3
Materials and Methods

3.1. Materials

3.1.1. Culture media

MRS agar (M641; HiMedia Laboratories Pvt. Ltd, Mumbai, India)
Plate Count Agar (HiMedia M091A)
Tryptone glucose yeast extract agar (HiMedia M014)

3.1.2. Chemicals

Acetonitrile (PubChem CID: 6342; 25251 L25; S.D. Fine-Chem Limited (SDFCL), Mumbai)
4-aminoantipyrine (PubChem CID 2151; A4382; Sigma-Aldrich, St Louis, MO)
Casein (PubChem CID 73995022; HiMedia RM087)
Catechin (PubChem CID 73160; Sigma-Aldrich C1251)
Diamine oxidase (EC 1.4.3.6; Sigma-Aldrich D7876)
di-Sodium hydrogen phosphate (PubChem CID 24203; HiMedia TC051)
Ferric chloride (PubChem CID 24380; SDFCL 38379)
Ferric nitrate (PubChem CID 25251; HiMedia RM1376)
Histamine dihydrochloride (PubChem CID 5818; Sigma-Aldrich H7250)

Horseradish peroxidise (EC 1.11.1.7; Sigma-Aldrich P6782)
Hydrochloric acid (PubChem CID 313; 61262325001046; Merck Specialities Pvt. Ltd, Prabhadevi, Mumbai)
Lactic acid (PubChem CID 612; HiMedia RM243)
Methanol (PubChem CID 887; Merck 1.06009.2500)
Nitric acid (PubChem CID 944; Merck 61752505001046)
Petroleum ether (PubChem CID 241; Merck 61782225001730)
Phenol (PubChem CID 966; Merck 82229605001046)
Potassium hydroxide (PubChem CID 14797; HiMedia RM1015)
Potassium thiocyanate (PubChem CID 516872; SDFCL 39666)
Raffinose (PubChem CID 10542) (Fluka Sigma-Aldrich 83400)
Sodium chloride (PubChem CID 5234; Merck 61751905001730)
Sodium dihydrogen phosphate (PubChem CID 23672064; HiMedia TC068)
Sodium hydroxide (PubChem CID 14798; Merck 61757305001046)
Stachyose (PubChem CID 439531) (Sigma-Aldrich S4001)
Sucrose (PubChem CID 5988) (Fluka Sigma-Aldrich 84100)
Trichloroacetic acid (PubChem CID 6421; Merck 82234205001730)
Tris buffer (PubChem CID 6503; 204982; Sisco Research Laboratories Pvt. Ltd, Mumbai)
Trypsin (EC 3.4.21.4; HiMedia RM618)
Vanillin (PubChem CID 1183; HiMedia RM616)
Verbascose (PubChem CID 441434) (Fluka Sigma-Aldrich 56217)
Xylose (PubChem CID 644160) (Sigma-Aldrich 95729)

3.1.3. Reagents

Acidified methanol

Conc. HCl	10 ml
Methanol	990 ml

Casein solution

Casein	2.0 g
Phosphate buffer (0.1 mol/l, pH 7.6)	100 ml

Ferric chloride solution

$FeCl_3$	5.78 mg
Trichloroacetic acid (30 g/l)	1 ml

Peptone physiological saline

Peptone	1.0 g
NaCl	8.5 g
Distilled water	1000 ml
pH 7.0	

Phenolphthalein solution

Phenolphthalein	0.1 g
Methanol	95 ml
Distilled water	5 ml

Phosphate buffer (0.1 mol/l)

$Na_2HPO_4.7H_2O$	23.3 g
$NaH_2PO_4.H_2O$	1.8 g
Distilled water	1000 ml
pH 7.6	

Stock trypsin solution

Trypsin	5 mg
HCl (0.001 mol/l)	100 ml

Vanillin reagent

Solution A	
Vanillin	1.0 g
Methanol	100 ml
Solution B	
Conc. HCl	8 ml
Methanol	92 ml

Solutions A and B were mixed in equal proportions just before use.

3.2. Experimental

3.2.1. Preparation of samples

Raw soybean, raw blackgram dal, raw bengalgram dal and raw rice were powdered using a blender (Bajaj Electricals, Mumbai). Soaked products (soybeans, dal and rice) and cooked soybean were made to paste. The dal-rice mixed (unfermented and fermented) batters were divided into two sets, one for the estimation of viable cell count, pH and titratable acidity, while the other set as well as pastes of cooked and fermented soybeans, raw substrate powders, soaked dal pastes and macerated dhokla and idli were left overnight at -20°C, lyophilized (Eyela freeze dryer, FDU-506, Tokyo Rikakikai Co. Ltd, Tokyo, Japan) and powdered. The lyophilized powdered samples were used for the evaluation of antinutritional factors.

3.2.2. Extraction and estimation of antinutritional factors

3.2.2.1. Tannins

The method described by Price *et al*. (1978) was followed for the estimation of tannins. Powdered sample (200 mg) was mixed with 10 ml acidified methanol and shaken at 25°C for 1 h. The mixture was centrifuged (3000 *g*, 15 min) and the clear supernatant was collected. The extraction process was repeated once and pooled. Fresh extract (1 ml) was added with 5 ml of vanillin reagent. The mixture was incubated at 30°C for 20 min before taking absorbance at 500 nm using a UV–Vis spectrophotometer (Type 118; Systronics, Ahmedabad, India). The tannins content was expressed as catechin equivalent using the standard curve of catechin and the formula:

$$\text{Tannins content (mg/g)} = \frac{\text{C x vol. of extract (ml)}}{\text{Wt of sample (g)}}$$

where, C was the concentration obtained from the standard curve.

3.2.2.2. Phytic acid

Phytic acid was extracted and estimated following the method of Wheeler and Ferrel (1971). A 3 g-sample was mixed with 50 ml of 30 g/l trichloroacetic acid (TCA) and shaken on a rotary shaker (Bti-43; Bio-Technics, Mumbai) at 25°C for 30 min followed by centrifugation (10,000 *g*, 10 min). A 4 ml-ferric chloride solution was added to 10 ml of the supernatant, boiled in a water bath for 45 min and centrifuged (10,000 *g*, 10 min). The supernatant was allowed to react with 3 ml of 1.5 mol/l sodium hydroxide solution. The red precipitate formed was dissolved with 40 ml of hot 3.2 mol/l conc. nitric acid and filtered using Whatman no. 2 paper. The iron content in the samples was estimated at 480 nm immediately after adding 20 ml of 1.5 mol/l potassium thiocyanate solution. Phytate phosphorus was calculated from the iron results, assuming 4:6 of iron:phosphorus molar ratio. Phytic acid was estimated by assuming that 0.282 g phosphorus was present per gram sample (Deshpande *et al*., 1982). Calculation of iron content was made from the standard curve prepared using ferric nitrate as standard.

3.2.2.3. Trypsin inhibitor activity

Approximately 5 g of lyophilized samples were defatted using petroleum ether, based on the method described by Sarkar *et al*. (1996). The solvent was evaporated off at room temperature. The procedure of Kakade *et al*. (1969) was then followed to assay trypsin inhibitor activity. About 1 g defatted powdered sample was mixed with 19 ml distilled water (pH 7.6). The suspension was shaken at 25°C for 1 h and centrifuged (11,000 *g*, 20 min). The supernatant was pooled and diluted to 50 ml using phosphate buffer. An aliquot (0.2–1.0 ml) of the extract was taken into each of a triplicate set of test tubes (one set for each level of extract). The volume was bought to 1.0 ml using phosphate buffer. To each tube, 1 ml of stock trypsin solution was added. The tubes were placed in a water bath at 37°C. One of the triplicate sets was added with 6 ml of 50 g/l TCA solution which served as a blank. To each of the other tubes, 2 ml of warmed (37°C) casein solution was added. All the tubes were allowed to stand at 37°C for 20 min and added with 6 ml of 50 g/l TCA solution to terminate the reaction. After standing for 1 h at room temperature, the suspension in the tubes was filtered using Whatman no. 1 paper. Absorbance of the filtrate was measured at 280 nm against the blank. 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.

3.2.2.4. Haemagglutinating activity

The method described by Liener and Hill (1953) was followed for the determination of haemagglutinating activity. A 1 g-sample was mixed with 10 ml of 9.0 g/l sodium chloride solution, blended for 1 min, allowed to stand for 15 min and centrifuged (10,000 *g*, 20 min). Human blood sample (B-group female), collected from the Health Centre of the University of North Bengal, was diluted four times with cold 9.0 g/l sodium chloride solution and the suspension was centrifuged (313 *g*, 10 min). The sediment, after washing with sodium chloride solution until the supernatant became colourless, was diluted with sodium chloride solution to obtain a final red blood cell (RBC) concentration of 4% (v/v).

The sample extract was diluted with sodium chloride solution to get 10 different serial 2-fold dilutions of the extract (1:0-1:528). A 0.2 ml of the RBC suspension was added to each of the 10 tubes having dimension of 10 x 75 mm containing 0.5 ml of the diluted sample extract. After incubating the mixture at 37°C for 1 h, agglutination was checked by observing settling of the cells down to the bottom of the test tube. The tubes were graded (0 to 4+) to measure the degree of agglutination. One haemagglutinating unit (U) is defined as the least amount of haemagglutinin which produced positive agglutination (1+) under the condition of the experiment. Haemagglutinating activity was calculated as follows:

$$U/g = \frac{D_a \times D_b \times S}{V}$$

where, D_a was dilution factor of the extract in tube 1 (which is 1), D_b was dilution factor of the tube containing 1 HU, S represented the volume of original extract per gram sample (which was 10 ml) and V represented the volume of extract in tube 1 (which was 0.5 ml).

3.2.2.5. Total biogenic amines

Total biogenic amines content was assayed according to the method described by Yeh *et al*. (2006). A 5 g-sample was mixed with 50 ml of 200 g/l TCA solution and homogenized for 10 min using a magnetic stirrer. The supernatant (10 ml) was diluted 10 times using distilled water and filtered using a Whatman no. 1 paper.

The filtrate was adjusted to pH 9.0 with 0.5 g/ml potassium hydroxide and centrifuged (700 *g*, 5 min). The clear supernatant (1 ml) was mixed with 0.45 ml of the colour-developing reagent (4 parts of 1.5 mol/l Tris buffer pH 9.0, 1 part of 400 mmol/l 4-aminoantipyrine and 1 part of 40 mmol/l phenol), 0.5 ml of 300 mU/ml diamine oxidase and 0.05 ml of 175 U/ml horseradish peroxidase type VI-A. After incubating the mixture at 50°C for 1 h, the absorbance was read at 505 nm and compared with the standard curve prepared using histamine dihydrochloride as standard.

3.2.2.6. Oligosaccharides

The powder samples of raw, soaked, mixed batters (both unfermented and fermented) and macerated idli were defatted with distilled petroleum ether in a Soxhlet extractors. The extract was evaporated at below 45°C in a rotary vacuum evaporator and quantified gravimetrically to use for fat correction during calculation of the oligosaccharide content of the defatted samples on dry weight basis. Defatting was followed by deproteination and oligosaccharide extraction by the procedure describe by Knudsen (1986). Defatted sample (approximately 1 g) was mixed with 10 ml distilled water and brought just to boiling. Then the mixture was shaken in a 60°C-water bath for 5 min, made up to 10 ml with distilled water and centrifuged at 1100 g for 10 min. The supernatant (3.5 ml) was mixed thoroughly with 6.5 ml HPLC grade acetonitrile and left overnight at 4°C. After filtering through a G3 sintered glass filter, an aliquot of the filtrate was placed in a 5-ml glass vial for HPLC analysis.

The estimation of oligosaccharides was done following the method described by Sarkar *et al*. (1997a). The chromatographic system (Waters Associates, Milford, MA) consisted of Waters isocratic 515 pump, a Rheodyne manual injector equipped with a 5-μl sample loop, a Waters column heating attachment, a Waters carbohydrate column (3.9 mm i.d. x 30 cm), a Waters guard-pak column, and a refractive index detector model 2414 (Waters). The mobile phase used for elution was acetonitrile-water (HPLC grade) (65:35, v/v) and the flow rate was constant at 1.0 ml/min. The column and detector were maintained at 31°C. The run time for the chromatogram was maintained for 13 min.

Identification and quantification of sugars present in the samples were done by comparing each peak retention time and area with those of the standards. The quantity of each sugar was corrected based on the recovery ratio of the internal standard. The standard sugars used were D(+)-sucrose, D(+)-raffinose, stachyose and verbascose. D(+)-xylose was used as an internal standard since it does not interfere with the oligosaccharides. The chromatographic data were collected and plotted using Waters Empower 2 software.

3.2.3. Microbiological analysis

A 10 g-sample of unfermented mixed batter and batters from each combination of fermentation conditions as per central composite rotatable design (CCRD) of idli and dhokla preparation was aseptically weighed and homogenized with 90 ml of sterile peptone physiological saline for 1 min at 'normal' speed using a Stomacher lab-blender 400 (Seward Medical, London, UK). Serial decimal dilutions were made using the same diluent. One millilitre of the appropriate dilutions was mixed with molten media and poured into plates. After incubation, the colonies appearing on the plates were counted as colony forming unit (cfu) per gram fresh weight sample.

3.2.3.1. Total aerobic mesophilic bacteria

The viable counts of total aerobic mesophilic bacteria were carried out in pour-plates of plate count agar, which were incubated at 37°C for 24 h.

3.2.3.2. Lactic acid bacteria

Lactic acid bacteria were enumerated in pour plates of Lactobacillus deMan, Rogosa and Sharpe (MRS) agar, incubated at 37°C for 48 h in an anaerobic culture jar (HiMedia LE002) with Anaerogas pack (HiMedia LE002A).

3.2.3.3. Yeasts

For yeast count, tryptone glucose yeast extract agar, supplemented with 10 IU/ml benzylpenicillin and 12 mg/ml streptomycin sulphate, was used. The plates were incubated at 37°C for 24 h.

3.2.4. Physicochemical analysis

3.2.4.1. pH

The pH of soak water of both dal and rice under different experimental conditions was measured using a CyberScan pH 510 meter (Eutech instruments, Thermo Fisher Scientific, Mumbai). For pH of mixed batter, a 100 g-sample was mixed with 20 ml of carbon dioxide-free distilled water for 1 min, equilibrated to 25°C before measuring pH (AOAC, 1990).

3.2.4.2. Titratable acidity

A 10 g-sample was blended with 90 ml of carbon dioxide-free distilled water for 1 min. The mixture was filtered. About 25 ml of the filtrate was titrated with 0.1 mol/l sodium hydroxide to an end point of phenolphthalein (AOAC, 1990).

$$\%\ \text{titratable acid content (as lactic acid content)} = \frac{100 \times \text{ml of NaOH} \times \text{N of NaoH} \times 0.09}{\text{Weight of sample (g)}}$$

3.2.5. Sensory analysis

Kinema prepared under optimized processing condition, and idli and dhokla prepared under different experimental conditions of fermentation and optimized steaming time were evaluated for organoleptic quality by a panel of 10 trained judges. An overall sensory quality was considered using a 100-point score card (Tables 2-4). Analysis of the samples was conducted in triplicate.

Table 2. Sensory score card for kinema

Name: Date: Time:

Please rate these samples for quality attributes according to the grade description and scoring:

Attribute	Defect	Slight	Distinct	Pronounced	Sample No. A	B	C	D
Flavour (smell) [50]	Flat	39	35	33				
Normal range: 38-47	Rotten	35	30	25				
	Raw beany	34	28	26				
Body and texture [45]	Dry	37	34	32				
Normal range: 38-44	Watery	35	30	20				
Colour [5]	Whitish	3	2	1				
Normal range: 4-5								
Total score [100]								

Signature of the Judge

Grading of kinema:

Total score	Grade
92-100	Excellent
82-91	Good
72-81	Fair
62-71	Poor
<61	Bad

Requirements of high-grade kinema:
Flavour: Nutty with ammoniacal odour
Body and texture: Highly sticky or mucilaginous and slightly pasty
Colour: Brown

Reproduced from Tamang (1993).

3.2.6. Minimization of antinutrients using response surface optimization of processing parameters

3.2.6.1. Experimental design

RSM was used to optimize numerically the processing variables of kinema, idli and dhokla making to minimize the level of antinutritional factors, namely tannins, phytic acid, trypsin inhibitor activity, haemagglutinating activity, total biogenic amines and oligosaccharides. The preliminary experimental trials and literature survey data were used for the selection of the levels of processing variables. Experimental design consisted of a

Table 3. Sensory score card for idli

Name: Date: Time:

Please rate these samples for quality attributes according to the grade description and scoring:

Attribute	Defect	Slight	Distinct	Pronounced	Sample No. A	B	C	D
Taste [35]	Bland	23	20	17				
Normal range: 28-34	Rancid	19	17	16				
Flavour (smell) [30]	Starchy	20	17	15				
Normal range: 22-28	Beany	18	16	13				
Body and texture [30]	Hard	20	17	15				
Normal range: 25-28	Sticky	18	15	14				
Colour [5]	Yellowish	3	2	1				
Normal range: 4-5								
Total score [100]								

Signature of the Judge

Grading of idli:

Total score	Grade
92-100	Excellent
82-91	Good
72-81	Fair
62-71	Poor
<61	Bad

Requirements of high-grade idli:
Taste: Sour
Flavour: Acidic
Body and texture: Spongy (honey-comb crumb interior)
Colour: White

Reproduced from Moktan (2011).

Table 4. Sensory score card for dhokla

Name: Date: Time:

Please rate these samples for quality attributes according to the grade description and scoring:

Attribute	Defect	Slight	Distinct	Pronounced	Sample No. A	B	C	D
Taste [35]	Bland	23	20	17				
Normal range: 28-34	Rancid	19	17	16				
Flavour (smell) [30]	Starchy	20	17	15				
Normal range: 22-28	Beany	18	16	13				
Body and texture [30]	Hard	20	17	15				
Normal range: 25-28	Sticky	18	15	14				
Colour [5]	White	3	2	1				
Normal range: 4-5								
Total score [100]								

Signature of the Judge

Grading of dhokla:

Total score	Grade
92-100	Excellent
82-91	Good
72-81	Fair
62-71	Poor
<61	Bad

Requirements of high-grade dhokla:
Taste: Slightly sour
Flavour: Slightly acidic
Body and texture: Spongy (honey-comb crumb interior)
Colour: Yellow

series of experimental runs, fitting the mathematical models and finally selecting variable levels by optimizing the response. The variables optimized were soaking, cooking and fermentation for kinema preparation, and soaking, fermentation and steaming for idli and dhokla preparation. The coded levels used for each stage of processing were: corner (±1), central (0) and axial (±1.682). Randomized experiments were conducted and an optimized processing condition was obtained using Design Expert version 8.0 (Stat-Ease Inc., Minneapolis, MN). After optimization of the first processing stage parameters, the successive stages were optimized. After model fitting of each processing stage, 3D response surface plots were generated to decipher the relation between independent and response variables. A separate experiment using MINITAB

15.1.1.0 (Minitab Inc., State College, PA) was performed to determine optimum soaking conditions for reduction of oligosaccharides in blackgram dal and rice. The optimized soaking condition was used to determine oligosaccharide contents in the subsequent stages, i.e. fermentation and steaming.

3.2.6.2. Fermented foods

3.2.6.2.1. Kinema

The three processing stages of kinema preparation, i.e. soaking, cooking and fermentation were optimized. The upper and lower limits of the independent variables selected for each stages of kinema preparation were shown in Table 5.

Table 5. Variables and their levels for the experimental design of kinema preparation

Experimental variable	Coded level*				
	-α† (augmented form)	-1 (factorial point)	0 (centre point)	1 (factorial point)	α† (augmented form)
Soaking					
Raw soybeans:water (w/w)	1:1	1:4	1:7	1:10	1:13
t (h)	0.5	7	13.5	20	26.5
T (°C)	2.5	10	17.5	25	32.5
Initial pH	2	4	6	8	10
Cooking of optimally soaked beans					
t (min)	6.59	10	15	18	23.41
Pressure (kg/cm^2)	0.5	0.7	1.0	1.3	1.5
Soaked soybeans:water (w/w)	1:0.98	1:2	1:3.5	1:5	1:6.02
Fermentation of optimally cooked beans					
t (h)	5.72	18	36	54	66.27
T (°C)	18.18	25	35	45	51.82
Inoculum load (log total cells/g)	1.98	3	4.5	6	7

*Low, middle and high levels of each variable were designated as -1, 0 and +1, respectively.
†α (1.682 for soaking and 2 for cooking and fermentation stage) is the axial distance from the centre point.

3.2.6.2.1.1. Soaking

Yellow variety soybeans were purchased from a retail shop in Kurseong town and kept in an air-tight aluminium container at room temperature until use (within 2 weeks). The beans were soaked in water under different conditions as per the CCRD generated. Soaking was performed by analyzing the effect of four independent variables, namely raw beans-water ratio (w/w), soaking time (h), soaking temperature (°C) and initial pH of soaking water. The pH of water was adjusted to a desired level using 0.1 mol/l lactic acid and 0.1 mol/l NaOH. The temperatures were controlled by placing the soaked beans in incubators, set at desired temperature levels. The soaking stage consisted of 30 experimental runs with 16 factorial points, eight axial points and six replicates at centre points (Table 6). Experiments were conducted and the antinutritional factors levels were evaluated. After numerical optimization, the optimally soaked beans were subjected to further processing and optimization.

3.2.6.2.1.2. Cooking

The optimally soaked beans were used for optimization of cooking. The cooking stage comprised of three independent variables, namely soaked beans-water ratio (w/w), cooking time (min) and cooking pressure (kg/cm^2). Experimental design consisted of 20 experimental runs with eight factorial points, six axial points and six replicates at the centre point (Table 7). The soaked bean-water ratio was maintained by adding fresh water to the bottles containing soaked beans. Then the bottles were loosely capped and autoclaved at different pressure levels according to the experimental design. After autoclaving, pressure was released immediately and the cook water in the bottles was drained out quickly under aseptic condition to minimize excess heating of beans. However, a little amount of free water was allowed to remain inside the bottles.

Table 6. Antinutritional factors of raw soybeans under different conditions of soaking as per central composite design

Run	Soaking condition				Antinutritional factor (per gram dry wt)*				
	Raw beans: water (w/w)	t (h)	T (°C)	Initial pH	TC (mg)	PAC (mg)	TIA (kU)	HA (U)	TBAC (µg)
1	1:7	13.5	17.5	6	0.94 ± 0.09	6.3 ± 0.1	38 ± 2.1	420 ± 27	442 ± 2
2	1:4	20	10	4	0.92 ± 0.03	6.3 ± 0.1	39 ± 0.5	373 ± 0	610 ± 2
3	1:7	13.5	17.5	6	0.99 ± 0.10	6.4 ± 0	38 ± 0	373 ± 0	466 ± 2
4	1:4	7	10	8	1.06 ± 0.03	6.3 ± 0.1	39 ± 0	480 ± 40	425 ± 2
5	1:13	13.5	17.5	6	0.82 ± 0.03	6.2 ± 0.1	37 ± 1.6	427 ± 40	535 ± 4
6	1:1	13.5	17.5	6	1.62 ± 0.12	6.3 ± 0	39 ± 1	427 ± 27	411 ± 3
7	1:7	13.5	17.5	6	0.90 ± 0.07	6.3 ± 0	39 ± 0	427 ± 0	446 ± 3
8	1:7	13.5	17.5	2	0.99 ± 0.06	6.6 ± 0.2	38 ± 0	320 ± 0	849 ± 2
9	1:4	20	10	8	1.09 ± 0.06	6.3 ± 0	38 ± 0	373 ± 0	421 ± 1
10	1:10	7	25	4	1.05 ± 0.02	6.2 ± 0	39 ± 0.7	320 ± 27	774 ± 1
11	1:4	20	25	8	1.02 ± 0.03	6.3 ± 0.1	39 ± 0	480 ± 27	534 ± 2
12	1:7	0.5	17.5	6	1.30 ± 0.11	6.3 ± 0.1	39 ± 0	627 ± 27	400 ± 2
13	1:10	20	10	4	0.79 ± 0.03	6.3 ± 0.1	37 ± 0	373 ± 0	678 ± 0
14	1:4	20	25	4	1.02 ± 0	6.2 ± 0	39 ± 0.7	320 ± 0	446 ± 1
15	1:7	13.5	17.5	6	1.00 ± 0.06	6.5 ± 0.1	38 ± 0	427 ± 0	500 ± 1
16	1:7	13.5	32.5	6	0.89 ± 0.07	6.3 ± 0	39 ± 0.8	320 ± 40	749 ± 2
17	1:10	7	25	8	1.16 ± 0.07	6.1 ± 0.1	39 ± 0	427 ± 27	593 ± 2
18	1:10	7	10	4	1.12 ± 0.03	6.2 ± 0	39 ± 0	427 ± 40	689 ± 1
19	1:7	26.5	17.5	6	0.76 ± 0.04	6.1 ± 0.1	36 ± 0	533 ± 0	509 ± 2
20	1:7	13.5	17.5	10	0.95 ± 0.13	6.7 ± 0	38 ± 0	320 ± 40	459 ± 2
21	1:4	7	25	4	1.24 ± 0.07	6.3 ± 0.1	39 ± 0	373 ± 27	585 ± 2
22	1:10	7	10	8	1.16 ± 0.03	6.2 ± 0.1	39 ± 0.9	320 ± 27	400 ± 1
23	1:4	7	10	4	1.06 ± 0.08	6.3 ± 0.1	39 ± 0	480 ± 40	649 ± 2
24	1:7	13.5	17.5	6	0.95 ± 0.03	6.3 ± 0.1	38 ± 0	427 ± 0	459 ± 2
25	1:10	20	25	8	0.72 ± 0.03	6.1 ± 0	37 ± 0.7	427 ± 0	640 ± 2
26	1:7	13.5	17.5	6	0.80 ± 0.03	6.3 ± 0	38 ± 0	427 ± 0	400 ± 1
27	1:4	7	25	8	1.20 ± 0	6.3 ± 0.1	38 ± 0.	373 ± 40	540 ± 1
28	1:10	20	25	4	0.72 ± 0.03	6.3 ± 0.1	38 ± 0	373 ± 27	714 ± 1
29	1:7	13.5	2.5	6	1.02 ± 0.07	6.4 ± 0.1	38 ± 0	320 ± 40	293 ± 2
30	1:10	20	10	8	0.76 ± 0.03	6.2 ± 0.1	38 ± 0	373 ± 27	341 ± 1

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content.

Table 7. Antinutritional factors of soaked soybeans under different cooking conditions as per central composite design

Run	Cooking condition			Antinutritional factor (per gram dry wt)*				
	t (min)	Pressure (kg/cm^2)	Soaked beans: water (w/w)	TC (mg)†	PAC (mg)	TIA (kU)	HA (U)†	TBAC (µg)
1	10	0.7	1:5	0.12 ± 0.03	5.8 ± 0.1	13 ± 0.9	20 ± 0	328 ± 1
2	15	0.5	1:3.5	0.16 ± 0.07	5.9 ± 0.1	17 ± 0.8	20 ± 7	324 ± 2
3	15	1.5	1:3.5	<dl	5.7 ± 0.1	12 ± 0	<dl	310 ± 2
4	6.6	1.0	1:3.5	0.19 ± 0	5.9 ± 0	17 ± 0.5	20 ± 0	328 ± 1
5	10	0.7	1:2	0.12 ± 0.03	5.9 ± 0.1	13 ± 0.4	20 ± 0	328 ± 2
6	20	1.3	1:2	<dl	5.7 ± 0.1	11 ± 0	<dl	307 ± 2
7	20	1.3	1:2	<dl	5.6 ± 0.2	10 ± 0	<dl	306 ± 2
8	15	1.0	1:0.98	0.12 ± 0.03	5.8 ± 0.2	13 ± 0.6	13 ± 7	325 ± 1
9	10	1.3	1:5	<dl	5.7 ± 0.1	13 ± 0.3	<dl	309 ± 1
10	15	1.0	1:6.02	0.09 ± 0	5.7 ± 0.1	13 ± 0	<dl	323 ± 2
11	15	1.0	1:3.5	0.09 ± 0.13	5.8 ± 0.1	15 ± 0.7	13 ± 7	323 ± 0
12	23.4	1.0	1:3.5	<dl	5.6 ± 0	9 ± 0.5	<dl	299 ± 1
13	15	1.0	1:3.5	<dl	5.9 ± 0.1	15 ± 0.6	20 ± 0	324 ± 2
14	10	1.3	1:2	<dl	5.7 ± 0	14 ± 0	<dl	314 ± 1
15	25	1.0	1:3.5	0.16 ± 0.07	5.8 ± 0.1	15 ± 0	13 ± 7	327 ± 2
16	20	0.7	1:2	<dl	5.8 ± 0.1	14 ± 0	<dl	316 ± 2
17	15	1.0	1:3.5	0.09 ± 0	5.7 ± 0.1	13 ± 0.7	20 ± 0	324 ± 1
18	20	0.7	1:5	0.09 ± 0	5.8 ± 0	14 ± 0.9	<dl	318 ± 1
19	15	1.0	1:3.5	0.09 ± 0	5.7 ± 0	15 ± 1.0	20 ± 0	320 ± 1
20	15	1.0	1:3.5	0.12 ± 0.03	5.7 ± 0.2	15 ± 0	13 ± 4	320 ± 1

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content.
†dl (detection limit), 0.003 mg/g dry wt for TC and 6.67 U/g dry wt for HA.

After cooling, the samples were prepared for the evaluation of antinutritional factors. Experiments for the evaluation of antinutritional factors were conducted and the optimum cooking condition was determined.

3.2.6.2.1.3. Fermentation

Optimally soaked and cooked beans were crushed aseptically to grits of mainly half to one-third of the original size. Using those grits, the fermentation stage was optimized. The independent variables selected for fermentation were inoculum load (log total cells/g), fermentation time (h) and fermentation temperature (°C). The fermentation stage consisted of 20 experimental runs with eight factorial points, six axial points and six replicates at the centre points (Table 8). Fermentation was initiated by inoculating the cooked

Table 8. Antinutritional factors of cooked soybeans under different fermentation conditions as per central composite design

Run	Fermentation condition			Antinutritional factor (per gram dry wt)*			
	t (h)	T (°C)	Inoculum load (log total cells/g)	TC (mg)†	PAC (mg)	TIA (kU)	TBAC (µg)
1	18	45	6	0.06 ± 0.02	3.5 ± 0	12 ± 1.1	898 ± 1
2	36	35	4.5	0.03 ± 0.03	3.6 ± 0	12 ± 0	1002 ± 1
3	54	25	6	<dl	3.4 ± 0	12 ± 0	935 ± 1
4	54	25	3	<dl	3.4 ± 0	12 ± 0.9	927 ± 1
5	36	35	4.5	<dl	3.4 ± 0	12 ± 0	918 ± 1
6	36	35	4.5	0.03 ± 0	3.2 ± 0	12 ± 0.6	979 ± 1
7	36	35	7.02	<dl	3.9 ± 0	12 ± 1.2	989 ± 2
8	5.7	35	4.5	0.12 ± 0.03	4.8 ± 0	12 ± 0	597 ± 1
9	18	25	6	0.09 ± 0	3.6 ± 0	13 ± 0	698 ± 2
10	36	35	4.5	<dl	3.6 ± 0	12 ± 0	925 ± 1
11	66.3	35	4.5	<dl	3.2 ± 0	11 ± 1.3	1310 ± 2
12	18	45	3	<dl	3.6 ± 0	12 ± 1.1	840 ± 3
13	36	35	4.5	0.09 ± 0	3.7 ± 0	12 ± 0	1010 ± 1
14	18	25	3	0.06 ± 0	3.5 ± 0	13 ± 0	725 ± 2
15	36	35	4.5	0.03 ± 0	3.4 ± 0	12 ± 1	930 ± 1
16	36	51.82	4.5	<dl	3.2 ± 0	11 ± 1	999 ± 1
17	36	35	1.98	0.03 ± 0	3.7 ± 0	12 ± 1.3	736 ± 3
18	36	18.18	4.5	0.03 ± 0	3.6 ± 0	12 ± 1	742 ± 1
19	54	45	6	<dl	3.3 ± 0	12 ± 0	1075 ± 1
20	54	45	3	<dl	3.3 ± 0	12 ± 2	1051 ± 2

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

beans with a culture of *Bacillus subtilis* DK-W1 (MTCC 1747) (Sarkar *et al.*, 1993). As per the condition of the experimental design, the inocula of different sizes were prepared and mixed with soybean grits to get a desired load of bacterial cells per gram grits. The beans (~ 50 g) were then distributed aseptically into sterile 250 ml glass bottles which were capped lightly and placed in the incubators, set at desired temperature levels. At selected fermentation times the bottles containing beans were removed for sample preparation and antinutrient analysis. Sensory analysis of the optimally fermented beans was carried out by a panel of 10 trained judges using a 100-point score card.

The flow sheet for the production of kinema has been shown, indicating the sites of sampling for antinutrients assay (Fig. 8).

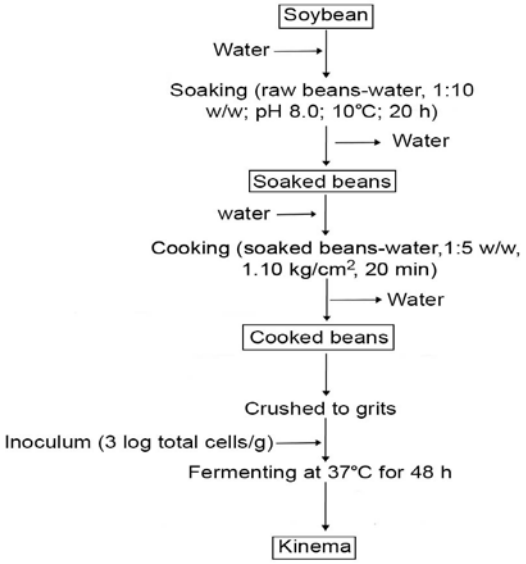

Fig. 8. Flow sheet for the production of kinema.
Boxed texts are the sites of sampling of antinutrients assay.

3.2.6.2.2. Idli

Blackgram dal and white polished rice were purchased from a retail shop in Siliguri. The ingredients were kept in an air-tight aluminium container at room temperature until use (within two weeks). The three processing stage parameters, selected for optimization of idli preparation, were soaking, fermentation and steaming. The levels of the variables for each of the stages were selected from preliminary experimental trials in the laboratory and literature survey data (Tables 9 and 10).

Table 9. Variables and their levels for the experimental design of idli preparation

Experimental variable	Coded level*				
	-α† (augmented form)	-1 (factorial point)	0 (centre point)	1 (factorial point)	α† (augmented form)
Soaking					
Dal/rice:water (w/w)	1:1	1:4	1:7	1:10	1:13
t (h)	0	6	12	18	24
T (°C)	4	15	26	37	48
Initial pH	2	4	6	8	10
Fermentation of optimally soaked dal/rice					
Added salt (g/kg)	0.9	7	16	25	31.1
t (h)	5.2	10	17	24	28.7
T (°C)	21.6	25	30	35	38.4
Steaming of optimally fermented batter					
t (min)		10	15	20	

*Low, middle and high levels of each variable were designated as -1, 0 and +1, respectively.
†α (1.682 for soaking and 2 for fermentation stage) is the axial distance from the centre point.

Table 10. Variables and their levels for the experimental design of soaking of blackgram dal

Experimental variable	Coded level*				
	-α† (augmented form)	-1 (factorial point)	0 (centre point)	1 (factorial point)	α† (augmented form)
Raw dal:water (w/w)	1:3	1:4.75	1:6.5	1:8.25	1:10
t (h)	0	6	12	18	24
T (°C)	15	20	25	30	35
Initial pH	4	5	6	7	8

*Low, middle and high levels of each variable were designated as -1, 0 and +1, respectively.
†α = 2 is the axial distance from the centre point.

3.2.6.2.2.1. Soaking

The independent variables of soaking were dal/rice:water (w/w), soaking time (h), soaking temperature (°C) and initial pH of soaking water. The soaking stage consisted of 30 experimental runs having 16 factorial points, eight axial points and six replicates at the centre points (Tables 11 and 12). Similarly, soaking condition for oligosaccharide estimation consisted of 31 experimental runs having 16 factorial points, eight axial points and seven replicates at the centre points (Table 13).

Blackgram dal and rice were dispensed into glass beakers and soaked in distilled water under different conditions as per the software-generated design. The pH of soaking water was adjusted to the desired values by using 0.1 mol/l lactic acid and 0.1 mol/l NaOH. The temperature was controlled by placing the glass beakers inside incubators, set at desired temperature levels. After desired times of soaking, the soaked beans were taken out and used for sample preparation. The final pH of soak water for each experimental run was noted. Experiments were conducted for antinutrient determination and the optimum soaking condition was calculated following numerical optimization procedure.

Table 11. Antinutritional factors of blackgram dal under different soaking conditions as per central composite design

Run	Soaking condition				Final pH	Antinutritional factor (per gram dry wt)*				
	Dal: water (w/w)	t (h)	T (°C)	Initial 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

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content.

Table 12. Antinutritional factors of rice under different soaking conditions as per central composite design

Run	Soaking condition				Final pH	Antinutritional factor (per gram dry wt)*		
	Rice:water (w/w)	t (h)	T (°C)	Initial 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

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

Table 13. Raffinose family oligosaccharides of blackgram dal under different soaking conditions as per central composite design

Run	Soaking condition				Oligosaccharide (mg/g dry wt)*				
	Dal:water (w/w)	t (h)	T (°C)	Initial pH	Raffinose	Stachyose	Verbascose	Ajugose	Total
1	1:6.5	12	25	6	0.14 ± 0.01	0.99 ± 0	4.2 ± 0	0.96 ±0	6.3 ± 0
2	1:6.5	0	25	6	0.14 ± 0.02	1.42 ± 0	6.9 ± 0	1.5 ± 0	10.0 ± 0
3	1:4.75	6	20	5	0.97 ± 0.02	2.12 ± 0.02	10.0 ± 0	2.4 ± 0	15.4 ± 0
4	1:8.25	18	20	7	0.81 ± 0.01	1.18 ± 0	2.0 ± 0	1.3 ± 0	5.3 ± 0
5	1:6.5	12	25	4	0.14 ± 0.01	1.45 ± 0.02	8.4 ± 0	1.8 ± 0	11.8 ± 0
6	1:4.75	18	20	5	0.15 ± 0.01	0.49 ± 0.01	2.7 ± 0	1.0 ± 0	4.4 ± 0
7	1:6.5	12	15	6	1.07 ± 0.01	0.83 ± 0	2.4 ± 0	1.0 ± 0.1	5.3 ± 0
8	1:8.25	18	30	5	0.11 ± 0.01	2.05 ± 0	8.2 ± 0	2.1 ± 0	12.4 ± 0
9	1:4.75	6	30	7	0.33 ± 0.01	0.37 ± 0	2.5 ± 0	0.8 ± 0	4.0 ± 0
10	1:4.75	18	30	7	0.18 ± 0	0.45 ± 0.01	2.1 ± 0	1.0 ± 0	3.7 ± 0
11	1:8.25	6	20	5	0.09 ± 0	0.46 ± 0	3.1 ± 0	1.0 ± 0	4.7 ± 0
12	1:8.25	6	30	5	0.51 ± 0.02	0.76 ± 0	3.8 ± 0	1.1 ± 0	6.2 ± 0
13	1:6.5	12	25	6	0.92 ± 0	1.09 ± 0.01	4.8 ± 0	1.3 ± 0.1	8.2 ± 0
14	1:4.75	6	30	5	0.59 ± 0.01	1.15 ± 0	2.3 ± 0	1.1 ± 0	5.1 ± 0
15	1:8.25	6	30	7	0.08 ± 0.01	1.00 ± 0.01	1.2 ± 0	1.2 ± 0	3.5 ± 0
16	1:6.5	12	25	6	0.16 ± 0.01	1.78 ± 0	7.0 ± 0	0.3 ± 0	9.3 ± 0
17	1:6.5	12	25	6	0.19 ± 0.01	1.17 ± 0	4.4 ± 0	1.5 ± 0	7.2 ± 0
18	1:4.75	18	20	7	0.21 ± 0.01	0.29 ± 0.01	1.3 ± 0	0.6 ± 0	2.3 ± 0
19	1:3	12	25	6	0.43 ± 0.06	1.99 ± 0.01	8.2 ± 0	1.5 ± 0	12.1 ± 0
20	1:8.25	18	30	7	0.97 ± 0	0.55 ± 0.01	4.7 ± 0	1.4 ± 0	7.6 ± 0
21	1:4.75	18	30	5	0.46 ± 0	1.83 ± 0	8.0 ± 0	1.5 ± 0	11.8 ± 0
22	1:6.5	12	25	6	0.22 ± 0.01	1.40 ± 0.01	5.2 ± 0	0.3 ± 0.1	7.2 ± 0
23	1:8.25	6	20	7	0.19 ± 0	1.97± 0	8.9 ± 0	2.1 ± 0	13.2 ± 0
24	1:4.75	6	20	7	0.97 ± 0.01	0.79 ± 0.01	4.9 ± 0	0.8 ± 0	7.5 ± 0
25	1:6.5	12	25	6	0.19 ± 0.01	0.94 ± 0.01	4.5 ± 0	1.5 ± 0	7.1 ± 0
26	1:6.5	24	25	6	1.25 ± 0.01	1.56 ± 0.01	9.0 ± 0	1.9 ± 0	13.7 ± 0
27	1:6.5	12	25	6	0.62 ± 0.04	0.64± 0.01	5.8 ± 0.1	1.3 ± 0	8.4 ± 0.1
28	1:6.5	12	25	8	0.23 ± 0.01	0.58 ± 0	1.7 ± 0	0.8 ± 0	3.3 ± 0
29	1:10	12	25	6	0.26 ± 0	0.53 ± 0	4.5 ± 0	1.0 ± 0	6.3 ± 0
30	1:8.25	18	20	5	0.23 ± 0.01	0.68 ± 0.01	4.5 ± 0	1.3 ± 0	6.7 ± 0
31	1:6.5	12	35	6	0.15 ± 0.01	1.91 ± 0.01	4.7 ± 0	1.1 ± 0	7.8 ± 0

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

3.2.6.2.2.2. Mixing of batters

While optimally soaked dal was ground to smooth paste, optimally soaked rice was coarsely ground. The paste and slurry were mixed in a ratio of 2:1 v/v to prepare unfermented mixed batter which was used for optimizing the fermentation stage parameters. One set of batter was used for the evaluation of antinutritional factors, while the other was used for the determination of microbial load (total aerobic mesophilic bacterial, lactic acid bacterial and yeast counts), pH and titratable acidity.

3.2.6.2.2.3. Fermentation

The fermentation stage consisted of three independent variables, namely added common salt (g/kg), fermentation time (h) and fermentation temperature (°C). The stage comprised of 20 experimental runs having eight factorial points, six axial points and six replicates at the centre points (Table 14). As per the experimental conditions, different amounts of common salt were added to the unfermented mixed batters. The batters were placed inside incubators, set at desired temperature levels and fermented for a stipulated time according to the design. The batters were used in two sections, one was used for sample preparation for antinutrient determination, while the other was used for the evaluation of microbial load, pH and titratable acidity. Oligosaccharide content of unfermented as well as fermented (30°C, 18 h) mixed batter containing 8 g salt/kg was estimated.

Table 14. Antinutritional factors, pH, titratable acidity and microbial load in mixed idli batter, fermented as per central composite design

Run	Fermentation condition			Antinutritional factor (per gram dry wt)*					Final pH*	TA*	Log cfu/g*		
	Added salt (g/kg)	t (h)	T (°C)	TC (mg)†	PAC (mg)	TIA (U)	HA (U)†	TBAC (µg)		(%)	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
Unf§ψ									5.6^{a} ± 0	0.03^{b} ± 0	6.7^{b} ± 0	6.2^{b} ± 0	3.3^{b} ± 0
Opt‡ψ									4.3^{b} ± 0	0.10^{a} ± 0	7.3^{a} ± 0	8.4^{a} ± 0	5.3^{a} ± 0

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content; TA, titratable acidity (as lactic acid); TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria.

†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 temp., 35°C).

§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 15. Antinutritional factors in fermented batter, steamed as per central composite design

Run	Steaming time (min)	Antinutritional factor (per gram dry wt)*				
		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

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content.
†dl (detection limit), 0.003 mg/g dry wt for TC and 6.67 U/g dry wt for HA.

3.2.6.2.2.4. Steaming

Optimally fermented idli batter was used for steaming which consisted of a single independent variable, namely steaming time (min) with seven experimental runs (Table 15). For steaming, the fermented batters were dispensed into cups of an idli steamer and steamed for desired steaming times as per CCRD. Experiments were performed from the samples prepared from steamed product and optimum condition was determined using numerical optimization procedure. Sensory analysis of the steamed product, i.e. idli was performed for each of the combinations of fermentation using optimum steaming time.

The flow sheet for the production of idli has been shown, indicating the sites of sampling for antinutrients assay (Fig. 9).

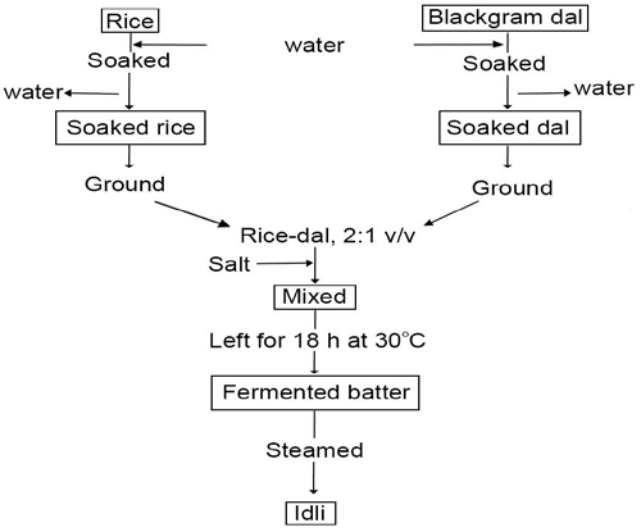

Fig. 9. Flow sheet for the production of idli. Boxed texts are the sites of sampling for antinutrients assay.

3.2.6.2.3. Dhokla

The preparation procedure of dhokla was similar to that of idli. The independent variables selected for soaking, fermentation and steaming for dhokla preparation were also similar to idli processing variables. The difference was that in case of dhokla bengalgram dal, instead of blackgram dal, was used. The level of independent variables selected from the preliminary experimental trials and literature survey data is shown in Table 16.

3.2.6.2.3.1. Soaking

Soaking was performed using four independent variables, namely dal/rice:water (w/w), soaking time (h), soaking temperature (°C) and initial pH of soaking water. The soaking stage consisted of 30 experimental runs, 16 factorial points, eight axial points and six replicates at centre points (Tables 12 and 17). The experiments were conducted, and predicted optimum soaking condition as produced by the software was selected.

Table 16. Variables and their levels for the experimental design of dhokla preparation

Experimental variable	Coded level*				
	-α† (augmented form)	-1 (factorial point)	0 (centre point)	1 (factorial point)	α† (augmented form)
Dal soaking					
Dal:water (w/w)	1:2	1:3	1:4	1:5	1:6
t (h)	5	10	15	20	25
T (°C)	10	20	30	40	50
Initial pH	4	5	6	7	8
Rice soaking					
Rice:water (w/w)	1:1	1:4	1:7	1:10	1:13
t (h)	0	6	12	18	24
T (°C)	4	15	26	37	48
Initial pH	2	4	6	8	10
Fermentation of optimally soaked dal/rice					
Added salt (g/kg)	1.6	5	10	15	18.4
t (h)	3.2	10	20	30	36.8
T (°C)	20.6	25	31.5	38	42.4
Steaming of optimally fermented batter					
t (min)		10	15	20	

*Low, middle and high levels of each variable were designated as -1, 0 and +1, respectively.
†α (1.682 for soaking and 2 for fermentation stage) is the axial distance from the centre point.

Table 17. Antinutritional factors of bengalgram dal under different soaking conditions as per central composite design

Run	Soaking condition				Final pH*	Antinutritional factor (per gram dry wt)*				
	Dal:water (w/w)	t (h)	T (°C)	Initial pH		TC (mg)	PAC (mg)	TIA (kU)	HA (kU)	TBAC (µg)
1	1:5	10	20	7.0	6.9 ± 0	1.06 ± 0.03	1.3 ± 0	11.1 ± 0.1	2.6 ± 0	363 ± 2
2	1:5	10	40	7.0	6.8 ± 0	0.96 ± 0.03	1.2 ± 0.1	10.8 ± 0.1	1.7 ± 0.4	433 ± 5
3	1:3	20	40	5.0	4.6 ± 0	0.49 ± 0.06	1.1 ± 0	8.1 ± 0.3	1.3 ± 0.4	404 ± 1
4	1:5	20	40	7.0	6.7 ± 0.2	0.48 ± 0.03	1.1 ± 0	8.1 ± 0.4	1.3 ± 0.4	393 ± 6
5	1:4	15	30	4.0	3.9 ± 0	0.59 ± 0	1.2 ± 0	9.1 ± 0.1	1.7 ± 0	436 ± 2
6	1:4	15	30	6.0	5.7 ± 0	0.58 ± 0.03	1.2 ± 0	9.7 ± 0.1	1.7 ± 0.4	387 ± 4
7	1:5	20	40	5.0	4.6 ± 0	0.46 ± 0.03	1.1 ± 0	8.1 ± 0.3	1.3 ± 0.4	401 ± 6
8	1:3	20	40	7.0	6.7 ± 0	0.49 ± 0	1.1 ± 0	8.1 ± 0.3	1.3 ± 0.4	407 ± 2
9	1:3	20	20	7.0	6.7 ± 0	0.56 ± 0.03	1.1 ± 0	8.1 ± 0.1	1.7 ± 0.4	348 ± 7
10	1:6	15	30	6.0	5.6 ± 0	0.46 ± 0.03	1.1 ± 0	8.9 ± 0	1.7 ± 0.4	371 ± 4
11	1:5	10	40	5.0	4.8 ± 0.1	0.96 ± 0.03	1.2 ± 0	10.6 ± 0	1.7 ± 0.4	432 ± 5
12	1:4	5	30	6.0	6.0 ± 0	1.70 ± 0.03	1.5 ± 0	12.1 ± 0	5.1 ± 0	425 ± 3
13	1:4	15	30	6.0	5.7 ± 0	0.49 ± 0	1.2 ± 0	9.2 ± 0.3	2.1 ± 0.4	372 ± 4
14	1:3	10	20	7.0	6.9 ± 0	1.16 ± 0.03	1.3 ± 0	11.1 ± 0.1	2.6 ± 0	366 ± 5
15	1:3	20	20	5.0	4.6 ± 0	0.56 ± 0.03	1.1 ± 0.1	8.1 ± 0	1.7 ± 0.4	353 ± 7
16	1:4	15	10	6.0	5.9 ± 0	1.16 ± 0.07	1.2 ± 0	11.2 ± 0	2.1 ± 0	376 ± 5
17	1:4	15	50	6.0	5.4 ± 0	1.16 ± 0.03	1.1 ± 0	9.5 ± 0.1	1.1 ± 0.4	443 ± 5
18	1:4	15	30	6.0	5.6 ± 0	0.52 ± 0.03	1.2 ± 0.1	9.8 ± 0.5	2.1 ± 0	387 ± 4
19	1:4	25	30	6.0	5.5 ± 0	0.46 ± 0.03	1.1 ± 0.1	8.1 ± 0	1.3 ± 0.4	345 ± 2
20	1:4	15	30	8.0	7.9 ± 0.2	0.56 ± 0.03	1.2 ± 0	9.3 ± 0.4	1.7 ± 0.4	368 ± 6
21	1:5	20	20	5.0	4.7 ± 0	0.59 ± 0.10	1.1 ± 0	8.2 ± 0	1.7 ± 0.4	353 ± 5
22	1:5	10	20	5.0	4.7 ± 0	1.06 ± 0.03	1.3 ± 0	11.1 ± 0.1	2.6 ± 0	390 ± 6
23	1:3	10	40	7.0	6.8 ± 0	1.04 ± 0.03	1.3 ± 0	10.8 ± 0	1.7 ± 0.4	436 ± 6
24	1:5	20	20	7.0	6.7 ± 0	0.59 ± 0.03	1.1 ± 0	8.1 ± 0	1.7 ± 0.4	349 ± 5
25	1:3	10	40	5.0	4.8 ± 0	1.06 ± 0.03	1.3 ± 0	10.9 ± 0	1.7 ± 0.4	436 ± 2
26	1:4	15	30	6.0	5.7 ± 0.2	0.59 ± 0.10	1.2 ± 0	9.5 ± 0.3	1.7 ± 0.4	389 ± 3
27	1:4	15	30	6.0	5.7 ± 0	0.50 ± 0.03	1.2 ± 0	9.7 ± 0	1.7 ± 0	398 ± 4
28	1:4	15	30	6.0	5.7 ± 0	0.50 ± 0.09	1.2 ± 0	9.6 ± 0.3	2.1 ± 0.4	382 ± 4
29	1:2	15	30	6.0	5.8 ± 0	0.76 ± 0.03	1.3 ± 0	10.1 ± 0.1	2.1 ± 0.4	431 ± 5
30	1:3	10	20	5.0	4.7 ± 0	1.10 ± 0.03	1.3 ± 0	11.2 ± 0	2.6 ± 0	380 ± 4

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content.

Table 18. Antinutritional factors, pH, titratable acidity and microbial load in mixed dhokla batter, fermented as per central composite design

Run	Fermentation condition			Antinutritional factor (per gram dry wt)*					Final pH†	TA (%)*	Microbial load (Log cfu/g)*		
	Added salt (g/kg)	t (h)	T (°C)	TC (mg)	PAC (mg)	TIA (kU)	HA (kU)	TBAC (µg)			TAMB	LAB	Yeasts
1	1.6	20	31.5	0.10 ± 0.03	0.33 ± 0.02	2.2 ± 0.3	0.19 ± 0.02	411 ± 2	4.8 ± 0	0.06 ± 0.01	7.2 ± 0	8.2 ± 0	5.2 ± 0
2	18.4	20	31.5	0.12 ± 0	0.34 ± 0.02	3.1 ± 0	0.16 ± 0.01	336 ± 2	4.9 ± 0	0.05 ± 0	7.1 ± 0	8.1 ± 0	4.9 ± 0
3	10	20	42.4	0.06 ± 0.01	0.30 ± 0	2.9 ± 0	0.09 ± 0.01	391 ± 2	4.3 ± 0	0.08 ± 0	7.2 ± 0	8.1 ± 0	4.8 ± 0
4	5	30	38	0.06 ± 0.01	0.25 ± 0	1.9 ± 0	0.06 ± 0	501 ± 3	4.4 ± 0	0.08 ± 0	7.1 ± 0	8.3 ± 0.1	5.1 ± 0
5	15	30	25	0.09 ± 0	0.29 ± 0	2.1 ± 0.1	0.10 ± 0	406 ± 2	4.5 ± 0	0.05 ± 0.02	7.2 ± 0	8.2 ± 0	5.1 ± 0
6	10	20	31.5	0.12 ± 0.03	0.42 ± 0.03	3.1 ± 0.1	0.26 ± 0.02	363 ± 3	4.9 ± 0	0.06 ± 0.02	7.2 ± 0	8.1 ± 0	5.1 ± 0
7	10	3.2	31.5	0.29 ± 0	1.00 ± 0	5.0 ± 0	0.90 ± 0.03	273 ± 3	5.6 ± 0	0.04 ± 0.01	6.1 ± 0	6.1 ± 0	3.1 ± 0
8	10	36.8	31.5	0.03 ± 0	0.23 ± 0	1.9 ± 0	0.06 ± 0	533 ± 6	3.1 ± 0	0.16 ± 0.01	7.4 ± 0	8.4 ± 0.1	5.3 ± 0.1
9	10	20	31.5	0.12 ± 0.03	0.43 ± 0.01	2.3 ± 0	0.19 ± 0.01	354 ± 6	4.9 ± 0	0.06 ± 0	7.2 ± 0	8.1 ± 0	5.1 ± 0
10	10	20	31.5	0.09 ± 0.03	0.34 ± 0.01	2.3 ± 0.3	0.18 ± 0.01	363 ± 5	4.9 ± 0	0.07 ± 0	7.2 ± 0	8.1 ± 0	5.1 ± 0
11	15	10	25	0.19 ± 0	0.44 ± 0.01	3.4 ± 0.2	0.34 ± 0.02	232 ± 7	4.9 ± 0	0.05 ± 0	6.9 ± 0	8.1 ± 0	4.8 ± 0
12	10	20	31.5	0.19 ± 0	0.30 ± 0	3.1 ± 0	0.18 ± 0.01	356 ± 2	4.9 ± 0	0.06 ± 0	7.2 ±0	8.1 ± 0	5.1 ± 0
13	10	20	20.6	0.19 ± 0.02	0.43 ± 0	3.4 ± 0	0.08 ± 0	316 ± 2	5.1 ± 0	0.05 ± 0	7.2 ± 0	8.1 ± 0	5.2 ± 0
14	10	20	31.5	0.10 ± 0	0.44 ± 0	2.3 ± 0	0.27 ± 0.02	356 ± 6	4.9 ± 0	0.07 ± 0	7.2 ± 0	8.2 ± 0	5.1 ± 0
15	5	10	25	0.19 ± 0	0.42 ± 0.04	3.6 ± 0.1	0.43 ± 0.03	319 ± 4	4.8 ± 0	0.06 ± 0	6.6 ± 0	8.2 ± 0	5.1 ± 0.1
16	15	30	38	0.06 ± 0.01	0.26 ± 0.03	1.9 ± 0.1	0.08 ± 0	483 ± 6	4.5 ± 0	0.07 ± 0	7.2 ± 0	8.2 ± 0	5.1 ± 0
17	15	10	38	0.12 ± 0	0.40 ± 0.04	2.8 ± 0.1	0.42 ± 0.03	280 ± 4	4.9 ± 0	0.05 ± 0	6.9 ± 0	8.2 ± 0	5.0 ± 0
18	10	20	31.5	0.10 ± 0	0.26 ± 0	3.1 ± 0	0.23 ± 0.02	357 ± 2	4.9 ± 0	0.07 ± 0.01	7.2 ± 0	8.1 ± 0	5.2 ± 0
19	5	30	25	0.09 ± 0.01	0.28 ± 0	2.1 ± 0	0.09 ± 0	460 ± 2	4.5 ± 0	0.05 ± 0	7.1 ± 0	8.4 ± 0	5.2 ± 0
20	5	10	38	0.12 ± 0.03	0.40 ± 0.02	2.0 ± 0.2	0.44 ± 0.03	327 ± 3	4.8 ± 0	0.06 ± 0	7.0 ± 0	8.2 ± 0	5.1 ± 0
Unf. †,§									5.6^{a} ± 0	0.04^{b} ± 0.01	6.1^{b} ± 0	6.0^{b} ± 0	3.1^{b} ± 0
Opt. ‡,§									4.7^{b} ± 0	0.10^{a} ± 0.01	7.2^{a} ± 0	8.3^{a} ± 0	5.1^{a} ± 0

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content; TA, titratable acidity (as lactic acid); TAMB, total aerobic mesophilic bacteria; LAB, lactic acid bacteria.

†Unfermented mixed batter prepared form optimally soaked dal and rice (3:1 v/v).

‡Batter, fermented under optimum condition (added salt, 8 g/kg; fermentation temp., 32°C; fermentation time, 18 h).

§Means, 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.

3.2.6.2.3.2. Mixing of batters

The optimally soaked dal and rice were ground, mixed in different ratios of dal batter-rice slurry (4:1, 3:1, 2:1, 1:1, 1:2, 1:3 and 1:4 v/v) with added common salt (8 g/kg batter), fermented at 32°C for 15 h and steamed for 15 min (Moktan et al., 2011). The prepared dhokla was subjected to sensory analysis (Table 4). Dhokla with highest sensory score was selected, and the selected ratio of unfermented mixed batter was used for optimization of the fermentation stage parameters.

3.2.6.2.3.3. Fermentation

For the optimization of fermentation stage parameters, three processing variables, namely added common salt (g/kg), fermentation time (h) and fermentation temperature (°C) were selected. The fermentation stage consisted of 20 experimental runs with eight factorial points, six axial points and six replicates at the centre points (Table 18). After performing the experiment, predicted optimum condition was selected. In addition, pH, acidity and microbial load in unfermented mixed batter and fermented batter according to CCRD were evaluated to study their effects on the levels of antinutritional factors and sensory attributes of prepared dhokla.

3.2.6.2.3.4. Steaming

The optimally fermented batter was used for the optimization of steaming stage with steaming time (min) as a single independent variable. The design comprised of seven experimental runs (Table 19). The fermented batter was dispensed in a dhokla steamer and steamed according to the CCRD. Steamed product

Table 19. Antinutritional factors in fermented batter, steamed as per central composite design

Run	Steaming time (min)	Antinutritional factor (per gram dry wt)*				
		TC (mg)†	PAC (mg)	TIA (kU)	HA (kU)†	TBAC (µg)
1	20	<dl	0.09 ± 0	1.1 ± 0	<dl	267 ± 1
2	15	<dl	0.14 ± 0	1.1 ± 0	<dl	295 ± 1
3	12.5	<dl	0.16 ± 0	1.1 ± 0	<dl	322 ± 2
4	20	<dl	0.09 ± 0	1.1 ± 0	<dl	267 ± 2
5	17.5	<dl	0.10 ± 0	1.1 ± 0	<dl	285 ± 2
6	10	<dl	0.17 ± 0	1.2 ± 0	<dl	354 ± 3
7	10	<dl	0.17 ± 0	1.2 ± 0	<dl	354 ± 2

*Values, showing mean ± *SE*, were obtained from triplicate sets. TC, tannins content; PAC, phytic acid content; TIA, trypsin inhibitor activity; HA, haemagglutinating activity; TBAC, total biogenic amines content.
†dl (detection limit), 0.003 mg/g dry wt for TC and 0.007 kU/g dry wt for HA.

sample was prepared and optimum condition was determined by following the procedure of numerical optimization. The prepared dhokla from each experimental run of the fermentation stage parameters and optimized steaming time were subjected to sensory analysis. Dhokla was evaluated by a panel of 10 trained judges, using a 100-point score card.

The flow sheet for the production of dhokla has been shown, indicating the sites of sampling for antinutrients assay (Fig. 10).

3.2.7. Statistical analysis

Regression analysis and analysis of variance (ANOVA) were conducted for fitting the models and to examine the statistical significance of the model terms using second order polynomial equation:

$$Y = \beta_0 + \beta_A A + \beta_B B + \beta_C C + \beta_D D + \beta_{AA} A^2 + \beta_{BB} B^2 + \beta_{CC} C^2 + \beta_{DD} D^2 + \beta_{AB} AB + \beta_{AC} AC + \beta_{AD} AD + \beta_{BC} BC + \beta_{BD} BD + \beta_{BC} BC$$

where, Y was the response, i.e. antinutritional factors (tannins content, phytic acid content, trypsin inhibitor activity, haemagglutinating activity, total biogenic amines content and oligosaccharides), and A, B,

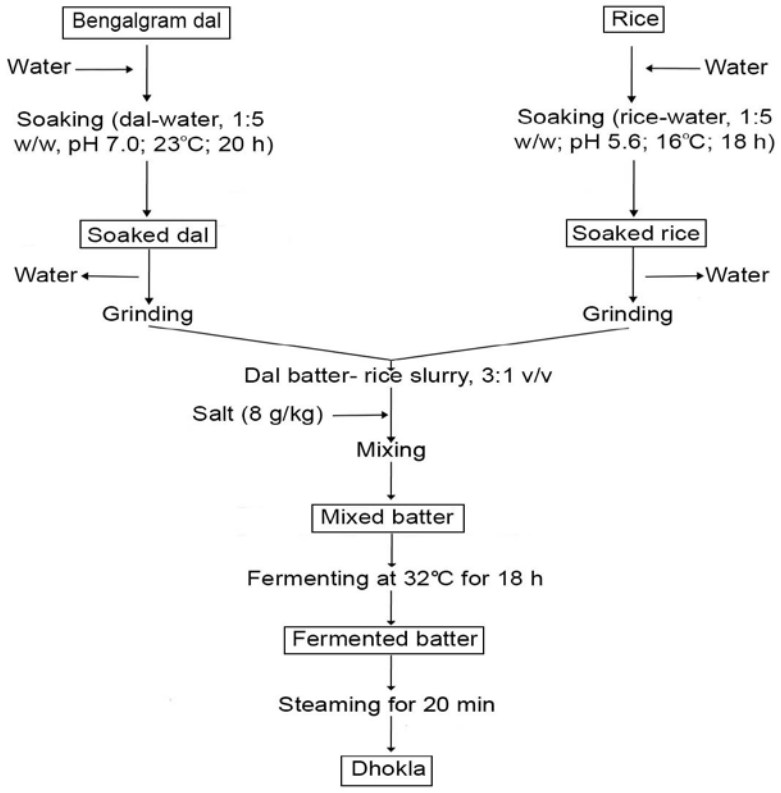

Fig. 10. Flow sheet for the production of dhokla. Boxed texts are the sites of sampling for antinutrients assay.

represented regression coefficients for the linear effect terms, β_{AA}, β_{BB}, β_{CC} and β_{DD} represented quadratic effect terms, and β_{AB}, β_{AC}, β_{AD}, β_{BC}, β_{BD} and β_{BC} were interaction effect terms. The regression coefficient produced by the software and the term combination of independent variables were selected and significance of the model was determined from the *P*-value given by the software. The insignificant (P >0.05) terms were removed and a reduced equation for optimizing the processing variables was drawn. The adequacy of the models was determined using R^2 (coefficient of determination) and lack of fit test. Non-significant lack of fit was good, which means that the model was adequate in describing the response. R^2 was defined as the ratio of the explained variation to the total variation and was a measure of the degree of fit. Higher R^2 signified good fit for the predicted model. The significance of the linear, quadratic and interaction effects of independent variables on response was determined at 5% level of confidence. After model fitting, the relation between independent and response variables was studied using 3D response surface plots.