CHAPTER - III
MATERIALS AND METHODS

3.0 STARTER CULTURES

Various starter culture yeasts *viz.* *Saccharomyces cerevisiae* MTCC4043 and *Debaryomyces hansenii* MTCC9882 were purchased from Microbial Type Culture Collection (MTCC), Chandigargh, India. *Hansenula anomala* NCL3341 and *Torulopsis candida* NCL3234 were purchased from National Chemical Laboratories (NCL), Pune, India.

3.1 STARTER CULTURES MAINTENANCE

The yeasts were grown for 48 hour at 28 °C on YM slants, shaken with 5 ml sterile water and the suspension used to inoculate each batch of batter comprising 200 g dry ingredients—rice (*Oryza sativa*) and black grams (*Phaseols mungo*), 3:1 w/w—which were then incubated at 28°C for 30 h. Samples were taken aseptically from each batch at intervals and bacteria and yeasts enumerated together with the determination of other parameters (Soni & Sandhu 1989a).

3.2 STEPS IN THE PREPARATION OF IDLI BATTER

3.2.1 Source of raw ingredients

The major ingredients for the preparation of Idli batter were parboiled (Ponmani and CO-43) rice (*Oryza sativa*) and black gram dhal (*Phaseolus Mungo*) purchased from local markets in Chidambaram. The nutrition Table of Ponmani rice, CO-43 rice and Black gram dhal are given in Table 3.2.1.
Table 3.2.1 Nutritional Value (Durgadevi et al., 2012; Sathe et al., 1983)

<table>
<thead>
<tr>
<th>Nutrition</th>
<th>Ponmani Rice</th>
<th>CO-43 Rice</th>
<th>Black gram dhal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10.70%</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Fat</td>
<td>0.12</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>0.88</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>77.16</td>
<td>75.0</td>
<td>60</td>
</tr>
<tr>
<td>Calorific value per 100g</td>
<td>350</td>
<td>320</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B1mg per 100g</td>
<td>0.21</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin B2mg per 100g</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Calcium mg</td>
<td>-</td>
<td>-</td>
<td>154</td>
</tr>
<tr>
<td>Phosphorous mg</td>
<td>-</td>
<td>-</td>
<td>385</td>
</tr>
</tbody>
</table>

3.2.2 Soaking

The raw ingredients rice and black gram dhal were cleaned to remove the stones and other dust particles and soaked in non-chlorinated water (use of chlorinated water may destroy wild yeast) separately for various periods in between 2 to 6 h.

3.2.3 Washing

After soaking, black husk was manually removed from unhusked black gram with hands by stirring and rubbing. During washing process the rice and black gram dhal were washed with good quality water for three times to remove the unwanted particles.

3.2.4 Grinding

The soaked and washed rice was added to the LG Ultra table top wet grinder (muller mill type) and ground for 30 min by adding required quantity of water, so as to get a paste. Similarly black gram dhal was ground separately in the wet grinder for 30 minutes by adding required quantity of water to get a fine paste.
3.2.5 Mixing

The ground rice flour and black gram flour were mixed thoroughly. By adding 1 to 3% common salt with or without starter cultures and additives.

3.2.6 Fermentation

After mixing the Idli batter in the closed stainless steel container (2000ml) the batter was kept for fermentation at room temperature for 6 h to 12 h depending on the climatic conditions.

3.3 IDLI BATTER PREPARATION

In this study 500 g of parboiled Ponmani rice was soaked in 1000 ml of water for 4 h and 150 g of black gram dhal was soaked in 500 ml of water for 4 h. After 4 h of soaking time the black gram dhal was decorticated and ground to a fine paste by adding sufficient water (500ml) for about 30 min. The soaked rice was ground separately to a fine paste by adding sufficient quantity of water (500ml) for 30 min. The grinding time depends upon the type of grinder being used. It may be a table top wet grinder or it may be normal single stone wet grinder. The grinding depends upon the rpm of the grinding and the roughness of the inner surface of the grinder. Normally the grinding time varies between 20 min to 45 min.

3.4 IDLI PREPARATION

The consistency of the Idli which indicates whether the batter is completely cooked or not, may be tested using a very thin stick. Generally the stick is inserted in the cooked Idli and taken out. If there is no paste sticking to the stick, indicates the batter is completely cooked and ready to serve. If it is over cooked for more time the Idli may became a semi solid or jelly type. The fermented batter was steamed in perforated cups (50 g of batter was taken in a stainless steel segmented cup of diameter 85 mm and depth 18 mm) in the Idli plates arranged in a stand in a pressure cooker for a period of 8-10 min, which gives rise to soft and spongy Idli. The flow chart for preparation of Idli is furnished in Fig 3.1.
Traditional Idli preparation

Black gram dhal
(200gm)

Soaked and washed (4 hours)

Grind finely in a wet grinder

30 min + 500ml water

Mix the slurries into a thick batter and mix well

Addition of salt (2% w/v)

Incubate it in a warm place at 30°C for 8 h

Pour batter into small cups in idli cooker

Steam for 8-10 min

Soft and spongy idli

Fig: 3.1 Preparation of Idli
3.5 EVALUATION OF IDLI BATTER FERMENTATION

3.5.1 Evaluation of whey separation

The stored batter samples were observed for whey separation by removing the separated whey in a measuring cylinder. Noting its volume % whey separation was evaluated as:

\[
\text{Volume of whey separated} \times 100
\]
\[
\% \text{ whey separation} = \frac{\text{Initial volume of the batter}}{\text{Initial volume of the batter}}
\]

3.5.2 Evaluation of Idli

Idlis made from the batters stabilized by the addition of different stabilizers and stored at different temperatures for different periods of time were evaluated for the following parameters.

3.5.3 Bulk density

Bulk density was measured by seed displacement method using mustard seeds as in the case of bread (g/cc)

3.5.4 Texture (ASTM MNL14)

Idli has a circular shape of approximately 7–10 cm diameter (depending on the mold size), flat with lower and upper surface bulging, so that the product is thick at the center (2–3 cm) and tapering towards periphery. Texture of the Idlis was analysed on Stevens-LFRA Texture Analyser. The cut test was conducted in the centre were the average thickness is 2–3 cm using knife probe (TA-8) in the normal mode at 2 mm/s up to a depth of 10 mm. Texture was expressed as the load in grams required to cut the product.

3.5.5 Colour (ISO 16820 Sensory Analysis)

Colour of the Idlis was measured using Hunter Lab Colorimeter model DP-9000 D25A (Hunter associates laboratory, Reston, VA, USA), in terms of Hunter L (lightness, ranging 0–100 indicating black to white), a (\(a^*\); redness and 2a; greenness) and b (\(b^*\); yellowness and 2b; blueness).
3.5.6 Aroma (ISO 5495 Sensory Analysis)

Aroma is the word for a fragrant scent, one that pleases the nose in a way that makes you lick your lips. Unlike its foul-smelling cousin the odor, an aroma smells but never stinks.

3.5.7 Taste (ISO 13302 Sensory Analysis)

Taste was normal sense of human being taste can be vary from person to person. The trained expert can evaluated the sensory analysis of food products. The taste play important role in the food products.

3.5.8 Acceptability of Idlis

Acceptability of Idlis made from the stored batter stabilized by addition of different stabilizers and stored at different temperatures for different periods of time was determined by sensory evaluation using 10-member panel on a 10-point hedonic scale as follows: very good 8–10, good 5–8, fair 3–5, poor 1–3

3.5.9 Estimation of increase in volume of the Idli batter (Rekha and Vijayalakshmi, 2011)

The increase in volume of the Idli batter was determined by keeping a known volume of the Idli batter in a graduated plastic container. The rise in volume of the Idli batter fermentation period were recorded for every 2 h increment. The subtraction of the initial volume from the final volume will give the actual increase in volume rise due to fermentation. Batter Volume Rise (BVR) was expressed in cc. The increase in volume of Idli batter indicates the extent of fermentation and also the quality of Idli, which may be expressed.

Actual increase in volume of Idli batter =  \( V_f - V_i \)

The percent increase in volume was calculated as detailed below.

\[
BVR = \frac{V_f - V_i}{V_i} \times 100
\]
BVR = % Batter Volume Rise

$V_f$ = final rise in Idli batter volume

$V_i$ = initial Idli batter volume

3.5.10 Estimation of total reducing sugars (TRS) by Dinitrosalicylic acid (DNS) method (Miller, 1972)

For sugar estimation an alternative to the Nelson - Somogyi method (Nelson, 1944; Somogyi, 1952) is the dinitrosalicylic acid method- simple, sensitive and adoptable during handling of a large number of samples at a time.

(i) Preparation of Dinitrosalicylic acid reagent (DNS)

About 1.0g of 3, 5-Dinitrisalicylic acid, 200mg of crystalline phenol and 50mg of Sodium sulphite is dissolved in 1000ml of 1% Sodium hydroxide solution. The reagent is stored at 4°C. Since the reagent deteriorates due to Sodium sulphite, if longer storage is required, Sodium sulphite may be added at the time of use.

(ii) Preparation of Rochelle salt solution (Potassium sodium tartrate)

About 40g of Potassium sodium tartrate is dissolved in 100ml of distilled water.

(iii) Preparation of the glucose stock solution

100mg of D-glucose is dissolved in 100ml of double distilled water. 10ml of the stock solution is diluted to 100ml of double distilled water to obtain working standard.

(iv) Estimation of Total Reducing sugars (TRS)

100mg of the sample is weighed and treated with hot 80% ethanol twice (5ml each time) to extract the reducing sugars. The supernatant is collected and evaporated it by keeping it in a water bath at 80°C. 10 ml of distilled water is added to dissolve the sugars. 0.5 to 3 ml of the extract is pipetted out in test tubes and equalized the volume to 3 ml with water in all the tubes. With this 3 ml of DNS reagent is added. The content in the tubes is heated in a boiling water bath for 5 min. 1 ml of 40%
Rochelle salt solution is added when the contents of the tubes are still warm. Finally it is cooled and the intensity of dark red colour product is spectrophotometrically read at 510 nm UV/V in-double beam Biospectrophotometer using blank as a reference. Similar procedures are followed for the other concentration containing a known quantity of sugars.

3.5.11 Estimation of total carbohydrate by phenol sulphuric acid method
(Dubios et al. 1956)

The phenol-sulphuric acid method to estimate total carbohydrates is described

(i) Preparation of Phenol solution

Redistilled (reagent grade) 50 gm of phenol is dissolved in water and diluted to 1.0 litre

(ii) Preparation of glucose stock solution

100 mg of D-glucose is dissolved in 100 ml of double distilled water in a standard flask. 10 ml of the stock solution is diluted to 100 ml of double distilled water to obtain working standard.

(iii) Estimation of Total Carbohydrate

100 mg of the sample is weighed and transferred into a boiling tube. The content is hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCL and is cooled to room temperature. It is neutralized with solid sodium carbonate until the effervescence ceases. And the volume is equalized to 100 ml with double distilled water and centrifuged. About 0.2 ml of the working standard and 0.8 ml of distilled water taken in a test tube with 1 m of phenol solution is added. With this solution 5 ml of 96% sulphuric acid is added and agitated well. After 10 min shake the contents of the tube are placed in a water bath at 25- 30°C for 20 min. The colour intensity of product is read spectrophotometrically at 490 nm in UV/V is-double beam Biospectrophotometer using blank reference. Similar procedures are followed for the other concentration containing a known quantity of sugars.
3.6 RESPONSE SURFACE METHODOLOGY (RSM)

The RSM has several classes of designs, with its own properties and characteristics. Central composite design (CCD), Box–Behnken design (BBD) and three-level factorial design are the most popular designs applied by the researchers. The CCD was used to study the effects of the variables towards their responses and subsequently in the optimization studies. This method is suitable for fitting a quadratic surface and it helps to optimize the effective parameters with a minimum number of experiments, as well as to analyze the interaction between the parameters. In order to determine the existence of a relationship between the factors and the response variables, the data collected is analyzed in a statistical manner, using regression. A regression design is normally employed to model a response as a mathematical function (either known or empirical) of a few continuous factors and good model parameter estimates are desired (Montgomery, 2001).

The coded values of the process parameters are determined by the following equation

\[ x_i = \frac{X_i - X_0}{\Delta x}, \]

where \( x_i \) – coded value of the \( i^{th} \) variable, \( X_i \) – uncoded value of the \( i^{th} \) test variable and \( X_0 \) – uncoded value of the \( i^{th} \) test variable at center point. The regression analysis was performed to estimate the response as a second order polynomial function which is given below.

\[ Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \]

Where \( Y \) is the predicted response, \( \beta_i, \beta_{ij} \) are coefficients obtained from the regression. They represent the linear, quadratic and cross products of \( X_1, X_2, X_3 \) on response.

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3.7 **MODEL FITTING AND STATISTICAL ANALYSIS**

The regression and graphical analysis with statistical significance are carried out using Design-Expert software (Version 8.0.3.1, Stat-Ease, Inc., Minneapolis, USA). In order to visualize the relationship between the experimental variables and responses, the response surface and contour plots are generated from the models. The optimum values of the process variables are obtained from the response surface.

The adequacy of the models was further justified through analysis of variance (ANOVA). Lack-of-fit is a special diagnostic test for the adequacy of a model that compares the pure error, based on the replicate measurements on the other lack of fit, based on the model performance (Noordin et al., 2004). F-Value, calculated as the ratio between the lack-of-fit mean square and the pure error mean square, is the statistical parameter used to determine whether the lack-of-fit is significant or not, at a significance level.

3.8 **SENSORY EVALUATION**

Sensory evaluation was carried out by the 5 expert panel members in the age group of 22-36 years and Idli samples were served to the members to taste the Idli and tick in the appropriate box by 9 hedonic scale. The member should mark the quality of the products such as Colour, Taste, Aroma, Texture, Softness, Hardness, Puffiness, Adhesiveness and Cohesiveness in the given preforma and all the data sheets are given in appendix 1. The products were analysed by the highest and lowest score obtained from the panel member of 9 hedonic scale in Food Processing and Technology at the Department of Technology, Annamalai university. The samples were evaluated based on colour, taste, aroma and texture using a 9 point hedonic scale where: 9 = Excellent, 8 = Very Good, 7 = Good, 6 = Just a little good, 5 = May be good or may be bad, 4 = Just a little bad, 3 = Bad, 2 = Very Bad and 1 = Worst.
3.9 GLASSWARES

Glass wares used for the laboratory experiments were of Borosil make and were cleaned with soap solution, rinsed with distilled water for 2-3 times, dried and sterilized in hot air oven at 160°C for 3 h before use.

3.9.1. Chemicals

Xanthan, and Carrageenan were purchased from Himedia, Mumbai - India. Guar gum and Pectin (high methoxypectin, degree of esterification, 66–70%) were supplied by Indian gum industries, India.