# BIOGENIC AMINES AND QUALITY CHARACTERISTICS OF CHINESE-STYLE SAUSAGE IMPLANTED IN RICE BRAN BED FERMENTED BY LACTIC ACID BACTERIA

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**Abstract - The objective was to investigate the biogenic amines and quality characteristics of Chinese-style sausage formulated with high sugar and low salt implanted in rice bran bed fermented by different lactic acid bacteria (P:*Lactobacillus plantarum*; A:*Lactobacillus acidophilus*; L:*Lactococcus lactis* subsp. *lactis*; M:mixed of three strains). The pH value and lactic acid bacteria counts (LAB) of fermented rice bran bed were analysed. Stuffed sausage was implanted in fermented rice bran bed for 72 hours then was dried by 10℃, RH 80%, and then vacuum packaged products were stored at 0-4℃ for 8 weeks. The Chinese-style sausage samples were collected for analysis of water activity, pH, TBARS value, biogenic amines, total plate count (TPC) and lactic acid bacteria count (LAB). The results showed that the pH of the fermented rice bran bed decreased while LAB counts was increased (P<0.05) at the first day of fermentation. Water activity of Chinese-style sausage decreased during storage. pH decreased from 5.42-5.70 to 4.13-4.51 (P<0.05) during 0-3 week storage. At 1st week, TBARS value increased (P<0.05). Biogenic amines were variable, and the total content of biogenic amines in group L and M was lower; tyramine could be detected in all groups; cadaverine was only detected at 0 week; putrescine was not detected; histamine content was low in all groups, and group P was not detected in any sample. TPC and LAB increased (P <0.05) at 2-4 week of storage, then gradually increased during storage, TPC of group P was lower (P <0.05) than other groups.**

**Key Words - Chinese-style sausage, rice bran, starter culture, biogenic amines**

## I. INTRODUCTION

Meat products through fermentation process can increase the nutrients and functional abilities. Fermented sausages usually add starter cultures for fermentation to produce organic acids rapidly [1]. Fermented sausages usually use lactic acid bacteria to produce acids to increase slice ability and preserve the products, produce antibacterial substances, prevent oxidation of fatty acids, and cause proteolysis and lipolytic activity to produce flavors and decrease residual nitrite, but they can also cause amino acid decarboxylation and form biogenic amines [2]. Biogenic amines are basic nitrogenous compounds. These substances have been proposed as possible indicators of food quality of fermented products [3]. The most important factors that influence the biogenic amine content of meat products are starter cultures [4], the quality of raw materials, the hygienic conditions of the processing environment, temperature or relative humidity also have been proposed as possible indicators of high contents of biogenic amines [5]. Rice bran is the outer layer of grain, and a by-product through grain processing. It contains carbohydrates, oils, most proteins in rice, vitamin $B_1$ and $B_2$, dietary fibers and some antioxidants, for example, γ-oryzanol, phytic acid, tocopherol and tocotrienol. In Japan, people always use rice bran bed called nukadoko to pickle vegetables, the microorganisms used in rice bran beds are lactobacilli [6]. The aim of this research was to study the biogenic amines and quality characteristics of Chinese-style sausage implanted in fermented rice bran formulated with high sugar and low salt and dried by 10℃, RH 80% condition.

## II. MATERIALS AND METHODS

*Fermented rice bran*

The fermented rice bran was prepared by the modified method of Nakayama et al. [6]. It was separated into four groups, P: *Lactobacillus plantarum* BCRC 12944; A:*Lactobacillus acidophilus* B161; L: *Lactococcus lactis* subsp. *lactis*; M: remixed 3 stains with 1:1:1, all strains were previously subcultured twice in MRS broth (Merck). Materials (Table 1) were mixed and sterilized at 120°C for 20 min, cooled and incubated with starter cultures for 7 d. To make the rice bran bed to reach $10^6$ CFU $g^{-1}$, P group was cultured at 30°C, other groups were incubated at 37°C. The fermented rice bran was stirred once every day.

Table 1. The materials of rice bran bed

| Material | (%) |
|---|---|
| distilled water | 120 |
| whey protein powder | 5 |
| skimmed milk powder | 5 |
| salt | 3 |

*Chinese-style sausage*

Chinese-style sausage was prepared by mixing 80％ground pork ham (95/5) and 20％ diced fat (0/100), with non-meat ingredients (Table 2). Each of the sausage batters was stuffed into hog casing (28 mm, Taiwan). Each sausage had a *ca.* length of

10 cm. The sausage was implanted into a rice bran bed and ripened for 72 h at 0-4℃, removed and dried for 72 h at 10°C and relative humidity (RH) at 80-85%, vacuum packaged (-0.92bar) and stored at 0-4°C for 8 wk, and analyzed each week. The fermented rice bran was analyzed for pH (SP-2200 Suntex pH meter, Taiwan), total plate counts (TPC) and lactic acid bacteria counts (LAB) (FDA Bacteriological Analytical Manual for Foods method [7]) every day. The Chinese-style sausages were analyzed for water activity (Rotronic Ag, HygroPalm AW 1-SET/14, Taiwan), pH, TBARS value (Salih et al. [8] with modifications), biogenic amines (extracted according to the method of Konosu et al. [9] and benzoylation of standard amine solution according to Yen and Hsieh [10] with modifications), total plate counts (TPC) and lactic acid bacteria counts (LAB) every day.

Table 2. The non-meat ingredients of Chinese-style sausage, meat weight as 100%

| Material | (%) |
|---|---|
| polyphosphate | 0.25 |
| salt | 1.5 |
| sugar | 12 |
| sodium nitrite | 0.015 |

*Statistical analysis*

Statistical analysis was performed with the SAS program for Windows V9.1 (SAS Institute, Cary, NC, USA). ANOVA with Turkey's multiple range tests was carried out to analyze the significant differences among treatments ($P<0.05$).

## III. RESULTS AND DISCUSSION

The pH of the rice bran bed in groups P and L was significant lower ($P<0.05$) (Fig. 1), and the pH value of group P was the lowest. The initial pH of all groups were between 6.08-6.22, and decreased significantly ($P<0.05$) at the first day. LAB counts of rice bran bed of group M was significant higher than other groups ($P<0.05$) during the fermentation period (Fig. 2). LAB counts of all groups increased significantly during 2 d ($P<0.05$). LAB counts reached 6.15-7.13 log CFU $g^{-1}$ after 7 d of storage. Lactic acid bacteria and other acid-producing bacteria could produce organic acids to cause the decreased of pH. Garriga et al. [11] found that *L. plantarum* could produce acid rapidly, but it could over acidify in some case.

The water activity of Chinese-style sausage was not significantly different among the groups (Fig. 3), and decreased during storage.

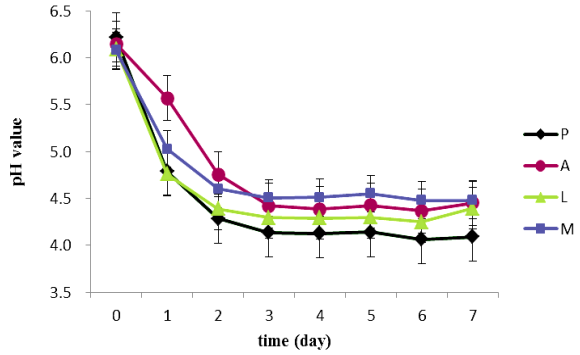


Figure 1. pH of rice bran beds fermented by lactic acid bacteria

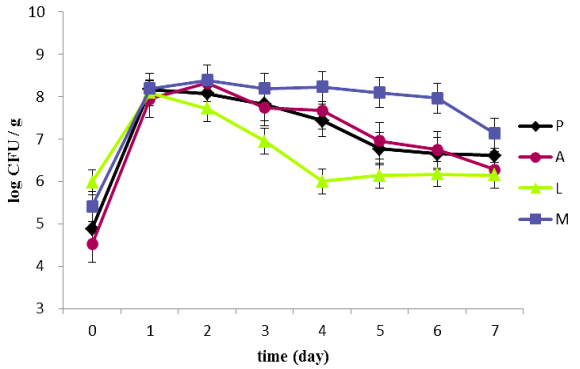


Figure 2. Counts of lactic acid bacteria in rice bran bed fermented by lactic acid bacteria

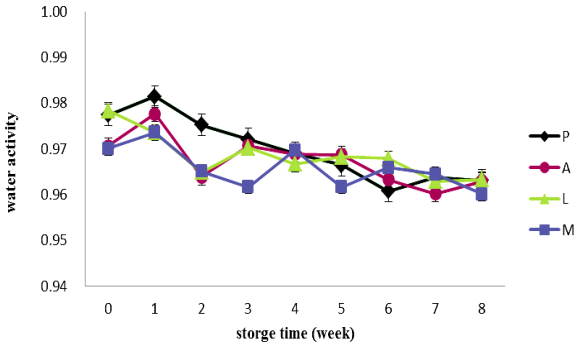


Figure 3. Changes in water activity of Chinese-style sausage implanted in rice bran bed fermented by lactic acid bacteria during 0-4°C storage

The pH of sausage was 5.42-5.70 at the first week (Fig. 4), and group P was significantly lower than other groups ($P<0.05$) at the early period of storage (0-3 week), the pH of group L and M decreased at the first week ($P<0.05$). Group A, L and M reached a minimum pH of 5.25-5.32 at 6-7 weeks of storage. In this experiment, the rice bran bed of group P fermented by *L. plantarum* had the lowest pH to cause the pH of sausage to be lower. This result agrees with that of other researchers [12, 13, 14].

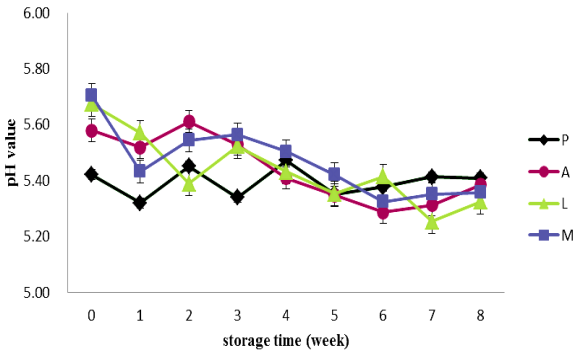


Figure 4. Changes in pH value of Chinese-style sausage implanted in rice bran bed fermented by lactic acid bacteria during 0-4°C storage

TBARS value of sausages were similar among treatments (Fig. 5), and The TBARS of all treatments increased significantly in the first week ($P<0.05$). Fermented sausage relies on the degradation of proteins and fats to produce special flavors, but it also could cause fat oxidation, to produce rancid substances during fermentation period. Starter cultures are the most important factor during fermentation [15].

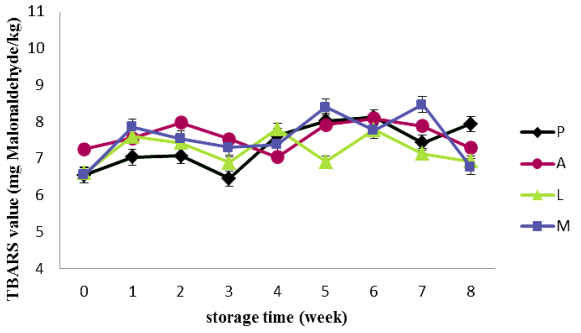


Figure 5. Changes in TBARS values of Chinese-style sausage implanted in a rice bran bed fermented by lactic acid bacteria during 0-4°C storage

Standard chromatograms of biogenic amines detected by HPLC are shown in Fig. 6. Putrescine was not detected in all groups during storage, cadaverine only detected at 0 week (267-403 mg/kg), spermine only detected in Group P, A and M and at 0-1 week, spermidine was below 8.90 mg/kg and 2-phenylethylamine was below 68 mg/kg in all groups during storage, tryptamine was 34-85 mg/kg during storage, histamine was not detected in group P, tyramine was detected in all groups during storage time. Putrescine, cadaverine and polyamines can be the indicator of pork spoilage, polyamines and putrescine may be naturally present in raw meat, but high concentration of putrescine and other amines may due to the growth of undesired microorganism [16]. Putrescine was not detected in this experiment and spermine was higher than other studies, but polyamines could exist in meat naturally, spermine was higher than spermidine usually [17]. Tyramine and histamine are the most important biogenic amines in toxicology [13]. Histamine is the only biogenic amine subjected to legal regulations in some fish species, with an upper limit of 100 mg/kg in Europe [18]. FDA guideline value of histamine in fish product is 50 mg/kg [19]. Nout [20] pointed out that histamine of fermented sausages must between 50-100 mg/kg. Tyramine is the most productive biogenic amine [21] and some researchers point out that levels below 500 mg/kg are safer [22]. The biogenic amines content in fermented meat varies, even if using the same starters, the same brand and the same batch fermented sausages, the content of biogenic amines may be significant different, showing that biogenic amines are affected by many factors [23].

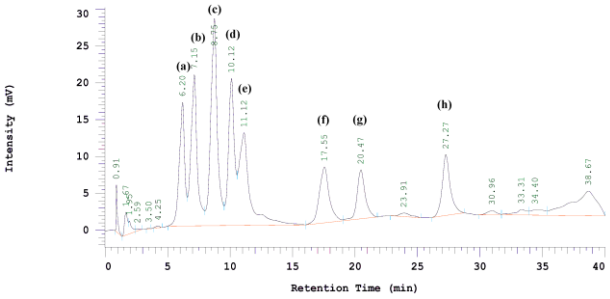


Figure 6. HPLC chromatograms of biogenic amine standards. (a) Putrescine, Put. (b) Cadaverine, Cad. (c) Tryptamine, Tryp. (d) Phenylethylamine, 2-phe. (e) Spermidine, Spd. (f) Spermine, Sper. (g) Histamine, His. (h) Tyramine, Tyr. HPLC: Hitachi (Japan) with RP-18 column (Merck). Mobile phase: methanol-water, 50:50 v/v at first, 60:40 v/v for 15 mins, 70:30 v/v for 3 mins, then maintain 50:50 v/v for 50 minute. Injected volumn: 20 µL, flow rate: 0.8 mL/min, UV-detector: 254 nm

The TPC of group P was significantly lower than other groups ($P<0.05$) (Fig. 7). TPC was between 3.28-4.12 log CFU $g^{-1}$ on first day, total plate counts of group A, L and M increased significantly to 5.20-5.84 log CFU $g^{-1}$ ($P<0.05$) at 2 wks; LAB counts of each group were not significantly different (Fig. 8). LAB counts were between 3.16-4.22 log CFU $g^{-1}$ on the first day, and the LAB counts was similar to TPC.

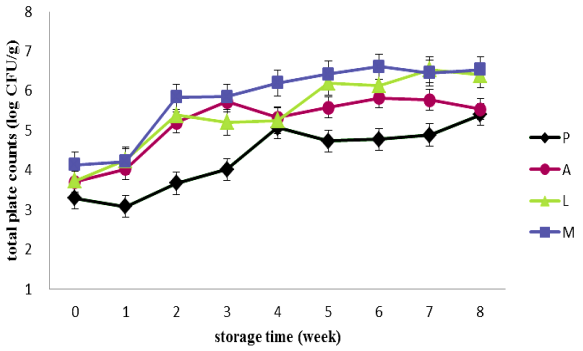


Figure 7. Changes in total plate counts of Chinese-style sausage implanted in rice bran bed fermented by lactic acid

bacteria during 0-4°C storage.

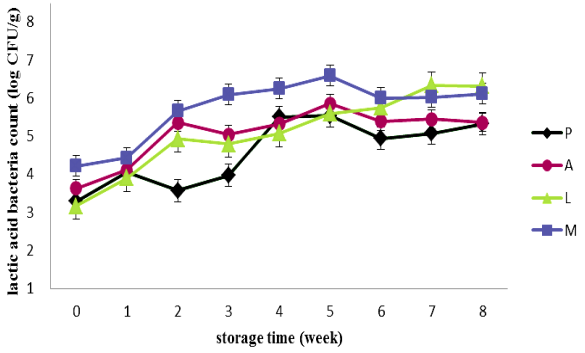


Figure 8. Changes in lactic acid bacteria counts of Chinese-style sausage implanted in rice bran bed fermented by lactic acid bacteria during 0-4°C storage.

## IV. CONCLUSIONS

The results from this study showed that the formation of biogenic amines in fermented sausage was effects by manufacturing methods. The biogenic amines and quality characteristics of Chinese-style sausage implanted in rice bran bed fermented by lactic acid bacteria were controlled by vacuum packaged and stored at refrigerated condition.

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