

Substitution of nitrite by Chinese red broken rice powder in Thai traditional fermented pork sausage (Nham)

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Abstract: The objectives of this study were to determine the physical, chemical and microbiological properties and sensory evaluation of Thai traditional fermented pork sausage (Nham) which added Chinese red broken rice powder from *Monascus purpureus* as a substitute for nitrite. In this experiment, 4 levels (0.1, 0.2, 0.3 and 0.4 g/100 g) of Chinese red broken rice powder were used. The sausages, then, were packed in polypropylene bags and were kept for 24 h at 30°C until sour taste occurred and subsequently stored at chilled temperature (5°C) for 30 days. The color values (L*, a* and b*), titratable acidity, pH, a_w values, moisture, protein and fat contents were analyzed. Samples were also examined microbiologically for total plate counts (TPC) and the presence of *Clostridium perfringens*, *Salmonella* spp. and *Staphylococcus aureus*. The results revealed that L* and b* values were the lowest and a* values was the highest in 0.4 g/100 g rice powder added treatment. Lactic acid contents of the samples ranged from 0.91 – 2.47% which was higher than that of the control. The pH of the samples ranged from 3.74 – 4.25 which was dropped to the lowest value (3.74) on day 24 and then increased until day 30. Moisture contents and a_w values were slightly changed with no significant difference ($P>0.05$). The results also indicated that the sausages containing rice powder were not different in protein and fat contents compared with the control. TPC of samples were between 8.65 – 10.99 log CFU/g which was increased to the highest numbers (10.99 log CFU/g) on day 24 until day 30 and pathogenic microorganisms were not found during storage. The appearance and color attribute liking scores in 0.1 g/100 g rice powder added treatment were the highest for up to 12 days.

Keywords: Chinese red broken rice powder, quality, Thai traditional fermented pork sausage; Nham

Introduction

Thai traditional fermented pork sausage or Nham has been popular among consumers in Thailand although it is originally mere food of the North-eastern of the country. It has to be added nitrite for pink color forming in cured meats. The primary function of nitrite is the effect of the pink color characteristic of cured meats desired by the consumer and is usually indicative of quality of cooked products (Shahidi and Pegg, 1991). In addition, nitrite shows antibacterial activity, particularly the inhibition of germination of spores and toxin formation by *Clostridium botulinum* (Cassens, 1997; Martin, 2001).

Nevertheless, nitrite causes a formation of carcinogenic *N*-nitrosamines in cured meat products due to its reaction with secondary amines and amino acids in muscle proteins. Furthermore, residual nitrite in fermented meats may form *N*-nitrosamines in the gastrointestinal tract (Shahidi and Pegg, 1991). Thus, the meat industry continues searching for alternative methods to produce nitrite-free meats that maintain the

color characteristics of nitrite cured meat products.

The red pigment from Chinese red rice can be used for food coloring and can be use as an alternative to nitrite or synthetic food color in smoked sausage and Chinese sausage (Inkioe, 2002). The chemical component isolated from this fungus is the secondary metabolites which has been demonstrated as hypocholesterolemic (Endo, 1979 and 1980), liver-protective and antitumor effects (Yasukawa *et al.*, 1996; Aniya *et al.*, 1998). Accordingly, Chinese red rice has been used as a functional dietary supplement to reduce cholesterol level in the human body (Tobert *et al.*, 1982). Citrinin produced from *Angkaka* production was known as monascidin A. The antibacterial effects of monascidin A was confirmed by Wong and Koehler (1981). The monascidin A was able to inhibit *Bacillus* spp., *Streptococcus* spp. and *Pseudomonas* spp. (Wong and Bau, 1977). Therefore, Chinese red broken rice powder is an interesting agent to use instead of nitrite in cured meat products.

The objective of this study was to compare the quality of fermented pork sausages that added with Chinese red broken rice powder and nitrite during storage at 5°C.

Materials and Methods

Culture preparation

Lyophilized culture of *Monascus purpureus* TISTR 3080 purchased from the Thailand Institute of Scientific and Technological Research, Thailand was used in this study. The culture was inoculated on potato dextrose agar (PDA) slant and incubated at 30°C for 14 days. Five ml of sterile distilled water was added to get the culture spore suspension of 1×10^6 spores/ml.

Chinese red broken rice preparation

The broken rice was purchased from local market in Phitsanulok Province, Thailand. One kg of broken rice was washed by tap water and filtered through pore basket for 10 min. The 100 g of soaked rice was put in 250 ml flask and sterilized by autoclave at 121°C for 15 min. The spore suspension of 5 ml (10^6 spores/ml) was added in the sterilized broken rice and incubated at 25°C for 21 days. The sample was dried at 60°C for 24 h and 20 mesh fined powder was obtained by using a mill. The sample was kept in an opaque bottle for further use.

Nham preparation

Lean meat purchased from a local market was trimmed of all visible fat and connective tissue and subsequently minced through a 2-mm plate. Pork skin was trimmed of all visible fat and soaked in boil water to remove pig hair from the follicle. The de-fatted skin was finely shredded. Nham was produced with following ingredients : 1 kg minced pork, 100 g shredded cooked pork rind, 100 g cooked glutinous rice, 100 g chopped garlic, and 40 g salt, which were thoroughly mixed. In the control, these ingredients and 0.01 g/100g of sodium nitrite was added. Whilst the treatments the same ingredients were used and red broken rice powder of 0.10, 0.20, 0.30, and 0.40 g/100g were added. The well-mixed pastes were stuffed into polypropylene bags with a diameter of 3.0 cm (approximately 150 g each) and sealed tightly. The samples were kept for 24 h at 30°C until sour taste occurred and subsequently stored at chilled temperature (5°C) for 30 days. Samples were analysed every 6 day until 30 days for physical, chemical and microbiological properties and sensory evaluation.

Color measurement

The color of samples was measured by using a HUNTER LAB (Model DP 9000). The instrument was calibrated using a white standard plate ($L^* =$

90.26, $a^* = -1.29$, $b^* = 5.18$). The values, expressed as L^* (lightness), a^* (redness) and b^* (yellowness) units were obtained from five different cut areas of each samples. For each treatment, three links of sausage were used for the analysis.

pH and lactic acid content

Aliquots of 10 g of minced sample were dispersed in 90 ml of distilled deionized water with a tissue grinder. The pH of the slurry was measured by using a pH meter (Thermo Orion model 420, USA). The measurement was carried out triplicate (Zhang *et al.*, 2007). The titratable acidity (TA) in samples was measured by the method of AOAC (1995) and expressed as % lactic acid contents based on dry weight.

Moisture, fat, and protein contents

The sausages were homogenized (Robot Coupe Blixer RSI BX3, Robot Coupe USA Inc., Ridgeland, MS, USA) and samples were taken for proximate analysis. Moisture and fat contents were determined following AOAC (1995) procedures. Crude protein percentage was measured following AOAC (1995) that was also analyzed in triplicate by the macro Kjeldahl method using digestion (Kjeldahl digestion system, B-435, Buchi Laboratoriums-Technik AG, Flawil, Switzerland) and distillation units (Kjeldahl distillation unit, B-316, Buchi Laboratoriums-Technik AG, Flawil, Switzerland).

Water activity

An adequate amount of grounded sample was placed in a holding cup (about one-half full) and the water activity value was read (AQUA LAB CX-2, Decagon Devices Inc., Pullman, WA, USA) after equilibrated at 25°C.

Microbiological analysis

Dilution sample for microbiological analysis was prepared by homogenized 25 g grounded sample with 225 ml sterile peptone water. The 10-fold serial dilution with pour plate technique was used for total plate counts (TPC) and *Clostridium perfringens* determination and spread plate technique for *Salmonella* spp. and *Staphylococcus aureus* determination. Total plate counts were enumerated on plate count agar (Merck Co., Darmstadt, Germany) at 35°C for 48 h. *Clostridium perfringens* counts were incubated anaerobically on TSC agar (Merck Co., Darmstadt, Germany) at 35°C for 24 h. *Salmonella* spp. and *Staphylococcus aureus* were incubated on

Salmonella Shigella (SS) agar and Mannitol salt (Phenol Red) agar(MSA)(Merck Co., Darmstadt, Germany) at 37°C for 48 h and 35°C for 48 h, respectively (AOAC, 1995).

Sensory evaluation

Samples were heated on fried-pan. A portion of sample (about 1 cm) from each treatment was prepared for panel evaluation. The panelists ($n = 25$) were untrained. At each evaluation, five samples were coded with random numbers and liking evaluation for color, appearance, firmness, odor, taste and overall on a 9-point hedonic scale (9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely). Another evaluation of overall acceptance was monitored which scored not less than 5.00 were assumed to be acceptable.

Statistical analysis

All the experiments were repeated three times. Statistical analysis performed by using analysis of variance (ANOVA) was done to determine the significance of the main effects in SPSS 14.0 for Windows (SPSS, Chicago, Ill., USA) software package. Significant differences ($P \leq 0.05$) between means were identified using Duncan's new multiple's range test procedures.

Results and Discussion

Fat and protein contents

Table 1 showed that fat and protein contents of all samples were in the range of 3.00 – 3.86% by weight and 17.83 – 20.82% by weight, respectively with no significant difference ($P > 0.05$). When compared to Visessanguan *et al.* (2006) reported that lipid and protein compositions of Nham before and after fermentation were in the range of 2.33 – 2.62% by weight and 20.3 – 20.4% by weight, respectively. The result showed that addition of Chinese red broken rice in Nham did not affect on fat and protein contents. Similar to the study reported by Lin and Lin (2002) found that the production of low-fat Chinese-style sausage containing potassium sorbate, sodium lactate, and trisodium phosphate which fat and protein contents had no different contents. Lin and Huang (2008) reported that the production of Chinese-style sausage containing different molecular-weight konjac gels that was found in all treatments had no difference in protein and fat contents.

Color measurement

The L^* , b^* and a^* values of samples were slightly changed during storage time. The L^* value of 0.4

Table 1. Fat and protein contents of Nham in control and different Chinese red broken rice powder levels added treatments (g/100g)

Treatment	Protein content ^{ns} (% by weight)	Fat content ^{ns} (% by weight)
Control	20.73 ± 1.22	3.80 ± 1.98
0.10	18.95 ± 1.45	3.44 ± 0.32
0.20	17.83 ± 0.44	3.25 ± 0.33
0.30	20.33 ± 1.02	3.33 ± 1.49
0.40	20.82 ± 0.12	3.86 ± 0.99

^{ns} Means with the same letters within a column are not significantly different ($P > 0.05$)

g/100g of Chinese red broken rice powder added Nham was the lowest whereas the a^* value of this treatments was the highest. (Figure1). The a^* values of samples increased with increasing rice powder, which the L^* and b^* values decreased. The change of color values during storage times was found because the pigment was sensitive to heat, unstable in the pH range of 2-10 and faded with light (Dufosse *et al.*, 2005). For these results, the color values of the sausages were unstable due to the pH values of Nham which were between 3.70 – 4.30. Nitrite is responsible for the formation of pink color characteristic in cured meats. The pink color was developed in a number of complicated reaction steps until NO-myoglobin (Fe^{2+}) was formed. Oxygen and other oxidizing agents like nitrite could oxidize the Fe^{2+} to Fe^{3+} (metmyoglobin : MetMb). The formed metmyoglobin (MetMb) was brown during storage (Honikel, 2008). It might be this reaction which caused the changes of color values in the control. Besides, samples were packed in polypropylene bag which is an oxygen permeable film and then brown color was rapidly formed (Seydim *et al.*, 2006). This result was similar to what was reported by Inkioe (2002) that the more Chinese red rice was added into Chinese sausages, the more red color appeared, though such color was unstable during storage.

Changes in pH and lactic acid contents

The pH values of all samples ranged from 3.74 – 4.25 which were lower than that of the control ($P \leq 0.05$). The pH values of the treatments dropped during day 12-24 (Figure 2). This was because of the produce of lactic acid contents which ranged from 0.91 – 2.47% (Figure 3). It might show that, during storage time at 5°C, lactic acid bacteria (LAB) could grow in the treatments and utilized starch from cooked glutinous rice (100 g/1 kg minced pork) which could obtain

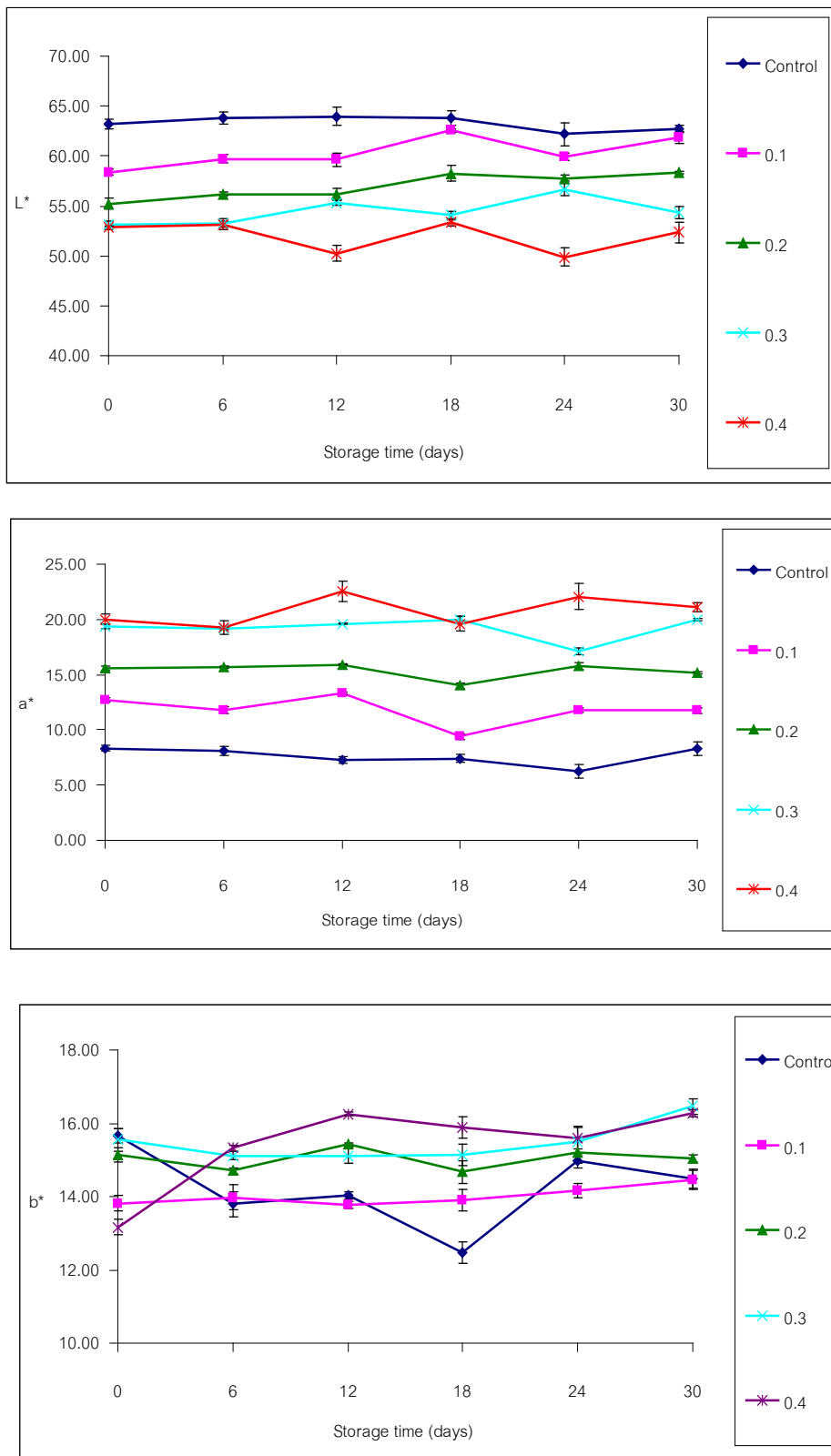


Figure 1. L*, a* and b* values of Nham in control and different Chinese red broken rice powder levels added treatments (g/100g) during storage for 30 days. Error bars indicate the means \pm standard error

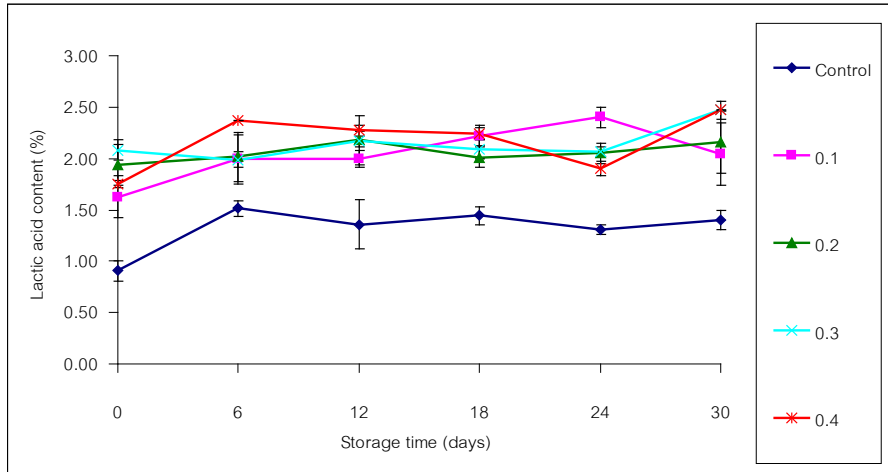


Figure 3. Lactic acid contents of Nham in control and different Chinese red broken rice powder levels added treatments (g/100g) during storage for 30 days. Error bars indicate the means \pm standard error.

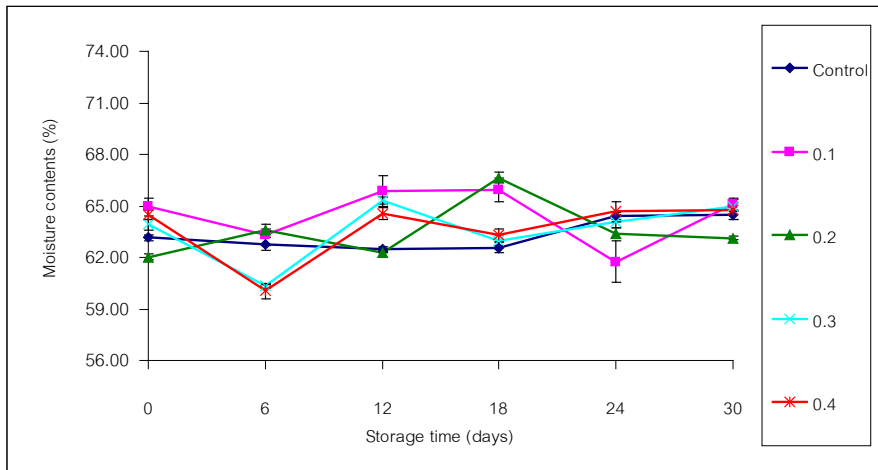


Figure 4. Moisture contents of Nham in control and different Chinese red broken rice powder levels added treatments (g/100g) during storage for 30 days. Error bars indicate the means \pm standard error.

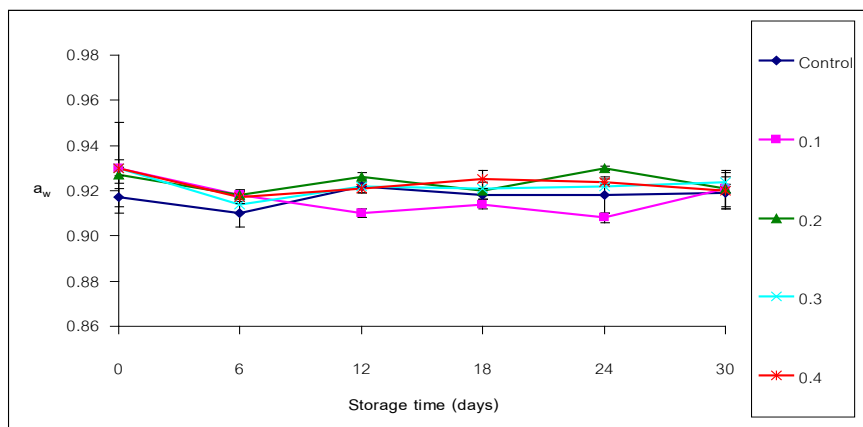


Figure 5. a_w values of Nham in control and different Chinese red broken rice powder levels added treatments (g/100g) during storage for 30 days. Error bars indicate the means \pm standard error.

Table 2. Total plate counts (Log CFU/ml) of Thai traditional fermented sausage in control and different Chinese red broken rice powder levels added treatments (g/100g) during storage for 30 days

Day	Control	¹ Levels of Chinese red broken rice ²			
		0.10	0.20	0.30	0.40
0	^a 8.65±0.55 ^a	^b 9.22±0.05 ^b	^b 9.30±0.12 ^b	^b 9.26±0.55 ^b	^b 9.14±0.33 ^b
6	^a 8.67±0.72 ^a	^{ab} 9.04±1.24 ^{bc}	^a 8.97±0.52 ^{ab}	^a 8.97±1.34 ^{ab}	^b 9.16±0.01 ^c
12	^a 8.66±0.32 ^a	^{ab} 8.91±1.72 ^a	^a 8.96±0.21 ^a	^a 8.77±0.35 ^a	^a 8.90±0.06 ^a
18	^a 8.90±1.72 ^a	^a 8.70±0.32 ^a	^{ab} 9.01±1.47 ^a	^a 8.78±0.67 ^a	^{ab} 9.04±1.04 ^a
24	^b 10.86±0.22 ^a	^c 10.75±0.28 ^a	^c 10.94±0.03 ^a	^c 10.80±1.93 ^a	^c 10.99±0.05 ^a
30	^b 10.76±0.86 ^a	^c 10.85±1.11 ^a	^c 10.02±0.18 ^a	^c 10.85±0.99 ^a	^c 10.99±0.39 ^a

¹ Means with different letters within a column are significantly different ($P \leq 0.05$)

² Means with different letters within a row are significantly different ($P \leq 0.05$)

Table 3. The liking scores of Thai traditional fermented sausage in control and different Chinese red broken rice powder levels added treatments (g/100g)

Score ^a	control	Levels of Chinese red broken rice powder ¹			
		0.10	0.20	0.30	0.40
appearance	7.19 ^a ±1.17	6.44 ^{ab} ±1.03	5.69 ^b ±1.49	5.69 ^b ±1.78	4.63 ^c ±1.26
firmness	7.38 ^a ±0.72	6.50 ^b ±1.21	6.13 ^{bc} ±1.09	5.75 ^c ±1.53	5.81 ^{bc} ±1.05
color	6.94 ^a ±1.34	6.88 ^a ±1.36	6.25 ^{ab} ±1.18	6.25 ^{ab} ±1.29	5.81 ^b ±1.56
odor ^{ns}	6.23±1.15	6.81±1.38	6.25±1.18	6.31±1.45	6.44±1.26
taste ^{ns}	6.63±1.50	6.81±1.17	6.31±1.45	6.44±1.67	6.50±1.32
overall ^{ns}	7.00±1.03	6.81±1.05	6.25±1.29	6.38±1.59	6.75±0.86

¹ Means with different letters within a row are significantly different ($P \leq 0.05$)

^{ns} Means with different letters within a row are not significantly different ($P > 0.05$)

^a Hedonic scale: 1 = extremely, dislike in appearance, firmness, color, odor, taste, and overall; 5 = neither like nor dislike; 9 = extremely like in appearance, firmness, color, odor, taste, and overall.

Table 4. Overall acceptance scores of Thai traditional fermented sausage in control and different Chinese red broken rice powder levels added treatments (g/100g) during storage for 18 days

Day ^a	control	Levels of Chinese red broken rice powder ¹			
		0.10	0.20	0.30	0.40
0	^a 7.00±1.03 ^a	^a 6.81±1.05 ^a	^b 6.25±1.30 ^a	^a 6.38±1.59 ^a	^a 6.75±0.86 ^a
6	^a 6.38±1.59 ^a	^a 6.81±1.05 ^a	^a 7.19±0.75 ^a	^a 6.50±1.32 ^a	^a 6.69±1.54 ^a
12	^a 6.38±1.20 ^a	^b 5.81±1.33 ^a	^b 6.38±1.02 ^a	^a 5.88±1.23 ^a	^b 5.69±1.45 ^a
18	^b 5.13±1.20 ^a	^c 4.81±1.33 ^a	^c 3.89±1.26 ^b	^b 3.38±1.20 ^b	^c 3.25±1.00 ^b

¹ Means with different letters within a column are significantly different ($P \leq 0.05$)

² Means with different letters within a row are significantly different ($P \leq 0.05$)

^a Hedonic scale: ≥ 5 score are overall acceptance and < 5 score are not overall acceptance.

sugar and finally produced many kinds of acid. It was similar to the report of Cerning (1990) who found that some specific species of *Lactobacillus* could utilize carbohydrate substrate and produced lactic acid in the treatments. Furthermore, Dufosse (2005) reported that *Monascus* spp. could utilize starch substrate to produce various kinds of acid such as succinic acid, citric acid, gluconic acid, and oxalic acid. This results agreed with previous study of Khieokhachee *et al.* (1977) who reported that the number of LAB in Thai fermented pork sausage increased drastically to a maximum of 8 – 9 log CFU/g within 24 h and remained constant until the fermentation were completed and Valyasevi *et al.* (2001) found that Lactobacilli produced lactic acid increasingly which related to the decrease in pH and the increase in acidity during the fermentation. These microorganisms also played a significant role in pigment (Vernam and Sutherland, 1995) and flavour compounds (Coretti, 1977) producing. Ockerman and Kuo (2001) reported that the pH value of dry cured hams decreased during storage and it was probably due to lactic acid. Banwart (1979) indicated that cured meat became sour (low pH) because of the fermentation of carbohydrates by lactic acid bacteria. Kuo and Chu (2003) reported that Chinese sausages contained 10% of sugar, which probably could be used by lactic acid bacteria, resulting in lower pH value during storage. The pH of meat could be affected by many factors, however, growth of lactic acid bacteria resulting in lactic acid production which is the major factor in pH decrease in packaged meats (Gill, 1996). One reason that would affect to the increase of pH and TPC during day 24-30 is protein hydrolysis by microorganisms in meat sausage which formed alkaline environment. The result was similar to the study of Cilla *et al.* (2006) who found that the pH and non protein nitrogen increased during storage of dry-cured ham in refrigerated temperature.

a_w values and moisture contents

The initial *a_w* values and moisture contents of samples were slightly changed during storage time (Figures 4 and 5). This was probably due to samples were packed in sealed polypropylene bags which is an oxygen permeable film (Inkioe, 2002). This results agreed with previous work of Inkioe (2002) reported that the smoked sausages in packed with polypropylene and stored at 5°C for 30 days were not different in moisture contents and *a_w* values during storage times. Wang (2000) also reported that *a_w* values of the Chinese sausage packed in a vacuum bag (NY/PE/PE) and stored at 20°C for 30

days were not different during storage times.

Microbiological analysis

The microbiological quality of Nham followed the Thai community product standard (Ministry of Industry, 2003). It showed that during storage *Salmonella* spp. was not found of 25 g/sample and *Staphylococcus aureus* and *Clostridium perfringens* were not found of 0.1 g/ sample. (data not shown).

Total plate counts (TPC) of Chinese red broken rice powder added treatments ranged from 9.22 – 9.30 log CFU/g which were more than that of the control at day 0 ($P \leq 0.05$) (Table 2). They were slightly decreased until day 18 and then increased to 10.75 – 12.02 log CFU/g after day 24 up to day 30 while that of control which was not different from day 0 to day 18 and then increased to 10.86 – 10.76 log CFU/ g during day 24 – 30. This was because of the growth of *Monascus* spp. in Chinese red broken rice powder and could produce antibacterial metabolite such as citrinin (monascidin A) (Wong and Koehler, 1981). Whereas, nitrite in the control might affect to lower TPC counts due to it was changed to nitrous acid and formed an acidic environment which inhibited the microbial growth (Honikel, 2008). Such antimicrobial agents affected the microorganism in limited range of time then the number of microorganism finally increased and the reason that mentioned in 3.3 is protein hydrolysis which formed alkaline condition (Cilla *et al.*, 2006).

Sensory evaluation

The color attribute liking scores of control and 0.1 – 0.3 g/100g of rice powder added treatments were not significantly different ($P > 0.05$) (Table 3). The appearance attribute liking score of control and 0.1 g/100g of rice powder added treatments were not significantly different ($P > 0.05$) and they were the highest. It was due to the color property of rice powder which affected on color liking. The firmness attribute of the control scored the highest followed with 0.1 % of rice powder added treatment. Whereas taste, odor and overall attribute scores of all samples were not significantly different ($P > 0.05$).

The overall acceptable of Chinese red broken rice powder added treatments were up to day 12 whereas the control was up to day 18 because the sour taste increased due to the more lactic acid produced (Table 4).

Conclusions

The most acceptable level for an alternative to nitrite was 0.1 g/100g of Chinese red broken rice powder and the fermented pork obtained the highest liking score of color and appearance attributes. The increase levels of rice powder produced the more pink color in the samples and affected lactic acid content which was higher than that of the control hence affected the consumer acceptance. The production of Nham containing different levels of Chinese red broken rice powder was not different in protein and fat contents and pathogenic microorganisms were not found in all treatments during storage times.

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