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Journal of Food Processing and Preservation

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https://doi.org/10.1111/jfpp.13099

MINIMIZATION OF ANTINUTRIENTS IN IDLI BY USING RESPONSE SURFACE PROCESS OPTIMIZATION

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Received for Publication January 3, 2016
Accepted for Publication May 3, 2016

doi:10.1111/jfpp.13099

ABSTRACT

Deploying response surface methodology, the stages of idli preparation were optimized for minimizing the level of antinutrients. Under optimum conditions of soaking blackgram dal (1:5 of dal and water at 16C, and pH 4.0 for 18 h) and rice (1:5 of rice and water at 16C, and pH 5.6 for 18 h), the tannins content, trypsin inhibitor activity and hemagglutinating activity reduced, while phytic acid content remained unchanged. The optimum conditions for fermentation of dal-rice (1:2) mixed batter were 16 g/kg common salt supplementation and 19 h at 35C, resulting in a decrease in all the antinutrient levels, except amines. Steaming for an optimized period of 20 min further reduced the phytic acid content and trypsin inhibitor activity. In idli, while total biogenic amines content increased by 339% over raw ingredients, tannins content, phytic acid content, trypsin inhibitor activity and hemagglutinating activity decreased by 100, 89, 58 and 100%, respectively.

PRACTICAL APPLICATIONS

For idli preparation, the optimization of processing stages using response surface methodology significantly minimized the level of antinutrients from both blackgram dal and rice without affecting the organoleptic attributes of the product. The optimized process parameters can be applied to household level and are also useful in scaling up idli production with a minimum level of antinutrients and better consumer acceptability. The outcome of this research can be exploited to other legume-based foods as well, particularly in developing regions where the consequences of antinutrients may exacerbate malnutrition and disease, thus effectively utilizing full potential of the legumes as human and animal foods.

INTRODUCTION

In countries, where scarcity of animal protein prevails, legumes play an important role in the human diet. Legumes fulfill the basic nutritional requirements, such as proteins, dietary fibers, unsaturated fatty acids, vitamins and minerals. Legumes, blended with cereals, provide nutritional and functional proteins having a well-balanced essential amino acid profile (Boye *et al.* 2010). A significant part of the world's human population relies on legumes as staple food for subsistence, particularly in combination with cereals.

However, besides having these beneficial qualities, legumes and cereals contain a considerable amount of antinutrients, such as galacto-oligosaccharides, nonprotein amino acids, tannins, phytates, trypsin inhibitors, hemagglutins or lectins and biogenic amines which limit their consumption (Hemalatha *et al.* 2007). Reduction of antinutrients in legumes can be achieved either by selection of plant genotypes with low levels of such factors or through postharvest processing. Since antinutrients are important to the plants as they function as potent defense compounds against herbivores and pathogens, postharvest processing has been the strategy for their elimination from seeds. As many of the antinutrients are toxic, unpalatable and/or indigestible for human consumption, the traditional domestic means of their reduction consists mainly of dehulling, leaching, germination, heating and fermentation. Structure of antinutrients and their chemical properties, especially heat

lability, dictate which physical process will be more effective in their reduction or removal.

Idli is a classic example of cereal-legume mixture food, consumed throughout India, especially in southern parts, and Sri Lanka (Aidoo *et al.* 2006). Traditionally, idli is prepared by soaking blackgram (*Vigna mungo* (L.) Hepper; synonym *Phaseolus mungo* L.) dal (dehulled split seeds) and white polished rice (*Oryza sativa* L.) separately. The soaked rice is ground coarsely, while soaked dal is ground to a smooth, viscous paste. The slurry and paste are mixed at different ratios with common salt to form a thick batter that is left overnight at ambient temperature. The leavened batter is poured into the cups of an idli steamer and steamed until starch is gelatinized to prepare soft and spongy cakes having a pleasant acid flavor (Aidoo *et al.* 2006; Rakshit *et al.* 2015). Idli is usually eaten with chutney (a batter made of coconut) and sambar (spicy vegetable soup containing tamarind juice). Idli is fermented naturally. Many times use of the same utensils helps to stabilize a mixed microbiota during fermentation. The leavening and flavor formation of idli are achieved by the activities of lactic acid bacteria, such as *Leuconostoc mesenteroides*, *Enterococcus faecalis* and *Pediococcus dextrinicus*, and yeasts such as *Saccharomyces cerevisiae*, *Pichia anomala*, *Debaryomyces hansenii*, *Trichosporon pullulans* and *Trichosporon cutaneum* (Nout *et al.* 2007).

The fate of oligosaccharides in blackgram during its processing for preparing idli has been reported elsewhere (Rakshit *et al.* 2015). However, reports on the other major antinutrients, such as tannins, phytic acid, total biogenic amines, trypsin inhibitor activity and hemagglutinating activity exhibited varied results under different conditions.

Response surface methodology (RSM) is a powerful tool that is useful for applications in which a response is influenced by several factors (Montgomery 2005). Since RSM also evaluates the interactions between one or more response variables with fewer experimental runs, it is used extensively for optimization of different food processes. Hence, the present study aimed to use RSM for optimization of the traditional domestic processing stages in achieving preparation of idli having a minimum level of antinutrients.

MATERIALS AND METHODS

Sampling

Blackgram dal and white polished rice were purchased from a retailer in Siliguri town, packed in an air-tight aluminum container and stored at ambient temperature (22–30C) for earliest use (within 2 weeks).

Experimental Design

Preliminary experimental trials conducted in the laboratory and literature survey (Rakshit *et al.* 2015) enabled us to choose the minimum and maximum values of the independent variables. The influence of variables of each stage was evaluated using a central composite rotatable design (CCRD). Experiments were performed in triplicate sets, according to combinations for each processing stage to obtain an optimized condition using Design Expert v. 8.0 (Stat-Ease Inc., Minneapolis, MN). The soaking stage consisted of 30 experimental runs having 16 factorial points, eight axial points and six replicates at central points (Tables 1 and 2). While optimally soaked dal was ground to a smooth paste, optimally soaked rice was coarsely ground. The dal paste and rice slurry were mixed to get a thick batter which was used for optimization of the subsequent (fermentation) stage employing three independent variables. This stage consisted of 20 experimental runs having eight factorial points, six axial points and six replicates at the central points (Table 3). As per the experimental condition, different amounts of common salt (sodium chloride) were added. At different fermentation times, the bottles were sampled for analysis of antinutrients. The results obtained were used to optimize fermentation condition. Optimally fermented idli batter was used in the final stage (steaming), where a single-independent variable consisted of seven experimental runs (Table 4). After model fitting of each processing stage, 3D response surfaces were generated to decipher the relation between independent and response variables.

Viable Cell Count

A 10 g sample was homogenized with 90 mL sterile peptone-physiological saline (1 g/L neutral peptone, 8.5 g/L sodium chloride, pH 7.2) in a stomacher lab-blender 400 (Seward Medical, London, UK) for 1 min at "normal" speed. One milliliter of the appropriate dilution was mixed with 15 mL molten (45C) plate count agar (M091A; HiMedia Laboratories, Mumbai, India) for total aerobic mesophilic bacterial count, MRS agar (HiMedia M641) for lactic acid bacterial count and tryptone glucose yeast extract agar (HiMedia M014) supplemented with 10 IU/mL benzylpenicillin and 12 μg/mL streptomycin sulfate for yeast count. The plate count agar and tryptone glucose yeast extract agar plates were incubated at 37C for 24 h, while MRS agar plates were incubated for 48 h at 30C in an anaerobic culture jar (HiMedia LE002A).

pH and Titratable Acidity

For determining pH and titratable acidity, the methods described in AOAC (1990) were followed.

TABLE 1. EXPERIMENTAL VALUES, BASED ON RSM DESIGN, OF ANTINUTRIENTS IN SOAKED DAL

	Soaking condition (variable)					Antinutrient (per g dry wt)*				
Run	Dal:water (w/w)	*t* (h)	*T* (C)	Initial pH	Final pH	TC (mg)	PAC (mg)	TIA (U)	HA (U)	TBAC (μg)
1	1:1	12	26	6.0	5.7 ± 0.3	0.46 ± 0.09	4.9 ± 0	111 ± 2.3	160 ± 0	296 ± 1
2	1:7	12	4	6.0	5.8 ± 0.3	0.23 ± 0.12	4.1 ± 0.3	81 ± 0.6	133 ± 27	305 ± 2
3	1:7	24	26	6.0	5.6 ± 0	0.19 ± 0	3.0 ± 0	65 ± 0	120 ± 40	306 ± 2
4	1:10	6	37	4.0	3.6 ± 0	0.32 ± 0.03	4.2 ± 0	85 ± 1.4	107 ± 27	324 ± 2
5	1:10	6	37	8.0	6.5 ± 0	0.32 ± 0.03	4.2 ± 0	83 ± 1.9	133 ± 27	304 ± 1
6	1:7	12	26	6.0	5.6 ± 0	0.26 ± 0.03	4.0 ± 0	83 ± 1.2	133 ± 27	317 ± 1
7	1:7	12	26	10.0	6.1 ± 0	0.26 ± 0.03	3.9 ± 0	88 ± 0	160 ± 0	290 ± 0
8	1:10	6	15	8.0	6.8 ± 0	0.32 ± 0.03	4.3 ± 0	84 ± 0	133 ± 27	305 ± 1
9	1:7	0	26	6.0	6.1 ± 0	0.46 ± 0.07	5.0 ± 0	111 ± 0.7	160 ± 0	296 ± 1
10	1:4	18	37	4.0	3.3 ± 0	0.29 ± 0	3.9 ± 0	73 ± 0.6	107 ± 27	326 ± 1
11	1:10	18	37	4.0	3.4 ± 0	0.23 ± 0.12	3.6 ± 0	77 ± 0	120 ± 0	344 ± 1
12	1:10	18	15	8.0	6.8 ± 0	0.22 ± 0.03	3.5 ± 0	79 ± 0	133 ± 27	300 ± 1
13	1:4	6	15	8.0	6.8 ± 0	0.36 ± 0.03	4.5 ± 0	87 ± 0.4	160 ± 0	306 ± 0
14	1:4	6	15	4.0	3.9 ± 0	0.36 ± 0.03	4.5 ± 0.1	95 ± 0.6	120 ± 40	311 ± 0
15	1:10	6	15	4.0	3.9 ± 0	0.26 ± 0.03	4.3 ± 0	82 ± 0.1	120 ± 40	306 ± 0
16	1:10	18	15	4.0	3.9 ± 0	0.23 ± 0.12	3.5 ± 0	75 ± 0.6	120 ± 40	304 ± 1
17	1:7	12	48	6.0	5.3 ± 0	0.29 ± 0	4.0 ± 0	84 ± 0.1	93 ± 13	362 ± 1
18	1:7	12	26	2.0	3.0 ± 0	0.32 ± 0.03	4.0 ± 0	80 ± 0.8	107 ± 27	322 ± 0
19	1:10	18	37	8.0	6.4 ± 0	0.22 ± 0.03	3.5 ± 0	79 ± 0.2	133 ± 27	310 ± 2
20	1:7	12	26	6.0	5.6 ± 0	0.26 ± 0.03	3.8 ± 0	79 ± 0.1	133 ± 40	314 ± 0
21	1:7	12	26	6.0	5.6 ± 0	0.23 ± 0.09	3.8 ± 0	85 ± 0.1	120 ± 27	313 ± 0
22	1:4	6	37	8.0	6.5 ± 0	0.36 ± 0.03	4.5 ± 0	88 ± 0.6	133 ± 40	300 ± 1
23	1:13	12	26	6.0	5.7 ± 0	0.09 ± 0	3.8 ± 0	67 ± 0	120 ± 27	298 ± 0
24	1:7	12	26	6.0	5.6 ± 0	0.29 ± 0.01	4.0 ± 0	86 ± 0	107 ± 27	336 ± 1
25	1:4	6	37	4.0	3.6 ± 0	0.36 ± 0.03	4.5 ± 0	92 ± 0.6	107 ± 40	329 ± 0
26	1:7	12	26	6.0	5.6 ± 0	0.26 ± 0.03	3.7 ± 0	72 ± 0	120 ± 27	308 ± 0
27	1:4	18	15	8.0	6.8 ± 0	0.23 ± 0.12	3.9 ± 0	75 ± 0.1	133 ± 27	299 ± 1
28	1:7	12	26	6.0	5.6 ± 0	0.32 ± 0.03	3.8 ± 0	78 ± 0.1	107 ± 27	313 ± 1
29	1:4	18	15	4.0	3.9 ± 0	0.23 ± 0.12	3.9 ± 0	77 ± 0	120 ± 40	298 ± 0
30	1:4	18	37	8.0	6.5 ± 0	0.26 ± 0.03	3.8 ± 0	77 ± 0.1	120 ± 40	311 ± 0

Note: TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, hemagglutinating activity; TABC, total biogenic amines content.

*Values, showing mean ± SE, were obtained from triplicate sets.

Sensory Analysis

Samples of idli, prepared under different experimental conditions of fermentation and optimized steaming time, were evaluated organoleptically by a panel of 10 trained judges. An overall sensory quality was considered using a 100-point score card (Table 5). Analysis of the samples was conducted in triplicate.

Evaluation of Antinutrients

While the samples of raw dal and rice were powdered, those of soaked dal and rice were made to paste using a blender (Bajaj Electricals, Mumbai, India). The powders, pastes and dal-rice mixed (unfermented and fermented) batters were left overnight at −20C, lyophilized (Eyela freeze dryer, FDU-506, Tokyo Rikakikai, Tokyo, Japan) and powdered.

The contents of tannins, phytic acid and total biogenic amines, and trypsin inhibitor and hemagglutinating activities were determined using the methods described by Price *et al.* (1978), Wheeler and Ferrel (1971), Yeh *et al.* (2006), Kakade *et al.* (1969) and Liener and Hill (1953), respectively. One unit (U) of trypsin inhibitor activity was defined as a decrease in A_{280} of 0.01, relative to the blank, in 20 min using a 10 mL assay volume, while one unit (U) of hemagglutinating activity was defined as the least amount of hemagglutinin which produced positive agglutination (1+) under the experimental condition. The analyses of the samples for estimating antinutrient levels were conducted in triplicate.

Following the traditional method of idli preparation, as described by Rakshit *et al.* (2015), the antinutrient levels of the product were evaluated to compare the efficiency of the optimized process conditions.

TABLE 2. EXPERIMENTAL VALUES, BASED ON RSM DESIGN, OF ANTINUTRIENTS IN SOAKED RICE

	Soaking condition (variable)					Antinutrient (per g dry wt)*		
Run	Rice:water (w/w)	*t* (h)	*T* (C)	Initial pH	Final pH	TC (mg)†	PAC (mg)	TBAC (μg)
1	1:1	12	26	6.0	5.8 ± 0	0.06 ± 0.03	1.3 ± 0	81 ± 2.2
2	1:7	12	4	6.0	5.9 ± 0	0.03 ± 0.03	1.1 ± 0	76 ± 1.7
3	1:7	24	26	6.0	4.9 ± 0	<dl	0.9 ± 0	78 ± 2.9
4	1:10	6	37	4.0	3.8 ± 0	<dl	1.3 ± 0	98 ± 0
5	1:10	6	37	8.0	6.4 ± 0	0.03 ± 0.03	1.3 ± 0	79 ± 0.9
6	1:7	12	26	6.0	5.8 ± 0	<dl	1.4 ± 0	77 ± 2.3
7	1:7	12	26	10.0	6.2 ± 0	0.03 ± 0.03	1.3 ± 0	79 ± 1.0
8	1:10	6	15	8.0	6.6 ± 0	<dl	1.3 ± 0	75 ± 0
9	1:7	0	26	6.0	6.1 ± 0	0.06 ± 0.03	1.4 ± 0	76 ± 1.0
10	1:4	18	37	4.0	3.3 ± 0	<dl	1.4 ± 0	96 ± 1.2
11	1:10	18	37	4.0	3.2 ± 0	<dl	1.1 ± 0	86 ± 0.9
12	1:10	18	15	8.0	6.6 ± 0	<dl	1.2 ± 0	73 ± 1.5
13	1:4	6	15	8.0	6.7 ± 0	0.06 ± 0.03	1.5 ± 0	80 ± 0
14	1:4	6	15	4.0	3.8 ± 0	0.03 ± 0	1.6 ± 0	87 ± 0.8
15	1:10	6	15	4.0	3.8 ± 0	0.03 ± 0.03	1.3 ± 0	82 ± 0.7
16	1:10	18	15	4.0	3.7 ± 0.1	<dl	0.7 ± 0	78 ± 1.0
17	1:7	12	48	6.0	4.4 ± 0	0.03 ± 0.03	1.0 ± 0	95 ± 1.1
18	1:7	12	26	2.0	3.0 ± 0	<dl	1.2 ± 0	97 ± 1.7
19	1:10	18	37	8.0	5.9 ± 0	<dl	1.0 ± 0	81 ± 0.9
20	1:7	12	26	6.0	5.9 ± 0	0.06 ± 0.03	1.1 ± 0	73 ± 6.0
21	1:7	12	26	6.0	5.9 ± 0	0.03 ± 0	1.3 ± 0	74 ± 0
22	1:4	6	37	8.0	6.6 ± 0	0.06 ± 0.03	1.4 ± 0	84 ± 0.2
23	1:13	12	26	6.0	5.9 ± 0	<dl	0.9 ± 0	80 ± 0.1
24	1:7	12	26	6.0	5.9 ± 0	<dl	0.9 ± 0	80 ± 0
25	1:4	6	37	4.0	3.7 ± 0	0.03 ± 0.03	1.4 ± 0.1	96 ± 0.2
26	1:7	12	26	6.0	5.9 ± 0	0.06 ± 0.03	1.0 ± 0	73 ± 0.9
27	1:4	18	15	8.0	6.6 ± 0	<dl	1.1 ± 0	82 ± 0.3
28	1:7	12	26	6.0	5.9 ± 0	<dl	1.1 ± 0	79 ± 0.1
29	1:4	18	15	4.0	3.8 ± 0	<dl	1.5 ± 0	79 ± 0.1
30	1:4	18	37	8.0	6.0 ± 0	<dl	1.4 ± 0	81 ± 0.4

Note: TC, tannins content; PAC, phytic acid content; TABC, total biogenic amines content.
*Values, showing mean ± SE, were obtained from triplicate sets.
†dl (detection limit), 0.003 mg/g dry wt.

Validation of the Model Equations

Following numerical optimization, the optimized models of each processing stage were subjected to validation. The deviation error was expressed as a percentage, which was computed by comparing the actual values with the predicted ones at the optimum condition to evaluate model performance.

RESULTS AND DISCUSSION

Raw Dal/Rice

While raw dal contained considerable amounts of antinutrients, rice had a lesser amount of those (Table 6). The values of tannins content, phytic acid content and hemagglutinating activity of raw dal and rice were similar to earlier reports (Hettiarachchy and Sri Kantha 1981; Hemalatha *et al.* 2007).

Soaking

Results for levels of antinutrients under different soaking conditions of dal according to RSM design are shown in Table 1. The models have significant *F* value, an insignificant lack-of-fit, low-standard deviation and coefficient of variance (Table 7). The low coefficients of determination (R^2) values for hemagglutinating and trypsin inhibitor activities show that 54 and 59% of variations are influenced by independent variables of soaking conditions, and the remaining variations can be attributed to other factors. The adjusted and predicted R^2 for models are in reasonable agreement. Adequate precision for models in the study has ratio greater than 4, which is desirable and indicates adequate model discrimination. The low values of *CV*, *SD* and *PRESS* for the models show adequacy with which the experiment is conducted and indicate a better prediction.

TABLE 3. EXPERIMENTAL VALUES* OF ANTINUTRIENTS, FINAL pH, TITRATABLE ACIDITY AND MICROBIAL LOAD IN MIXED BATTER, FERMENTED ACCORDING TO RSM DESIGN

	Fermentation mileu			Antinutrient (per g dry wt)†							Log cfu/g		
Run	Added salt (g/kg)	*t* (h)	*T* (C)	TC (mg)	PAC (mg)	TIA (U)	HA (U)	TBAC (μg)	Final pH	TA (%)	TAMB	LAB	Yeasts
1	7	10	35	0.12 ± 0.03	1.2 ± 0	23 ± 0.6	40 ± 0	638 ± 2	4.6 ± 0	0.08 ± 0	7.1 ± 0	8.3 ± 0	5.1 ± 0
2	7	24	25	0.09 ± 0	1.0 ± 0	21 ± 0.1	27 ± 7	701 ± 2	4.2 ± 0	0.06 ± 0.01	7.4 ± 0	8.3 ± 0	5.3 ± 0
3	25	10	25	0.16 ± 0.03	1.5 ± 0	25 ± 0.3	53 ± 13	516 ± 2	4.7 ± 0	0.08 ± 0	7.0 ± 0	8.0 ± 0	5.0 ± 0.1
4	16	5.2	30	0.19 ± 0.06	1.7 ± 0	28 ± 0.1	80 ± 0	450 ± 2	4.4 ± 0	0.06 ± 0	6.4 ± 0	7.4 ± 0	4.2 ± 0
5	16	17	30	0.09 ± 0.03	1.2 ± 0.3	23 ± 0	33 ± 7	672 ± 2	4.3 ± 0	0.06 ± 0.01	7.5 ± 0	8.4 ± 0	5.3 ± 0
6	25	10	35	0.12 ± 0.03	1.3 ± 0	23 ± 0	40 ± 0	561 ± 2	4.6 ± 0	0.07 ± 0	7.0 ± 0	8.0 ± 0	5.0 ± 0
7	16	17	38.4	0.03 ± 0.03	0.8 ± 0	21 ± 0.9	<dl	720 ± 6	4.4 ± 0	0.06 ± 0.01	7.5 ± 0	8.4 ± 0	5.3 ± 0.1
8	25	24	25	0.09 ± 0.05	1.2 ± 0	21 ± 0.6	27 ± 7	585 ± 5	4.4 ± 0	0.08 ± 0.01	7.0 ± 0	8.1 ± 0	5.0 ± 0
9	7	10	25	0.16 ± 0.03	1.3 ± 0	25 ± 0.1	53 ± 13	811 ± 2	4.6 ± 0	0.08 ± 0	7.1 ± 0	8.0 ± 0	5.0 ± 0
10	31.1	17	30	0.09 ± 0.05	1.5 ± 0	21 ± 0	40 ± 0	411 ± 4	4.9 ± 0	0.08 ± 0	7.1 ± 0	7.3 ± 0	4.5 ± 0
11	16	28.7	30	<dl	0.9 ± 0	20 ± 0.5	20 ± 0	713 ± 2	3.3 ± 0	0.10 ± 0.01	7.1 ± 0	8.3 ± 0	5.3 ± 0
12	16	17	30	0.09 ± 0	0.9 ± 0.3	22 ± 1.1	27 ± 7	631 ± 4	4.3 ± 0	0.07 ± 0.01	7.5 ± 0	8.3 ± 0	5.2 ± 0
13	7	24	35	0.03 ± 0.03	0.4 ± 0	20 ± 0.1	<dl	798 ± 2	4.2 ± 0	0.09 ± 0	7.2 ± 0	8.3 ± 0	5.3 ± 0
14	16	17	30	0.06 ± 0.03	1.0 ± 0	22 ± 0.6	33 ± 7	636 ± 5	4.2 ± 0	0.11 ± 0	7.5 ± 0	8.4 ± 0	5.2 ± 0
15	16	17	30	0.06 ± 0.03	1.2 ± 0	21 ± 0	33 ± 7	687 ± 4	4.2 ± 0	0.10 ± 0	7.5 ± 0	8.3 ± 0	5.3 ± 0
16	16	17	30	0.09 ± 0	0.9 ± 0	22 ± 0.1	20 ± 0	661 ± 6	4.3 ± 0	0.10 ± 0	7.5 ± 0	8.4 ± 0	5.2 ± 0
17	16	17	21.6	0.13 ± 0.06	1.3 ± 0	23 ± 0	53 ± 13	612 ± 3	4.3 ± 0	0.07 ± 0	7.0 ± 0	8.3 ± 0	5.1 ± 0
18	25	24	35	0.03 ± 0.03	0.4 ± 0	20 ± 0.6	<dl	607 ± 3	4.2 ± 0	0.07 ± 0	7.3 ± 0	8.0 ± 0	5.1 ± 0
19	0.9	17	30	0.09 ± 0	1.1 ± 0	22 ± 0	40 ± 0	811 ± 3	3.0 ± 0	0.17 ± 0	7.2 ± 0	8.8 ± 0	5.0 ± 0
20	16	17	30	0.06 ± 0.03	1.2 ± 0	23 ± 0	33 ± 7	697± 4	4.2 ± 0	0.07 ± 0	7.5 ± 0	8.3 ± 0	5.3 ± 0
Opt‡ψ									4.3^{b} ± 0	0.10^{a} ± 0	7.3^{a} ± 0	8.4^{a} ± 0	5.3^{a} ± 0
Unf§ψ									5.6^{a} ± 0	0.03^{b} ± 0	6.7^{b} ± 0	6.2^{b} ± 0	3.3^{b} ± 0

Note: TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, hemagglutinating activity; TABC, total biogenic amines content; TA, titratable acidity (as lactic acid); TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria.

*Values, showing mean ± SE, were obtained from triplicate sets.

†dl (detection limit), 0.003 mg/g dry wt for TC and 6.67 U/g dry wt for HA.

‡Batter, fermented under optimum condition (added salt, 16 g/kg (set as the target value); fermentation time, 19 h; fermentation temperature, 35C).

§Unfermented mixed batter prepared from optimally soaked dal and rice (1:2 v/v).

ψMeans, followed by different superscripted letters in each column for unfermented mixed batter (Unf) and optimally fermented batter (Opt), differ significantly ($P < 0.05$), as determined by Student's *t*-test.

TABLE 4. EXPERIMENTAL VALUES OF ANTINUTRIENTS IN FERMENTED BATTER, STEAMED ACCORDING TO RSM DESIGN

		Antinutrient (per g dry wt)*				
Run	Steaming time (min)	TC (mg)†	PAC (mg)	TIA (U)	HA (U)†	TBAC (μg)
1	20	<dl	0.3 ± 0.02	17 ± 0.1	<dl	636 ± 2
2	15	<dl	0.4 ± 0	18 ± 0	<dl	639 ± 1
3	12.5	<dl	0.6 ± 0	18 ± 0	<dl	644 ± 5
4	20	<dl	0.3 ± 0.02	17 ± 0.1	<dl	636 ± 0
5	17.5	<dl	0.3 ± 0.02	18 ± 0	<dl	635 ± 3
6	10	<dl	0.4 ± 0	19 ± 0.1	<dl	643 ± 1
7	10	<dl	0.4 ± 0	19 ± 0.1	<dl	643 ± 4

Note: TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, hemagglutinating activity; TABC, total biogenic amines content.

*Values, showing mean ± SE, were obtained from triplicate sets.

†dl (detection limit), 0.003 mg/g dry wt for TC and 6.67 U/g dry wt for HA.

The reduction ($P < 0.05$) of tannins content, phytic acid content and trypsin inhibitor activity was influenced by dal-water ratio and time. Although the reduction of hemagglutinating activity was dependent on temperature, initial pH had no effect ($P < 0.05$). While initial pH played a significant ($P < 0.05$) role in the reduction of total biogenic amines content, temperature had an opposite ($P < 0.05$) effect. Interestingly, interaction of initial pH and temperature reduced ($P < 0.05$) the total biogenic amines content. After removing insignificant terms for the coded variables of each response, the reduced polynomial equations were:

Tannins content (mg/g) $= 0.283 - 0.044A - 0.054B$

Phytic acid content (mg/g) $= 3.844 - 0.189A - 0.378B + 0.119A^2$

Trypsin inhibitor activity (U/g) $= 82.598 - 4.380\text{A} - 7.327B$

Hemagglutinating activity (U/g) $= 125.756 - 6.611C + 10.972D$

Total biogenic amines content (μg/g) $= 316.816 + 9.709\text{C} - 7.042D - 5.547CD - 4.724A^2 - 3.615B^2 + 4.454C^2$

where A was dal-water ratio, B was time, C was temperature and D was initial pH.

An increase in time and dal-water ratio caused a linear reduction in tannins content (0.19 mg/g; Fig. 1a), phytic acid content (3.4 mg/g; Fig. 1c), trypsin inhibitor activity (71 U/g; Fig. 1e) and hemagglutinating activity (118 U/g; Fig. 1f) when temperature and initial pH were kept constant at 26C and 6.0, respectively. The predicted reduction of tannins content, phytic acid content, trypsin inhibitor activity

TABLE 5. SENSORY SCORES OF IDLI, OBTAINED THROUGH DIFFERENT FERMENTATION CONDITIONS

	Fermentation condition			Sensory attribute					
Run	Added salt (g/kg)	Time (h)	Temperature (C)	Taste (max. score, 35)	Flavor (max. score, 30)	Body and texture (max. score, 30)	Color (max. score, 5)	Overall quality (max. score, 100)	Grade*
1	7	10	35	23 ± 1	24 ± 1	28 ± 0	4 ± 0	79 ± 2	F
2	7	24	25	22 ± 1	29 ± 0	28 ± 0	3 ± 0	82 ± 1	G
3	25	10	25	29 ± 0	25 ± 0	28 ± 0	3 ± 0	85 ± 0	G
4	16	5.2	30	27 ± 0	17 ± 1	28 ± 0	4 ± 0	76 ± 1	F
5	16	17	30	32 ± 0	28 ± 1	28 ± 0	3 ± 0	91 ± 1	E
6	25	10	35	28 ± 0	24 ± 1	27 ± 0	4 ± 0	83 ± 1	G
7	16	17	38.4	31 ± 1	26 ± 0	28 ± 0	4 ± 0	89 ± 1	G
8	25	24	25	27 ± 0	28 ± 0	28 ± 0	4 ± 0	87 ± 0	G
9	7	10	25	22 ± 0	24 ± 0	28 ± 0	4 ± 0	78 ± 0	F
10	31.1	17	30	26 ± 1	28 ± 0	28 ± 1	3 ± 0	85 ± 2	G
11	16	28.7	30	33 ± 0	28 ± 0	28 ± 0	4 ± 0	93 ± 0	E
12	16	17	30	33 ± 0	28 ± 0	28 ± 0	3 ± 0	92 ± 0	E
13	7	24	35	31 ± 0	29 ± 0	28 ± 0	4 ± 0	92 ± 0	G
14	16	17	30	32 ± 0	28 ± 0	28 ± 0	4 ± 0	92 ± 0	G
15	16	17	30	31 ± 0	28 ± 0	29 ± 0	4 ± 0	92 ± 0	E
16	16	17	30	32 ± 1	29 ± 0	28 ± 0	3 ± 0	92 ± 1	E
17	16	17	21.6	32 ± 0	29 ± 0	29 ± 0	3 ± 0	93 ± 0	E
18	25	24	35	29 ± 0	29 ± 0	29 ± 0	4 ± 0	91 ± 0	G
19	0.9	17	30	24 ± 1	28 ± 0	27 ± 0	3 ± 0	82 ± 1	G
20	16	17	30	31 ± 1	28 ± 0	29 ± 0	3 ± 0	91 ± 1	E
Opt.†	16	19	35	32 ± 0	29 ± 0	29 ± 0	4 ± 0	94 ± 0	E

Note: Values are mean ± SE ($n = 30$).

*F, fair; G, good; E, excellent.

†Optimum condition, set by targeting 16 g/kg and 19 h as the desired salt level and fermentation time, respectively.

TABLE 6. ANTINUTRIENTS IN SUBSTRATES AT OPTIMUM CONDITION OF EACH STAGE OF IDLI PREPARATION

	Antinutrient (per g dry wt)				
Parameter	TC (mg)‡	PAC (mg)	TIA (U)‡	HA (U)‡	TBAC (μg)
Raw dal*	$0.49^{A} \pm 0.06$	$5.1^{A} \pm 0$	$120^{A} \pm 18.5$	$160^{A} \pm 0$	$295^{B} \pm 3$
Soaking (Raw Dal-Water of 1:5 w/w, pH 4.0, 16C, 18 h)					
Predicted values	0.25	3.8	78	119	301
Experimental values*	$0.26^{B} \pm 0.03$	$3.8^{A} \pm 0$	$81^{B} \pm 8.6$	$120^{B} \pm 0$	$303^{A} \pm 3$
Error %	3.85	0	3.7	0.83	0.66
% change†	−49.0	−25.5	−35.0	−25.6	+2.0
Raw rice*	$0.06^{X} \pm 0.03$	$1.5^{X} \pm 0$	<dl	<dl	$70^{Y} \pm 4$
Soaking (Raw Rice-Water of 1:5 w/w, pH 5.6, 16C, 18 h)					
Predicted values	0.01	1.2	0	0	75
Experimental values*	$<dl^{Y}$	$1.1^{X} \pm 0$	<dl	<dl	$76^{X} \pm 2$
Error %	0	9.09	0	0	1.32
% change†	−83.3	−20.0	0	0	+7.1
Unfermented Mixed Batter (Dal Batter: Rice Slurry; 1:2 v/v)					
Experimental values*	$0.09^{1} \pm 0$	$1.7^{1} \pm 0.1$	$33^{1} \pm 4.8$	$40^{1} \pm 0$	$127^{3} \pm 3$
Fermentation (Salt Concentration of 16 g/kg, 35C, 19 h)					
Predicted values	0.05	0.8	21	14	681
Experimental values*	$0.05^{2} \pm 0$	$0.7^{2} \pm 0$	$21^{2} \pm 2.7$	$14^{2} \pm 0$	$681^{1} \pm 1$
Error %	0	14.3	0	0	0
% change†	−44.4	−53	−36.4	−65	+436.2
Steaming (20 min)					
Predicted values	0	0.3	17	0	636
Experimental values*	<dl	$0.3^{3} \pm 0$	$17^{2} \pm 0.4$	<dl	$636^{2} \pm 0$
Error %	0	0	0	0	0
% change†	−100 (−100)	−82.4 (−62.5)	−48.5 (−19)	−100 (−100)	+400.8 (−6.6)

Note: TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, hemagglutinating activity; TABC, total biogenic amines content.

*Values are mean ± SE of triplicate determinations in raw/processed samples. Means followed by the different superscripts in each column for raw and soaked dal (A and B)/rice (X and Y) differ significantly ($P < 0.05$) as determined by *t*-test, and for unfermented, fermented and steamed dal-rice mixture (1, 2 and 3) differ significantly ($P < 0.05$) as determined by Duncan multiple range test.

†− and + indicate percent decrease and increase, respectively, of predicted values over raw dal/rice for the soaking stage, and over unfermented mixed batter for fermentation and steaming stages (values within parentheses indicate percentages calculated over the predicted values of fermented batter).

‡dl (detection limit), 0.003 mg/g dry wt for TC, 15.26 U/g dry wt for TIA and 6.67 U/g dry wt for HA.

and hemagglutinating activity over raw dal was 61, 33, 41 and 26%, respectively. The minimum total biogenic amines content (308 μg/g; Fig. 1g) was obtained when dal was soaked in water of initial pH 8.0 and 20.5C, keeping dal-water ratio and time constant at 1:7 and 12 h, respectively.

The loss of tannins content is attributed to the binding of tannins with carbohydrates and proteins, and also to the activation of enzyme polyphenol oxidase (Saharan *et al.* 2002). Therefore, an increase in dal-water ratio and time might have enhanced leaching of tannins and phytate from dal (beans having no external barrier, i.e., seed coat) into the soak water. The loss of trypsin inhibitor activity might be due to leaching of trypsin inhibitors. Since trypsin inhibitors are low-molecular weight proteins, they are likely to leach out of the seeds easily (Grewal and Jood 2006). The reduction of tannins content, phytic acid content, trypsin inhibitor activity and hemagglutinating activity during soaking in the present study is in agreement with the earlier reports (Grewal and Jood 2006; Khandelwal *et al.* 2010; Kalpanadevi and Mohan 2013). The level of total biogenic amines content reduced with an increase in initial pH and a decrease in temperature. For the production of decarboxylase, a low pH (3.0–6.0) is favorable to bacteria (Silla Santos 1996). A higher temperature favors proteolysis and decarboxylation of amino acids, resulting in an increased concentration of amines (Joosten and van Boekel 1988).

Results for levels of antinutrients under different soaking conditions of raw rice according to RSM design are shown in Table 2. In case of rice soaking, the models for different antinutrients are significant with nonsignificant lack of fit (Table 7). For the contents of tannins and phytic acid, model terms explain 50% of variations. On the other hand, R^2 value of total biogenic amines indicates a good fit between predicted values and the experimental data points. This implies that 92% of the variations for reduction in the antinutrient levels are

TABLE 7. GOODNESS OF FIT OF THE MODELS GENERATED

Model	R^2	Adj R^2	Pred R^2	Adeq precision	*SD*	Mean	*CV*%	*PRESS*
Soaking of Blackgram								
TC	0.724	0.680	0.587	13.033	0.043	0.283	15.113	0.068
PAC	0.934	0.872	0.683	14.289	0.149	4.010	3.732	1.605
TIA	0.593	0.528	0.391	10.344	6.940	82.590	8.402	1803.151
HA	0.539	0.466	0.331	9.839	12.641	125.756	10.052	5803.069
TBAC	0.873	0.754	0.564	11.069	7.727	311.766	2.479	3063.573
Soaking of Rice								
TC	0.500	0.42	0.351	8.018	0.018	0.020	91.652	0.011
PAC	0.501	0.421	0.301	7.279	0.163	1.217	13.419	0.934
TBAC	0.915	0.836	0.636	10.642	3.064	81.799	3.746	603.469
Fermentation								
TC	0.861	0.835	0.805	17.745	0.019	0.091	21.658	0.009
PAC	0.747	0.699	0.569	13.001	0.183	1.085	16.856	0.912
TIA	0.953	0.911	0.769	17.947	0.626	22.469	2.784	19.268
HA	0.892	0.871	0.817	19.751	7.469	31.667	23.567	1510.470
TBAC	0.793	0.754	0.653	15.425	50.357	633.707	7.947	67966.570
Steaming								
PAC	1	1	NA	–	0	1.187	0	NA
TIA	0.981	0.977	0.963	28.253	0.329	22.416	1.470	1.054
TBAC	0.998	0.996	0.983	52.986	0.234	639.366	0.037	1.571

Note: TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, hemagglutinating activity; TABC, total biogenic amines content; *SD*, standard deviation; *CV*, coefficient of variation; *PRESS*, predicated residual error sum of squares; NA, not applicable.

explained by independent variables studied in the present study. Regression coefficient data for the soaking of rice indicate rice-water ratio and time caused a reduction ($P < 0.05$) of the contents of tannins and phytic acid. The main influencing factors for reduction of total biogenic amines content were rice-water ratio, initial pH and interaction of temperature and initial pH. However, temperature alone showed an opposite ($P < 0.05$) effect. The reduced polynomial equations were:

$$\text{Tannins content (mg/g)} = 0.020 - 0.010A - 0.015B$$

$$\text{Phytic acid content (mg/g)} = 1.217 - 0.119A - 0.116B$$

$$\text{Total biogenic amines content (μg/g)} = 75.946 - 1.467A + 4.331C - 4.390D - 2.242CD + 2.486C^2 + 3.191D^2$$

where A was rice-water ratio, B was time, C was temperature and D was initial pH.

Keeping temperature and pH constant at 26C and 6.0, respectively, an increase in time and rice-water ratio caused a complete reduction in tannins content (Fig. 1b); the level of phytic acid content reduced to 0.98 mg/g (Fig. 1d), indicating 35% reduction over raw rice. The minimum content of total biogenic amines (74 μg/g; Fig. 1h) was obtained by soaking rice at 20.5C in water (pH 7.0), and keeping the rice-water ratio fixed at 1:7 and time at 12 h.

Optimized Soaking Condition

After numerical optimization, the optimum condition for soaking of dal was 1:10 of dal-water ratio, time of 18 h, temperature of 16C and initial pH of 4.0, and that for soaking of rice was 1:10 of rice-water ratio, time of 18 h, temperature of 22C and initial pH of 7.1. To minimize wastage of water, desirability of various target ratios was compared ($P < 0.01$) and a ratio of 1:5 for dal/rice-water was selected, Therefore, the optimized soaking condition for dal was 1:5 of dal-water, 18 h, 16C and initial pH 4.0. Similarly, the optimum soaking condition for rice was 1:5 of rice-water, 18 h, 16C and initial pH 5.6. Predicted reduction ($P < 0.05$) of antinutrient levels for soaked dal and rice is shown in Table 6.

The initial pH (set under different experimental conditions) for both dal and rice ranged from 4 to 10. The final pH of soak water of dal and rice ranged from 3.0 to 6.8 and 3.0 to 6.6 (run numbers: 18 to 8), respectively (Tables 1 and 2). In the present study, the decrease in pH might be due to accumulation of organic acids by the growth of microbiota during soaking (Ashenafi and Busse 1991).

Unfermented Mixed Batter

Optimally soaked dal paste and rice slurry were mixed in a ratio of 1:2 v/v to prepare unfermented mixed batter. Compared to dal, rice contained negligible amounts of antinutrients. Since in the mixed batter dal constituted one-third by volume, the level of antinutrients in mixed batter was approximately one-third of the level of those in soaked dal (Table 6).

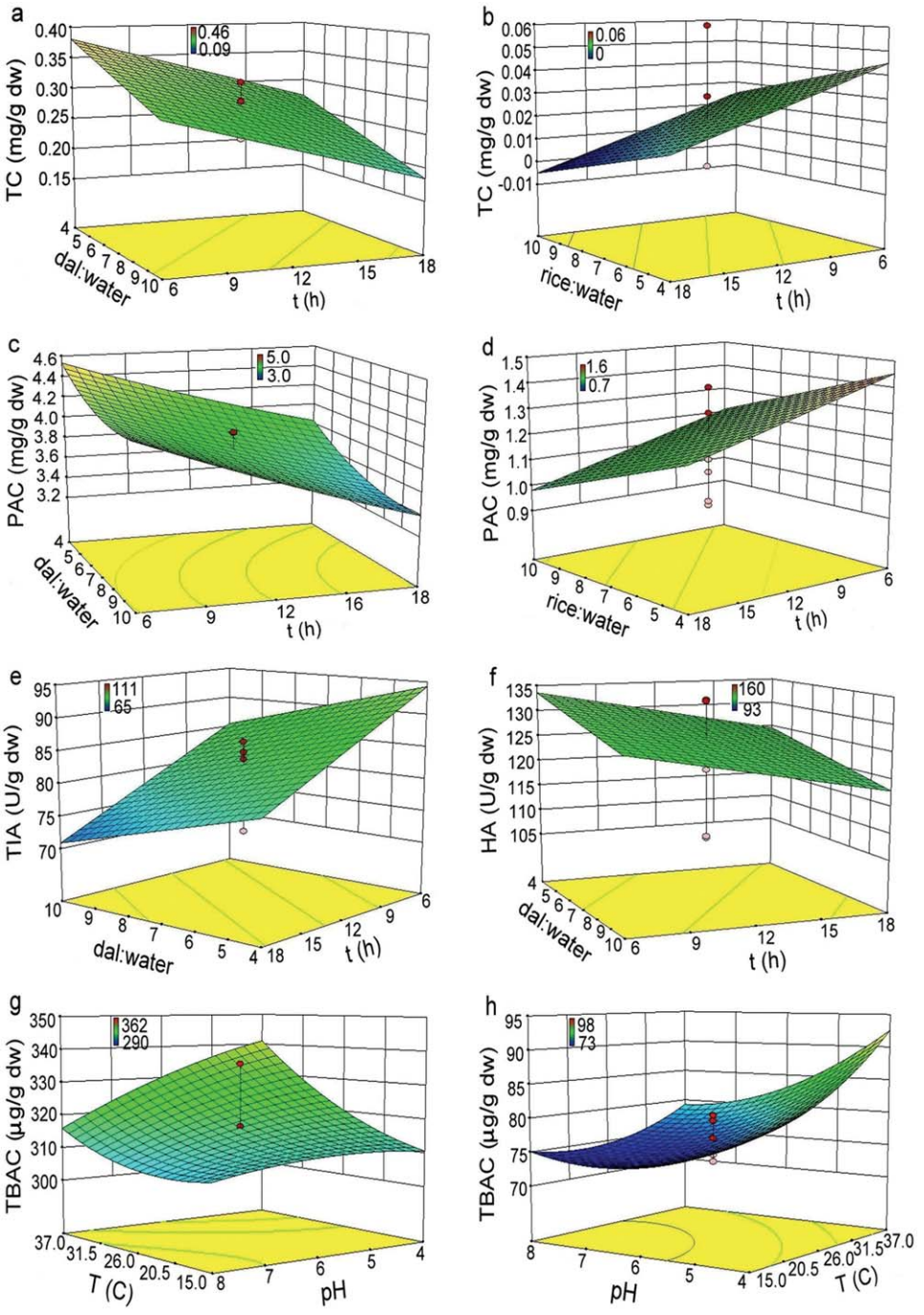

FIG. 1. RESPONSE SURFACE 3D PLOTS SHOWING THE INFLUENCE OF BLACKGRAM DAL/RICE-WATER RATIO, SOAKING TIME (*t*), SOAKING TEMPERATURE (*T*) AND INITIAL pH OF SOAKING WATER ON ANTINUTRIENTS: INFLUENCE OF DAL/RICE-WATER RATIO AND *t* ON TANNINS CONTENT (TC; a and b), PHYTIC ACID CONTENT (PAC; c and d), TRYPSIN INHIBITOR ACTIVITY (TIA; e) AND HEMAGGLUTINATING ACTIVITY (HA; f) WHEN *T* AND pH WERE KEPT CONSTANT AT 26C AND 6.0, RESPECTIVELY; INFLUENCE OF *T* AND pH ON TOTAL BIOGENIC AMINES CONTENT (TBAC; g and h) WHEN DAL/RICE-WATER RATIO AND *t* WERE KEPT CONSTANT AT 1:7 AND 12 h, RESPECTIVELY

Fermentation

The levels of antinutrients under different fermentation conditions as per RSM design are shown in Table 3. The models for fermentation stage are significant with nonsignificant lack of fit (Table 7). The R^2 values show that independent variables explain more than 75% variation for each antinutrient. The adjusted and predicted R^2 values for models are in reasonable agreement, and adequate precision for models has ratio greater than 4. The low values of *CV*, *SD* and *PRESS* for models show the models are fit for prediction.

Fermentation time and temperature were significant for minimizing the levels of all the antinutrients, except total biogenic amines. Although salt concentration had a reducing effect on total biogenic amines content, it did not affect the other antinutrients. Reduced equations for significant

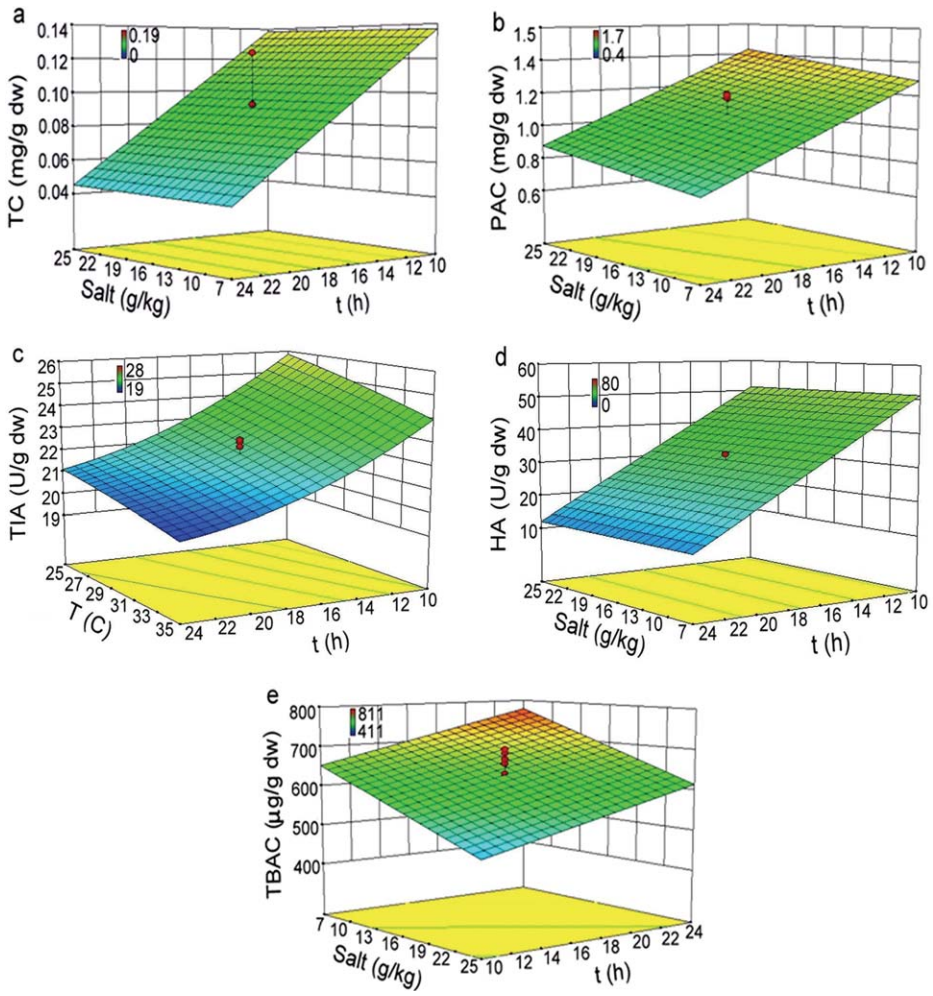

FIG. 2. RESPONSE SURFACE 3D PLOTS SHOWING THE INFLUENCE OF SALT CONCENTRATION, FERMENTATION TIME (*t*) AND FERMENTATION TEMPERATURE (*T*) ON ANTINUTRIENTS: INFLUENCE OF ADDED SALT AND *t* ON TANNINS CONTENT (TC; a), PHYTIC ACID CONTENT (PAC; b), HEMAGGLUTINATING ACTIVITY (HA; d) AND TOTAL BIOGENIC AMINES CONTENT (TBAC, e) WHEN *T* WAS KEPT CONSTANT AT 30C; INFLUENCE OF *t* AND *T* ON TRYPSIN INHIBITOR ACTIVITY (TIA; c) WHEN ADDED SALT CONCENTRATION WAS KEPT CONSTANT AT 16 g/kg

($P < 0.05$) linear terms for tannins content, phytic acid content, hemagglutinating activity and total biogenic amines content and quadratic terms for trypsin inhibitor activity were:

Tannins content (mg/g) $= 0.091 - 0.047B - 0.026C$

Phytic acid content (mg/g) $= 1.085 - 0.277B - 0.185C$

Hemagglutinating activity (U/g) $= 31.667 - 19.615B - 12.426C$

Total biogenic amines content (µg/g) $= 633.707 - 81.090A + 62.260B + 30.337C$

Trypsin inhibitor activity (U/g) $= 22.189 - 2.183B - 0.751C + 0.630B^2$

where A was salt concentration; B was fermentation time and C was fermentation temperature.

With an increase in fermentation time from 10 to 24 h at 30C, there was a linear reduction in tannins content (0.04 mg/g; Fig. 2a), phytic acid content (0.7 mg/g; Fig. 2b) and hemagglutinating activity (12 U/g; Fig. 2d). The predicted reduction of tannins content, phytic acid content and hemagglutinating activity was 56, 59 and 70%, respectively, over unfermented mixed batter. When the salt concentration was fixed at 16 g/kg, the minimum level of trypsin inhibitor activity (20 U/g; Fig. 2c) obtained at 24 h and 35C was due to its reduction by 39%. The reduction of tannins in fermented batter signifies an extensive leaching during soaking and fermentation. Reduction in the level of tannins content might be due to polyphenol oxidase, produced by the microorganisms during fermentation (Reddy and Pierson 1994). The reduction of phytic acid content in the present study might be due to phytase activity of the fermenting microorganisms. Microbial fermentation can enhance bioavailability of dietary minerals by hydrolyzing phytic acid using their own phytase (Kumar *et al.* 2010). The reduction of hemagglutinating activity and trypsin inhibitor activity during fermentation has been reported earlier (Reddy and Pierson 1994; Holzapfel 2002).

The minimum level of total biogenic amines content (493 µg/g; Fig. 2e) was obtained at 10 h of fermentation when the salt concentration was increased to 25 g/kg, keeping fermentation temperature fixed at 30C.

The total biogenic amines content in fermented batter was almost three times more than that of mixed

unfermented batter. This is because biogenic amines are formed by several microbial groups possessing decarboxylase activity during fermentation (Holzapfel 2002). Interestingly, in this study, a reduction in the level of total biogenic amines content was observed when the salt concentration was increased. Similar results were reported by Chander *et al.* (1989). The high concentration of salt leads to reduced cell yield and progressively disturbs the membrane-located microbial decarboxylase (Sumner *et al.* 1990).

Optimized Fermentation Condition

Although maximum reduction of antinutrients was observed at salt concentration of 25 g/kg, presence of such a high concentration of salt in finished products may affect organoleptic quality and not be acceptable to consumers. Thus, based on the desirability ($P < 0.01$) of various concentrations of salts, 16 g salt/kg was selected. Similarly, although fermentation for 24 h gave maximum reduction of antinutrients, a period of 19 h was selected as the duration of fermentation. Therefore, the optimized fermentation condition was 16 g/kg added salt at 35C for 19 h. The predicted reduction of antinutrient levels is shown in Table 6. The experimental values agreed with the predicted ones.

Steaming

Regression coefficient data show models for steaming stage to be significant with nonsignificant lack of fit (Table 7). This implies that model terms could explain more than 98% of variation for reduction in antinutrient levels. These results indicate a high precision in predicting reduction in antinutrient levels. The adjusted and predicted R^2, adequate precision, *CV*, *SD* and *PRESS* indicate adequate model discrimination.

Phytic acid content, trypsin inhibitor activity and total biogenic amines content reduced ($P < 0.05$) with the increase in steaming time. The reduced significant linear equations were:

Phytic acid content (mg/g) $= 0.410 - 0.288A + 0.236A^2 + 0.271A^3 - 0.302A^4$

Trypsin inhibitor activity (U/g) $= 17.650 - 1.047A + 2.303 + 0.427\mathrm{A}^3 - 2.013\mathrm{A}^4$

Total biogenic amines content (μg/g) $= 639.577 - 11.357A - 0.329A^2 + 7.877A^3$

where A was steaming time.

When steaming time was raised to 20 min, the levels of antinutrients reduced sharply (Table 4). The phytic acid content increased initially (run numbers: 7 to 3), however after 12.5 min (run numbers: 3 to 1) it decreased. Such a time-dependent change in phytic acid content during heating at 100C was observed earlier (Kim and Kim 1998). The decrease in phytic acid content can be due to its degradation by heat or formation of insoluble complexes between phytate and proteins or minerals (Vijayakumari *et al.* 2007). During steaming, destruction of disulfide bonds, splitting of covalent bonds or hydrolysis of peptide bonds might have caused a reduction of trypsin inhibitor activity (Adams 1991). Since hemagglutinin is heat-sensitive, it reduced below the limit of detection during the steaming process. Total biogenic amines content reduced by 6.6%, indicating steaming for 20 min is effective in removal of total biogenic amines content in the final product.

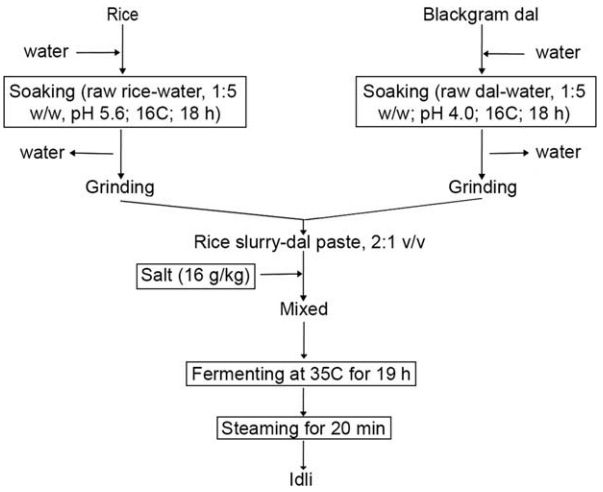

FIG. 3. THE OVERALL OPTIMIZED AND EXPERIMENTALLY VALIDATED PROCESS CONDITIONS (WITHIN BOXES) OF IDLI-MAKING

Optimized Steaming Condition

The predicted optimum time for steaming condition was 20 min. Reduction of antinutrient levels is shown in Table 6. Although there was an increase ($P < 0.05$) of total biogenic amines content during optimum fermentation condition, it reduced during steaming. Since the levels of total biogenic amines content (636 μg/g) in optimally produced idli are below the hazardous level of 1000 μg/g food (Silla Santos 1996), such a product is safely consumable.

Optimum production processes of idli with minimum level of antinutrients are shown in Fig. 3 and Table 6. Traditionally prepared idli contained 0.06 mg, 0.7 mg, 24 U, 0 U and 641 μg of tannins, phytic acid, trypsin inhibitor activity, hemagglutinating activity and total biogenic amines, respectively, per gram dry weight of idli.

Microbiological Study

The counts of total aerobic mesophilic bacteria, lactic acid bacteria and yeasts were studied for both unfermented and fermented mixed batters under different experimental conditions (Table 3). The microbial content, initial pH (5.6) and titratable acidity (0.03%) values of unfermented batter changed rapidly during the course of fermentation.

With the increase in salt concentration, lactic acid bacterial and yeast counts reduced compared to total aerobic mesophilic bacterial count, indicating a low salt concentration favored the growth of lactic acid bacteria and yeasts (Table 3). Again, with the increase in fermentation time not only lactic acid bacterial and yeast counts but total aerobic mesophilic bacterial count also increased. At the onset of fermentation, the population of total aerobic mesophilic bacteria exceeded ($P < 0.05$) that of lactic acid bacteria (1:0.9), indicating the presence of a relatively large number of nonlactic acid bacteria. However, after fermentation, the situation reversed (1:1.2), indicating that lactic acid bacteria had outgrown (dominated) other (nonlactic acid) bacteria, which – in combination with the demonstrated acidification – is a sign of successful lactic acid fermentation. A similar situation was observed during lactic acid fermentation of various food grains (Agarwal *et al.* 2000; Shimelis and Rakshit 2008). Reduction ($P < 0.05$) in pH of batter by lactic acid bacteria allowed yeasts to grow (Soni and Sandhu 1991).

Sensory Analysis

Idli of good quality should have a slight sour aroma and a spongy texture with honey-comb crumb interior. Idli prepared from batter (having 16 g/kg salt) fermented under optimized conditions of 35C for 19 h scored maximum (94), reaching to the "excellent" quality level (Table 5).

CONCLUSION

Traditionally, idli is prepared by soaking blackgram dal and rice (dal/rice:water, 1:4–1:10) at room temperature (15–37C) overnight (6–18 h), grinding soaked dal and rice, mixing of those (1:1–1:4) along with common salt (7–25 g/kg), fermenting at room temperature (25–35C) for 6–24 h and steaming for 15–20 min. Whereas the optimum condition for idli preparation in the present study was soaking of dal and rice (dal/rice:water, 1:∼5) at 16C for 18 h followed by grinding and mixing of those (1:2) along with 16 g/kg common salt, fermenting at 35C for 19 h and steaming for 20 min.

In case of traditionally prepared idli, there was reduction of tannins content, phytic acid content, trypsin inhibitor activity and hemagglutinating activity by 70, 74, 40 and 100%, respectively, over raw ingredients. On the other hand, the optimization of processing treatments caused a reduction ($P < 0.05$) of their respective levels by 100, 89, 58 and 100%. There was no difference ($P < 0.05$) between the two processes with respect to changes in total biogenic amines content. Thus, by deploying CCRD, idli-making was optimized towards a minimum level of antinutrients without affecting the organoleptic attributes of the product. The outcome of this study has a potential of scaling up idli production.

ACKNOWLEDGMENT

This work was supported by grant, F.3-4/2013 (SAP-II), from the University Grants Commission, New Delhi, India.

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