ABSTRACT

Changes in pH, total reducing sugar content, and total monomeric anthocyanin (cyanidin-3-glucoside) content of Khaow-Maak from black glutinous rice during fermentation were investigated. During fermentation, the decrease in pH and total monomeric anthocyanin content with the concomitant increase in total reducing sugar content were observed (p<0.05). As fermentation time increased, a* value of sample increased (p<0.05). After the fermentation was completed (pH<4.5), fermented Khaow-Maak contained 58.57% moisture, 10.55% protein, 0.81% lipid, 0.44% ash, 1.84% crude fiber, 27.90% carbohydrate and 2.28% ethyl alcohol. Khaow-Maak showed acceptable scores in appearance, color and overall liking, while the scores in odor liking, texture liking and flavor liking ranged from 6.63 to 6.87. Therefore, black glutinous rice could be an alternative source for Khaow-Maak production with anthocyanin, protein and crude fiber.
INTRODUCTION

Khaow-Maak, a Thai fermented rice, is produced by fermentation of cooked glutinous rice with look-pang. Look-pang is a semicircle starch ball which contains yeasts and the white hyphae of mold (Manosroi et al., 2011). The mixture is packed in banana leaves or plastic cup and left to ferment for 2-3 days at ambient temperature (TCPS 162, 2003). Generally, Khaow-Maak can be served as a dessert (Blandio et al., 2003). Traditionally, Khaow-Maak usually produced from cooked white glutinous rice (Manosroi et al., 2011) or those mixed with cooked black glutinous rice. In Thai folklore wisdom, Khaow-Maak has been considered to promote the growth development of malnutritioned children, active bacterial activity, and used as a dietary supplement (Pitiporn, 2008 cited by Manosroi et al., 2011). It is a probiotic which consists of live microorganisms and can have a health benefit on the host when administered in adequate amounts, since the fermentation process of Khaow-Maak has used mold and yeast such as Saccharomyces cerevisiae strain which had been approved to be probiotics (de Llanos et al., 2006). It also increases and maintains a healthy bacterial gut flora by providing and increasing amount of food for these bacteria. Black glutinous rice is the most famous one and generally used as an ingredient is snacks and desserts (Tananuwong and Tewaruth, 2010). Most colored rice, especially black glutinous rice, consists of anthocyanin pigments, cyaniding-3-glycoside and peonidin-3-glucoside afford enriched medicinal effects such as the protection against cytotoxicity (Chen et al., 2005), anti-neurodegenerative activity (Kim et al., 2005), glycogen phosphorylase inhibition (Jakob et al., 2006) and antioxidative activity (Kano et al., 2005). Thus, it was advantageous for these compounds to be antioxidants in the acidic environment of the fermentation sap. This has supported the nutritional facts and health benefits of the fermented Thai folk wisdom dessert which can be used for food supplements because of their high content of anthocyanin. However, no information in fermentation, chemical composition and changes in total anthocyanin content as well as acceptability of Khaow-Maak produced from black glutinous rice has been reported. Thus, the objectives of this study were to investigate changes in pH, total reducing sugar content, total monomeric anthocyanin content and color during fermentation and to monitor the chemical composition and acceptability of fermented Khaow-Maak.

MATERIALS AND METHODS

Production of Khaow-Maak from black glutinous rice

Black glutinous rice (Oryza sativa L.: Raitip®) was procured from supermarket in Bangkok, Thailand. For rice fermentation, black glutinous rice (1 kg) was soaked in hot water (95°C) (1.5 kg) for 16 h and steamed in an electric rice cooker (Sharp, Bangkok, Thailand) until it was done (40 min). Then
cooked rice was cooled to room temperature (28-30°C). Look-pang (12 g) was sprinkled on the cooked rice and mixed well. The mixture (100 g) was put in a cup and closed tightly and kept at room temperature (28 – 32°C) for 10 days. Sample was taken at day 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 for analyses.

Analyses

The pH of samples was determined by the method as described by Benjakul et al. (1997). The total reducing sugar content was determined according to the method of James (1999). The anthocyanin content was evaluated in term of total monomeric anthocyanin as described by Giusti and Wrolstad (2005). The color of samples was measured as L* a* b* in the mode of CIE by Hunter Lab (ColorFlex 45/0, USA). Fermented sample (pH 4.0 – 4.5) was presented to untrained 30-member panel for acceptance test. The panelists consisted of people familiar with fermented food. Samples were evaluated for appearance, color, odor, taste, sweetness, flavor, texture, and overall liking using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) (Chamber IV and Wolf, 1996). Moisture, lipid, protein, ash, crude fiber and carbohydrate were determined according to the method of AOAC (2000). Ethyl alcohol of fermented sample was measured according to the method of AOAC (2000). For statistical analysis, data were subjected to Analysis of Variance (ANOVA) and differences between means were evaluated by the Duncan’s Multiple Range Test using SPSS statistic program (Version 10) (SPSS, 1.2, 1998).

RESULTS AND DISCUSSION

Changes during fermentation

pH

Change in pH of black glutinous rice during 10-day fermentation indicating the progress of fermentation process is shown in Figure 1A. At 0 day of fermentation, the pH of cooked black glutinous rice was 5.83. During fermentation, yeast could play an important role for fermentation system which ferments monosaccharide or sugar to alcohols and acids (Manosroi et al., 2011). The reduction of pH of fermented rice was probably due the formation of acids by yeasts utilized carbohydrate. The pH of sample decreased to 4.5 within 4 days of fermentation. Additionally, Manosiri et al. (2011) reported that the pH of purple glutinous rice decreased to 4.4 within 6 days of fermentation. The lowered pH indicated the safety of the product as determined by TCPS 162 (2003).

Total reducing sugar

An increase in total reducing sugar of Khaow-Maak occurred during fermentation as shown in Figure 1B. During 3 days of fermentation, total reducing sugar content in Khaow-Maak was notably
increased. Generally, the starch molecules in the rice seeds are first fermented to monosaccharides by molds in the hydrolysis reaction (Cheng et al., 2008). Additionally, mold in Look Pang could transform the fermented condition for the flourish growth of yeast by providing the monosaccharides such as glucose, fructose and galactose as the carbon sources to support the growth and metabolism of yeast (Buglass, 2011). Thereafter, yeast could be an important role for fermentation system which ferments monosaccharide or sugar to alcohol and acids. Yeasts and mold were found in fermented Khaow-Maak including *Rhizopus*, *Mucor*, *Saccharomyces*, *Hansenula* (Blandino et al., 2003). From the result, increase in total reducing sugar content was resulted from the metabolite activity of molds and yeasts.

**Total monomeric anthocyanin content**

Decrease in total monomeric anthocyanin content in Khao-mak from black glutinous rice during fermentation is depicted in Figure 1C. Generally, cyaniding-3-glucoside is the most widespread anthocyanin from fruit, vegetable and plants (Kong et al., 2003). Acid may cause partial or total hydrolysis of the acyl moieties of acylated anthocyanins that are present in some plants (Kong et al., 2003). In addition, degradation of anthocyanins in the presence of weak acids, such as ascorbic acid, consists of direct condensation of acid on the carbon 4 of the anthocyanin molecule, causing the loss of both (Poei-Langston and Wrolstad, 1981). On the other hand, according to Lacobucci and Sweeny (1983), the loss of anthocyanin color, caused by acid, occurs due to the oxidative cleavage of the pyrillum ring by a free radical mechanism in which the acid acts as a molecule oxygen activator, producing free radicals. From the results, the decrease in total monomeric anthocyanin content resulted from pH lowering during fermentation.

**Color**

$L^* a^*$ and $b^*$ values of Khaow-Maak produced from black glutinous rice during fermentation are shown in Figure 2. Fermented Khaow-Makk (at day 5) had the higher $a^*$, compared with Khaow-Maak at 0 day ($p<0.05$). Generally, color of cooked black glutinous rice is black purple. After fermentation was completed, color of Khaow-Maak tended to be red-purplish. Manosroi et al. (2011) reported that the anthocyanin affected on color of black glutinous rice. Additionally, the anthocyanin is responsible for attractive colors, varying from red to blue (Stract and Wray, 1993). However, changes in color of anthocyanin could be influenced by pH, temperature, oxygen, light, polymeric forms, concentration, the presence of phenolic compounds and the chemical structure. Thus, changes in color of fermented Khaow-Maak resulted from the decrease in pH and total monomeric anthocyanin content during fermentation (Figure 1).
Figure 1 Changes in pH (A), total reducing sugar (B) and total monomeric anthocyanins content (C) of Khaow-Maak from black glutinous rice during 10-day fermentation at room temperature. Bars represent standard deviation from triplicate determinations.

Figure 2 Changes in L* (A), a* (B), and b* (C) of Khaow-Maak from black glutinous rice during 10-day fermentation at room temperature. Bars represent standard deviation from triplicate determinations.

Chemical composition of fermented Khaow-Maak produced from black glutinous rice

Chemical composition of fermented Khaow-Maak produced from black glutinous rice (at pH < 4.5) is summarized in Table 1. Khaow-Maak contained 58.57% moisture, 0.44% ash, 10.55% protein, 0.81% lipid, 1.83% crude fiber, and 27.80% carbohydrate. Generally, Khaow-Maak could be served as a source of protein and crude fiber. Fermented sample had minute amount of lipid content. In addition, it has been reported that gamma linolenic acid and linoleic acid were found in fermented purple glutinous rice (Manosroi et al., 2011). For ethyl alcohol content, sample contained 2.28% which was higher than standard ethyl alcohol content of fermented Khaow-Maak as determined by TCPS 162 (2003). In general, the ethyl alcohol content of Khaow-Maak must less than 0.5% by weight (TCPS 162...
Ethyl alcohol is a volatile compound contributed to flavor of the product. In addition, ethyl alcohol formation might be resulted from metabolized products from sugar by yeasts.

### Table 1 Chemical composition of fermented Khaow-Maak produced from black glutinous rice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture</td>
</tr>
<tr>
<td>Khao-mak</td>
<td>58.57 ± 0.29*</td>
</tr>
</tbody>
</table>

* Mean ± SD from triplicate determinations.

### Acceptance of Khaow-Maak produced from black glutinous rice

The acceptance score of fermented Khao-Maak produced from black glutinous rice was monitored based on appearance liking, color liking, odor liking, texture liking, flavor liking and overall liking, which varied from 6.63 to 7.03 (Table 2). Due to the lowering pH, sample had some released water which purplish-pink. Nevertheless, both flavor and odor were influenced by ethyl alcohol (Table 1) and some low molecule weight volatile compounds. Due to cooked glutinous rice was hydrolyzed by enzymes from molds and yeast. Thus, the textural characteristic of fermented rice might be affected by yeast and mold fermentation. Therefore, changes in pH, total reducing sugar content, total monomeric anthocyanin content as well as chemical composition may have significant influence on characteristics of fermented Khaow-Maak.

### Table 2 Acceptance score of fermented Khaow-Maak produced from black glutinous rice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Appearance</td>
</tr>
<tr>
<td>Khao-mak</td>
<td>7.23 ± 0.97*</td>
</tr>
</tbody>
</table>

* Mean ± SD from thirty determinations.

### CONCLUSION

During fermentation, changes in pH, total reducing sugar content, and total monomeric anthocyanin content of Khaow-Maak produced from black glutinous rice affected the characteristics of fermented Khaow-Maak. Additionally, Khaow-Maak produced from black glutinous rice had a great acceptability with good appearance and color.
REFERENCES


