การเปรียบเทียบระหว่างปริมาณสารประกอบเอมีนในแหนมที่ใส่และไม่ใส่ขิง
Comparison of Biogenic Amine Contents in Nham Sausage with and without Ginger

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บทคัดย่อ
สารประกอบเอมีนพบได้ในอาหารหลายชนิดและมักมีอยู่ในปริมาณสูงในอาหารหมัก สารประกอบเอมีนชนิดวงแหวนพบว่ามีผลต่อความดันโลหิตหรือระบบประสาทและและมักเกิดร่วมกับอาหารที่มีสารประกอบฮีสตามีน อาหารที่ก่อให้เกิดไมเกรน และความดันโลหิตสูง กิจกรรมเอมีนออกซิเดสได้รับความสนใจโดยเฉพาะการนำมาลดปริมาณสารประกอบเอมีนในผลิตภัณฑ์อาหารเช่น แหนมหรือเนื้อหมูหมัก วัตถุประสงค์ของการศึกษานี้เพื่อลดการสะสมของสารประกอบเอมีนระหว่างการหมักแหนมที่ใส่ขิง การวัดกิจกรรมของเอมีนออกซิเดสทำได้โดยใช้วิธีปฏิกิริยาร่วมกันระหว่างเอนไซม์เปอร์ออกซิเดสและกัวคอลโดยใช้พรูเทรสซีนเป็นซับสเตรทที่พีเอช 7.5 อุณหภูมิ 30 องศาเซลเซียสเป็นเวลา 30 นาที กิจกรรมเอมีนออกซิเดสจากขิงเท่ากับ 33.34 หน่วยต่อกรัมน้ำหนักสด โดยเมื่อเติมสารละลายสารสกัดจากขิงปริมาตร 10-24 มิลลิลิตรในแหนมน้ำหนัก 100 กรัมก่อนการหมัก กิจกรรมเอมีนออกซิเดสของแหนมที่มีสารสกัดจากขิงก่อนการหมักเท่ากับ 7.86 หน่วยต่อกรัมแหนม หลังการหมักเป็นระยะเวลา 7 วัน สารประกอบเอมีนทั้งหมดในแหนมที่ใส่ขิงมีปริมาณต่ำกว่าคิดเป็น 64.7% เมื่อเปรียบเทียบกับตัวอย่างที่ไม่ใส่ขิง ผลการศึกษานี้แสดงให้เห็นว่าขิงที่มีเอมีนออกซิเดสในการหมักแหนมมีประสิทธิสูงในการลดการสะสมสารประกอบเอมีน

คำสำคัญ: ขิง เอมีนออกซิเดส เนื้อหมูหมัก

Abstract
Biogenic amines (BAs) are naturally present in many foods and relatively high contents of some BAs can be present in fermented foods. The aromatic BAs have been reported as vasoactive or psychoactive amines and they have been associated with food histaminic intoxications, food-induced migraines and severe hypertensive crisis. Amine oxidase activity has become a particular interest to reduce BAs concentration in food products such as Nham or fermented pork. The aim of this study was to reduce BAs accumulation during Nham fermentation by ginger. Amine oxidase activity was measured by coupled reaction with peroxidase and guaiacol with putrescine as substrate at pH 7.5, 30 °C for 30 min. The amine oxidase activity of ginger was 33.34 U/ g fresh weight. The ginger of 10-24 ml was added in 100 g Nham before fermentation. Amine oxidase activity of Nham with ginger before fermentation was 7.86 U/ g Nham. After 7 days of fermentation, total Bas concentration was 64.7 % less in samples added with ginger extract, as compared to control samples. These results indicated that ginger with amines oxidase activity in Nham fermentation was effective in reducing BAs accumulation.

Keywords: ginger, amine oxidase, fermented minced pork

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Introduction

Biogenic amines (BAs) are basic nitrogenous compounds produced mainly by bacterial decarboxylation activity toward amino acids in foods.[1,2] BAs can cause adverse health effect to consumers when ingested at considerable amounts or when the natural mechanisms for their catabolism are inhibited or genetically deficient in the human body. Nham is a popular fermented pork product and commonly used as a condiment in Thailand. Nham is prepared by fermenting pork with cooked rice, minced garlic and salt at ambient temperature for 3–7 days. Typically, no starter culture is applied in pork sauce fermentation, since it merely relies on indigenous bacteria from raw materials. BAs are naturally present in many foods and relatively high contents of some BAs can be present in fermented foods. BAs are organic molecules with low molecular weight. These compounds are usually generated by microbial decarboxylation of amino acids present in foods. The aromatic amines (histamine, tyramine, serotonin, β-phenylethylamine, tryptamine) have been reported as vasoactive or psychoactive amines and they have been associated with food histaminic intoxications, food-induced migraines, and severe hypertensive crisis due to monoamine oxidase inhibitor drug interactions. Moreover, amines such as putrescine and cadaverine could generate carcinogenic nitrosamines in the presence of nitrites.[3] Interest in cadaverine, putrescine, tyramine and histamine also lies in their potential as spoilage indicators of food. In addition, they may have unpleasant odours and it was also found that putrescine and cadaverine could inhibit the activity of muscle.[4] Amine oxidases catalyze the oxidative deamination of primary amine groups of several BAs in the presence of molecular oxygen, by accepting two electrons from the substrate and transferring them to oxygen, according to Equation 1:

$$R\text{-}CH_2NH_2 + O_2 + H_2O \longrightarrow R\text{-}CHO + NH_3 + H_2O_2 \quad (1)$$

Amine oxidases are frequently referred as semicarbazide sensitive amine oxidases, due to their characteristic sensitivity to inhibition by this compound. Many reports have demonstrated that the involvement of amine oxidases enzymes in amine catabolism and the products derived from their degradation are involved in a variety of important physiological processes. These include: cell wall maturation and lignification during cell development,[5] wound-healing and cell wall reinforcement during pathogen invasion[6] and abiotic stresses, such as osmotic stress, phytohormones and salinity.[7]

Amine oxidase activity was found in legumes pea epicotyls, Yiciafubn leaves, *Lathyrus satiuus Euphorbiu* latex though the enzymes have been in animals bacteria and fungi.[8] Amine oxidase was found in cereal seedling, tea leave and ginger. Amine oxidase in ginger was high activity, comparing to the cereal seedling and tea leave, with the enzyme activities of 151.46 U/g dry wt and 156.75 U/g dry wt, for monoamine oxidase and diamine oxidase, respectively.[9]

Monoamine and diamine oxidases had been described from cereal seedlings such as maize, mung bean and soybean and local plants such as green tea leaves and ginger monoamine oxidase and diamine oxidase from cereal seedling, green tea leaves and ginger. It was found that ginger contained higher both monoamine oxidase and diamine oxidase more than the others.[9]

Kala *et al*.[10] reported a reduction of BAs concentration in fermented food products by using lactic acid bacteria producing amine oxidase as inoculants and Zaman *et al*.[11] also reported that application of starter cultures with amines oxidase activity in pork sauce fermentation was found to be effective in reducing BAs accumulation.

Meanwhile, only little information is available on the effect of local plant amine oxidase on biogenic amine reduction in fermented pork. Therefore, the objective of this study was to investigate the effectiveness of ginger amine oxidase in inhibiting biogenic amine accumulation during pork fermentation.

Material and Methods

Nham preparation

Nham[12] was prepared to make a total 100 g

Nham by mixing 52 g minced pork, 35g cooked pork rind, 1.9 g curing salt, 0.2 g sodium erythrobate, 0.2g sodium tripolyphosphate, 4.3 g minced garlic, 4.3 g minced cooked rice, 2 g chilli, 0.4 g sucrose, 0.2 g monosodium glutamate, 0.01

g potassium nitrite and 0.6 g sodium chloride. Ginger (200 g) was added into the mixture and mixed thoroughly for 10 min. The mixture without ginger addition was used as the control. The mixture was then stuffed into a polyethylene casing with a diameter of 1.5 inch and the fermentation was conducted for 7 day. From studies of monoamine oxidase activities from ginger,[9] it was represented catalytic activity in the range of 30-50 °C which the optimum temperature was about 45-50°C. These characteristics were slightly different from the diamine oxidase activities which represented the temperature activity of 30-40°C. The enzyme activities decreased rapidly when incubation in the temperature higher than 40 °C. So in this study, the temperatures in the range of 30-50°C were used for the enzymes to react with BAs during fermentation.

Sample collection

Nham samples were incubated in the incubator at 20-50°C for 7 days. During incubation samples were taken every 24 h of fermentation for analyses. In order to prepare the samples for analysis, the casings were removed. Samples were cut up and ground in a meat grinder for 2 min and kept at 4°C for further analysis.

Sample preparation and extraction

Four grams of sample was mixed with 10 ml of 5% trichloroacetic acid and extracted using a homogenizer. The homogenate was centrifuged at 17,212 × g for 10 min at 4°C, the supernatant was collected and the precipitate was extracted again for three times with 10 ml of 5% trichloroacetic acid. After centrifugation, the supernatant was kept at -20°C.

Derivatization of sample extracts and mixed standards

A 100 µl of 2 N NaOH and 150 of µl saturated $NaHCO_3$ were added to 0.5 ml of the extract, mixed with 1 ml of dansyl chloride (Sigma, Analytical grade, USA) (10 mg/kg in acetone) and incubated at 40°C in a water bath for 45 min. To remove residual dansyl chloride, 50 µl of 100% ammonia was added and the solution was centrifuged at 500 × g for 30 min and the supernatant was filtered through a 0.45 mm filter. Dansyl derivatives of the calibration standards were mixed with the samples as previously described.[13]

Biogenic amine determination

HPLC method with ultraviolet-visible spectrophotometry were performed with a LC 10 AD Shimadzu LC (Japan) using a 20 µl loop. LC column C18-Hypersil BDS (200 mm. 4.6 mm, 5 µm particle size) was used. The detection wavelength was 254 nm for the dansyl derivatives. Amine standard solutions were prepared in DI water to a final concentration of 5 mg/kg for each biogenic amine. Putrecine, cadaverine, histamine and tyramine (Sigma, Analytical grade, USA) were used. Internal standard solution was prepared by diluting 15 mg of 1, 7-diaminoheptane in 5 ml of water. A gradient elution programme was used with the mobile phase of 100% methanol (solvent A) and nanopure distilled water (solvent B), starting with 55% solvent A and 45% solvent B and finishing with 100% solvent A and 0% solvent B after 45 min. The flow rate was 1.5 ml/min.

Statistical analysis

Data were expressed as mean ± standard error of mean of three experiments.

Results and Discussion

Putrescine profile of Nham with ginger during fermentation was presented in Figure 1 (A). Putrescine concentration slowly decline from 41.7 mg/kg at day 0 to 18.4 mg/kg during 7 days of fermentation in sample at 50°C, respectively. For sample at 30°C, putrescine concentration progressively highly decreased from 41.7 mg/kg at day 0 to 7.2 mg/kg during 7 days of fermentation. In sample at 20, 25, 35, 40 and 45 °C, putrescine concentration also decreased during 7 days of fermentation.

Cadaverine profile of Nham with ginger during fermentation was presented in Figure 1 (B). Cadaverine concentration was decreased from 37.2 mg/kg at day 0 to 16.6 mg/kg at day 7 of fermentation in sample at 50°C but cadaverine concentration at 30°C, highly declined to

9.4 mg/kg at day 7 of fermentation. However, cadaverine contents incubated at 20, 25, 35, 40 and 45 °C also slowly declined during fermentation.

Histamine profile of Nham with ginger was presented in Figure 1 (C). Sample incubated at 50°C, the concentration of histamine was decreased from 64.2 mg/kg to 44.3 mg/kg during 7 days of fermentation. The sample at 30°C, histamine concentration showed maximal reduction during fermentation. However, incubation at the other temperatures, histamine concentration slowly decreased.

Tyramine profile of Nham with ginger during fermentation was presented in Figure 1 (D). Tyramine concentration was slowest decreased comparing to the temperature 20-45°C. The incubation at 30°C showed tyramine concentration maximum decrease.

Putrescine content in control was higher than that of treated samples throughout fermentation at 30°C. The results showed putrescine concentrations during 7 days of fermentation were increase from 41.7 mg/kg to 153.6 mg/kg, (Figure 2 (A)) in control, while samples treated with ginger, putrescine concentrations decreased to 7.2 mg/kg while putrescine concentration was observed to be higher in control than samples added with ginger during 7 days of fermentation.

Final concentration of putrescine reached 153.6 and 7.2 mg/kg in control and samples with ginger, respectively. Cadaverine concentration in control was progressively increased from initially around 37.2 mg/kg to 127.4 mg/kg (Figure 2 (B)) in control during fermentation at 30°C while samples added with ginger, cadaverine concentration decreased to 9.5 mg/kg.

Histamine concentration in control was increased from initially around 64.2 mg/kg to 148.0 mg/kg (Figure 2 (C)) during fermentation at 30°C while samples added with ginger homogenate, histamine concentration decreased to 30.6 mg/kg. For tyramine in control, the concentration was increased from 87.2 mg/kg to 216.9 mg/kg (Figure 2 (D)) while samples added with ginger, tyramine concentration decreased to 44.0 mg/kg.

Six temperatures were tested for the antibiogenic amine activity of the enzyme: 30-50°C was found to be the optimum temperature of ginger amine oxidase according to the report of Yeunyongsuwan and Kongkiattikajorn[9] and 40°C, since the probable application of the enzyme in pork processing fermentation might occur at incubator during fermentation. Antibiogenic amine activity was determined by measuring the decrease in amine concentration (Figure 2). In contrast to the control group, ginger amine oxidase could reduce histamine content of sample in pH 5.0 (in neither 25°C nor 40°C. However, ginger amine oxidase represented a significant reduction in amine content after 1 d (in either 20°C -50 °C) in comparison to control group. This is consistent with previous studies[9] and suggests that this temperature is suitable for ginger amine oxidase antibiogenic amine activity.

Whereas high enzyme activity at 30-40 °C was observed, it is plausible to apply ginger amine oxidase in high temperature (such as a pork processing fermentation). The initial putrescine, cadavarine, histamine and tyramine levels were 41.7, 37.2, 64.2 and 87.2 mg/kg, respectively. Putrescine, cadavarine, histamine and tyramine contents of in control were elevated to 153.6, 127.4, 148.0 and 216.9 mg/kg, respectively, after 7 d at 30°C while putrescine, cadavarine, histamine and tyramine contents in Nham added with ginger were reduced to 7.2, 9.4, 30.6 and 43.9 mg/kg , respectively. In the report that porcine amine oxidase fitted into an exponential decline model of amine degradation in fermented pork sauce,[11] ginger amine oxidase also show significance difference in reduction of biogenic amine in fermented pork.

BAs were regarded as the main factor causing the increase in pH, since their concentration increased in accordance with the increase in pH. It had been considered that formation of BAs by bacteria is a physiological mechanism to counteract the acidic condition.[2] *Enterobacter cloacae*, *Pantoea agglomerans* and *Bacillus megaterium* were putrescine and cadaverine producers isolated from fermented pork.[14,15] *Paenibacillus tyramigenes* was reported as tyramine producing bacteria.[16] *Lactobacillus brevis* is also known as a tyramine producing bacterium.[17] BAs concentration namely histamine, putrescine, cadaverine and tyramine were found to increase throughout the

fermentation. The increase was progressive during the 7 days of fermentation. Veciana-Nogues *et al*.[18] also reported that these four amines have increased during manufacture of semipreserved anchovies. BAs formation in pork was mainly due to the activity of bacterial amino acid decarboxylation.[1]

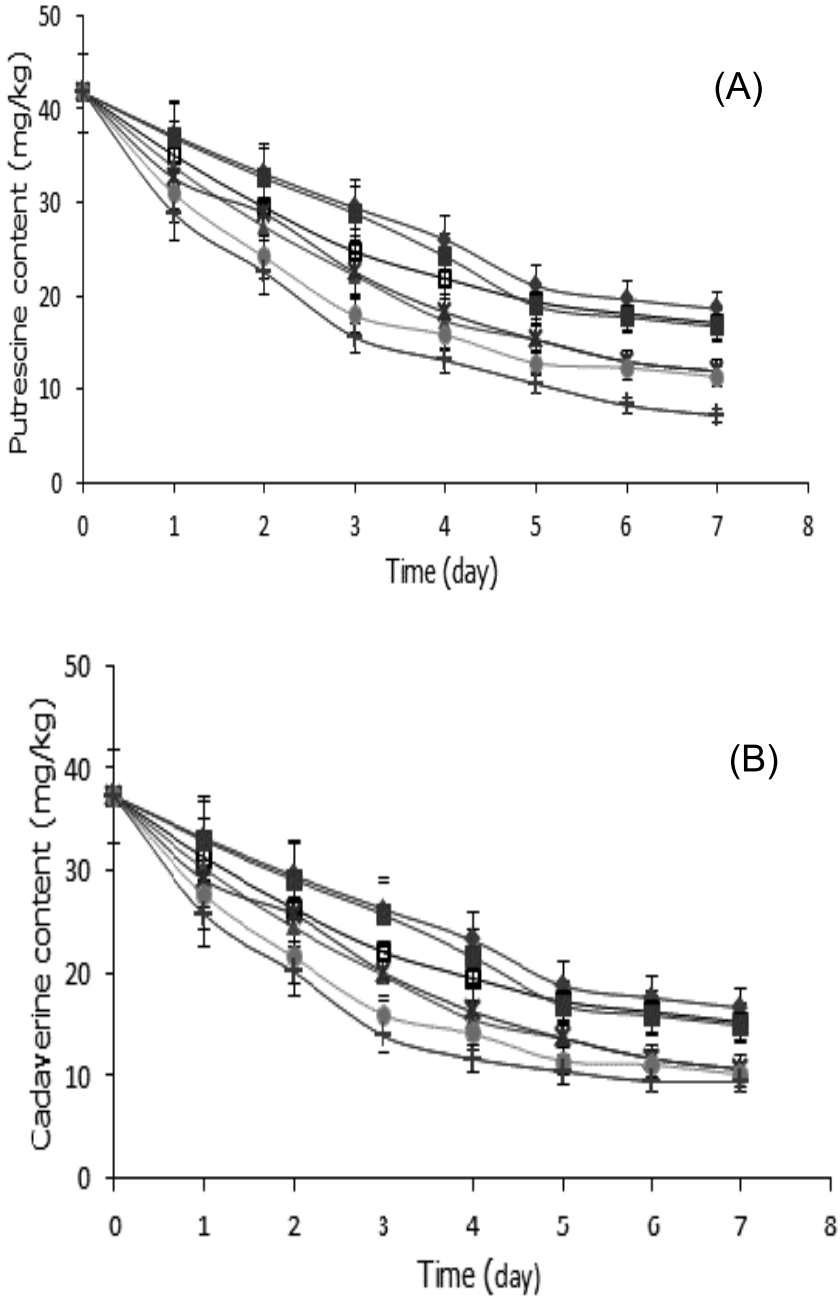
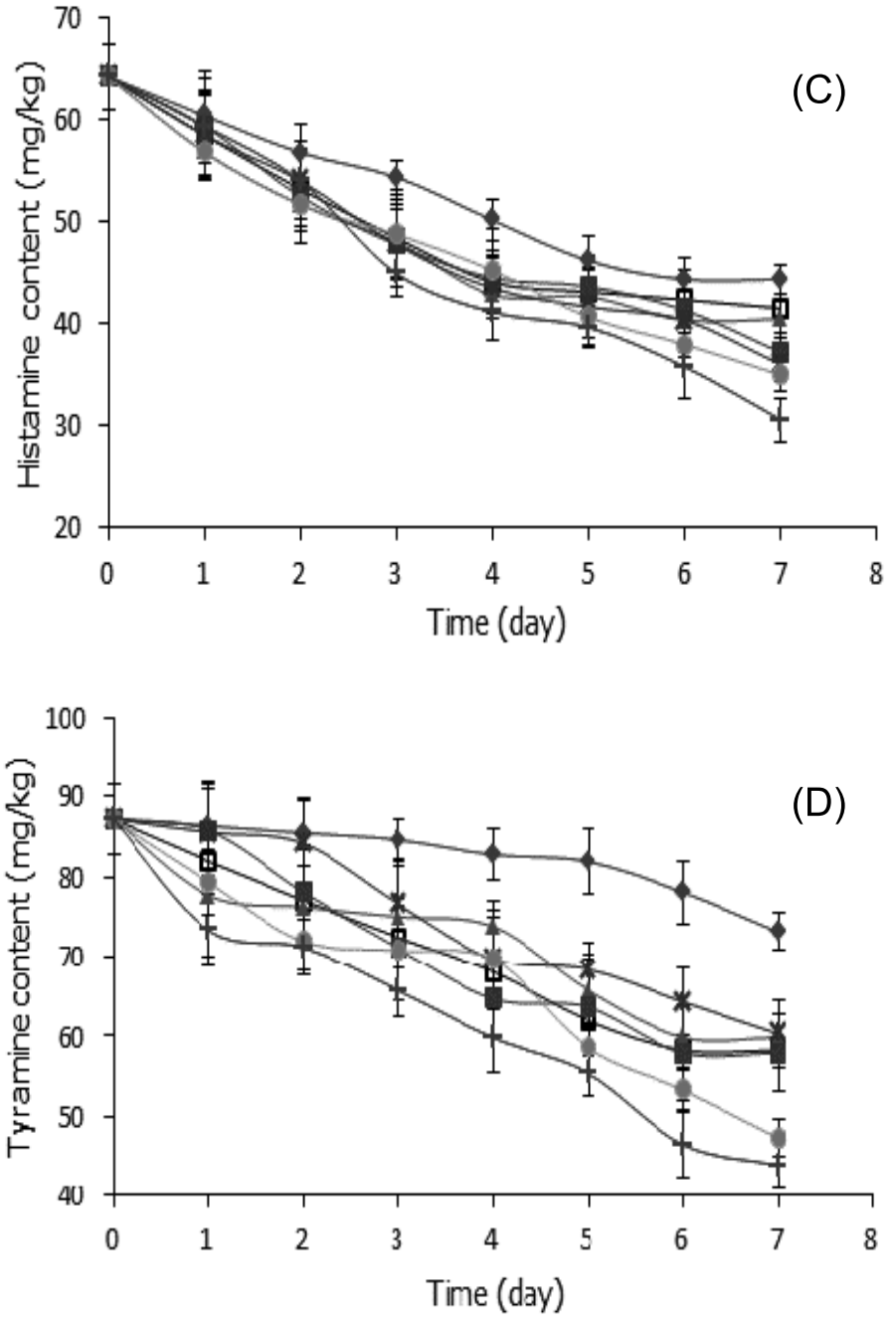


Figure 1 Effect of temperature on biogenic amine during fermentation of pork at pH 6.0 for 7 days. (A): putrescine, (B): cadaverine, (C): histamine and (D): tyramine. ▲: 20°C, ■: 25°C, +: 30°C, ●: 35°C, ┼: 40°C, ✳: 45°C, ◆: 50°C.



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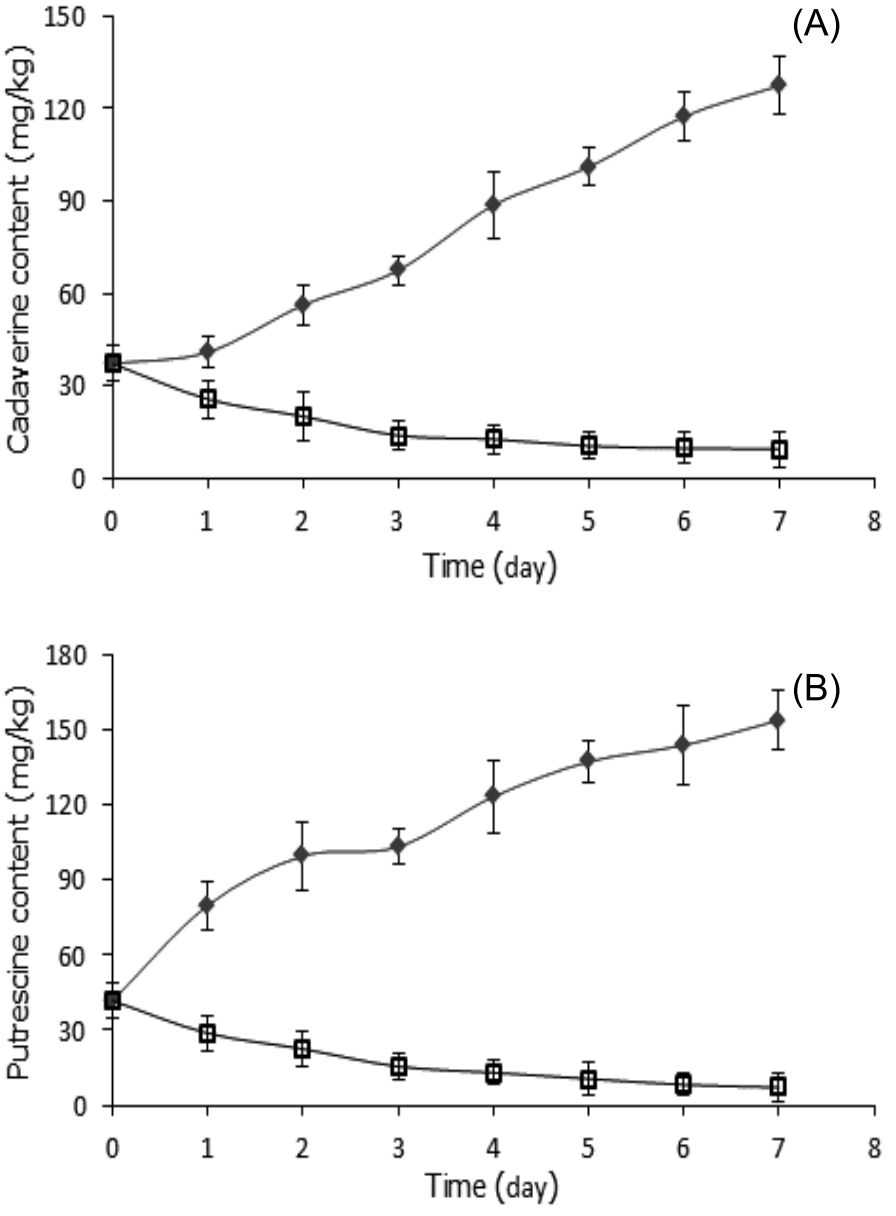
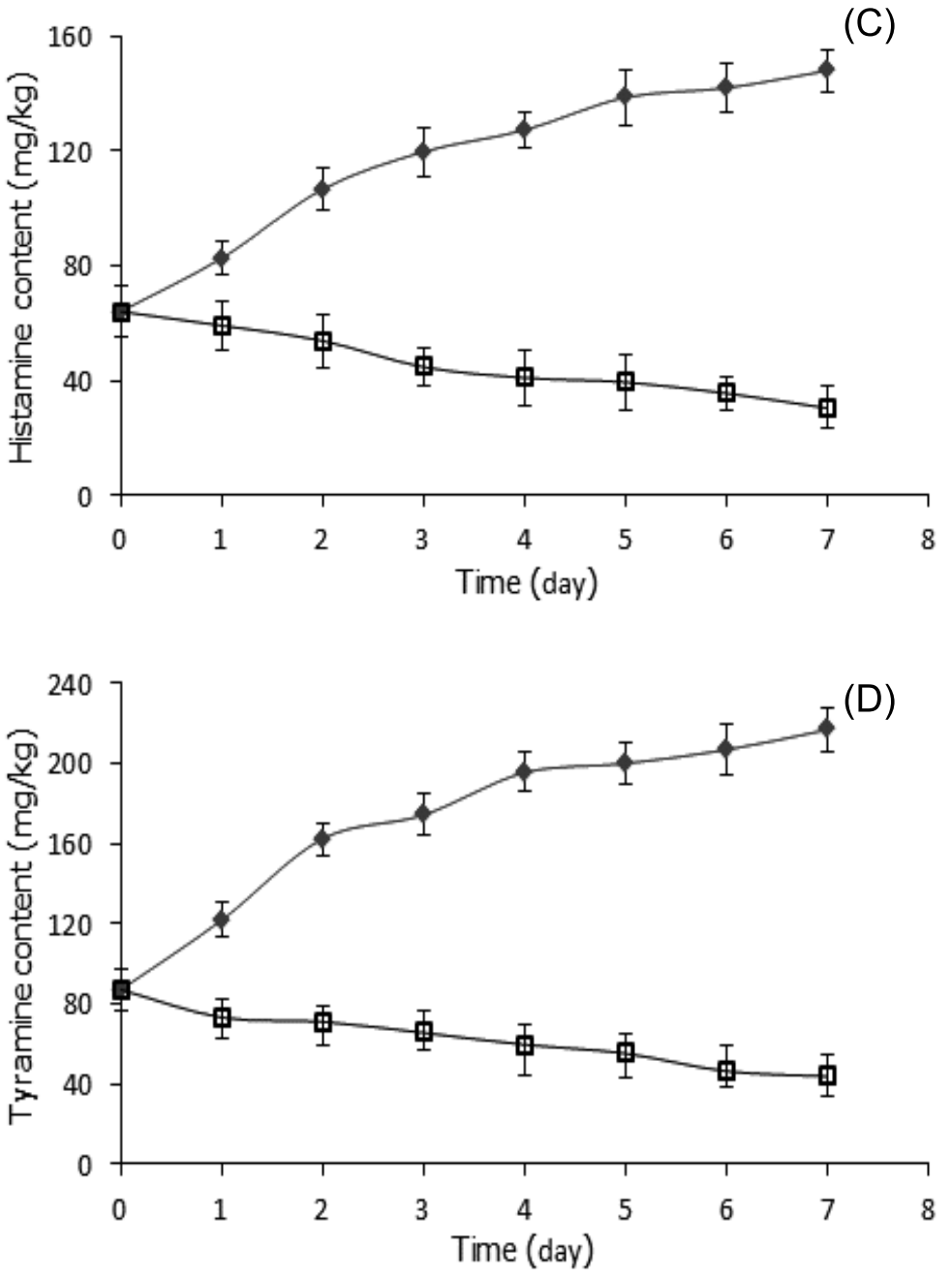


Figure 2 Biogenic amine profiles (n=3) during fermentation of pork at 30 °C for 7 days. (A): putrescine, (B): cadaverine, (C): histamine and (D): tyramine. ■: fermented pork added with ginger, ◆: fermented pork without ginger (control).



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This will consequently lead to the accumulation of BAs during pork fermentation when the environmental conditions were suitable for decarboxylase activity. Histamine has been found to be the most active amine; therefore, most of research on BAs has been focused on histamine. It was found that BAs concentration increased markedly during 7 days (Figure 2) of fermentation and therefore resulted in a high rise in the level of histamine within the same period (Figure 2 (C)). Ginger which had amine oxidase activity and it was used in this study, indigenous bacteria in fermentation process were deemed responsible for biogenic amine production. So, biogenic amine concentration was higher in control than samples added with ginger throughout the fermentation period. Pork contains high amount of tyramine. In this study, after 7 days of fermentation, tyramine concentration was 43.9 mg/kg (Figure 2 (C)) in samples with ginger, as compared to control samples (216.9 mg/kg). This proved that ginger could reduce histamine accumulation. It should be noted that histamine is quite resistant to degradation once it is formed in food products. Heat treatment such as autoclaving was found not to be effective to degrade histamine and other amines.[19]

In this study, amine oxidase activity in ginger could be estimated to about 34.3 U/g fresh wt according to previous study[9] and the total enzyme activity could be estimated about 6866 U in 1 kg of minced pork. So, amine oxidase could reduce the BAs in fermented food samples as shown in the mechanism of reaction (Equation 1).[5,10,11] After 7 days of fermentation, the overall BAs concentration (sum of putrescine, cadaverine, histamine and tyramine contents) was 91.1 mg/kg in samples added with ginger, as compared to control samples (645.9 mg/kg). This investigation emphasized that application of local plant with amine oxidase in pork fermentation was effective in inhibiting BAs accumulation. However, further investigation is needed to clarify the factors influencing BAs degradation by the amine oxidase in local plant so that optimal degradation can be obtained. The application of ginger amine oxidase in homogenized pork not only prevented the amine raise - more frequent in the homogenized texture of meat through bacterial action-but also reduced the already existent amine to the initial level. Accordingly, it is suggested that the ginger has the feasibility of amine reduction in foods. The antibiogenic amine properties assigned to ginger amine oxidase, elicits a novel enzymatic strategy for amine degradation in fermented pork. Its application in fermented pork is more practical because the preparation process is less time and energy consuming.

Conclusions

In this study, amount of BAs in Thai fermented pork samples of Nham made by homogenized fresh pork with and without ginger, under laboratory sanitized conditions was determined. It was found that Nham samples with ginger contained BAs less than Nham without ginger. Based on the this findings, it can be urged that an approach of using ginger for the production of good quality Nham could be recommended as a safer alternative for its commercialization. Hence, it is concluded that Nham samples produced in this study can be recommended for human consumption at commercial level. However, research is in progress on the development of more combinations of beneficial local plants as amine oxidase in order to further improve the quality and safety of Nham samples in terms of reduced contents of BAs.

Acknowledgements

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