Optimization of Jackfruit Sauce Formulations Using Response Surface Methodology

Pitiporn Ritthiruangdej*, Wannisa Srikanmnoy and Thanut Amatayakul

ABSTRACT

The study aimed to develop a jackfruit sauce product using response surface methodology (RSM), one of the most commonly used optimization techniques in food science. RSM with a three-level, three-factor mixture design was used to optimize the formula of the product. The effects of three independent variables: jackfruit puree (X₁; 45–55%), sugar (X₂; 30–40%) and vinegar (X₃; 5–15%) on the chemical, physical and sensory qualities of jackfruit sauce were investigated. The behavior of the response surface (Yᵢ) was also investigated for the response function by performing a regression analysis procedure with no intercept option. Moreover, graphical optimization was carried out to determine the optimum formula of jackfruit sauce in terms of sensory attributes. The three independent variables had significant (P < 0.05) effects for all response variables. The optimum formula of jackfruit sauce consisted of 45.9% jackfruit puree, 32.4% sugar, 11.7% vinegar, 0.6% pickled chili, 0.3% pickled garlic, 0.9% salt and 8.2% water. The developed jackfruit sauce had an orange-yellow color with CIELAB system values for L*, a*, b*, H° and C° of 37.7, 2.6, 18.9, 97.8° and 19.0, respectively. The total soluble solids, pH, moisture content, titratable acidity and reducing sugar were 38.57 °Brix, 3.72, 62.13, 0.074 and 4.13%, respectively. As calculated by the power law model, the flow behavior index (n) and consistency index (k) were 0.127 and 83.07 mPa.sⁿ, respectively. The evaluation of the developed jackfruit sauce showed that 74% of consumers accepted the product and the overall liking was at the moderate level.

Keywords: response surface methodology (RSM), jackfruit, optimization, sauce, mixture design

INTRODUCTION

Jackfruit (Artocarpus heterophyllus Lam.) is one of the most popular tropical fruits grown in Asia, particularly in Thailand where jackfruit is consumed both as a vegetable in the unripe stage and also as a fruit when ripe. Generally, there is very little research available on jackfruit. The gross composition of jackfruit, its vitamin content, water-soluble sugars, starch, free sugars, fatty acids and flavor volatiles have been documented (Ong et al., 2006). The energy available to humans in jackfruit has been calculated to provide approximately 2 MJ/kg of wet weight ripe perianth. For this reason, it is commonly referred to as the poor man's food (Matior et al., 1995).

Jackfruit is a climacteric fruit with a short shelf life, which quickly becomes unfit for human consumption. During harvesting, the fruit is...
sometimes allowed to fall and must be collected daily as it has a shelf life of only a few days, while mature undamaged fruit can be stored at 12 °C for about 3 wk and ripens in 3–7 d (Pua et al., 2007). Therefore, shelf-stable products using jackfruit as the main ingredient should be developed in order to reduce post-harvest losses as well as to add value to the fruit. In addition, many authors point out that most consumers do not prefer jackfruit due to its intense flavor (Schnel et al., 2001; Jagadeesh et al., 2007). Productivity of the crop is relatively high (25.71 t/ha). However, as the varieties are local types and are mostly of seed origin, the quality of most fruit is not accepted by consumers. Nonetheless, jackfruit is gaining popularity, even in the United States of America, due to emerging ethnic and mainstream marketing opportunities (Jagadeesh et al., 2007). Therefore, it is challenging to provide new jackfruit products that can provide an appealing taste, texture and appearance to meet consumer desires. In the market, a few jackfruit products such as jackfruit with honey, canned jackfruit and jackfruit flavors are available (Pua et al., 2007). Among various categories of new products, the jackfruit sauce developed in this study falls under the category of Invention meaning it has not been previously marketed or produced but is unique and distinctly untried, unfamiliar or even previously nonexistent (Segall, 2000).

Response surface methodology (RSM) is one of the most commonly used optimization techniques in food science, probably because of its comprehensive theory, reasonably high efficiency and simplicity. RSM has been successfully applied to optimize food processing operations. It can be used in solving problems that concern ingredients and/or processing conditions as variables. RSM is a collection of mathematical and statistical techniques useful for designing experiments, building models and analyzing the effects of several independent factors. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple factors and their interactions. Also, the study of the individual and interactive effects of these factors will be helpful in finding the target value. Therefore, RSM provides an effective tool for investigating the aspects affecting the desired response if there are many factors and interactions in the experiment. To optimize the process, RSM can be employed to determine a suitable polynomial equation for describing the response surface (Deshpande et al., 2008; Yin et al., 2009).

The objective of this study was to find an optimum jackfruit sauce formulation using RSM. RSM employing a mixture design was used to determine the optimum ratio of the three ingredients (jackfruit puree, sugar and vinegar) in the jackfruit sauce formulations.

MATERIALS AND METHODS

Materials

Fresh ripe jackfruit was obtained from a local market in Kanchanaburi province, Thailand. Jackfruit ripeness was determined by estimating the skin’s firmness and the number of days after harvest. The maturity of jackfruit used in the present study ranged from 80% to 90%, which was approximately 5–6 d after harvest. Deseeded jackfruit pulp samples were kept in sealed laminated aluminum foil at -20 °C and used throughout the study. Good quality raw materials (vinegar, sugar and salt) were purchased from the local market. Pickled garlic and pickled chili were supplied by Cityfoods Co., Ltd., Thailand.

Experimental design

RSM with a three-level three-factor mixture design was used to optimize the formula of the product. The effects of three independent variables: jackfruit puree (X₁; 45°55%), sugar (X₂; 30°40%) and vinegar (X₃; 5°15%) on the chemical, physical and sensory qualities of jackfruit sauce were investigated. The
characteristic feature of a mixture as described by Castro et al. (2005) is that the sum of all its components add up to 100%. This means that the components (X_i) of the mixture cannot be manipulated completely independently of one another and that their proportions must lie somewhere between 0 and 1. In this study, the lower and upper bound constraints for each mixture component were used to generate the extreme vertices design for the mixture experiment. To facilitate the statistical interpretation of the mixture models, the component proportions were converted to L-pseudo-components (X'_i) using the usual equation (Deshpande et al., 2008) and shown in Equation 1:

\[
X'_i = \frac{(X_i - L_i)}{(1 - L)} \tag{1}
\]

where: 

- \( i = 1,2,3, \ldots, q \)
- \( L = L_1 + L_2 + L_3 + \ldots + L_q \)
- \( X'_1 + X'_2 + X'_3 + \ldots + X'_q = 1 \).

In the definition, the L-values represent the lower bound constraints of the three components (\( L_1 = 0.45, L_2 = 0.30 \) and \( L_3 = 0.05 \)), and the range of X-values represent the original lower and upper bound constraints (\( X_1: 0.45^\circ 0.55; X_2: 0.30^\circ 0.40; \) and \( X_3: 0.05^\circ 0.15 \)). Substituting these values in Equation 1 resulted in redefined constraints for the mixture design as \( X'_1 = 0-0.50, X'_2 = 0-0.50, \) and \( X'_3 = 0-0.50 \). These redefined experimental design points are given in Table 1.

In this study \( q = 3 \), which described the number of mixture variables in the design (\( X_1, X_2 \) and \( X_3 \)).

### Jackfruit sauce preparation

Jackfruit sauces were prepared in the laboratory. Each sample amount (g/100 g) was based on the total weight of the main three ingredients (jackfruit puree, sugar and vinegar). The other ingredients used for making the jackfruit sauces were: 0.7 g pickled chili, 0.3 g pickled garlic, 1.0 g salt and 9.0 g water. The jackfruit puree was mixed with the amount of sugar and vinegar added to jackfruit puree according to the experimental design in Table 1.

In brief, the deseeded jackfruit pulp was blanched in boiling water for 5 min. After cooling in an ice bath, the pulp was placed in a blender (Panasonic, model MX-J210GN) for 30 s at low speed and for 1 min at high speed. The pickled garlic and pickled chili were dissolved in the vinegar and this solution was filtered and added to the jackfruit puree in an open pan. The mixture was then heated on a hot plate, set at a moderate temperature, and stirred continuously until the mixture reached the desired temperature of 80 °C. Then, sugar and salt were added to the mixture and the sauce was heated for 5 min. While still hot, jackfruit sauce samples were poured into glass jars, sealed with rubber seal screw caps and then stored at ambient temperature (25 °C) in the dark for 24 h before the analyses.

### Chemical analysis

Moisture content, reducing sugar, pH and titratable acidity were determined in triplicate.

### Table 1  Experimental design for jackfruit sauce formulations.

<table>
<thead>
<tr>
<th>Formulation number</th>
<th>X_1 (Actual value (%))</th>
<th>X_2</th>
<th>X_3</th>
<th>X'_1</th>
<th>X'_2</th>
<th>X'_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55.00</td>
<td>35.00</td>
<td>10.00</td>
<td>0.50</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>55.00</td>
<td>30.00</td>
<td>15.00</td>
<td>0.50</td>
<td>0.00</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td>50.00</td>
<td>35.00</td>
<td>15.00</td>
<td>0.25</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>45.00</td>
<td>40.00</td>
<td>15.00</td>
<td>0.00</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>5</td>
<td>50.00</td>
<td>40.00</td>
<td>10.00</td>
<td>0.25</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>55.00</td>
<td>40.00</td>
<td>5.00</td>
<td>0.50</td>
<td>0.50</td>
<td>0.00</td>
</tr>
</tbody>
</table>

\( X_1 = \) jackfruit puree; \( X_2 = \) sugar; \( X_3 = \) vinegar.
according to the AOAC methods (1990). Titratable acidity was determined as the percent of citric acid by titration with 0.1 N NaOH solution using phenolphthalein as the indicator. The pH values were measured with a digital pH meter.

**Physical analysis**

Total soluble solids (TSS) of jackfruit sauce samples were measured using a hand refractometer and expressed as °Brix. The color of all samples was measured using a Chroma meter (Konica Minolta, model CR400). In the CIELAB system, L* indicates the degree of lightness or darkness (L* = 0 indicating perfect black and L* =100 indicating perfect white), a* and b* indicate the degree of redness or greenness and yellowness or blueness, respectively, while C* indicates the chroma and H* is a measure of the hue angle (°). The viscosity of the jackfruit sauce samples was determined at a constant temperature of 25 °C with a Brookfield viscometer (Model DV-II, Brookfield Engineering Labs, Inc, Stoughton, MA) equipped with a temperature control unit. Spindle number 34 for small samples was used. Measurements were performed over a shear rate range of 2.5–7.0 rpm. The obtained empirical data were converted into shear stress and shear rate data as described by Sahin and Ozdemir (2004). The shear rates versus shear stress data were interpreted using the power law expression \( \tau = k\gamma^n \) where \( \tau \) is the shear stress, \( \gamma \) is the shear rate, \( n \) is the flow behavior index, and \( k \) is the consistency index, mPa.s\(^n\).

**Sensory analysis**

For the sensory test, the panelists used throughout this study were recruited through personal communication and their willingness to undertake this research. They were all students at Mahidol University, Kanchanaburi Campus, Kanchanaburi, Thailand, who had eaten ripe jackfruit. In order to find an optimum jackfruit sauce formulation using RSM, thirty untrained panelists evaluated color, flavor, taste, texture and overall liking of the 6 sauce samples using a 9-point hedonic scale, where 9 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely. The sauce samples were presented monadically for each panelist. Subsequently, the developed jackfruit sauce obtained from RSM was subjected to the consumer sensory test in order to assess consumer preference and buying criteria for the jackfruit sauces. One hundred consumers (22 males and 78 females) evaluated the color, flavor, taste, texture, overall liking, purchase intention and global impression of the developed jackfruit sauces. Each consumer evaluated the samples using the same nine-point hedonic scales.

**Statistical analysis**

The STATISTICA 5.0 (StatSoft Inc., Tulsa, OK, USA) software was used for the experimental design and SPSS 17.0 for Windows (SPSS, Chicago, IL, USA) software was used for data analysis. The behavior of the response surface \( Y_i \) was investigated for the response function by performing a regression analysis procedure with no intercept option because the model components were required to sum to 100. An alternative system of coordinates involving lower bound pseudo-components, or L-pseudo-components, was utilized to set up a design for fitting a model over a sub-region. A first-degree model was fitted to the responses (chemical, physical and sensory data). The statistical significance of the terms in the regression equations was examined by ANOVA for each response and the significance test level was set at 5% \( (P < 0.05) \).

Graphical optimization was carried out to determine the optimum formula of jackfruit sauce in terms of sensory attributes. Six sensory attributes (appearance, color, viscosity, flavor, taste and overall acceptability) were considered as response variables whereas jackfruit puree, sugar and vinegar were considered as independent variables.

The fitted models for all the attributes
RESULTS AND DISCUSSION

Chemical compositions, physical and sensory qualities

The mean values of the chemical compositions and physical and sensory qualities of the jackfruit sauces are shown in Table 2. All responses were significantly different among assays, which is a basic requirement for further RSM application (Granato et al., 2010). The chemical compositions for all samples ranged from 57.62 to 66.45% for moisture content, 3.42 to 3.97 for pH, 0.047 to 0.102% for citric acid and 4.46 to 6.80% for reducing sugar. The total soluble solids values ranged from 34.5 to 41.8 °Brix. The color characteristics for all sauce samples ranged from 34.20 to 37.26 for L* (lightness), -2.44 to -1.46 for a* (greenness), 13.99 to 18.86 for b* (yellowness), 95.27 to 97.37° for H* and 14.09 to 19.02 for C*. It was observed that the color of the jackfruit sauce samples was characterized as yellow because the H* value was close to 90° and b* was positive indicating a yellow color. Ong et al. (2006) reported that the color H* parameter could be used to express the changes in jackfruit pulp color during ripening. The significant increase in the hue value with ripening may be attributed to an increase in the carotenoid content in the ripe fruit pulp.

The viscosity of fluid foods is an

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Chemical, physical and sensory qualities of jackfruit sauces.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>61.53 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>3.67 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total soluble solid (%)</td>
<td>0.070± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reducing sugar (%)</td>
<td>5.67 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Color CIE L*,a*,b*</td>
<td></td>
</tr>
<tr>
<td>- L*</td>
<td>35.80 ± 0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>- a*</td>
<td>-1.61 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>- b*</td>
<td>15.74 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>- C*</td>
<td>15.83 ± 0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>- H*</td>
<td>95.88 ± 1.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Viscosity behavior&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>- Flow behavior index (n)</td>
<td>0.366± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Consistency index (k,Pa.S&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>67.20 ± 1.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sensory attributes</td>
<td></td>
</tr>
<tr>
<td>- Color</td>
<td>7.2 ± 1.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Appearance</td>
<td>7.0 ± 1.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Viscosity</td>
<td>6.9 ± 1.43&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Flavor</td>
<td>6.7 ± 1.68&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Taste</td>
<td>6.5 ± 1.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Overall acceptability</td>
<td>6.8 ± 1.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are shown as mean ± standard deviation.

Different lower-case letters in a row indicates the mean values differ significantly at P = 0.05.

<sup>+</sup>Viscosity behavior parameters, n and k, were obtained by fitting a power law model to the experimental data.
important parameter of their texture as it determines to a great extent the overall feel in the mouth and influences the intensity of the flavor (Sharoba et al., 2005). The power law equation was found to be an adequate model to describe the flow behavior of the samples in this study. The flow behavior index \((n)\) and consistency index \((k)\) values obtained by fitting the power law model to the experimental shear stress-shear rate data are given in Table 2. The \(n\) and \(k\) values were in the ranges 0.331–0.434 and 38.06–78.80 mPa.S \(n\), respectively. Viscosity function data showed that all jackfruit sauce samples under examination were non-Newtonian fluids, since the values for flow behavior indices \((n)\) were below 1, which was indicative of pseudoplasticity (shear thinning). The pseudoplastic behavior is a characteristic of tomato ketchups (Koocheki et al., 2009).

The mean hedonic ratings of the 6 sensory attributes are presented in Table 2. It was observed that the mean ratings for overall acceptability were between 6 (like slightly) and 7 (like moderately). Jackfruit sauce formulation #5 \((X_1 = 50.0 \text{ g/100g}; X_2 = 40.0 \text{ g/100g}; X_3 = 10.0 \text{ g/100g})\) had the significantly highest mean ratings for color (7.3), viscosity (7.2), flavor (6.9) and overall acceptability (7.0) of the jackfruit sauce formulations. Jackfruit sauce formulation #3 received the highest mean ratings for appearance (7.7), while jackfruit sauce formulation #4 received the highest mean ratings for taste (6.8). It was noted that jackfruit sauce formulation #6, which had a low level of vinegar \((5.0 \text{ g/100g})\) was rated low (less than 6.0) for the appearance, taste, flavor and overall acceptability attributes. This indicated that low amounts of vinegar \((5.0 \text{ g/100g})\) were not preferred.

**Model fitting**

The effects of the three independent variables: jackfruit puree, sugar and vinegar on the chemical, physical and sensory qualities of jackfruit sauce are shown in Table 3. The independent and dependent variables were fitted to the first order model equation and examined for goodness of fit. The correlation coefficients for all chemical, physical and sensory qualities were quite high for a response surface \((R^2 > 0.99)\). Analysis of variance was used to assess how well the model represented the data. To evaluate the goodness of fit of the model, F-value tests were conducted. The F-values for all responses were significant at the 95% confidence level (Table 3). The analysis of variance indicated that the predictive models adequately represented the data for the chemical, physical and sensory qualities of jackfruit sauce.

**Generation of contour plots**

Some important stages in the application of RSM as an optimization technique are: 1) the selection as independent variables of the major effects on the system through screening studies and the delimitation of the experimental region, according to the objective of the study and the experience of the researcher; 2) the choice of the experimental design and experimentation according to the selected experimental matrix; 3) the mathematical-statistical treatment of the obtained experimental data through the fit of a polynomial function; 4) the evaluation of the model’s fitness; 5) the verification of the necessity and possibility of performing a displacement in direction to the optimal region; and 6) obtaining the optimum values for each studied variable (Bezerra et al., 2008).

According to the predictive models for sensory attributes (Table 3), optimization was carried out by generating contour plots for each attribute as shown in Figure 1. In the present study, the lower limit of consumer liking ratings for all of the attributes was set at consumer ratings greater than or equal to 6.0 (like slightly) on a 9-point hedonic scale.
Optimization of jackfruit sauce formulation

In order to determine the optimum formulation, the regions of acceptability in the contour plot for each attribute were superimposed. Superimposition of contour plot regions of interest (within which, each attribute received hedonic ratings greater than or equal to 6.0) resulted in optimum regions for jackfruit sauce formulation. The area of overlap obtained is thus represented as the shaded region in jackfruit sauce formulations in Figure 2. The shaded regions indicate that any point within this area represents a combination of jackfruit puree, sugar and vinegar that would result in consumer acceptance for all of the sensory attributes (hedonic ratings greater than or equal to 6.0). The optimum jackfruit sauce formulations were determined as all combinations from 45.0 g/100 g to 57.0 g/100 g jackfruit puree, from 30.0 g/100 g to 50.0 g/100 g sugar and from 5.0 g/100 g to 25.0 g/100 g vinegar, which in each case totaled 100 g. Based on the superimposed plots, the selected optimal ingredient (independent variable) levels were 51.0 g/100 g jackfruit puree ($X'_1 = 0.30$), 36.0 g/100 g sugar ($X'_2 = 0.30$), and 13.0 g/100 g vinegar ($X'_3 = 0.40$).

In order to verify the optimum formulation, the jackfruit sauce using the optimal ingredient level was analyzed and the results were listed in Table 3.
Figure 1 Contour plots for hedonic ratings of 6 sensory attributes obtained using L-pseudo-components for jackfruit sauce formulations.

Figure 2 Optimum regions (shaded areas) obtained by superimposing contour plots for all 6 attributes for jackfruit sauce formulation.

statistically compared to the predicted values of the mathematical model. The predicted response values and the actual obtained response values for the optimized products were within the range and found to be not statistically different at the 95% confidence level. The analysis showed that the jackfruit sauce developed had an orange-yellow color with CIELAB system L*, a*, b*, H* and C* values of 37.7, -2.6, 18.9, 97.8°, and 19.0, respectively. The total soluble solids, pH, moisture content, titratable acidity, and reducing sugar were 38.57 °Brix, 3.72, 62.13, 0.074, and 4.13%, respectively. As calculated by the power law model, the flow behavior index (n) and consistency index (k) were 0.127 and 83.07 mPa.S^n, respectively.

Consumer sensory test

Overall, 100 people participated in the consumer test. Consumers were asked to complete a short questionnaire following the acceptance test. The questionnaire asked for demographic information and examined jackfruit consumption habits. Of the respondents, 74% accepted the product and the overall liking was at the moderate level. The consumer liking ratings for color, appearance, viscosity, flavor, taste and overall acceptability were 7.37, 7.26, 7.08, 6.07, 7.14, and 7.25, respectively. According to this consumer test,
CONCLUSION

RSM was successfully used to identify the best combination of jackfruit puree, sugar and vinegar for a jackfruit sauce. The final goal was to obtain an innovative jackfruit sauce with a high sensory acceptance and suitable physicochemical properties. The optimum formula of jackfruit sauce consisted of 45.9% of jackfruit puree, 32.4% sugar, 11.7% vinegar, 0.6% pickled chili, 0.3% pickled garlic, 0.9% salt and 8.2% water. The developed jackfruit sauce had an orange-yellow color with $L^*$, $a^*$, $b^*$, $H^*$ and $C^*$ values of 37.7, -2.6, 18.9, 97.8° and 19.0, respectively. As calculated by the power law model, the flow behavior index ($n$) and consistency index ($k$) were 0.127 and 83.07 mPa·s, respectively. Moreover, the modeling of experimental data allowed the generation of useful equations for general use in predicting the quality of jackfruit sauce under different combinations of factors.

ACKNOWLEDGEMENTS

This research study was funded by the Industrial and Research Projects for Undergraduate Students (IRPUS), Thailand Research Fund (TRF) under financial support for project I352A10014. The authors would like to thank Cityfoods Co., Ltd., Thailand for supplying the pickled chili and pickled garlic used throughout the study. Thanks are also recorded to Ms. Ornnattha Supatham, Mahidol University, Kanchanaburi Campus, Thailand, for helpful suggestions on the manuscript.

LITERATURE CITED


