FOOD INNOVATION ASIA CONFERENCE 2010

"INDIGENOUS FOOD RESEARCH AND DEVELOPMENT TO GLOBAL MARKET"

POSTER PRESENTATION PROCEEDINGS

SP1: Indigenous food with high market potential raw material, innovative product, chemical and physical properties and functional ingredient.

SP2: Industrial process development of indigenous food including conventional and innovative processing method, scale-up process, storage and handling.

SP3: Food safety issue of indigenous food including regulation, standard, traceability, safety, hazard and quality system.

SP4: Nutrition quality and health benefit of indigenous food

SP5: Sensory and consumer research on indigenous food

SP6: Halal indigenous food

SP7: Other food related agro-industry
FOOD INNOVATION ASIA CONFERENCE 2010

“INDIGENOUS FOOD RESEARCH AND DEVELOPMENT TO GLOBAL MARKET”

POSTER PRESENTATION PROCEEDINGS

SP1: Indigenous food with high market potential raw material, innovative product, chemical and physical properties and functional ingredient.
ABSTRACT

Dadih is a traditional Malaysian milk product consisting of milk, sugar and salt which has been acidified with whey and steamed to form a gel. The whey is obtained by fermenting milk overnight with dried asam gelugur (Garcinia atroviridis). In this study, jackfruit flavored dadih was made by a modification of the traditional method. Jackfruit was added to improve the flavor and nutritional value of dadih. Citric acid (0.1M) was used to adjust the pH close to the isoelectric point of milk so as to destabilize the casein complex and facilitate gel formation upon steaming. This study investigated the effects of milk, sugar and initial pH on the chemical and organoleptic characteristics of jackfruit flavoured 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 and then sugar and salt (0.1%) were added. Jackfruit puree, in a ratio of 50:80 (jackfruit pulp: water), was sieved to obtain the juice. Jackfruit juice (10 ml) was then added to the mixture and the pH was adjusted using citric acid. The mixture was then poured into little containers, steamed and refrigerated. 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 an opposite effect. Sensory analysis showed that jackfruit flavored Malaysian dadih formulated with 15% milk, 6% sugar and adjusted to pH 5.8 was significantly preferred (p<0.05) over other formulations. 

Keywords: Dadih, jackfruit, chemical, citric acid, milk powder

Introduction

The term dadih in Malaysia refers to a dairy based dessert which has custard like texture and is sweet in taste. Traditional Malaysian dadih has a typical flavor from the asam gelugur used in the making of whey, which is one of the ingredients used in dadih making. Whey used in dadih is obtained by fermenting a small amount of milk overnight with agam gelugur. The whey is then added to a mixture of milk, sugar and salt which is then steamed to form a gel. In Indonesia, the term dadih refers to a product which is like yoghurt and sour in taste. Modern dadih production makes use of either hydrocolloids, enzymes (rennilase) or acids. The flavor of modern dadih varies depending on the flavouring agents used like chocolate, strawberry, corn.

Jackfruit (Artocarpus heterophyllus Lam.) is a good source of vitamins (especially vitamin A from β-carotene) fiber and minerals. It can be incorporated into a product to increase the nutritional content of the product. Rahman et al. (2001) reported that high
amount of jackfruit spoilage happened during peak fruiting season and this will cause food wastage and stated that the addition of jackfruit to yogurt improved the aroma, taste, colour and texture of the yogurt.

In the tropics, milk powder is preferred over fresh milk due to the difficulty in obtaining fresh milk. In the manufacture of dairy dessert, milk powder is often used because it has good protein functionality. By reconstituting with water, powdery milk provides better control to obtain the desired amount of total solid content which ultimately determines the texture of the product. Sugar has been a part of Malaysian dadih formulation because most of the consumers prefer sweet dadih as opposed to Indonesian dadih which is sour and taste more like yoghurt. The use of sugar in dairy dessert such as ice cream was investigated by Koeferli et al. (1996); these authors studied about ice cream related to the use of sugar with fat and non fat milk solid. They found that sugar acted as a flavor profile modifier. PH adjustment is an important aspect in acidified milk preparations. In this study, milk need to be acidified close to the isoelectric point of casein in order to induce gel formation upon heating. Since jackfruit is low in acid, citric acid is used to obtain the desired pH. Anema (2008) noted that amounts of non-sedimentable denatured whey protein and κ-casein on heating is improved by increasing the pH prior to heat treatment.

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

**Materials and Methods**

**Materials**

Milk powder (Fern Leaf), sugar, and jackfruit were purchased from a hypermarket in Penang. 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   A = milk powder   B = sugar   C = pH
A1B1C1           15%           4%           5.6
A1B1C2           15%           4%           5.8
A1B2C1           15%           6%           5.6
A1B2C2           15%           6%           5.8
A2B1C1           20%           4%           5.6
A2B1C2           20%           4%           5.8
A2B2C1           20%           6%           5.6
A2B2C2           20%           6%           5.8

1. Preparation of Jackfruit Puree

Jackfruit puree was made by macerating cut jackfruit with water in a ratio of 50:80 and sieving the sample through a muslin cloth to get the juice.

2. Preparation of jackfruit dadih

200 mL milk was heated to 80-90°C for 10 min. The heated milk was then cooled to 40°C. Sugar, salt (0.1%) and 10 mL jackfruit puree was then added. pH was adjusted using citric acid (0.1M). After that the milk mixture was poured in little containers and steamed to form gel.

3. Chemical analysis

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

4. Organoleptic evaluation

The samples were served in small cups and evaluated by 22 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).

5. 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

Data related to pH, brix, moisture and TS content is shown in Table 1. pH of products ranged from 6.02-6.23. The highest pH of jackfruit dadih was found in formulation A2B2C2 (20% milk, 6% sugar and 5.8 pH). Higher initial pH led to higher final pH of products, although in some formulations, the differences were not significant.

Brix value of samples ranged from 16.60-24.20 where A1B1C1 was the lowest and A2B2C2 was the highest. The use of 20% milk powder resulted in significantly higher (P<0.05) brix value compared to 15% milk powder. However, 4 and 6% sugar levels had no significant effect on brix.

Moisture content of samples ranged from 74.59-81.55 where A2B2C2 was the lowest and A1B2C2 was the highest. The result showed a trend of higher moisture content with lower percentage of milk powder. Moisture content was significantly (P<0.05) affected by sugar levels, where higher sugar content caused significantly lower (P<0.05) moisture content, except for the pair A1B1C2 and A1B2C2 where the difference was not significant.

Table 1 pH, brix, moisture, and TS of jackfruit 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>6.1±0.0</td>
<td>16.6±0.5</td>
<td>81.5±0.1</td>
<td>18.5±0.1</td>
</tr>
<tr>
<td>A1B1C2</td>
<td>6.2±0.0</td>
<td>18.9±0.9</td>
<td>81.2±0.3</td>
<td>18.8±0.3</td>
</tr>
<tr>
<td>A1B2C1</td>
<td>6.0±0.0</td>
<td>18.0±0.6</td>
<td>79.8±0.4</td>
<td>20.3±0.4</td>
</tr>
<tr>
<td>A1B2C2</td>
<td>6.1±0.1</td>
<td>18.2±1.1</td>
<td>81.6±1.2</td>
<td>18.5±1.2</td>
</tr>
<tr>
<td>A2B1C1</td>
<td>6.1±0.0</td>
<td>21.8±0.7</td>
<td>78.5±2.0</td>
<td>21.5±2.0</td>
</tr>
<tr>
<td>A2B1C2</td>
<td>6.2±0.0</td>
<td>23.5±1.2</td>
<td>76.9±0.2</td>
<td>23.1±0.2</td>
</tr>
<tr>
<td>A2B2C1</td>
<td>6.1±0.0</td>
<td>23.4±1.1</td>
<td>75.6±0.1</td>
<td>24.4±0.2</td>
</tr>
<tr>
<td>A2B2C2</td>
<td>6.2±0.0</td>
<td>24.2±1.3</td>
<td>74.6±0.8</td>
<td>25.4±0.8</td>
</tr>
</tbody>
</table>

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

TS showed negative correlation to moisture content. TS content of sample showed increasing trend with higher milk powder content (20%). Except for the formula A1B2C2, higher sugar (6%) led to significantly higher TS content.

From those result, it was found that milk powder played a more important role than sugar and pH on moisture and TS content. Robinson and Tamime (1986) concluded that higher firmness and viscosity of yoghurt is caused by higher total solids content of milk. The association of higher or lower total solid content with the final milk product properties was also noted by Allmere et al. (1999). They found that rheological properties of acidified skim milk gels was influenced by the concentration of protein, fat, protein and casein in milk added in a formulation. A study on plain stirred yoghurt was reported by Penna et al. (2006) who noted that the increase of total solids content (9.3–22.7 %)
resulted in a significant increase in consistency index and a decrease in flow behaviour index.

Milk powder had a significant (P<0.05) effect on all parameters, except pH. Sugar had a significant effect on moisture and TS content, while initial pH had significant effects on final pH and brix. Interaction between milk and sugar had a significant effect on all parameters except brix. Moisture and TS content were influenced by the interaction between milk and pH.

2. Color, odor, taste, texture, and overall acceptability of jackfruit dadih

The organoleptic results are shown in Table 2. According to panelists, color, taste, texture, and overall acceptability showed significant difference (P<0.05) among samples. However, odor showed no significant difference (P<0.05) with different combinations of milk powder, sugar and initial pH.

For color, A2B1C2 had the lowest score whereas A1B2C2 had the highest score. There was no clear trend for color preference in jackfruit dadih samples. A1B1C1 scored lowest whereas A2B1C2 and A1B2C2 scored highest for taste. Significantly (P<0.05) higher scores were found in samples with higher initial pH (pH 5.8). The 2 levels of milk powder and sugar had no significant effect on the taste of jackfruit flavored dadih. Panellists preferred samples which are less sourish. There was no clear trend in the preference for texture. For overall acceptability, A1B2C2 had the highest score. Many factors contribute to the organoleptic evaluation in milk products. Pohjanheimo and Sandell (2009) noted that subjects who considered natural content, ethical concern, and health as important food choice motives perceived sourer, thicker, and more genuine yoghurt flavour as more pleasant, compared to subjects who considered convenience, price, mood, and familiarity more important, evaluated sweeter and smoother yoghurt as more pleasant. Addition of other uncommon additives also affect the acceptability of milk product such as found in the study of Kip et al. (2006) whereby they noted that the use of inulins can improve the creamy mouthfeel of low-fat yoghurts.

<table>
<thead>
<tr>
<th>Code</th>
<th>Color</th>
<th>Odor</th>
<th>Taste</th>
<th>Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B1C1</td>
<td>4.4ab±1.5</td>
<td>4.7±1.1</td>
<td>4.1a±1.4</td>
<td>4.2ab±1.5</td>
<td>4.1a±1.1</td>
</tr>
<tr>
<td>A1B1C2</td>
<td>4.7ab±1.3</td>
<td>4.7±1.1</td>
<td>4.8b±1.3</td>
<td>5.0bc±1.4</td>
<td>4.6±1.3</td>
</tr>
<tr>
<td>A1B2C1</td>
<td>4.5ab±1.1</td>
<td>4.5±1.2</td>
<td>4.2a±1.4</td>
<td>4.0ab±1.5</td>
<td>4.1a±1.3</td>
</tr>
<tr>
<td>A1B2C2</td>
<td>5.3b±1.4</td>
<td>5.0±1.2</td>
<td>5.3b±1.3</td>
<td>5.3c±1.2</td>
<td>5.4b±1.2</td>
</tr>
<tr>
<td>A2B1C1</td>
<td>5.1ab±1.1</td>
<td>4.8±1.1</td>
<td>4.6ab±1.3</td>
<td>4.9bc±1.2</td>
<td>4.5a±1.4</td>
</tr>
<tr>
<td>A2B1C2</td>
<td>4.2±1.7</td>
<td>4.6±1.3</td>
<td>5.3b±1.4</td>
<td>3.5a±1.8</td>
<td>3.8a±1.9</td>
</tr>
<tr>
<td>A2B2C1</td>
<td>4.6ab±1.4</td>
<td>4.9±1.4</td>
<td>4.3b±1.4</td>
<td>3.9ab±1.7</td>
<td>4.2a±1.3</td>
</tr>
<tr>
<td>A2B2C2</td>
<td>4.7ab±1.0</td>
<td>4.9±1.2</td>
<td>5.2b±1.5</td>
<td>3.5a±1.7</td>
<td>3.9b±1.6</td>
</tr>
</tbody>
</table>

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

**Conclusions**

Generally, addition of milk powder and sugar significantly affected the chemical properties of jackfruit dadih analyzed in this study. In the making of jackfruit dadih, the interaction between milk and sugar has a significant impact on the chemical characteristics.
analyzed in this study. The formula of A1B2C2 containing lower levels of powdered milk, and higher level of sugar and initial pH was significantly (P>0.05) preferred by panelists.

Acknowledgement

This work was funded by the RU grant 1001/PTEKIND/815032.

References


SP1-02

Extraction optimization of antioxidant from passion fruit seeds using response surface methodology

Samart Sai-U1, Akkasit Jongjareonrak2, Phanuphong Chaiwut3, Saroat Rawdkuen1*
1Food Technology Program, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand
2Nutraceutical and Functional Food Research and Development Center, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
3School of Cosmetic Science, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand
*To whom correspondence should be addressed. Tel: +66-5391-6752. Fax: +66-5391-6739.
*Corresponding e-mail address: saroat@mfu.ac.th

ABSTRACT

Response surface methodology (RSM) was applied to optimize the condition for phenolic antioxidant extraction from passion fruit seed. The extraction yield, extractable phenolic content (EPC) and antioxidant activities (AAO; DPPH, ABTS and FRAP assay) were determined. The extraction yield, EPC and AAO increased with the decrease in the solid-to-liquid ratio. Ethanol concentrations and temperatures significantly affected to AAO and also to extraction recovery and EPC (p<0.05). Effect of ethanol and temperature gave an increase in extraction yield, EPC and radical scavenging activity. The optimal condition of 87% ethanol, 94°C and 186 min were obtained using desirability function analysis of phenolic antioxidant extraction from passion fruit seeds. Under those optimum condition, the corresponding predicted response values (experimental values) for extraction yield, EPC, DPPH, ABTS and FRAP of the extract was 13.7% (14.4%), 3,140 (3,235), 1,328 (1,344), 5,793 (5,564) and 1,567 (1,509) mg GAE/100 g dry passion fruit seed, respectively. The values agreed with those predicted, thus indicating suitability of the model employed and the success of RSM in optimizing the extraction conditions.

Keywords: antioxidant, extraction, fruit seed, passion fruit, response surface methodology

Introduction

Utilization of wastes and by-products from the food industry has become widespread. By-products and wastes generated from the processing of fruits and vegetables are favorable raw materials for obtaining extracts rich in phenolic compounds with good antioxidants properties. The availability of phenolic compounds from agricultural and industrial residues, their extraction and antioxidant activity have been widely investigated. They have a particular interest as food preservatives or as agents to protect human health against various diseases (Pompeu, Silva & Rogez, 2009).

Passion fruit is a perennial woody creeper which is indigenous to the tropical regions of America. The two main commercial varieties are purple passion fruit (*Passiflora edulis* L.), and yellow passion fruit (*P. edulis f. flavicarpa*) that mainly cultivated in the northern part of Thailand. The preliminary results showed that one ton of passion fruits produced about 300 kilograms of juice, 110 kilograms of seed, 320 kilograms of inner peel and 225 kilograms outer peel. However, there are considerably higher ratios of seed and other by-products arising from passion fruit juice processing industry and derived products. It would be beneficial, in improving the complete utilization of the seeds and other wastes, if they could be used as a source of natural food additives and ingredients.
Extraction is a very important step in isolating these bioactive compounds. Many factors such as solvent composition, extraction time and temperature, solvent to solid ratio and pressure may significantly influence the extraction efficacy (Liyana-Pathirana & Shahidi, 2005; Karacabey & Mazza, 2010; Wijngaard & Brunton, 2010). In general, optimization of a process could be achieved by either empirical or statistical methods; the former having limitations toward complete optimization. Response surface methodology (RSM) enables evaluation of the effects of several process variables and their interactions on response variables (Box & Wilson, 1951). To optimize the extraction phenolic antioxidant from passion fruit seed, extraction condition and RSM were applied.

Materials and Methods

Chemicals

Absolute ethanol was purchased from Merck (Darmstadt, Germany). 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), and Folin–Ciocalteu phenol reagent were obtained from Fluka (Steinheim, Germany). 2,2’-diphenyl-pirclyhydrazyl (DPPH•) and Gallic acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Plant materials

Passion fruits were obtained from The Royal Project Foundation, Chiang Rai, Thailand. The fruits were washed with tap water, removed their peels and edible proportion, and then dried in hot air oven at 50°C for 24 hrs. The fruits seeds were powdered with a hammer mill through a 20-mesh (0.84 mm) sieve. The sample powder was stored in a plastic bag and kept in a freezer at ~20°C until use.

Effect of solid-to-liquid ratio on extraction of phenolic antioxidant

The influence of the solid-to-liquid ratio on the extraction was investigated, by considering six ratios (1:5, 1:10, 1:15, 1:20, 1:30, 1:40; g:ml). The total volume of the extraction was fixed at 30 ml of an EtOH:H₂O (50:50, v/v) solution. The mixtures were agitated (150 rpm) at room temperature for 4 h. Extraction solution was centrifuge at 8,000 rpm for 15 min and then supernatant was filtrated through filter paper. Residual yield and antioxidant activities of the extract were determined. The ratio gave the highest value of EPC and AAO (FRAP assay) was chosen for RSM.

Response surface methodology for extraction of phenolic antioxidant

The optimization of the extraction of phenolic compounds from the passion fruit seeds by RSM was carried out using an experimental plan based on a three factors/five levels design referred to as a rotatable central composite design, which consisted of seventeen experimental runs, including three replicates at the center point. The independent variables were the ethanol concentration (X₁; 40-80%, v/v ethanol/water), the extraction time (X₂; 60-180 min), and extraction temperature (X₃; 40-80°C). Five levels of values for the independent variables were expressed in their coded and uncoded forms (Table 1). The experimental data were fitted to a second-order polynomial model (Eq. 1) and the regression coefficients were obtained by multiple linear regression.

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=2}^{3} \beta_{ij} X_i X_j
\]  

(1)
where $X_1$, $X_2$ and $X_3$ are the independent variables affecting the responses $Y$'s; $\beta_0$, $\beta_i$, ($i = 1, 2, 3$), $\beta_{ij}$, ($i = 1, 2, 3; j = 2, 3$) are the regression coefficients for the intercept, linear, quadratic and cross-product terms, respectively.

The optimal extraction conditions were obtained by the desirability function approach using Minitab statistical software. The response surface plots were developed using the STATISTICA Kernel Release 7.0.61.0 EN (StatSoft Inc., Tulsa, OK) for Windows.

### Table 1 Independent variables and their coded and actual values used for optimization

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Units</th>
<th>Symbol</th>
<th>Code levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol proportion</td>
<td>% (v/v)</td>
<td>$X_1$</td>
<td>-1 40 60 80 93.64</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>$X_2$</td>
<td>-1 40 60 80 93.64</td>
</tr>
<tr>
<td>Time</td>
<td>min</td>
<td>$X_3$</td>
<td>19.09 60 120 180 220.9</td>
</tr>
</tbody>
</table>

Determinations

**Extractable phenolic content (EPC)**

Extractable phenolics content was determined by the Folin–Ciocalteu method, which was adapted from Swain and Hillis (1959). The absorbance was measured at 760 nm using a UV-spectrophotometer and the results were expressed in gallic acid equivalents (GAE; mg/100 g dry mass) using a gallic acid (0–200 μg/mL) standard curve.

**Ferric reducing antioxidant power (FRAP)**

FRAP was assayed according to Benzie and Strain (1996). A sample (90 μl) was mixed with 810 μl of FRAP solution and kept for 30 min in the dark at room temperature. The ferrous tripyridyltriazine complex was measured by reading the absorbance at 595 nm. A sample blank at each concentration was prepared by omitting FeCl$_3$ from the FRAP solution and distilled water was used instead. The standard curve was prepared using gallic acid. The activity was expressed as gallic acid equivalents (GAE) mg/100g dry mass.

**DPPH radical scavenging activity**

DPPH radical scavenging activity was determined as described by Brand-Williams, Cuvelier and Berset (1995) with a slight modification. The absorbance of the resulting solution was measured at 520 nm using a UV-spectrophotometer. Results are expressed as gallic acid equivalents (GAE) mg/100g dry mass.

**ABTS radical scavenging activity**

ABTS radical scavenging activity was assayed as per the method of Arnao, Cano, and Acosta (2001) with a slight modification. Samples (50 μl) with a concentration range of 0.5–10 mg/l were mixed with 950 μl of ABTS solution and the mixture was left at room temperature for 120 min in dark. The absorbance was then measured at 734 nm using the spectrophotometer. A standard curve of Gallic acid ranging from 2 to 50 μg/ml was prepared. The activity was expressed as gallic acid equivalents (GAE) mg/100g dry mass.

**Statistical analysis**

All chemical analyses were preformed in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan’s multiple-range test. Analysis was performed by using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL, USA).
Results and Discussion

Selection of solid-to-liquid ratio

Preliminary studies were performed in order to determine the required solid-to-liquid ratio for the extraction of the phenolic compounds from passion fruit seed using an aqueous alcoholic solution (50% EtOH). Solvent extraction is more frequently used for isolation of antioxidants and both extraction yield and antioxidant activity of extracts are strongly dependent on the solvent (Moure et al., 2001). Ethanol and water are the most widely employed solvents for hygienic and abundance reasons. The results showed that the extraction of the phenolic compounds was dependent on the solid-to-liquid ratio (Table 2). At the ratio of 1:15 to 1:30, no significant difference of extraction yield was observed (p>0.05). Slightly increased of the yield was obtained at the ratio of 1:40 (w/v). The yield increased with decrease in the solid-to-liquid ratio. Thus, as an economic logistic, the 1:20 solid-to-liquid ratio was chosen. In the extraction of milled berries (Cacace & Mazza, 2003) and milled black currants (Cacace & Mazza, 2002), the yields of total phenolics and anthocyanins were maximized with 19 L of solvent per kg of material, which is similar to the ratio used in this experiment.

<table>
<thead>
<tr>
<th>Sample: ethanol ratio (w/v)</th>
<th>Extraction yield (g/100g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:5</td>
<td>6.87 ± 0.90 (^a)</td>
</tr>
<tr>
<td>1:10</td>
<td>7.91 ± 0.47 (^b)</td>
</tr>
<tr>
<td>1:15</td>
<td>9.02 ± 0.40 (^c)</td>
</tr>
<tr>
<td>1:20</td>
<td>9.56 ± 0.17 (^{cd})</td>
</tr>
<tr>
<td>1:30</td>
<td>10.16 ± 0.27 (^d)</td>
</tr>
<tr>
<td>1:40</td>
<td>11.59 ± 0.08 (^e)</td>
</tr>
</tbody>
</table>

*Values are given as mean ± SD from triplicate determinations.
**Different superscripts in the same column indicate significant differences (p<0.05).

Extractable phenolic content and ferric reducing antioxidant power measured in the extracts at different solid-to-solvent ratio are presented in Figure 1. EPC markedly increased when the volume of extractant increased and reached the maximum value at the ratio of 1:30. However, FRAB of the extract continuously increased as the amount of extractant increased. They have some correlation between EPC and antioxidant power at the low level of extractant ratio. The antioxidant activity depends on the extract concentration. As a general trend, increased antioxidant activity was found with increasing extract concentration (Moure et al., 2001). Increasing of EPC, the antioxidant activity by FRAP assay also increased. The high solubility of polyphenols in hydro alcoholic solution, especially when they are in a glycoside form may explain the absence of variability for the higher ratios (Silva, Rogez & Larondelle, 2007). According to the result, whichever the ratio chosen above 1:20, the quantity of phenolic compounds extracted will not significantly increase, while the energy that consumes to remove solvent would be increase. This allows choosing any value above this limit, but one should avoid the use of an excessive quantity of solvent and energy consuming in the design of a process.
Figure 1 Effect of the solid-to-solvent ratio on the extraction of extractable phenolics content and ferric reducing antioxidant power from passion fruit seed using aqueous ethanol (50:50, v/v), at 25°C for 4 h.

Extraction optimization by RSM

The extraction condition would be optimum if the extraction yields of phenolic compounds, EPC and AAO reached maximum values. The RSM analysis demonstrated a high regression value ($R^2 = 0.9701$) for the extraction yield of phenolic compounds in the passion fruit extract. The extraction temperature and time had positive linear and negative quadratic effects on the extraction yield whereas ethanol concentration had no significance. In addition, the interaction between ethanol concentration and extraction temperature had positive effects on the total phenolic yields (data not shown).

Figure 2 showed response surface model plot of predicted models showing the effects of ethanol proportion and temperature and effects of ethanol concentration and time on the extraction yield (mg/100 g dry mass), EPC and DPPH (mg GAE/g dry mass) from passion fruit seed extract. Figure 3 is also shown the ABTS and FRAB of the extract from passion fruit seed. Optimum zone was generated, in which every point would represent a combination of extraction parameters that would give the optimum yields for the five dependent variables. According to practical (cost-saving) considerations, the point representing possible combination of the lowest levels of factors within the optimum zone would be preferred over other combinations (Xu et al., 2008).

According to the parameters in the experiment, the higher the ethanol proportion, the higher the values for extraction yield, EPC and AAO were observed. There were two distinct regions: an increasing region until approximately 60% of ethanol, followed by a decrease in the phenolic yield, a negative quadratic effect being observed ($p < 0.05$).
Figure 2  Response surface model plot of predicted models showing the effects of ethanol proportion and temperature (A, B and C) and effects of ethanol proportion and time (D, E and F) on extraction yield (mg/100 g dry mass), EPC and DPPH (mg GAE/g dry mass) from passion fruit seed extracts.

Increased in temperature, EPC and AAO seemed to be decrease. This is possibly due to their decomposition at high temperatures (Liyana-Pathirana & Shahidi, 2005). The modification in diffusion coefficient of the phenolic compounds and an increase in their solubility in the solvent probably caused of the phenomenon. Increasing ethanol proportion results in a reduction in the dielectric constant of the solution and a consequent
reduction in the energy required separating the solvent molecules, allowing the solute molecules to enter between them (Cacace & Mazza, 2002, 2003).

Figure 3  Response surface model plot of predicted models showing the effects of ethanol proportion and temperature (A and B) and effects of ethanol proportion and time (C and D) on ABTS and FRAP (mg GAE/g dry mass) from passion fruit seed extracts.

Validation of the optimized condition

The optimized conditions obtained by RSM were used to validate the predictive model of extraction for phenolic yield, EPC and AAO from passion fruit seeds. The results (Table 3) showed that the experimental data were within the 95% confidence interval of the fitted second-order polynomial model used to optimize the extraction of phenolic compounds from passion fruit seed. Thus, 87% ethanol, 94°C and 186 min were obtained using desirability function analysis of phenolic antioxidants extraction from passion fruit seed. Under those optimum conditions, the corresponding predicted response values (experimental values) for extraction yield, EPC, DPPH, ABTS and FRAP of the extracts was 13.7% (14.4%), 3,141 (3,235), 1,327 (1,344), 5,793 (5,564) and 1,567
(1.509)mg GAE/100 g dry passion fruit seed. The experimental values agreed with those predicted, thus indicating suitability of the model employed and the success of RSM in optimizing the extraction conditions.

**Table 3** Response values of phenolic antioxidants from passion fruit seed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Condition</th>
<th>Response Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yield</td>
</tr>
<tr>
<td>Passion fruit</td>
<td>Predicted</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>14.4 ± 0.66</td>
</tr>
</tbody>
</table>

**Conclusions**

The RSM was found to be most effective in prediction the optimal condition for phenolic antioxidant extraction from passion fruit seed. The conditions for extraction of phenolics from passion fruit seed was 87%, 94°C and 186 min for ethanol concentration, temperature and extraction time, respectively.

**Acknowledgements**

The authors would like to thank Mae Fah Luang University and Thailand Research Fund (TRF-MAG) for financial support under the project No. MRG-WII 525 S 088, to Mr. Samart Sai-Ut.

**References**


Antioxidant and tyrosinase inhibitory activities of RD6 and black glutinous rice soaking water

Phanuphong Chaiwut1*, Tisakorn Dumrongphuttidecah1, Chutamas Maneewong1, Punyawatt Pintathong1, Saroat Rawdkuen2

1School of Cosmetic Science, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand
2Food Technology Program, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand

*Corresponding e-mail address: phanuphong@mfu.ac.th

ABSTRACT

Before cooking, glutinous and black glutinous rice will be soaked with water overnight for complete water absorption and easy cooking. After soaking, the rice grain is separated, while the soaking water was considered as waste and will be discarded. In view of interest to value-add, this glutinous rice soaking water was subjected to analyze its antioxidant capacity by ABTS method and tyrosinase inhibitory activity. Antioxidant from rice soaking water of Black glutinous and RD6 rices were studied by using rice to water ratios of 1:1, 1:2, 1:3 and 1:5 (w/v) and soaking time of 0, 3, 6, 9, 12 and 15 h. The rice to water ratio of 1:1 provided the soaking water with the highest antioxidant power of IC\textsubscript{50} 0.64 and 6.84 mg/ml for black glutinous and RD6 rice, respectively. The time of 12 h was the most suitable to give adequate antioxidant amount in the glutinous rice soaking water. Though the glutinous rice soaking water showed quite high antioxidant activity, it exhibited rather low tyrosinase activity when compared to kojic acid with IC\textsubscript{50} of 92 and 670 μg/mL, respectively. This study showed preliminary result that the glutinous rice soaking water would be a potential source of antioxidant extraction.

Keywords: antioxidant activity, black glutinous rice, RD6 glutinous rice, soaking water, tyrosinase inhibitory activity

Introduction

Glutinous rice (\textit{Oryza sativa} L.) cv. RD6 and black glutinous rice also called waxy or sweet rice, are the staple food of Asian people. They are widely grown in the Northern and Northeastern of Thailand. Generally, large quantities of glutinous rice are consumed as principal food for daily meals in the north and northeastern parts of the country. Unlike non-glutinous rice, glutinous rice is cooked by steaming after overnight soaking instead of boiling. The long soaking time needed is due to less water uptake value determined in glutinous rice than in non-glutinous rice (Juliano & Perez, 1983; Sowbhagya, Ramesh, & Ali, 1994).

The rice soaking water or “Nam Saow Khaow” is the surplus water from bringing the milled rice to soak before cooking or ripping. In addition, the rice soaking before crushing the rice flour for the raw materials to produce other products such as dessert, sweets and cracker for the next step (Keeratipibul \textit{et al}., 2008). Soaking the milled rice is the important step in the dried rice flour processing process. For that step, rice can absorb the water to make the rice has the soft structure, which gives the usefulness for crushing the rice seed and it is easy for dividing the flour from the rice seed easily. Furthermore, soaking rice helps in increase of the food value and the mineral quantity such as zinc, calcium and iron after soaking the rice seed (Liang \textit{et al}., 2008; Liang \textit{et al}., 2009).
There have been reported that soaking the rice for a long time, increased capability of the β-glucosidase enzyme and releasing the phenolic compounds which were attached with the glucoside (Shahidi and Naczk, 2004). The time for soaking the rice has a result with the losing of these important substances. In addition, soaking the rice seed for a long time was announced that it made the rice seed loses some type of protein, sugar, lipid, ashes (Chen et al., 1999; Chiang et al., 2002) and phenolic compounds such as ferulic acid and p-coumaric acid (Shahidi and Naczk, 2004). It also can decrease the losing rate of the important substance. Decreasing the losing rate of these important substances by increasing the temperature of soaking rice is also important because increasing the temperature helps hasten the seeping of water through the rice seed. Chiang et al. (2002) reported that the time for soaking rice seeds to absorb water until saturation was long for 8 hours at temperature of 25°C.

Phenolic compounds in rice seed mainly act as antioxidant (Chi et al., 2007), most of which are flavonoids, isovitexin, cyaniding, ferulic acid and coumaric acid. The other abundant antioxidants in rice seed are oryzanol, α-tocopherols and phytic acid (Ramarathnam et al., 1989; Wu K., 1994). Isovitexin and phytic acid are highly effective to resist lipid peroxidation (Ramarathnam et al., 1989). It has been reported that made soaking rice in the ratio of 1:5 (rice : water) by mass-volume percentage (%w/v), it made the phytic acid quantity in the rice seed decreases 42-49 % and the report estimate that the phytic acid quantity was blended out in the soaked rice water. From that information, it indicated that soaking the rice of the household in the Northern and Eastern part of Thailand or in the dessert factory will soak the rice in the room temperature. It takes more than 10 hours for soaking the rice and the bioactive compound such as phenolic compound is blended out into the rice soaking water in the high quantity. Previous study also reported that antioxidant can be found in black glutinous rice (Liang et al., 2008; Liang et al., 2009).

Therefore, in view of interest to value-add of the waste from soaking rice, the purpose of the present study was to investigate and evaluate antioxidant and tyrosinase inhibitory activity in RD6 glutinous rice and black glutinous rice.

**Materials and Methods**

1. Chemicals & raw materials

ABTS (2, 2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), BHT (Butylated hydroxyl toluene), L-DOPA (3,4-Dihydroxy-L-phenylalanine), mush room tyrosinase and kojic acid were purchased from Sigma-Aldrich. 95%ethanol was obtained from Merck. Dipotassium hydrogen phosphate and sodium di-hydrogen phosphate were purchased from Fluka.

Black glutinous rice (Oryza sativa L.) and glutinous rice (RD 6) (Oryza sativa L.) were obtained from Phayao province during March 2009.

2. Effect of rice to water ratio on extraction of antioxidant

Glutinous rice (RD6) and black glutinous rice were soaked with water at the ratio of 1:1, 1:2, 1:3 and 1:5 (w/v) at room temperature for 12 h. Then, rice soaking water was rinsed and centrifuged at 5,000 rpm, 4°C for 20 min. Supernatant was then lyophilized. Antioxidant activity of rice soaking water sample was analyzed by using ABTS radical scavenging assay.

3. Effect of soaking time on extraction of antioxidant
The ratio of rice to water providing the highest antioxidant activity was chosen for study of influence of soaking time. Glutinous rice and black glutinous rice were soaked with water by using the best ratio from section 2. at various soaking times of 0, 3, 6, 9, 12 and 15h. The rice soaking water was rinsed and centrifuged at 5,000 rpm, 4°C for 20 min. Supernatant was then lyophilized. Antioxidant activity of rice soaking water sample was analyzed by using ABTS radical scavenging assay.

4. ABTS radical scavenging assay

The ABTS assay was performed followed the method of Re et al., (1999) with a slight modification. ABTS (0.0384g.) was dissolved in 10 ml of distilled water to give a 7 mM concentration. ABTS radical cation (ABTS\(^{•+}\)) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate. The mixture was stored at room temperature in the dark for 16h. For the study, ABTS\(^{•+}\) was diluted with phosphate buffer saline, pH 7.4 to an absorbance 0.70-0.80 at 734 nm. Sample (50 μL) was mixed with 1450 μL of ABTS solution and the mixture was left at room temperature for 20 min in dark. The absorbance was then measured at 734 nm using the spectrophotometer. The antioxidant activity was expressed as IC\(_{50}\) which was obtained from plotting between % ABTS scavenging and concentration of the sample. The percentage ABTS scavenging was calculated as:

\[
\text{% ABTS scavenging} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%
\]

where; \(A_{\text{control}}\) is the absorption at 734 nm of ABTS solution without sample
\(A_{\text{control}}\) is the absorption at 734 nm of ABTS solution with sample

5. Determination of tyrosinase inhibition

Tyrosinase inhibitory activity of freeze dried glutinous rice soaking water was determined by a spectrophotometric method as describe in Lim et al. (2009) with a modification. L-DOPA was used as a substrate. Sample of 50 μL was mixed with 20 μl of mushroom tyrosinase, 20 μl of L-DOPA (15 mM) and 110 μL of 0.1 M phosphate buffer (pH 6.8) in a 96-well microtitre plate. The reaction mixture was then left at room temperature for 10 min and then the absorbance was measured at 475 nm. The tyrosinase inhibitory activity was expressed as IC\(_{50}\) compared to standard kojic acid. The IC\(_{50}\) was obtained from plotting between % tyrosinase inhibition and concentration of the inhibitor. The percentage of tyrosinase inhibition was calculated as:

\[
\text{% Tyrosinase inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100\%
\]

where; \(A_{\text{control}}\) is the absorption at 475 nm of reaction mixture without inhibitor
\(A_{\text{control}}\) is the absorption at 475 nm of reaction mixture with inhibitor

6. Statistical analysis

All chemical analyses were preformed in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan’s multiple-range test. Analysis was performed by using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL, USA).
Results and Discussion

1. Effect of rice to water ratio on extraction of antioxidant

The effect of rice to water ratio on soaking glutinous rice for extracting antioxidants is shown in Table 1. The rice to water ratio had significantly effect to the antioxidant extraction. Soaking water from black glutinous rice gave the IC$_{50}$ ranged 0.64-0.74 mg/ml, while the soaking water from RD6 rice exhibited the IC$_{50}$ in the range of 6.84-8.18 mg/ml. The lowest IC$_{50}$ is response to greater antioxidant activity. The most suitable ratio of rice to water on soaking the black glutinous and RD6 rice was 1:1(w/v) which exhibited the IC$_{50}$ of 0.64 and 6.84 mg/ml, respectively. It was found that high amount of rice to water provided the higher antioxidant activity in the soaking water. The antioxidant activity of black glutinous rice to water ratio 1:1 was significantly different from those of 1:2, 1:3 and 1:5, respectively. So, there were different significant between each rice to water ratio. The antioxidant capacity containing in the soaking water might be due to water solubilization of some bioactive compounds containing in rice such as anthocyanin, vitamin E, ferulic acid, coumaric acid and another phenolic compounds (Zhang et al., 2006). However, the result clearly showed that black glutinous rice soaking water gave a much higher antioxidant than that of RD6 rice.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio(w/v)</th>
<th>IC$_{50}$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black glutinous rice soaked water</td>
<td>1:1</td>
<td>0.64±0.01</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>0.72±0.01</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>0.74±0.01</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>RD6 rice soaked water</td>
<td>1:1</td>
<td>6.84±0.03</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>7.14±0.02</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>8.18±0.04</td>
</tr>
<tr>
<td></td>
<td>1:5</td>
<td>8.05±0.50</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>0.30±0.01</td>
</tr>
</tbody>
</table>

* Different superscripts in the same column of each rice cultivar indicate the significant differences (P< 0.05).

2. Effect of soaking time on extraction of antioxidants

Time of rice soaking affecting the extraction of antioxidant was studied. The glutinous rice samples were soaked with water using the ratio of 1:1 with various time soaking of 0, 3, 6, 9, 12 and 15 h. Effect of time was evaluated by measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. As shown in Table 2, the best time of soaking glutinous rice was 12h with IC$_{50}$ of 0.64 and 6.84 mg/ml for black and RD6 glutinous rice, respectively. From the result, soaking time significantly affected to release of antioxidant in the rice soaking water. Time of soaking increase, the higher of antioxidant capacity in the soaking water was found. For the RD6 glutinous rice, the IC$_{50}$ were ranged from 6.79 to 17.98 mg/mL with the lowest belonged to 12 h of soaking time. Similarly to black glutinous rice, the IC$_{50}$ were ranged from 0.64 to 1.45 mg/mL with the lowest belonged to 12 h of soaking time. Therefore, 12 h was the best for soaking glutinous rice in according to optimal release the antioxidant to the soaking water.
Table 2  Effect of soaking time on extraction of antioxidant in rice soaking water.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soaking time (h)</th>
<th>IC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black glutinous rice soaked water</td>
<td>0</td>
<td>1.45±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.00±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.85±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.83±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.64±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.68±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RD6 rice soaked water</td>
<td>0</td>
<td>17.98±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.54±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>11.07±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10.98±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.84±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6.89±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>0.30±0.01</td>
</tr>
</tbody>
</table>

<sup>*</sup> Different superscripts in the same column of each rice cultivar indicate the significant differences (P< 0.05).

As shown in Figure 1, 0 h of soaking provided the lowest antioxidant capacity in the soaking water which needed 6 and 0.7 mg/mL for inhibiting the ABTS radical of 20% for RD6 and black glutinous rice, respectively. In contrast to the higher soaking time, especially more than 12 h, only 3.2 and 0.25 ml/mL were needed for 20% inhibition from RD6 and black glutinous rice, respectively. Chiang and Yeh, (2002) reported that during soaking, protein, lipid, ash, and some compounds leached out from the rice kernel. For 1 h at 25 °C, less than 7% of protein was loosen. Nevertheless, less than 30% of lipid and ash leached out at 25 °C (Chiang and Yeh, 2002). Therefore, soaking times and temperature during soaking are the effect of leaching out of bioactive compound in rice grain and confused in water. Ferulic acid. and p-coumaric acid are the major phenolic compounds in rice and exist in the form of free, soluble conjugated, and insoluble bound that were separated and identified from a methanol extract of rice (Bello et al, 2005). Black glutinous rice is a special cultivar of rice that contains rich anthocyanins (Guo et al, 2007) which is different from RD6 glutinous rice. This reason was related with the result in the results, which the IC₅₀ of black glutinous rice was lower than RD6 rice.

Anthocyanins are responsible for the red to black color of plant organs. They are water-soluble pigment presented in vacuole of epidermis/coat and mesophyll/flesh cells of some colorful leaves, flowers, fruits and seeds. Anthocyanins are produced from flavonoids via the shikimic acid pathway in cytoplasm. The RD6 rice appeared as white grain and therefore has no or little containing of anthocyanin. As the reason, RD6 glutinous rice soaking water had the antioxidant activity less than black glutinous rice soaking water. Hence, Black glutinous rice soaking water were high effective in antioxidant activity than RD6 glutinous rice soaking water.

From the result, black glutinous rice soaking water from 1:1 ratio of rice to water and 12h of soaking time provided the highest antioxidant activity. This condition was chosen for further study of tyrosinase inhibitory activity.
Figure 1 ABTS radical scavenging activity of black (A) and RD6 (B) glutinous rice when soaking the rice at various times.

3. Tyrosinase inhibitory activity of black glutinous rice soaking water

The tyrosinase inhibitory activity of the lyophilized black glutinous rice soaking water was also investigated by using mushroom tyrosinase. The black glutinous rice extract from the optimum condition of rice to water ratio of 1:1 (w/v) and 12 h of soaking was used. After lyophilization, 0.553% yield of 1,550 mL black glutinous rice soaking water was obtained. As result shown in Table 3, the black glutinous rice extract showed IC\textsubscript{50} of 670 μg/mL compared with that 92 μg/ml of standard tyrosinase inhibitor kojic acid. It can be seen that though the antioxidant activity from glutinous rice soaking water was comparable to the standard BHT, its tyrosinase inhibitory activity was rather low. This the result relate to reported by Manosroi \textit{et al.} (2008) that the purple glutinous rice showed tyrosinase inhibition activity lower of about 140 times of kojic acid. Although, it has high content of anthocyanin. The anthocyanins have two hydroxyl groups on the benzene ring, the hydroxyl substituent at the C-3 position enhanced the activity, but the presence of the glucose moiety reduced the activity. The main active components for antioxidation in black rice were four anthocyanidin of malvidin, pelargonidin-3, 5-diglucoside, cyaniding-3-glucoside and cyaniding-3, 5-diglucoside. Cyanidin were the best inhibitors of tyrosinase activity. For pelargonidin-3, 5-diglucoside had only one hydroxyl substituent on the benzene ring, had a weak effect. When anthocyanins are added to the reaction mixture, the structure would also be broken, and the reaction products may still retain the inhibitory effect of the tyrosinase (Takanori \textit{et al.}, 1997). Nevertheless, the chemical structure of the breakdown products of the anthocyanins and their inhibitory effect on tyrosinase of black glutinous rice can inhibit.
Table 3 Tyrosinase inhibitory activity of lyophilized black glutinous rice soaking water.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kojic acid</td>
<td>92</td>
</tr>
<tr>
<td>Black glutinous rice</td>
<td>670</td>
</tr>
</tbody>
</table>

Conclusions

The optimum condition for soaking the glutinous rice was 1:1 ratio of rice to water with 12 h of soaking time. This condition provided the highest antioxidant capacity in the soaking water with the lowest IC$_{50}$ for scavenging the ABTS radical accompanying with the rice substantially absorbed the water. Though the glutinous rice soaking water exhibited substantial antioxidant capacity, it showed rather low tyrosinase inhibitory activity when compared to that of kojic acid.

Acknowledgments

The authors would like to express their sincere thanks to Mae Fah Luang University for partially financial support.

References


Antioxidant activity applying an improved ABTS radical cation decolorization 

Lim, T.Y., Lim, Y.Y. and Yule C.M. (2009) Evaluation of antioxidant, antibacterial and 

Zhang, M. W., Guo, B. J., Zhang, R. F., Chi, J. W., Wei, C., Xu, Z. H., Zhang, Y and 
Tang, X. J. (2006) Separation, purification and identification of antioxidant 


Effect of Anthocyanin-Rich Extract from Black Rice (Oryza sativa L. 
indica) on Hyperlipidemia and Insulin Resistance in Fructose-Fed Rats, Plant 

Manosroi, A., Ruksiriwanich, W. and Manosroi, J. (2008) Free radical Scavenging and 
tyrosinase inhibition activities of fermented Thai rice for cosmeceuticals. Faculty 
of Pharmacy, Chiang Mai University.

Tsudai, T. and Osawa, T. (1997) Inhibition of Tyrosinase Activity by the Phaseolus 
vulgaris L., Food Sci. Technol. 3: 82-83.
Comparative study on extraction of phenolic compounds from some edible Thai fruit seeds

Punyawatt Pintathong1*, Saroat Rawdkuen2, Nukrak Pratumpho1, Pataporn Duangchit1, Phanuphong Chaiwut1

1School of Cosmetic Science, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand
2Food Technology Program, School of Agro-Industry, Mae Fah Luang University, Muang, Chiang Rai 57100, Thailand

*Corresponding e-mail address: punyawatt.pin@mfu.ac.th

ABSTRACT

Seeds of rambutan, lychee and longan were value-added by using them as raw materials for phenolic antioxidant extraction. Effect of extractant, method and time affecting the phenolic content extraction were investigated. The result showed that water was the best solvent to extract the phenol compounds from longan seeds, whereas ethanol was better solvent for rambutan and lychee seeds. Extractable phenolic content (EPC) increased when increase of extraction time in all seeds and methods used. Sonication method showed the most powerful method for extracting the phenol contents from longan and lychee seeds. Longan seed extract exhibited the highest EPC (2,043 µg/g) and highest antioxidant activity (IC50 0.41 µg/ml), while the rambutan and lychee extracts provided 309 and 1,103 µg/g for the EPC and gave 16.84 and 0.64 µg/ml for IC50 value, respectively. This study comprises that the fruit seeds, especially longan are attracting for using them as a new source of active ingredients in food and cosmetic applications.

Keywords: antioxidant, lychee, longan, phenolic compound, rambutan, seed

Introduction

Antioxidants are both natural and artificial (synthetic) compounds which are capable to scavenge free radicals and to inhibit oxidation processes. Epidemiological studies and intervention trials on prevention of cancer and cardiovascular disease in people taking antioxidant supplements are suggestive that dietary intake of antioxidants can help scavenge free radicals and oxidants and protect the body against diseases. However, artificial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) that have been widely used for preventing lipid peroxidation in several food products are gradually limited and controlled in the food industry because they are suspected to be toxic and carcinogenic (Namiki, 1990). Therefore, the development and isolation of natural antioxidants from natural plants are more attractive and have been highly studied for new antioxidants. Many plants such as fruits and vegetables contain various phenolic compounds, which can possess antioxidant activity (Maisuthisakul et al., 2007).

The phenolic compounds and their oxidant properties have been widely studied from many plant species, including Thai plants. The commercial Thai fruits such as rambutan, lychee and longan are wildly grown and are also popular for consumption in Thailand because they have a good taste and favors. Recently, there are over on demand and there are more wastes which are consisted of pericarps and seeds from the processing of preservative foods. This study selected fruit seeds for researching because there have been many reports on fruit seeds found a rich source of antioxidant compounds, including carotenoids, thiols, vitamins such as ascorbic acid and tocopherols, flavonoids, and
phenolics especially in longan seed. The rambutan (Nephelium lappaccum Linn.), lychee (Lichi chinensis Sonn.) and longan (Dimocapus longan Lour.) are classed as Sapindaceae family which are more potential source for extraction of the bioactive compounds for many applications.

In the present, different extraction methods such as maceration, percolation, soxhlet and supercritical fluid extraction have been used to isolate antioxidants (Grigonis et al., 2005). In addition, ultrasonic and microwave assisted-methods are also introduced for antioxidant extraction (Huang et al., 2009). However, none of them can not be estimated that which one is an appropriate method for extraction. Moreover, other factor such as solvent composition, extraction time, temperature, solvent to solid ratio and pressure could be significantly influenced for extraction efficacy (Liyana-Pathirana and Shahidi, 2005, Karacabey and Mazza, 2010). The aim of this study was targeted to extract phenolic compounds from the Thai fruit seeds (rambutan, lychee and longan) using different conditions of extractants, extraction methods, and extraction time.

**Materials and Methods**

1. Chemicals
   All chemicals and solvents used were of analytical grade. 1,1-diphenyl-picyrilhydrazyl (DPPH) was purchased from Sigma Chemical CO. (St. Louis, MO, USA). Butylate hydroxyl toluene (BHT) and Gallic acid were obtained from Fluka (Buchs, Switzerland). The Folin-Ciocaltu reagent and absolute ethanol were purchase from Merck (Darmstadt, Germany).

2. Plant materials
   The fruits (rambutan, litchee and longan) were purchased from Ban Doo Market, Chiang Rai, Thailand. Seeds were separated from pericarp and cleaned with trapping water. The seeds were then dried at 55°C until their weights were constant. The sample were subsequently ground into powder and kept at -20°C until used.

3. Extraction of phenolic compounds
   Three grams of dry sample powder were transferred into a 50 ml erlenmeyer flask with 15 mL of extractant (ethanol or DI water) in which the ratio of sample:extractant was represented to 1:5 (w/v). The samples were used for extraction of phenolic compounds with 3 different methods, i.e., maceration, ultrasonic and microwave-assisted extraction. For conventional (maceration) method, extraction was done using an incubatolr shaker with 100 rpm speed under 55°C with various extraction times of 0, 15, 30, 45 and 60 min (Prasad et al., 2009). Ultrasonic (sonication)-assisted extraction was carried out by placing the samples into an ultrasonic bath which were sonicated with frequency of 35 kHz at 55°C for 0, 15, 30, 45 and 60 min(Huang at al., 2009). For microwave-assisted extraction, the samples were cooked in a domestic microwave oven (890 watt working power) for 0, 0.5, 1, 2 and 3 min (Grigonis et al., 2005). The water loss during the microwave process was prevented by covering the sample flask with a beaker. The all extracts obtained from various extraction methods were filtered through Whatman No. 1 and the filtrates were then employed to determine for extractable phenolic content (EPC). DPPH assay was employed for determination of antioxidant capacity.
4. Determinations

4.1 Extractable phenolic content (EPC) assay

The total phenolic content in the sample was determined by using Folin-Ciocalteu’s phenol reagent (Swain and Hillis, 1959). The absorbance was measured at 760 nm by UV-VIS spectrophotometer. The results were expressed in term of gallic acid equivalent (µg GAE/ g dry weight (DW)) using gallic acid as a standard.

4.2 DPPH radical scavenging activity

The total free-radical scavenging capacity of sample was determined by using the DPPH method (Thitilertdecha et al., 2010). The absorbance of sample was measured at 520 nm by UV-VIS spectrophotometer. The inhibition of DPPH radical by the sample was calculated according to the following equation:

DPPH inhibition (%) = [(absorbance of control – absorbance of sample)/absorbance of control ] x 100.

IC50 value (the concentration required to scavenge 50% DPPH free radicals) was also calculated using BHT as standard.

4.3 Statistical analysis

The all experiments were performed in triplicate. One way analysis of variance (ANOVA) and Duncan’s New Multiple-range test were determined for the differences among the means using SPSS package version 11.5 for Windows (SPSS Inc., Chicago, USA). P-values less than 0.05 (<0.05) were regarded as significant.

Results and Discussion

1. Effect of extractant on phenolic compounds extraction

Study on extraction of fruit seeds was based on 3 parameters that were type of solvent, extraction time and extraction method. The fruit seed extracts were collected for determination of total phenolic contents using Folin-Ciocalteu method. When effect of extractants was studied, ethanol and water were used to compare for phenolic content extracted in seed samples. Gallic acid was used as a standard for determining the extractable phenol contents. The result shown in Fig. 4.1 indicated that the extractable phenolic contents ranged from 77.00 µg GAE/g (from lychee seed extracted with water and microwave assisted-method) to 2,043.62 µg GAE/g (from longan seed extracted with water and sonicate assisted-method). For rambutan seed, ethanol gave the significantly higher phenolic content than that of water in Maceration and microwave method, while the phenolic content was not significantly observed in sonication method. Moreover, it seems to be that ethanol had more effective in the extraction by maceration and microwave assisted method. Similarity results were found in the phenolic extraction from lychee seed extract. Ethanol had more significantly efficient to extract those compounds, especially by microwave-assisted method. In contrast for longan seed extract, water significantly exhibited more potential to extract the phenolic compounds from that seed. This extractant gave the extractable phenolic contents ranging of about 1,225 to 2,043 µg GAE/g, whereas the ethanol showed that ranged from about 179 to 328 µg GAE/g. The results implied that extractable phenolic contents in rambutan and lychee seeds are less polarility than that consisting in the longan seeds. Therefore, ethanol is better to extract the phenolics from rambutan and lychee seeds, while water was better for that from longan seeds.
Figure 1  Extractable phenolic contents from rambutan, lychee and longan seed extracts extracted with 95% ethanol (EtOH) or DI water under different extraction methods.
2. Effect of extraction method on phenolic compounds extraction

The fruit seeds (rambutan, litchee and longan) were extracted by 3 different methods that were maceration, sonication and microwave. The result of extractable phenol contents is shown in Fig. 2. The extraction method had no significant influence for phenolic compound extracted with water or ethanol from rambutan seeds. The low contents of EPC (133.01-351.37 µg GAE/g) were obtained in rambutan seeds, although the extraction method was changed. However, the different EPCs were significantly found in lychee and longan seeds when extraction with different methods. The highest EPC was significantly observed by sonication-assisted method, in which the EPC of 226.17 and 2,043.62 µg GAE/g was obtained in lychee and longan seeds when extraction with water, while the EPC were 226.17 and 2,043.62 µg GAE/g in ethanol extraction, respectively. Therefore, it can be summarized that extraction method had an a role for phenolic compound extraction from lychee and longan seeds, while there has no an effect on among phenolic extraction methods for rambutan seeds.

**Figure 2** Extractable phenolic contents extracted with different methods (maceration, sonication and microwave-assistant methods) from rambutan, lychee and longan seed extracts using water or ethanol as extractants. Different letters on the bars compared under the same fruit seed indicate the significant differences (P<0.05).
3. Effect of extraction time on phenolic compounds extraction

The fruit seeds were extracted with various extraction time (0, 15, 30, 45 and 60 min) by sonication and maceration methods, while extraction times of 0, 0.5, 1, 3 and 5 min were selected for microwave extraction. In this study, ethanol was used as extractant for rambutan and lychee seeds, while water was offered for longan seed according to the previous result shown in Figure 1. The EPCs in the fruit seeds, which were extracted at various times by using various methods (sonication, maceration and microwave) were represented in Fig. 3. From the figure, it proposed that time for extraction had significantly an effect on EPC. For maceration, the extraction time was achieved at 15, 30 and 45 min for rambutan, lychee and longan seeds, respectively. The highest EPC of rambutan was found in 45 min of time extraction in ethanol (330.64 µg/g) of sonication. For microwave assisted extraction, the highest total phenolic content of rambutan was found in 5 min (347.60 µg/g) of Ethanol. The highest phenolic content of lychee was found in 45 min of time extraction in EtOH (1,103.93 µg/g) of sonication. For maceration extraction, the highest total phenolic content of lychee was found in 60 min (241.79µg/g) of Ethanol. For microwave assisted extraction, the highest EPC of lychee was found in 5 min of time extraction in EtOH (522.35 µg/g). The highest phenolic content of longan was found in 30 min of time extraction in water (2,043.62 µg/g) of sonication. For maceration extraction, the highest total phenolic content of longan was found in 15 min of time extraction in H₂O (1,755.52µg/g) and microwave assisted extraction, the highest total phenolic content of longan was found in 3 min of time extraction in H₂O (1,591.27 µg/g).

4. Comparison of extractable phenolic contents (EPC) in the 3 fruit seeds.

The EPC in the 3 fruit seeds were compared. From the result in Fig. 4.8, it was found that longan seed exhibited higher EPC (2,043.62 µg/g) than litchee and rambutan with water. In contrast, longan seed in all conditions with 95% EtOH exhibited lower EPC than longan seed with water. The extract of rambutan exhibited higher EPC (351.37 µg/g) >longan (328.22 µg/g)>litchee (255.52 µg/g) with 95% EtOH, respectively. In this study, all fruits had high % yield of EPC was nearly which it under condition at 95% EtOH. It mean there are high phenolic content such as five compound namely; gallic acid, (-)-epicatechin, (-)-gallocatechin, procyanidin B2, and (-)-epicatechin-3-gallate (Prasad et al., 2009). It was possible reason that longan seed was highest EPC with water because there were another potential source of potent natural antioxidants (Rangkadilok., et al. 2007) and there were uncharacterized polyphenols from other fruits (Zheng et al., 2009). When their compared extractable phenolic contents and antioxidant capacities. The result showed longan much lower IC50 than litchee and rambutan, respectively which it mean longan highest antioxidant power and rambutan lowest of antioxidant power. So, it can be dedicated that the phenolic content was relationship with antioxidant power by the high level of phenolic compounds that contain hydroxyl group can inhibit extensive free-radical scavenging activities as hydrogen donor or electron-donating agents, and metal ion-chelating properties (Prasad et al., 2009). In this study, that was the highest of hydroxyl group in the phenolic effect to the higher antioxidant activity.
Figure 3 Extractable phenolic content of rambutan, lychee and longan seed extracted with different method at various time of extraction. Different letters on the bars compared under the same fruit seed indicate the significant differences (P<0.05).
Table 1 The 50% DPPH scavenging activity and EPC of rambutan, litchee and longan seed extracts

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fruit</th>
<th>Method</th>
<th>Time(min)</th>
<th>Solvent</th>
<th>EPC(μg/g)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rambutan</td>
<td>Sonicate</td>
<td>45</td>
<td>95% EtOH</td>
<td>330.64±35.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.83±0.76&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Rambutan</td>
<td>Maceration</td>
<td>15</td>
<td>95% EtOH</td>
<td>295.64 ± 119.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.84±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Rambutan</td>
<td>Microwave</td>
<td>1</td>
<td>95% EtOH</td>
<td>309.64 ± 13.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.46±8.09&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Litchee</td>
<td>Sonicate</td>
<td>45</td>
<td>95% EtOH</td>
<td>1,103.93±84.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.64±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Litchee</td>
<td>Maceration</td>
<td>30</td>
<td>95% EtOH</td>
<td>192.51 ± 23.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.45±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>Litchee</td>
<td>Microwave</td>
<td>5</td>
<td>95% EtOH</td>
<td>522.35 ± 29.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.13±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>Longan</td>
<td>Sonicate</td>
<td>30</td>
<td>Water</td>
<td>2,043.62±21.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.41±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>Longan</td>
<td>Maceration</td>
<td>15</td>
<td>Water</td>
<td>1,755.52±702.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.94±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>Longan</td>
<td>Microwave</td>
<td>1</td>
<td>Water</td>
<td>1,545.50±77.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values indicate mean±S.D of three replicates. Means with different superscript letters in same column are significantly different (p<0.05).

Conclusions

Ethanol had effective to extract the phenolic compounds from rambutan and litchee seeds, while the water had the most potential for that longan seeds. When time of extraction was increased, the extractable phenolic contents trended to be increased in all seed extracts. Sonication and microwave assisted extraction seemed to be substantial for total phenolic extraction from fruit seeds. When using water as extractant, longan seed extract showed the highest EPC of 1,225, 2,043 and 1,545 μg/g for extraction by sonication, maceration and microwave, respectively. When using ethanol as extractant, all 3 seed extracts exhibited the ranging from 146 to 351 μg/g. Longan seed extract from the optimum narrow EPC amount. Extraction condition showed the highest antioxidant activity with IC<sub>50</sub> ranged of 0.41-0.94 μg/ml. The antioxidant activity of all seed extracts was correlated with EPC in the all extracts.

Acknowledgments

The authors would like to express their sincere thanks to Mae Fah Luang University for partially financial support.

References


SP1-05
Classification of Thai honey origins by their mineral contents
Nongnuch Sungayuth*, Jitranut Leewatchararongjaroen, Pitiporn Ritthiruangdej
Mahidol University, Kanchanaburi Campus, 199, Lumsun, Saiyok, Kanchanaburi 71150
*Corresponding e-mail address: scntt@mahidol.ac.th

ABSTRACT

Honey is a product of the elaboration of flower nectar by bees. The general features and elemental composition of honey depend on its botanical origin. In this study, five elements (Na, K, Mg, Ca and Zn) were determined to relate with the origins of four types of Thai honey where as (1) longan flower (Dimocarpus sp.), (2) lychee flower (Litchi sp.), (3) sunflower (Helianthus sp.) and (4) wild flower (Eupotorium sp.). The 91 Thai honey samples from different types and places of Thailand (harvests in 2007-2008) were wet-digested before determining the five elements (Na, K, Mg, Ca and Zn) using flame atomic absorption spectrometry. The results showed three different concentration ranges of the five minerals. The first, the K showed the highest levels with concentrations ranged between 103.6-1137.4 mg/kg. The second, the Na (17.4-130.2 mg/kg), the Mg (8.4-40.4 mg/kg) and the Ca (25.9-177.8 mg/kg) showed the moderate mineral contents of honey samples. The third, the Zn (0.8-4.2 mg/kg) was the lowest level for all honey samples. The characteristics of the five mineral contents and the color in 91 samples of the four botanical origins were classified using a principal component analysis (PCA) concept. The contents of Ca (PC1,0.945), K (PC1,0.826) and Mg (PC1,0.635) in honey are the major indicators for classifying the four honey origins where the percentage of variance is 43.11%. The contents of the three minerals in the origins of Thai honey are: sunflower > wild flower > longan flower > lychee flower. While as the contents of Zn (PC2,0.733) and Na (PC2,-0.708) in honey are the minor indicators where the percentage of variance is 25.21%. The contents of the Zn and Na minerals in the Thai honey can classify the origin of honey of the longan flower from the others. 

Keywords: Classification, Minerals, Origins, Honey, Sources

Introduction

Honey is one of the most complex foods produced by nature, and is the only sweetening agent that can be used by humans without processing. Each honey is unique based on nature of the botanical origins or the apiculture. A review report mentions that sources of honey are the major parameters which are effects on their chemical and physical properties such as pH, acidity, ash content, color, electrical conductivity, aroma, precipitation, as well as metals reported by Sanz, M.L. et.al in 2005. Chemical and physical properties of honey are importance to their applications and their values or their prices. Some of many kinds of Thai famous honey products are from longan flower, lychee flower, sun flower, and wild flowers.

Many articles have been attempted to classify and characterize the origin of honeys around the world by their compositions such as moisture, total sugars, ash and total acid reported by Nagai, T. et al. in 2006. However, metal contents are a significantly key indicator that can be used to classify the origin of honeys especially Na, K, Mg, Ca and Zn reported by Hernandez, O.M. et al. in 2005, Spano, N. et al. in 2008 and Rashed, M.N. and Soltan, M.E. in 2004. These parameters may not depend on age of honey compared to the
others. Moreover, the several metals can be referred to the source and type of contaminations and locations of the honey as well using statistical techniques.

Several statistical techniques have been applied to correlate the parameters and honey origins namely partial least-squares (PLS) regression, neural networks, cluster analysis, linear discriminant analysis (LDA), the K nearest neighbour (KNN), including principle component analysis (PCA) mentioned by Latorre, M.J. et al. in 2000.

The purpose of this work was to determine the color and the metal compositions of Na, K, Mg, Ca and Zn in 91 honey samples from four Thai botanical origins where as (1) sunflower (*Helianthus* sp.), (2) lynchee flower (*Litchi* sp.), (3) longan flower (*Dimocarpus* sp.) and (4) wild flower (*Eupotorium* sp.). The data of colors and metal contents were related with the botanic origins of the honey samples using principle component analysis (PCA).

**Materials and Methods**

**Materials**

The four botanic origins of the 91 Thai honey samples obtained from different locations and harvested by 2007-2008: (1) longan flower honey 30 samples, (2) lynchee flower honey 13 samples, (3) sunflower honey 30 samples and (4) wild flower honey 18 samples. Standard stock solutions of the five metals (Na, K, Mg, Ca and Zn) are 1000 μg/mL concentrations were atomic absorption spectroscopic grade. The stock solutions were diluted to make working solutions. Reagent-grade nitric acid and hydrogen peroxide, ultrapure deionized Milli-Q water was used.

**Methods**

1. **Sample digestion**

   About 0.5 mL of warm honey sample at 50 °C for 15 min in water bath was placed in Erlenmeyer flask. Then the 1.5 mL of 1:2 ratios of HNO₃:H₂O₂ were placed in the Erlenmeyer flask, and sonicating for 30 min. after that make volume to 50.0 mL by deionized water. All samples were prepared similar for 3 times.

2. **Determination of colors**

   Fives parameters of color namely (1) L*(lightness, 0-100), (2) a*(red), (3) b*(yellow), (4) C*<sub>ab</sub> (chroma) and (5) h<sub>ab</sub> (hue angle) were determined using the Minolta CR-400 colorimeter. The averages of the five color parameters in each botanic type of Thai honey were presented in table 1.

3. **Determination of metals**

   All metal were determined by comparing the atomic absorption signal for each sample with the standard solution signal of the same metal. The flame atomic absorption spectrometry used was GBC Model 932 Plus. The metal contents in all honey samples were obtained with the standard deviation from three times per each sample. The ranges of metal concentrations in each botanic type of Thai honey were shown in table 2.

4. **Data processing by principal component analysis (PCA)**

   The results of colors and metal contents were processed using principle component analysis to search for data trends by combining the original variables. The multivariate technique provides a partial view of the data in a space with a reduced number of dimensions while preserving most of their variability. The three principal components (PCs) were obtained and explained their relationship in the results and discussion section.
Results and Discussion

1. Determination of colors

The average values of each color parameter were presented in table 1. From the results, the color parameters of the lychee flower honey presented a significant different than the other types of honey. The lychee flower honey presented the highest \( L^* \) (76.0), \( h_{ab} \) (89.3), and the lowest \( a^* \) (0.9), \( b^* \) (32.6), and \( C^* \) (32.6). It referred that the lychee flower honeys were pale brown color than the other honeys. While as the color parameters from the longan flower, sunflower, and wild flower honeys showed less different average values. However, these results of all color parameters were combined with the metal contents for classifying process of the botanic origins of honeys using the principal component analysis.

Table 1 The average values of the five color parameters of \( L^* \), \( a^* \), \( b^* \), \( C^* \), and \( h_{ab} \) which were obtained from the 91 honey samples from the different botanic types and locations in Thailand.

<table>
<thead>
<tr>
<th>Botanical origin</th>
<th>Average values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( L^* )</td>
</tr>
<tr>
<td>Lynchee flower (C) (13 samples)</td>
<td>76.0</td>
</tr>
<tr>
<td>Longan flower (G) (30 samples)</td>
<td>65.7</td>
</tr>
<tr>
<td>Sunflower (S) (30 samples)</td>
<td>65.</td>
</tr>
<tr>
<td>Wild flower (W) (18 samples)</td>
<td>59.0</td>
</tr>
</tbody>
</table>

2. Determination of metal contents

The concentrations of metal in the four botanic types were presented in table 2. The results showed that the concentrations of K were a major indicator to classify the type of honeys because the ranges of K concentrations quite different in each botanic honey by the K concentration range of honeys from wild flower > longan flower > sunflower > lychee flower as shown in table 2. However, the relationship between metal concentrations and the sources of botanic were analyzed using principal component analysis.

Table 2 Concentration ranges of Na, K, Mg, Ca and Zn (mg/kg) which were obtained from the 91 honey samples from the different botanic types and locations in Thailand.

<table>
<thead>
<tr>
<th>Botanical origins</th>
<th>Concentration range of metals (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Lynchee flower (C) (13 samples)</td>
<td>17.9-49.4</td>
</tr>
<tr>
<td>Longan flower (G) (30 samples)</td>
<td>17.4-33.9</td>
</tr>
<tr>
<td>Sunflower (S) (30 samples)</td>
<td>19.2-130.2</td>
</tr>
<tr>
<td>Wild flower (W) (18 samples)</td>
<td>19.4-103.3</td>
</tr>
<tr>
<td>Range (mg/kg)</td>
<td>17.4-130.2</td>
</tr>
</tbody>
</table>
2. Principal component analysis (PCA)

Ten parameters from five color parameters and concentrations of five metals were reduced to three principal components. The three principal components accounted for 78.38% of total variance; the first (PC1) accounted for 44.33%, the second (PC2) accounted for 21.58%, and the third (PC3) accounted for 12.47%. List of loading based on the original variables in the first three PCs as presented in table 3.

Table 3 Loading variables in the first three principal components and percent variance of the components.

<table>
<thead>
<tr>
<th>Variable parameters</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>0.205</td>
<td>-0.244</td>
<td>-0.722</td>
</tr>
<tr>
<td>K</td>
<td>0.833</td>
<td>0.099</td>
<td>-0.036</td>
</tr>
<tr>
<td>Mg</td>
<td>0.595</td>
<td>-0.141</td>
<td>0.375</td>
</tr>
<tr>
<td>Ca</td>
<td>0.816</td>
<td>-0.241</td>
<td>-0.118</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.103</td>
<td>0.282</td>
<td>0.704</td>
</tr>
<tr>
<td>L*</td>
<td>-0.926</td>
<td>-0.265</td>
<td>-0.040</td>
</tr>
<tr>
<td>a*</td>
<td>0.927</td>
<td>0.277</td>
<td>0.011</td>
</tr>
<tr>
<td>b*</td>
<td>0.234</td>
<td>-0.934</td>
<td>0.192</td>
</tr>
<tr>
<td>Chroma</td>
<td>0.466</td>
<td>-0.834</td>
<td>0.180</td>
</tr>
<tr>
<td>Hue</td>
<td>0.824</td>
<td>0.465</td>
<td>-0.065</td>
</tr>
<tr>
<td>% variance</td>
<td>44.33</td>
<td>21.58</td>
<td>12.47</td>
</tr>
</tbody>
</table>

The first two greatest weights (PC1 and PC2), 65.91% variance, should suffice to identify any differences of the four botanic origins. The relationship between PC1 and PC2 were showed in figure 1. From the figure 1, the lychee flower (C) honeys showed the highest L*(PC1) but the low values of K(PC1), Mg(PC1), Ca(PC1), a*(PC1), b*(PC2), chroma(PC2), and hue angle were lower than the other types of honeys. The sunflower honey were the highest and similar b*(PC2) and chroma(PC2).

Figure 1 Score plot of 91 honey samples from different four botanic honeys and different locations (PC1; K, Mg, Ca, L*, a*, and hue angle and PC2; b* and chroma) where as (C) is Lychee flower honey (13 samples), (G) Longan flower honey (30 samples), (S) Sunflower honey (30 samples), and (W) Wild flower honey (18 samples)
From table 3, PC1 and PC3 were complementary, 56.80% variance; the variables with the greatest weights in PC3 were also presented in PC1 as showed in figure 2.

**Figure 2** Score plot of 91 honey samples from different four botanic honeys and different locations (PC1; K, Mg, Ca, L*, a*, and hue and PC3; Na and Zn ) where as (C) is Longan flower honey (30 samples), (G) Lynchee flower honey (13 samples), (S) Sunflower honey (30 samples), and (W)Wild flower honey (18 samples)

From the figure 2, the lynchee flower honey are the highest and similar $L^*(PC1)$ but the value of K(PC1), Mg(PC1), Ca(PC1), $a^*(PC1)$, and hue are lower that the other types of honeys. The longan flower honeys are the highest and similar Zn (PC3) but the lowest Na (PC3). While as the wild flower honeys are the lowest and similar Zn (PC3) but the highest Na (PC3).

**Conclusions**

The five metal contents and the five color parameters in 91 samples of the four botanical origins were the potential key indicators that can successfully classified by a various multivariate techniques namely principal component analysis (PCA) using the first three principal components.

Based on the results, the PC1 (K, Mg, Ca, L*, a*, and hue angle) can classified the lynchee flower honeys from the other honeys because they presented the highest value of $L^*$ and also showed the lowest K, Mg, Ca, $a^*$, and hue angle compared to the other Thai honeys. The PC2 ($b^*$ and chroma) can identified the sunflower honeys from the others because the highest $b^*$ and chroma. While as the PC3 can characterize the longan flower and the wild flower honeys from the others. The longan flower honeys presented the highest Zn concentrations but showed the lowest Na concentrations. In the other hand, the wild flower honeys presented the lowest Zn concentrations but showed the highest Na concentrations.
Acknowledgments

We would like to thanks Thailand Research Fund (TRF), Young Scientist Research Fund from faculty of Science Mahidol University and Center of Excellence for Innovation in Chemistry (PERCH-CIC) for funding this research as a part of project.

References


Effect of damaged starch content on glass transition temperature and gelatinization temperature of Cassava starch

Udomrat Samsi, Montira Nopharatana, Ruenrom Lerdlattaporn, Sahachai Suwannakan, Sawanit Aichayawanich

Department of Food Engineering, King Mongkut's University of Technology Thonburi,
126 Pracha u-tid road, Bangkok 10140, Thailand

*Corresponding e-mail address: udomrat5@hotmail.com

ABSTRACT

Effect of damaged starch content on glass transition temperature ($T_g$) and gelatinization temperature ($T_{gel}$) of cassava starch was investigated by differential scanning calorimetry (DSC). Cassava starch was milled by wet milling to obtain the damaged starch contents of 0 – 1 %. The results indicated that amylose and moisture content of all cassava starch samples were not significantly different ($P \geq 0.05$). However, the results revealed that $T_g$ decreased and the midpoints of $T_{gel}$ slightly increased with increasing damaged starch content. However, the endsets of $T_{gel}$ were not significantly different ($P \geq 0.05$).

Introduction

Cassava or tapioca is one of the most important crops in Thailand. It is grown mainly for commercial purposes. Approximately one million hectares (2.5 million acres) were devoted to cassava, producing about 20 million tons of roots. This makes Thailand the first producer and exporter of cassava in 2009 (Thai Tapioca Development Institute). Approximately one half of the cassava is processed into cassava starch.

Nowadays, cassava starch is one of the most important raw materials in the food industry because of its unique characteristics such as low gelatinization temperature, high viscosity, and high water binding capacity, translucent paste, relatively good stability, bland taste, neutral flavor and easy degradation (Balagopalan et al., 1996; Glicksman, 1969). Cassava starch production process starts from receiving cassava starch roots from supplier Then the roots are sent to screening, washing, chopping, milling, extracting, separating, dewatering, sieving and packing units (Thai Tapioca Development Institute or TTDI). During milling process, some starch granules are damaged from mechanically disruption (Han et al., 2002; Zhang et al., 2010).

Damaged starch is the change of starch granule structure such as cracked or split granules and flat granules (Jone, 1940). Some part of starch granules is mechanically disrupted resulted in loss of the crystalline structure and hydrogen bonds. Some amylopectin was broken down into low molecular weight fragment during milling processes (Han, et al., 2002; Zhang, et al., 2010; Yin and Stark, 1988; Morrison and Tester, 1994). Moreover, it was reported that the molecular size of fragments decreased with increasing milling time (Morrison and Tester, 1994). Damaged starch granules swell more than intact starch granule at low temperature but intact starch granules tend to have a high swelling factor at higher temperature (Morrison and Tester, 1994; Tester, 1997). There are many disadvantages of damaged starch in food processing. Biscuit processes require flours with low damaged starch content to lower the loss of water during baking (Dubat and Chopin, 2004).
Glass transition temperature ($T_g$) is a very important physical parameter, which serves to describe the physical and chemical behavior of food systems (Bell and Touma, 1996). It is defined as the temperature at which the amorphous phase of the polymer is converted between rubbery and glassy states. When starch is heated, it changed from glassy to rubbery state (Perdomo et al., 2009). As starch contains amorphous and crystalline regions, the exact thermal event representing $T_g$ is difficult to detect. However, high sensitivity differential scanning calorimetry (DSC) allowed the measurement of $T_g$ in amorphous and native starches with various levels of crystallinity (Bencze et al., 1998; Mizuno et al., 1998; Zeleznak et al., 1987).

Gelatinization is a term used to explain the molecular events associated with heating starch in water. Starch is converted from a semi-crystalline, relatively indigestible form to an amorphous form (Richard et al., 2000). The gelatinization temperature ($T_{gel}$) is the critical temperature at which the starch granules start to lose crystallinity and birefringence by irreversible expanding (Zhenyu et al., 2003).

As the glass transition temperature and the gelatinization temperature depended on the structure of starch granule, damaged structure of starch granule from milling process may alter glass transition temperature and gelatinization temperature. Therefore, the objective of this study is to study the effect of damaged starch content on glass transition temperature and gelatinization temperature of cassava starch.

**Materials and Methods**

1.1. Materials
Moist cassava starch was purchased from Choncharean cassava starch factory, Thailand. Its initial moisture content was approximately 32.8% ± 0.1 (d.b.).

1.2. Sample preparation
During milling process, the starch temperature was controlled to be lower than 50°C to prevent gelatinization with starch/water ratio of 1:1 by weight. Cassava starch was milled for 0, 5, 10, 15, 20 and 25 minutes by wet milling. Their slurry was sieved pass 270 mesh screens and placed in a 1 µm nylon filter bag before being centrifuged at 1,450 rpm for 15 minutes. Then, the moisture content was adjusted to 60.6 ± 0.4% (d.b.) by placing the starch samples over saturated salt solution ($K_2CrO_4$) for 2 weeks at ambient temperature until its weight is constant (Greenspan, 1977).

2.2.1 Moisture content
Moisture content of cassava starch was measured following AOAC 1984.

2.2.2 Damaged starch content
Damaged starch content was determined by using enzymatic digestion (AACC method 76-31). $T_g$ and $T_{gel}$ of cassava starches with 0 to 1% of the damaged starch contents were determined by using the differential scanning calorimetry (DSC).

2.2.3 Amylose content
The amylose content of sample damaged starch content was determined by the method given by Juliano (1971).

1.3. Differential scanning calorimetry (DSC)
The experimental procedure starts with calibration of DSC 1 star$^e$ system (PerkinElmer Co., USA) with indium with the melting point of 156.6°C. 15±0.5 mg of
cassava starch samples were placed in an aluminum pan (20 µg) with lids. Then, the sample thermal history was collected by heating from -20°C to 120°C with nitrogen condition at 10°C/min. The characteristics of midpoint of glass transition temperature ($T_m$) and gelatinization temperatures including, onset temperature ($T_o$), peak temperature ($T_p$), and conclusion temperature ($T_c$) were recorded.

1.4. Statistical analysis

The analysis of variance in this experimental data was determined and P-values were considered at P < 0.05.

**Result and Discussion**

3.1 Effect of damaged starch content on the amylose content

Table 1 shows the amylose content of cassava starch at different damaged starch contents (0.36, 0.41, 0.47, 0.65 and 0.76%). The amylose contents of all cassava starch were not significantly different. The result indicated that damage of starch granule did not affect on amylose.

<table>
<thead>
<tr>
<th>Damaged starch content (%)</th>
<th>Amylose content ** (% db.) ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.36</td>
<td>20.87±0.41</td>
</tr>
<tr>
<td>0.41</td>
<td>21.29±0.13</td>
</tr>
<tr>
<td>0.47</td>
<td>20.89±0.34</td>
</tr>
<tr>
<td>0.65</td>
<td>20.717±0.19</td>
</tr>
<tr>
<td>0.76</td>
<td>21.01±0.17</td>
</tr>
</tbody>
</table>

* Moisture Content of 41.5 ± 0.2 % w.b
** Values followed by the same letter are not significantly different (P > 0.05)

**Figure 1** Glass Transition Temperature ($T_g$) of cassava starch (Moisture content = 41.5 ± 0.2 % w.b) at different damaged starch content
3.2 Effect of damaged starch content on glass transition temperature ($T_g$)

The glass transition temperature ($T_g$) is one of the most important characteristic properties of a material. Figure 1 and Table 2 shows glass transition temperatures ($T_g$) of cassava starch containing different damaged starch content.

**Table 2** Glass transition temperature at different damaged starch content

<table>
<thead>
<tr>
<th>Damaged starch* (%)</th>
<th>$T_g$ (°C)**</th>
<th>onset</th>
<th>midpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.36</td>
<td>19.84 ± 0.71</td>
<td>21.41a ± 0.15</td>
<td></td>
</tr>
<tr>
<td>0.41</td>
<td>19.44b ± 0.13</td>
<td>19.86b ± 0.03</td>
<td></td>
</tr>
<tr>
<td>0.47</td>
<td>19.59b ± 0.11</td>
<td>19.65b ± 0.13</td>
<td></td>
</tr>
<tr>
<td>0.65</td>
<td>17.02c ± 0.28</td>
<td>18.44c ± 0.08</td>
<td></td>
</tr>
<tr>
<td>0.76</td>
<td>13.51d ± 0.44</td>
<td>14.93d ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

* Moisture Content of 41.5 ± 0.2% w.b.
** Values followed by the same letter are not significantly different ($P > 0.05$)

3.3 Effect of damaged starch content on gelatinization temperature ($T_{gel}$)

As damaged starch content of cassava starch increased from 0.36 to 0.76%, the onset and midpoint of glass transition temperatures decreased from 19.84±0.71°C to 13.51±0.44°C and 21.41±0.15°C to 14.93 °C, respectively. Normally, glass transition temperature of material depends on its molecular weight. The glass transition decreased with decreasing molecular weight of material (Thoung et al., 2003). In case of starch, Han, Campanella, Guan, Keeling, and Hamaker (2002) reported that the molecular weight of starch increased when material more damaged during milling. Therefore, the depression of glass transition temperature of cassava starch sample with high damaged starch content may due to its lower molecular weight.

Table 3 shows the gelatinization temperatures ($T_{gel}$) of cassava starch at different damaged starch contents. The values of $T_{gel}$ of all samples were within the range of 64.11 to 72.62°C. These values were in the same range with the result of Sriroth and Piyachomkwan, (2000) who studied gelatinization of cassava starch. Their DSC results showed that cassava starch had a gelatinization temperature range of 55 to 70°C. The results of this study also showed that the onset and midpoint of the gelatinization
temperatures of cassava starch slightly increased with increasing damaged starch content. However, the endset of gelatinization temperatures of cassava starch with different damaged starch content were not significantly different.

**Table 3** Gelatinization temperature ($T_{gel}$) of cassava starch at different damaged starch content

<table>
<thead>
<tr>
<th>Damaged starch content (%)</th>
<th>Gelatinization temperature (°C) **</th>
<th>**onset</th>
<th>midpoint</th>
<th>endset (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.36</td>
<td>64.49 $^b$ ±0.03</td>
<td>67.58 $^c$ ±0.34</td>
<td>72.05±0.01</td>
<td></td>
</tr>
<tr>
<td>0.41</td>
<td>64.31$^{bc}$ ±0.01</td>
<td>67.89$^{bc}$ ±0.00</td>
<td>72.20±0.01</td>
<td></td>
</tr>
<tr>
<td>0.47</td>
<td>64.11 $^c$ ±0.01</td>
<td>68.01$^{abc}$ ±0.04</td>
<td>72.03±0.36</td>
<td></td>
</tr>
<tr>
<td>0.65</td>
<td>64.76 $^a$ ±0.15</td>
<td>68.32$^{ab}$ ±0.25</td>
<td>72.27±0.24</td>
<td></td>
</tr>
<tr>
<td>0.76</td>
<td>64.83 $^a$ ±0.04</td>
<td>68.54 $^a$ ±0.00</td>
<td>72.62±0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Moisture Content of 41.5 ± 0.2 % w.b
** Values followed by the same letter are not significantly different ($P > 0.05$)

**Conclusions**

Cassava starch sample with various percentage of damaged starch content is prepared by wet milling. The result reviews that the amylose content of all sample were not significantly affected by milling process. For thermal analysis, $T_{gel}$ of cassava starch decreased with increasing damaged starch content. The onsets and midpoints of $T_{gel}$ of the samples slightly increased with increasing damaged starch content. However, endsets of $T_{gel}$ of all samples were not significantly different.

**References**


