

Abstract - starter culture and 7% of cooked rice and different levels of anka rice were used to produce nham sausage, and evaluated the microbiological, chemical and physical properties. Both of level of anka rice and storage time factors weren't significantly affected on the chemical contents, total plate counts and pH value ($P>0.05$). Whereas, mold and yeast counts in each treatment were increased during storage period ($P<0.05$) and showed the maximum count number at the 3rd day of storage. The higher level of anka rice resulted in the significant decrease for nitrite residue and L value, but the significant increase for a value ($P<0.05$). However, added 0.25-0.5% anka rice could significantly improve the appearance and color scores of nham sausage ($P<0.05$), but showed a reversed effect when the level of anka rice was higher than 0.5% in this study.

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Index Terms - starter culture, anka rice, Thai fermented sausage, cooked rice.

I. INTRODUCTION

In fermented sausages, the pigment is responsible for the characteristic cured color that is the bright red nitrosylmyoglobin, which is consumers used for indication of its freshness. Most of the natural colorants are extracts from plants, but some are derived from animals or microorganisms. Among the latter, red yeast rice, also know as ang khak or hong qu in China, koji, ang-khak, beni-koji, red-koji in Japanese or red mould in the USA (Erdo and Azirak, 2005). *Monascus* belongs to the class of Ascomycetes and family of Monascaceae in particular. The genus of *Monascus* can divided into four species: *M. purpureus*, *M. pilosus* and *M. rubber* (Hawksworth and Pitt, 1983). *Monascus* can produce six major pigments: yellow pigment (Monascin and Ankaflavin), orange pigment (Monascorubin and Rubropunctatin), and the red pigment (Monascorubramin and Rubropunctamin) (Chen and John, 1993). The *Monascus* fungus is a source of natural food colorants and can produce hydrolytic enzymes which are importantly used in food

process such as alcoholic rice wine beverage, cheese, and tofu (Pichayanguras, 1979). The objective of this study was to study the effect of various levels anka rice (0.25, 0.50 and 0.75%) on microbiological, chemical and physical properties of Thai fermented pork sausages (nham sausage) during storage at 4°C for 7 days.

II. MATERIALS AND METHODS

A. Starter culture

Pure cultures of *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus* were obtained from FIRDI of Taiwan. A 0.015 mL of *S. thermophilus* and *L. rhamnosus* were inoculated in 7 ml MRS broth (Merck) then incubated at 37°C for 48 h; *L. bulgaricus* was inoculated in 7 ml MRS broth and also incubated at 37°C for 120 h. The starter cultures were kept at 4°C about 1 week for liquid cultures.

B. Preparation of anka rice (*Monascus purpureus*)

Monascus purpureus (CCRC No. 31499) was obtained from FIRDI of Taiwan. *Monascus purpureus* culture from PDA agar was inoculated into medium culture (sucrose 10%, yeast extract 0.8%) at pH 5.5, 35°C and shook at 120 rpm for 7 days. The liquid culture was homogenized by a blender 2 times (5s per time) then mixed with cooked rice to produce anka rice and the processing was described by Wu (2003). Finally, anka rice was dried at 45°C for 24 hr.

C. Preparation of Thai fermented pork sausage (nham)

Raw pork was obtained from a local meat factory and all visible fats and connective tissues were trimmed off. The raw meat was minced through a 2 mm plate. Pork skins without all visible fat were cooked in an autoclave (121°C) about 3 minutes After chilling, the cooked pork skin was finely shredded by a slicing machine. Rice (Thai Jasmine rice) was cooked in an autoclave (121°C) about 1 minute then chilled until 5°C. Nham was prepared by mixing of ground pork: cooked pork rind (70 : 30), cooked rice (7%), garlic (5%), salt (2%), bird chilli (2%), starter culture (0.075%), sucrose (0.4%), monosodium glutamate (0.2%), PPT (0.2%) and sodium nitrite

(0.015%). At last step, different levels of anka rice (0%, 0.25%, 0.50% and 1.5% by weight, respectively) were designed to add into the formula. The mixtures were thoroughly mixed with a blender, then stuffed into a plastic casing of diameter 2.5 cm and sectioned (approximately 45 g each) and sealed tightly prior to incubate at 30°C for 36 h. (pH about 4.6) then stored at 4°C for 7 days.

D. Chemical and quality determination

During storage, the samples were taken at day 0 and 7 for chemical composition (moisture, crude fat, crude protein and ash) analyses (AOAC, 1990) and sensory evaluation (appearance, color, sourness, flavor, tenderness and overall). The pH, microorganism, nitrite residue, color (L, a) of nham sausage were determined at the day 0, 1, 3, 5 and 7. E. Statistical analysis Data were analyzed using a statistics software package (SAS, version 9.1, 2002). The ANOVA system was used to test the significance of treatment effect. When significant ($P < 0.05$) differences were found, means were separated by the Duncan's multiple range test.

III. RESULTS AND DISCUSSION

A. Chemical composition

Moisture, crude protein, crude fat and ash of nham sausage at the 0 day and the 7th day are shown in Table 1. All chemical contents were not significantly different among all treatments and during storage ($P > 0.05$). This result may be caused by a less than 1% anka rice and plastic casing were used in this experiment. The reports of several studies also showed that there was no significant difference in all chemical contents in their meat products such as non-fermented cured ducks, Chinese-style sausages and roast beef (Cheng and Ockerman, 1998; Wu, 2003 ; Lee, 2005), when compared with anka rice treatment.

B. Microbiological change

In this study, Table 2 showed the total plate counts kept stable during storage for 7 days and there were no significant differences among all anka rice treatments ($P > 0.05$). Simultaneously, a higher count of mold and yeast was found in all nham sausages with anka rice but there was no significant difference. During storage, the count was significantly increased as time increased up to the day 3 then decreased down as the initial. However, Chuang (2008) also reported that the mold and yeast of chicken jerky treated with 3% *Monascus purpureus* increased during storage at 25°C for 21 days.

C. pH value

The pH values of nham sausage with different concentrations (0%, 0.25%, 0.50% and 0.75%) of anka rice are showed in Table 3. The pH value was showed the lowest value in 0.75% anka rice treatment (4.59 at the 1st day and 4.35 at the 7th days). Moreover, the pH value increased as the content of anka rice decreased but no significant difference ($P > 0.05$) was found among all treatments because the active microorganism (*Monascus purpureus*) is existed in anka rice and the same quantity of carbohydrate (cooked rice) and starter culture are used in this study.

D. Nitrite residue value

Several studies indicated that anka rice could make a contribution of a slowly decline rate of residual nitrite content in their products during storage (Wu, 2003; Lee, 2005; Hsu, 2006). Table 4 is showed the nitrite residue value in different anka rice treated nham sausages. The nitrite reduction was not significantly different during storage ($P > 0.05$) in each treatment. Added anka rice in nham sausages significantly reduced the value of nitrite residue ($P < 0.05$). Combination of 0.75% anka rice and 150 ppm nitrite treatment was showed the maximum of nitrite reduction. Moreover, the diminution of the nitrite contents was decreased following by 0.50%, 0.25% and 0% of anka rice concentration.

E. Color change

Anka rice can be contributed as a red colorant for foods (Bacus and Deibel, 1972; Pichayanguras, 1979; Verluyten et al, 2003). The changes of color (L, a) of the control and anka rice treatments showed in figure 1 and 2, respectively. The data of Figure 1 showed that anka rice in all treatments were significantly decreased L value ($P < 0.05$) compared with the control treatment. Regarding lightness evolution throughout storage, L values increased slowly from 0-5 days of storage and then decreased slightly after 5 days ($P < 0.05$). The L value showed a significant decrease as level of anka rice increasing and the result was the following: 0, 0.25, 0.5 and 0.75% anka rice, respectively, was 51.80, 46.85, 42.81 and 40.57 at the 1st day and was 53.06, 48.07, 44.46 and 42.74 at the 7th day during storage.

Figure 2 was showed the change in a value of nham sausage. All anka rice treatments obtained higher a value than the control treatment ($P < 0.05$). These results also responded to the higher anka concentration and the higher redness color in nham sausages. At 0% of anka rice treatment showed

the lowest a value.. Moreover, a value also significantly increased ($P<0.05$) as level of anka rice increasing and the result was the following: 0, 0.25, 0.5 and 0.75% anka rice, respectively, was 10.38, 18.98, 23.62 and 27.84 at the 1st day and was 11.53, 20.66, 25.82 and 30.44 at the 7th day during storage. F. Sensory evaluation The scores of sensory characteristics of nham sausage are showed in Table 5. Although the results indicated that added anka rice and storage time didn't significantly affect on the sourness, flavor, tenderness and overall acceptance of nham sausage, it was obtained significantly the highest appearance and color scores in nham sausage with 0.25% anka rice at 0 day and 7th day during storage. However, in this study, added 0.25-0.5% anka rice exactly could improve all sensory evaluations, especially on appearance and color. Moreover, added higher than 0.5% anka rice had reversed effects on all sensory items of nham sausage.

IV. CONCLUSION

Overall conclusion, added 0.25-0.75% anka rice didn't affect on the chemical composition, total plate count of nham sausage during storage. However, added 0.25-0.5% anka rice exactly could improve pH, nitrite residue, color and all sensory items of nham sausage in this study.

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