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Effect of Fermentation Parameters to Reduce Fermentation Time for making Idli Batter

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ABSTRACT

Fermentation of natural foods as a method of improving the digestibility and nutritional quality has been known from the earliest times. Fermented foods help in eliminating protein calorie malnutrition and other nutritional deficiencies in the developing countries. Cereals and legumes have been commonly used for the preparation of fermented food products like idli. Even these legumes can be substituted with the millets of Uttarakhand like barnyard millet and black soybean. The investigations were carried out to develop standard process and reduce fermentation time for idli batter using fermentation technique. For the preparation of idli batter, the predicted 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). The pH of the batter decreased by increasing the level of alpha amylase while titratable acidity increased. 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: Fermentation, alpha amylase, starter culture, fermented food, pH and idli batter.

Introduction

Millet is one of the most important drought-resistant crops and the 6th cereal crop in terms of world agriculture production. Also, millet has resistance to pests and diseases, short growing season, and productivity under drought conditions, compared to major cereals. The 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).**

In the current scenario of price rise, there is strong need for using low-cost vegetable sources of protein in the world economy. This has changed the focus on vegetable proteins like soy and millets in food formulations. Soybean and millet have great potential for use as human food because of their high level of good quality protein and their unique functional and nutritional properties. One of the most promising uses of soybean is the fortification of cereal based products, because the profile in essential amino acids in soy is complementary to that in most cereals. To seek alternative use of protein other than animal foods, an attempt has been made to utilize these crops, for human food consumption as in the preparation of traditional fermented food such as idli, a fermented steamed low calorie product with a soft and spongy texture. In the traditional idli batter, fermentation takes place due to the micro flora present in the raw materials and in the environment leading to the several changes that has an impact on digestibility and nutritional value bringing about desirable changes **(Soni and Sandhu, 1989).**
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. The three predicted variables which influences the fermentation parameters; Blend ratio, alpha amylase enzyme, and starter culture ratio. The statistical method using response surface methodology (RSM) has been proposed to determine the influences of predicted variables and the influence of their interactions. RSM 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 **(Meyers and Montgomery, 1995).** The response surface design was used in this study to: 1) determine how pH and titrable acidity (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.

Materials and Methods

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. 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 from Chandigarh.

Fresh grains were cleaned, sorted and soaked separately in water for 8h. These were then ground separately with dehulled black gram dhal and black soybean into a fine paste and barnyard to a coarse consistency. The preparation of batter was followed by addition of 0.9% w/w salt, levels of alpha amylase enzyme and two different starter cultures were used to prepare idli batter samples in different combination of LAB (*Lactobacillus plantarum*) and yeast (*Saccharomyces cerevisiae*). Samples were allowed to ferment for 9 h at 30°C. The batter samples were analysed for pH and titrable acidity as per method described by **(AOAC, 1984).**

Experimental design

Response surface methodology (RSM) was used for the design and analysis of all experiments for three predicted 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 influencing factors on the response. 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 by **(Bezerra et al, 2008.).**

Table 1 Levels of predicted variables

Predicted variables	Range		
	-1	0	1
Blend ratio (BM:BG:BS) (X_1)	3:1:0.5:0.5,	3:1:1:1,	3:1:1.5:1.5
Alpha–amylase enzyme concentration (X_2) (U/100g)	5	10	15
Starter culture ratio (X_3)	1:0	0:1	1:1

Based on literatures and preliminary experiments conducted the level chosen for blend ratio, X_1 alpha amylase enzyme, X_2 and starter culture ratio,X_3 were X_1: (BM:BG:BS) 3:1:0.5, 3:1:1, and 3:1:1.5; X_2: 5.10 and 15U and X_3: 1:0, 0:1 and 1:1 for preparing idli batter. Once the desired value ranges of the predicted variables had been defined, they were coded as =1 for the factorial points, 0 for the center points. The ranges of variables in coded form for the design of experiments are shown in Table 1.

Analysis of idli batter

pH

The pH of different sets of batters was determined after 9 h of fermentation **(Steinkraus et al, 1967).**

Titrable acidity

The acidity produced during fermentation was measured by titrimetric method **(AOAC, 1984)**. Five gram of batter was dissolved in 10 ml distilled water and titrated against 0.1N NaOH using phenolphthalein as indicator Titrable acidity was calculated by the following formula

$$\text{Percent lactic acid/g} = \frac{\text{ml of 0.1N NaOH} \times 0.009}{\text{Weight of sample (g)}} \times 100 \quad (1)$$

Second order polynomial model

Statistical analysis was conducted to see the effect of predicted variables i.e. blend ratio, alpha amylase, starter culture ratio on various responses considered during the study **(Khuri and Cornell, 1987).** A full second order model was fitted into each response, according to the equation presented below

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

Where, Y is the predicted response (pH and titrable acidity) used as a dependent variable; k the number of predicted variables, *xi* (*i* = 1, 2) the input predictors or controlling variables (factors); β_0 the constant coefficient of regression, and β_i, β_{ij}, β_{ii} the coefficients of regression of linear, interaction and quadratic terms, respectively. Multiple linear regression analysis was used for determining the coefficient parameters by employing the software Design-Expert (version 8). Design-Expert was also used to find the 2-D contour plots of the response models.

Results and Discussion

Model fitting

The adequacy of the model was tested using coefficient of determination (R^2), Fisher's F-test and Lack of fit. The best fit equations were developed in order to draw contour plots for showing the effect of predicted variables on those responses. After fitting experimental data, quadratic regression models were developed, as shown in Eqn (3) and (4).

$pH\ (Y_1) = 0.0075X_1 - 0.23X_2 - 0.21X_3 - 0.03X_1X_2 + 0.015X_1X_3 - 0.025X_2X_3 - 0.026X_1^2 - 0.036X_2^2 + 0.82X_3^2 + 3.49$ (R^2 = 98.95 %, R^2_{adj} = 97.60%) (3)

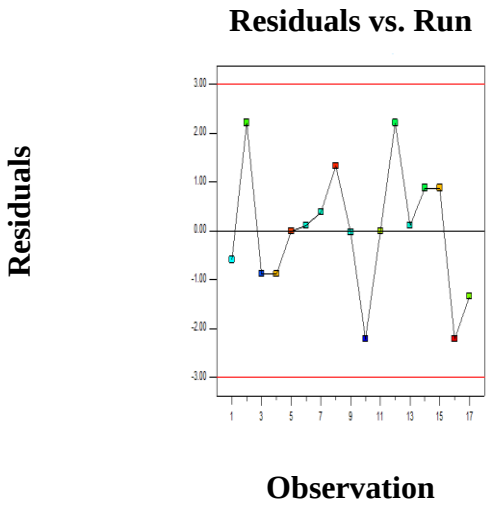
$\text{Titrable acidity}\ (Y_2) = -0.003X_1 + 0.37X_2 + 0.15X_3 + 0.13X_1X_2 - 0.030X_1X_3 + 0.022X_2X_3 + 0.11X_1^2 + 0.16X_2^2 - 0.75X_3^2 + 1.38$ (R^2 = 97.57 %, R^2_{adj} = 94.4%) (4)

Where, X_1, X_2 and X_3 are the blend ratio, alpha amylase enzyme and starter culture ratio. The coefficients with one factor (the ones in front of X_1 or X_2 or X_3) represent the effects of that particular factor, while the coefficients with two factors (the ones in front of X_1X_2 or X_1X_3 or X_2X_3) and those with quadratic terms (the ones in front of X^2_1 or X^2_2 or X^2_3) represent the

interaction between the two factors and the quadratic effects, respectively. The positive sign in front of the terms indicates a synergistic effect, while the negative sign indicates an antagonistic effect.

Validation of the model

The developed quadratic model is usually checked for ensuring it provides an approximation to the real system. As a primary tool and confirmation for graphical techniques and numerical methods were used to validate the models in this study **(Trinh and Kang, 2010).** The graphical method characterizes the nature of residuals of the models. A residual is defined as the difference between an observed value and its fitted values. The first plot, residual versus order, as shown in Fig. 1, each residual is plotted against an index of observation orders of data, which was used to check for any drift in the process. Normal probability plot, shown in Fig. 2, the data were plotted against a theoretical normal distribution in such a way that the points should form an approximate straight line, and a departure from this straight line would indicate a departure from a normal distribution, which was used to check the normality distribution of the residuals. As shown in Fig. 2, it is necessary that the assumptions of normality were satisfied for the data. Lastly, residuals versus the fitted values, as shown in Fig. 3, were used to test the efficiency of the functional part of the developed model.



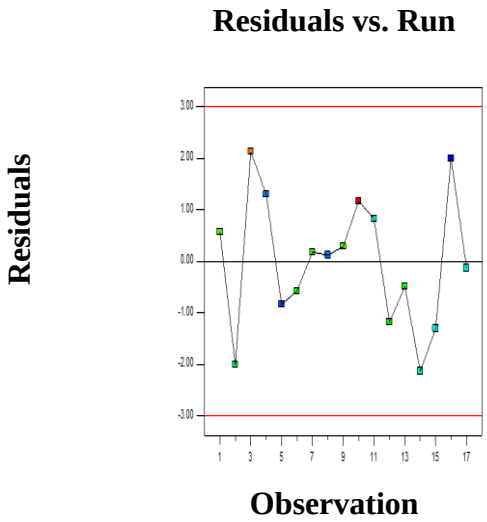


Fig.1: Residuals vs. observation orders of data for (a) pH

(b) Titrable acidity

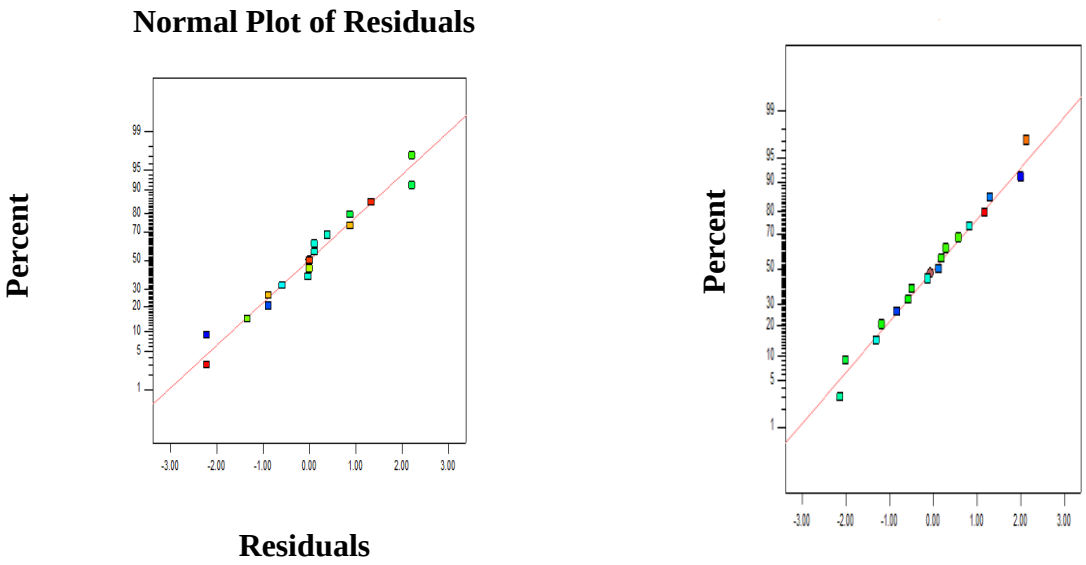


Fig.2: Normal probability plots for (a) pH **(b) Titrable acidity**

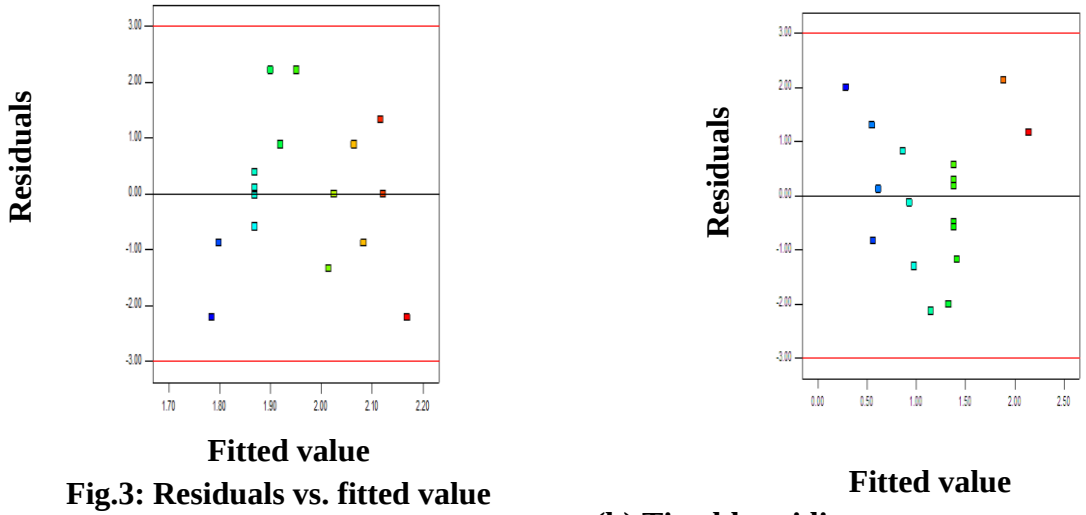


Fig.3: Residuals vs. fitted value (a) pH **(b) Titrable acidity**

The developed models were then examined using a numerical method employing the coefficient of regression (R^2), adjusted R^2 (R^2adj), and then calculated as shown in Eqn (5) and (6) **(Haber and Runyun 1977),** the coefficient of determination R^2 indicates how much of the observed variability in the data was accounted for by the model, while R^2adj modifies R^2 by taking into account the number of covariates or predictors in the model.

$$R^2 = 1 - \frac{SS_{residual\ error}}{SS_{model} + SS_{residual}} \quad (5)$$

$$R^2adj = 1 - \frac{\frac{SS_{residual}}{Df_{residual}}}{\frac{SS_{residual} + SS_{model}}{Df_{residual} + Df_{model}}} \quad (6)$$

Where, SS is the sum of the squares, Df is degree of freedom. The quadratic model of experimental data was developed in this study with the values of R^2 higher than 90%, say 98.95 and 97.57% for pH, and titrable acidity of idli batter, respectively. Furthermore, an R^2adj very close to the R^2 values for the response pH than titrable acidity insures a satisfactory adjustment of the quadratic models to the experimental data and having least residual error in model. Therefore, the regression models explained to check the effect of each response on predicted variables well.

pH

In fermented idli batter samples, pH ranged from 4.6 to 3.1. Maximum pH was observed at experiment no. 9 in which (X_1= 0), (X_2= -1) and (X_3= -1). The minimum pH was observed at experiment no. 4 in which (X_1= 1), (X_2= 1) and (X_3= 0).

Table 2 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 1and 5 % level of significance respectively

Table 3 Analysis of variance for titrable 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 1and 5 % level of significance respectively

ANOVA on pH model, as shown in Table 2, demonstrates that the models were highly significant ($p<0.01$). The predicted R^2 of 98.95% is in reasonable agreement with the adj R^2 of 97.60%. The lack of fit test describes the variation in the data around the fitted model. If the model does not fit the data well, the lack of fit will be significant. Effect of response on blend ratio, alpha amylase enzyme, and starter culture ratio, at linear, quadratic and interactive levels are reported in Table 2. It shows that 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 predicted 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

highly the model was significant. The highest effect of starter culture on pH was observed and the effect of alpha amylase was found significant, while no effect of blend ratio was observed on pH. It was clear that both starter culture and alpha amylase reduces the pH and fermentation time.

The range for change in titrable acidity for idli batter from 0.401 to 2.211%. Maximum acidity was found 2.211% for experiment no.4, in which (X_1= 1), (X_2= 1) and (X_3= 0). The minimum acidity was observed 0.401% for experiment no.9 in which (X_1= 0), (X_2= -1) and (X_3= -1). The model of titrable 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 titrable acidity was highest at quadratic level due to highest calculated F-value (62.2) followed by linear level and no effects on titrable acidity was observed at interactive level. The highest effect of starter culture on titrable acidity was observed and followed by the effect of alpha amylase, while no effect of blend ratio was observed on acidity. The number of experiments and results for the different predicted variables were showed in Table 4. Optimized condition for the predicted variables to prepare the idli batter was reported in Table 5.

Table 4 Box behnken method and results obtained

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

Table 5 Optimum value of parameters for idli batter

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

Fig. 4 shows the three-dimensional surface contour plot. The response surface and contour is the graphical representation of regression analysis used to visualize the response between the response and experimental level of each input factor as shown in these plots. pH of fermented idli batter slightly decreased with the increase in level of unit of alpha amylase whereas the effect of starter culture ratio was significantly decreasing the pH till the centre point at optimum level of conditions. The interactive effect of alpha amylase and substrate ratio on acidity was shown in Fig. 4 (b) increasing the level of alpha amylase gradually increased the total acidity while increasing the substrate ratio decreased the total acidity of idli batter. Optimum levels of predicted variables were obtained by analyzing the response surface contour and the derivative of Eqn (3) and (4). The optimum conditions were a set of X_1 (substrate ratio), X_2 (alpha amylase) and X_3 (starter culture ratio) where the derivative becomes zero, as shown in Eqn (7)

$$\frac{dY}{dX1} = \frac{dY}{dX2} = \frac{dY}{dX3} = 0 \qquad (7)$$

pH

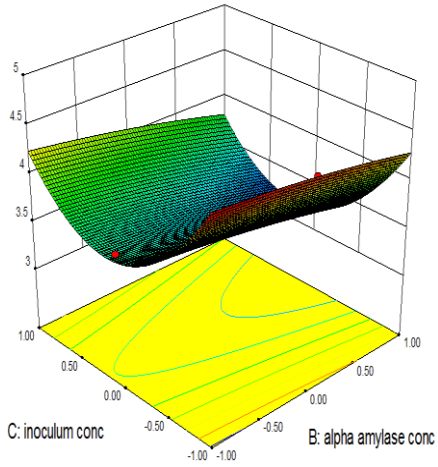


Fig. 4 Three dimensional surface plots for (a) pH

Titrable acidity

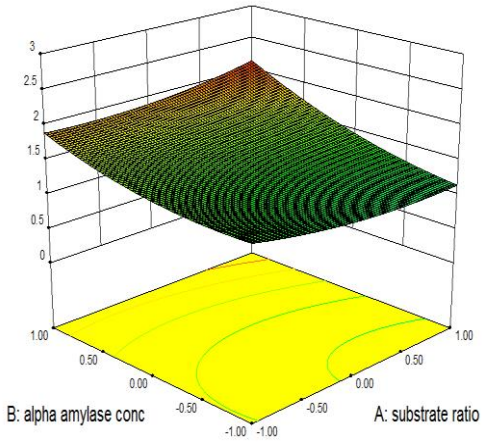


Fig. 4 Three dimensional surface plots for (b) Titrable acidity

Conclusions

On the basis of experimental and analytical data, it could be concluded that Response Surface Methodology application could be beneficial for seeking the optimal conditions for the preparation of idli batter. With the use of appropriate quantity of blend ratio (3:1:0.5), alpha amylase concentration (15U) and starter culture ratio (0.05:1.95) it can be prepared and recommended for making quality idli batter within 9h. The future scope of this study in this area

is of great commercial importance due to the reduction in fermentation time for large scale idli production. The use of culture can help to extend the shelf-life of the batter and product can be developed in the ready to eat form

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References

1 AOAC. 1984. "Official methods of analysis" 14th ed. Association of Official Analytical Chemists. Washington, DC.

2 Bezerra M.A. Santelli E.R., Oliveira P.E., Villar L.S. and Escaleira A.E. 2008. RSM as volumetric tool for optimization in analytical chemistry. Talanta, 76: 965-977.

3 F.A.O. 2012. Economic and Social Department: The Statistical Division. Statistics Division, posted on September, p58.

4 Haber A., Runyun R.P. 1977. General statistics. 3rd ed. Reading, MA: Addision Wesley, p98

5 Khuri A.I. and Cornell J.A. 1987. Response surface design and analysis, Marcel Dekker, Inc, ASQC Quality Press, New York, 112-139.

6 Meyers R.H. and Montgomery D.C. 1995. Response surface methodology: Process and product optimization using designed experiments, John Wiley and sons, Inc. New York. p178.

7 Soni S.K. and Sandhu D.K. 1989. Indian fermented foods: microbiological and biochemical aspects. Indian Journal of Microbiology, 30: 135–157.

8 Steinkraus K.H., Van Veck A.G. and Theireau D.B. 1967. Studies on idli-an Indian 311 fermented black gram rice food. Food Technology, 21: 110-111.

9 Trinh T.K. and Kang L.S. 2010. Application of response surface method as an experimental design to optimize coagulation test. Environmental Engineering Research,15(2): 63-70.