Optimisation of wheatgrass fortified steamed rice cake using response surface methodology

Arpita Das, Utpal Raychaudhuri and Runu Chakraborty*

Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-700032, India.

Abstract

The present work is focused on the process parameter optimization of 1% wheatgrass fortified steamed rice cake (SRC). The parameters like fermentation time (16-24 h) and temperature (26-34 °C) were optimized using response surface methodology (RSM). The design contained a total of 13 experimental runs involving replications of the central points. The experimental data were subjected to the analysis of variance (ANOVA) and fitted to a second order polynomial equation. Increase in batter volume, total lactic acid bacteria content, L*-value and hardness of SRC samples was varied significantly with the fermentation time and temperature.

Keywords: Steamed rice cake (SRC), response surface methodology (RSM), wheatgrass, L*-value, hardness.

Introduction

Rice is one of the leading food crops in South East Asia including China, and the production of rice in this part of the world is much higher than that of wheat. It can be ground into powder and utilized to produce many kinds of foods, including several types of steamed cakes (Ying et al., 2007). Rice cake is one of that types which is shaped and condensed. A wide variety of rice cakes exist in different countries where rice is eaten. It is particularly prevalent in Asia. They are perhaps best utilized in Japan and the countries of the Pacific region where rice is an economic staple food and the grain is the basis for multiple types of meals and foods. The cakes are usually two to three inches in diameter and are made by steaming a batter, which is fermented overnight. People with celiac disease are unable to consume certain gluten proteins from cereals – such as wheat, rye, barley, kamut– and hybrids like triticale. The most common cereal flours used for gluten free bread production are rice. The only effective treatment for celiac disease is a strict adherence to a gluten free diet throughout the patient’s lifetime, which, in time, results in clinical and mucosal recovery (Gallagher et al., 2004). Rice flour is one of the most suitable cereal flour for gluten free-products because it has a low level of prolamin. Besides, rice possesses unique nutritional, hypoallergenic, colorless, and bland taste properties (Sanchez et al., 2002). There are many nutritional impacts of steamed rice cake (SRC). When people start looking for ways to cut calories, their one of the preferred food is rice cakes. It is one food which is usually eaten by weight loss strugglers, especially those who are dieting. A re-design of the gluten-free bakery goods is needed for obtaining gluten-free baked products with similar nutritional composition to that of their gluten counterparts. Those products would allow celiac patients and/or population with other allergic reactions and intolerances caused by proteins or another component of cereals to meet dietary guidelines without changing their dietary pattern. Research on gluten-free cakes has focused on the effect of wheat flour replacement by rice flour in traditional recipes, as steamed leavened rice cakes (Mohamed and Hamid, 1998). Rice cakes became even more popular as treats for festivals and as local specialties. It is required to supplement food materials with nutritionally rich items for e.g. fruits like citrus, banana and grapes, sprouts and herbs like wheatgrass. They contain antioxidants in addition to the compounds of nutrient elements (Niwa et al., 1998). Tender wheatgrass and its juice are consumed for healthy growth of human body. Tender wheatgrass is renowned for its therapeutic value since ancient times and contains vitamins, minerals, enzymes, chlorophyll and 17 amino acids (Pardeshi et al., 2013). Tender wheatgrass and its juice are consumed for healthy growth of human body. Wheatgrass was also reported to be helpful in curing certain diseases such as thalassemia (Marwaha et al., 2004) and distal ulcerative colitis (Ben-Arye et al., 2002). A large proportion of the world cereals production is processed by fermentation prior to consumption. The enhancement of attractive flavour and texture, and the improved shelf-life and
digestibility as a result of fermentation are important reasons for this (Nout, 2009).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques used for development, improvement and optimization of processes or formulations (Malcolmson et al., 1993; Bas and Boyaci, 2007; Turabi, 2008). It is used to examine the relative significance between a set of quantitative experimental factors and the response variables. In food research studies, response surface methodology (RSM) is very frequently used to optimize the efficiency of the ingredients such as fibres, improvers (Collar et al., 1999; Collar et al., 2007; Clarke et al., 2003; Sabanis et al., 2009), composite flours (Shittu et al., 2007), optimization in food processes like product development, functional food preparation etc (Seog et al., 2008; Gupta et al., 2007; Flander et al., 2007) as well as in optimizing processing conditions (Ghodke et al., 2009; Mondal and Datta, 2010).

Therefore, the aim of this research was to study the effect of different fermentation time and temperature combinations on characteristics of 1% wheatgrass powder fortified steamed rice cake (Idli) followed by optimization of the process parameters.

Materials and methods

Raw materials: The major food ingredients used for the preparation of SRC are, split dehusked (SD) black gram dhal (Phaseolus mungo Roxb.) and parboiled rice (Oryza sativa) which was purchased from a local market. Seed of wheat (Triticum aestivum) and common salt, NaCl (Tata salt, India) were purchased from the local grocery stores at Kolkata.

Processing of fortifying ingredients: The seeds of wheat (Triticum aestivum L.), collected from Kolkata, West-Bengal, were authenticated by the Taxonomists of the Botanical Survey of India, Kolkata (Ref. CNH/1-1/10/2010/Tech II/176). Those seeds were first washed with tap water and then with distilled water. The seeds were soaked in distilled water (24-25°C) for 5 h. The steeped seeds were then covered with a clean moistened cloth to promote the onset of germination for 24 h (120 mL of water was sprayed onto the cloth every 8 h to keep the cloth moistened). The seeds were then transferred to the tray. The wheat plants were grown in sterilized soil. The seeds were sowed in trays (length 35 cm × breadth 25 cm × depth 7 cm), containing 4 kg of soil (pH 7.5±0.4). The soil was provided with sufficient tap water regularly and was placed in a room where normal air flow and sunlight were available. Samples were collected from the plant on the eighth day. The samples collected were washed, wiped and cut into small pieces (2 cm length). Wheatgrass samples were frozen at −78°C (Ultra-Low Temperature Freezer, Model no-C340-86, New Brunswick Scientific, England) for 12 h and then freeze-dried in a vacuum (2.4×10⁻² mB) for 6h. The condenser temperature was −49°C. The dried material had moisture content of 5.4% (AOAC 1984). Then dried wheatgrass was ground in kitchen mixer at 8000 rpm for 5 min to get a fine, smooth wheatgrass powder. This wheatgrass powder was used directly for fortification in SRC.

SRC batter preparation process: SRC were prepared with parboiled rice and split dehusked black gram dhal. The ideal ratio of rice and black gram dhal for the product is 2:1. The ingredients, rice and black gram dhal, were washed several times with water to remove adhering dirt and dust particles from the surface, soaked separately for 4 h at 30±1°C temperature and ground in a kitchen mixer blender separately. Rice was ground coarsely and black gram finely to a smooth batter and both were mixed together with common salt (2.0%). The batter was then allowed to ferment for different assay condition according to RSM in glass beakers covered with cotton cloths. The mean particle size of parboiled rice and decorticated black gram batter prepared was 400μm-250μm measured by 40-60 mesh size sieves.

Evaluation of SRC batter

Evaluation of increase in volume: For evaluation of percent increase in volume, the batter was placed in a measuring cylinder (Nisha et al., 2005).

\[
\text{% increase in volume} = \left( \frac{\text{Final volume} - \text{Initial volume}}{\text{Initial volume}} \right) \times 100
\]

pH measurement: The pH of the batter at different fermentation times was recorded using a pH meter (Thermo Orion Basic pH Meter, Model 420A pH/mV/ORP/temperature meter).

Microbial analysis: Fermented SRC batters prepared in all assay combination were used for plating to determine lactic acid bacteria. The microbial load at different assay condition was measured by suspending the respective batter in 0.5% autoclaved saline and plating it out at appropriate dilutions. For lactic acid bacteria, de Man, Rogos and Sharpe (MRS) media was used. The technique employed is pour plate method. Counts of the colonies were made after incubation for 24 hr for MRS at 37°C.
Evaluation of SRC

Textural Profile Analysis: SRC has a circular shape of approximately 5–10 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 SRC was analyzed with Instron Universal Testing Machine, Table Model 4301 (Instron Ltd., High Wycombe, Bucks, UK) in the compression mode fitted with a 50N load cell. All test samples are 20mm thick. Double compression test was performed by compressing axially each sample with a 40 mm diameter flat plate probe attached to the moving crosshead. The testing conditions were: compression ratio of 50% deformation from the initial height of the sample; 10mm/min crosshead speed 10mm/min chart speed. The force-distance curve obtained was used to derive the various textural properties (Bourne, 1978).

Color: Color of the SRC was measured using Hunter Lab Colorimeter model DP-9000 D25A (Hunter associates laboratory, Reston, VA, USA), in terms of Hunter parameters L (lightness, ranging 0–100 indicating black to white). Five replicates of color measurements were taken.

Experimental Design and Statistical Analysis

ANOVA was done to determine the effects of fermentation time and temperature on various physical properties of the SRC followed by optimization of the process using response surface methodology. A central composite design was employed with two independent variables (factors) namely, fermentation time (X₁) and fermentation temperature (X₂) and five dependent variables (responses) namely, Increased batter volume (Y₁), pH of batter (Y₂), lactic acid bacteria (Y₃), L* value (Y₄) and hardness (Y₅). Thirteen experiments were performed with five centre points. The combination of the two factors (baking time and temperature), their actual and coded values are given in Table 1. Five replicates at the centre of the design were used.

For each response variable, model selection for linear, quadratic or cubic models were made on the basis of sequential model sum of squares (SMSS), lack-of-fit tests and the multiple correlation coefficients (R²). The cubic model was aliased because there were not enough points for this type of model. From this information, the most accurate model was chosen which in all cases appeared to be quadratic. The second-order response functions for the experiments were fitted to the following quadratic regression equation:

\[ Y_o = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2 \]

Where X₁ is the fermentation time; X₂ is the fermentation temperature; \( \beta_0 \) is the value of the fitted response at the centre point of the design i.e. point (0, 0); \( \beta_1 \) and \( \beta_2 \) are the linear regression terms; \( \beta_{11} \) and \( \beta_{22} \) are the quadratic regression terms; and \( \beta_{12} \) is the cross-product regression term.

All the studies were replicated three times and the mean was individually calculated for scores obtained for all quality attributes of each product. Statistical analysis of variance (ANOVA) and multiple regressions were performed using the software, Design Expert® Version 7.1.6 (Stat-Ease, Inc., Minneapolis, USA) to fit the equation. The results included the estimated model coefficients, the regression coefficients and the lack-of-fit test.

Result and Discussion

Effect of fermentation time and temperature on batter volume and pH of SRC batter

The effects of fermentation time and fermentation temperature on 1% wheatgrass fortified SRC increased batter volume and pH are shown in Table 2. Increase in batter volume and pH ranged from 50 to 77(%) and 4.01 to 5.72 respectively. Minimum pH (4.1) was found to be at fermentation for 20 h and fermentation temperature 38°C, while maximum pH (5.72) was recorded at fermentation for 24 h and fermentation temperature 30°C (Table 2). It can be observed from Table 3 that fermentation time and fermentation temperature were significant (at 95% confidence level) for batter volume and pH. Regression models explained 77.6 and 97.01% of the total variability in batter volume and pH of wheatgrass fortified SRC, respectively. Batter volume depended on the two principal factors namely, amount of gas production and gas holding capacity of the batter. The rise in CO₂ production can be correlated with the increase in batter volume (Sridevi et al., 2010).

Response surface models: Regression coefficients were estimated by multiple regression analysis (Table 3) and the correlations were obtained which are given below:

Increased batter volume (Y₁) = 70.79 + 3.58X₁ + 1.08X₂ – 4.28X₁² + 1.11X₂² – 4.30X₁X₂ (2)

pH of batter (Y₂) = 4.87 + 0.15X₁ – 0.11X₂ - 0.16X₁² – 0.17X₂² + 0.28X₁X₂ (3)

Lactic acid bacteria (Y₃) = 29.67 + 0.67X₁ + 0.33X₂ – 3.80X₁² – 4.30X₂² – 4.50X₁X₂ (4)
Table 1: Independent variables and their levels in the central composite design

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Unit</th>
<th>Symbol</th>
<th>Coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation time</td>
<td>h</td>
<td>$X_1$</td>
<td>-2  -1  0  +1  +2</td>
</tr>
<tr>
<td>Fermentation temperature</td>
<td>ºC</td>
<td>$X_2$</td>
<td>22  26  30  34  38</td>
</tr>
</tbody>
</table>

Table 2: The central composite design with actual values of independent variables and responses

<table>
<thead>
<tr>
<th>Fermentation time (h)</th>
<th>Fermentation temp. (ºC)</th>
<th>Increased batter volume (%)</th>
<th>pH of batter</th>
<th>Lactic acid bacteria (cfu/mL)</th>
<th>L*-value</th>
<th>Hardness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>30</td>
<td>70</td>
<td>4.8</td>
<td>29</td>
<td>45.64</td>
<td>23</td>
</tr>
<tr>
<td>24</td>
<td>30</td>
<td>62</td>
<td>5.72</td>
<td>18</td>
<td>44.65</td>
<td>23</td>
</tr>
<tr>
<td>22</td>
<td>34</td>
<td>64</td>
<td>5.22</td>
<td>16</td>
<td>52.9</td>
<td>37</td>
</tr>
<tr>
<td>20</td>
<td>22</td>
<td>58</td>
<td>4.34</td>
<td>12</td>
<td>30.13</td>
<td>34</td>
</tr>
<tr>
<td>18</td>
<td>34</td>
<td>56</td>
<td>4.29</td>
<td>28</td>
<td>45.78</td>
<td>17</td>
</tr>
<tr>
<td>22</td>
<td>26</td>
<td>61</td>
<td>5.01</td>
<td>24</td>
<td>37.02</td>
<td>30</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>69</td>
<td>4.9</td>
<td>34</td>
<td>46.65</td>
<td>31.5</td>
</tr>
<tr>
<td>16</td>
<td>30</td>
<td>50</td>
<td>5.2</td>
<td>11</td>
<td>47.37</td>
<td>16</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>72</td>
<td>4.82</td>
<td>27</td>
<td>48.64</td>
<td>29</td>
</tr>
<tr>
<td>18</td>
<td>26</td>
<td>50</td>
<td>5.2</td>
<td>18</td>
<td>36.08</td>
<td>37</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>75</td>
<td>4.78</td>
<td>30</td>
<td>49.64</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>77</td>
<td>4.92</td>
<td>28.5</td>
<td>47</td>
<td>29.5</td>
</tr>
<tr>
<td>20</td>
<td>38</td>
<td>60</td>
<td>4.01</td>
<td>13</td>
<td>46.06</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 3: Estimated coefficients of the fitted quadratic equation for different responses such as Increased batter volume ($Y_1$), pH of batter ($Y_2$), lactic acid bacteria ($Y_3$), L*-value ($Y_4$) and hardness ($Y_5$)

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>$Y_1$</th>
<th>$Y_2$</th>
<th>$Y_3$</th>
<th>$Y_4$</th>
<th>$Y_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>70.59</td>
<td>4.87</td>
<td>29.67</td>
<td>47.01</td>
<td>28.24</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>3.58</td>
<td>0.15</td>
<td>0.67</td>
<td>0.22</td>
<td>2.25</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>1.08</td>
<td>-0.11</td>
<td>0.33</td>
<td>4.79</td>
<td>-0.083</td>
</tr>
<tr>
<td>$\beta_{11}$</td>
<td>-4.28</td>
<td>0.16</td>
<td>-3.80</td>
<td>-0.41</td>
<td>-1.98</td>
</tr>
<tr>
<td>$\beta_{12}$</td>
<td>-3.53</td>
<td>-0.17</td>
<td>-4.30</td>
<td>-2.39</td>
<td>2.39</td>
</tr>
<tr>
<td>$\beta_{22}$</td>
<td>-0.75</td>
<td>0.28</td>
<td>-4.50</td>
<td>1.55</td>
<td>6.75</td>
</tr>
</tbody>
</table>

Lack of fit \( P > 0.05 \) \( \text{P} > 0.05 \) \( \text{P} > 0.05 \) \( \text{P} > 0.05 \) \( \text{P} > 0.05 \)

R² \( 0.776 \) \( 0.9701 \) \( 0.9235 \) \( 0.8845 \) \( 0.8093 \)

\( L*-\text{value} (Y_4) = 40.01 +0.22X_1 + 4.79X_2 - 0.41X_1^2 - 2.39X_2^2 + 1.55X_1X_2 \) \( (5) \)

Hardness (Y₅) = 28.24 + 2.25X₁ – 0.083X₂ – 1.98 X₁² + 2.39X₂² + 6.75X₁X₂ \( (6) \)

Analysis of variance (ANOVA) of the effect of fermentation time and temperature on wheatgrass fortified SRC (linear, quadratic and interaction terms on the response variables, the lack-of-fit test and the regression coefficients) are given in the Table 4. F-test values of 4.85, 45.36, 16.89, 10.72 and 5.94 for increased batter volume, pH of batter, lactic acid bacteria, L*-value and hardness respectively, obtained demonstrated that the models are significant. The P-value checked the significance of each of the coefficient to understand the interaction between the independent variables. The lower the P-values, the more significant are the model terms. Values of P less than 0.05 indicate that the model term is significant (Ghodke et al., 2009). The regression coefficient (R²) checks the goodness of fit of the models. The closer the R² values to 1.0, the more fit are the models.
Table 4: Analysis of variance table showing the effect of treatment variables on increased batter volume (Y₁), pH of batter (Y₂), lactic acid bacteria (Y₃), L*-value (Y₄) and hardness (Y₅)

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Degree of freedom</th>
<th>Y₁</th>
<th>Y₂</th>
<th>Y₃</th>
<th>Y₄</th>
<th>Y₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>5</td>
<td>0.0310*</td>
<td>&lt; 0.0001*</td>
<td>0.0009*</td>
<td>0.0035*</td>
<td>0.0185*</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>0.0571*</td>
<td>0.0015*</td>
<td>0.4417*</td>
<td>0.7942*</td>
<td>0.1132*</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>0.5139*</td>
<td>0.0065*</td>
<td>0.6957*</td>
<td>0.0006*</td>
<td>0.9484*</td>
</tr>
<tr>
<td>AB</td>
<td>1</td>
<td>0.7914*</td>
<td>0.0009*</td>
<td>0.1555*</td>
<td>0.3048*</td>
<td>0.0165*</td>
</tr>
<tr>
<td>A²</td>
<td>1</td>
<td>0.0072*</td>
<td>0.0002*</td>
<td>0.0004*</td>
<td>0.5069*</td>
<td>0.0631*</td>
</tr>
<tr>
<td>B²</td>
<td>1</td>
<td>0.0175*</td>
<td>0.0001*</td>
<td>0.0002*</td>
<td>0.0046*</td>
<td>0.0326*</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>3</td>
<td>0.0814 NS</td>
<td>0.0773 NS</td>
<td>0.3747 NS</td>
<td>0.0627 NS</td>
<td>0.2282 NS</td>
</tr>
<tr>
<td>Regression (R²)</td>
<td></td>
<td>0.776</td>
<td>0.9701</td>
<td>0.9235</td>
<td>0.8845</td>
<td>0.8093</td>
</tr>
</tbody>
</table>

**Not significant, *Significant at (p>0.05), **Significant at (p>0.01)**

**Response surface plots:** The relationship between independent variables and response is shown by the response surface plots (Figs. 1-5) generated using equations 2-6. The contour plots below response surface show multiple asymmetric saddling indicating a rather complex relationship between the dependent and the independent variables.

**Effect of fermentation time and temperature on lactic acid bacteria of SRC batter:** Colony count (cfu/mL) of lactic acid bacteria on MRS agar plate after different fermentation time and temperatures of incubation is given in Table 2. The colony count ranged from 11 to 34 cfu/mL. Total lactic acid bacterial load depended both upon fermentation time and fermentation temperature as it had positive linear effect (Table 3). The linear fermentation time and temperature terms and quadratic temperature term has significant P values (Table 4). The regression coefficient value of this model is 0.9235 which means that 92.35% of the variability in the response data fitted the model. Fig 3 depicts the combined effect of fermentation time and fermentation temperature on total lactic acid bacteria content (cfu/mL) 3D surface.

**Effect of fermentation time and temperature on L*-value of SRC:** The linear, quadratic and interaction model terms were found to have significant P-values (Table 4). Thus L*-value depended upon fermentation time and temperature; the linear variables...
are positive whereas the quadratic effect of the variables are negative. The regression coefficient value of 0.8845 suggests that the raw data had a good fit to the model.

**Effect of fermentation time and temperature on hardness of SRC:** Texture of *idli* is very critical from consumer point of view; it should be spongy, soft and fluffy. The texture of *idli* is influenced by many variables like raw material, quantity, soaking time, grinding conditions, fermentation temperature and time and adjuncts on quality of *idli* (Desikachar et al., 1960; Radhakrishnamurthy et al., 1961; Ramakrishnan, 1979). Table 4 indicates that all the linear and quadratic model terms have significant P values. The regression coefficient value is 0.8093 stating that 80.93% of the data fits with the model. The hardness values ranged between 16N to 40N (Table 2). Fig 5 defines the 3D plot of hardness of SRC with respect to fermentation time and fermentation temperature. An optimum hardness of SRC has been reached at the middle points of fermentation time and temperature.

**Optimization:** The next step involved the detection of the best combination of factors that are able to produce the expected characteristics in the final product. Numeric and graphic optimizations were carried out. Each variable response was chosen to be in the range, maximized or minimized based on the preferred preference of the 1% wheatgrass fortified SRC. The widely accepted SRC quality criteria are large volume, soft texture and optimum color content. Thus, to obtain such product, batter volume should be maximized, hardness has to be minimized and whiteness content to be controlled. As a result of optimization, the best fermentation conditions for the expected responses were a fermentation time 20 h and fermentation temperature 30°C.

**Conclusions**
This study showed that varying temperature-time combination during fermentation leads to significant changes in the physical characteristics of wheatgrass fortified SRC. All the responses were significantly affected by the varying factors. Increased batter volume, pH of batter, total lactic acid bacteria content, L*-value and hardness of SRC fitted well with the quadratic model. The fermentation conditions obtained from optimization were incubated at 30°C for 20 h. Therefore, the study was useful in interpreting the basic fermentation conditions of steamed rice cake fortified with herbal constituents.

**References**
Clarke CI, Schober TJ, Angst E and Arendt EK (2003). Use of response surface methodology to investigate...
the effects of processing conditions on sourdough wheat bread quality. *European Food Research and Technology*, 217: 23-33.


