SP5: Sensory and consumer research on indigenous food
Development of jackfruit sauce using response surface methodology

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ABSTRACT

Jackfruit (Artocarpus heterophyllus L.) is a climacteric fruit with short shelf life that quickly becomes unfit for human consumption. Therefore, shelf stable products using jackfruit as a main ingredient should be developed in order to reduce post-harvest losses as well as to add value to the fruit. The aim of the present study was to develop the jackfruit sauce product by using Response Surface Methodology (RSM), one of the most commonly used optimization technique in food science. RSM with a three-level three-factor mixture design was used to optimize the formula of product. The effects of three independent variables; jackfruit puree (X1: 45-55%), sugar (X2: 30-40%), and vinegar (X3: 5-15%) on 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 the 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 were significant at p≤0.05 for all response variables. 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, -4.5, 47.8, 97.5°, 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. The evaluation on the developed jackfruit sauce showed that 74% of consumers accepted the product and the overall acceptability was at the moderate level. The present study indicated that RSM is a useful experimental technique in determining the optimum formula of jackfruit sauce. Percentages of jackfruit, sugar, and vinegar incorporated significantly affected the chemical, physical and sensory qualities. The model equation developed can be used for predicting the quality of jackfruit sauce.

Keywords: 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 and 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 wet weight ripe perianth. For this reason, it is commonly referred to as “the poor man’s food” (Matior Rahman et.al., 1995).
Jackfruit is a climacteric fruit with 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 weeks and ripen in 3–7 days (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. Productivity of the crop is relatively high (25.71 t/ha). But as the varieties are of local types and are mostly of seed origin, the quality of most fruits is not accepted by consumers. However, jackfruit is gaining popularity, even in the United States, due to emerging ethnic and mainstream marketing opportunities (Jagadeesh et al., 2007). Therefore, it is challenging to provide new jackfruit product that ensures appealing taste, texture, and appearance to 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 effort to find 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 by using response surface methodology (RSM). RSM employing 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 this study ranged from 80% to 90%, which was approximately 5–6 days after harvest. Deseeded jackfruit pulps were kept in a sealed laminated aluminium foil at -20°C and used throughout this study. Good-quality raw materials i.e., vinegar, sugar, and salt were purchased from the local market. Pickled garlic and pickled chili were supported by Cityfoods Co., Ltd., Thailand.
Methods

1. Experimental design

RSM with a three-level three-factor mixture design was used to optimize the formula of product. The effects of three independent variables: jackfruit puree ($X_1$: 45-55%), sugar ($X_2$: 30-40%), and vinegar ($X_3$: 5-15%) on 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 these components ($x_i$) mixture factors 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):

$$X'_i = \frac{(X_i - L_i)}{(1 - L)}$$

where $i = 1, 2, 3, \ldots, q$, $L = L_1 + L_2 + L_3 + \ldots + L_q$, and $X'_1 + X'_2 + X'_3 + \ldots + X'_q = 1$. In the definition, the $L$-values represented lower bound constraints of three components ($L_1 = 0.45$, $L_2 = 0.30$, and $L_3 = 0.05$), and the range of $X$-values represented the original lower and upper bound constraints ($X_1$: 0.45-0.55; $X_2$: 0.30-0.40; and $X_3$: 0.05-0.15). Substituting these values in the definition resulted in redefined constraints for the mixture design as $X'_1 = 0.50$, $X'_2 = 0.50$, and $X'_3 = 0.50$. These redefined experimental design points are given in Table 1. In this study $q = 3$; described as the number of mixture variables in the design ($X_1$, $X_2$, and $X_3$).

<table>
<thead>
<tr>
<th>Formulation number</th>
<th>Actual values (%)</th>
<th>$L$-pseudo-component values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$X_1$</td>
<td>$X_2$</td>
</tr>
<tr>
<td>1</td>
<td>55.00</td>
<td>35.00</td>
</tr>
<tr>
<td>2</td>
<td>55.00</td>
<td>30.00</td>
</tr>
<tr>
<td>3</td>
<td>50.00</td>
<td>35.00</td>
</tr>
<tr>
<td>4</td>
<td>45.00</td>
<td>40.00</td>
</tr>
<tr>
<td>5</td>
<td>50.00</td>
<td>40.00</td>
</tr>
<tr>
<td>6</td>
<td>55.00</td>
<td>40.00</td>
</tr>
</tbody>
</table>

$X_1$: jackfruit puree, $X_2$: sugar, $X_3$: vinegar

2. Jackfruit sauce preparation

Jackfruit sauces were prepared in the laboratory. An amount (g/100 g) based on the total weight of the main three ingredients (jackfruit puree, sugar, and vinegar), the other ingredients used for making 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 de-seeded jackfruit pulps were blanched in boiling water for 5 min. After cooling by using an ice bath, these were blended with a blender 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 darkness for 24 h before the analyses.

3. Chemical analysis

Moisture content, reducing sugar, pH, and titratable acidity were determined according to the AOAC methods (1990) in triplicate. Titratable acidity was determined as 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.

4. Physical analysis

Total soluble solids (TSS) of jackfruit sauce samples were measured by using a hand refractometer and expressed as °Brix. The color of all samples was measured by using a Chroma meter (Konica Minolta), model CR400. In the CIE L*a*b* system, L* indicates degree of lightness or darkness (L* = 0 indicating perfect black and L* =100 indicating perfect white); a* and b* indicate degree of redness or greenness and yellowness or blueness, respectively. C* indicates chroma and H* is a measure of hue angle (°) (Mukprasirt and Sajjaanantakul, 2004). The viscosity of jackfruit sauce samples was determined at constant temperature of 25 °C with a Brookfield viscometer (Model: DV-II, Brookfield Engineering Labs, Inc, Stoughton, MA) equipped with a temperature control unit. The spindle number 34 for small sample 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 (τ = kγ^n; where τ is the shear stress, γ is the shear rate, n is the flow behavior index, and k is the consistency index, mPa.s^n).

5. Sensory analysis

For 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, Thailand, who had eaten ripe jackfruit. In order to find an optimum jackfruit sauce formulation by using RSM, thirty untrained panelists evaluated color, flavor, taste, texture, and overall acceptability of the six sauce samples by using eight-point hedonic scales, 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 jackfruit sauce. One hundred consumers (22 males and 78 females) evaluated the color, flavor, taste, texture, overall acceptability, purchase intention and global impression of the developed jackfruit sauce. Each consumer evaluated the sample using the same nine-point hedonic scales.

6. Statistical analysis

The behavior of the response surface (Y_i) was investigated for the response function by performing the 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 was fitted to the responses (chemical, physical, and sensory data).
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 independent variables.

The fitted models for all the attributes were used to generate three-dimensional response surfaces as well as contour plots using STATISTICA statistical software. Superimposition of contour plot regions of interest (within which each attribute received hedonic ratings > 6.0) resulted in optimum regions for jackfruit sauce formulation.

Results and Discussion

1. Chemical compositions, physical and sensory qualities

The mean values of the chemical compositions, physical, and sensory qualities of the jackfruit sauces are shown in Table 2. All responses presented statistical difference (p < 0.05) among assays, basic requirements for further RSM application (Granato et al., 2010).

Table 2 Chemical, physical, and sensory qualities of jackfruit sauces.

<table>
<thead>
<tr>
<th>Jackfruit sauce qualities</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Moisture (%)</td>
<td>61.53±0.20b</td>
<td>66.45±0.12a</td>
<td>61.56±0.26b</td>
<td>58.29±0.27b</td>
<td>58.57±0.21b</td>
<td>57.62±0.21b</td>
</tr>
<tr>
<td>2. pH</td>
<td>3.67±0.01f</td>
<td>3.62±0.01f</td>
<td>3.63±0.01f</td>
<td>3.42±0.01f</td>
<td>3.62±0.00d</td>
<td>3.97±0.00c</td>
</tr>
<tr>
<td>3. Titratable acidity (%)</td>
<td>0.070±0.00f</td>
<td>0.094±0.00f</td>
<td>0.102±0.10f</td>
<td>0.092±0.00f</td>
<td>0.069±0.00f</td>
<td>0.047±0.00f</td>
</tr>
<tr>
<td>4. Reducing sugar (%)</td>
<td>5.67±0.01c</td>
<td>5.7±0.27b</td>
<td>6.8±0.25a</td>
<td>6.04±0.12a</td>
<td>4.96±0.42c</td>
<td>4.46±0.04d</td>
</tr>
<tr>
<td>5. Total soluble solid (% Brix)</td>
<td>37.9±0.12b</td>
<td>34.5±0.00d</td>
<td>38.6±0.06d</td>
<td>41.8±0.00d</td>
<td>41±0.00d</td>
<td>41.6±0.06b</td>
</tr>
<tr>
<td>6. Color CIE L<em>a</em>b*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>- L*</td>
<td>35.80±0.27b</td>
<td>37.02±0.44a</td>
<td>35.91±0.63b</td>
<td>34.51±0.38b</td>
<td>34.20±0.09f</td>
<td>37.26±0.15d</td>
</tr>
<tr>
<td>- a*</td>
<td>-1.61±0.30b</td>
<td>-2.39±0.03b</td>
<td>-1.56±0.12b</td>
<td>-1.46±0.09b</td>
<td>-1.62±0.08b</td>
<td>-2.44±0.09b</td>
</tr>
<tr>
<td>- b*</td>
<td>15.74±0.82a</td>
<td>18.56±0.28a</td>
<td>17.01±0.82a</td>
<td>14.75±0.59a</td>
<td>13.99±0.52a</td>
<td>18.86±0.24a</td>
</tr>
<tr>
<td>- C*</td>
<td>15.83±0.79a</td>
<td>17.89±0.27a</td>
<td>17.08±0.81a</td>
<td>14.82±0.58a</td>
<td>14.09±0.51a</td>
<td>19.02±0.24a</td>
</tr>
<tr>
<td>- H*</td>
<td>95.88±1.38b</td>
<td>97.34±0.18b</td>
<td>95.27±0.66b</td>
<td>95.68±0.56b</td>
<td>96.63±0.52b</td>
<td>97.37±0.30b</td>
</tr>
<tr>
<td>7. Viscosity behavior</td>
<td></td>
<td></td>
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<tr>
<td>- Flow behavior index (n)</td>
<td>0.366±0.02b</td>
<td>0.331±0.01b</td>
<td>0.411±0.01b</td>
<td>0.333±0.01b</td>
<td>0.434±0.02c</td>
<td>0.423±0.00c</td>
</tr>
<tr>
<td>- Consistency index (k)</td>
<td>67.2±1.16b</td>
<td>64.66±5.91b</td>
<td>58.36±1.09b</td>
<td>38.06±0.22d</td>
<td>55.65±0.46d</td>
<td>78.8±1.08d</td>
</tr>
<tr>
<td>8. Sensory attributes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Color</td>
<td>7.2±1.24a</td>
<td>6.8±1.27a</td>
<td>7.1±1.47a</td>
<td>7.1±1.38a</td>
<td>7.3±1.11a</td>
<td>6.6±1.58b</td>
</tr>
<tr>
<td>- Appearance</td>
<td>7.0±1.27a</td>
<td>6.2±1.65b</td>
<td>7.7±1.60a</td>
<td>6.7±1.51b</td>
<td>6.9±1.32b</td>
<td>5.9±1.74a</td>
</tr>
<tr>
<td>- Viscosity</td>
<td>6.9±1.43b</td>
<td>6.5±1.43b</td>
<td>6.8±1.61ab</td>
<td>6.3±1.82b</td>
<td>7.2±1.39a</td>
<td>6.8±1.49b</td>
</tr>
<tr>
<td>- Flavor</td>
<td>6.7±1.68a</td>
<td>6.2±1.54bc</td>
<td>5.9±1.62b</td>
<td>6.6±1.81bc</td>
<td>6.9±1.36b</td>
<td>5.6±1.86d</td>
</tr>
<tr>
<td>- Taste</td>
<td>6.5±1.77a</td>
<td>6.1±1.19a</td>
<td>6.4±1.58a</td>
<td>6.8±1.95b</td>
<td>6.6±1.63a</td>
<td>5.1±1.71b</td>
</tr>
<tr>
<td>- Overall acceptability</td>
<td>6.8±1.64a</td>
<td>6.4±1.03b</td>
<td>7.0±1.32a</td>
<td>6.8±1.53b</td>
<td>7.0±1.35b</td>
<td>5.7±1.48b</td>
</tr>
</tbody>
</table>

Different letters in a row means the values differ significantly at P = 0.05.

Viscosity behavior parameters, n and k, were obtained by fitting Power law model to the experimental data.

The chemical compositions for all samples ranged from 57.62 to 66.45% moisture, 3.42 to 3.97 pH, 0.047 to 0.102% citric acid, 4.46 to 6.80% reducing sugar, respectively. The total soluble solids values ranged from 34.5 to 41.80°Brix. The color
characteristics for all sauce samples ranged from 34.20 to 37.26 L* value (lightness), -2.44 to -1.56 a* value (greenness), 13.99 to 18.86 b* value ( yellowness), 95.27 to 97.37° H*, and 11.99 to 18.79 C*, respectively. It was observed that the color of jackfruit sauce samples were characterized as yellow because the H* value was closed to 90° and b* was positive indicating 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 hue value, which increased significantly with ripening, may be attributed to an increase in carotenoid contents in ripe fruit pulp. The viscosity of fluid foods is an important parameter of their texture. 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) obtained by fitting of the Power law model to the experimental shear stress-shear rate data are given in Table 2. The n and k values ranged between 0.331 to 0.434, and 38.06 to 78.8, respectively. Viscosity functions 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 the pseudoplastic (shear thinning). The pseudoplastic behavior is the nature of tomato ketchups (Koocheki et al., 2009).

The mean hedonic ratings of six sensory attributes 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 (X₁ = 50.0 g/100g, X₂ = 40.0 g/100g, X₃ = 10.0 g/100g) received significantly the highest mean ratings for color (7.3), viscosity (7.2), flavor (6.9), and overall acceptability (7.0) of the jackfruit sauce formulations. The result also showed that 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 formulations #6 having low level of vinegar (5.0 g/100g) were rated low for appearance, taste, flavor, and overall acceptability attributes (<6.0). This indicated that lower extremes of vinegar (5.0 g/100g) were not preferred.

2. Model fitting

The effects of three independent variables: jackfruit puree, sugar, and vinegar on 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 the goodness of fit. The correlation coefficient for all chemical, physical, and sensory qualities were quite high for a response surface (R² >0.99). An analysis of variance was calculated to assess how well the model represents the data. To evaluate the goodness of the model, the F-value tests were conducted. The F-value for all responses was significant at the 95% level, as shown in Table 3. On the basis of the analysis of variance, the conclusion is that the predictive models adequately represent the data for chemical, physical, and sensory qualities of jackfruit sauce.

3. Generation of contour plots

Some stages in the application of RSM as an optimization technique are as follows: (1) the selection of independent variables of 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 carrying out the experiments according to the selected
experimental matrix; (3) the mathematic–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), the optimization was done by generating contour plots for each attribute as shown in Figure 1. In this study, the lower limit of consumer acceptability ratings for all of the attributes was decided to be consumer ratings \( \geq 6.0 \) (like slightly) on a 9-point hedonic scale.

**Table 3** Regression coefficients, \( R^2 \), and \( P \) or probability values for chemical, physical, and sensory variables for jackfruit sauce formulation

<table>
<thead>
<tr>
<th>Dependent variables ((Y_i))</th>
<th>Predictive models (Y_i = a_1X'_1 + a_2X'_2 + a_3X'_3)</th>
<th>(R^2)</th>
<th>(P) or probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Moisture ((%))</td>
<td>65.446* 50.020* 66.542*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>2. pH</td>
<td>4.071* 3.724* 3.170*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>3. Titratable acidity ((%))</td>
<td>0.048* 0.043* 0.145*</td>
<td>0.998</td>
<td>0.000*</td>
</tr>
<tr>
<td>4. Reducing sugar ((%))</td>
<td>4.469* 4.445* 7.901*</td>
<td>0.995</td>
<td>0.001*</td>
</tr>
<tr>
<td>5. Total soluble solid ((^\circ\text{Brix}))</td>
<td>34.174* 48.310* 35.166*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>6. Color CIE L<em>a</em>b*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- L*</td>
<td>38.986* 33.690* 34.674*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>- a*</td>
<td>-2.876* -1.396 -1.268</td>
<td>0.997</td>
<td>0.006*</td>
</tr>
<tr>
<td>- b*</td>
<td>20.837* 13.341* 15.277*</td>
<td>0.994</td>
<td>0.001*</td>
</tr>
<tr>
<td>- C*</td>
<td>21.010* 13.410* 15.330*</td>
<td>0.994</td>
<td>0.001*</td>
</tr>
<tr>
<td>- H*</td>
<td>98.030* 96.110* 94.910*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>7. Viscosity behavior†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- flow behavior index ((n))</td>
<td>0.400* 0.457* 0.292*</td>
<td>0.993</td>
<td>0.001*</td>
</tr>
<tr>
<td>- consistency index ((k, \text{mPa.s}^n))</td>
<td>101.807* 50.007* 29.551*</td>
<td>0.997</td>
<td>0.000*</td>
</tr>
<tr>
<td>8. Sensory attributes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Color</td>
<td>6.590* 7.150* 7.310*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>- Appearance</td>
<td>5.950* 6.670* 7.230*</td>
<td>0.997</td>
<td>0.000*</td>
</tr>
<tr>
<td>- Viscosity</td>
<td>7.070* 6.990* 6.190*</td>
<td>0.999</td>
<td>0.000*</td>
</tr>
<tr>
<td>- Flavor</td>
<td>5.757* 6.509* 6.669*</td>
<td>0.995</td>
<td>0.001*</td>
</tr>
<tr>
<td>- Taste</td>
<td>4.970* 6.170* 7.610*</td>
<td>0.997</td>
<td>0.000*</td>
</tr>
<tr>
<td>- Overall acceptability</td>
<td>5.710* 6.510* 7.630*</td>
<td>0.997</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

\(X'_1\): L-pseudo-component values, \(X'_2\): Jackfruit, \(X'_3\): Sugar, \(X'_4\): Vinegar

* Significant at \(p < 0.05\).
4. 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 ≥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 (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 ≥6.0). The optimum formulations for jackfruit sauce formulations were determined as all combinations of 45.0 g/100 g – 57.0 g/100 g jackfruit puree, 30.0 g/100 g – 50.0 g/100 g sugar, and 5.0 g/100 g – 25.0 g/100 g vinegar, adding up to 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 experimentally analyzed and the results were statistically compared to the predicted values of the mathematical model. We found that 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 5% level. As experimentally analyzed, the developed jackfruit sauce had an orange-yellow color with L*, a*, b*, H*, and C* values of 37.7, -4.5, 47.8, 97.5°, 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, respectively.

Figure 1 Contour plots for hedonic ratings of six sensory attributes obtained using L-pseudo-components for jackfruit sauce formulations.
5. Consumer sensory test

Overall, 100 persons participated in the consumer test. Consumers were asked to complete a short questionnaire following the preference test. This questionnaire asked demographic information, and examined jackfruit consumption habits. It was reported that 74% of consumers accepted the product and the overall acceptability was at the moderate level. The consumer acceptability rating 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, the category of invention product is the main reason for the consumption of jackfruit sauce.

Conclusions

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 8.1% water. The developed jackfruit sauce had an orange-yellow color with L*, a*, b*, H*, and C* values of 37.7, -4.5, 47.8, 97.5°, 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 factor combinations.

Acknowledgments

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SP5-02

Development of slowly digestible dried noodle products supplemented with unripe banana flour

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ABSTRACT

Unripe banana is both a source of carbohydrates and interesting bioactive nutrients. Unripe banana flour is a starchy food that contains a high proportion of indigestible compounds such as resistant starch, and non-starch polysaccharides which includes in the dietary fiber content. The objective of this study was to use unripe banana flour as an ingredient to make dried noodle products of high nutritional quality with low carbohydrate digestibility and rich resistant starch. The effect of wheat flour substitution with unripe banana flour was investigated in terms of physicochemical, textural, cooking, and sensory qualities of dried noodles. The ratios of the mixtures of banana flour per wheat flour studied were 0:100, 10:90, 20:80, 30:70, 40:60, and 50:50. The optimal ratio of noodle from banana flour was investigated using sensory qualities in comparison to the controlled (0:100). The results of noodle formula development indicated that as the amount of banana flour increased, the stickiness of noodle decreased and the appearance grew darker. The optimum formula consisted of 20.45% banana flour, 47.72% wheat flour, 20.45% water, 2.04% salt, 1.02% sodium carbonate, 6.82% egg powder, 0.14% polyphosphate, and 1.36% propylene glycol. Thirty percent of banana flour was used to replace wheat flour in the formula for total flour 100%. Uncooked dried noodle was composed of 13.7% protein, 0.12% fat, and 4.8% dietary fiber (including 2.8% resistant starch). The optimal cooking time and cooking loss were 14.5 min and 11.15%, respectively. Tensile strength and breaking length of cooked noodle were 16.4g and 67.2 mm, respectively. The results of consumer evaluation showed that the overall liking of uncooked and cooked dried noodle was at the moderate level. The present study indicated that unripe banana flour is a potential source of fiber when substituted to wheat flour in dried noodle products. The incorporation of 30% unripe banana flour in the noodle ingredients significantly increased their total dietary fiber and resistant starch content. Therefore, we were able to produce a good quality noodles in terms of nutritional values and taste quality.

Keywords: dried noodle, unripe banana flour, digestion, resistant starch

Introduction

Noodle products are the staple food in many parts of Asia. Asian noodles made from wheat may be divided into two general classes based on the ingredients used: white salted noodles (WSN, made from flour, sodium chloride and water) and yellow alkaline noodles (YAN, made from flour, alkaline salts such as sodium and potassium carbonate and water) (Asenstorfer et.al., 2006). The type of salt, properties of the flour and manufacturing process lead to a wide array of noodle types (Martin et.al., 2008). Traditional noodle is claimed to lack other essential nutritional components such as dietary fiber, vitamins and minerals, which are lost during wheat flour refinement. Thus, noodle products which represent a major end-use of wheat, are suitable for enhancing
health after incorporating sources of fiber and essential nutrients (Choo and Aziz et. al., 2010). The development of new products is a strategic area of the food industry. Consumers are demanding for foods that show two main properties: the first-one deals with the traditional nutritional aspects of the food, whereas, as a second feature, additional health benefits are expected from its regular ingestion. These kinds of food products are often called nutraceutical foods. In a rapidly changing world, with altered food habits and stressful life styles, it is more and more recognized that a healthy digestive system is essential for the overall quality of life (Brouns et al., 2002).

Several studies have suggested that consumption of unripe bananas exerts a beneficial effect on human health, associated with undigestible components. Unripe bananas are a source of carbohydrates and nutritionally interesting bioactive compounds. There is an excess of production and large quantities of fruits are lost during commerce, as a consequence of deficient postharvest handling. New economic strategies are considered for banana as a food ingredient (Ovando-Martinez et.al., 2009). According to literature, green banana is very rich in starch and its flour may present 61.3–76.5 g/100 g of starch and also has a fiber content of 6.3–15.5 g/100 g. Moreover, a great part of the starch found in green banana flour is the resistant starch type 2 (RS2 – from 52.7 to 54.2 g/100 g (Tribess et. al., 2009). Resistant starch (RS) has attracted interest because of its positive effects in the human colon and implications for health. It can be found in both processed and raw food materials. According to various RS types, RS2 are native uncooked granules of some starches such as those in raw potatoes and green bananas, whose crystallinity makes them scarcely susceptible to hydrolysis (Englyst et. al., 1992).

Some research has been recently carried out to improve the nutritional properties of food products adding to its supplements from unripe banana flour. Choo and Aziz et. al. (2010) reported that green banana flour has potential as a source of fiber when substituted in yellow alkaline noodle products. The incorporation of 30% banana flour significantly increased the total dietary fiber of noodle, resistant starch, total starch and some essential minerals, including phosphorus, magnesium, potassium, and calcium. Aparicio-Saguilán et.al. (2007) found that a resistant starch-rich powder prepared from banana starch as a potential ingredient for bakery products containing slowly digestible carbohydrates. Ovando-Martinez et.al.(2009) reported that pasta products containing banana flour exhibit a low rate of carbohydrate enzymatic hydrolysis and they could help broaden the range of low-glycaemic index foods available to the consumer. However, there is no information on the use of unripe banana flour in dried noodle-making. Therefore, the objective of this study was to use unripe banana flour as an ingredient to make dried noodle products of high nutritional quality with low carbohydrate digestibility and rich resistant starch. The effect of wheat flour substitution with unripe banana flour was investigated in terms of physicochemical, textural, cooking, and sensory qualities of dried noodles.

Materials and Methods

Materials

Banana flour preparation

Unripe banana (Musa paradisiaca L.) fruits were purchased from a local market in Kanchanaburi province, Thailand. Unripe banana flour was prepared using procedure described by Ovando-Martinez et.al. (2009). Fruits were peeled and cut into 3 mm slices and immediately rinsed in sodium metabisulfite (0.25% w/v). The slices were dried at
50°C, ground using commercial grinder and stored at 25°C in sealed plastic containers prior to further analyses. The commercial wheat flour used for this study was also obtained from the local market.

Methods

1. Dried noodle processing

Noodles were prepared in the laboratory following the procedures of Bui et al. (2007). The basic ingredients used for making dried noodles were: 100.0 g flour, 30.0 g water, 10.0 g egg powder, 3.0 g salt, 2.0 g propylene glycol, 1.5 g sodium carbonate, and 0.2 g polyphosphate. Control dried noodle was prepared from 100% wheat flour. Five additional dried noodles were prepared by substituting wheat with 10%, 20%, 30%, 40%, and 50% banana flour.

The different formulations were processed into noodle using a Kenwood mixer and a small spaghetti maker (Imperia brand, Italy) consisting of two rolls with adjustable gap settings and a cutting roll attachment. In brief, salt, egg powder, propylene glycol, sodium carbonate, and polyphosphate were dissolved in the water and this solution was added to the flour in the mixer set on speed one. After that, the speed of mixer was increased; the resultant dough had a crumbly consistency similar to that of moist breadcrumbs. The dough was first formed into a dough sheet by a process of folding and passing the crumbly dough through the rollers of the noodle machine several times. Then this combined sheet was allowed to rest in a plastic bag, at room temperature for 30 min. The sheet was cut into strands using the cutting roll attachment of the noodle machine to a width of 2.0 mm. The noodle strands were then cut to 25 cm in length before steaming over boiling water for 2 min. Subsequently, the steamed noodles were dried at 50°C for a total drying time of 1.5 h in an oven.

2. Chemical analysis

The chemical proximate compositions of unripe banana flour, wheat flour, and different raw dried noodle samples were determined as follows. Moisture content was determined by gravimetric heating (130 °C for 2 h) using a 2–3 g sample. Ash and protein were analyzed according to methods 08-01, and 46-13, respectively (AACC, 2000). Total dietary fiber (DF) was evaluated using the 985.29 AOAC method (AOAC, 1999). Resistant starch was measured by the method proposed by González et al. (1996). All analyzes were performed in triplicate.

3. Cooking qualities

The qualities of the cooked dried-noodles, cooking time and cooking loss, were evaluated according to Chillon et al. (2008). Optimal cooking time was evaluated by observing the time of disappearance of the core of the noodle strand during cooking (every 30 s) by squeezing the noodle between two transparent glass slides. The cooking loss was determined by measuring the amount of solid substance lost to cooking water. A 10 g sample of noodle was placed into 300 ml of boiling distilled water in a 500 ml beaker. Cooking water was collected in an aluminium vessel, placed into an air oven at 105 °C and evaporated to dryness. The residue was weighed and reported as a percentage of the starting material. For each optimal cooking time and cooking loss values, five determinations were performed to obtain the mean values.
4. Noodle color analysis

Dried noodle sheet and optimally cooked noodle colors were measured with a Chroma-meter (Minolta, Tokyo, Japan) equipped with a D65 illuminant using the CIE L*a*b* system. The L*, a*, and b* readings were obtained directly from the instrument provided measures of lightness, redness, and yellowness, respectively. All measurements were performed at least in triplicate.

5. Noodle texture analysis

Noodles were optimally cooked, cooled for 1 min under running distilled water, drained, stored for exactly 10 min at 25°C as described by Kruger et al., (1994), and submitted to tensile test using the TA-XT2i texture analyzer (Lu et.al, 2000). Instrument settings were extension mode, trigger type, auto-0.5g; pretest speed, 2.0 mm/s; posttest speed, 10 mm/s; test speed, 3.0 mm/s; trigger distance, 80 mm. From force-distance curves, two texture parameters were obtained: tensile strength (maximum force, g) and breaking length (distance at maximum force, mm). Three replicates of cooked noodles at each level of unripe banana flour were determined.

6. Sensory evaluation

All dried noodle samples were prepared for sensory evaluation. The samples were boiled using tap water for the optimum cooking time. The samples were then stored for not more than one half an hour in tightly covered plastic food containers before testing.

Thirty untrained panelists consisting of faculty, staff and students of Mahidol University, Kanchanaburi Campus, Thailand, evaluated optimally cooked noodle samples. The panelists evaluated appearance, flavor, taste, texture, and overall acceptability of the samples by using nine-point hedonic scales, where 9 = like extremely and 1 = dislike extremely. The optimal ratio of noodle from banana flour was investigated using sensory qualities in comparison to the control noodle.

7. Statistical analysis

The means and standard deviations were determined for all the physicochemical, textural, cooking, and sensory qualities studied. The significant difference of their mean values was assessed with one-way analysis of variance (ANOVA) followed by a Duncan’s test of SPSS software (significant level $p < 0.05$).

Results and Discussion

1. Chemical compositions of raw noodles, banana and wheat flours

The chemical compositions of raw dried noodles with added banana flour, the control raw dried noodle and their respective flours (banana and wheat flour) are presented in Table 1. It can be observed that the moisture content of dried noodle decreased when the level of banana flour in the noodle increased. As described by Ovando-Martinez et.al. (2009), this pattern is related to the decrease in the protein content with the increase of banana flour in the noodle where the network produced by the gluten is reduced; consequently, the separation of water during the drying is higher. Low-moisture content is important in the shelf-life of food products. The fat content did not change with the addition of banana flour, but the ash content increased when the banana flour amount in the noodle increased. The ash content depends on the quality of the flour and thus corresponds to the higher mineral content, especially potassium (Kim, 1996).
Banana contains the high potassium (400 mg/100 g pulp) and magnesium (34 mg/100 g edible portion contents) (McCance and Widdowson, 2002).

An important increase in dietary fiber (DF) and resistant starch (RS) level were obtained in the noodle with the addition of banana flour. The control sample showed the lowest value of DF (3.70%) and RS (0.40%), while the noodle containing 50% of banana flour possessed the highest value of DF (5.94%) and RS (4.71%). These are related with the high DF and RS content of banana flour (7.8% and 56.17%, respectively). Faisant et al. (1995) reported that unripe banana is the natural product with the highest RS content. The RS values of 57.2 and 47.3% were determined in unripe banana flour using two different methods.

**Table 1** Chemical compositions of raw materials and raw dried noodles supplemented with banana flour (%).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (w/w)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Dietary fiber (%)</th>
<th>Resistant starch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>11.1±0.000</td>
<td>4.98±0.01</td>
<td>16.4±0.03</td>
<td>0.04±0.01</td>
<td>3.70±0.01</td>
<td>0.40±0.05</td>
</tr>
<tr>
<td>10%</td>
<td>10.07±0.040</td>
<td>4.98±0.02</td>
<td>15.75±0.02</td>
<td>0.04±0.03</td>
<td>4.44±0.06</td>
<td>2.13±0.13</td>
</tr>
<tr>
<td>20%</td>
<td>10.31±0.030</td>
<td>5.20±0.00</td>
<td>14.87±0.02</td>
<td>0.22±0.09</td>
<td>4.51±0.05</td>
<td>2.76±0.02</td>
</tr>
<tr>
<td>30%</td>
<td>9.42±0.011</td>
<td>5.31±0.02</td>
<td>13.68±0.02</td>
<td>0.12±0.03</td>
<td>4.77±0.03</td>
<td>2.77±0.15</td>
</tr>
<tr>
<td>40%</td>
<td>9.98±0.004</td>
<td>5.44±0.00</td>
<td>13.40±0.03</td>
<td>0.16±0.01</td>
<td>4.98±0.05</td>
<td>3.62±0.05</td>
</tr>
<tr>
<td>50%</td>
<td>8.67±0.026</td>
<td>5.35±0.01</td>
<td>12.35±0.02</td>
<td>0.14±0.01</td>
<td>5.94±0.13</td>
<td>4.71±0.00</td>
</tr>
<tr>
<td>Banana</td>
<td>7.58±0.010</td>
<td>3.31±0.04</td>
<td>3.18±0.02</td>
<td>0.38±0.08</td>
<td>7.80±0.06</td>
<td>56.17±1.50</td>
</tr>
<tr>
<td>Wheat</td>
<td>11.86±0.050</td>
<td>0.59±0.00</td>
<td>11.64±0.02</td>
<td>3.00±0.07</td>
<td>0.45±0.01</td>
<td></td>
</tr>
</tbody>
</table>

Different letters in a column indicate significant differences (p < 0.05).

2. Cooking qualities

Cooking time and cooking loss of dried noodles supplemented with banana flour are shown in Table 2. Degree of cooking can be observed either by eye or image analysis (Sozer et al., 2007). In this study, it was determined by the disappearance of the core of the noodle strand during cooking. The optimum cooking times of all noodle samples ranged from 13.0-14.5 min. Cooking loss is the amount of dry matter into the cooking water of optimally cooked noodle. An increase in cooking loss with banana flour containing noodle (Table 2) may be due to weakening of protein network by the presence of banana flour. This may allow leaching out more solids from the noodle into the cooking water (Rayas-Duarte et al., 1996). These results are agreement with Ovando-Martinez et al. (2009). They reported that partial or complete substitution of durum wheat semolina with fiber material can result in negative changes to pasta quality, including increased cooking loss.

**Table 2** Cooking time and cooking loss of noodles supplemented with banana flour (%).

<table>
<thead>
<tr>
<th>Sample (%)</th>
<th>Cooking time (min)</th>
<th>Cooking loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>13.0</td>
<td>9.35±0.12^d</td>
</tr>
<tr>
<td>10%</td>
<td>13.5</td>
<td>8.71±0.06^d</td>
</tr>
<tr>
<td>20%</td>
<td>14.0</td>
<td>9.31±0.09^d</td>
</tr>
<tr>
<td>30%</td>
<td>14.5</td>
<td>11.15±0.00^b</td>
</tr>
<tr>
<td>40%</td>
<td>13.5</td>
<td>11.09±0.02^b</td>
</tr>
<tr>
<td>50%</td>
<td>13.0</td>
<td>11.49±0.08^a</td>
</tr>
</tbody>
</table>

^Different letters in a column indicate significant differences (p ≤ 0.05).
3. Color characteristics

Color is a key quality trait (Mares and Campbell, 2001) because of the visual impact at the point of sale. It provides some indication of quality of the starting materials and in some cases the age of the product. Asian customers prefer bright yellow alkaline noodles that retain a stable color for 24–48 h after preparation and perceive red or dull grey colors as undesirable (Asenstorfer et.al., 2006). Factors controlling color stability, which include alkaline formulation, flour refinement, and enzymatic browning associated with polyphenol oxidase, have been extensively investigated (Hatcher et.al., 2008).

Color characteristics of raw sheet and optimally cooked noodles supplemented with banana flour are shown in Table 3. The results indicated that as the amount of banana flour increased, the appearance of raw sheet and cooked noodles supplemented with banana flour grew darker. The darkness of both raw sheet and cooked noodles supplemented with banana flour is a product of the Maillard reaction between reducing sugars and proteins (Mohamed et al., 2010).

Table 3 Color characteristics of raw sheet and optimally cooked noodles supplemented with banana flour (%).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw sheet noodle</th>
<th>Optimally cooked noodle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>a*</td>
</tr>
<tr>
<td>Control</td>
<td>83.52±0.70⁹</td>
<td>-1.44±0.14⁹</td>
</tr>
<tr>
<td>10%</td>
<td>76.65±1.68⁸</td>
<td>0.24±0.11⁸</td>
</tr>
<tr>
<td>20%</td>
<td>70.39±1.23⁹</td>
<td>2.23±0.19⁹</td>
</tr>
<tr>
<td>30%</td>
<td>72.07±0.60⁸</td>
<td>2.25±0.26⁸</td>
</tr>
<tr>
<td>40%</td>
<td>71.01±0.52⁹</td>
<td>1.31±0.10⁹</td>
</tr>
<tr>
<td>50%</td>
<td>69.02±1.18⁹</td>
<td>2.28±0.18⁹</td>
</tr>
</tbody>
</table>

Different letters in a column indicate significant differences (p < 0.05).

4. Noodle texture analysis

Instrumental texture tests on cooked noodles have been well reviewed by Ross (2006). Despite profound difference in the “test” environments, repeated studies have shown the ability of instrumental texture tests, of a variety of types, to correlate closely with related sensory texture attributes. Tensile tests used to measure elasticity and related attributes are amenable to interpretations of the raw time/force data that can derive fundamental rheological parameters (Ross, 2006). Tensile parameters, tensile strength and breaking length, of cooked noodles made from wheat flour and supplemented with banana flour were compared in Figure 1. The tensile strength of cooked noodles was significantly (p < 0.05), while breaking length of noodles was insignificantly (p > 0.05) different with the variation of banana flour content. The tensile strength decreased when banana flour content increased. It can be noted that the addition of banana flour, one of non-gluten flours, in the fabrication of dried noodle diluted the gluten strength of the wheat flour and interrupted as well as weaken the overall structure of the noodle. These results are in line with Kovacs et al. (2004). They reported that about 80% of the total protein of wheat flour is gluten. Gluten proteins are composed of gliadins and glutenins,
which are responsible for gluten or dough extensibility (viscosity) and strength (elasticity), respectively. Therefore, removal of wheat flour would impair the gluten matrix, hence leading to the weakening of noodle texture.

![Graph](a) Effect of banana flour (%) on tensile strength (a) and breaking length (b) of optimally cooked noodles supplemented with banana flour.

5. Sensory evaluation

Asian consumers normally purchase noodle product daily from either convenience stores or local manufacturers, basing their purchasing decisions upon their initial assessment of noodle quality by its visual appearance i.e. color, brightness and the absence of undesirable specks (Hatcher et.al., 2009). In this study, the sensory evaluation of optimally cooked noodles supplemented with banana flour was carried out with the objective to select the best-suited banana flour (%) for preparation of the noodles. The means sensory acceptability scores for appearance, flavor, taste, texture, and overall acceptability of optimally cooked noodles supplemented with banana flour are shown in Figure 2. Most panelists scored the optimally cooked noodles supplemented with 10%, 20%, and 30% banana flour to be equally acceptable as the control noodle. No statistically significant (p>0.05) difference could be found for all sensory attributes. It is noted that the sensory acceptability scores for flavor and taste attributes of noodles were insignificantly (p > 0.05) different with the variation of banana flour content. In order to make dried noodle products of high nutritional quality with low carbohydrate digestibility and rich resistant starch, the noodle supplemented with 30% banana flour was selected and subsequently used for the consumer test.
One hundred consumers (22 males and 78 females) participated in the consumer test. Consumers were asked to complete a short questionnaire following the preference test. This questionnaire asked demographic information and examined yellow alkaline noodle consumption habits. It was reported that 88% of consumers accepted the product and the overall liking of uncooked and cooked dried noodle were at the moderate level. According to this consumer test, the category of invention and health product is the main reason for the consumption of dried noodles supplemented with banana flour.

**Figure 2** Effect of banana flour (%) on sensory acceptability for appearance (a), flavor (b), taste (c), texture (d), and overall acceptability (e) of optimally cooked noodles supplemented with banana flour.

Conclusions

It can be inferred from the present study that banana flour could be added to dried noodle up to the level of 30% without any significant change in organoleptic characteristics. Dried noodle made from banana flour, up to 30% level, were considered as most acceptable, organoleptically and nutritionally as they contained appreciable amount of 13.7% protein, 0.12% fat, and 4.8% dietary fiber (including 2.8% resistant
starch). The development of such functional foods not only improves the nutritional status of the general population but also helps those suffering from degenerative diseases associated with today’s changing life styles and environment.

Acknowledgments

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References

Characterization and classification of Thai steamed pork sausages by using principal component analysis

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ABSTRACT

The objective of this study was to characterize and classify Thai steamed pork sausages, called “Mu-Yor”, by using principal component analysis (PCA). Chemical, color, and texture analyses were performed on different commercial brands of Thai steamed pork sausages. The Thai sausages with ten different commercial brands were purchased from open markets (n=4) and supermarkets (n=6). All samples were subjected to chemical composition, color, and texture analyses. Moisture, ash, protein, fat, carbohydrate, NaCl, and pH were determined according to the standard AOAC procedures. The surface color of sausage slices (CIE L*, a*, b*, hue, and chroma) was measured by using a Chroma meter. Texture profile analysis (TPA) was performed by using a texture analyzer. From the resulting force/deformation curves, the textural parameters of hardness, cohesiveness, springiness index, and chewiness were calculated.

Four separated PCA using cross-validation were performed on chemical, color, textural, and all data by mean values. All brands are shown to indicate significant differences in chemical, textural, and color parameters (p<0.05), which were analyzed by PCA. The results are as follows. First, PCA performed on 7 chemical parameters showed that the first two principal components (accounted for 83.02% of the total variance) could determine 3 groups of samples based on carbohydrate and moisture contents. Second, PCA performed on five color parameters could determine 3 groups of samples based on red color (a*) using the first PC, which were accounted for 77.05% of the total variance. Third, PCA performed on 4 textural parameters could determine 4 groups of samples based on hardness and springiness index using the first two PCs, which were accounted for 82.08% of the total variance. Finally, PCA performed on all parameters (chemical, color, and textural parameters) showed that the first two PCs, which were accounted for 65.86% of the total variance, could determine 2 groups of samples according to the market locations; open markets and supermarkets, with the differences in carbohydrate, protein, ash, pH, L*, a*, b*, chroma, and cohesiveness values. The present study indicated that PCA proves to be the very convenient method for multivariate characterization of Thai steamed pork sausages in terms of chemical, color, and textural properties.

Keywords: Principal component analysis (PCA), Thai steamed pork sausage, Mu-Yor, Characterization, Classification

Introduction

Thai steamed pork sausage or “Mu-Yor” is one of the most popular processed meat products in Thailand. It is also very popular as a souvenir, especially in the Northern and Northeastern parts. These products are also greatly appreciated by the consumers due to their sensory properties and their image of traditional products.
The quality of steamed pork sausage is influenced by many factors. One of these factors is the different chemical constituents of pork meat used as raw material. Each constituent affects the physical quality either independently or in combination with other constituents. Therefore, it is rational to assume that there are some relationships between chemical constituents, i.e. water, protein, fat, salt and ash, and physical attributes of meat products, i.e. tenderness, hardness, springiness, cohesion, gumminess, chewiness and color (Václková et.al, 2007). According to Thai Industrial Standard of Thai steamed pork sausage, the most desirable physical properties of a high quality steamed pork sausage are homogeneous appearance, textural cohesiveness and springiness (Thai Industrial Standard Institute, 1996). To understand these relationships in the meat system is very important not only for the prediction of physical properties from chemical constituents, but also for the control of the quality of meat products (Cheng and Sun, 2005). However, there is a lack of information about the relationship among quality attributes of Thai steamed pork sausages.

Many authors have suggested the use of multivariate analysis to describe or classify cooked ham and sausages (Casiraghi et.al, 2007). Principal component analysis (PCA), one of the techniques of multivariate analysis, has been widely used to characterize processed meat and meat products such as cooked pork hams (Cheng and Sun, 2005; Václková et.al, 2007), frankfurters (Ordóñez et.al, 2001; González-Viñas et al., 2004), Greek traditional sausages (Papadima et.al, 1999; Ambrosiadis et al., 2003), Greek cooked meat product (Arvanitoyannis et al., 2000), Italian dry-cured sausages (Dellaglio et.al, 1996), and traditional Spanish blood sausages (Santos et al., 2003).

PCA is a multidimensional modeling method, which bases on the calculation of linear combinations between the variables that explain the most variance of the data. It provides an interpretable overview of the key information through two plots, i.e., the loading plot and the score plot. In the loading plot, components (so-called principal components) that are close together are positively correlated, while those lying opposite to each other tend to have negative correlation. In the score plot, samples to the right have high values compared with samples placed to the left, the same holds for those at the top or bottom (Naes et al., 1996).

The objective of the study was to characterize and classify Thai steamed pork sausages by means of chemical, color, and textural parameters, and to investigate the comprehensive effects of different purchasing locations (open markets and supermarkets) on the quality parameters of steamed pork sausage, and the relation among these parameters. The PCA method was applied to analyze the results.

Materials and Methods

Materials

Ten commercial brands of Thai steamed pork sausage were purchased from open markets (L1, L2, L3 and L4) and supermarkets (S1, S2, S3, S4, S5 and S6). The common ingredients in this type of product are lean pork meat, pork backfat, salt, sugar, starch and spices. The samples were transported to the laboratory in portable, insulated cold-boxes and stored at approximately 4°C until they were analyzed, normally between 1 and 5 days after collection.
Methods

1. Chemical analysis

   Moisture, protein (Kjeldahl N×6.25), fat, ash, and sodium chloride were determined according to the AOAC methods 950.46, 928.08, 960.39 and 971.27 (AOAC, 1990). Total carbohydrate was calculated as the residue by difference from the total of moisture, protein, fat, protein, and ash values. The pH values were measured with a digital pH meter (Sartorius, UK, model PP-15E) after mixing 10 g of sample with 10 mL of distilled water in a homogenizer. The result stated for each sample is the mean value of 3 measurements.

2. Color analysis

   The internal color of the sausages was analyzed with a Chroma meter (Konica Minolta), model CR400 (D65/10°). CIELAB L*, a* and b* values were determined as indicators of lightness, redness and yellowness. Chroma (C) and Hue (H°) values were obtained using the following equations: 
   \[ C = (a^*^2 + b^*^2)^{1/2} \]
   \[ H^o = \arctan b^*/a^* \]
   Measurements were carried out immediately after slicing and repeated three times in different portions of a 10 mm-thick slice. The result stated for each sample is the mean value of 5 measurements.

3. Texture analysis

   Texture profile analysis (TPA) was performed with a Lloyd Texture Analyzer (Lloyd Instrument Ltd., UK), model LR5K, equipped with a standard cylindrical plate of 5 cm in diameter. The samples were prepared in a cubic shape of size 20 mm×20 mm with a thickness of 20 mm, after discarding the external layer of the sausage. All samples were equilibrated to room temperature and compressed twice to 40% of their original thickness at a constant speed of 30 mm/min. Parameters of hardness, springiness index, cohesiveness and chewiness from the force-time curves were determined as described by Bourne (1978). The result stated for each sample is the mean value of 5 measurements.

4. Statistical analysis

   Data collected for chemical, color, and texture analyses were analyzed by one way analysis of variance (ANOVA) with SPSS® software (version 17.0) (SPSS Inc., Chicago, IL, USA). Significant differences between means were determined by Duncan's Multiple Range tests. Principal component analysis (PCA) was applied on the significant descriptors to distinguish different samples by using the Unscrambler® software (Ver. 7.8: CAMO AS, Norway). Four separated PCA using cross-validation were performed on chemical, color, textural, and all data by mean values. All data were standardized prior to the PCA analysis by weighing variables by their standard deviations. For interpretation of the results, the optimal number of the principal components (PCs) that explained most of the information in the data was determined (that is, we used a model with a total residual variance close to 0 or a large total explained variance).

Results and Discussion

1. Chemical compositions, color and texture properties

   The chemical compositions of Thai steamed pork sausages are shown in Table 1. The steamed pork sausages from all brands ranged from 54.22 to 64.25% moisture, 2.20 to 3.38% ash, 7.05 to 11.53% protein, 1.43 to 9.96% fat, 18.06 to 32.28% carbohydrate, 2.40 to 3.27% NaCl, respectively. The pH values ranged from 6.25 to 6.86. Significant differences in those values were observed (p ≤ 0.05). The color characteristics for all samples ranged from 64.89 to 75.05 L* value (lightness), 2.48 to 5.64 a* value (redness), 2.89 to 6.27 b* value (yellowness).
11.73 to 18.01 b* value (yellowness), 59.86 to 78.46° hue, and 11.99 to 18.79 chroma (Table 2). Texture profile analysis (TPA) is the most commonly used instrumental method for food textural property determination. The compression parameters obtained with TPA have been employed by many authors in their evaluations of meat products, such as dry fermented sausages, as an index to determine the quality of the finished product or to select the best functional ingredients (Herrero et al., 2007). Textural properties of the different steamed pork sausages are shown in Table 3. Significant differences (p ≤ 0.05) were found. Results show that the hardness, cohesiveness, springiness index, and chewiness of steamed pork sausage samples ranged from 15.69 to 43.76N, 0.44 to 0.55, 0.79 to 0.94, and 57.36 to 146.41 N.mm, respectively.

Results from the chemical, color, and TPA analysis showed many variations (p ≤ 0.05) indicating a great data dispersion for all the parameters studied. As expected, the sausages were different in terms of basic chemical composition and simultaneously the very strong effect of the sausage brand on most variables derived from color and texture tests was stated.

### Table 1 Chemical proximate compositions (Means ± SD) of Thai steamed pork sausages.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Carbohydrate (%)</th>
<th>NaCl (%)</th>
<th>pH (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>58.75±0.25d</td>
<td>2.32±0.10g</td>
<td>9.70±0.02c</td>
<td>9.96±0.00f</td>
<td>19.28±0.07d</td>
<td>2.68±0.01i</td>
<td>6.81±0.00bc</td>
</tr>
<tr>
<td>L2</td>
<td>64.15±0.03a</td>
<td>2.86±0.00c</td>
<td>7.73±0.01e</td>
<td>1.93±0.00c</td>
<td>23.33±0.02g</td>
<td>0.00</td>
<td>3.16±0.09abc</td>
</tr>
<tr>
<td>L3</td>
<td>54.22±0.03f</td>
<td>3.38±0.01a</td>
<td>7.28±0.09f</td>
<td>2.84±0.17f</td>
<td>32.28±0.11a</td>
<td>0.00</td>
<td>6.79±0.01abc</td>
</tr>
<tr>
<td>L4</td>
<td>56.66±0.00b</td>
<td>2.89±0.02b</td>
<td>7.78±0.10a</td>
<td>1.43±0.12b</td>
<td>31.24±0.07b</td>
<td>0.00</td>
<td>6.86±0.01abc</td>
</tr>
<tr>
<td>S1</td>
<td>58.64±0.04d</td>
<td>2.20±0.01h</td>
<td>11.53±0.25a</td>
<td>9.57±0.01b</td>
<td>18.06±0.08a</td>
<td>0.00</td>
<td>2.40±0.02abc</td>
</tr>
<tr>
<td>S2</td>
<td>61.62±0.00c</td>
<td>2.60±0.01e</td>
<td>11.52±0.07a</td>
<td>2.07±0.08b</td>
<td>22.19±0.04d</td>
<td>0.00</td>
<td>6.58±0.01abcde</td>
</tr>
<tr>
<td>S3</td>
<td>58.75±0.00</td>
<td>2.23±0.01g</td>
<td>11.01±0.14b</td>
<td>7.71±0.01c</td>
<td>20.30±0.08e</td>
<td>0.00</td>
<td>3.01±0.11bc</td>
</tr>
<tr>
<td>S4</td>
<td>64.25±0.06a</td>
<td>2.67±0.00d</td>
<td>7.05±0.03d</td>
<td>2.72±0.19b</td>
<td>23.31±0.07d</td>
<td>0.00</td>
<td>6.45±0.03abcde</td>
</tr>
<tr>
<td>S5</td>
<td>61.71±0.10c</td>
<td>2.52±0.15f</td>
<td>8.28±0.19d</td>
<td>4.14±0.05f</td>
<td>23.35±0.09c</td>
<td>0.00</td>
<td>3.07±0.01abcde</td>
</tr>
<tr>
<td>S6</td>
<td>62.84±0.06b</td>
<td>2.54±0.05f</td>
<td>9.47±0.23c</td>
<td>5.17±0.03e</td>
<td>20.00±0.08e</td>
<td>0.00</td>
<td>3.00±0.01e</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences (p ≤ 0.05).
The chemical composition and NaCl values are expressed in % of wet weight.

### Table 2 Color properties (Means ± SD) of Thai steamed pork sausages.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Hue angle (°)</th>
<th>Chroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>68.44±0.63</td>
<td>5.34±0.34</td>
<td>18.01±0.55</td>
<td>73.47±1.47d</td>
<td>18.79±0.43a</td>
</tr>
<tr>
<td>L2</td>
<td>70.89±0.34d</td>
<td>4.21±0.28bc</td>
<td>14.85±0.44d</td>
<td>74.16±1.37cd</td>
<td>15.44±0.37ad</td>
</tr>
<tr>
<td>L3</td>
<td>64.89±0.46b</td>
<td>4.51±0.33b</td>
<td>16.80±0.44b</td>
<td>74.99±1.16c</td>
<td>17.40±0.42b</td>
</tr>
<tr>
<td>L4</td>
<td>66.08±0.38b</td>
<td>5.64±0.96e</td>
<td>15.39±0.39h</td>
<td>69.86±3.54e</td>
<td>16.42±0.25c</td>
</tr>
<tr>
<td>S1</td>
<td>70.22±0.60a</td>
<td>4.26±0.12d</td>
<td>16.07±0.23f</td>
<td>69.50±0.44d</td>
<td>15.41±0.24f</td>
</tr>
<tr>
<td>S2</td>
<td>72.46±0.71c</td>
<td>2.48±0.11f</td>
<td>11.73±0.25g</td>
<td>78.04±0.72b</td>
<td>11.99±0.23b</td>
</tr>
<tr>
<td>S3</td>
<td>74.33±0.71b</td>
<td>2.60±0.11f</td>
<td>12.76±0.23f</td>
<td>78.46±0.47c</td>
<td>13.02±0.23f</td>
</tr>
<tr>
<td>S4</td>
<td>67.94±0.51f</td>
<td>3.39±0.28e</td>
<td>14.13±0.29g</td>
<td>76.50±1.23b</td>
<td>14.53±0.23c</td>
</tr>
<tr>
<td>S5</td>
<td>68.76±0.39f</td>
<td>3.30±0.44e</td>
<td>14.35±0.46f</td>
<td>77.00±2.03b</td>
<td>14.73±0.36e</td>
</tr>
<tr>
<td>S6</td>
<td>70.54±0.16d</td>
<td>3.40±0.06d</td>
<td>14.34±0.22e</td>
<td>76.65±0.29b</td>
<td>14.74±0.22c</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences (p ≤ 0.05).
Table 3 Textural properties (Means ± SD) of Thai steamed pork sausages.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (N)</th>
<th>Springiness index(-)</th>
<th>Cohesiveness(-)</th>
<th>Chewiness (N.mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>29.36 ± 1.45c</td>
<td>0.94 ± 0.05a</td>
<td>0.46 ± 0.03abc</td>
<td>102.39 ± 13.76bc</td>
</tr>
<tr>
<td>L2</td>
<td>28.02 ± 1.75c</td>
<td>0.91 ± 0.00bc</td>
<td>0.44 ± 0.01c</td>
<td>91.02 ± 5.69c2</td>
</tr>
<tr>
<td>L3</td>
<td>43.76 ± 4.39c</td>
<td>0.83 ± 0.03abc</td>
<td>0.47 ± 0.02bc</td>
<td>146.40 ± 9.62c</td>
</tr>
<tr>
<td>L4</td>
<td>29.62 ± 1.76bc</td>
<td>0.79 ± 0.02ac</td>
<td>0.44 ± 0.02abc</td>
<td>89.57 ± 6.97cd</td>
</tr>
<tr>
<td>S1</td>
<td>22.87 ± 1.24a</td>
<td>0.79± 0.01ac</td>
<td>0.53 ± 0.02a</td>
<td>77.40 ± 3.63c</td>
</tr>
<tr>
<td>S2</td>
<td>34.11 ± 0.44b</td>
<td>0.84 ± 0.05abc</td>
<td>0.49 ± 0.02abc</td>
<td>113.98 ± 7.17b</td>
</tr>
<tr>
<td>S3</td>
<td>30.08 ± 0.83c</td>
<td>0.85 ± 0.04abc</td>
<td>0.54 ± 0.01a</td>
<td>110.26 ± 7.74b</td>
</tr>
<tr>
<td>S4</td>
<td>22.57 ± 0.84d</td>
<td>0.91 ± 0.07abc</td>
<td>0.55 ± 0.05a</td>
<td>100.23 ± 9.09bc</td>
</tr>
<tr>
<td>S5</td>
<td>24.12 ± 0.46d</td>
<td>0.86 ± 0.07abc</td>
<td>0.52 ± 0.06ab</td>
<td>87.32 ± 18.04ad</td>
</tr>
<tr>
<td>S6</td>
<td>15.69 ± 0.43c</td>
<td>0.83 ± 0.04abc</td>
<td>0.55 ± 0.02a</td>
<td>57.36 ± 4.27c</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant differences (p < 0.05).

2. PCA of chemical compositions

A first PCA was performed on the concentration of the 7 chemical compositions of steamed pork sausage samples listed in Table 1 in order to define the most appropriate descriptors of chemical compositions of sausage samples. The first two principal components, PC1 and PC2, accounted for 83.02% of total variance (59.97 and 23.05%, respectively). The relationship between the attributes and the principal components can be seen by their location in PCA loading plot (Figure 1A). The first PC appeared to be strongly related to carbohydrate, fat, ash, NaCl, protein and pH. Carbohydrate, ash, NaCl and pH gave high positive loadings, while fat and protein gave high negative loadings. Cheng and Sun (2005) obtained same results in pork ham and concluded that fat content was positively correlated with protein content (p≤0.01). The second PC described variation among the steamed pork sausages with respect to moisture content. Fat in sausages plays an important role in product quality, including texture and flavor. In order to increase the stability, quality, texture, flavor and acceptability of low-fat meat products, carbohydrates have been added to the products (Hughes et.al, 1996).

The PCA score plot for the first two principal components is given in Fig. 1B. Samples to the right in the score plot have a large value for response variables than those to the right in the loading plot. The same holds true for samples on the left, at the top and at the bottom of the plot. Samples lying close together have similar properties and the variables close together are positively correlated. Variables close to different axes have a low correlation and variables lying opposite to each other in the loading plot tend to have a negative correlation (Naes et al., 1996).

It is apparent from Figure 1B that there are three different groups of sausage samples (Group 1, 2 and 3). PC1 and PC2 discriminated those groups by carbohydrate and moisture contents, respectively. Sausages from group 1 (coded by L3 and L4) are characterized by carbohydrate content higher than 31%, and moisture content lower than 58%. Sausages from group 2 (coded by L2, S2, S4, S5 and S6) are characterized by carbohydrate content of 20-23% and moisture content of 61-64%. Sausages from group 3 (coded by L1, S1 and L3) are characterized by carbohydrate content lower than 20% and moisture content of 58-60%.
Figure 1  PCA loading plot (A) and PCA score plot (B) for the first two principal components obtained by PCA for 7 chemical compositions of 10 steamed pork sausages.

3. PCA of color properties

In order to define the most appropriate descriptors of color properties of materials sausage samples, the second PCA was carried out using data listed in Table 2. The first principal component accounted for 77.05% of the variance, with a negative contribution of $a^*$, chroma, and $b^*$ and a positive one of $L^*$ and hue (Figure 2A). The color $a^*$ is the most sensitive parameter of color measurement, characterizing red color and color stability (García-Esteban et al., 2003). This is an agreement with Dvorak et.al. (2001) previous work in pork quality evaluation on a production line in a large slaughterhouse using color measurements with CIEL*a*b* system, who found that $a^*$ value was the most important aspect of color, although its correlation coefficients with quality parameters such as pH and drip loss were very low.

Figure 2  PCA loading plot (A) and PCA score plot (B) for the first two principal components obtained by PCA for 5 color properties of 10 steamed pork sausages.

Figure 2B illustrates how samples separate throughout their color properties. The first PC of the color parameters sorted the sausage samples out into three different groups (Group 1, 2 and 3) with different in $a^*$ value levels. Sausages from group 1 (coded by L1, L3 and L4) are characterized by high $a^*$ value (4.51-5.64). Sausages from group 2 (coded
by L2, S1, S4, S5 and S6) are characterized by intermediate $a^*$ values (3.30-4.26). Sausages from group 3 (coded by S2 and L3) are characterized by low $a^*$ values (2.48-2.60). Válková et.al. (2007) characterized cooked pork hams using instrumental measurements of color and texture. The evaluation was performed by PCA, which separated three samples that differ from the other (of a total of 13) with a higher $a^*$ value.

4. PCA of textural properties

According to the quality of cooked sausage, textural properties are among the important attributes affecting consumers’ palatability and acceptability. A third PCA was carried out using data listed in Table 3 in order to define the most appropriate descriptors of textural quality of sausage samples. The first two components explained 82.08% of the variance of the experiment data. As shown in Figure 3A, the first PC accounted for 56.75% of the variance, with a positive contribution of hardness and chewiness, and a negative contribution of cohesiveness. The second PC described 25.33% of the total variance and had a high positive loading for springiness index. The positive correlation between hardness and chewiness of the steamed pork sausage found in this study was similar to the report of Spanish dry-cured sausages by González-Fernández (2006).

It is apparent from Figure 3B that there are four different groups of sausage samples (Group 1, 2, 3 and 4). PC1 and PC2 discriminate those groups by hardness and springiness index, respectively. Sausages from group 1 (coded by L3) are characterized by hardness higher than 43 N, and springiness index lower than 0.86. Sausages from group 2 (coded by L1, L2, S4 and S5) are characterized by hardness of 22-34 N and springiness index not less than 0.86. Sausages from group 3 (coded by L4, S1, S2 and S3) are characterized by hardness of 22-34 N and springiness index lower than 0.86. Sausages from group 4 (coded by S6) are characterized by hardness lower than 15N and springiness index lower than 0.86. Some previous studies on food texture tended to apply PCA to reduce the numbers of textural attributes such as Ordóñez et al. (2001), Rahman and Al-Farsi (2005) and Probola and Zander (2007). As conclusions of these researches, two or three principal components might group total variables measured, and PCA proved to be a good statistical method in reducing and explaining textural factors.

Figure 3 PCA loading plot (A) and PCA score plot (B) for the first two principal components obtained by PCA for 4 textural properties of 10 steamed pork sausages.
5. PCA of all properties

The fourth PCA was performed on the basis of all variables related to chemical composition, color and texture properties. Table 4 contains the loadings for the first four principal components with their variances. The result showed that the first four principal components described about 90.44% of the variation of the 16 original variables. The first PC described 46.24% of the total variance in the data set and had high negative loadings for L*, cohesiveness, protein, and hue and high positive loadings for carbohydrate, pH, ash, chroma, b*, and a*. The second PC described 19.62% of the total variance and had a high positive loading for fat, and a high negative loading for NaCl. The third PC accounted for 14.28% of the variance, with a positive contribution of moisture and a negative contribution of hardness and chewiness. The fourth PC described 10.30% of the total variance and had a high positive loading for springiness index. Texture depends on the amount of collagen content in a connective tissue, and the intramuscular connective tissue and its role in meat quality has been reviewed by Purslow (2005). The negative correlation between moisture content and hardness of the steamed pork sausage found in this study were similar to the report of dry-cured hams by Virgili et al. (1995). Moreover, Gimeno et al. (2000) concluded that the cohesiveness of a dry fermented sausage showed significant negative correlations with pH. Matulis et al. (1995) pointed out that if pH was reduced below the isoelectric point of muscle protein, solubilization of protein would be higher, producing firmer sausages.

Table 4 PCA correlation loadings between each principal component and each quality properties.

<table>
<thead>
<tr>
<th>Quality properties</th>
<th>Parameters</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical properties</td>
<td>Moisture</td>
<td>-0.495</td>
<td>-0.331</td>
<td>0.734</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>0.808</td>
<td>-0.486</td>
<td>-0.074</td>
<td>-0.057</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>-0.743</td>
<td>0.298</td>
<td>-0.494</td>
<td>-0.020</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>-0.450</td>
<td>0.819</td>
<td>-0.157</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>Carbohydrate</td>
<td>0.852</td>
<td>-0.394</td>
<td>-0.213</td>
<td>-0.224</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>0.530</td>
<td>-0.726</td>
<td>0.291</td>
<td>-0.135</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>0.822</td>
<td>0.340</td>
<td>0.046</td>
<td>0.055</td>
</tr>
<tr>
<td>Color properties</td>
<td>L*</td>
<td>-0.875</td>
<td>0.153</td>
<td>-0.219</td>
<td>-0.043</td>
</tr>
<tr>
<td></td>
<td>a*</td>
<td>0.759</td>
<td>0.585</td>
<td>0.135</td>
<td>-0.235</td>
</tr>
<tr>
<td></td>
<td>b*</td>
<td>0.761</td>
<td>0.458</td>
<td>0.240</td>
<td>0.263</td>
</tr>
<tr>
<td></td>
<td>Hue (°)</td>
<td>-0.577</td>
<td>-0.553</td>
<td>-0.061</td>
<td>0.538</td>
</tr>
<tr>
<td></td>
<td>Chroma</td>
<td>0.782</td>
<td>0.486</td>
<td>0.229</td>
<td>0.193</td>
</tr>
<tr>
<td>Textural properties</td>
<td>Hardness</td>
<td>0.593</td>
<td>-0.167</td>
<td>-0.691</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td>Cohesiveness</td>
<td>-0.777</td>
<td>-0.148</td>
<td>0.083</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>Springiness index</td>
<td>0.095</td>
<td>0.070</td>
<td>0.556</td>
<td>0.791</td>
</tr>
<tr>
<td></td>
<td>Chewiness</td>
<td>0.484</td>
<td>-0.250</td>
<td>-0.606</td>
<td>0.556</td>
</tr>
</tbody>
</table>

The biplot for the first two principal components is given in Figure 4. The main information given in the PCA biplot was the difference between the sausage samples purchased from open markets (coded by L1, L2, L3 and L4) and supermarkets (coded by S1, S2, S3, S4, S5 and S6). Therefore, the quality difference was mainly caused by the L*, carbohydrate, pH, ash, chroma, cohesiveness, b*, a* and protein values within sample. Samples purchased from supermarkets were located on the left hand side of the
score plot (Figure 4), which showed variables of $L^*$, protein, and cohesiveness had higher values compared to the variables of sausages purchased from open markets.

![PCA biplot](image)

**Figure 4** PCA biplot for the first two principal components obtained by PCA for chemical, color and textural properties of 10 steamed pork sausages.

**Conclusions**

In this study, we have presented a possible strategy for characterization and classification of Thai steamed pork sausage samples by using Principal component analysis (PCA). Four separated PCA were performed on chemical, color, textural, and all data by mean values. Our results indicated that PCA performed on all the parameters measured showed better results. The result showed that the first principal component could sort out the difference between the sausage samples purchased from open markets and supermarkets, which meant that the quality difference was mainly caused by the $L^*$, carbohydrate, pH, ash, chroma, cohesiveness, $b^*$, $a^*$ and protein values within sample. The sausage samples purchased from supermarkets showed higher values of protein content, cohesiveness, and color $L^*$ than those of the sausage samples purchased from open markets.

**Acknowledgments**

The authors are grateful to the Department of Food Engineering, Faculty of Engineering Kasetsart University, Kamphaeng Saen Campus, Thailand, for giving us the opportunity to use a Lloyd Texture Analyzer. This research was supported by Faculty of Science, Mahidol University, Thailand. We would also like to thank Ms. Ornnattha Supatham, Mahidol University, Kanchanaburi Campus, Thailand, for helpful suggestions on the manuscript.

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Effect of nisin on the survival of *Staphylococcus aureus* inoculated in fish ball

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**ABSTRACT**

Inhibitory effect of nisin at concentrations of 0-20 µg/ml was determined against *Staphylococcus aureus* (approximately 7 log cfu/ml) and the suitable inhibitory concentration was incorporated with fish ball for shelf-life study. The results showed that inhibition of *S. aureus* increased as nisin concentrations and incubating time increased (p<0.05). The number of *S. aureus* decreased from 4.94±0.08 log cfu/ml to 2.73±0.18 log cfu/ml at 4±2°C and 5.37±0.04 log cfu/ml to 3.49±0.12 log cfu/ml at 10±2°C after incubating for 42 h when the concentration of nisin increased from 5 to 20 µg/ml. D-values for *S. aureus* were between 17.92-10.74 h at 4±2°C and 18.58-12.10 h at 10±2°C. According to the D-value results, the suitable nisin concentration of 15 µg/ml was incorporated with fish ball inoculated with 7 log cfu of *S. aureus*. The samples were packed in polyethylene bags before being kept at 4±2°C and 10±2°C. Microbiological results indicated that *S. aureus* of both control groups increased from 7.27 ± 0.33 log cfu/g to 9.53 ± 0.34 log cfu/g and 10.28 ± 0.39 log cfu/g when the samples were kept at 4±2°C and 10±2°C, respectively while that of nisin treated samples reduced to 5.70±0.53 log cfu/g on day 12 and 6.43±0.27 log cfu/g on day 6 when the samples were kept at 4±2°C and 10±2°C respectively. In addition, the total plate count of control samples increased to 10.2±0.36 log cfu/g and 11.25±0.31 log cfu/g at 4±2°C and 10±2°C, respectively whereas that of nisin treated samples reduced to 6.86±0.20 log cfu/g on day 12 and 7.27±0.44 log cfu/g on day 6 at 4±2°C and 10±2°C, respectively. For chemical results, total acid of fish ball was ranged from 0.0046% to 0.0049% and their pH values were between 6.77 and 6.92 during keeping for 30 days at both storage temperatures. In conclusion, the use of nisin at 15 µg/ml would be an alternative to decrease the contamination level and extending shelf life of fish ball.

**Keywords:** Nisin, *Staphylococcus aureus*, total plate count, D-value, fish ball

**Introduction**

Fish ball is a commonly cooked food in southern China and oversea Chinese communities. It is also very popular in Thailand. Fish ball is made of fish meat that has been finely pulverized. It is cooked in noodle soup, fried or grilled to be eaten as snack. The product is generally for local consumption as its shelf-life is not very long. Since fish ball requires considerable handling during preparation and is often kept at slightly elevated temperatures after preparation, it is frequently involved in microbial contamination. Meechai (2005) reported that average total bacteria count in fish ball ranged from 3.4x10⁴ to 2.11x10⁸ colonies/g and most of them were *Staphylococcus aureus*. Staphylococci not only exist in air, water, and food but on food equipment, environmental surfaces, humans, and animals as well. It grows and reproduces at...
temperatures from 10°C (50°F) to 48.89°C (120°F), with the most rapid growth occurring near body temperature (about 37°C). The toxin produced by *S. aureus* is very heat-stable. Symptoms of staphylococcal food poisoning are usually rapid and in many cases serious. The most common symptoms are nausea, vomiting, abdominal cramping, and prostration. In more severe cases, headache, muscle cramping, and changes in blood pressure and pulse rate may occur (Jay, 2000).

Nisin is a polycyclic peptide antibacterial produced by fermentation using the bacterium *Lactococcus lactis*. It is used as a food preservative in many foods to extend shelf life by suppressing Gram-positive spoilage and pathogenic bacteria (Solomakos et al, 2008). As cited by Gallo, Pilosof and Jagus (2007), the action of nisin against bacteria is that it binds electrostatically to the negatively charged phospholipids then inserts itself into the cytoplasmic membrane resulting in pore formation. The efflux of essential intracellular constituents through those forming pores causes a complete collapse of the proton motive force and subsequently results in cell death. Nisin is soluble in water and can be effective at levels nearing the parts per billion range. It has been approved as a food additive in Europe and achieved GRAS (Generally recognized as safe) status in USA (Schillinger et al., 2001). In foods, it is common to use nisin at levels ranging from 0.25-37.7 mg/l, depending on the food type and regulatory approval (Delves-Broughton, 1990).

Many researches has been studied on the efficacy of nisin against pathogenic and spoilage microorganisms such as *Listeria monocytogenes* (Nilsson, Huss and Gram, 1997; Pranoto, Rakshit, and Salokhe, 2005; Neetoo et al., 2008), *Clostridium sporogenes* (Jamuna, Babusha, and Jeevaratnam, 2005; Naim et al., 2008), *Bacillus* sp. (Delves-Broughton, 1990; Pranoto, Rakshit, and Salokhe, 2005) and *Staphylococcus aureus* (Jamuna, Babusha, and Jeevaratnam, 2005; Pranoto, Rakshit, and Salokhe, 2005). However, inactivation kinetics of nisin on *S. aureus* and the application of nisin in fish ball have not been studied so far. Since the number of *S. aureus* in fish ball is too low to use for determining a major inhibitory effect on its growth. Hence, the challenge for the fish ball was conducted. This action was not only to limit contamination of end product with *S. aureus* as much as practically possible, but to provide a control strategy that suppresses the pathogen growth (Phillips, 1996). The present study deals with the evaluation of this potential to preserve and/or extend the shelf life of this meat product. The objective of this investigation was to determine the effective inhibitory of nisin on the growth of *S. aureus* in liquid medium and in fish ball.

**Materials and Methods**

Effect of nisin on the survival of *Staphylococcus aureus* in culture media

**Preparation of nisin stock solution**

The stock solution was prepared as described by Paik et al. (2006). Briefly, 0.0012 g of commercial nisin (Sigma-Aldrich, Germany) was dissolved in 1 ml of 0.02 N HCl. The solution volume was adjusted to 10 ml with distilled water before subjecting to heating for 10 min and kept at -20°C until used.

**Bacterial strain and culture media**

*Staphylococcus aureus* TISTR 29 was purchased from the culture collection at Thailand Institute of Scientific and Technological Research (TISTR). The bacteria was
cultured on the nutrient agar slant and kept at 4±2°C. In the preparation of seeding culture for antimicrobial test, the bacteria from agar slant was inoculated in nutrient broth (Britania, Argentina) and incubated at 37±2°C for 24 h. A serial dilution was taken to meet required bacterial population by sterile peptone water (Fluka, Germany).

Inhibitory effect of nisin against Staphylococcus aureus

The nisin stock solution was diluted with distilled water to obtain nisin concentrations of 0, 5, 10, 15 and 20 µg/ml. To establish sensitivity test, 2 ml of each nisin concentration was added to bacterial suspension in nutrient broth to obtain the microbial concentration of 7 log CFU/ml. Samples were incubated at 4±2°C and 10±2°C. For each incubation group, aliquots were taken every 6 h interval and estimated for S. aureus count using direct plating on nutrient agar (Britania, Argentina).

Kinetics determination

For each temperature, the logarithm of survivors was plotted against incubation times. D-values were calculated as the inverse negative slope of the regression line. Z-values were determined from the regression as the inverse negative slope when plotting the logarithm of D-values against the treatment concentrations.

Effect of nisin on the survival of Staphylococcus aureus inoculated in fish ball

Fish ball treatment

The fish ball was made in the Department of Microbiology at the King Mongkut’s University of Technology Thonburi. The ingredients of the fish ball are given in Table 1. Nisin at appropriate concentration obtained from the previous section was added to the fish mass prior to cooking. The fish ball was set in warm water (40±1°C) for 20 min and then cooked until the core temperature reached 90±2°C. Cooked samples were inoculated by dipping in calibrated bacterial suspension at approximately 7 log cfu of bacterial cells/ml for 10 seconds. The treated samples were packed in polyethylene bags and then incubated separately at 4±2°C (refrigerated temperature) and 10±2°C (abused storage temperature). Fish ball without nisin treatment was used as the control. All samples were taken for analysis every 3 days.

Table 1 Ingredients of the fish ball

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meat</td>
<td>94.0</td>
</tr>
<tr>
<td>Ice</td>
<td>1.0</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.8</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.9</td>
</tr>
<tr>
<td>Other spice assortment</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Analysis

Microbiological analysis

A sample (25 g) was removed aseptically and transferred to 225 ml of sterile 0.1% peptone water solution. The sample was homogenized in a stomacher (AES Smasher, Australia) for 1 min. A 10-fold dilution was made of the peptone water as needed for plating. The bacterial counts in the resultant slurry were determined by pour plate technique on manitol salt phenol red agar for S. aureus (Fluka, Germany) and on plate
count agar for total plate count (Merck, Germany). The samples were incubated at 35±2 °C for 24-48 h.

Chemical analysis

Total acidity determination

Total acidity was determined as the amount of standardized 0.1 M sodium hydroxide (NaOH) required to neutralize the meat using phenolphthaleine as indicator (AOAC, 1995). Titratable acidity was expressed as lactic acid in grams per 100 g sample.

pH measurement

The pH value was recorded using pH meter (Metrohm, Switzerland). Samples (10 g) were thoroughly homogenized with 100 ml of distilled water and the homogenate was used for pH determination.

Statistical evaluation

Analysis of variance of the data was performed using the ANOVA procedure by Duncan’s multiple range test. Significant differences (p<0.05) between mean values of triplicate samples were determined.

Results and Discussion

Inhibitory effect of nisin against Staphylococcus aureus

Inhibitory efficacy of nisin (0-20 µg/ml) against Staphylococcus aureus in nutrient broth at 4±2°C (Figure 1a) and 10±2°C (Figure 1b) was investigated. In this study, the inhibitory activity was measured based on the reduction of microbial population. Figure 1a demonstrates that S. aureus in control sample (0 µg/ml) slightly increased from 7.51±0.18 log cfu/ml to 7.73±0.08 log cfu/ml as the incubation time increased which meant that no inhibitory effect was detected. The results also revealed that addition of different concentration of nisin (5-20 µg/ml) in bacterial suspension affected S. aureus population significantly (P≤0.05). S. aureus counts were reduced by approximately 4.5-4.8 log cfu/ml when nisin at the concentrations of 15-20 µg/ml was applied whereas there was only a slight decrease by 2.5-3.3 log cfu/ml at nisin concentrations of 5-10 µg/ml at the end of the test period. This finding indicated that the degree of microbial inactivation was directly related to the concentration of nisin and incubation period. This behavior was consistent with previous findings. Jamuna, Babusha, and Jeevaratnam (2005) found that bacterial growth decreased from 10.3 to 8.1 log cfu/ml as nisin concentration increased from 40 to 80 AU/ml. This is in good agreement with Pranoto, Rakshit, and Salokhe (2005) who observed that inhibitory zone (clear zone) of chitosan films containing nisin markedly increased by the increase of nisin incorporated. The similar result was found when the experiment was conducted at 10±2°C. However, higher incubation temperature tended to slow down the inhibitory effect. As observed in Figure 1b, adding nisin at the concentrations of 5-10 and 15-20 µg/ml could reduce the bacteria counts by 2.2-2.6 log cfu/ml and 3.5-4.1 log cfu/ml, respectively. For each nisin concentration, the number of S. aureus incubated at 10±2°C was relatively higher than that incubated at 4±2°C after 42 h. The counts were 7.73±0.08, 4.94±0.08, 4.25±0.07, 2.97±0.1 and 2.77±0.18 log cfu/ml at 4±2°C and 7.83±0.03, 5.37±0.04, 4.83±0.01, 4.09±0.08 and 3.49±0.12 log cfu/ml at 10±2°C for 0 µg/ml, 5µg/ml, 10µg/ml,15µg/ml and 20µg/ml, respectively. This implied that higher temperature enhanced bacterial growth and thus lowering nisin efficacy.
Kinetics determination

In this study, D-Value refers to the time required at a certain nisin concentration to kill 90% of the *S. aureus*. This property is unique for each microorganism hence it measures a disinfectant's efficiency to reduce the number of microbes at a given nisin concentration. Table 2 summarizes D-values for *S. aureus* inoculated in the nutrient broth. The results showed that D-values significantly decreased as nisin concentration increased (p<0.05). As compared to the incubation temperature, D-values decreased from $17.92 \pm 1.09$ to $10.74 \pm 0.83$ h at $4 \pm 2^\circ$C and from $18.58 \pm 1.12$ to $12.10 \pm 0.49$ h at $10 \pm 2^\circ$C when the concentration of nisin increased from 5 to 20 µg/ml. The results explicated that a higher efficacy of nisin was observed when the sample was incubated at $4 \pm 2^\circ$C. This is to be expected as nisin activity depends upon its concentration and incubation temperature (Delves-Broughton, 1990). However, the results from statistical analysis revealed that there was no significant difference of D-values between the sample treated with nisin at concentration of 15 µg/ml and that of 20 µg/ml at both temperatures. Therefore, the concentration of nisin at 15 µg/ml was used for further study.

Plotting the log D-values against nisin concentrations provided straight line (Figure 2) from which the Z-values were estimated. In the recent study, the Z-value is defined as the concentration of nisin that is required for inhibition curve to move one log cycle. This value can relate the resistance of *S. aureus* to different nisin concentration. From Figure 2, the Z-values of nisin treated samples were 1.05 µg/ml at $4 \pm 2^\circ$C and 1.24 µg/ml at $10 \pm 2^\circ$C.

**Figure 1** Effect of nisin concentration and incubation time on *Staphylococcus aureus* at $4 \pm 2^\circ$C (a) and $10 \pm 2^\circ$C (b)

(a) (♦)0 µg/ml (Control), (●) 5µg/ml, (▲) 10µg/ml , (×)15µg/ml , (★) 20µg/ml

(b)
Table 2 D-values of *Staphylococcus aureus* at nisin concentration of 5-20 µg/ml

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>Nisin concentration (µg/ml)</th>
<th>D-value (h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4±2</td>
<td>5</td>
<td>17.92±1.09</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>14.50±0.71</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>11.16±0.59</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>10.74±0.83</td>
</tr>
<tr>
<td>10±2</td>
<td>5</td>
<td>18.58±1.12</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.89±0.04</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>13.47±0.97</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12.10±0.49</td>
</tr>
</tbody>
</table>

* means with different letters (a, b,...) in the same row are significantly different (p<0.05)

Figure 2 Z-values of *Staphylococcus aureus* at nisin concentration of 5-20 (µg/ml)

Effect of nisin on the survival of *Staphylococcus aureus* inoculated in fish ball

The effect of nisin on the *Staphylococcus aureus* counts in fish ball samples was investigated. All fish ball samples had no detectable *S. aureus* before inoculation. As shown in Figure 3, the control sample (without nisin) allowed *S. aureus* on fish ball to grow rapidly, especially at higher storage temperature. The increase of *Staphylococcus* population in log cfu from 7.27±0.33 to 9.53±0.34 and from 7.27±0.33 to 10.28±0.39 was detected at 4±2°C and at 10±2°C, respectively. On the other hand, *S. aureus* in treatments incorporating nisin decreased by approximately 1.6 log cfu on day 12 at 4±2°C and 0.84 log cfu on day 6 at 10±2°C after which the inverse effect was observed. There was a slight increase by 1.3 and 2.0 log cfu in fish ball kept at 4±2°C and at 10±2°C, respectively at the end of the test period. The initial decrease in number of *S. aureus* was due to nisin activity while the subsequently increment was due to the lowering of nisin activity and the presence of nisin-tolerant strain (Gallo, Pilosof and Jagus, 2007; De Vuyst and Vandamme, 1994).
The experimental results also inferred that nisin at the concentration of 15 µg/ml was not effective enough to prevent the contamination. However, it delayed the growth of *S. aureus* population at both incubation temperatures. In addition, the growth inhibition was more prominent at 4±2°C than at 10±2°C. This also pointed out that nisin inhibited this microorganism in a temperature dependent fashion.

The effect of nisin on the total aerobic counts is shown in Figure 4. The initial bacterial number was approximately 7.55±0.42 log cfu/g at day 0. This indicated that there was other microflora besides *S. aureus* in the samples. Meechai (2005) reported that the contaminated microorganisms on fish ball were *S. aureus*, *Escherichia coli*, *coli form*, *Clostridium perfringens* and *Salmonella sp*. For the control samples (without nisin), the microbial population increased with storage time. The total counts were 10.2±0.36 and 11.25±0.31 log cfu/g after kept at 4±2°C and 10±2°C for 30 days. Unlike aforementioned samples, nisin in treated sample was attributed to microbial reduction. The samples incorporating nisin had the minimum counts of 6.86±0.2 and 7.27±0.44 log cfu/g on day 12 at 4±2°C and on day 6 at 10±2°C, respectively. Similar to previous results, the total counts became propagated afterwards. At the end of the experiment, total flora increased to 9.18±0.42 log cfu/g for 4±2°C and 9.68±0.37 log cfu/g for 10±2°C. Observation on microbial counts, nisin treated samples stored at 4±2°C were consistently lower than those stored at 10±2°C.

The changes in pH values and acidity of the samples are shown in Figure 5. The initial pH ranged between 6.91 and 6.93 and the pH on the 30th day varied from 6.75 to 6.80 whereas the acidity of the samples was about 0.0046-0.0047% at the beginning and was 0.0048-0.0049% at the end of storage period. Statistical analysis indicated that these parameters were not significantly affected by nisin treatment, storage temperature and time (p>0.05).
Figure 4 Effect of nisin on total plate counts of fish ball inoculated with *Staphylococcus aureus* kept at 4±2°C (a) and 10±2°C (b)

Figure 5 Effect of nisin on total acidity and pH of fish ball inoculated with *Staphylococcus aureus* kept at 4±2°C (a) and 10±2°C (b)

Conclusions

Inhibition of *Staphylococcus aureus* increased as nisin concentration and incubating time increased (p<0.05). D-values for *S. aureus* were between 17.92-10.74 h at 4±2°C and 18.58-12.10 h at 10±2°C. Nisin at the concentration of 15 µg/ml did not achieve a satisfactory degree of *S. aureus* inhibition in fish ball. The fact that there were initial reduction of microorganisms and the inhibition action of nisin depended upon temperature indicated that nisin might be used together with other preservation methods to control post-processing contamination of *S. aureus* in this product.
References


Muesli development from local Thai raw materials
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ABSTRACT
Muesli development from local Thai raw materials (germinated rice, green rice, brown rice, pop rice, papaya, banana, pineapple and rambutan) was conducted in this study. The optimal process was 1) stepwise preserved fresh fruits in syrup upto 70%Brix concentration before drying by hot air dryer at 55°C for 9 hr, 2) germinated rice by soaking 5-10 hours then dried by drum dryer at 130°C, speed 2 rpm before frying at 130°C 5 sec (same as frying green rice). 100 g of each cereals and dried fruits were mixed together with 50 g peanut butter, 50 g coconut sugar, 100 g glucose syrup and 2 g salt. These were heat, mixed together and pressed in a mold, then cut down to a bar stick of 20 g. Its nutrition facts was illustrated a high content of carbohydrate (69±0.03%), including protein and fat 17.04±2.29% and 11.02±6.21%, respectively. Moreover, it was found high content of fiber (1.43±0.60%) and minerals (ash) (1.51±0.04%) also. The results indicated that it was sufficient nutrition as Thai RDI (requirement daily intake) food.

At the beginning of storage period, its moisture content and water activity (a_w) were 2.85±0.35% and 0.39±0.01. It obtained high acceptance of all sensory characteristics: crispy, adherence, odor, and total acceptance from 25 panelists. During storage period, a total acceptable score was rapidly decreased by the time (p<0.05). Total microorganisms, yeast and mold were significantly increased by the time also (P<0.05) but not affected the product acceptances. The expiration date was on day 15th with total acceptance score of 6.56±0.59, high in moisture content 7.70±0.02% and a_w 0.697±0.05%. Therefore, drying process and packing technique were developed. The total acceptance was raise from 10 days to over 26 days after increased drying process from 9 hours to 15 hours. It was illustrated the feasibility to be an economical product with production cost of 1.27 baht/20 g (market sale price was 20-25 baht each).

Keywords: Muesli, local Thai raw materials, germinated rice, brown rice, cereals

Introduction
Thai life style has been changed to easy going style for a few decades. Fast food is rising up in sale number and most of them contained low nutrient. On the other hand, a number of sales of import cereals (and/or muesli) or high nutrient fast food were growing up dramatically around 10-20% a year. In 2006, the market value of cereals product was about 600 billion baht which continuously increased every year. Muesli is a kind of high nutrient snack bar which mixed cereals and dried fruits together. Recently, Thailand has been rising in number of health concern people and number of imported goods (like muesli). Normally, local Thai food materials have many kinds of high nutrient stuffs which can be used as muesli raw materials. Most of them were cheap and sufficient all year. Muesli development from local Thai raw materials (germinated rice, green rice, brown rice, pop rice, papaya, banana, pineapple and rambutan) was conducted in this study to create nutritional food stuff.
Materials and Methods

Raw materials: from Papayom, Phattalung
1. Fruits: papaya, banana, pineapple and rambutan
2. Cereals: pop rice, green rice, new harvest Suongyod brown rice

Methods
1. Germinated brown rice
   1.1. New harvested Suangyod brown rice was soaking for 5-10 hours
   1.2. Drained and incubated in wet cloth, left for 10-24 hours
   1.3. Stop germinated by drying until moisture content was below 14%
   1.4. Dried by drum dryer at speed 1, 1.5 and 2 rpm (round per minute) at 120, 130 and 140 °C
   1.5. Selected a treatment which was achieved completely gelatinized seeds (transparency)
2. Pop rice and fried rice
   1.1. Pop rice was a ready to eat product from Papayo market, Phattalung
   1.2. Fried rice: green rice and dried germinated rice was fry at 100, 130 and 150 °C for 5, 10 and 15 second and drain oil
   1.3. Selected a treatment which was occured the highest volume after pop up
   1.4. Seal product in a low polyethylene plastic bag at ambient condition (27±3 °C, 1 atm)
3. Fruits: from Papayom market, Phattalung
   1.1. Babana: fried plain babana chip was used as an ingredient.
   1.2. Half ripe papaya, pineapple core and rambutan were used. They were cut as cubic or chunk then soaked in 1% calcium solution. Afterward, preserved them in glucose syrup from 30°Brix to 70°Brix stepwise of concentration
   1.3. Dried in hot air dryer at various temperatures (45, 50 and 55 °C) for 7, 8 and 9 hr, respectively
   1.4. Selected a treatment that was observed as the highest quality (good in color and texture) of dry fruit
   1.5. Seal product in a low polyethylene plastic bag at ambient condition (27±3 °C, 1 atm)
4. Muesli bar
   1.1. 100 g of each cereals and dried fruits were mixed together
   1.2. Added 50 g peanut butter, 50 g coconut sugar, 100 g glucose syrup, 2 g salt and a little water in a pot. Cured on heater until sticky, turn off heater
   1.3. Well mixed (a) and (b) together rapidly
   1.4. Place it in square mold and well pressed by cylindrical wood
   1.5. Baked at 180°C for 10-15 min
   1.6. Left it cool, turn the mold out and cut it as a bar stick of 20 g each
   1.7. Seal product in a low polyethylene plastic bag at ambient condition (27±3 °C, 1 atm)
   Product was evaluated in physical, chemical, biological and sensory characteristics on day 0, 5, 10 and 15 of storage as follows:
   1.1. Physical analysis: moisture content, water activity
   1.2. Chemical analysis: carbohydrate, protein, fat, fiber and ash
   1.3. Biological analysis: total bacteria, yeast and mold
1.4. Sensory evaluation: line scale test by 25 trained panelists on crispy, adhesion, flavor and total acceptance characteristics

1.5. ANOVA was used as statistic analytical method

Results and Discussion

It was found that the optimal process was preserved fresh fruits stepwise to 70°Brix of syrup concentration before drying by hot air oven at 55°C 9 hr, germinated brown rice by soaking 5-10 hours then dried by drum dryer at 130°C, speed 2 rpm before frying at 130°C 5 sec same as green rice. Dried fruits, pop rice, butter, sugar and salt were heat mixed and pressed in a mold, then cut as a 20 gram bar stick.

Muesli nutrition

Muesli nutrition (Table 1) illustrated a high content of carbohydrate (69±0.03%), followed by protein and fat 17.04 ±2.29% and 11.02 ± 6.21%, respectively. Moreover, it was found higher content of fiber and minerals (ash) 1.43 ± 0.60% and 1.51 ± 0.04% than biscuit. It indicated that it was a sufficient nutritional food as Thai RDI (requirement daily intake). Muesli was able to be suggested as energy and fiber supplemented food for sport players, student, officer etc.

<table>
<thead>
<tr>
<th>Table 1 Muesli nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutrition per 100 g</strong></td>
</tr>
<tr>
<td>product</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Muesli</td>
</tr>
<tr>
<td>Biscuit</td>
</tr>
</tbody>
</table>

*Nutrition Division (1978)*

* The percentage of nutrients that was suggested to intake daily for Thai people older than 6 years, base on 2,000 kcal/day

Muesli sensory evaluation during storage

The sensory characteristics were evaluated and demonstrated that it obtained high score of all sensory characteristics: crispy, adherence, odor, and total acceptance from 25 trained panelists. During 15 days of storage, total acceptable score was rapidly decreased by the time (p<0.05). The main effect was from loose adhesive product. It possibly due to the uncompleted dried fruits including the low density polyethylene bag allowed moisture diffuse in. These made rapid increasing of moisture content and a_w by the time (Table 4).

<table>
<thead>
<tr>
<th>Table 2 Sensory evaluation on day 0, 5 and 15 of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>line scale</strong></td>
</tr>
<tr>
<td>Storage (day)</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>15</td>
</tr>
</tbody>
</table>
Microorganism determination during storage

Total bacteria, yeast and mold was determined during storage at 0, 5, 10 and 15 day. It demonstrated that total bacteria, yeast and mold were less than regulation limit (<25-30 CFU/g)(Table 3). Consequently, this result was not the reason that affected on product acceptable.

Table 3 Total bacteria, yeast and mold in muesli on day 0, 5 and 15 of storage

<table>
<thead>
<tr>
<th>Storage (day)</th>
<th>Microorganism Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Bacteria</td>
</tr>
<tr>
<td>0</td>
<td>0.3513 ± 0.66^a</td>
</tr>
<tr>
<td>5</td>
<td>0.5187 ± 0.98^b</td>
</tr>
<tr>
<td>10</td>
<td>0.9487 ± 1.83^c</td>
</tr>
<tr>
<td>15</td>
<td>1.3447 ± 2.57^d</td>
</tr>
</tbody>
</table>

Moisture content and $a_w$ during storage

The moisture content and $a_w$ on the day 0, 5, 10 and 15 of storage was illustrated in Table 4 that rapid increasing of moisture content and $a_w$ by the time. Therefore, this problem was solved by increase drying time to 10, 13 and 15 hr. It was found that $a_w$ below 0.4 (close to cereals) was obtained at 15 hr. After process, product moisture content and $a_w$ during storage was determined again as shown in the right column of Table 4. The result showed that this product life cycle increased from 15 days to over 26 days (base on moisture content).

Table 4 Moisture contents and water activity ($a_w$) during storage

<table>
<thead>
<tr>
<th>Storage (day)</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture</td>
<td>$a_w$</td>
</tr>
<tr>
<td>0</td>
<td>2.856 ± 0.35^a</td>
<td>0.398 ± 0.01^a</td>
</tr>
<tr>
<td>5</td>
<td>4.053 ± 0.03^b</td>
<td>0.454 ± 0.02^b</td>
</tr>
<tr>
<td>10</td>
<td>5.376 ± 0.03^c</td>
<td>0.566 ± 0.02^c</td>
</tr>
<tr>
<td>15</td>
<td>7.700 ± 0.02^d</td>
<td>0.697 ± 0.05^d</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*a–f Means in a column not having a common superscript letter are different (P < 0.05)
Production cost

A production cost was 38.24 baht of total recipe 602 g (31.88 baht of material cost plus 20% of operation cost) (Table 5) which was 1.27 baht per 20 g bar stick. The market price was 20-25 baht per piece. It is feasible to develop as an economical product of Thailand.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Price/unit(baht/kg)</th>
<th>Recipe(g)</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pop rice</td>
<td>170</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>Green rice</td>
<td>60</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>papaya</td>
<td>10</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>Fried banana</td>
<td>10</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>Pineapple core</td>
<td>15</td>
<td>50</td>
<td>0.75</td>
</tr>
<tr>
<td>rambutan</td>
<td>18</td>
<td>50</td>
<td>0.9</td>
</tr>
<tr>
<td>Glucose syrup</td>
<td>25</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>Coconut sugar</td>
<td>24</td>
<td>50</td>
<td>1.2</td>
</tr>
<tr>
<td>Butter</td>
<td>50</td>
<td>50</td>
<td>2.5</td>
</tr>
<tr>
<td>Salt</td>
<td>13.33</td>
<td>2</td>
<td>0.027</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>602</td>
<td>31.877</td>
</tr>
</tbody>
</table>

Conclusions

The optimal process of this muesli product was – preserved fresh fruits stepwise to 70°C Brix of syrup concentration before drying by hot air oven at 55°C 15 hr, germinated brown rice by soaking 5-10 hours then dried by drum dryer at 130°C, speed 2 rpm before frying at 130°C 5 sec same as green rice. Dried fruits, pop rice, butter, sugar and salt were heat mixed and pressed in a mold, then cut as a 20 gram bar stick. It was found high content of fiber and minerals (ash) which indicated that it was a sufficient nutritional food for sport players, student, officer, etc. unacceptable on day 15 was relevance to the moisture content which probably came from uncompleted dried of fruits and/or it was kept in the low density polyethylene plastic package. The amount of yeast, mold, and total microorganisms, significantly increased by the time (P<0.05) but not affected the product acceptable. The expiration date was on day 15th with total acceptance score of 6.56 ± 0.59, high in moisture content 7.70 ± 0.02% and a_w 0.697 ± 0.05%. Therefore, drying process and packing technique were developed. The total acceptance was raise from 15 days to over 26 days after increased drying process from 9 hours to 15 hours.

It was illustrated the feasibility to an economical product that its production cost was 1.27 baht/ 20 g while market sale price was 20-25 baht each.

Acknowledgements

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References

Nutritional Division. 1978. Table of edible food quality in 100 g. The Government of Pharmaceutical Organization. 48. 135-140
Chemical and organoleptic properties of orange (Citrus sinensis) flavored Malaysian Dadih
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ABSTRACT

In peninsular Malaysia, dadih refers to a kind of milk gel dessert with a soft texture, and a sweet milky taste. Dadih is made from milk, sugar and salt to which whey (obtained by fermenting milk overnight with asam gelugur (Garcinia atroviridis)) has been added to acidify it to a pH range of 5.6-5.8, which is close to the isoelectric point of milk. The acidified milk mixture with the destabilized casein complex is then steamed to form a gel. In this study whey was substituted with orange juice to be used as the acidifying agent. Orange juice contributed aroma and also improved the nutritional value of the dadih. This study investigated the effects of milk, sugar and initial pH on the chemical and organoleptic characteristics of orange flavored Malaysian dadih. Two levels each of whole milk powder (15 and 20%), sugar (4 and 6%) and initial pH (5.6 and 5.8) were used in a factorial experiment to assess their effects on pH, brix, moisture and total solid content of the product. Milk was heated to 80-90°C for 10 min followed by sugar and salt (0.1%). Orange juice was obtained by squeezing and sieving fresh orange puree. The initial pH and total soluble solid of orange juice were 3.36 and 11.8 respectively. The juice was then added to the milk-sugar mixture until a certain pH (5.6 or 5.8) was reached. The mixture was then steamed to form a soft gel. The organoleptic properties were evaluated using a 7-point hedonic scale. Milk powder had a significant effect on brix, moisture and total solid but not on pH of product. The effect of sugar on all the parameters was significant except for pH and brix, while initial pH had no significant effect on all the chemical properties. Sensory evaluation showed that all the samples were acceptable. Color, taste, texture, and overall acceptability showed significant differences (P<0.05) among treatments. There was no significant differences (P>0.05) among treatments for odor.

Keywords: Dadih, orange, milk powder, Garcinia atroviridis, soft gel

Introduction

Dadih is traditional fermented milk from Malaysia. However, it is also found in West Sumatra, but prepared in a different way. Originally, dadih was made from buffalos’ milk, but now cows’ milk is more commonly used, since modern machineries have replaced buffalos in the padi fields. In Malaysia, dadih refers to a gelled milk dessert, which is sweet in taste, and has custard like texture. Traditional dadih are made by adding whey to a mixture of milk, sugar and salt. The whey is obtained by fermenting a small amount of milk overnight with asam gelugur (Garcinia atroviridis). Whey is added to the milk mixture until the pH reaches close to the isoelectric point of casein, which will
destabilize the casein, thus inducing gel formation upon heating. Dadih made this way acquire the asam gelugur flavour typical of traditional dadih. Most commercial dadih now makes use of stabilizers like agar to solidify the milk and various flavours are added. Fresh fruit, chocolate, or synthetic flavouring agents were used as the source of flavor in dairy dessert. The use of strawberry, pineapple, peach, yellow peach, red peach, mixed fruit and wild berry fruit (Sánchez-Segarra et al., 2000), mango (Kumar and Mishra, 2004), peanut (Isanga and Zhang, 2009) are some examples of flavoring agents used in dairy dessert. In this study, milk powder was used instead of fresh milk. Milk powder was reconstituted with different amounts of water to certain percentages. The reconstituted milk was preheated prior to dadih making. As noted by Anema et al. (2004), prior to acidification, in order to denature the whey proteins, milk is often heated to above 70°C. Besides its use as a sweetening agent, sugar used in an acid caseinate-stabilized emulsion can enhance the strength of protein-protein interaction (Dickinson and Merino, 2002). Tournier et al. (2006) found that higher sucrose content increased the acceptability of aroma in custard desserts. The adjustment of pH (acidification of milk) was achieved by the addition of orange juice to replace whey. Schkoda et al. (1999) found that pH influences the structure of fermented dairy products besides milk protein, fat, and various additives. Similar observation was found by Anema (2008) who noted that the final acid gel properties were influenced by pH adjustment of milk prior to heat treatment.

Orange (Citrus sinensis) is a fruit with high vitamin C content. Besides contributing to acidity, oranges also provide some nutrients like vitamin C, β-carotene, minerals as well as flavour. Two levels each of whole milk powder (15 and 20%), sugar (4 and 6%) and initial pH (5.6 and 5.8) were used in a factorial experiment to assess their effects on pH, brix, moisture and total solid content of the product.

The aim of this study was to investigate the effects of different formulations using different concentrations of milk powder, sugar, and orange juice (pH) on the chemical and organoleptic properties of orange flavored Malaysian dadih.

Materials and Methods

Materials

Milk powder (Fern Leaf), sugar, and oranges were purchased from a hypermarket in Penang, Malaysia. Ingredients used in the formulation of dadih were of food grade, while chemicals used for analysis were of analytical grade.

Methods

A factorial experiment was used with different treatments of milk powder, sugar, and pH at 2 different levels. Samples were coded as follows:
A: percentage of milk powder, A1 and A2 (15% and 20%)
B: percentage of sugar, B1 and B2 (4% and 6%)
C: pH value, C1 and C2 (5.6 and 5.8)
### Combinations of Ingredients

<table>
<thead>
<tr>
<th>Combinations</th>
<th>A = milk powder</th>
<th>B = sugar</th>
<th>C = pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1C1</td>
<td>15%</td>
<td>4%</td>
<td>5.6</td>
</tr>
<tr>
<td>A1B1C2</td>
<td>15%</td>
<td>4%</td>
<td>5.8</td>
</tr>
<tr>
<td>A1B2C1</td>
<td>15%</td>
<td>6%</td>
<td>5.6</td>
</tr>
<tr>
<td>A1B2C2</td>
<td>15%</td>
<td>6%</td>
<td>5.8</td>
</tr>
<tr>
<td>A2B1C1</td>
<td>20%</td>
<td>4%</td>
<td>5.6</td>
</tr>
<tr>
<td>A2B1C2</td>
<td>20%</td>
<td>4%</td>
<td>5.8</td>
</tr>
<tr>
<td>A2B2C1</td>
<td>20%</td>
<td>6%</td>
<td>5.6</td>
</tr>
<tr>
<td>A2B2C2</td>
<td>20%</td>
<td>6%</td>
<td>5.8</td>
</tr>
</tbody>
</table>

1. **Preparation of orange flavored Malaysian dadih**

   200 mL milk was heated to 80-90°C for 10min and cooled. Sugar and salt were then added and mixed well. Fresh orange was squeezed and sieved to separate solid and juice. The juice was added to the mixture of milk, sugar, and salt until the desired pH was reached and poured into containers to be steamed until a gel is formed. The dadih was then refrigerated.

2. **Chemical analysis**

   Moisture, Total Solids (TS) content, and ascorbic acid analysis of dadih were carried out using the AOAC (2005) method. Determination of brix was made using the refractometer (Hanna, HI 96801 USA) and pH using pH meter (Sartorius, PB 10 Germany).

3. **Organoleptic evaluation**

   The samples were served in small cups and evaluated by 23 untrained panelists from among the students of the food technology division of the School of Industrial Technology, Universiti Sains Malaysia. The samples were evaluated on color, odor, texture, taste and overall acceptability on a 7 point hedonic scale (dislike very much=1, like very much=7).

4. **Statistical analysis**

   The data collected were analyzed using Statistical Package for Social Science (SPSS), version 17.0. Means of the treatment showing significant differences (P<0.05) were subjected to Duncan Multiple Range Test.

### Results and Discussion

1. **Chemical Properties**
The results of chemical properties of samples are shown in Table 1. Final pH of products ranged from 5.74-6.30 where A1B1C2 was the lowest and A1B2C2 was the highest. A1B1C2 was significantly lower (P<0.05) compared to A1B2C2 whereas among treatments A2B1C1, A2B1C2, A2B2C1, A2B2C2, A1B1C1, and A1B2C1 showed no significant difference (P>0.05). pH of data did not show any trend.

Table 1  pH, brix, moisture, and TS of orange flavored Malaysian dadih

<table>
<thead>
<tr>
<th>Code</th>
<th>pH</th>
<th>Brix</th>
<th>Moisture</th>
<th>TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1C1</td>
<td>5.9a±0.1</td>
<td>16.8a±0.8</td>
<td>80.7c±0.5</td>
<td>19.3a±0.5</td>
</tr>
<tr>
<td>A1B1C2</td>
<td>5.7a±0.6</td>
<td>16.7a±0.3</td>
<td>80.9c±0.2</td>
<td>19.1a±0.2</td>
</tr>
<tr>
<td>A1B2C1</td>
<td>5.9ab±0.1</td>
<td>16.8a±2.1</td>
<td>79.5bc±0.5</td>
<td>20.5ab±0.5</td>
</tr>
<tr>
<td>A1B2C2</td>
<td>6.3b±0.5</td>
<td>18.5a±1.7</td>
<td>77.7ab±2.3</td>
<td>22.3bc±2.3</td>
</tr>
<tr>
<td>A2B1C1</td>
<td>5.9ab±0.1</td>
<td>21.9b±1.5</td>
<td>78.7ab±0.1</td>
<td>21.3bc±0.1</td>
</tr>
<tr>
<td>A2B1C2</td>
<td>6.0ab±0.1</td>
<td>22.0b±1.5</td>
<td>77.6ab±1.7</td>
<td>22.4bc±1.7</td>
</tr>
<tr>
<td>A2B2C1</td>
<td>5.9ab±0.1</td>
<td>22.5b±0.7</td>
<td>76.9a±0.5</td>
<td>23.1c±0.5</td>
</tr>
<tr>
<td>A2B2C2</td>
<td>6.1ab±0.1</td>
<td>21.9b±1.3</td>
<td>77.8ab±1.6</td>
<td>22.2bc±1.6</td>
</tr>
</tbody>
</table>

*aMeans in the same column followed by different letters were significantly different (P<0.05)

Brix values of the samples ranged from 16.73-22.53 where A1B1C2 was the lowest and A2B2C1 was the highest. Treatments using 20% milk powder showed significantly higher (P<0.05) brix compared to the treatments with 15% milk powder. Different sugar levels and initial pH of products had no significant effect on the samples.

Moisture content of samples ranged from 76.92-80.86% where A2B2C1 was the lowest and A1B1C2 was the highest. A2B2C1 had significantly higher (P<0.05) moisture compared to A1B1C1, A1B1C2, and A1B2C1 but was not significantly different (P>0.05) compared to A2B1C1, A2B1C2, A2B2C2. From the result, it can be seen that higher amounts of milk powder (20%) led to significantly lower (P<0.05) moisture content of dadih.

TS content showed negative correlation to moisture content. TS of the samples ranged from 19.14-23.08% where A1B1C2 was the lowest and A2B2C1 was the highest. All the treatments except A1B2C2 showed higher total solids content with higher milk powder (20%). Although some samples showed no significant difference, it can be seen that higher sugar levels (6%) increased the total solids content of the products.

From the moisture and TS content results, it can be seen that milk powder concentration has an effect on the final moisture and TS content of the product which ultimately affect the texture of the product. As noted by Lucey and Singh (1998), increasing milk concentration or dry-matter enrichment can increase the protein or solid non fat (SNF) of milk. In acidified skim milk, concentration of fat, protein, and casein concentration as representation of total solid will influence the rheological properties (Allmere et al., 1999).

Milk powder concentration had a significant effect on brix, moisture and total solid but not on pH of product. The effect of sugar on all the parameters was significant.
except for pH and brix, while initial pH had no significant effect on all the chemical properties. Interaction between milk and sugar, milk and pH, and sugar with pH was not significant. Interaction between milk, sugar and pH had a significant effect on moisture and total solid but not on pH and brix value.

2. Organoleptic evaluation of orange flavored dadih

The organoleptic results of orange flavored traditional dadih are shown in Table 2. Color, taste, texture, and overall acceptability were significantly different (P<0.05) among treatments whereas odor did not show any significant difference (P>0.05). Color scores of samples ranged from 3.61-5.61 where A2B2C2 was the lowest and A2B1C2 was the highest. A2B2C1 and A1B2C2 were significantly lower (P<0.05) than A2B1C1 and A2B1C2. Formulations A2B2C1, A1B1C1, A1B1C2, and A1B2C2 showed no significant difference (P>0.05). From the color data, it can be seen that the formula using higher milk powder (20%) and lower sugar (4%) found in A2B1C1 and A2B1C2 were significantly preferred (P<0.05) by panelists.

Table 2 Color, odor, taste, texture, and overall acceptability of orange flavored dadih

<table>
<thead>
<tr>
<th>Code</th>
<th>Color</th>
<th>Odor</th>
<th>Taste</th>
<th>Texture</th>
<th>Overall Acceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1C1</td>
<td>5.0bc±1.2</td>
<td>5.2±1.2</td>
<td>4.3±1.6</td>
<td>5.1bc±1.5</td>
<td>5.0bcd±1.2</td>
</tr>
<tr>
<td>A1B1C2</td>
<td>4.9bc±1.5</td>
<td>5.0±1.3</td>
<td>4.6dbc±1.6</td>
<td>4.4±1.4</td>
<td>4.3ab±1.3</td>
</tr>
<tr>
<td>A1B2C1</td>
<td>4.8bc±1.3</td>
<td>5.0±1.2</td>
<td>5.0bc±1.7</td>
<td>4.8bc±1.5</td>
<td>4.7bc±1.4</td>
</tr>
<tr>
<td>A1B2C2</td>
<td>3.8±1.8</td>
<td>4.7±1.6</td>
<td>4.9abc±1.6</td>
<td>3.3±1.8</td>
<td>4.1ab±1.6</td>
</tr>
<tr>
<td>A2B1C1</td>
<td>5.4±1.3</td>
<td>5.4±1.3</td>
<td>4.9abc±1.7</td>
<td>4.9bc±1.6</td>
<td>5.0bcd±1.5</td>
</tr>
<tr>
<td>A2B1C2</td>
<td>5.6±1.1</td>
<td>5.2±1.3</td>
<td>5.4bc±1.4</td>
<td>5.7±1.0</td>
<td>5.7bc±1.4</td>
</tr>
<tr>
<td>A2B2C1</td>
<td>4.4ab±1.7</td>
<td>5.1±1.2</td>
<td>5.5c±1.0</td>
<td>5.6±1.4</td>
<td>5.5cd±1.0</td>
</tr>
<tr>
<td>A2B2C2</td>
<td>3.6a±1.7</td>
<td>4.8±1.4</td>
<td>4.4ab±1.9</td>
<td>2.9±1.5</td>
<td>3.8±1.4</td>
</tr>
</tbody>
</table>

*a, b, c, d Means in the same column followed by different letters were significantly different (P<0.05)

The score for taste of the samples ranged from 4.26-5.48 where A1B1C1 was the lowest and A2B2C1 was the highest. A1B1C1 was significantly lower (P<0.05) than A2B2C1. Formulations A2B1C1, A2B1C2, A2B2C2, A2B1C2, A1B2C1, and A1B2C2 showed no significant difference (P>0.05) among them. However, there was no clear trend for taste.

Texture of the samples ranged from 2.91-5.65 where A2B2C2 was the lowest and A2B1C2 was the highest. A2B2C2 and A1B2C2 were significantly lower (P<0.05) than A2B1C1, A2B1C2, A2B2C1, A1B1C1, A1B1C2, and A1B2C1. A2B1C2 and A2B2C1 were significantly higher (P<0.05) than the other treatments except A2B1C1. However, again, no clear trend can be found in the texture data. Pereira et al. (2003) noted that gels obtained by acidification of heated and unheated reconstituted skim milk, at different TS
concentration, can be texturally discriminated using a trained sensory panel and visual and in-hand attributes.

Overall acceptability of the samples ranged from 3.83-5.70 where A2B2C2 was the lowest and A2B1C2 was the highest. A2B2C2 was significantly lower (P<0.05) than A2B1C1, A2B1C2, A2B2C1, A1B1C1, A1B2C1. A2B1C2 was significantly higher (P<0.05) than A2B2C2, A1B1C2, A1B2C1, and A1B2C2. Although no clear trend can be found in overall acceptability, the treatment using 20% milk powder, 4% sugar, and initial pH of 5.8 was preferred. Perception of dadih organoleptic characteristics in this study may relate to their composition of total solid, sourness, and sweetness. Pereira et al. (2003) said that different chemical compounds of dairy products’ behaviour in the mouth influence the enjoyment of eating.

Conclusions

Generally, levels of milk powder and sugar significantly affected chemical and organoleptic characteristics of the orange dadih. Initial pH of product did not play a significant role in influencing the properties of the final product. Products with formulation using 20% milk powder, 4% sugar, and initial pH of 5.8 was most preferred by the panelists. Sensory evaluation showed that all the samples were acceptable. Color, taste, texture, and overall acceptability showed significant differences (P<0.05), whereas odor showed no significant differences (P>0.05) among treatments.

Acknowledgement

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References


SP5-07

Nutrient value in processing of traditional snack food for elementary school children made from mixture of maize flour and Tempeh flour

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ABSTRACT

Blended food was a based food that is formulated from several ingredients to increase quantity and quality of the nutritional content. The aim of the research was to develop a blended food from local ingredients (maize and tempeh) to be use in supplementary feeding program for children in order to increase their nutritional status. The objectives were to find the acceptable maximal proportion of tempeh flour that could substitute maize flour, and to find the nutritional content of the product and the acceptability. The study used conventional procedure in making blended flour from maize and tempeh into snack foods (cookies). Snack food (cookies) made from this blended flour by adding fat, eggs, and milk. Study result is presented by descriptive quantitative method. Research showed that the best nutritional content was in the formula of maize-tempeh flour (70:30). Carbohydrates were around 62 gram, protein 20 and fat 8 gram per 100 gram formula. Total calories were around 400 kcal/100 gram blended flour. Crude fiber content was less than 2%. The nutritional content furthermore increases to more than 500 kcal/100 gram cookies as a result of additional fat. From the cookies produced, carbohydrate content was 45.78 gram, fats 30.15 gram, and protein 14.61 gram. From the organoleptic test it was found that average panelists (children) quite liked the product by giving score between 3 – 3.5 for color, flavor, and texture. However, for taste, most of the panelists did not like the formula by giving score 2.1-2.1 in 1 to 5 scale.

Keywords: blended food, supplementary feeding, snack food, tempeh flour, maize flour

Introduction

One of the strategic efforts in order to improve nutrition of children is through the provision of supplementary food. For children aged 6 - 12 months are given MP-ASI (complementary food for breastfed infant) in the form of food ingredient mixture with the optimum formulation to obtain certain nutrients. For school-age children do promotions through PMT-AS (additional feeding school children). The supplementary food for both programs was made from local ingredients which fortified with vitamin and mineral. The program is particularly designed to address nutritional problems in the Indonesian’s districts with high prevalence of moderate and severe malnutrition especially in rural area. The programs have some limitations: the continuity, physical and nutritional quality of foods which rely on the availability on local raw materials as well as the sustainability of the program.

Corn is the potential of local food products were used as sources of carbohydrates. In addition to its abundant availability and so far only used as fodder, it is also has valuable nutrient content of food sources of carbohydrates such as wheat and rice. Lack
of protein and micro components can be mitigated by the substitution of other uses local food such as tempeh.

Tempeh is a fermented product, which has a high nutritional quality of the availability of both macro and micro nutrient components and ease of the body to utilize these nutrients (bioavailability). Research showed that food with tempeh formulation may cure chronic diarrhea in patients with poor nutrition and malnutrition. Besides those affects, food with tempeh formulation may increase weight loss due to high protein content than other grains, as well raising the hemoglobin concentration due to Fe content availability and other bioactive components which are available in tempeh (Apriyantono, et al., 1996). Therefore, substitution will be examined on corn meal soybean meal as a food ingredient mixture (BMC) for children under five. It is expected that this research obtain the optimal mixture of BMC formulas and various forms of BMC products such as cakes and cookies. This product was recommended to be used as complementary breastfeeding and supplementary food for infants and school children and can be produced commercially.

Materials and Methods

Materials

Yellow corn (Zea mays L. ssp) and tempeh (soyfood) were used for production of instant corn flour (maize) and tempeh flour. The ingredients were bought from the same shop in the local market to minimize the variation of consistency, microbial contamination.

Methods

1. Product Formulation
   1.1 Production of instant corn flour (maize).
   Raw materials were chosen from parboiled and partially-de-hulled yellow corn. Yellow corn were then washed and soaked for 24 hours. After soaking, corns were drained and grind with an electric grinding machine which was available from the laboratory to make corn flour (maize). The corn flour (maize) was then sifted using an 80-mesh sieve then boiled to form slurry; characterized by the consistency of the dough. Furthermore, the dough (porridge) was cooled down until 20°C then they were packaged in a plastic bag, put into freezer -20°C temperature. After being frozen for 24 hours, the corn flour (maize) package was thawed with running cold water and then dried on 60-70°C temperature oven for 3 hours. The corn flour (maize) was then sieved using 80 mesh sieves.

   1.2 Production of instant tempeh flour.
   Fresh tempe bought from local market was cut and blanched with 100°C water for 10 minutes, removed, drained, settled at room temperature for 10 minutes, then wrapped in plastic. Tempe were packed frozen in a freezer at -20°C temperature for approximately 48 hours, then thawed for 30 minutes with running cold water, then dried in a oven at a temperature of 60-70°C for 8 hours. Dried tempeh was then grind with an electric grinder from the lab and then sieved using 80 mesh sieves.

2. The research matrix.
   The treatments were done by mixing of instant corn flour (maize) and tempeh flour with 3 matrixes. The matrixes were the combination mixture of yellow corn flour
(maize): tempeh flour was: 90:10; 80:20; and 70:30. Only the most preferable preference of those combination mixtures was selected to make the cookies.

3. Biochemical parameters
   In this, nutrients have been determined on dry weight basis. Estimations for following biochemical parameters have been carried out:
   3.1 Proximate composition
   Moisture content was estimated by the standard method of AOAC (1980). Fat, total nitrogen, and crude protein (N · 6.25) contents (AOAC, 1984), ash, and crude fiber contents of corn and tempeh mixture were estimated following the method of Sudarmadji, et. al. (1996). Total carbohydrates and energy contents were calculated by the formula given in Winarno (2003).
   3.2 Sensory evaluation
   For the assessment of acceptability, all samples were selected except the control raw sample. A 5-point hedonic scale test was performed to measure the degree of likeness or dislike for different corn and tempeh flour samples, which were rated on a scale ranging from ‘like extremely’ to ‘dislike extremely’ (Larmond, 1982). Composite scoring test was carried out to know acceptability of various quality attributes, such as color, taste, texture, flavor and acceptability of cookies made from corn samples separately.
   3.3 A Quantitative Descriptive Analysis (QDA)
   A panel of 50 school students of 5th grade was trained in the process of sensory analysis. A questionnaire was developed. Reliability of these students in the use of questionnaire was evaluated by presenting several foods twice. The process took 4 weeks.

Results and Discussion

1. Nutritional content
   The selection of the tempeh flour and corn flour (maize) combination was conducted to see suitable nutrition needed in order to formulate cookies to increase nutritional intake of elementary students. Table 1 showed the variety of nutritional content of each mixture. Based on the highest percentage of carbohydrate, fats, protein, and crude fiber, the mixture of maize and tempeh flour (70:30) combination was selected to formulate into cookies.

   **Table 1** Nutrient value found in tempeh and corn (maize) instant flour.

<table>
<thead>
<tr>
<th>Mixture of tempeh and corn (maize) instant flour</th>
<th>Carbohydrate %</th>
<th>Fats %</th>
<th>Protein%</th>
<th>Crude fiber %</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 : 0</td>
<td>84.32</td>
<td>0.74</td>
<td>7.19</td>
<td>0.57</td>
</tr>
<tr>
<td>90 : 10</td>
<td>79.16</td>
<td>2.765</td>
<td>10.425</td>
<td>0.965</td>
</tr>
<tr>
<td>80 : 20</td>
<td>74.846</td>
<td>5.155</td>
<td>12.915</td>
<td>1.595</td>
</tr>
<tr>
<td>70 : 30</td>
<td>66.836</td>
<td>7.64</td>
<td>19.21</td>
<td>1.73</td>
</tr>
</tbody>
</table>

2. Sensory evaluation
   2.1 Evaluation of instant flour combination (maize and tempeh flour).
   A panel of ten judges was selected from triangle test who were well acquainted with organoleptic qualities of flour quality. Samples were prepared in a-100
gram package after has become instant flour. The flour combination was scored in the range of liked slightly to like moderately in the hedonic scale test, using a "hedonic-scale" consisting of a 5 point scale: 1-dislikes extremely, 3-neither likes nor dislikes, and 5-likes extremely. From organoleptic test it is found that average panelists quite liked the product by giving score between 3 – 3.5 for color, flavor, and texture.

2.2 Sensory evaluation of cookies made from selected flour combination.

From the selected instant flour combination, cookies were produced to form the way to reduce the aroma and flavor from soybean (tempeh). However, for taste, most of the panelists did not like the formula by giving score 2.1-2.1 in 1 to 5 scale.

3. Total nutrient value and total energy evaluation on the cookies produced

Total energy resulted from 100 gram cookies produced can be seen at Table 2 below:

<table>
<thead>
<tr>
<th>Mixture of Maize andTempeh Instant Flour (70:30)</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>45.76</td>
</tr>
<tr>
<td>Fats</td>
<td>30.15</td>
</tr>
<tr>
<td>Protein</td>
<td>14.61</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.23</td>
</tr>
<tr>
<td>Total Calories</td>
<td>528.54</td>
</tr>
</tbody>
</table>

Discussions

1. Nutritional content

The nutrition content in the product showed increasing protein value due to tempeh addition. In addition, enzymatic process produced by microorganisms influenced quality and nutritional value, digestion process and shorter cooking time (Hesseltine & Wang, 1982). In the research showed moisture may be increased as a result of absorption of water during fermentation, but after certain period, drop in water content may be because of utilization of water in metabolic activities. Increase in ash content may be as a result of addition of salt. Ash, protein content, fat, and crude fiber increased. Crude protein may be increased because of proteolytic activity of bacteria during fermentation that degrades protein into simple proteins, peptides and amino acids which are readily utilizable by bacteria (Zamora & Fields, 1979).

Crude fiber content of food ingredients on the product formula especially for children is very important. Crude fiber content in food should not be too high because it affects the digestive process of children's food. According to the USDA standard for fiber should not exceed of 2.0% solids (USDA, 2009). Fiber analysis showed that the maximum crude fiber content in the resulting formula was still below 2%. It showed that the increase in soybean meal substitution was cause an increase in the proportion of coarse fibers in the formula is produced, from these results, we may conclude that the crude fiber in tempeh flour role in increased fiber formula resolve BMC instant flour is produced, but the highest proportion in the substitution of crude fiber still be accepted which is less than 2%.
2. Total nutrient value

   Energy content (calories) in a formula of ingredients is very important for the additional food to improve nutritional status. Foodstuffs with high energy density are very beneficial to meet the caloric needs of children every day. It's also important to note that high energy density; the child does not need to eat huge portions to meet daily calorie needs. BMC corn meal: soybean (70:30) with the energy density of about 410 grams kcal/100 considered quite high considering using 2 - 3 servings of this BMC (800-1200kcal) child's daily calorie needs can be met. Besides, from the proximate analysis results described previously, the composition of macro nutrients (proteins, fats and carbohydrates) on the BMC corn; tempeh 70:30 formula is most appropriate to the standard MP breastfeeding and supplementary food (MOH, 2006; de Pee et. al. 2008; USDA 2009).

Conclusions

   The mixture of tempeh flour and maize flour of (30 : 70) are considered functional foods and there has been an increased interest on fulfilling energy content and nutrient value status of the children. The research result has obtained the optimal mixture of BMC formulas and various forms of BMC products of cookies. This product was recommended to be used as supplementary food for infants and school children and can be produced commercially. When tempeh flour and maize flour were incorporated into the cookies, there resulted a higher protein content and total calorie firmer crumb, and darker crumb and crust color. Soya contains natural antioxidants and the 10% substitution level could have had an effect by lowering peroxide value development, but negatively affecting sensory quality of the cookie.

   This is a new and important topic that needs further investigation. The joining together of these two kinds of flour has limited exposure in literature. The health aspects that they offer are unique. There appears to be a possible synergistic relationship between these two flour combination.

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References


SP5-08

Development of an acceptable formula of herbal
Jaew – Horn (Isan hot pot) soup
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ABSTRACT

The objective of this study was to develop an acceptable formula of Herbal Jaew-Horn (Isan Hot Pot) soup. Jaew-Horn is a traditional dish for the Northeastern of Thailand and now is very popular among the Thai. It is similar to the hot pot but the soup has a unique distinctive taste and much spicier. It would be more convenient for the housewife if the soup could be in a form of a ready to cook soup. It would also be more health benefits if some herbal spices and leaves were added. The study was first used a focus group interviewing to determine a form of ready to cook soup and kind of herbal plants and spices to be added to this traditional soup recipes. Design Expert (Mixture Design-D Optimal) was used to optimize the formulation of soup based, herbal spices and herbal leaves soup together with the 9 point Hedonic scale for the acceptability test. The focus group preferred the soup which was in the dried powder form. They recommended 5 herbal spices and leaves in the dried form to be added to the soup. The final acceptable formula were dried chilly powder 1.7 %, ground roasted glutinous rice 36.125 %, dried pork bouillon 47.175 % (soup based) %, ground dried galangal 3.5 %, ground dried lemongrass 5.5 %, ground dried kaffir lime leaves 1 %, dried mulberry leaves powder 4.25 % and dried Vietnamese mint powder 0.75 % (herbal spice and leaves) by weight with the overall liking score of 8.50±0.57.

Keywords: Jaew-Horn (Isan hot pot), herbal soup powder, product formulation, mixture design, acceptability test.

Introduction

Jaew-Horn is a traditional dish for the Northeastern of Thailand and now is very popular among the Thais. It is similar to the hot pot but the soup has a unique distinctive taste and much spicier. The soup of Jaew-Horn is usually made of dried chilly powder, ground roasted glutinous rice and some herbs. Meats, vegetables and some herbs are cooked in soup. Jaew-Horn is usually for the eaten out or to be taking away, hardly cook at home. This may be due to unknown recipes to make a delicious soup and inconvenience to prepare the soup. It would more convenient for the housewife if the soup could be in the form of ready to cook such as a concentration, paste and dried powder forms. Which form would the consumer prefer have to investigate. It would also be more health benefits if some herbal spices and leaves were added. Herbal spices such as ginger (Zingiber officinale), galangal (Alpinia galanga), lemongrass (Cymbopogon citratus D.C.), kaffir lime (Citrus hystrix DC), basils (Ocimum sanctum Linn) have been used as the condiment in flavoring the Thai foods. These herbs have functional properties and health benefits. Ginger, galangal and lemongrass are commonly used for curing stomach ache and have reported that their essential oil contain antimicrobials (Mayachiew and Devahastin, 2008; Oonmetta-aree et al., 2006) while dried galangal powder and holy basil (Ocimum sanctum Linn) powder are believed to have antioxidant activity, inhibits
lipid oxidation in pork (Jantachote et al., 2006). Ginger have been reported to have anticarcinogenic, antibacterial, antifungal, hypoglycemic, and anti-atherosclerotic activity (Shukla and Sing, 2007). Mulberry leaves (Morus indica L.) and chilies (Capsicum frutescens) have promising antihyperglycemic and antioxidant role. (Chen at al, 1985; Rosa et al., 2002) These herbal spices and leaves have their distinctive flavors. Some may be enhance the flavor of the Jaew-Horn soup. Some may cause unfamiliar flavor to the soup. This study was firstly to determine the form of Jaew-Horn soup which consumer could preferred and accepted, the suitable herbal spices and leaves that could be used for soup and the product descriptive. Then secondly the study was developed an acceptable formula of Herbal Jaew-Horn (Isan Hot Pot) soup.

**Materials and Methods**

**Materials**

The Ingredients used for a soup based, herbal spices and leaves were purchased from the market in Mahasarakham town district, Mahasarakham Province. Dried chilly powder used Brand Rai Tip, dried pork bouillon used Brand Rod Dee, ground roasted glutinous rice used local made mulberry leaves used Burirum 60 variety from Charerm Pakiet Mulberry and Silk Center, Roiet Provence.

**Methods**

1. Drying of fresh herbal spices and leaves

   The fresh herbal spices and leaves were washed, leaved for dry and then finely shredded. Dry in hot air oven at 55°C until moisture content reduced to 9%, then ground and sieved through 70 Mesh.

2. Determination of the form of Jaew-Horn soup, suitable herbal spices and leaves and the product descriptive by focus group interview

   A focus group of 8 Jaew-Horn experienced consumers was used. The focus group interview was conducted using method of Resurreccion (1998), the form of Jaew-Horn soup, suitable herbal spices and leaves for flavor and functional properties and the required product descriptive were discussed and determined.

3. Optimum formulation of Jaew-Horn for soup base

   The ingredients were based on the local commercial recipes of Jaew-Horn in Mahasarakham province and the ingredients determined by the focus group were used for the formulation of soup base. The Mixture Design-D Optimal (Hu, 1999) of the Design Expert programmed Version 7.0 for the optimization. The 9 point hedonic scale was used to determine the liking of saltiness, aroma of ground roast rice, well blend taste, hotness of chili and overall liking (Macfile and Others, 1989). The hedonic scale was: 1 = dislike extremely to 9= like extremely. 30 consumers who have eaten Jaew-Horn regularly were used for the acceptability test.

4. Optimum formulation of herbal spices for Jaew-Horn soup

   From results of the optimum formulation of Jaew-Horn for soup base in 3(Optimum formulation of Jaew-Horn for soup base) and herbal spices determined by the focus group were added for the next step of formulation. The Mixture Design-D Optimal
of the Design Expert and 9 point hedonic scale acceptability test were used. The test was for liking of spicy aroma, liking of taste and overall liking. The same group of 30 consumers was used.

5. Optimum formulation of herbal spices and leaves for Jaew-Horn soup

From the results of the optimum formulation of herbal spices for Jaew-Horn soup in 4, herbal leave determined by the focus group were added for the next formulation. The Mixture Design-D Optimal of the Design Expert and 9 point hedonic scale acceptability test were used. The test was for liking of herbal aroma, liking of taste, liking of flavor intensity and overall liking. The same group of 30 consumers was used.

Results and Discussion

1. Result of the determination of the form of Jaew-Horn soup, suitable herbal spices and leaves and the product descriptive by the focus group interview

The group required Jaew-Horn soup in powder form, which was not too hot of chili taste, has a spicy flavor. The focus group determined three ingredients for soup base which were: dried chili powder dried, roasted glutinous rice and pork bouillon. The group recommended galangal, ground dried lemongrass, and ground dried kaffir lime leaves as herbal spices and mulberry and Vietnamese mint as herbal leaves.

2. Result of the optimum formulation of Jaew-Horn for soup base

The dried chili powder at the level of 2 - 5 %, dried roasted glutinous rice at the level of 37 - 45 % and pork bouillon at the level of 50 - 58 % were used in the formulation. Jaew-Horn soup of these formula made of boiling 100 g of dried soup base powder with 1,000 g of water was used for the acceptability test. The formulation design and the liking score are show in table 1. There were no differences in the score of liking for hotness of chili among formulas. For the overall liking formula number 7 had the highest score (7.17±0.99). This formula used dried chili powder at the level of 2%, dried roasted glutinous rice at the level of 42.5% and pork bouillon at the level of 55.5%. This formula was chosen for the soup base.

3. Result of the optimum formulation of herbal spices for Jaew-Horn soup

By using the formula number 7 as the soup base, the next optimum herbal spice formulation was comprised of the grounded dried galangal, grounded dried lemongrass and grounded dried kaffir lime leaves at the levels of 15 - 35 %, 15 - 60 % and 10-30% respectively. These three herbal spices were used in the proportion of 10% in the soup. The formulation design and the liking score are show in table 2. The formulation number 5 had the highest spicy aroma liking score (8.30±0.60), taste liking score (8.60±0.56) and overall liking score (8.60±0.56). This formula comprised of grounded dried galangal at the level of 35 %, grounded dried lemongrass at the level of 45% and grounded dried kaffir lime leaves at the levels of 20%. It was therefore grounded dried galangal 3.5 %, grounded dried lemongrass 4.5% and grounded dried kaffir lime leaves 2% were used in the soup recipe.

4. Result of the optimum formulation of herbal spices and leaves for Jaew-Horn soup

Grounded dried Mulberry leave powder at the levels of 75 - 95 % and grounded dried Vietnamese mint leave powder at the levels of 5 - 25 % were used for the
formulation of herbal spices and leaves Jaew-Horn soup. These herbal leaves were used in the proportion of 5% in the soup.

Formula number 3 was rated the highest herbal aroma liking score (8.30±0.70), taste liking score (8.53±0.57), flavor intensity liking score (8.27±0.52) and overall liking (8.50±0.57) among the other formulas. This formula used grounded dried mulberry leaves at the levels of 85% and grounded dried Vietnamese mint leaves at the levels of 15% (Table 3). So grounded dried mulberry leaves 4.25 % and grounded dried Vietnamese mint leaves 0.75 % were used in the soup recipe. This formula number 3 was the final optimized recipe which contained all ingredients: soup base, herbal spices and herbal leaves. This formula was accepted by the consumers with the rating of all the attribute liking and overall liking at the liking level of “very much liking. This final recipe for used dried chili powder 1.7 %, ground roasted glutinous rice 36.125 %, dried pork bouillon 47.175 %, ground dried galangal 3.5 %, ground dried lemongrass 5.5 %, ground dried kaffir lime leaves 1 %, dried mulberry leaves powder 4.25 % and dried Vietnamese mint leave powder 0.75 % for Jaew-Horn soup power.

Conclusions

The required ready to cook Jaew – Horn soup was in the form of soup power. The ingredients for soup base were dried chilli powder, ground roasted glutinous rice and dried pork bouillon. The herbal spices were ground dried galangal, ground dried lemongrass and ground dried kaffir lime leaves. The herbal leaves were dried mulberry leaves powder and dried Vietnamese mint powder. The recipe for of herbal Jaew – Horn soup power was comprised of dried chilli powder 1.7 %, ground roasted glutinous rice 36.125 %, dried pork bouillon 47.175 %, ground dried galangal 3.5 %, ground dried lemongrass 5.5 %, ground dried kaffir lime leaves 1 %, dried mulberry leaves powder 4.25 % and dried Vietnamese mint power 0.75 % dry weight.

Acknowledgments

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References


SP5-09

Influence of coconut milk mixing proportion on sensory characteristics of frozen sweet sticky rice (Kao Naew Moon)

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ABSTRACT

Sweet sticky rice or Kao Naew Moon is Thai desert enriched with tasty coconut milk. When the product is frozen, its eating quality after thawing is usually lowered by hard texture due to retrogradation which is usually related to water content. The aim of this study was therefore to investigate the effect of separated coconut milk proportion mixed before and after freezing on sensory attributes of sweet sticky rice. The prepared coconut milk used in the experiment contained 40% coconut milk, 40% sugar and 2% salt, respectively. Proportion of coconut milk mixed with steamed sticky rice before freezing was varied at 50%, 75% and 100% of its total amount in the recipe, respectively. The rest of its amount was separated, filled in plastic (PE) bag, packed and frozen altogether with sticky rice in cylindrical plastic container with diameter and height of 10 cm and 4 cm, respectively, which was subsequently mixed after thawing. After being frozen at -30 °C and kept at -18 ºC for 1 week, the samples were tasted and compared with unfrozen sample served as a control by 50 untrained panelists, using nine-point hedonic score and Just about right scale (JAR). Amongst frozen samples, it was found that sticky rice mixed with 50% of coconut milk before freezing was the most preferred (liking score = 5.88) and accepted by most consumers (80%). Furthermore, JAR indicated that this sample gave the most satisfactory sensory attribute intensity including coconut flavor, glossiness, stickiness and hardness. Nevertheless, all frozen samples were still less preferred as compared with the unfrozen control sample (p ≤ 0.05).

Keywords: sticky rice, Thai desert, frozen, sensory quality, coconut milk

Introduction

The ready-meal market has grown and expanded worldwide. Concerning the preservative technologies applied to ready to eat meals, freezing has been recognized as an excellent method of preserving the quality characteristics of food, i.e. flavor, color, texture and nutrition.

Cooked rice is known to become hard and decline in texture and tastes overtime. Freezing the cooked rice has been associated with certain problems including dehydration of the food surfaces with time, loss of moisture and glossiness, and clumping within the foods. This phenomenon is generally called retrogradation. Amylose retrogradation rapidly increases the hardness of cooked rice in a short time and amylopectin retrogradation contributes to increasing hardness during storage within at least 3 days (4 °C) (Yu et al., 2009).

Controlling the moisture content of cooked rice is one of the common methods used to reduce the rate of retrogradation which is sensitive to water content of starch paste or starch gel. The rate of starch crystallization is reduced with increasing moisture content
above 50%, presumable due to dilution of the crystallizable component in the water-plasticized starch matrix (Biliaderis, 2009).

Another method for preserving retrogradation is controlling freezing rate and storage temperature at -10°C and -20 °C to obtain a homogeneous structure without a spongy network because amylose and amylopectin chain in concentrated matrix have a reduced mobility that limits the molecular association responsible for the crystallization (retrogradation) of starch molecules (Zaritzky, 2009). In addition, adding the small molecules, i.e. sugars, enzymes, water-soluble polysaccharides, modified starch, using emulsions also retard the rate of retrogradation.

The objective of this study was to investigate the influence of coconut milk mixing proportion into the sticky rice before freezing on the product eating quality.

Materials and Methods

Materials
Sticky rice was purchased from Chaitip Co., Ltd. Coconut milk without added water was purchased from local market (Nakhon Pathom). Sugar was purchased from Thai Roong Ruang Sugar Co., Ltd. Salt was purchased from K.C. International CO., Ltd. The cylindrical PE plastic container with diameter and height of 10 cm and 4 cm, respectively was used for packing the product. The PE plastic bag with size of 6×12×0.01 cm was used to fill the separated mixing coconut milk.

Methods
Sticky rice was washed for 3 minutes before soaking for 4 hours (water: rice = 3:1). It was then followed by draining the excess water and steaming for 40 minutes. The coconut milk containing 40 % coconut milk, 40% sugar and 2% salt was heated to 70 ºC and stood for 1 minute. The steamed sticky rice was then mixed with the prepared coconut milk at various mixing proportions of 50%, 75% and 100%. The mixed sticky rice was stood for about 30 minute or until it reached the room temperature before packing in the PE plastic container. The rest of its amount (50% and 25% for the premixed proportions of 50% and 70%, respectively) was filled in the PE plastic bag, separately and put on the top of rice. The stuff were frozen at -30 ºC for 3 hours and kept at -18 ºC for 1 week. The frozen products were thawed by microwave at 900W for 2.30 minutes. For the samples with separate coconut milk, the mixing was done after thawing. The samples were tested and compared with unfrozen sample served as a control by 50 untrained panelists using nine-point hedonic score for overall liking, just about right scale for aroma, glossiness, adhesiveness, hardness and stickiness attribute.

Results and Discussion

1. Overall liking and perception of the product
According to the affective sensory evaluation using nine-point hedonic score, the unfrozen sample (control) received the highest liking score (p≤0.05). Amongst the frozen samples, sticky rice premixed with 50% portion of coconut milk was the most preferred but not significantly different from that with 75% portion.
Table 1 Sensory Characteristics in overall liking and perception by 50 untrained panelists.

<table>
<thead>
<tr>
<th>Premixed coconut milk proportion (%)</th>
<th>Overall Liking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfrozen (Control)</td>
<td>6.82±1.77a</td>
</tr>
<tr>
<td>50%</td>
<td>5.88±1.63b</td>
</tr>
<tr>
<td>75%</td>
<td>5.40±1.88bc</td>
</tr>
<tr>
<td>100%</td>
<td>4.94±1.76c</td>
</tr>
</tbody>
</table>

Note: Means in the same column followed by the same letters are not different (p> 0.05).

2. Product attribute intensity

Product attribute intensity including aroma, glossiness, adhesiveness, hardness and stickiness was evaluated using just about right (JAR) scale as shown in Figure 1-5. The satisfactory product attributes were determined by the proportion of judges (percentage) who perceived the intensity of each product attribute as satisfactory or JAR. It was found that all frozen products received less satisfactory attributes (Non-JAR, either too much or not enough strong intensity) specifically in aroma and hardness. Most panelists perceived the frozen products as not enough strong aroma and too much hard in texture. It was noticed that the premixed coconut milk at proportions of 50% and 70% improved the product adhesiveness while at proportion of 100% (no separation of coconut milk proportion) seemed to bring about more adhesiveness to the frozen product (Figure 3). The premixed coconut milk proportion of 50% gained the JAR percentage in glossiness and stickiness (Figure 2 and 5) closer to the control sample as compared to other samples. These results were in accordant with the liking scores presented in Table 1 and also indicated that the separation of coconut milk portion was necessary to improve the product attributes, especially in adhesiveness. As reported in literatures, the texture of frozen rice products is controlled by retrogradation which is associated with amount of water absorbed in the starch granules. According to these experimental results, the most suitable premixed coconut milk proportion for frozen sweet sticky rice was 50%.

![Figure 1 Aroma intensity of frozen sweet sticky rice justified by 50 untrained panelists](image-url)
**Figure 2** Glossiness intensity of frozen sweet sticky rice justified by 50 untrained panelists

**Figure 3** Adhesiveness intensity of frozen sweet sticky rice justified by 50 untrained panelists

**Figure 4** Hardness intensity of frozen sweet sticky justified by 50 untrained panelists
The experimental results indicated that eating quality of frozen sweet sticky rice was degraded by loss of aroma and increasing hardness. The separation of premixed coconut milk proportion was found improve the product quality after thawing. The premixed coconut milk proportion of 50% was the most suitable in this study.

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References

