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Optimization of fermenting parameters for idli batter

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Abstract
The work focused on the development of idli batter by reducing the conventional fermentation time period of 24h, utilizing the lesser known crops of Uttarakhand**.** Cereals and legume based foods are a major source of economical dietary energy and nutrients, worldwide. One of the popular among them is idli which is widely consumed as a snack food in India. For the preparation of idli batter, variables were taken for the experiments as blend ratio (3:1:0.5, 3:1:1, 3:1:1.5), alpha amylase (5, 10, 15U) and starter culture ratio (1:0, 0:1, 1:1). Designed experiments were conducted randomly to find the effect of these variables on pH, titrable acidity and batter volume. For development of acceptable quality of idli batter, the best suitable fermentation condition of predicted variables were, blend ratio 3:1:0.5, alpha amylase 15U, and starter culture 0.05:1.95 and the highest leavening action of idli batter (350 ml) was obtained at temperature 30°C using 9 h of fermentation.

Keywords: Starter culture, idli batter, fermented food, pH and alpha amylase.

1. Introduction

The production of fermented foods is one of the oldest food processing technologies known to man. The diversity of the population of India has given rise to a large number of traditional fermented foods which have been extensively reviewed (Soni and Sandhu 1990, Achaya 1994) [12, 1].

Cereals are used world-wide as staple food, they are considered to be one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for people all over the India. Most commonly cereals are utilized in combination with legumes to improve the overall protein quality of the fermented product. This combination can be replaced by the small seeded grains that are known as millets in breakfast food, convenience foods, and snack foods. The total production of millet grains of world was 762712 metric tons (FAO 2012) [6].

In Uttarakhand, underutilized crops like millets are grown in huge amount, so these crops can be utilized to formulate a fermented food product by applying processing technique "Fermentation", most simple and economical way of improving the nutritional value, sensory and textural properties and functional qualities of final product. Barnyard millet (*Echinochloautilis*) locally known as jhangora is a fair source of protein, which is highly digestible and is an excellent source of dietary fiber. The carbohydrate content is low and slowly digestible. It has recorded highest protein content (15.07%) (Veena *et al*. 2005) [16]. Black gram dhal (*Phaseolusmungo*) commonly known as urd is rich in vitamins, minerals and devoid of cholesterol. It is store house of calcium (154 mg), potassium, iron (4 mg) and protein (24 g) (Hulse *et al*. 1980) [7]. Black soybean (*Glycine max*) locally known as bhat in kumaon region of Uttarakhand, it is rich source of protein, fiber and iron (36.5 g, 9.3g, 15.7 mg per 100 g) (Mukherjee *et al*. 1965) [8].

Due to the presence of anti-nutritional factors such as phytic acid and polyphenols which form complex with divalent cations, the availability of the nutrients from the millet to humans is restricted (Yee 2000, Tuoying 2011) [17, 15]. Starch and protein digestibility also considerably get reduced as a result of the activity of phytic acid (Soetan and Oyewole 2009; Arlete *et al*. 2004) [13, 3]. This could be overcome by fermentation with pure cultures of yeast and lactobacilli. Fermentation enhances the protein and starch digestibility and also increases the bio-availability of minerals by cutting down the activity of phytic acid and polyphenols (Babalola and Giwa 2012) [5].

In recent years attempts have been made by several research workers to obtain idli like products by replacing traditionally used rice and black gram with different substitutes. However the use of the barnyard millet and black soybean in combination in the preparation of idli has not been reported. The value addition of these crops is required, because commonly the local people of Uttarakhand used to make local dishes like bhat ki churkaani, dubke and from barnyard millet khicchadi, laddu, chapati etc which are popularly famous in kumaon region and they are not aware of the possibilities of different value added products like idli, dosa, halwa, weaning food and many more can be formulated by using these crops. Therefore an attempt to focus an attention on the use of underutilized crops like barnyard millet and black soybean as an alternative to rice and black gram has been made in preparation of idli. The conventional fermentation time from 14 to 24 hour to prepare a fermented food product; idli is a time consuming process mostly preferred with overnight fermentation. Therefore, in view of reduction of fermentation time and maximization of underutilized and vegetable protein sources for human food, the current interest will be directed towards the reduction of conventional fermentation time period by using an external source, and the use of barnyard millet and soybean as a cheaper source of protein in preparation of fermented food, idli.

Response surface methodology is a collection of statistical and mathematical techniques useful for developing, improving, optimizing processes and achieving the optimum conditions for desirable responses with a limited number of planned

experiments, it also provides a mathematical model, which describes the relationships between the independent and dependent variables (Myers and Montgomery 1995) [9]. The response surface design was used in this study to: 1) determine how pH, titrable acidity and batter volume (as responses) are affected by changes in the level of blend ratio, alpha amylase enzyme, and starter culture ratio, 2) determine the optimum combination of blend ratio, alpha amylase enzyme, and starter culture ratio.

2. Materials and Methods

For the preparation of idli batter, fresh raw grains i.e. Barnyard millet (*Echinochloa frumentacea*), Black gram (*Phaseolus mungo)* and Black soybean (*Glycine max)* were procured from the local market of Haldwani, District Nainital. Alpha amylase enzyme was procured from Hi-media Pvt. Ltd. India, Udham Singh Nagar. Strains of *Lactobacillus plantarum* (MTCC 6160) *and Saccharomyces cerevisiae* (MTCC 4794) were purchased from the Microbial Type Test Culture in Chandigarh (Punjab), India.

2.1. Experimental design

Experimental design, defined as a specific set of experiments which are defined by a matrix, a matrix composed by the different level of combination variables. Response surface methodology was used to design and analysis of all experiments for three independent variables at three levels. It also helps to reduce the number of experiments without affecting the accuracy of results and to decide the interactive effects of independent variables on the response. Box-Behnken model was selected for the optimization of the process variables (Khuri and Cornell 1987). Box-Behnken is a class of rotatable second order design based on three levels incomplete factorial design. This design does not contain for which all factors are simultaneously at their highest and lowest levels. So this design is useful in avoiding experiments performed under extreme conditions for which unsatisfactory results might occur (Bezerra *et al*. 2008) [4]. Total numbers of experiments were found seventeen shown in Table1. The number of experiments (N) required for development of Box-Behnken Design.

$$N=2K\,(K-1) + C_0 \qquad (1)$$

Where, N= Total no. experiments
K= No. of variables
C_0= Centre point`

In order to determine a critical point (maximum, minimum or saddle) it is necessary for the polynomial function to contain the quadratic terms. According to the equation presented below

$$y = \beta_0+\sum_{i=1}^{K}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii}Xi^2+\sum_{1\le i\le j}^{k}\beta_{ij}X_iX_j \qquad (2)$$

Where, β_{ii}, β_0 and β_{ij} are the coefficients of regression. (i= 1, 2….n) (j= 1, 2….n)

Table 1. Experimental value of the variables

	Coded	values		Actual	values	
Expt No	**X_1**	**X_2**	**X_3**	**Blend ratio**	**Alpha amylase conc.**	**Starter culture ratio**
1	-1.00	-1.00	0.00	3:1:0.5	5	0:1
2	1.00	-1.00	0.00	3:1:1.5	5	0:1
3	-1.00	1.00	0.00	3:1:0.5	15	0:1
4	1.00	1.00	0.00	3:1:1.5	15	0:1
5	-1.00	0.00	-1.00	3:1:0.5	10	1:0
6	1.00	0.00	-1.00	3:1:1.5	10	1:0
7	-1.00	0.00	1.00	3:1:0.5	10	1:1
8	1.00	0.00	1.00	3:1:1.5	10	1:1
9	0.00	-1.00	-1.00	3:1:1	5	1:0
10	0.00	1.00	-1.00	3:1:1	15	1:0
11	0.00	-1.00	1.00	3:1:1	5	1:1
12	0.00	1.00	1.00	3:1:1	15	1:1
13	0.00	0.00	0.00	3:1:1	10	0:1
14	0.00	0.00	0.00	3:1:1	10	0:1
15	0.00	0.00	0.00	3:1:1	10	0:1
16	0.00	0.00	0.00	3:1:1	10	0:1
17	0.00	0.00	0.00	3:1:1	10	0:1

2.2. Procedure for preparation of idli batter

Soaked grains were then wet grinded separately with dehulled black gram dhal and black soybean into a fine paste and barnyard to a coarse consistency in a mixer grinder with 2-3 ml addition of water (grinded up to 2 min and 5 min to get a coarse and fine paste respectively). With the seventeen (experimental design) different blend ratio batter samples were prepared taking, three levels for blend ratio {BM:BG:BS (3:1:0.5, 3:1:1, 3:1:1.5)}, The preparation of batter was followed by addition of 0.9% w/w salt, further the calculated amount of alpha amylase enzyme powder was added to obtain i.e. 5, 10, 15U activity per 100g of batter respectively and three levels of alpha amylase enzyme powder were (5U, 10U, 15U) and finally two different starter cultures (Probiotic strains) were used to prepare idli batter samples in different combination of LAB (*Lactobacillus plantarum*) and yeast (*Saccharomyces cerevisiae*) were added in hygienic conditions under the laminar air flow. Idli batter prepared using the traditional method (without any added culture and amylase enzyme) was used as a control. Optimization was done using RSM box behnken technique on the basis of dependent parameters. Further with the optimized set of independent parameters, the experiment was conducted and all the fermentation conditions were checked.

2.3. Physico-chemical analysis

2.3.1. pH: The pH values of different sets of batters were measured after 9 h of fermentation using a digital Triode India pH meter (Steinkraus *et al*. 1967) [11].

2.3.2. Titrable acidity: The acidity produced during fermentation was measured by titrimetric method. Five gram of material/batter was dissolved in 10 ml distilled water and titrated against 0.1N NaOH using phenolphthalein as indicator (AOAC 1984).

2.3.3. Batter volume: The prepared batter sample was placed in 500 ml measuring cylinders up to the mark of 100 ml, covered with the aluminium foil and kept at 30°C to observe the rise in the volume during fermentation (Sridevi *et al*. 2010) [10]. Initial and final readings for the leavening action of batter were noted.

3. Results and discussion

3.1. Results of preliminary trials

Few preliminary trials were conducted in the month of October at ambient conditions to set the value of levels for the predicted variables. Results of prelim experiments for natural fermentation i.e control sample (A), the value of pH was found 4.85 before incubating the batter, and after 24 h it was 4.42 and batter volume obtained (200ml). While in the second induced batter (B) which was inoculated with starters (*Lactobacillus plantarum and Saccharomyces cerevisiae*) and alpha amylase with (15U) was added to make idli batter. It was seen that the pH dropped up to the value of (3.3) within 9-10 h of fermentation and batter volume was obtained (300ml). Hence based on this experimental trial, it was concluded that inoculation of idli batter with starter cultures (*Lactobacillus plantarum and Saccharomyces cerevisiae*) (0:1) and addition of alpha amylase enzyme aided in reducing the conventional fermentation time period (24 h).

Table 2. Results of responses for fermented idli batter

Expt. No	Coded levels			pH	Titratable acidity (%)	Batter volume (ml)
	Blend ratio (X_1)	Alpha amylase (X_2)	Starter culture ratio (X_3)			
1	-1.00	-1.00	0.00	3.7	1.345	220
2	1.00	-1.00	0.00	3.72	1.022	290
3	-1.00	1.00	0.00	3.2	2.011	340
4	1.00	1.00	0.00	3.1*	2.211**	350**
5	-1.00	0.00	-1.00	4.5	0.511	120
6	1.00	0.00	-1.00	4.54	0.621	120
7	-1.00	0.00	1.00	4	0.921	150
8	1.00	0.00	1.00	4.1	0.91	140
9	0.00	-1.00	-1.00	4.6**	0.401*	100*
10	0.00	1.00	-1.00	4.3	0.9	170
11	0.00	-1.00	1.00	4.3	0.625	150
12	0.00	1.00	1.00	3.9	1.211	200
13	0.00	0.00	0.00	3.5	1.33	270
14	0.00	0.00	0.00	3.45	1.441	300
15	0.00	0.00	0.00	3.52	1.4	290
16	0.00	0.00	0.00	3.49	1.411	270
17	0.00	0.00	0.00	3.5	1.321	240

*Minimum value and **Maximum value

3.2. Effect of variables on pH

Experimental data in Table 2 shows that in case of fermented idli batter, the pH ranged from 4.6 to 3.1. Maximum pH was found 4.6 for experiment no. 9, having blend ratio 3:1:1, alpha amylase unit 5U and starter culture ratio 1:0 (*saccharomyces*: *LAB*). Minimum pH was found 3.1 for the samples having blend ratio 3:1:1.5, alpha amylase unit 15U and starter culture ratio 0:1 (*saccharomyces*: *LAB*). Due to high unit of alpha amylase pH got decreased during fermentation. Addition of alpha amylase enzyme in varied concentrations aided in decreasing the pH of a fermented idli batter. The statistical analysis of pH was given in Table 3. The model of pH was found highly significant (P<0.01) because it had higher F-value (80). It was also observed that the effect of variables on pH was highest at quadratic level due to highest calculated F-value (188) followed by linear level and no effects on pH was observed at interactive level it means that higher the residual error.

Table 3. Analysis of variance for pH

SOURCE	DF	SS	MS	F-value
Model	9	3.6	0.4	80***
Linear	3	0.75	0.25	50***
Quadratic	3	2.82	0.94	188***
Interactive	3	0.007	0.002	0.4
Residual error	7	0.037	0.005	
Lack of fit	3	0.035	0.011	
Total	16	3.367	0.405	

***, **,* indicates significant at 1, 5 and 10 % level of significance

- At 1% $F_{tab}(9, 7)=6.71$, $F_{tab}(3, 7)=8.45$,
- At 5% $F_{tab}(9, 7)=3.67$, $F_{tab}(3, 7)=4.34$
- At 10% $F_{tab}(9, 7)=2.72$, F_{tab} $(3, 7)=3.07$

The pH of fermented idli batter sample significantly decreased from 4.3 gradually to 3.3 with the increase in the value of level of starter culture ratio at centre point (0.00) (0:1*Saccharomyces cerevisiae*: *Lactobacillus plantarum*) after that increased the ratio of starter culture, increased the pH from 3.25 to 3.7 at optimum value of blend ratio (3:1:0.5) and alpha amylase enzyme (15U) showed in Fig 1. It was due to the increase growth of yeast (*Saccharomyces cerevisiae*) which is helpful for the formation of alcoholic compounds.

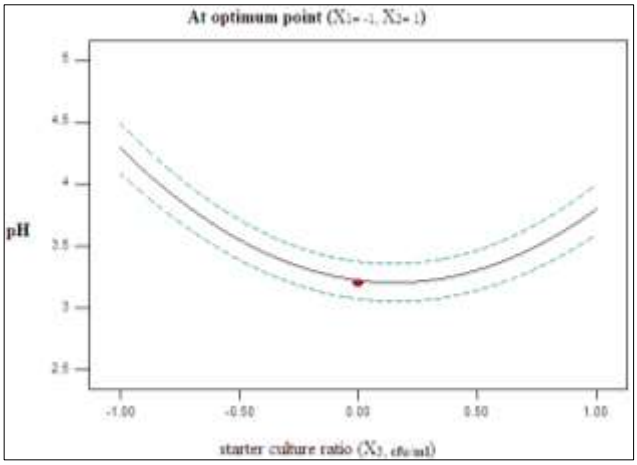

Fig 1. Variation of pH with starter culture ratio

3.3. Effect of variables on titratable acidity

Data tabulated in Table 2 shows that titratable acidity ranged from 0.401 to 2.211%. Maximum acidity was found 2.211% for experiment no.4, having blend ratio 3:1:1.5, alpha amylase unit 15U and starter culture ratio 0:1 (*saccharomyces*: *LAB*). Minimum acidity was observed 0.401% for experiment no.9 having combination of 3:1:1, alpha amylase unit 5U and starter culture ratio 1:0 (*saccharomyces*: *LAB*). Since alpha amylase unit (15) was high and it effects the pH to drop during the fermentation. Different combination of cultures provided an acidic medium to enhance the growth of microbes and hence pH decreases and acidity increases.

The statistical analysis of titratable acidity was given in Table 4. The model of titratable acidity was found highly significant (P<0.01) because it had higher F-value (31.1). It was also observed that the effect of predicted variables on titratable acidity was highest at quadratic level due to highest calculated F-value (62.2) followed by linear level and no effects on titratable acidity was observed at interactive level it means that higher the residual error.

Table 4. Analysis of variance for titratable acidity

SOURCE	DF	SS	MS	F-value
Model	9	3.8	0.42	31.1***
Linear	3	1.2	0.4	29.6***
Quadratic	3	2.53	0.84	62.2***
Interactive	3	0.07	0.02	1.4
Residual error	7	0.094	0.013	
Lack of fit	3	0.083	0.027	
Total	16	3.894	0.433	

***, **, * indicates significant at 1, 5 and 10 % level of significance respectively

The total acidity of fermented idli batter increased with the increase in level of unit of alpha amylase at optimum values of blend ratio (3:1:0.5) and starter culture ratio i.e. *Saccharomyces*: *Lactobacillus plantarum* (0.05:1.95). It was found that acidity increased from 1.3% to 1.8% and was highest at these point (1.00) (15U) shown in Fig.2.

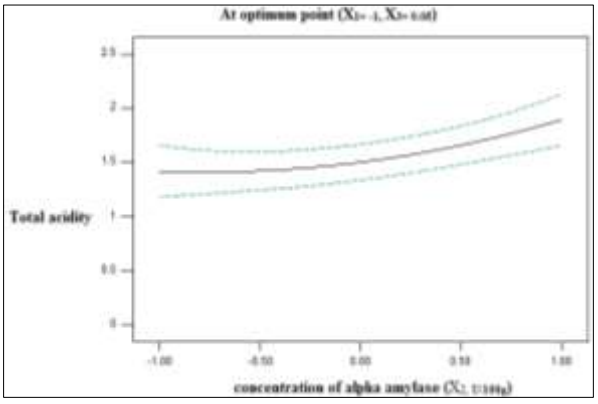

Fig 2. Effect of alpha amylase on total acidity

3.4. Effect of variables on batter volume

Experimental data for batter volume were reported in Table 2. In case of fermented idli batter samples, the increase in batter volume in ml ranged from 100 to 350 ml. Maximum volume make up (350 ml) was found in case of experiment no. 4, having blend ratio 3:1:1.5, alpha amylase unit 15U and starter culture ratio 0:1 (saccharomyces: LAB). Minimum volume make up (100 ml) was found for the samples having blend ratio 3:1:1, alpha amylase unit 5U and starter culture ratio 1:0 (saccharomyces: LAB). Since alpha amylase unit (15) was high and therefore it effected the pH to drop during the fermentation and eventually it helped microorganisms to grow faster in the batter, and produced the leavening action of batter due to which volume of batter raised. The statistical analysis of batter volume was given in Table 5. The model of batter volume was found highly significant (P<0.01) because it had higher F-value (21.6). It was also observed that the effect of variables on batter volume was highest at quadratic level due to highest calculated F-value (56.1) followed by linear level and no effects on batter volume was observed at interactive level it means that higher the residual error.

Table 5. Analysis of variance for batter volume

SOURCE	DF	SS	MS	F-Value
Model	9	102681.5	11409.05	21.6***
Linear	3	13975	4658.3	8.83**
Quadratic	3	88891.2	29630.4	56.1***
Interactive	3	1025	341.6	0.65
Residual error	7	3695	527.857	
Lack of fit	3	1575	525	
Total	16	210267.7	46567.2	

The batter volume at linear level, increased from 240 ml to 343 ml with the increase in unit of alpha amylase at the optimum point for blend ratio (3:1:0.5) and the starter culture ratio (0.05) from Fig.3. Rise in batter volume appears from the centre point 0.00 (10) and then it significantly rises in volume with the maximum value at 1.00 (15) level of variable. From Fig 4, It was observed that batter volume increases with the increase in starter culture ratio from -1.00 (1:0 *saccharomyces cerevisiae*) to 0.00 (0:1 *Lactobacillus plantarum*) point. It was due to the LAB, prominent in the batter and responsible for the leavening action of batter. After centre point the gas which was entrapped in the pores of batter starts to escape then suddenly the volume decreases with the increase in the value of the level of starter culture ratio.

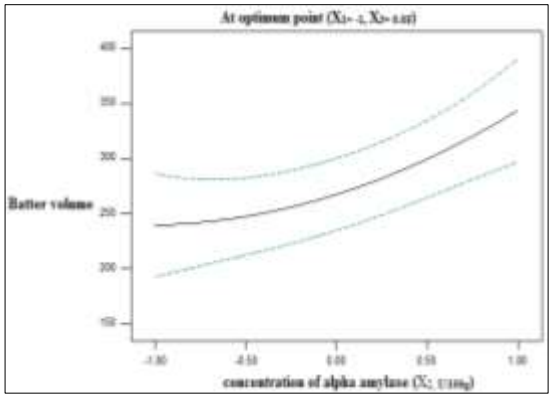

Fig 3. Effect of unit of alpha amylase on batter volume

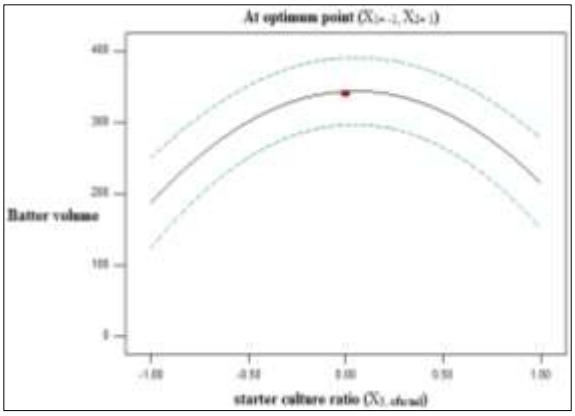

Fig 4. Effect of starter culture ratio on batter volume

3.5. Compromise optimization of response

To get optimum variables, the numerical optimization was carried out using Design-Expert 8.0.7.1 statistical software. The goal was fixed in range for blend ratio, unit of alpha amylase and starter culture ratio, while among the responses pH, titratable acidity was taken in range and batter volume was taken maximum. The goal seeking begins at a random starting point and proceeds up and down the steepest slope on the response surface for maximum and minimum value of the response respectively. All responses and variables were given similar (+++) importance. The optimized values of the variable for making idli batter are given in Table 6 and idli batter at optimized values of fermenting variables was shown in Plate 1.

Table 6. Optimum value of variables for idli batter to reduce fermentation time

Predicted variables	Coded level	Actual level
Blend ratio (X_1)	-1	3:1:0.5
Unit of alpha amylase (X_2, U/100 gm)	1	15
Starter culture ratio (X_3,cfu/ml)	0.05	0.05:1.95

Plate 1. Idli batter at optimized conditions

4. Conclusions

On the basis of experimental and analytical data, it was concluded that the use of appropriate quantity of blend ratio (3:1:0.5), alpha amylase concentration (15U) and starter culture ratio (0.05:1.95), the best quality idli batter was prepared within 9h. The use of culture could help to extend the shelf-life of the batter and product can be developed in the ready to eat form

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