Kokum Fruit Bar Development via Response Surface Methodology (RSM).

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Abstract
A response surface methodology (RSM) was used for the determination of optimum ingredients level to prepare kokum fruit bar. Kokum pulp was extracted using water extraction method at temperature (24.97°C) and time (30.42 min). The effects of ingredients levels on sensory parameters like texture, overall acceptability and calcium content of the prepared fruit bar were studied by employing a Box- Behenken Design (BBD). During the experimental trials the pulp quantity is kept fixed. The coefficient of determination R² for texture, overall acceptability and calcium content were 0.8298, 0.9239 and 0.9842 respectively. Analysis of variable (ANOVA) performed on the experimental values showed that sugar and milk powder were the most important factors that affected characteristics of the kokum fruit bar as it exerted a highly significant influence (p < 0.05) on all the dependent variables. Based on surface and contour plots, optimum ingredients level for formation of kokum fruit bar were pulp, sugar, milk powder; 50g, 40g and 9.39g respectively.

Key words: Kokum, Fruit Bar, Response Surface Methodology, Box- Behenken Design, Calcium, Optimization

I. Introduction
Garcinia indica Choisy belonging to the family Guttiferae (in the mangosteen) is an indigenous tree of India. It was originally found only in the western peninsular coastal regions and the adjoining Western Ghats in the states of Maharashtra, Goa, Karnataka and Kerala, India as well as parts of Eastern India in the states of West Bengal, Assam and North Eastern Hill regions. Studies have shown that the rind contains moisture (80.0 g/100 g), protein (1%), tannin (1.7%), pectin (0.9%). Total sugars (4.1%) and fat (1.4%). The seed is very rich in stearic, oleic and stearic triglycerides Phytochemical studies have shown that when compared with any other natural sources, kokum rind contains the highest concentration of anthocyanins (2.4 g/100 g of kokum fruit) (Nayak, Rastogi et al., 2010;Nayak, Srinivas, et al., 2010). The anthocyanins cyanidin-3-glucoside and cyanidin-3-sambubioside are the major pigment present in kokum and is reported to occur in the ratio of 4:1. Studies have shown that (-)-hydroxycitric acid (HCA) is the major organic acid in kokum leaves and rinds.

In summer the ripe rinds are ground in a blender with sugar and cardamom and consumed as a cooling drink. Addition of kokum is supposed to enhance the taste of coconut-based curries and to remove the unpleasant smell of mackerel and sardines. They are also used in some vegetable dishes and to prepare chutneys and pickles. The Goans regularly prepare kokum kadi or birinda solkadhi. This curry is used with rice or like an after meal digestive drinks. Both birindi saar and kokum kadi are supposed to be digestive and to relieve gastric problems. As kokum is a highly under-utilized fruit, it can be preserved by making it as a processed food to use it during off-season. (Krishnamurthy, Ravindranath, B. 1982)

1.2 Objective of Research:
Perishable nature of kokum and unavailability of the preserved product in the market gave rise to this research. This study was planned keeping in view the nutritional importance of kokum and to utilize it by preserving as Fruit bar. This kokum fruit bar provides both nutritional and phytochemical benefits to human population. Also its industrial application will help to catch worldwide consumers of kokum.

II. Materials and Methods
2.1 Collection of Fruits
Salt rubbed kokum rinds (completely blackish red in colour) sugar and cumin powder were purchased from a local shop in Parys Market, Chennai.

2.2 Methodology
Fruit bar was prepared by drying the boiled kokum pulp with addition of sufficient quality of sugar and other ingredients. The process involves in fruit bar preparation is shown in Fig 1.
2.1 Equipment used
The laboratory scale mixer consists of two parts—mixing jar and motor (Preethi Himaachal & Co. Solan, India). A grinding blade made up of stainless steel (1.6 mm thick) with three cutting edges was used to grind the material in the jar.

2.2 Extraction of Kokum Pulp
Purchased kokum rinds were washed in cold water three times for the removal of salt. 100 g of the samples was used for each treatment. Equal amount (100 ml) of water was added to the washed rinds. The washed rinds were soaked in water at 24.97°C temperature for 30.42 min of time. The kokum pulp was extracted by grinding into the mixer operated at 5000 rpm for 5 min at controlled room temperature (28-32 °C). After extracted, the pulp was strained through 20 mesh stainless steel sieve to get the smooth pulp.

2.3 Experimental Design Layout
The levels of ingredients were selected based on basic trials. By keeping the pulp quantity constant as 50 g, the independent variables considered were: Sugar quantity (40-60 g) and milk powder quantity (5.0-15.0 g). A two-variable (three levels of each variable) 3-level factorial design and a response surface methodology (RSM) were used to understand the interactions of sugar and milk powder quantity on the texture overall acceptability and calcium content of the prepared fruit bar in 13 runs (Table 2.2), of which 4 were centre edge, four were factorial, and five were at centre point.

Table 2.1 - Processing ingredients and their levels

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar (g)</td>
<td>40, 50, 60</td>
</tr>
<tr>
<td>Milk powder (g)</td>
<td>5, 10, 15</td>
</tr>
</tbody>
</table>

2.4 Fruit Bar Development Trials
The fruit bar development trials were carried out and the responses observed also shown in the table 2.2

2.5 Determination of Sensory Parameters
The sensory parameters of the prepared fruit bar samples like texture and overall acceptability were evaluated on the basis of 9 point hedonic scale using B2 Monadic Test of sensory. The samples were given to 20 semi trained panel members.

<table>
<thead>
<tr>
<th>Run</th>
<th>Factor 1 Sugar (g) $X_1$ (x1)</th>
<th>Factor 2 Milk powder (g) $X_2$ (x2)</th>
<th>Response 1 Texture</th>
<th>Response 2 Overall Acceptability (OA)</th>
<th>Response 3 Ca content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40 (-1)</td>
<td>10 (0)</td>
<td>8.52</td>
<td>8.69</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>50 (0)</td>
<td>10 (0)</td>
<td>8.34</td>
<td>8.5</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>60 (+1)</td>
<td>10 (0)</td>
<td>8.17</td>
<td>8.27</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>50 (0)</td>
<td>10 (0)</td>
<td>8.32</td>
<td>8.43</td>
<td>92</td>
</tr>
<tr>
<td>5</td>
<td>50 (0)</td>
<td>10 (0)</td>
<td>8.3</td>
<td>8.5</td>
<td>93</td>
</tr>
<tr>
<td>6</td>
<td>40 (-1)</td>
<td>5 (-1)</td>
<td>8.1</td>
<td>8.32</td>
<td>84</td>
</tr>
<tr>
<td>7</td>
<td>60 (+1)</td>
<td>5 (-1)</td>
<td>7.27</td>
<td>7.86</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>60 (+1)</td>
<td>15 (+1)</td>
<td>8.36</td>
<td>8.38</td>
<td>105</td>
</tr>
<tr>
<td>9</td>
<td>50 (0)</td>
<td>10 (0)</td>
<td>8.37</td>
<td>8.41</td>
<td>92</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Run</th>
<th>df</th>
<th>Estimated Variables</th>
<th>F- values</th>
<th>P value Prob. &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>5</td>
<td>Texture: 8.39, OA: 8.44, Ca cont: 92.55</td>
<td>Texture: 6.83, OA: 17.01, Ca cont: 238.88</td>
<td>Texture: 0.0128, OA: 0.0009, Ca cont: 0.0001</td>
</tr>
<tr>
<td>X₁</td>
<td>1</td>
<td>-0.11, -0.16, 0.17</td>
<td>2.39, 28.35, 0.32</td>
<td>0.1661, 0.0011**, 0.5920</td>
</tr>
<tr>
<td>X₂</td>
<td>1</td>
<td>0.17, 0.13, 10.17</td>
<td>5.43, 18.24, 1172.92</td>
<td>0.0526, 0.0037**, 0.0001***</td>
</tr>
<tr>
<td>X₁ X₂</td>
<td>1</td>
<td>0.34, 0.095, 0.001</td>
<td>14.33, 6.66, 0.001</td>
<td>0.0068*, 0.0364*, 0.9875</td>
</tr>
<tr>
<td>X₃²</td>
<td>1</td>
<td>-0.22, 0.058, 0.57</td>
<td>4.45, 1.73, 1.69</td>
<td>0.0728, 0.2297, 0.23465</td>
</tr>
<tr>
<td>X₄²</td>
<td>1</td>
<td>-0.18, -0.25, 1.57</td>
<td>3.01, 31.03, 12.86</td>
<td>0.0128, 0.0008***, 0.0089**</td>
</tr>
<tr>
<td>Lack-of-fit</td>
<td></td>
<td></td>
<td></td>
<td>0.0786, 0.1954, 0.1743</td>
</tr>
</tbody>
</table>

| R² | Pred. R² | 0.8298, 0.9239, 0.9842 | 0.7883, 0.8696, 0.9616 |

**Significant at 0.01 level; ***Significant at 0.001 level; *Significant at 0.05 level

3.2.1 Textured Products

3.2 Effect of Ingredients on Response Variables

2.5 Calcium Content Determination

Calcium is determined as calcium pectate. Pectin extracted from plant material is saponified with alkali and precipitated as calcium pectate from an acid solution by addition of calcium chloride. The calcium pectate precipitate is washed until free from chloride, dried and weighed.

2.6 Statistical Analysis for Optimization of Ingredients Quantity

The response functions y (dependent variables) were sugar quantity and milk powder quantity. The following second-order polynomial model was fitted to the dependent variables with the experimental data (Eq.1.1)

\[ y = b_0 + b_1 X_1^1 + b_2 X_2^1 + b_{11} X_1^2 + b_{12} X_1 X_2 + b_{22} X_2^2 \]

The coefficients of the polynomial were represented by \( b_0 \) (constant term), \( b_1 \) and \( b_2 \) (linear effects), \( b_{11} \) and \( b_{22} \) (quadratic effects), and \( b_{12} \) (interaction effects). The analysis of variance (ANOVA) tables were generated and the effects, regression coefficients and ANOVA of the second-order polynomial model for the response variable were utilized. The response functions y (dependent variables) were sugar quantity and milk powder quantity for the optimum ingredients level of kokum pulp, overall acceptability and calcium content were 0.8298, 0.9239 and 0.9842 respectively. The closer the value of \( R^2 \) to unity, the better is the empirical models fit the actual data. On the other hand, the smaller the value of \( R^2 \) the less relevance the dependent variables in the model have in explaining the behaviour of variations (Little & Hills, 1978; Mendehall, 1975).

2.7 Optimization

Numerical and graphical optimization was carried out for the independent variables to obtain the fruit bar with Maximum texture, Overall acceptability scores and calcium content using Design-Expert software. Conventional graphical method was applied to obtain Maximum pulp of all responses. Predictive models were used to graphically represent systems. Contour plots of the response variables were utilized to select optimum ingredients level of kokum pulp sugar, milk powder quantity for the production of kokum fruit bar.

III. Results and Discussions

3.1 Statistical Analysis

Table 3.1 summarizes the results of each dependent variable with their coefficients of determination (\( R^2 \)). The statistical analysis indicates that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the \( R^2 \) for all the responses. The \( R^2 \) values for texture, overall acceptability and calcium content were 0.8298, 0.9239 and 0.9842 respectively. The closer the value of \( R^2 \) to unity, the better is the empirical models fit the actual data. On the other hand, the smaller the value of \( R^2 \) the less relevance the dependent variables in the model have in explaining the behaviour of variations (Little & Hills, 1978; Mendehall, 1975).

3.2 Effect of Ingredients on Response Variables

3.2.1 Texture

x represents the coded level of variables; X represents the actual level of variables.
ANOVA (Table 3.1) indicated that the texture was highly significant at 1% level on interaction term of Sugar and milk powder quantity. Quadratic terms of process variables were not having any significant effect. By neglecting the non significant terms in Eq. 1.1 and with the coded values of independent variables, the following equation (Eq. 1.2) describes the effect of significant process variables on texture of the produced fruit bar.

\[
\text{Texture} = 8.39 + 0.34 \times X_1 \times X_2 (R^2 = 0.82)
\]

Where, \(X_1\) is the sugar quantity in grams; \(X_2\) is the milk powder quantity. The interaction term of pulp quantity, (Eq. 1.2) indicated that colour increased with increase of this variable. The quadratic terms suggested that increase of these variables resulted in increase of colour. The variation of colour with pulp, sugar level were graphically presented in the 3-D surface plot and contour plots (Fig. 3.1a, 3.2b).

### 3.2.2 Overall Acceptability

ANOVA (Table 3.1) indicated that the Anthocyanin content was significant at 1% level on linear terms of sugar quantity and milk powder quantity. Quadratic terms of process variable; milk powder quantity was having significant effect at 0.1% level on overall acceptability of the fruit bar.

\[
\text{O.A.} = 8.44 - 0.16 \times X_1 + 0.13 \times X_2 + 0.095 \times X_1 \times X_2 - 0.25 \times X_2^2 (R^2 = 0.92)
\]

The positive coefficients of the linear term of milk powder quantity (Eq. 1.3) indicated that overall acceptability content increased with increase of milk powder. The quadratic terms suggested that increase of this variable resulted in decrease of overall acceptability. The interaction term showed that increase in the term causes increase of overall acceptability. The variation of Overall acceptability with sugar and milk powder were graphically presented in the 3-D surface plot and contour plots (Fig. 3.3a, 3.4b).

### 3.2.3 Calcium Content

ANOVA (Table 3.1) indicated that the calcium content was significant at 0.1% level on linear term of milk powder quantity. Quadratic terms of process variable; milk powder quantity was having significant effect at 5% level on calcium content. No Interaction term also had any significant effect. By neglecting the non significant terms in Eq. 1.1 and with the coded values of independent variables, the following equation (Eq. 1.4) describes the effect of significant process variables on calcium content of the produced fruit bar.

\[
\text{Calcium content} = 92.55 + 10.17 \times X_2 + 1.57 \times X_2^2 (R^2 = 0.98)
\]

The positive coefficients of the linear term of process variables (Eq. 1.4) indicated that calcium content increased with increase of this variable. The quadratic terms suggested that increase of milk powder quantity.
powder resulted in increase of calcium content. The variation of calcium content with sugar and milk powder were graphically presented in the 3-D surface plot and contour plots (Fig. 3.5a, 3.6b).

![3-D Response surface plots](image1)
![Contour plots](image2)

Fig. 3.3a 3-D Response surface plots
Fig. 3.3b Contour plots
Showing the effect of sugar, milk powder quantity on overall acceptability of fruit bar

Fig. 3.5a 3-D Response surface plots
Fig. 3.6b Contour plots
Showing the effect of sugar, milk powder quantity on calcium content of fruit bar

### 3.3 Numerical Optimization

Figs. 3.7 – 3.9 show the optimum levels of the process parameters to obtain the fruit bar with maximum texture, overall acceptability score and calcium content, respectively by numerical optimization. The contours indicate that when the fruit bar was prepared with pulp, sugar, milk powder; 50g, 40g and 9.39g respectively gives the predicted value of maximum texture score 8.29, overall acceptability score 8.68 and calcium content 91.73mg/100g.
3.4 Optimized Ingredients Level for Kokum Fruit Bar Production

In graphical optimization overlay plot (Fig. 3.10) was obtained by applying superimposing surface methodology to contour plots of the response variables to select optimum levels of ingredients. The optimum was obtained at pulp, sugar, milk powder; 50g, 40g and 9.39g respectively from the overlay plot.
IV. Conclusions

The different levels of ingredients for preparation of kokum fruit bar showed that all these variables markedly affect texture, overall acceptability score and calcium content of the prepared fruit bar. These can be related to the ingredients levels by using second order polynomials. Using the contour plots, the optimum set of the ingredients are obtained graphically for the production of kokum fruit bar. The optimized ingredients level achieved after the numerical and Graphical optimization for maximum texture, overall acceptability score and calcium content were, pulp, sugar, milk powder, 50g, 40g and 9.39g respectively. The desirability of 0.87 was achieved at this optimum point. The predicted values of texture, overall acceptability score and calcium content were 8.29, 8.68, 91.73mg/100g respectively, at optimum ingredients level.

References


