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Product Development of Sweet Fermented Rice (Khoa-Mak) Supplemented with Red Yeast Rice

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Abstract A new healthy food product, sweet fermented glutinous rice (Khoa-Mak) supplemented with red yeast rice was developed in this study. Significant source of Loop-Pang (rice starter culture) for Khoa-Mak fermentation were investigated, and effect of supplementation of red yeast rice on quality of Khoa-Mak during storage was further carried out. Total soluble solid (TSS), pH, ethanol content, yeast and mold, total viable count, *Escherichai coli*, sensory evaluation of sample were determined. Glutinous rice was fermented with various sources of Loog-Pang (Chachoengsao, Chanthaburi, Trad and Prachinburi) at 30°C for 72 hours. Microbial populations in various Loog-Pang sources increased rapidly during fermentation. Rice fermented by Loog-Pang sources from Trad for 48 hours had the highest sensory acceptability by elderly consumers, and contained 0.12% of ethanol, 40°Brix of TSS, pH of 4.2 and less than 3 MPN/g of *E. coli*. During storage at 4°C for 2 weeks, microbial populations survived at low pH in Khoa-Mak (without red yeast rice, and supplemented with 0.1, 0.2 and 0.3% of red yeast rice). The supplementation of red yeast rice from 0 to 0.3% resulted in the increasing of *Monascus* pigments concentrations and antioxidative properties in Khoa-Mak ($p<0.05$). Sensory scores of Khoa-Mak supplemented with 0.3% of red yeast rice were higher than those for other samples, except Khoa-Mak without red yeast rice ($p<0.05$). The results indicated that Khoa-Mak supplemented with 0.3% of red yeast rice could be a new healthy food product, particularly for elderly consumers.

Keywords: Sweet fermented rice, Red yeast rice, Loog-Pang, Fermentation, Storage

Introduction

Sweet fermented glutinous rice or Khoa-Mak is one of traditional Thai dessert, is favored especially for the elderly. Traditionally, Khoa-Mak was prepared from steamed white glutinous rice mixed with Look-Pang-Khoa-Mak or Look-Pang, a culture of microorganisms including molds (*Aspergillus* spp., *Rhizopus* spp., *Mucor* spp. and *Amylomyces rouxii*), yeasts (*Saccharomyces*

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cerevisiae, *S. fibuligera* and *Candida* spp.) and bacteria in rice flour mixed with herbs such as pepper, garlic and galangal as anti-pathogenic microbial agent (Manosroi *et al*., 2011; Mongkontanawat and Lertnimitmongkol, 2015; Khamkeaw and Phisalaphong, 2016). During fermentation, enzymes including α-amylase and glucoamylase from mold cultures in Look-Pang hydrolyze starch to sugars such as oligosaccharide and glucose and the latter is then converted to alcohol by yeasts. In addition, organic acids and volatile compounds are also synthesized by major microbial including molds and yeasts in Look-Pang. Fermentation is continued for 2-3 days at ambient temperatures. After fermentation, the product has a soft texture, sweet taste, low alcohol, organic acid flavors, lumps of steamed glutinous rice and a pale white color (Plaitho *et al*., 2013; Mongkontanawat and Lertnimitmongkol, 2015). On completion, Khoa-Mak should contain <0.5% of ethanol, 40-50°Brix of total soluble solid (TSS), 4.0-4.5 of pH and <3 MPN/g of *Escherichai coli* (Community Product Standard (TCPS) No. 162, Ministry of Industry, 2013).

Khoa-Mak is considered a health food for humans. It contains living *S. cerevisiae*, a probiotic with health benefits when consumed in adequate amounts (Manosroi *et al*., 2011; Nagpal *et al*., 2012; Mongkontanawat and Lertnimitmongkol, 2015). Recently, Khoa-Mak produced from Thai pigmented rice varieties was shown to have antioxidant and antimutagenicity activities relative to unfermented rice (Plaitho *et al*., 2013), whereas products from glutinous rice and/or germinated native black glutinous rice exhibited antioxidant activities and contained γ-amino butyric acid (GABA) (Mongkontanawat and Lertnimitmongkol, 2015). Quality of Khoa-Mak is influenced by various factors such as rice preparation method, fermentation period and, in particular, starter cultures in Look-Pang. Differences of Khoa-Mak characteristic occur also in the different ingredients and microorganism strains in Look-Pang, which obtain from the different producer sources.

Red yeast rice is rice fermented by the red mould, *Monascus*. Species of *Monascus* produce six major pigments, two each of red, orange and yellow. *Monascus* pigments have long been used in food coloring agent in Asian with no adverse health effects (Carvalho *et al*., 2003; Feng *et al*., 2012).These pigments exhibit anti-inflammatory activity (Hsu *et al*., 2013). Moreover, yellow pigments including monascin and ankaflavin exhibited various physiological functions such as anticancer and antioxidation agents (Su *et al*., 2005; Lee *et al*., 2006). Recently, monascin and ankaflavin also showed anti-hyperlipidemic and anti-atherosclerosis effects by significant reducing total cholesterol, triglyceride and low-density lipoprotein cholesterol (LDL-C) levels in serum and liquid plaque in the heart aorta of hamsters. Monascin also increased significantly high-density lipoprotein cholesterol (HDL-C) concentrations (Lee *et al.*, 2013). To the present, no effort has been made to select appropriate Look-Pang for Khoa-Mak fermentation and supplement Khoa-Mak with red yeast rice to enhance its health benefits without diminishing its microbiological and sensory properties. The objective of this study was to investigate the significant of Look-Pang for Khoa-Mak fermentation and further investigate the effect of red yeast rice supplementation on quality of Khoa-Mak during storage.

Materials and methods

Cultures

Monascus purpureus TISTR 3541 was purchased from Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research, Thailand and maintained at 4°C on potato dextrose agar (PDA). Loog-Pang samples were obtained from Chachoengsao, Chanthaburi, Trad, and Prachinburi provinces and stored at 4°C. Each sample was ground into a fine powder for the starter culture.

Khoa-Mak fermentation

White glutinous rice, Khiaw-ngoi was acquired from a local market in Chonburi, Thailand and used as a solid substrate for fermentation. Rice (250 g) was washed, soaked in distilled water for 1 h, steamed (100°C, 20 min), cooled at room temperature and twice washed with the sterilized water to remove viscous material. Then, rice was mixed with 0.2% (w/w) of Loog-Pang (0.2 g per 100 g of raw rice). A 50 g of sample was put in a plastic cup, covered tightly and fermented at 30°C for 72 hours (Manosroi *et al*., 2011; Plaitho *et al*., 2013; Mongkontanawat and Lertnimitmongkol, 2015).

Red yeast rice fermentation

Red yeast rice was prepared according to Jirasatid *et al*. (2013) but cultivated at 30°C.

Effect of Look-Pang culture for Khoa-Mak fermentation

Four treatments of Khoa-Mak were prepared from four sources of Loog-Pang cultures. During fermentation, ethanol content, TSS, pH, total viable count, yeast and mold count were measured at 12 hours intervals. Sensory attribution was evaluated for replicate samples at 48 and 72 hours of fermentation. *E. coli* count was determined in the final Khoa-Mak products. The experimental was carried out in two replications. The appropriate Loog-Pang culture and fermentation period for Khoa-Mak production were selected from consumer preference and chemical and microbial properties of product.

Effect of supplementation of red yeast rice on the quality of Khoa-Mak during storage

Red yeast rice powder (0.1, 0.2 and 0.3% w/w) was added to the selected Khoa-Mak (Glutinous rice fermented for 2 days with Loog-Pang from Trad). Then, samples were mixed gently, placed in a tightly covered plastic container. Khoa-Mak without red yeast rice was used as control. Samples were stored at 4°C in a refrigerator for 14 days. During storage, chemical properties (ethanol content, TSS and pH), microbiological characteristics (total viable count, yeast and mold, and *E. coli*), sensory evaluation, DPPH radical scavenging activity, and pigment concentration including yellow, orange and red were determined every 7 days. Experimental was conducted in duplicate.

Chemical analysis

Total soluble solid (TSS) of ground sample was measured with a refractometer (Master-M, Atago, Japan) and pH value was measured with a calibrated pH meter (Lab 850, Schott, Germany). Sample was crushed by motar after dilution to 10% (w/v) with distilled water (Johns and Stuart, 1991). Ethanol was determined by redox titration (Amerine and Ough, 1974).

Microbial analysis

Samples (25 g) were homogenized with 225 ml of 0.1% sterile peptone and diluted in a 10-fold serial. Total viable count was enumerated on compact dry total count (TC), which was incubated at 37°C for 48 hours. The yeast and mold count was examined using compact dry yeast and mold, which was incubated at 30°C for 4-5 days. *E. coli* count was determined using Most Probable Number (MPN) method (Yousef and Carlstrom, 2003).

Sensory evaluation

Sensory evaluation of Khoa-Mak was performed by 20 untrained elderly panelists (> 60 years old) because target consumer group of this product focus to the elderly. All panelists were experienced consumers of Khoa-Mak. Samples were provided in white plastic cups and served at room temperature.

Panelists were asked to evaluate appearance, color, taste, odor, flavor and overall acceptability using a 9-point hedonic test (1=extremely dislike to 9=extremely like).

Analysis of DPPH radical scavenging activity

The DPPH radical scavenging effect of sample was determined according to the method of Mongkontanawat and Lertnimitmongkol (2015). The absorbance was measured at wavelengths of 517 nm using UV/vis spectrophotometer (Genesys 20, Thermo Scientific, USA). % DPPH radical scavenging activity was expressed as:

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Analysis of pigment concentration

The pigments concentrations including red, orange and yellow were determined as described by Johns and Stuart (1991). Yellow, orange and red pigments were measured at wavelengths of 400, 470 and 500 nm, respectively with a UV/vis spectrophotometer (Genesys 20, Thermo Scientific, USA). The concentration of each pigment (OD units/g substrate dry weight; sdw) was calculated as:

$$\text{pigment concentration} = \frac{\text{OD}_{\text{nm}} \times \text{Dilution factor} \times \text{volume of alcohol (50 ml)}}{\text{weight of sample (dry basic)}}$$

where OD_{nm} is the absorbance of the sample and dilution factor is fold of dilution.

Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA). Differences among means were tested by Duncan's multiple range tests. Significance was accepted at p<0.05.

Results

Effect of Look-Pang culture for Khoa-Mak fermentation

Time courses of viable counts of yeast and mold in the fermented rice using Loog-Pang from Chachoengsao, Chanthaburi, Trad and Prachinburi provinces are presented in Figure 1. The yeast and mold cultures in fermented rice from various sources of Loog-Pang grew rapidly in Khoa-Mak samples from 2.4-3.1 to 5.7-6.5 log cfu/g after 72 hours of fermentation. The highest counts of yeast and mold occurred in fermented rice using Loog-Pang from Prachinburi (6.5 log cfu/g), followed by Chanthaburi (5.9 log cfu/g), Trad (5.8 log cfu/g) and Chachoengsao (5.7 log cfu/g), respectively.

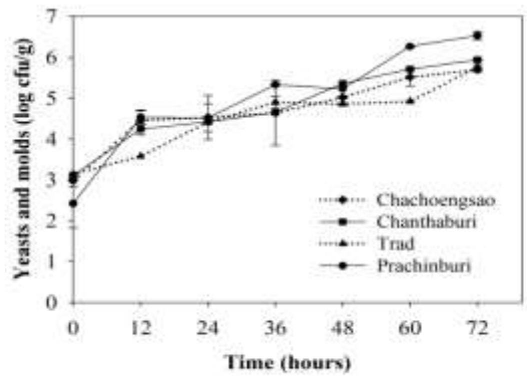

Figure 1. Changes in yeast and mold counts during Khoa-Mak fermentation by various sources of Loog-Pang

Increasing the total viable counts occurred throughout fermentation for rice from each of the four sources (Figure 2). The initial total viable counts varied between 2.5-2.8 log cfu/g. After cultivation for 72 hours, total viable counts for fermented rice using Loog-Pang from Chachoengsao, Chanthaburi, Trad and Prachinburi increased significantly to 5.7, 6.2, 5.7 and 6.3, respectively.

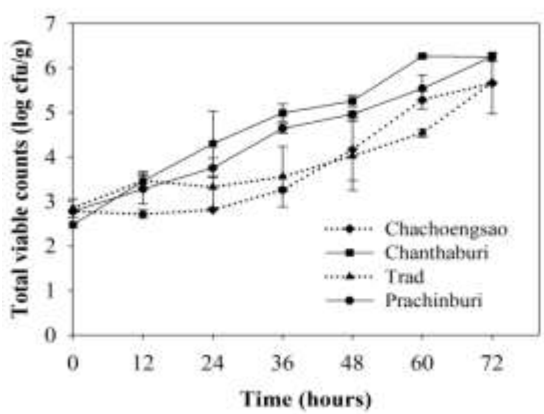

Figure 2. Changes in total viable counts during Khoa-Mak fermentation by various sources of Loog-Pang

Initially, TSS was absent in Khoa-Mak but dramatically increased to 38-39°Brix for 12 hours of fermentation, and thereafter changed little for the remaining of 48 hours of fermentation (39-40°Brix). TSS decreased slightly in the final stage of fermentation (48-72 hours). Final TSS of samples were 31-35°Brix after 72 hours of fermentation (data not shown).

Regarding pH values of Khoa-Mak, initial pH values of Khoa-Mak ranged from approximately 5.2-5.8. Mold and yeast cultures in Loog-Pang produced organic acids during growth resulting in pH reduction to 3.9-4.3 after 72 hours of fermentation (data not shown). The profiles of ethanol content showed the typical primary metabolite from yeast (data not shown). Initial ethanol content in Khoa-Mak increased during fermentation. After 72 hours of fermentation, ethanol content in Khoa-Mak using Loog-Pang from Prachinburi was the highest (0.16%), followed by Chanthaburi (0.14%), Chachoengsao (0.14%) and Trad (0.13%) respectively.

Table 1. Sensory attributes in Khoa-Mak during fermentation at different Loog-Pang sources and different fermentation period.[a]

Source of Loog-Pang	**Time of fermentation (hours)**	**Sensory attributes (scores)**					
		Appearance	Color	Taste	Odor	Flavor	Overall acceptability
Chachoengsao	48	7.9[bc]	7.7[bc]	6.6[c]	5.7[b]	5.7[c]	6.5[b]
	72	7.8[bc]	7.3[c]	6.1[cd]	3.1[e]	3.0[f]	4.2[d]
Chanthaburi	48	7.5[cd]	6.0[d]	6.6[c]	6.2[c]	6.3[b]	6.4[b]
	72	7.3[d]	4.2[e]	5.8[d]	4.0[f]	4.0[e]	4.9[c]
Trad	48	9.0[a]	8.9[a]	8.9[a]	9. 0[a]	8.9[a]	9.0[a]
	72	8.1[b]	8.1[b]	7.3[b]	4.1[d]	4.1[e]	6.4[b]
Prachinburi	48	6.3[e]	3.9[f]	5.8[d]	5.2[c]	5.1[d]	5.3[c]
	72	5.4[f]	1.6[g]	2.9[e]	1.4[f]	1.4[g]	1.6[e]

[a], Means; Values with different small letters in a column are significantly different at $p<0.05$.

Sensory attribution in fermented rice for 48 and 72 hours using various sources of Loog-Pang presents in Table 1. It was found that the scores of sensory attributes in each Khoa-Mak sample including appearance, color, taste, odor, flavor and overall acceptability decreased significantly from 48 to 72 hours of fermentation. It was probable that the flavor and smell of alcohol in Khoa-Mak was more intense during fermentation. For 48 hours of fermentation, all Khoa-Mak were sensorial accepted from elderly consumers as indicated by higher score than 5 of overall acceptability. Moreover, sensory scores in fermented rice using different sources of Loog-Pang were significant difference. The highest score of overall acceptability of Khoa-Mak was observed in fermented rice for 48 hours using Loog-Pang from Trad (p<0.05). In addition, *E. coli* counts were <3 MPN/g in Khoa-Mak samples, which were fermented using Loog-Pang from Chachoengsao, Chanthaburi, Trad and Prachinburi for 48 hours. Therefore, Khoa-Mak was fermented with Loog-Pang from Trad for 48 hours was selected for further experiment.

Effect of supplementation of red yeast rice on the quality of Khoa-Mak during storage

The viable counts of yeast and mold in Khoa-Mak (with and without red yeast rice) decreased insignificantly during storage at 4°C for 2 weeks (p≥0.05) (Table 2). In addition, yeast and mold counts did not differ significantly in Khoa-Mak without red yeast rice (0%), and supplemented with 0.1, 0.2 and 0.3% of red yeast rice (p≥0.05) under the same storage time.

Table 2. Viability of yeast and mold counts in Khoa-Mak without red yeast rice, and supplemented with red yeast rice at different concentrations during storage.[a]

Storage time (weeks)	**Yeast and mold counts (log cfu/g)**			
	0%	0.1%	0.2%	0.3%
0	$3.9 \pm 0.4^{ns, NS}$	4.6 ± 0.5^{ns}	4.3 ± 0.2^{ns}	4.5 ± 0.0^{ns}
1	3.8 ± 0.0^{NS}	4.1±0.1	3.8±0.4	3.8±0.2
2	3.7 ± 0.0^{NS}	4.2±0.0	4.2±0.0	4.3±0.5

[a], Means ±Standard deviation; [ns], not significant in a column at p<0.05; [NS], not significant in a row at p<0.05.

Total viable count in each Khoa-Mak sample was almost constant throughout storage (p≥0.05) (Table 3). Total viable counts in Khoa-Mak samples (with and without red yeast rice) were >4 log cfu/g after 2 weeks of storage. Moreover, at 1 and/or 2 weeks of storage, total viable counts increased significantly (p<0.05) when the supplementation of red yeast rice increased (from 0 to 0.3% w/w). Additionally, the counts of *E. coli* were <3 MPN/g in Khoa-Mak samples (with and without red yeast rice) throughout 2 weeks of storage period.

Ethanol of each Khoa-Mak sample (with and without red yeast rice) declined insignificantly with storage time (p≥0.05) (data not shown). Initial ethanol contents of Khoa-Mak varied within the range of 0.09-0.11%, while final ethanol contents were observed between 0.08-0.09%. Moreover, ethanol varied slightly but not significantly among Khoa-Mak samples supplemented with 0.1, 0.2 and 0.3% of red yeast rice, and without red yeast rice at either weeks of storage ($p \geq 0.05$) (data not shown). Initial pH and final pH of Khoa-Mak samples were within the range 3.7-3.9 and 4.3-4.4, respectively (data not shown). TSS increased significantly in each sample from 31-32°Brix to 37-38°Brix during storage at 4°C for 2 weeks ($p<0.05$) (data not shown). This is attributed to hydrolysis of starch to sugars by enzymes from molds during cold storage.

Table 3. Viability of total viable counts in Khoa-Mak without red yeast rice, and supplemented with red yeast rice at different concentrations during storage.[a]

Storage time (weeks)	Total viable counts (log cfu/g)			
	0%	0.1%	0.2%	0.3%
0	4.3±0.0 $^{ns, NS}$	4.3±0.1^{ns}	4.3±0.1^{ns}	4.5±0.0^{ns}
1	4.2±0.1^{CD}	4.4±0.0^{BC}	4.6±0.0^{AB}	4.7±0.1^{A}
2	4.1±0.2^{CD}	4.4±0.1^{BC}	4.5±0.1^{AB}	4.6±0.0^{A}

[a], Means±Standard deviation; Values with different capital letters in a row are significantly different at $p<0.05$; [ns], not significant in a column; [NS], not significant in a row.

The concentrations of *Monascus* pigments including yellow, orange and red increased significantly as red yeast rice powder was added in Khoa-Mak from 0 to 0.3% ($p<0.05$). The pigments concentrations in Khoa-Mak supplemented with red yeast rice declined according to storage time (Table 4). After storage at 4°C for 2 weeks, the concentrations of yellow and orange pigments were below detection limit, while red pigment concentrations remained approximately 24-50%.

DPPH radical scavenging activities of Khoa-Mak improved significantly when the supplementation of red yeast rice powder increased ($p<0.05$). However, DPPH radical scavenging activities of each Khoa-Mak sample diminished continuously throughout storage period ($p<0.05$). The remaining of antioxidant activity was 92, 93, 93 and 91% in Khoa-Mak without red yeast rice, and supplemented with 0.1, 0.2 and 0.3% of red yeast rice, respectively after storage (Table 5).

Table 4. Concentrations of yellow, orange and red pigments in Khoa-Mak without red yeast rice, and supplemented with red yeast rice at different concentrations during storage.[a]

St (weeks)	Yellow pigment concentrations (OD units/g sdw)				Orange pigment concentrations (OD units/g sdw)				Red pigment concentrations (OD units/g sdw)			
	0%	0.1%	0.2%	0.3%	0%	0.1%	0.2%	0.3%	0%	0.1%	0.2%	0.3%
0	0.0 nsD	4.8 aC	14.8 aB	27.6 aA	0.0 nsC	1.7 aC	4.2 aB	14.3 aA	0.0 nsD	6.3 nsC	10.3 aB	25.3 aA
1	0.0 C	0.9 bABC	1.3 bAB	2.2 bA	0.0 NS	0.0 b	0.0 b	0.0 b	0.0 C	4.3 BC	7.7 bB	13.5 bA
2	0.0 NS	0.0 bc	0.0 bc	0.0 bc	0.0 NS	0.0 b	0.0 b	0.0 b	0.0 D	3.1 BC	4.9 cAB	6.2 cA

Abbreviation: St, storage time; sdw, substrate dry weight; [a], Means±Standard deviation; Values with different small letters in a column and capital letters in a row of each pigment are significantly different at $p<0.05$; [ns], not significant in a column; [NS], not significant in a row.

Table 5. DPPH radical scavenging activities in Khoa-Mak without red yeast rice, and supplemented with red yeast rice at different concentrations during storage.[a]

Storage time (weeks)	DPPH radical scavenging activities (%)			
	0%	0.1%	0.2%	0.3%
0	62.0±0.4[aD]	68.8±0.1[aC]	72.4±0.4[aB]	80.6±0.2[aA]
1	60.4±0.3[abD]	66.8±0.3[bC]	69.8±0.6[bB]	75.2±0.4[bA]
2	56.9±0.3[cD]	64.0±0.6[cC]	67.4±0.3[cB]	73.2±0.2[cA]

[a], Means±Standard deviation; Values with different small letters in a column and capital letters in a row are significantly different at $p<0.05$.

Mean scores of sensory attributes including appearance, color, taste, odor, flavor and overall acceptability of Khoa-Mak supplemented with red yeast rice (0.1, 0.2 and 0.3%) deteriorated significantly as compared to the control sample ($p<0.05$) (Table 6). This was problematic due to odor, taste and color of red yeast rice affecting in Khoa-Mak characteristics. However, sensory scores of Khoa-Mak supplemented with 0.3% of red yeast rice were higher than other samples supplemented red yeast rice (0.1 and 0.2%). In addition, scores of all sensory attributes declined continuously with storage time. For one week of storage, only control sample (7.4 score of overall acceptability) and Khoa-Mak supplemented with 0.3% of red yeast rice (6.4 score of overall acceptability) were accepted favorably by the elderly consumers. No Khoa-Mak samples were received favorably after cold storage for 2 weeks.

Table 6. Sensory attributes in Khoa-Mak without red yeast rice, and supplemented with red yeast rice during storage.[a]

Storage time (weeks)	Concentration of red yeast rice (% w/w)	Sensory attributes (scores)					
		Appearance	Color	Taste	Odor	Flavor	Overall acceptability
0	0	8.8[a]	8.7[a]	9.0[a]	8.5[a]	9.0[a]	9.0[a]
	0.1	6.1[c]	6.6[b]	7.1[c]	7.0[c]	6.4[c]	6.4[c]
	0.2	5.7[d]	6.0[c]	5.2[d]	5.5[d]	5.2[d]	5.6[d]
	0.3	6.8[b]	6.7[b]	8.2[b]	7.6[b]	8.6[b]	7.9[b]
1	0	7.4[a]	7.0[a]	7.6[a]	6.6[a]	6.6[a]	7.4[a]
	0.1	3.8[d]	4.0[d]	3.8[d]	4.4[d]	4.4[d]	3.8[d]
	0.2	5.6[c]	5.2[c]	4.8[c]	4.6[c]	4.6[c]	4.8[c]
	0.3	6.2[b]	6.4[b]	6.8[b]	6.2[b]	6.2[b]	6.4[b]
2	0	4.6[a]	3.2[a]	3.6[b]	3.0[a]	3.0[a]	4.2[a]
	0.1	1.8[d]	2.2[c]	3.2[d]	2.8[b]	2.8[b]	2.2[d]
	0.2	2.4[c]	2.3[c]	3.4[c]	2.8[b]	2.8[b]	2.8[c]
	0.3	3.2[b]	3.0[b]	4.8[a]	2.6[c]	3.0[a]	3.6[b]

[a], Means; Values with different small letters in a column at the same storage time are significantly different at $p<0.05$.

Discussion

The microbial in Khoa-Mak contained yeast, mold and bacteria. The enzymes from mold cultures hydrolyzed starch in rice to sugars. Yeasts, molds and bacteria continuously consumed sugars for synthesis of cell and their metabolites (Khamkeaw and Phisalaphong, 2016). Growth patterns of yeast and mold cultures in Khoa-Mak followed that of ethanol production in relation to the respective sources of Loog-Pang and were the highest with that from Prachinburi. The changes in ethanol content, TSS and pH during fermentation of Khoa-Mak of this study were in agreement with those of Mongkontanawat and Lertnimitmongkol (2015), who fermented Khao-Mak from glutinous rice and the germinated native black glutinous rice for 7 days. In addition, the sensory properties of Khoa-Mak including appearance, color, taste, odor, flavor and overall acceptability were depended on sources of Loog-Pang as well as fermentation period. The results suggested sensory attribution of Khoa-Mak was the highest with glutinous rice fermented with Loog-Pang from Trad for 48 hours. Under this condition, count of *E. coli* (<3 MPN/g) and chemical properties including ethanol content (0.12%), TSS (40°Brix) and pH (4.2) were according to Thai Community Product Standard (Ministry of Industry, 2013).

The yeast and mold cultures were capable of survived at low pH in Khoa-Mak during storage at 4°C for 2 weeks. Total viable counts in Khoa-Mak were almost constant throughout storage. This result agreed with the finding of Tochampa *et al*. (2009), who found that total viable counts in Khoa-Mak was nearly constant during storage at 10°C for 8 days at low pH. The result revealed that red pigment was more stable under acidic condition than that of yellow and orange pigments. Feng *et al*. (2012) suggested that *Monascus* pigments were very stable at pH value of 6.0-8.0. *Monascus* pigments by *M. purpureus* JR were more stable at alkaline pH (9-11) than at acidic pH (3-5). The DPPH radical scavenging activities of Khoa-Mak were improved by the supplementation of red yeast rice. It was suggested that red yeast rice powder had appreciable potent antioxidation activity. Our finding on benefit of Khoa-Mak is consistent with earlier studies. Plaitho *et al.* (2013) found that Khoa-Mak made from Thai pigmented rice varieties including Sung Yod, Mon Poo, Hom Mali, Riceberry and black glutinous rice exhibited higher DPPH antioxidant activity than the non-fermented varieties. DPPH antioxidant activity of the fermented Thai pigmented rice varieties varied between 3.4-5.3 mmol Trolox equivalent per 100 g dry weight of sample. Mongkontanawat and Lertnimitmongkol (2015) reported that Khoa-Mak producing from the germinated native black glutinous rice (IC_{50} 59.7 mg/ml) showed higher DPPH radical scavenging activity than that of Khoa-Mak produced from glutinous rice (IC_{50} 199.37 mg/ml). The result also indicated that the supplementation of red yeast rice affected sensory attributes of Khoa-Mak. The sensory scores of Khoa-Mak supplemented with 0.3% of red yeast rice were higher than other samples supplemented red yeast rice (0.1 and 0.2%), but lower than control sample. However, DPPH antioxidant activity in Khoa-Mak supplemented with 0.3% of red yeast rice was the highest. Therefore, the present study suggested that Khoa-Mak supplemented with 0.3% of red yeast rice exhibited potential as a new healthy food product with a shelf life of approximately 1 week at 4°C because the product was still accepted favorably.

In conclusion, Loog-Pang from Trad was a suitable starter in culturing Khoa-Mak that could be fermented for 48 hours. Interestingly, red yeast rice can be promoted in functional food products as higher bioactive compounds such as *Monascus* pigments, and stronger antioxidant activity of this Khoa-Mak. Khoa-Mak supplemented with 0.3% of red yeast rice was also favored by elderly consumers. This study offers a new healthy food product that is particularly well suited for the elderly consumers.

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