# Biofortification of riboflavin and folate in idli batter, based on fermented cereal and pulse, by Lactococcus lactis N8 and Saccharomyces boulardii SAA655

Rajendran, S.C.Chandrasekar

2017-05-03

Rajendran , S C C , Chamlagain , B , Kariluoto , S , Piironen , V & Saris , P E 2017 , ' Biofortification of riboflavin and folate in idli batter, based on fermented cereal and pulse, by Lactococcus lactis N8 and Saccharomyces boulardii SAA655 ' , Journal of Applied Microbiology , vol. 122 , no. 6 , pp. 1663-1671 . https://doi.org/10.1111/jam.13453

http://hdl.handle.net/10138/258270
https://doi.org/10.1111/jam.13453

acceptedVersion

Received Date : 15-Aug-2016
Revised Date : 09-Mar-2017
Accepted Date : 20-Mar-2017
Article type : Original Article

# Biofortification of riboflavin and folate in idli batter, based on fermented cereal and pulse, by *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655 in idli batter

Suresh Chander C.R., Bhawani Chamlagain, Susanna Kariluoto, Vieno Piironen, Per E.J. Saris

Department of Food and Environmental Sciences, University of Helsinki, Finland

**Address for correspondence**
Suresh Chander C.R.
Department of Food and Environmental Sciences,
University of Helsinki, Finland
Suresh.chander@helsinki.fi

## Abstract

### Aims

*Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655 were investigated for their ability to synthesize B-vitamins (riboflavin and folate) and their functional role as microbial starters in idli fermentation.

### Methods and results

In this study, ultra-high performance liquid chromatography (UHPLC) and microbiological assay were used to determine the total riboflavin and folate content, respectively. Increased levels of folate were evident in both *L. lactis* N8 and *S. boulardii* SAA655 cultivated medium. Enhanced riboflavin levels were found only in *S. boulardii* SAA655 grown medium whereas decreased riboflavin level was found in *L. lactis* N8 cultivated medium. To evaluate the functional role of microbial starter strains, *L. lactis* N8 and *S. boulardii* SAA655 were incorporated individually and in combination into idli batter, composed of wet grounded rice and blackgram. For the experiments, naturally fermented idli batter was considered as control. The results indicated that natural

idli fermentation did not enhance the riboflavin level and depleted folate levels by half. In comparison with control, *L. lactis* N8 and *S. boulardii* SAA655 incorporated idli batter (individually and in combination) increased riboflavin and folate levels by 40 – 90 %. Apart from compensating the folate loss caused by natural fermentation, *S. boulardii* SAA655 fermented idli batter individually and in combination with *L. lactis* N8 also showed the highest leavening character. Moreover, the microbial starter incorporation did not significantly influence the pH of idli batter.

**Conclusion**

Incorporation of *L. lactis* N8 and *S. boulardii* SAA655 can evidently enhance the functional and technological characteristics of idli batter.

**Significance and Impact of Study**

UN General Assembly declared 2016 the International Year of pulses emphasizing the importance of legumes as staple food. Further this is the first experimental report of *in situ* biofortifcation of riboflavin and folate using microbes in pulse based fermented staple food. The current study suggests possible avenues for research towards an economical strategy to reduce B-vitamin deficiency among the consuming population.

*Keywords – idli, fermentation, folate, riboflavin, Saccharomyces boulardii, Lactococcus lactis, biofortification*

## 1 Introduction

Idli is a traditional cereal-legume based steamed cake widely consumed in the Indian subcontinent. The conventional preparation process of idli at households involves soaking of rice and dehulled black gram, followed by grinding and fermentation of wet batter. The fermented batter is steamed and consumed. Steamed idlis are white in color, round, soft and porous in texture with its characteristic mild-sour taste, resembling the attributes of sour dough bread. Nutritionally, idli is a balanced source of carbohydrate, protein and amino acids

(Reddy et al. 1982). On an everyday basis, idli is consumed in bulk quantities within households, restaurants, hospitals and corporate arrangements.

The beneficial aspects of idli fermentation have been a discipline of research for more than three decades (Khandwala et al. 1962, Ananthachar & Desikachar 1962, Agrawal et al. 2000, Sridevi et al. 2010, Saravanan & Shetty 2015).

Lactic acid bacteria (LAB) and yeasts play a predominant role in idli fermentation. *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus delbrueckii*, *Lb. fermentum*, *Lb. plantarum*, *Saccharomyces cerevisiae, Torulopsis candida*, *Kluyveromyces marxianus* and *Candida kefyr* (Mukherjee et al. 1965, Reddy et al. 1982, Soni et al. 1986, Iyer et al. 2013) are some LAB and yeast species that form the natural microbiota of idli batter. These organisms originate during soaking of rice and black gram aiding spontaneous fermentation accompanied by two notable changes in idli batter - acidification and leavening (Mukherjee et al. 1965). Studies also report that natural fermentation evidently increases B-vitamins, amino acids and reduces anti-nutrients like phytates in idli batter (Reddy et al. 1982). Traditionally idli has been prepared through natural (spontaneous) fermentation process (Mukherjee et al. 1965). Back slopping strategy or use of microbial starter cultures for fermentation has not been in practice so far. In food fermentations, benefits of microbial starter cultures have been well documented (Holzapfel 2002). However, nutritional improvement of idli batter utilizing microbial starters remains understudied.

B-vitamins such as riboflavin and folate are essential micronutrients vital for human development and metabolism. In India, the prevalence of B- vitamin deficiency is high due to socioeconomic reasons (Swaminathan et al. 2013). Biofortification through fermentation is an efficient way to reduce the B-vitamin deficiency and has been intensively studied in recent years (Kariluoto et al. 2006, Capozzi et al. 2012a, Capozzi et al. 2012b, Kariluoto et al, 2014,

Russo et al. 2014; del Valle et al. 2014, Ibrahim et al. 2015). Recently, several LAB and yeasts have been identified for their ability to produce riboflavin (Sybesma et al. 2003; LeBlanc et al. 2011; Arena et al. 2014, Thakur et al. 2015; Thakur & Tomar 2015; Thakur & Tomar 2016; Thakur et al. 2016; Silva et al. 2016) and folate ( Sybesma et al. 2003; Hjortmo et al. 2005; Iyer & Tomar 2009; Iyer et al. 2010; Iyer & Tomar 2011).

Employment of vitamin-producing LAB and yeasts in cereal substrate seems to be a convenient economical alternative over synthetic addition (Capozzi et al. 2012b). Identification of such strains is a key challenge, as riboflavin and folate production is an individual strain specific property (LeBlanc et al. 2011). An alternative would be to overproduce folate and riboflavin through metabolic engineering and it has been successfully demonstrated in different LAB and yeasts ( Jiménez et al. 2005, Burgess et al. 2009). Nevertheless, the general negative opinion on genetically manipulated foods among consumers has resulted in increased preference for natural vitamin producers. The strategy of fermentation of cereal-based substrates for B vitamin fortification has succeeded in wheat, rye, oat, and soy based substrates (Kariluoto et al. 2004, Hjortmo et al. 2008, Rekha and Vijayalakshmi 2010, Korhola et al. 2014).

In this study, the ability of *Lactococcus lactis* N8 and probiotic *Saccharomyces boulardii* SAA655 to produce riboflavin and folate was assessed and their potential role as microbial starter cultures in idli batter was evaluated.

## 2 Materials and methods

### 2.1 Strains, media and culture conditions

*Lactococcus lactis* N8 (Graeffe et al. 1991) was cultured on M17 (Oxoid Ltd., Basingstoke, England) agar for the propagation and or in M17 broth supplemented with 0.5% glucose (w/v) (named as M17G) for the culture preparation. In our previous study, this strain was successfully used to ferment soymilk (Beasley et al. 2003) and was presumed to be competitive in a partly legume based idli fermentation.

*Saccharomyces boulardii* SAA655 was procured as a supplement capsule (Precosa, Biocodex) from a local pharmacy (Helsinki, Finland) and activated in yeast peptone dextrose (YPD) broth. This yeast was chosen as microbial starter because idli fermentation typically includes yeast and lactic acid bacteria. In addition, the chosen yeast is a well-known probiotic with a long history of use (McFarland 2010). The incubation for both cultures was performed at 30 °C for 12 h.

### 2.2 Ability of *L. lactis* N8 and *S. boulardii* SAA655 to enhance riboflavin and folate

The strains, *Lactococcus lactis* N8 (Ll N8) and *Saccharomyces boulardii* SAA655 (Sb), were inoculated (8 log cfu/g) in M17G broth and YPD broth, respectively, and incubated at 30 °C for 24 h. For the analysis of the riboflavin and folate producing ability, 20 ml of *L. lactis* N8 and *S. boulardii* SAA655 cultured broth samples were collected in 50 ml Falcon tubes at three time points ($0^{th}$, $12^{th}$ and $24^{th}$ hour). The uninoculated M17G and YPD broths incubated at 30 °C were considered as controls. Riboflavin and folate contents were analyzed as reported in section 2.5.

### 2.3 Preparation of idli batter

Parboiled rice (*Oryza sativa*) and dehulled black gram (*Phaseolus mungo*) were procured from local market (Helsinki, Finland) for the experiments. Rice and dehulled black gram were taken in 3:1 ratio separately and thoroughly washed in potable water and then soaked in sterile water for 6 h at 28 ± 2°C. Soaked ingredients were finely and coarsely grounded separately, with periodic additions of water in an electrical wet grinder (Sowbaghya, India). The batters were blended together with table salt (0.85% w/v).

### 2.4 Microbial starter addition and fermentation

For incorporation of microbial starters into the idli batter, 30 ml of overnight grown cultures of *L. lactis* N8 (9.8 log cfu/g) and *S. boulardii* SAA655 (9.5 log cfu/g) were taken in 50 ml Falcon tubes and centrifuged (5804 R, Eppendorf, Germany) at 7000 rpm for 10 min at 4 °C. The supernatant was discarded without disturbing the pellet formed and the cells were resuspended in distilled water. The pellet suspension with microbial starter culture was added to idli batter individually and in combination. Naturally fermented idli batter (without microbial starter) was used as control. The idli batter samples were allowed to ferment for 14 h at 28 ± 2°C. For the vitamins and the physicochemical analyses, the following samples were considered: (1) Unfermented idli batter (2) naturally fermented idli batter (control) (3) *L. lactis* N8 fermented idli batter (4) *S. boulardii* SAA655 fermented idli batter (5) *L. lactis* N8 and *S. boulardii* SAA655 (in combination) fermented idli batter.

**2.5 Riboflavin and folate analyses**

Broths were analysed for riboflavin and folate as such, whereas idli batter samples (150 g each) were freeze-dried before vitamin analyses. All the steps were performed in subdued light as these B-vitamins are sensitive to light. Riboflavin was analyzed using ultra-high performance liquid chromatographic (UHPLC) method reported by Chamlagain et al. (2016). In short, samples (about 1 ml of broth or 0.1 g of the freeze-dried idli batter) were extracted using acid hydrolysis, followed by enzymatic treatment with β-amylase and Taka-diastase. Separation was achieved on Acquity BEH C18 column (2.1 mm ×100 mm; 1.7 µm particles) with 20 mM ammonium acetate in 30% aqueous methanol as the mobile phase, and riboflavin was detected by fluorescence detection with 444 nm as the excitation and 520 nm as the emission wavelength (Chamlagain et al. 2016).

For folate analysis, 5 ml of broth or about 0.8–1.5 g of freeze-dried idli batter was taken as the sample. Extraction procedure included heat extraction in a boiling water bath followed by trienzyme treatment with amylase, hog kidney conjugase and protease. Total folate contents were determined by microbiological assay on microtiter plates using *Lactobacillus rhamnosus* ATCC 7469 as the growth organism and 5-formyltetrahydrofolate as the calibrant (Kariluoto et al. 2004).

**2.5 Change of pH and batter volume**

The pH of the different idli batter samples was measured directly with the pH meter (model 420A+, Thermo Orion, USA) before and after fermentation of natural and microbial starter fermented batters.

For the measurement of the change in batter volume, 100 ml of the unfermented idli batter was taken into 250 ml beaker. After 14 h of fermentation, the change in the batter volume for each sample was recorded in 'ml'.

**2.6 Total plate counts**

The changes in the microbial counts (log cfu/g) of the idli batter before and after fermentation were determined by the total-plate count method (TPC), using the spread plate technique. LAB and yeast counts were enumerated on MRS agar and YPD, respectively.

**2.7 Statistical analysis**

The results presented were obtained from two independent experiments and each sample was measured in duplicate (n = 2 x 2 = 4). The values were expressed as means ± standard deviations. The microbiological cell counts were initially obtained as colony forming units (CFU/g) and reported in log units. Analysis of variance and significant differences for the mean values of samples were determined by one-way ANOVA and Tukey's test. The data were processed through Microsoft Excel (Microsoft, USA) and Statgraphics Centurion XVI (Version16.1, Statgraphics).

## 3 Results

### 3.1 Ability of *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655 to enhance riboflavin

The initial riboflavin content of M17G medium was approximately 280 ng/g. When *L. lactis* N8 was grown for 24 h in this medium the riboflavin content decreased about 25 % to 210 ng/g (Fig. 1A), showing that in M17G medium *L. lactis* N8 did not produce riboflavin. In contrast, with *S. boulardii* SAA655 incubated in YPD medium the riboflavin content increased by about 50 % to 500 ng/g (Fig. 1B), demonstrating that *S. boulardii* SAA655 has the ability to synthesize riboflavin.

### 3.2 Ability of *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655 to enhance folate

The initial folate content of M17G and YPD medium was found to be around 87 ng/g and 137 ng/g, respectively. An increase in folate content was noted in both M17G medium incubated with *L. lactis* N8 and YPD medium incubated with *S. boulardii* SAA655 (Fig. 2A and B), suggesting that both strains synthesize folate. At the end of fermentation with *L. lactis* N8 and *S. boulardii* SAA655, the folate content increased by around 130 % and 40 %, respectively.

### 3.3 Changes in riboflavin content of idli batter on addition of *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655

The initial riboflavin content of the unfermented idli batter was 540 ng/g (dry matter basis). Natural fermentation without addition of the microbial starters did not significantly affect the initial amounts of riboflavin. Fermentation with *L. lactis* N8 could not significantly improve the riboflavin content of the idli batter (Fig. 3A). However, a notable and significant increase in riboflavin level (40%; $p < 0.05$) was recorded in idli batter by the addition of *S. boulardii* SAA655. Idli batter incorporated with *S. boulardii* SAA655 in combination with *L. lactis* N8 did not further enhance the noted rise in riboflavin content of idli batter fermented with *S. boulardii* SAA655 alone. These results suggest that *S. boulardii* SAA655 can be used to increase the riboflavin content of the idli batter.

### 3.4 Changes in folate content of idli batter on addition of *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655

Natural idli fermentation process without addition of microorganisms decreased (by 50 %) the initial folate amounts (344 ng/g) of the idli batter (Fig. 3B). This decrease in folate content was compensated by the addition of *L. lactis* N8 or *S. boulardii* SAA655 (Fig. 3B). The idli batter fermented with the combination of the microbial starters had the highest amount of folate (408 ng/g). The folate amount present in combinational microbial starter fermented idli was statistically ($p > 0.05$) similar to *S. boulardii* SAA655 fermented idli (352 ng/g) (Fig. 3B). This clearly shows that the addition of *S. boulardii* SAA655 with or without *L. lactis* N8 to the idli batter fermentation restores the initial folate content of the idli batter compared to a significant loss of folate in the natural fermentation.

### 3.5 Change of pH during the idli fermentation

Freshly grounded, unfermented idli batter had an initial pH of 5.91 (± 0.10). After 14 h of fermentation, the pH of all (control and microbial starter added) idli batter samples decreased to 4.35 (± 0.14). The pH value recorded in the microbial starter added idli batters was statistically ($p > 0.05$) similar to the pH value of the naturally fermented batter.

### 3.6 Microbiological changes of the idli batter during the fermentation

In naturally fermented idli batter, rice and dehulled black gram were first soaked (6 h) to allow the rice and black gram to soften. During the soaking period the indigenous microbial population grew and the measured microbial counts were around 7 log cfu/g of LAB and yeasts (Table 1) before the actual fermentation, which followed the wet grinding and mixing of the grounded rice and black gram. The idli batters with added *L. lactis* N8 and/or *S. boulardii* SAA655 yielded higher LAB and yeast counts. After the fermentation, the final population of the yeast and LAB ranged between 9.7 – 9.9 log cfu/g in all of the idli batters (Table 1). The cell numbers increased by 2.8 log units in naturally fermented idli batter. In the *S. boulardii* SAA655 fermented idli batter, a 10-fold rise from the initial counts (8.6 log) was observed. In the idli batter fermented with both microbial starters, the initial and final populations were similar (Table 1).

### 3.7 Change in the idli batter volume

In all the idli batter samples there was an increase in the batter volume at the end fermentation compared to the starting volume (Fig. 4). Statistically similar batter volume increase was observed between the naturally fermented and *L. lactis* N8 added idli batter.

Among the fermented idli batter samples, a higher expansion was observed in the batter with the addition of *S. boulardii* SAA655 individually and in combination with *L. lactis* N8 (by 70-85%) compared to the naturally fermented batter. The *S. boulardii* SAA655 fermented batter showed a similar rise of batter volume, suggesting that *S. boulardii* SAA655 in combination or alone was responsible for significantly improving the batter volume by producing a higher amount of carbon dioxide.

## 4 Discussion

B–vitamin levels of *L. lactis* N8 and the probiotic *S. boulardii* SAA655 cultured in their respective growth media suggest that *L. lactis* N8 is a riboflavin consumer but a folate enhancer, whereas *S. boulardii* SAA655 is both a riboflavin and folate producer. These results are in agreement with the previous reports on the B-vitamin (riboflavin and folate) producing ability of *L. lactis* and *S. boulardii* SAA655 (Sybesma et al. 2003, Burgess et al. 2004; Hjortmo et al. 2005). Burgess et al. (2004) reported *L. lactis* NZ9000 produced riboflavin in a chemically defined medium. Similar riboflavin synthesizing trend was not evident, when *L. lactis* N8 was cultivated in M17G medium, as vitamin synthesizing ability may be strain specific (LeBlanc et al. 2011).

LAB and yeast counts steadily rise from period of soaking rice and black gram resulting in $10^6$ - $10^7$ log cfu/g (Mukherjee et al. 1965), as observed (Table 1). In this study, microbial starter cultures were supplemented along with natural microbiota of the batter and no efforts were taken to eliminate the natural microbiota originated during the soaking process resulting in high initial counts (Table 1). High counts (~9.5 log cfu/g) of *L. lactis* N8 and *S. boulardii* SAA655 were used to inoculate batters, as the microbial starter's ability to co-exist with indigenous organisms was previously unknown. Therefore, high counts of *L. lactis* N8 and *S.*

*boulardii* SAA655 were incorporated to as an advantage for the added microbial starters to compete with the natural microbiota.

Successful progression of fermentation is marked by two pivotal changes - acidification and leavening (Mukherjee et al. 1965) and these changes were evident in all fermented batter samples. Reduction in pH can be attributed to an increase of lactic acid content in the batter (Sridevi et al. 2010).

Microbial starter additions did not result in a significant reduction of the batter pH compared to the naturally fermented idli. This is desired attribute, as too sour idlis are not generally accepted for consumption.

Increase in idli batter volume is reported due to action of heterofermentative *Leuconostoc mesenteroides* (Mukherjee et al. 1965) and different yeasts involved in the natural fermentation (Soni & Sandhu 1991). The maximum batter volume increase was apparent in the batters supplemented with *S. boulardii* SAA655 individually and in combination with *L. lactis* N8. Higher gas production is beneficial as it results in porous idlis. In terms of taste, idlis prepared from microbial starter fermented samples resembled the control (naturally fermented). With respect to texture, idlis from yeast added samples were found to be more porous and softer over control (results not shown).

The analysis of B-vitamin in fermented (microbial starter and natural) and unfermented idli batter revealed LAB and yeast activity had a significant influence on final riboflavin and folate contents of idli batter. An initial riboflavin and folate levels is contributed by the cereal matrix (rice and dehulled black gram) (Reddy et al. 1982; Soni & Sandhu 1991). In the present study we observed that natural idli fermentation did not affect riboflavin content but reduced folate content by 25%. Contrarily, Ghosh & Chattopadhyay (2011) reported that natural idli fermentation could enhance folate levels. The differences in these folate results

may partly be due to the composition of natural microbiota as well as dominance of individual strains during idli fermentation.

An interesting observation in our study was that *L. lactis* N8 did not produce riboflavin in the M17G medium but showed increased riboflavin content (22%) in the idli sample fermented with this organism.

This observation may suggest that *L. lactis* N8 may have influenced the metabolic activity of natural microbiota towards an enhanced riboflavin production. On the other hand, *S. boulardii* SAA655 individually and in combination with *L. lactis* N8 showed a riboflavin enhancement trait. Moreover, the co-fermentation of microbial starters does not necessarily have a negative impact on the riboflavin enhancement during fermentation. *S. boulardii* has been reported to improve the riboflavin level during fermentation of soymilk (Rekha and Vijayalakshmi, 2010).

In conclusion, idli batter fermented with *S. boulardii* SAA655 alone or in combination with *L. lactis* N8 had higher riboflavin levels than the naturally fermented idli batter. Folate producing LAB (Sybesma et al. 2003) and yeast (Hjortmo et al. 2005) possessed different levels of intracellular and extracellular folate. The added microbial starters *L. lactis* N8 and *S. boulardii* SAA655 cultures could have also contained intracellular folates, thereby increasing the folate levels even without an increase of *S. boulardii* SAA655 cells during fermentation. Further, the presence of the microbial starters may compensate the folate losses occurring in natural fermentation.

The maximum amount of riboflavin (880 ng/g) and folate content (408 ng/g) was found in *S. boulardii* SAA655 and in mixed microbial starter culture added idli batter over naturally fermented idli batter. The dietary reference value of riboflavin and folate is 1.4 mg/day and 200 µg/day, respectively (EC 2011). On average, people consume around 2-3 idli/day

(approximately 100 g). By consumption of microbial starter fermented idli batter people could meet approximately 6.3% and 8% of daily needed riboflavin and folate (approximation based on 40% dry weight of idli as the vitamin analyses of idli were made from freeze dried idlis), respectively. On the other hand, natural fermentation can only contribute 4.2% and 3.6% of required riboflavin and folate content.

In future, strains isolated from idli and currently identified B-vitamin producers could be subjected and explored with the vitamin overproduction strategies (genetic or chemical induced with roseoflavin) that has been successfully demonstrated in bread and pasta (Capozzi et al. 2011; Capozzi et al. 2012a) for *in situ* B-vitamin enrichment in idli batter. Thus, bioprocessing of idli batter with microbial starters would increase the nutritional value and the quality (Sridevi et al. 2010).

In our knowledge, this is the first report that demonstrates the enhancement or retention of two B-vitamins in idli by an incorporation of microbial starters in the cereal/legume based idli fermentation. *In situ* B-vitamin enriched foods like idli could serve as a convenient strategy to reduce B-vitamin deficiencies in densely populated countries like India where fortification programs are yet to be effectively implemented.

## 5 Acknowledgements

This work was supported by the Academy of Finland project number 1268922. We would also like to thank Maheshwor Dev Gautam and Ushanandini Mohanraj for their statistical assistance.

## 6 Conflict of interest

The authors declare no conflicts of interest.

## 7. References

Agrawal, R., Rati, E., Vijayendra, S.V.N., Varadaraj, M., Prasad, M. and Nand, K. (2000) Flavour profile of idli batter prepared from defined microbial starter cultures. *World J Microbiol Biotechnol* **16**, 687–690.

Ananthachar, T. K. and Desikachar, H.S.R. (1962) Effect of fermentation on the nutritive value of idli. *J Sci Ind Res* **21**, 191–192.

Arena, M.P., Russo, P., Capozzi, V., López, P., Fiocco, D. and Spano, G. (2014) Probiotic abilities of riboflavin-overproducing *Lactobacillus* strains: a novel promising application of probiotics. *Appl Microbiol Biotechnol* **98**, 7569–7581.

Beasley, S., Tuorila, H. and Saris P.E.J. (2003) Fermented soymilk with a monoculture of *Lactococcus lactis. Int. J. Food Microbiol* **81**, 159–162.

Burgess, C.M, O'connell-Motherway, M., Sybesma, W., Hugenholtz, J. and van Sinderen, D. (2004) Riboflavin production in *Lactococcus lactis:* potential for in situ production of vitamin-enriched foods. *Appl. Environ. Microbiol* **70**, 5769–5777.

Burgess, C.M., Smid, E.L. and van Sinderen D. (2009) Bacterial vitamin B2, B11 and B12 overproduction: An overview. *Int J Food Microbiol* **133**, 1–7.

Capozzi, V., Menga, V., Digesu, A.M., De Vita, P., van Sinderen, D., Cattivelli, L., Fares, C. and Spano, G. (2011) Biotechnological production of vitamin B2-enriched bread and pasta. *J Agri Food Chem* **59**, 8013–8020.

Capozzi, V., Russo, P., Fragasso, M., De Vita, P., Fiocco, D. and Spano, G. (2012a)

Biotechnology and pasta-making: lactic acid bacteria as a new driver of innovation. *Front Microbiol* **3**, 94.

Capozzi, V., Russo, P., Dueñas, M.T., López, P. and Spano, G. (2012b) Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products. *Appl Microbiol Biotecnol* **96**, 1383–1394.

Chamlagain, B., Deptula, P., Edelmann, M., Kariluoto, S., Grattepanche, F., Lacroix, C., Varmanen, P. and Piironen, V. (2016) Effect of the lower ligand precursors on vitamin B12 production by food-grade Propionibacteria. *LWT-Food Sci. Technol* **72**, 117–124.

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

EC (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to co nsumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. *Official Journal of the European Union* L **304**, 18-63.

Graeffe, T., Rintala, H., Paulin, L. and Saris, P. (1991) A natural nisin variant. *In* Nisin and Novel Lantibiotics. Jung, G. and Sahl, H.G. pp. 260–268**.** Leiden: ESCOM, Science Publishers B.V.

Hjortmo, S., Patring, J., Jastrebova, J. and Andlid, T. (2005) Inherent biodiversity of folate content and composition in yeasts. *Trends Food Sci. Technol*. **16**, 311–316.

Hjortmo, S., Patring, J., Jastrebova, J. and Andlid, T. (2008) Biofortification of folates in white wheat bread by selection of yeast strain and process. *Int. J. Food Microbiol* **127**, 32–36.

Holzapfel, W.H. (2002) Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int. J. Food Microbiol* **75**, 197–212.

Ibrahim, G.A., El-Sayed, H.S., El-Shafei, K. and Sharaf, O.M. (2015) Riboflavin and folate production in different media using encapsulated *Streptococcus thermophilus* and *Lactobacillus plantarum. Middle East J Appl Sci*. **5**, 663–669.

Iyer, B.K., Singhal, R.S. and Ananthanarayan, L. (2013) Characterization and in vitro probiotic evaluation of lactic acid bacteria isolated from *idli* batter. *J Food Sci Technol* **50**, 1114-1121.

Iyer, R. and Tomar, S.K. (2011) Dietary effect of folate-rich fermented milk produced by *Streptococcus thermophilus* strains on hemoglobin level. *Nutrition* **27**, 994–997.

Iyer, R. and Tomar, S.K.(2009). Folate: a functional food constituent. *J Food Sci* **74**, R114–R122.

Iyer, R., Tomar, S.K. and Singh, A.K. (2010). Response surface optimization of the cultivation conditions and medium components for the production of folate by *Streptococcus thermophilus*. *J Dairy Res* **7**, 350–356.

Jiménez, A., Santos, M.A., Pompejus, M. and Revuelta, J.L. (2005) Metabolic engineering of the purine pathway for riboflavin production in *Ashbya gossypii*. *Appl Environ Microbiol* **71**, 5743–5751.

Kariluoto, S., Vahteristo, L., Salovaara, H., Katina, K., Liukkonen, K.-H. and Piironen,

V. (2004) Effect of baking method and fermentation on folate content of rye and wheat breads. *Cereal Chem* **81**, 134–139.

Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L. and Piironen, V. (2006) Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. *Int J Food Microbiol* **106**, 137–143.

Kariluoto, S., Edelmann, M., Nyström, L., Sontag-Strohm, T., Salovaara, H., Kivelä, R., Herranen, M., Korhola, M. and Piironen, V. (2014) *In situ* enrichment of folate by microorganisms in beta-glucan rich oat and barley matrices. *Int J Food Microbiol* **176**, 38–48.

Khandwala, P. K., Ambegaokar, S. D., Patel, S. M. and Rao, M.V.R. (1962) Studies in fermented foods. 1. Nutritive value of idli. *J Sci Ind Res*. **21**, 275–278.

Korhola, M., Hakonen, R., Juuti, K., Edelmann, M., Kariluoto, S., Nyström, L., Sontag-Strohm, T. and Piironen, V. (2014) Production of folate in oat bran fermentation by yeasts isolated from barley and diverse foods. *J. Appl Microbiol* **117**, 679–689.

LeBlanc, J.G., Laiño, J.E., del Valle, M.J., Vannini, V., van Sinderen, D., Taranto, M.P., de Valdez, G., de Giori, G.S. and Sesma, F. (2011) B-Group vitamin production by lactic acid bacteria–current knowledge and potential applications. *J Appl Microbiol* **111**,1297–1309.

McFarland, L.V. (2010). Systematic review and meta-analysis of *Saccharomyces boulardii* in adult patients. *World J Gastroenterol* **16**, 2202–2222.

Mukherjee, S.K., Albury, M.N., Pederson,C.S., van Veen, A.G and Steinkraus, K.H.(1965) Role of *Leuconostoc mesenteroides* in leavening the batter of idli, a fermented food of India. *Appl Microbiol* **13**, 227–231.

Reddy, N.R., Sathe, S.K., Pierson, M.D. and Salunkhe, D.K. (1982) Idli, an Indian fermented food: A review. *J Food Qual* **5**, 89–101.

Rekha, C., and Vijayalakshmi, G. (2010) Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. *J Appl Microbiol* **109**, 1198–1208.

Russo, P., Capozzi, V., Arena, M.P., Spadaccino, G., Dueñas, M.T., López, P., Fiocco, D. and Spano, G. (2014) Riboflavin-overproducing strains of *Lactobacillus fermentum* for riboflavin-enriched bread. *Appl Microbiol Biotechnol* **98**, 3691–3700.

Saravanan, C. and Shetty, P.K.H. (2015) Isolation and characterization of exopolysaccharide from *Leuconostoc lactis* KC117496 isolated from idli batter. *Int J Biol Macromol.* **90**, 100–106.

Da Silva, F.F.P., Biscola, V., LeBlanc, J.G. and de Melo Franco, B.D.G. (2016) Effect of indigenous lactic acid bacteria isolated from goat milk and cheeses on folate and riboflavin content of fermented goat milk. *LWT-Food Sci Technol* **71**, 155–161.

Soni, S.K., Sandhu, D.K., Vilkhu, K.S. and Kamra, N. (1986) Microbiological studies on dosa fermentation. *Food Microbiol* **3**, 45-53.

Soni, S. and Sandhu, D. (1991) Role of yeast domination in Indian idli batter fermentation. *World J Microbiol Biotechnol* **7**, 505–507

Sridevi, J., Halami, P. and Vijayendra, S. (2010) Selection of starter cultures for idli batter fermentation and their effect on quality of idlis. *J food Sci* **47**, 557–563.

Swaminathan, S., Edward, B. and Kurpad, A. (2013) Micronutrient deficiency and cognitive and physical performance in Indian children. *Eur J Clin Nutr* **67**, 467–474.

Sybesma, W., Starrenburg, M., Kleerebezem, M., Mierau, I., de Vos, W.M. and Hugenholtz, J. (2003) Increased production of folate by metabolic engineering of *Lactococcus lactis*. *Appl Environ Microbiol* **69**, 3069–3076.

Thakur, K., Gosh, S., Kadam, J. and Tomar, S.K. (2015) Adhesion properties of riboflavin producing *Lactobacillus mucosae* isolated from human faeces. *J Cell and Tissue Res* **15***,* 5095–5100.

Thakur, K. and Tomar, S.K. (2015) Exploring indigenous *Lactobacillus* species from diverse niches for riboflavin production. *J Young Pharm* **7**, 122–127.

Thakur, K. and Tomar, S.K. (2016) *Invitro* study of riboflavin producing *Lactobacilli* as potential probiotic. *LWT*-Food Sci Technol **68**, 570–578.

Thakur, K., Tomar, S.K. and De, S. (2016) Lactic acid bacteria as a cell factory for riboflavin production. *Micro Biotechnol* **9**, 441–451.

del Valle, M.J., Laiño, J.E., de Giori, G.S. and LeBlanc, J.G. (2014) Riboflavin producing lactic acid bacteria as a biotechnological strategy to obtain bio-enriched soymilk. *Food Res Int* **62**, 1015–1019.

**8 Table titles**

Table 1. Microbiological profile of idli batters fermented with different microbial starters (n=4).

**9 Figure captions**

**Figure 1.** A) Changes in riboflavin content in M17G media by growth of *Lactococcus lactis* N8. B) Changes in riboflavin content in YPD media by growth of *Saccharomyces boulardii* SAA655. Different alphabets indicate significant differences ($p<0.05$) between the samples (n=4). Ll – *Lactococcus lactis* N8, Sb – *Saccharomyces boulardii* SAA655, control – media before addition of cells and fermentation.

**Figure 2.** A) Changes in folate content in M17G media by growth of *Lactococcus lactis* N8. B) Changes in folate content in YPD media on presence of *Saccharomyces boulardii* SAA655. Different alphabets indicate significant differences ($p<0.05$) between the samples (n=2). Ll - *Lactococcus lactis* N8, Sb – *Saccharomyces boulardii* SAA655, control – media before addition of cells and fermentation.

**Figure 3.** Change in riboflavin (A) and folate (B) contents in idli batter before and after fermentation by different microbial starters (individually and in combination). Different alphabets indicate significant differences ($p<0.05$) between the samples (n=4). UF – unfermented idli batter, NF – naturally fermented idli batter, Ll – *L. lactis* N8 added fermented idli batter, Sb – *S. boulardii* SAA655 added fermented idli batter, Ll & Sb – *L. lactis* N8 and *S. boulardii* SAA655 added fermented idli batter.

**Figure 4.** Rise in idli batter volume fermented with LAB and yeast starter (individually and in combination). Different alphabets indicate significant differences ($p<0.05$) between the samples (n=4). NF – naturally fermented idli batter, Ll – *L. lactis* N8 added fermented idli batter, Sb – *S. boulardii* SAA655 added fermented idli batter, Ll & Sb – *L. lactis* N8 and *S. boulardii* SAA655 added fermented idli batter.

**Table 1.** Microbiological profile of idli batters (before and after) fermented with different microbial starters (n=4). LAB and yeast counts do only represent colonies on the plates (not verified to be LAB or yeast).

| Idli batter fermented with microbial starters | Colony forming units before fermentation (log cfu/g) | | | Colony forming units after fermentation (log cfu/g) | | |
|---|---|---|---|---|---|---|
| | LAB count | | Yeast count | LAB count | | Yeast count |
| | MRS | M17G | YPD | MRS | M17G | YPD |
| Natural fermentation | 7.08 ± 0.61 | 7.21 ± 0.56 | 7.15 ± 0.39 | 9.88 ± 0.05 | 9.92 ± 0.05 | 9.89 ± 0.08 |
| *L. lactis* N8 | 10.2 ± 0.15 | 10.2 ± 0.15 | 10.08 ± 0.02 | 9.82 ± 0.85 | 9.99 ± 0.06 | 9.94 ± 0.07 |
| *S. boulardii* SAA655 | 8.67 ± 0.13 | 8.72 ± 0.17 | 8.61 ± 0.07 | 9.79 ± 0.17 | 9.71 ± 0.28 | 9.76 ± 0.17 |
| *L. lactis* N8 & *S. boulardii* SAA655 | 9.44 ± 0.34 | 9.14 ± 0.57 | 9.61 ± 0.06 | 9.86 ± 0.18 | 9.79 ± 0.16 | 9.86 ± 0.15 |