tyramine · Spermidine

Introduction

Biogenic amines are organic nitrogenous compounds of low molecular weight which have potential biological significance in microbial, vegetable, and animal cells. These organic compounds are not toxic except when large quantities are taken or the natural mechanism of their catabolism are suppressed (Peñas et al. 2010). Biogenic amines are mainly formed by the reactions involving decarboxylation of amino acids as well as transamination of aldehydes and ketones (Santos 1996; Bai et al. 2013). Based on their chemical structure, it can be classified as heterocyclic (histamine and tryptamine), aliphatic (putrescine and cadaverine) or aromatic (tyramine and phenylethylamine) (Spano et al. 2010). Biogenic amines in food systems are formed by the inherent enzymes (Histidine decarboxylase, putrescine carbamoyl transferase, carbamate kinase, lysine decarboxylase, etc.) present in the raw material or are generated by the indigenous microflora (Spano et al. 2010; Collins et al. 2011). Therefore, they are likely to occur in fermented foods where they can be formed by the metabolic activity of the associated microbes.

Histamine and tyramine are the most toxic biogenic amines in food when present in excess. Food containing more than 1000 mg/kg of histamine is considered toxic and unsafe for human consumption (Witczak and Sikorski 2017). The consumption of foods rich in histamine can cause a headache, flushing, itching, hypotension, nasal secretion, bronchospasm, tachycardia, urticarial, pruritus, and asthma. Tyramine rich food can also cause hypertension and headache along with other symptoms such as a migraine, vomiting, perspiration, and pupil dilatation (Collins et al. 2011; Del Rio et al. 2017). Putrescine and cadaverine are less potent than histamine and tyramine.

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✉ Laxmi Ananthanarayan
l.ananthanarayan@ictmumbai.edu.in
Baburaj Regubalan
baburaj1326@gmail.com

¹ Department of Food Engineering and Technology, Institute of Chemical Technology, Matunga, Mumbai 400019, India

Spermidine and spermine are not toxic in themselves but they can increase the effect of histamine and tyramine (Naila et al. 2010; Peñas et al. 2010). Therefore, analysis of biogenic amines in fermented foods is important because of their potential toxicity and the possibility of using them as indicators of the degree of spoilage or freshness of food. High-performance liquid chromatography (HPLC) based methods are reliable, sensitive and can be used to quantify the concentrations of all biogenic amines present in various fermented food (Onal 2007).

“Idli” is a fermented food widely consumed in the Indian subcontinent and recently gaining recognition as part of the global cuisine (Durgadevi and Shetty 2012; Shrivastava and Ananthanarayan 2015). It is a steamed product, renowned for its appearance, characteristic sour taste, spongy texture, and typical flavor. As a part of traditional nutritional heritage and practices, *idli* is a favored food for both babies and elderly, especially with those of compromised digestion. It is prepared from a spontaneously fermented batter of rice (*Oryza sativa* L.) and split black gram *dal* (*Phaseolus mungo*), in a proportion of 2:1–4:1 (w/w). (Steinkraus 1995; Shrivastava and Ananthanarayan 2015). Lactic acid bacteria (LAB) utilize the available carbohydrates into organic acids such as lactic acid, reducing the pH of the batter, and favoring the growth of yeast (Sridevi et al. 2010; Shrivastava and Ananthanarayan 2015). The shelf life of the fermented *idli* batter is short, at 30 °C for 1 day, before becoming unpalatable, and it cannot be stored more than 5 days under refrigerated conditions (Nisha et al. 2005; Regubalan and Ananthanarayan 2018).

LAB and yeast are the dominant indigenous microbes playing a vital role in *idli* batter fermentation (Shrivastava and Ananthanarayan 2015). The indigenous microbes responsible for the fermentation of *idli* batter are *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii*, *Lactobacillus lactis*, *Lactobacillus casei*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Pediococcus cerevisiae*, and *Enterococcus faecalis*. Yeasts such as *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, *Candida glabrata*, *Candida tropicalis*, *Candida fragilola*, *Hansenula anomala*, *Torulopsis holmii*, *Geotrichum candidum*, and *Trichosporon beigelli* have also been reported in *idli* fermentation (Steinkraus 1995; Sridevi et al. 2010; Saravanan et al. 2015). LAB have the ability to produce biogenic amines in fermented foods (Arena and Manca de Nadra 2001). However, yeast and molds cannot massively form biogenic amines (Collins et al. 2011).

Fermentation of *idli* batter leads to an increase in free sugar, amino acid, nicotinic acid, methionine and choline content (Ghosh and Chattopadhyay 2011; Shrivastava and Ananthanarayan 2015). The availability of free amino acid is essential for decarboxylase positive microorganism to form biogenic amines (Santos 1996). The black gram *dal*

contains 21.57% of protein (Smartt and Nwokolo 2012) which provide major protein source to *idli* batter. Increase in the proportion of black gram *dal* could increase the free amino acid in the fermented *idli* batter which may act as precursors to increase the level of biogenic amines in *idli* batter. In general, market *idli* batter samples may contain various proportion of the raw materials used and it may be stored under varying storage conditions with potential for temperature abuse. According to Sharma et al. (2017), *idli* batter fermented at 35 °C for 19 h showed total biogenic amines content of 636 µg/g using a spectrophotometric technique with the accuracy level of 200–2000 ppm.

There is not much research reported about the formation of individual biogenic amines in the *idli* batter as a function of storage conditions and time. In order to ensure food safety of *idli* batter, there is a great need to determine the levels of biogenic amines formed both in freshly fermented and stored sample. The objective of this research involves estimation of different biogenic amines formed in the *idli* batter prepared from rice and black gram *dal* taken in different proportions of 1:1, 2:1, 3:1 and 4:1 (w/w) as a function of storage temperature.

Materials and methods

Chemicals and solvents

Spermine tetrahydrochloride (SPR), putrescine dihydrochloride (PUT), histamine dihydrochloride (HIS), cadaverine dihydrochloride (CAD), tyramine hydrochloride (TYR) and spermidine trihydrochloride (SPD) were procured from SRL Chemicals, Mumbai, India. Dansyl chloride was purchased from Sigma Chemical Co. (St. Louis, MO) Mumbai. Tri-chloroacetic acid, sodium bicarbonate, sodium hydroxide, glutamic acid, acetonitrile, and acetone were obtained from Merck, Mumbai. All solvents used were of HPLC grade.

Preparation of *idli* batter

The raw materials, parboiled rice (*O. sativa*), split black gram (*P. mungo*), TATA salt, and six marketed *idli* batters were all purchased from the local supermarket of Mumbai. Parboiled rice and black gram were taken individually in 1:1, 2:1, 3:1, 4:1 (w/w) ratio and soaked in distilled water at 30 °C for 4 h. The rice was ground to a coarse consistency and black gram *dal* was ground separately to a fine paste. These two batters were mixed with addition of 0.9% w/w table salt. The resulting batter was incubated at 30 °C for half a day for fermentation (Regubalan and Ananthanarayan 2018). The fermented batter was transferred to plastic containers and stored at 30 °C and 4 °C for 7 days

to monitor the level of biogenic amines formation. Six *idli* batter samples were obtained from the local market, coded as IB1, IB2, IB3, IB4, IB5, and IB6 and immediately analyzed.

Measurement of pH

The pH of stored (30 °C and 4 °C) *idli* batter was measured using a digital EUTECH pH meter.

Measurement of acid content

Generally, 10 g of *idli* batter was taken in 25 ml of distilled water and titrated against freshly prepared 0.1 N NaOH using phenolphthalein as an indicator. The acid content of the *idli* batter was calculated in terms of anhydrous lactic acid produced during fermentation (Horwitz 1980).

Preparation of standard solutions of biogenic amines

In general, 5000 mg L⁻¹ stock solutions of each tested biogenic amines were prepared in deionized water and kept in amber colored glass bottles at - 20 °C. Standard solutions were prepared by diluting this stock solution and used for plotting the standard curve.

Extraction and derivatization of biogenic amines from *idli* batter samples

The biogenic amines were extracted from 5 g of *idli* batter sample by mixing in 25 mL of 5% tri chloro acetic acid. The mixture was homogenized for 20 min, centrifuged at 6,296 g at 4 °C for 5 min and the supernatant was filtered through Whatman No. 1 filter paper. The dansyl chloride derivatives of the extracted biogenic amines were prepared by taking one mL of above extract in a test tube, to which 200 µL of a saturated solution of NaHCO₃, 20 µL of 2 N NaOH and 2 mL of dansyl chloride (5 mg/mL) was added. The test tube was sealed and mixed properly and incubated at 70 °C for 15 min in dark. The unbound dansyl chloride was removed by addition of 1 mL of glutamic acid (2 mg/mL in deionized water) and this solution was left in the dark for 1 h. The final volume was adjusted to 5 mL with acetonitrile and filtered through 0.22 µm PVDF membrane filter (Saaid et al. 2009).

Analytical method

All the biogenic amines quantification was performed using Jasco HPLC system (UV-1575, PU-1580) by a standardized method reported by Saaid et al. (2009). Chromatographic separation was done by injecting 20 µL of pre-derivatized and filtered (0.22 µm) sample into

Waters Spherisorb 5 µm ODS2 column (250 × 4.5 mm). The analysis was carried out in isocratic mode with the mobile phase composition of acetonitrile:water; 70:30 (v/v) at a flow rate of 1 mL/min. The column effluent was monitored at 254 nm. Reproducibility and repeatability were evaluated by injecting five times each of the six standard mixtures on the same day. Limit of detection and limit of quantification were determined based on the method described by Campos et al. (2017). Biogenic amines were spiked at 10, 40, and 80 ppm in the unfermented *idli* batter samples which were extracted, derivatized, and analyzed by HPLC to determine the percentage recovery. All the experiments were done in triplicates and results are expressed in mean and percentage relative standard deviation (%RSD).

Statistical analysis

All experiments were done in triplicates and results are expressed as mean ± SD. Statistical differences between samples were compared by Duncan's test using IBM®SPSS® version 20 at a *p* value of 0.05.

Results and discussion

Analytical characteristics of the method for estimation of biogenic amines

The HPLC method developed for simultaneous estimation of biogenic amines revealed well resolved chromatographic peaks. The representative chromatogram of the standard biogenic amines (A) and *idli* batter made from 3:1 (w/w) rice:black gram *dal* ratio stored at 30 °C for 7 day sample extract (B) are shown in Fig. 1. The analytical characteristics of the HPLC method used for quantification of biogenic amines in *idli* batter are shown in Table 1. The HPLC method was found to be linear over concentration range of 5–140 µg/mL with regression coefficients > 0.997.

Limit of detection and limit of quantification were determined to know the sensitivity of the method. Good repeatability was achieved in both retention times (%RSD < 1.31) and the peak area (%RSD < 2.39) (Table 1). Acceptable recovery of all biogenic amines was obtained (91.52–106.53%).

Metabolic activity in relation to pH and titratable acidity

pH and titratable acidity are important parameters which decide the sour taste of *idli* and also represent the metabolic activity of the microbes. The observed trends for decrease in pH and increase in titratable acidity during the

Fig. 1 Chromatogram of **a** standard biogenic amines and **b** *idli* batter made from 3:1 (w/w) rice:black gram *dal* ratio stored at 30 °C for 7 day. Peak identity: spermine (1), putrescine (2), cadaverine (3), histamine (4), tyramine (5), spermidine (6)

storage of *idli* batter samples are depicted in Fig. 2. pH drastically decreased in all four variants of *idli* batter when stored at 30 °C for 7 days, whereas, refrigerated *idli* batter samples showed a slow reduction of pH and slow increase

in acidity throughout the storage period of 7 days because of the inhibition of the metabolic activity of microbes at low temperature.

Biogenic amines in *idli* batter sample stored at 30 °C

The biogenic amine profile of prepared *idli* batter samples stored at 30 °C for 7 days is shown in Fig. 3. Putrescine,

Table 1 Analytical characteristics of the HPLC method used for quantification of biogenic amines

Biogenic amines	tR (min)	R ²	LOD (µg/ml)	LOQ (µg/ml)	Linear range (µg/ml)	Intraday (% RSD) n = 3		% Recovery		
						tR	Area	10 (µg/ml)	20 (µg/ml)	40 (µg/ml)
Spermine	4.93	0.997	0.77	2.31	5–80	0.06	0.68	91.52 (1.80)	103.34 (1.26)	97.62 (0.40)
Putrescine	8.92	0.998	0.78	2.37	5–80	0.34	0.56	102.68 (0.56)	103.24 (1.15)	104.72 (1.53)
Cadaverine	10.05	0.999	0.42	1.28	5–80	0.16	1.13	99.05 (5.20)	99.39 (3.52)	100.3 (1.64)
Histamine	11.24	0.999	0.85	2.59	5–120	0.21	0.84	106.53 (0.22)	102.52 (0.84)	99.10 (2.42)
Tyramine	19.23	0.999	0.59	1.79	5–140	0.64	2.39	93.48 (3.42)	99.64 (2.39)	96.66 (2.69)
Spermidine	23.41	0.997	1.38	4.19	5–100	1.31	1.27	95.65 (4.40)	100.80 (3.16)	97.91 (2.28)

Values in bracket is relative standard deviation (%RSD)

tR Retention time, R² square of regression coefficient, LOD limit of detection, LOQ limit of quantification, RSD relative standard deviation

Fig. 2 Change in the pH and titratable acidity in different fermented *idli* batter samples (0.5 day) stored at 30 and 4 °C for 7 days

Fig. 3 The amount of the individual and total biogenic amines detected in fermented *idli* batter (0.5 day) stored at 30 °C for 7 days. Putrescine (a), cadaverine (b), tyramine (c), spermidine (d), and total biogenic amines (e). Different letters indicate statistically significant differences ($p < 0.05$)

Fig. 3 continued

cadaverine, tyramine, and spermidine were the major biogenic amines observed in *idli* batter. Histamine and spermine were not detected in the *idli* batter samples stored under these conditions. The biogenic amines were not formed in any variant of unfermented *idli* batter.

Parboiled rice contains 6% of protein and rice proteins are deficient in lysine (Shakuntala and Shadaksharaswamy 1987) whereas, the proximate analysis of black gram shows 21.57% of protein (Smartt and Nwokolo 2012) which contributes major proteins to the *idli* batter. According to Padhye and Salunkhe (1979), black gram contains lysine (6.0 mol%), arginine (5.2 mol%), and tyrosine (2.5 mol%) which are precursors for the biogenic amines formed during *idli* batter fermentation. Arginine is the precursor for the formation of putrescine, spermidine, and spermine. Similarly, lysine is the precursor for the formation of cadaverine (Santos 1996). Increase in the black gram *dal* proportion led to an increase in putrescine and cadaverine formation in the fermented *idli* batter (0.5 day). Tyramine was formed from tyrosine by tyrosine decarboxylase enzyme (Collins et al. 2011). Tyramine was detected in 1:1 and 2:1 (w/w) ratio of fermented *idli* batter (0.5 day), however, it was found on the second day of storage for 3:1 and 4:1 (w/w) ratio. The decrease in the proportion of black gram *dal* delays the formation of tyramine in *idli* batter. The highest concentration of tyramine was found to be 64.91 µg/g in 1:1 variant stored at 30 °C for 3 days. Spermidine was the only polyamine detected in 1:1 and 2:1 (w/w) ratio of *idli* batter from the second day of storage at 30 °C; similarly it was found in 3:1 and 4:1 (w/w) ratio from the fourth day of storage. The highest concentration of spermidine was found to be 25.91 µg/g in 1:1 variant stored at 30 °C for 5 days. Putrescine is the precursor for the formation of spermidine, and spermine. The conversion of putrescine into spermidine may be one of the reasons for the observed reduction of putrescine concentration in all the variants of *idli* batter stored at 30 °C beyond 3 days.

Putrescine and cadaverine were the predominant biogenic amines found in all four variants of *idli* batter after

fermentation (0.5 day). These biogenic amines may be potentially used as a bio-marker to quantify the proportion of black gram *dal* in *idli* batter. Biogenic amines detected from all four variants of *idli* batter were significantly reduced on the seventh day of storage at 30 °C. The maximum concentration of cadaverine, putrescine, tyramine, and spermidine were produced in 1:1 (w/w) ratio of *idli* batter stored at 30 °C. *Idli* batter is known to have wide varieties of LAB strains. The LAB culture that could produce amine oxidizing enzymes namely mono amine oxidase (MAO, EC 1.4.3.4) and diamine oxidase (DAO, EC 1.4.3.6) may be able to metabolize more than one biogenic amine leading to the degradation of biogenic amines in *idli* batter stored at 30 °C for 7 days. Tosukh Wong et al. (2011) reported that *L. plantarum* BCC 9546, has an ability to decrease tyramine formation in a Thai fermented sausage called *Nham*.

The fermented *idli* batter (0.5 day) made up of 1:1, 2:1, 3:1 and 4:1 (w/w) ratios of rice:black gram *dal* showed total biogenic amine concentration of 163.19, 125.93, 57.87 and 38.71 µg/g respectively. The highest concentration of total biogenic amines was 366.87 µg/g in 1:1 variant of *idli* batter stored at 30 °C for 3 days. Total biogenic amine levels of 1000 mg/kg in food were considered harmful to human health (Kim et al. 2012). Thus under adverse conditions of storage (30 °C), the total biogenic amine content in *idli* batter was found to be less than harmful level.

Biogenic amines in *idli* batter sample stored at 4 °C

The biogenic amines content measured from fermented *idli* batter (0.5 day) stored at 4 °C for 7 days is depicted in Fig. 4. Histamine, spermidine, and spermine were not detected throughout the storage at 4 °C in all variants of *idli* batter. Putrescine, cadaverine, and tyramine were detected during the storage of fermented *idli* batter (0.5 day) at 4 °C for 7 days. In the present work, the highest concentration of putrescine obtained was 140 µg/g

which was same ($p < 0.05$) for 1:1 ratio of *idli* batter stored at 4 °C for 3, 4 and 5 days. Cadaverine concentration was gradually increased in all variant of *idli* batter up to 3 days when stored at 4 °C. The highest concentration of cadaverine was found to be 58 µg/g in 1:1 variant of *idli* batter. Tyramine was formed in fermented *idli* batter (0.5 day) in 1:1 and 2:1 variants. It was formed on the second day of storage in 3:1 ratio and third day of storage in 4:1 ratio of *idli* batter. It can be concluded that the reduction in the proportion of black gram *dal* delayed the formation of tyramine under refrigerated storage conditions. According to Patat et al. (1995), 1100 mg of tyramine was found to be an effective dose (ED₅₀) at which 50% of the individuals responded. Highest tyramine content was found to be 44.31 µg/g in 1:1 variant of batter stored at 4 °C for 4 days, which is far lesser than the effective dose.

The highest total biogenic amine concentration was found to be 249.13 µg/g in *idli* batter prepared from 1:1 (w/w) ratio after 4 days of storage at 4 °C. Overall, *idli* batter stored under refrigerated conditions showed a gradual increase in the individual and total biogenic amines concentrations in the initial period of storage (up to 4 days) and subsequently a slight decline was observed. Refrigerated storage condition (4 °C) may lead to the reduction in enzymatic and metabolic activity of the microbes which could explain the slow increase and decline of biogenic amine formation at 4 °C as compared to storage of *idli* batter at 30 °C. The same phenomenon was observed by Peñas et al. (2010), who reported that the individual biogenic amine levels underwent a gradual increase in the sauerkraut when stored at 4 °C for 3 months.

Correlation of pH, titratable acidity and LAB count vs total biogenic amines

Despite pH, titratable acidity, LAB count and concentration of biogenic amines, all being output effects of fermentation, R² value was found to be 0.039 between pH and total biogenic amines formed in *idli* batter stored at 30 °C

for 7 days (Supplementary Fig. 1). The regression value was 0.026 for *idli* batter stored at 4 °C for 7 days. Similarly, the regression value was found to be less than 0.1 between titratable acidity and total biogenic amines under both storage conditions (Supplementary Fig. 2). Similarly, R² value was found less than 0.35 between LAB count and total biogenic amines in *idli* batter stored at 30 °C and 4 °C. It appears that formation of total biogenic amines is not correlated with pH and percent titratable acidity which are indicators of metabolic activity of the fermenting microflora. The Japanese fermented food “*nattao*” also showed the R² value less than or around 0.1 between percent titratable acidity and total biogenic amines found in fermented *nattao* (Kim et al. 2012).

Biogenic amines in marketed samples of *idli* batter

The levels of pH, titratable acidity and biogenic amines of six marketed *idli* batter samples are shown in Table 2. LAB plays a significant role in the fermentation of *idli* batter producing lactic acid as a result of their metabolic activity. The formation of lactic acid during fermentation leads to a drop in pH and increase in titratable acidity. An optimally fermented *idli* batter generally demonstrates a pH of 4.0 to 4.5. Thus pH and titratable acidity are good measures of degree of fermentation of *idli* batter. The market samples of *idli* batter procured for this study showed pH in the range of 4 to 4.5 implying that they were adequately fermented. This could support our findings of biogenic amines in these batters as these amines are formed due to the metabolic activity of LAB. Putrescine and cadaverine were the two predominant amines detected in all market samples except IB4 which may be due to the low proportion of black gram *dal* used in the preparation of batter which is also implied by the lower price of this batter sample. Tyramine was found only in IB6 sample. Tyramine formation is highly influenced by the proportion of black gram *dal* used in batter preparation (Figs. 3c, 4c). Tyramine formation was very less during the refrigerated condition of storage as shown in Fig. 4c.

Table 2 Levels of pH, % titratable acidity, and biogenic amines in market samples of *idli* batter

Market sample	Price (INR/kg)	pH	Acidity (%)	Biogenic amines (µg/g)			Total biogenic amines (µg/g)
				PUT	CAD	TYR	
IB1	60	3.96 ± 0.03 ^b	1.05 ± 0.02 ^d	35.27 ± 1.98 ^d	19.87 ± 1.56 ^b	N.D.	55.14 ± 3.54 ^b
IB2	60	4.42 ± 0.01 ^d	0.75 ± 0.03 ^a	10.99 ± 1.65 ^a	15.43 ± 2.11 ^a	N.D.	26.42 ± 3.76 ^a
IB3	70	4.19 ± 0.02 ^c	0.91 ± 0.01 ^c	29.22 ± 2.70 ^c	34.89 ± 1.99 ^d	N.D.	64.11 ± 0.71 ^c
IB4	30	3.87 ± 0.04 ^a	1.16 ± 0.03 ^e	N.D.	N.D.	N.D.	N.D.
IB5	70	4.21 ± 0.01 ^c	0.82 ± 0.02 ^b	24.11 ± 3.07 ^b	30.77 ± 2.45 ^{cd}	N.D.	54.88 ± 5.52 ^b
IB6	60	4.00 ± 0.03 ^b	1.03 ± 0.04 ^d	21.67 ± 2.37 ^b	28.65 ± 3.19 ^c	5.08 ± 2.76	55.40 ± 1.94 ^b

PUT putrescine, CAD cadaverine, TYR tyramine. Histamine, spermine, and spermidine were not detected; ND not detected

Different letters indicate a significant difference ($p < 0.05$) within the same column

Fig. 4 The amount of the individual and total biogenic amines detected in fermented *idli* batter (0.5 day) stored at 4 °C for 7 days. Putrescine (a), cadaverine (b), tyramine (c), and total biogenic amines (d). Different letters indicate statistically significant differences ($p < 0.05$)

Histamine, spermidine, and spermine were not detected in all four variants of *idli* batter stored at 4 °C for 7 days likewise these three biogenic amines were not detected in any of the six market samples. The IB3 market sample showed the highest concentration of total biogenic amines (64.11 µg/g), while IB4 showed no formation of biogenic amines. Overall, the total biogenic amines concentration estimated in market batter samples was far below the toxic dosage level.

Conclusion

The present work was conducted to investigate the biogenic amines formed in different variants of fermented *idli* batter stored at 30 °C and 4 °C for 7 days. Cadaverine and putrescine were the predominant amines found in the fermented *idli* batter (0.5 day) which also showed formation of tyramine. Histamine, spermidine, and spermine were not found in all variants of batter stored under refrigerated conditions. Traces of tyramine were found in only one of the market batter samples. The total biogenic amines content was increased for 3 days and decreased from the fourth day of storage at 30 °C for 7 days, whereas a gradual increase of total biogenic amines content was observed at 4 °C for 7 days. It was found that total biogenic amines quantified in the *idli* batter was substantially less than the toxic dosage which showed that *idli* is a safe food to consume. Thus biogenic amines found in *idli* batter were majorly influenced by the proportion of raw materials used and the storage conditions.

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