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The Effects of Starter Cultures on Biogenic Amine and Free Amino Acid Contents in Nham during Fermentation

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ABSTRACT

Fermented pork sausage, or Nham, is a Thai-style fermented food which relies mainly on adventitious microorganisms, normally found in raw materials. The fermented foods usually contain biogenic amines produced by the microorganisms which caused the reaction of amino acids decarboxylation. These compounds are associated with toxicological symptoms. The objective of this study was to study the influence of two decarboxylase negative starter cultures in the presence of biogenic amines and free amino acid contents in Nham. Derivative biogenic amines by dansyl chloride were determined by high performance liquid chromatography (HPLC). Cadaverine and tyramine were determined during ripening process. The highest concentrations of biogenic amines were found at the end of the ripening process in the control sausage with no starter culture. Starter cultures test showed that *Lactobacillus sakei* BCC102 and *Debaryomyces hansenii* BCC 106 were efficient in reducing the amine production since these strains caused a quick pH drop during sausage fermentation. Total free amino acids after fermentation process decreased and the high decreases in the contents were glutamine and arginine while tyrosine and lysine, precursors for tyramine and cadaverine, respectively, increased in all batches. This study suggested that the use of decarboxylase negative lactic acid bacteria as starter cultures, which produced a rapid decrease on the pH of the meat mixture, was important factor to be considered to reduce the levels of biogenic amines in Nham.

Key words: starter culture, Nham, fermentation, biogenic amine, amino acid

INTRODUCTION

Biogenic amines (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 BAs (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 (MAOI) drug interactions. Moreover, diamines such as putrescine and cadaverine could generate carcinogenic nitrosamines in the presence of nitrites (Scanlan, 1983). Interest in cadaverine, putrescine, tyramine and histamine also lies in their

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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 aminopeptidases (Flores *et al*., 1996).

Nham is a Thai traditional fermented pork sausage. Nham fermentation generally takes 3-5 days. The microorganisms involved in the fermentation process can yield much higher BA amounts than those found in the corresponding raw materials, because some BAs are the result of amino acid decarboxylation by microbial enzymes. BAs may represent a food-poisoning hazard in conjunction with additional promoting factors such as MAOI antidepressant drugs, alcohol, other food amines, gastrointestinal diseases and genetically deficient detoxification systems (Vidal-Carou *et al*., 1990).

Meat fermentation offers favourable conditions for BA formation, since the main required factors are present, i.e. there is growth of microorganisms over several days, a certain degree of proteolysis takes place giving rise to the presence of free amino acids as precursors of BA and, finally, the existence of an acidic environment can favour the amino acid decarboxylase activity of microorganisms. It has been reported that bacteria could be encouraged to produce decarboxylase enzymes in such acidic environments as part of their defense mechanisms against adverse conditions (Eitenmiller *et al*., 1978). Since microbial flora naturally present in the raw materials seem to have a strong influence on BA formation during ripening, the choice of good quality raw materials helps to minimize the number of amine-producing bacteria (Halasz *et al*., 1994). An important factor suggested for preventing amine accumulation is the addition of adequate starter cultures to complete the fermentation. Starter cultures usually consist of one or several strains such as lactic acid bacteria (LAB). LAB are being widely used as starter cultures in fermented sausages. The absence of BA formation of LAB was proposed as a selection criterion for starter cultures (Buckenhuskes, 1993).

Proteolysis during the fermentation of meat products is favoured by the denaturation of proteins as a consequence of the acidity, dehydration and the action of sodium chloride (DeKetelaere *et al*., 1974). During the fermentation, production of free amino acids from proteolysis might occur. Therefore, the determination of free amino acid contents can be useful in studying the potential relationship between proteolysis and BA formation in fermented sausages.

The objectives of the present study were: (1) to study the changes in BA levels during the fermentation processes (2) to examine the effect on BA formation of *L. sakei* BCC 102 *and D. hansenii* BCC 106 which are nondecarboxylase activity used as starter cultures added naturally fermented Nham and (3) to determine the effects of starter culture on the formation of free amino acid during the fermentation of Nham (4) to compare the formation of BAs in control Nham (naturally fermented) and in Nham fermented with starter microorganisms.

MATERIALS AND METHODS

Microorganisms

Two starter culture strains (*Lactobacillus sakei* BCC 102 and *Debaryomyces hansenii* BCC 106) isolated from Nham were chosen after screening for nondecarboxylase activities. Both strains were gift from the Culture Collection Laboratory, National Center for Genetic Engineering and Biotechnology (BIOTEC), Patumthani, Thailand. Cultures were stored at -80°C in 20% glycerol. One loopful of a stock culture was cross-streaked on Man, Rogosa and Sharpe (MRS) agar and then incubated at 30°C for 48 h. A single colony on MRS agar was grown in MRS broth at 30°C for 18-24 h. Cell-free supernatants were obtained after centrifugation of

the cultures at 10,200 × g for 15 min at 4°C. The starter culture was prepared to obtain an approximate cell concentration of 10^7 CFU/ml in sterile deionized water.

Preparation of Nham

Nham 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. The ingredients were thoroughly mixed and divided into three fractions for three different batches, i.e. control or naturally fermented without the addition of starter cultures (NC) and batches I and II processed through a starter-mediated fermentation, with a single starter culture of 10^4 CFU/g *L. sakei* BCC 102 (LS batch) and mixed starter culture consisting 10^4 CFU/g *L. sakei* BCC 102 combined with 10^6 CFU/g *D. hansenii* BCC 106 (LSY batch), respectively. Approximately 200 g of Nham was stuffed into a plastic casing 3 cm diameter and sealed tightly prior to incubation at 30°C for 120 h. Samples were taken every 24 h for chemical and microbiological analysis.

pH Measurement

pH of Nham samples of 0-5 days of fermentation were measured directly using a microcomputerized pH meter (Mettler Teledo 320, UK; Mettler Toledo, Inlab® 427) by inserting the electrode into the centre of Nham and recorded as the mean value of three measurements.

Microbiological analysis

For microbial analysis, 25 g of Nham was aseptically removed from the casing, cut into small pieces, placed in sterile Stomacher bag and homogenized using a Stomacher (IUL Instrument, Spain) with 225 ml of sterile diluent containing 0.1% peptone. Serial decimal dilutions were prepared. LAB were enumerated on MRS agar adding with 0.5 % calcium carbonate and incubated anaerobically at 30°C for 48 h.

Biogenic amine determination

HPLC determinations were performed with a LC 10 AD Shimadzu LC using a 20 µl loop. Detection was at 254 nm with UV detector. LC column C18-Hypersil BDS (200 mm.× 4.6 mm, 5 µm particle size) was used. Amine standard solutions were prepared in water to a final concentration of 5 mg/ml for each biogenic amine. Tyramine, putrecine, cadaverine, tryptamine, phenylethylamine and histamine were used. Biogenic amine concentrations in the working standard solutions chosen for the calibration curve were 0.005, 0.01, 0.05, 0.1, 0.5 and 1 mg/mL. These working solutions were made by further dilution of the stock solution with water. Internal standard solution was prepared by diluting 15 mg of 1, 7-diaminoheptane in 5 ml of water. The gradient-elution system was methanol as solvent A and water as solvent B. The system was equilibrated for 5 min before the next analysis. The flow rate was 1.5 ml/min.

Sample preparation and extraction

Four grams of sample was mixed with 10 ml of 5% trichloroacetic acid and extracted using 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 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 (10 mg/ml 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 (Eerola *et al*., 1994).

Free amino acid (FAA)determination

Free amino acids were determined according to the method of Benjakul and Morrissey (1997) using an amino acid analyzer (Waters 2690 Alliance with 280 nm Fluorescent detector). The column was an AccQ-Tag™ C18, 4 µm. The solvent system consisted of three eluents: (A) AccQ Tag Eluent pH 5.02; (B) HPLC-grade acetonitrile and (C) Nanopure distilled water. The flow rate was set at 1.0 ml/min. Five g of Nham blend was mixed with 20 ml of 5% trichloroacetic acid, then stomachered at 200 rpm/min for 8 min and centrifuged at 12,000 × g for 15 min. A 100 µl of supernatant was mixed with 20 µl of 2.5 mM ABAA and 800 ml nanopure distilled water and filtered through 0.45 µm (Minisart RC4, Sartorious), then 10 µl aliquot of filtrate was transferred into a vial, and 70 µl of Waters AccQ Fluor borate buffer was added. A 20 µl of AccQ Fluor reagent was added and the mixture was incubated at 55°C for 10 min before HPLC analysis.

Statistical analyses

The differences between the results of physical, chemical and microbiological analysis of Nham fermented by different strains were tested using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

pH determination

Changes of pH in Nham during ripening are shown in Figure 1. The pH of the control sample and the batch with *L. sakei* and *D. hansenii* decreased after 4 h of incubation while that of batch with *L. sakei* decreased after 8 h of

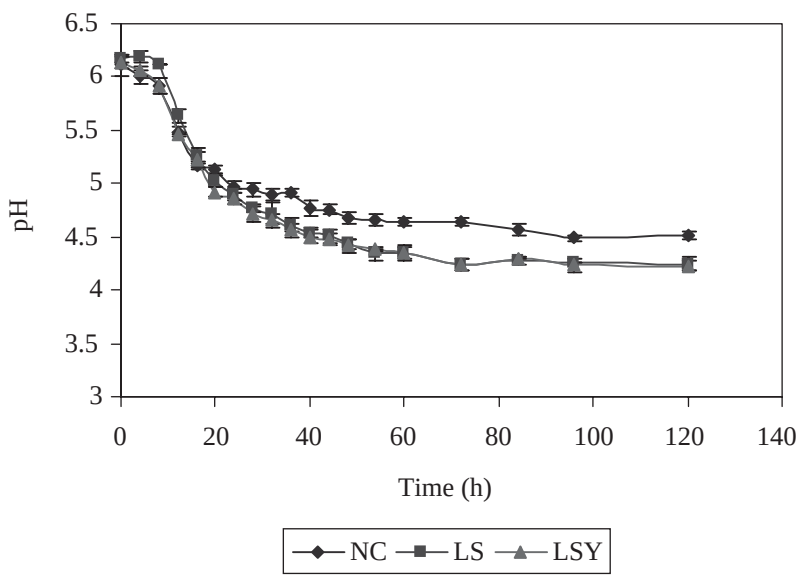

Figure 1 Changes in pH values during the ripening of Nham from different batches. Nham without added cultures (NC), Nham fermented with *L. sakei* BCC102 (LS), *L. sakei* BCC102 and *D. hansenii* BCC106 (LSY), for 120 h at 30°C (each data point represents the mean and standard deviation of three independent trials).

incubation and reached the final pH values of 4.5, 4.2, and 4.2 for the control, batch with *L. sake*i and batch with *L. sakei* and *D. hansenii*, respectively. The initial pH of all batches of Nham was pH 6.1, thereafter it decreased rapidly in the batch with starter cultures to pH 4.7, at 28 h. The pH of the control Nham was decreased to 4.9 at 28 h of fermentation. After 28 h, the pH in all batches gradually decreased throughout of incubation and pH values of the control slightly decreased less than that of the batches with starter cultures. The pH reduction during processing probably due to organic acid production by the inoculated starter cultures as well as the lactic flora. Statistical analysis of pH values recorded throughout ripening revealed significant differences between treatments at 24 h.

Microbiological analyses

Microbial counts increased in both the controls and starter added samples during fermentation (Figure 2). Initial lactic acid bacteria (LAB) counts in the starter added samples were higher than in the control samples. LAB counts increased during fermentation for both control and starter added samples. Initial counts of LAB (7.08 ± 0.24 log, 8.04 ± 0.32 log and 8.11 ± 0.21 log CFU/g for control, *L. sakei* added samples and mixed cultures of *L. sakei* and *D. hansenii* added samples, respectively) increased during fermenting, till the microorganism being at 9.48 ± 0.22 log, 9.45 ± 0.15 log and 9.66 ± 0.19 log CFU/g in the control, *L. sakei* added samples and mixed culture of *L. sakei* and *D. hansenii* added samples, respectively. This was an increase due to the environmental conditions which made gram-negative bacterial grow. After 16 h of fermentation, no significant differences were observed in total LAB counts in all batches. LAB increased during the ripening process, reaching maximum levels at day five in all types of sausages.

Changes in microbial counts in Nham inoculated with single starter culture of *L. sakei* and mixed starter cultures of *L. sakei* and *D.*

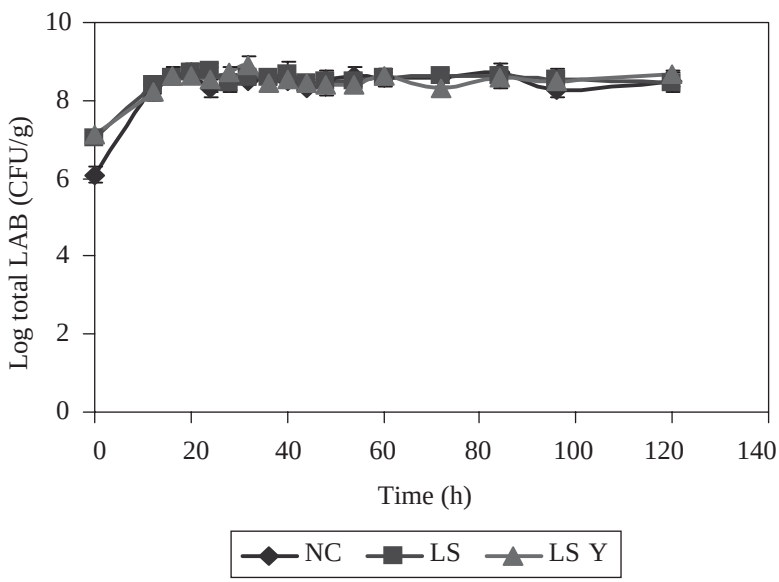

Figure 2 Changes in the population levels of LAB in Nham without added cultures (NC), Nham fermented with *L. sakei* BCC102 (LS), *L. sakei* BCC102 and *D. hansenii* BCC106 (LSY), for 120 h at 30°C (each data point represents the mean and standard deviation of three independent trials).

hansenii were similar to that of naturally fermented Nham (Figure 1). This microbial group rapidly increased after casing and reached the values of about 10^9 CFU/g in all the sausages, even in the samples to which starter cultures were not added. These high values remained relatively constant during ripening. Due to relatively high microbial load in Nham raw mix(10^7 CFU/g), inoculation of starter cultures at levels of 10^4 and 10^6 CFU/g had no significant effects on the LAB counts during fermentation. Similar to the results of Khieokhachee *et al*. (1997), initial flora of the Nham derived mainly from the raw materials. The number of LAB increased drastically to a maximum of 10^9 CFU/g within 18 h and remained the same until the fermentation was completed at 24 h for all batches . Thus, fermentation of Nham involving successive growth of different groups of microorganisms was dominated by LAB. Various metabolic products of LAB, such as short-chain organic acids, carbon dioxide, hydrogen peroxide, diacetyl, and bacteriocin, are known as antimicrobial agents (Rowan *et al*., 1998). Accumulation of organic acids also resulted in a decrease of pH. Thus, the dominance of LAB is likely to contribute to the inhibition of other microorganisms.

The addition of *L. sakei* and *D. hansenii* as starters might prevent the development of flora LAB that were present naturally in the initial mixture, and *L. sakei* might be able to dominate the whole ripening period in the batches while the control predominated with LAB flora.

Biogenic amines determination

Tyramine and cadaverine were present exclusively in 24 h fermented samples in typical quantitative sequence: cadaverine content was more than tyramine content (Table 1), other amines were not detectable. Both biogenic amines increased after 24 h of ripening until the final ripening of 120 h.

The differences between Nham elaborated with and without starter culture were observed, and the control Nham had significantly higher values of cadaverine and tyramine than the Nham inoculated with starter cultures. The *L. sakei* had significantly lower concentrations of biogenic amines than *L. sakei* and *D. hansenii*. Cadaverine and tyramine in fermented sausages were produced

Table 1 Biogenic amine contents (mg/kg) in Nham.

Time (h)	Control		*L. sakei*		*L. sakei* and *D. hansenii*	
	CAD	TYR	CAD	TYR	CAD	TYR
0	ND	ND	ND	ND	ND	ND
24	135.47 ± 14.85^{aA}	74.28 ± 5.71^{aB}	174.54 ± 18.22^{aC}	57.28 ± 7.96^{aD}	197.41 ± 16.34^{aF}	67.91 ± 7.14^{aG}
48	197.32 ± 18.92^{bA}	95.72 ± 8.42^{bB}	204.69 ± 23.85^{bA}	68.46 ± 4.85^{bC}	201.36 ± 14.89^{aA}	94.85 ± 5.54^{bB}
72	216.41 ± 21.56^{cA}	97.61 ± 7.48^{bB}	225.87 ± 19.51^{cC}	75.37 ± 6.29^{bcD}	223.52 ± 26.25^{bAC}	96.47 ± 8.20^{bB}
96	218.82 ± 24.63^{cA}	102.32 ±12.85^{bB}	218.34 ± 25.18^{cA}	77.16 ± 7.28^{cC}	227.28 ± 21.94^{bA}	104.62 ± 7.87^{bcB}
120	235.95 ± 17.44^{dA}	138.59 ± 14.74^{cB}	224.74 ± 21.82^{cC}	82.23 ± 5.45^{dD}	232.86 ± 25.17^{cA}	107.58 ± 6.41^{cE}

Mean values and standard deviations with different letters (a, b, c) in the same column indicate significant differences ($P<0.05$) during fermentation, and different letters (A, B, C) in the same row indicate significant differences ($P<0.05$) among Nham formula. (ND = Not detectable, CAD = cadaverine and TYR = tyramine)

by lysine- and tyrosine-decarboxylase activities, of *Enterobacteriaceae*, respectively. So, *L. sakei* and *D. hansenii* in Nham might inhibit the growth of *Enterobacteriaceae* resulting in decrease the lysine- and tyrosine-decarboxylase activity and biogenic formation (Bover-Cid *et al*., 2001b).

The main factors seemed to be a suitable starter culture and good quality raw materials (Bover-Cid *et al*., 2001a). However, in the present study, the high quality raw materials used were not effective in preventing the production of cadaverine and tyramine in control Nham (Table 1), and low contents of these amines were obtained only when a starter culture was included in sausage formulation.

In conclusion, to avoid the presence of high concentrations of biogenic amines in Nham, it was advisable to use a competitive starter culture such as *L. sakei*, a negative-decarboxylase strain, which might be predominant throughout the process, thus it would prevent the growth of bacteria which could produce biogenic amines.

Low occurrence of biogenic amines in raw pork meat: usually tyramine did not exceed a few mg kg^{-1} (tyramine less than 3.5 mg kg^{-1} as observed by Hernandez-Jover *et al*., 1997) while ripened and cured meat showed a general increase of amines (Bover-Cid *et al*., 1999). The choice of starters could be useful tool to control and reduce the development of some *Enterobacteriaceae* strains. However, the presence of biogenic amines in ripened dry uncooked fermented meat was fundamentally a consequence of the activity of decarboxylase-positive strains of *Lactobacillaceae* and *Enterococcaceae*.

Biogenic amines content depended also on an equilibrium between the decarboxylating and amine oxidizing activity of microflora (Gardini *et al*., 2002).

Therefore, to obtain Nham with low amine concentrations besides the high quality raw materials and good manufacturing practices, it is necessary to employ highly competitive amino acids decarboxylase negative starters cultures and the starter culture should be able to compete and grow well at the temperature intended for processing of the product (Maijala *et al*., 1995).

Analysis of FAA

The main differences in the content of total FAA among batches were detected at the end of the processing (5 days), where lower quantities were detected in all batches. From Table 2, after 5 days of incubation at 30°C, NC caused decrease of 36.8 % in total FAA while Nham with *L. sakei* and the Nham with mixed cultures of *L. sakei* and *D. hananii* caused decrease of 13.3% and 19.73%, respectively, in the concentration of FAA. The total FAA of Nham with starter culture was higher than that of the control (Table 2).

This suggested that the starter cultures batches might have higher proteolytic activity than the non-inoculated control batch and /or catabolized free amino acids to be the other products such as biogenic amines less than the control due to the batches added with starters lagged of amino acid decarboxylase.

Free amino acids precursors of biogenic amines were detected by HPLC. During fermentation step, the increases of tyrosine and lysine which were the precursors of tyramine and cadaverine, respectively were obsereved in all batches. However, after 5 days of ripening, the concentrations of tyrosine and lysine in Nham with starter cultures were more than that of the control, while the biogenic amines, tyramine and cadaverine in the control were more than that of the batches with starter cultures.

Evaluation of FAA during the ripening of fermented Nham sausages indicated an increase in most FAA over the 0-5 day fermentation period.

The main changes observed in the decrease of free amino acids at the end of processing showed a higher decrease proportion of glutamic acid and arginine in the control than that of the batch with *L. sakei* and the batch with

L. sakei and *D. hansenii* after 5 days of ripening. The decreases in glutamic acid and arginine contents might be due to these amino acids were used for the growth of the microorganisms and might be metabolized to flavours. The quantities of alanine, aspartic acid, glycine, isoleucine, leucine, methionine, phenylalanine, tyrosine, valine and lysine in the control after 5 days of ripening were higher than those before ripening. Some of these amino acids in the batches with *L. sakei* and the batches with *L. sakei* and *D. hansenii* also increased after ripening.

Some amino acids, especially those branched-chain amino acids, have been metabolised to generate volatile compounds (Dura *et al*., 2004). The contents of alanine, isoleucine, histidine and proline were similar in the three batches at the end of processing. Alanine, contributors of sweet taste was found in higher contents after ripening of fermented sausages. Therefore, the balance of these free amino acids would affect the sensory characteristics of the product (Ordonez *et al*., 1999). The addition of starter culture produced a limited effect on the free amino acid generation although the effect was different depending on the quantity of microorganisms inoculated. Many factors could affect the generation of free amino acids such as the presence of different substrates, the pH, the presence of different microorganisms and their evolution during processing. The significant ($P < 0.05$) reduction in the concentration of free amino acids could be produced by a more intense microorganism metabolism than their production

Table 2 Total and free amino acid contents (mg/100 g) in Nham during fermentation.

Amino acid	Starter culture					
	NC, 0 h	NC, 120 h	*L. sakei* 0 h	*L. sakei* 120 h	*L. sakei and D. hansenii* 0 h	*L. sakei and D. hansenii* 120 h
Ala	1.59 ± 0.16^{a}	3.13 ± 0.29^{b}	1.98 ± 0.04^{c}	3.59 ± 0.12^{b}	1.96 ± 0.05^{c}	3.15 ± 0.36^{b}
Asp	0.28 ± 0.04^{a}	0.49 ± 0.04^{b}	0.27 ± 0.01^{a}	0.81 ± 0.10^{c}	0.25 ± 0.01^{a}	0.76 ± 0.03^{c}
Gly	0.84 ± 0.10^{a}	1.47 ± 0.07^{b}	0.95 ± 0.02^{c}	1.87 ± 0.18^{d}	0.93 ± 0.01^{c}	1.69 ± 0.08^{d}
Ile	0.14 ± 0.01^{a}	0.51 ± 0.04^{b}	0.16 ± 0.00^{a}	0.59 ± 0.04^{b}	0.16 ± 0.00^{a}	0.56 ± 0.02^{b}
Leu	0.22 ± 0.01^{a}	1.35 ± 0.13^{b}	0.23 ± 0.00^{a}	0.11 ± 0.01^{c}	0.26 ± 0.01^{a}	1.70 ± 0.07^{d}
Met	0.04 ± 0.02^{a}	0.39 ± 0.03^{b}	0.61 ± 0.01^{c}	0.53 ± 0.03^{c}	0.05 ± 0.00^{a}	0.49 ± 0.02^{c}
Phe	0.10 ± 0.01^{a}	0.55 ± 0.04^{b}	0.12 ± 0.01^{a}	0.73 ± 0.04^{c}	0.12 ± 0.01^{a}	0.61 ± 0.03^{c}
Tyr	ND	0.14 ± 0.01^{a}	0.14 ± 0.01^{a}	0.29 ± 0.03^{b}	0.15 ± 0.01^{a}	0.17 ± 0.01^{a}
Val	0.27 ± 0.02^{a}	0.72 ± 0.04^{b}	0.33 ± 0.01^{a}	0.24 ± 0.02^{a}	0.33 ± 0.00^{a}	0.66 ± 0.05^{b}
Arg	12.89 ± 1.71^{a}	3.66 ± 0.51^{b}	13.63 ± 0.28^{c}	7.71 ± 0.04^{c}	13.66 ± 0.06^{d}	5.93 ± 0.20^{e}
Cys	1.26 ± 0.20^{a}	0.32 ± 0.07^{b}	1.45 ± 0.05^{c}	1.43 ± 0.05^{c}	1.49 ± 0.03^{c}	1.34 ± 0.04^{a}
Glu	7.65 ± 0.99^{a}	2.57 ± 0.49^{b}	8.62 ± 0.27^{c}	5.37 ± 0.39^{d}	7.57 ± 0.04^{a}	4.37 ± 0.15^{e}
His	0.79 ± 0.11^{a}	0.40 ± 0.05^{b}	0.93 ± 0.02^{a}	0.53 ± 0.02^{c}	0.93 ± 0.01^{a}	0.44 ± 0.05^{b}
Ser	0.96 ± 0.16^{a}	0.40 ± 0.12^{b}	1.08 ± 0.02^{a}	0.67 ± 0.09^{c}	1.08 ± 0.02^{a}	0.40 ± 0.09^{b}
Lys	0.55 ± 0.07^{a}	1.01 ± 0.05^{b}	0.61 ± 0.00^{c}	1.48 ± 0.02^{d}	0.62 ± 0.01^{c}	1.34 ± 0.04^{d}
Pro	0.28 ± 0.04^{a}	0.22 ± 0.01^{a}	0.29 ± 0.01^{a}	0.33 ± 0.02^{ab}	0.37 ± 0.01^{b}	0.28 ± 0.01^{a}
Thr	0.98 ± 0.13^{a}	0.28 ± 0.01^{b}	1.05 ± 0.02^{a}	1.15 ± 0.02^{c}	1.04 ± 0.00^{a}	0.99 ± 0.05^{a}
Total	28.84 ± 3.66^{a}	17.59 ± 1.79^{b}	31.83 ± 0.71^{a}	27.43 ± 0.90^{a}	30.97 ± 0.20^{a}	24.86 ± 1.13^{c}

Mean values and standard deviations with different letters (a, b, c) in the same column indicate significant differences ($P<0.05$) during fermentation among Nham formula. (ND = Not detectable)

during the stages of ripening as suggested by Hughes *et al*. (2002) and Ordonez *et al*. (1999). The changes of free amino acid contents represented the degradation of protein and the conversion of these free amino acids to the other compounds such as biogenic amines and flavours as well as growth metabolism of the microorganisms.

In conclusion, this study determined the effect of the starter cultures on biogenic amine formation in fermented Nham sausages. In addition to these, amino acid contents were analyzed to note changes of amino acids in Nham sausages. The starter culture, *L. sakei* BCC102, decreased pH quickly and suppressed the accumulations of tyramine. To avoid the formation of high concentration of biogenic amine in Nham, it is advisable to inoculate starter culture with negative-decarboxylate activity such as *L. sakei* BCC102 and use to top-quality raw meat materials for the manufactured food products.

CONCLUSIONS

The production of biogenic amines is dependent of several variables, such as the growth of the microorganisms, their proteolytic and decarboxylase activities, which interact with each other. Furthermore, there is not a univocal rule linking these variables with the different metabolic mechanisms necessary for the formation of biogenic amines. The results indicated that inoculation of starter cultures with decarboxylase negative should be carried out to initiate fermentation process. Inoculation with appropriate starter may lead to the decrease of biogenic amine as fermentation progressed. This study suggests that the use of *L. sakei* as starter culture was effective to reduce the accumulation of biogenic amine; cadaverine, during the ripening of fermented Nham.

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