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Original research paper

EFFECTS OF PURE AND COMMERCIAL PROBIOTIC CULTURES ON GAMMA-AMINOBUTYRIC ACID (GABA) FORMATION AND QUALITY ATTRIBUTES OF THAI FERMENTED PORK SAUSAGE (NHAM)

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Abstract: This study investigated the effects of pure and commercial probiotic starter cultures on gamma-aminobutyric acid (GABA) formation and key quality attributes of Thai fermented pork sausage (Nham). Four treatments were prepared: a control without inoculation, a commercial probiotic culture (Starter V3), *Lactobacillus plantarum* TISTR 543, and *Lactococcus lactis* subsp. *lactis* TISTR 1520, with all cultures immobilized on cooked germinated rough rice. Physicochemical properties, including pH, total acidity, and water activity, as well as color, texture, GABA content, and microbial quality, were evaluated during fermentation at 30 °C and during storage at 4 °C. The results showed that probiotic starter cultures significantly enhanced GABA production, with Starter V3 yielding the highest levels, followed by *L. plantarum* TISTR 543. Probiotic inoculation accelerated acidification, resulting in consistently lower pH values and higher total acidity compared to the control, indicating more efficient fermentation. Lactic acid bacteria counts increased markedly during fermentation and remained high during storage, with *L. plantarum* TISTR 543 showing the strongest survival. Textural properties, particularly hardness and gumminess, improved in probiotic-supplemented samples, reflecting enhanced gel formation associated with protein and acid interactions. Although color attributes differed among treatments on the first day of fermentation, these differences diminished thereafter. All treatments met microbiological safety standards throughout fermentation and storage, with no detection of *Staphylococcus aureus, Salmonella* spp., or *Escherichia coli*. Overall, the findings confirm that probiotic starter cultures, especially Starter V3 and *L. plantarum* TISTR 543, can improve fermentation performance, enhance GABA accumulation, and support the development of safe, high-quality Nham with functional health-related potential.

Key words: *Nham, probiotic, Starter V3, Lactobacillus plantarum TISTR 543, Lactococcus lactis subsp. lactis TISTR 1520*

INTRODUCTION

Nham is a Thai-style fermented pork sausage and the most popular fermented meat product in Thailand. It is usually consumed without cooking. The sausage mix normally contains raw minced pork, cooked pork rind, cooked rice, curing salt, garlic, chili, and is tightly packed into pork intestines or other casings. After stuffing, the sausage is left to ferment at room temperature in a dry place for 2-5 days until it becomes acidified by the lactic acid bacteria (LAB) such as lactobacilli and pediococci present in the raw materials. In terms of safety in

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consuming the product, the pH at the time of consumption should be no higher than 4.6, thereby preventing the growth of foodborne bacterial pathogens such as *Staphylococcus aureus, Salmonella* spp., and *Listeria monocytogenes* (Santiyanont et al., 2019). Consuming raw sausage of poor quality and contaminated foodborne bacterial pathogens poses a safety risk. According to a report by Suksai and Chowwanapoonpohn (2021), some manufacturers' production of Nham was found to be contaminated with these pathogens. However, if improperly handled, the production of fermented meat products with naturally occurring LAB often results in inconsistent qualities and unsafe outcomes. Furthermore, if the concentration of LAB is inadequate, the fermentation will be incomplete, leading to a lack of sourness, crumbly texture, or spoilage caused by undesirable microorganisms. To improve consistency in fermentation, the development of controlled fermentation processes with starter cultures from defined LAB components is necessary to provide safe, consistent products and ensure the standardization of product properties.

Starter cultures are used primarily to reduce fermentation time by accelerating the rate of pH reduction for inhibiting the growth of food-spoiling and pathogenic bacteria, which helps lower the risk of food contamination while still maintaining the desired flavors of the product (Laranjo, Potes & Elias, 2019). The predominant LABs found in Nham are lactobacilli, pediococci and lactococci (Pakwan et al., 2020).

These LAB's can rapidly produce lactic acid and are considered microbially safe. Some strains, such as *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Lactococcus lactis*, can produce bacteriocins that inhibit pathogenic microorganisms, helping to prevent food spoilage (Woraprayote et al., 2016). The three strains of LAB are also classified as probiotic bacteria (Swetwiwathana & Visessanguan, 2015; Woo, Hyun, Jang, Lee & Paik, 2024), which are a group of microorganisms that confer health benefits on the host. Probiotics play a significant role in protecting the host against harmful microorganisms and in strengthening the immune system. Numerous studies on gut microflora suggest that probiotic LAB from food can colonize the intestine and thus play a crucial role in its proper functioning (Neffe-Skocińska, Okoń, Kołożyn-Krajewska & Dolatowski, 2017).

Therefore, the three strains of probiotic bacteria can be used as starter cultures in the production of Nham. The amount of starter culture used must be appropriate to ensure complete fermentation activity. The Nham product should contain at least 10^7 CFU/g of probiotic microorganisms to be considered a probiotic, or a product that can provide health benefits (Meybodi & Mortazavian, 2017). In this study, the researchers aimed to improve the quality of Nham by utilizing probiotic bacterial starter cultures, including *L. plantarum* TISTR 543, *L. lactis* subsp. *lactis* TISTR 1520, and a commercial starter culture (Starter V3, containing *P. acidilactici* and *Staphylococcus xylosus*) to produce probiotic Nham. It was found that the use of pure strains of certain LAB as starter cultures in Nham production has not been extensively studied, particularly in the case of lactococci. The starter cultures used in this research were immobilized prior to use. Microbial cell immobilization is a technique that enhances both the concentration of microorganisms and their survival during the fermentation process (Xiudong, Ying, Xiaoli, Ying & Jianzhong, 2016). Cooked germinated rough rice can be used as a material for microbial cell immobilization. Due to this, it can serve as a carbohydrate source for LAB, which plays a significant role in fermentation processes. In Nham production, cooked rice is typically used as an ingredient, making microbial cells immobilized on germinated rough rice suitable as a starter culture. Furthermore, germinated rough rice is used as a material for microbial cell immobilization to enhance the gamma-aminobutyric acid (GABA) content in the product. GABA is a neurotransmitter known for its inhibitory effects, promoting relaxation and improving sleep quality. According to Jabeen et al. (2024), germinated rough rice processed under optimal temperature and time conditions contains a relatively high amount of GABA. Additionally, GABA can be produced by LAB during fermentation. Research by Redruello et al. (2021) and Krongkeha (2022) indicates that strains of *L. plantarum* and *L. lactis* can produce GABA during fermentation, potentially increasing the GABA content in the resulting Nham product when these starter cultures are used. However, limited information is available on the ability of pure and commercial

probiotic cultures to enhance GABA production in Nham. Previous studies have mainly focused on GABA formation in dairy or plant-based fermented products, while research on meat-based fermentation, particularly using probiotic starter cultures, remains scarce. Moreover, the comparative effects of pure versus commercial probiotic strains on both GABA synthesis and the overall quality attributes of Nham have not yet been systematically investigated.

Therefore, this study aims to fill this research gap by evaluating the effects of pure and commercial probiotic cultures on GABA formation, physicochemical characteristics, and microbial quality of Nham.

MATERIALS AND METHODS

Materials

Fresh pork meat, pork rind, fresh garlic, fresh chili, salt, and monosodium glutamate (MSG) were obtained from a local grocery store. A mixed powder of phosphate and sodium nitrite (Super N) was procured from Vicchi Enterprise Co., Ltd. All other reagents used in this study were of research-grade quality. Additionally, chromatography-grade reagents were employed for all substances analyzed via high-performance liquid chromatography (HPLC).

Preparation of germinated brown rice

Jasmine rice (*Oryza sativa* L. cv. KDML 105), cultivated in Maha Sarakham province, Thailand, was dehulled and germinated using a modified method as reported by Chen and Chen (2009). The rice grains were soaked in water at 7 °C for 6 h. The soaked grains were then placed on eight layers of cheesecloth saturated with a 150 mL solution containing 1 g of calcium chloride and 6 g of monosodium glutamate. The grains were placed in a partially sealed plastic container and germinated at 37 °C for 4 days. Following germination, the sprouted grains were dried at 40 °C to a constant weight.

Preparation of starter culture

In this study, two probiotic bacterial strains, *L. plantarum* TISTR 543 and *L. lactis* subsp. *lactis* TISTR 1520, were employed. These strains, originally isolated from Nham, were obtained as lyophilized cultures from the Thailand Institute of Scientific and Technological Research. Prior to application, individual colonies were isolated via cross streak on MRS agar (Himedia™, India) and subsequently sub-cultured in MRS broth (Himedia™, India) for 48 h. *L. plantarum* TISTR 543 was incubated at 30 °C, while *L. lactis* subsp. *lactis* TISTR 1520 was incubated at 37 °C. Cultures were stored at 4 °C and activated by sub-cultured twice before use.

For the preparation of starter cultures, *L. plantarum* TISTR 543 and *L. lactis* subsp. *lactis* TISTR 1520 were separately inoculated into 60 mL of MRS broth at a 5% (v/v) concentration. The cultures were incubated for 48 h under optimal conditions, achieving a final bacterial concentration of approximately 10^9 CFU/mL. The cells were centrifuged at 13,000 rpm for 1 min. The pellets were then washed twice with sterile saline solution (0.85% w/v) and subsequently resuspended in 1 mL of the same solution. The prepared bacterial cells were then utilized as immobilized cells within support matrices.

In addition, this study utilized a commercial Nham starter culture, Starter V3, which was kindly provided by Vicchi Enterprise Co., Ltd. The starter culture was supplied in the form of bacterial cells suspended in a solution and contained the probiotic bacteria *P. acidilactici* and *S. xylosus*. The culture was stored at 4 °C until further use.

Preparation of immobilized cells

The immobilization of cells within germinated brown rice was adapted from the method of Phuapaiboon, Leenanon and Levin (2013). Germinated brown rice was cooked using a rice cooker at a ratio of 130 g of rice to 325 mL of water. The cooked brown rice was allowed to cool and then finely ground. Subsequently, the rice was mixed with each bacterial starter culture at a ratio of 37.5 g of rice to 1 mL of starter culture. The mixtures were incubated for 3 h in an incubator, with *L. plantarum* TISTR 543 incubated at 30 °C, and *L. lactis* subsp. *lactis* TISTR 1520, along with the commercial starter culture, incubated at 37 °C. After incubation, the immobilized cells within the germinated brown rice were used as starter cultures for Nham production. For the control group, finely ground germinated brown rice without immobilized cells was used as an ingredient in Nham production.

Producing Nham

Nham was produced according to the traditional Thai fermentation process. Briefly, lean pork was minced and mixed with cooked rice, garlic, salt, and cooked pork rind. The mixture was then stuffed into casings, tightly wrapped, and fermented at ambient temperature for several days until a characteristic sour flavor and texture was developed (Visessanguan, Benjakul, Riebroy & Thepkasikul, 2004; Visessanguan et al., 2006). The production was conducted through four distinct treatments to simulate the variability of inoculation. Treatment 1 (T1) involved Nham produced through natural fermentation without the addition of starter culture (control). Treatment 2 (T2) utilized a commercial starter culture (Starter V3), Treatment 3 (T3) used *L. plantarum* TISTR 543, and Treatment 4 (T4) incorporated *L. lactis* subsp. *lactis* TISTR 1520 as a starter culture to produce Nham. For each treatment, ground pork hip (53%) was kneaded with Super N (0.75%) to achieve a cohesive texture. Salt (1%) and MSG (0.3%) were added, followed by cooked germinated brown rice or probiotic bacteria immobilized within cooked germinated brown rice (3.85%), ensuring thorough integration through kneading. Finely crushed garlic (4.2%) was then added and kneaded into the mixture, followed by cooked, thinly sliced pork rind (34.9%). Lastly, whole bird chilies (2%) were incorporated into the blend. The mixture was extruded through a stuffing horn into polyethylene casings (3 cm in diameter, approximately 200 g each) and tightly sealed.

Nham sausages were fermented at 30 °C for 3 days, after which the samples were stored at 4 °C for 27 days. Samples of sausages were randomly collected for GABA determination, physicochemical characterization, and microbiological analysis during fermentation (days 1 and 3) and subsequent storage (days 4 and 27).

Proximate analysis

Proximate compositions of samples including moisture contents, crude protein, crude fat, crude fiber and ash content were measured according to the AOAC (2000) methods. Moisture contents were measured using drying samples at 105 °C. Crude protein was measured by the Kjeldahl method and multiplying by factor 6.25. Crude fat was measured by the Soxhlet method with petroleum ether as a solvent and extracted for 3 h. Crude fiber was measured by the Weende method, and ash content were measured using sample mineralization at 550 °C.

Determination of pH, titratable acidity (TA) and water activity (a_w)

The pH and TA of samples was measured following the method of AOAC (2000). Briefly, 10 g of sample was soaked in 90 mL of deionized water. The mixture was blended in a laboratory blender for 1 min. The filtered solution was used for measurement with a pH meter (Sartorius, series Docu-pH+ Meter, Sartorius AG, Germany). TA was determined as a percentage of lactic acid by titrating with 0.1 N NaOH, using phenolphthalein as an indicator. Water activity (a_w) was measured using a water activity analyzer (Aqualab, series 3TE, De-cagon Devices Inc., USA).

GABA analysis

GABA was extracted from the sample as described by Komatsuzaki et al. (2007), with a slight modification. Briefly 5 g of the sample were extracted by a vigorous mix with a vortex mixer for 1 min with 25 mL of 70% ethanol. The extracts were centrifuged at 13,000 rpm for 2 min and the supernatant was collected. The same volume of 70% ethanol solution was added and the extraction was repeated twice. The collected supernatant was dried in a vacuum evaporator at 70 °C. Then, the residue was dissolved using 25 mL of 70% ethanol and a centrifuge at 13,000 rpm for 2 min. The GABA content of samples was analyzed using HPLC as described by Phuapaiboon, Leenanon and Levin (2013).

Color evaluation

The color of the samples was measured in the *CIELAB* color space (L*, a*, b*) using a ColorFlex EZ 45°/0° spectrophotometer (HunterLab, USA). The instrument was first standardized with a white and black calibration plate. Five measurements of surface reflectance, expressed as L* (lightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) values were performed on the slices of sausages with a thickness of approximately 2 cm and averaged.

Texture profile analysis (TPA)

TPA measurements were carried out using a TA.XTplus texture analyzer (Stable Micro

System, Godalming Surrey, UK) with a cylindrical aluminium probe P/36R (36 mm diameter) and were applied to determine texture parameters, including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience of samples. The sausages were cut into five cubes (20 mm high) after discarding the external layer and placed on the instrument's base. TPA parameters were measured at room temperature with the following testing conditions: pretest speed 5.0 mm/s, test speed 5.0 mm/s, post-test speed 5.0 mm/s, 40% strain, time 5 s. The Texture Exponent 32 software was used to collect and process the data. TPA analyses were defined and calculated as previously described by Bourne (1978).

Microbiology analysis

Samples of Nham (25 g) were aseptically placed in a sterile stomacher bag (Seward Stomacher® bags) and pummelled for 1 min in a stomacher (Seward Stomacher® 400 circulator), with 225 mL of 0.1% sterile peptone water. Serial dilutions were then made. Appropriate decimal dilutions of the samples were prepared using the same diluent and 0.1 mL aliquot of each dilution was plated in triplicate on different growth media. LAB was counted on MRS agar incubated at 37 °C for 48 h. Total viable counts were determined by plating samples on standard plate count agar (Oxoid™, UK) at 35 °C for 48 h. Yeast and mold were numbered on potato dextrose agar (Himedia™, India) adjusting to pH 3.5 by a sterile solution of tartaric acid (10%) and incubated at 25 °C for 5 days. *Staphylococcus aureus* was enumerated on a selective medium Baird Parker agar, supplemented with egg yolk tellurite emulsion (Himedia™, India), and incubated at 37 °C for 48 h. The microbial counts were expressed as colony forming units per gram of sample (CFU/g). For detection of *Salmonella* spp., a modified BAM method based on Andrews, Jacobson and Hammack (2014) was used. Salmonella had to report the final confirmed results of the samples by indicating if Salmonella was 'detected' or 'not detected' per 25 g. The presence of *Escherichia coli* was determined using the Most Probable Number (MPN) technique. Identification of *E. coli* isolates was subsequently confirmed by a series of IMViC biochemical tests. This procedure was conducted in accordance with the protocol described by Andrews (1992), providing an estimate of the MPN of *E. coli* in the samples analyzed.

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as means ± standard deviations (SD). The proximate analysis was conducted using a completely randomized design (CRD). The experiments for GABA, physicochemical, and microbiological analyses were arranged in a completely randomized factorial design (CRD factorial) with two factors (culture × time). Data were analyzed by analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test at a significance level of $p \leq 0.05$. Statistical analyses were carried out using SPSS software version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate composition of Nham

The proximate composition of Nham after 3 days of fermentation is presented in Table 1. Moisture content ranged from 72.34% to 73.24%, with the *L. lactis* subsp. *lactis* TISTR 1520 treatment (T4) showed the highest value ($p \leq 0.05$). The increase in moisture content could be attributed to the metabolic activity of LAB, which may cause protein denaturation and the release of bound water during fermentation (Zheng et al., 2025). Protein content varied from 19.27% to 21.71%, with the Starter V3 sample (T2) exhibiting the highest level, possibly due to enhanced proteolytic activity of the mixed starter culture, resulting in the breakdown of complex proteins into peptides and amino acids (Visessanguan et al., 2006).

In contrast, the lowest protein content was found in T4, possibly due to higher microbial utilization of nitrogen compounds for growth and metabolism. Crude fat and fiber contents significantly decreased in inoculated samples, which may be attributed to microbial lipolysis and degradation of fibrous components (Visessanguan et al., 2006; Hwang et al., 2023). Ash content slightly decreased in T4, while carbohydrate content increased in T3 and T4, likely due to microbial enzymatic activity releasing fermentable sugars. Overall, inoculation with probiotic starter cultures influenced the proximate composition of Nham, indicating that

Table 1.
Proximate composition of Nham samples on day 3 of fermentation

Parameters	T1	T2	T3	T4
Moisture (%)	72.34±0.60^{b}	72.99±0.09^{ab}	72.43±0.50^{b}	73.24±0.02^{a}
Crude protein (%)	20.24±1.60^{ab}	21.71±0.81^{a}	20.39±1.35^{ab}	19.27±0.57^{b}
Crude fat (%)	1.27±0.12^{a}	0.85±0.02^{c}	0.97±0.00^{c}	1.07±0.02^{b}
Crude fibers (%)	0.96±0.01^{a}	0.71±0.06^{b}	0.54±0.00^{c}	0.60±0.02^{c}
Ash (%)	2.38±0.07^{a}	2.32±0.01^{a}	2.29±0.11^{a}	2.11±0.04^{b}
Carbohydrate (%)	2.81±0.17^{b}	1.41±0.13^{c}	3.38±0.26^{a}	3.71±0.12^{a}

T1 = control; T2 = inoculated with Starter V3; T3 = inoculated with L. plantarum TISTR 543; T4 = inoculated with L. lactis subsp. lactis TISTR 1520. Mean values within a row with different letters are significantly different ($p \leq 0.05$)

LAB metabolism affected nutrient distribution during fermentation (Leroy & De Vuyst, 2004).

Change in pH, TA and a_w

The changes in pH, TA and a_w of Nham samples during fermentation and storage are shown in Figure 1. A pronounced decrease in pH was observed in all Nham treatments during fermentation, followed by a slight reduction during storage. This trend is mainly associated with the accumulation of organic acids produced by lactic acid bacteria during fermentation, resulting in increased acidity of the fermented sausages (Tangwatcharin, Nithisantawakhup & Sorapukdee, 2020). The pH values of all samples fermented on day 1 and day 3 ranged from 4.68 to 5.13 and 4.27 to 4.56, respectively. After subsequent storage at 4 °C on day 4 and day 27, the pH values of all samples ranged from 4.40 to 4.55 and 4.28 to 4.34, respectively. On day 1 of fermentation, Nham inoculated with *L. plantarum* TISTR 543 (T3) exhibited the lowest pH value, which was significantly different from the other treatments. This is consistent with the study by Van Ba et al. (2018), which reported that the highest pH drop rate was observed during the fermentation of sausages with *L. plantarum* at 30 °C.

However, on day 3 of fermentation, the T2 Nham sample had the lowest pH value, also significantly different from the other treatments. Therefore, the T3 and T2 samples presented a stronger acidifying ability. It is plausible since the presence of starter cultures drives the fermentation process. Additionally, on day 4 of storage, no significant differences in pH were observed among the treatments, which was also the case on day 27 of storage. Storage for four days did not significantly alter the pH compared to the pH values observed on day 3 of fermentation. However, on day 27 of storage, the pH values of all samples decreased significantly compared to their respective values on day 3. Since Nham is normally consumed without cooking, the Thai Industrial Standards Institute (TISI) (2004) recommends that a rapid decrease to an acidic pH (≤4.6 at the end of fermentation) be achieved to ensure product safety and shelf life by inhibiting pathogenic microorganisms naturally present in pork. Zhang et al. (2023) reported that lower pH could inhibit the growth of spoilage bacteria for improving product safety, promote the decomposition of nitrite, and reduce the generation of N-nitrosamines in fermented sausages. Based on the study results, the pH values of Nham samples on day 3 of fermentation and during refrigerated storage on days 4 and 27 in all treatments were found to be low enough. According to Visessanguan, Benjakul, Panya, Kittikun and Assavanig (2005), Nham fermentation generally takes 3 to 5 days at room temperature (30 °C), during which Nham usually has a pH ranging between 4.4 and 4.8.

The values of TA showed an inverse relationship to pH values during fermentation and storage. On day 1 of fermentation, the lactic acid content in all treatments ranged between 0.29% and 0.42%, with T3 Nham exhibiting the highest lactic acid concentration, which was significantly different from the other three treatments. This finding indicates that *L. plantarum* TISTR 543 exhibited robust growth during the initial stage of Nham fermentation. This result is consistent with the findings reported by Tangwatcharin et al. (2020), who observed that *L. plantarum* produced acid more rapidly during the fermentation of traditional Thai fermented meat. On day 3 of fermentation, the TA in all treatments ranged from 0.55% to 0.62%, with no statistically significant differences observed among treatments. On day 4 and 27 of storage, the TA levels ranged between 0.58% to 0.66% and 0.69% to 0.74%, respectively, without significant differences among treatments. It is noteworthy that the TA levels of the control sample (T1) did not significantly

differ from those of T3 and T4 during day 3 of fermentation and storage periods. This suggests that the lactic acid formation in the control group resulted from the natural lactic acid bacteria present in the raw materials used in Nham production to produce lactic acid from carbohydrates. Lactobacilli are the primary producers of lactic acid, leading to an increase in acidity during the fermentation process. Lactic acid and acetic acid are frequently suggested as the key contributors to acid aromas and tastes, as well as the development of the texture in Nham (Visessanguan et al., 2005).

The a_w values of Nham samples across the four treatments ranged from 0.93 to 0.98 during fermentation on days 1 and 3, as well as during storage on days 4 and 27. The high a_w values in the Nham samples could be related to their high moisture content (Table 1). This observation is consistent with the findings of Ratanaburee, Kantachote, Charernjiratrakul and Sukhoom (2013), who reported that after a fermentation period of four days, all Nam samples exhibited an a_w value of 0.99. On day 1 of fermentation, there were no statistically significant differences in a_w values among all treatments. This trend was similarly observed on day 3 of fermentation and during storage on days 4 and 27. On day 3 of fermentation, the a_w values of all treatments significantly decreased compared to day 1. The decrease in a_w was consistent with the reduction in pH value. A lower pH induces protein denaturation, which subsequently reduces the water-holding capacity of the product, ultimately resulting in a decreased a_w value (Zhang et al., 2023). On day 3 of fermentation, the a_w value of all treatments was recorded as 0.93, which is consistent with the findings of Visessanguan et al. (2004), who reported that high-quality Nham should have an a_w ranging between 0.80 and 0.95. However, on days 4 and 27 of storage, the a_w values of all treatments significantly increased compared to those observed on day 3 of fermentation. The observed increase in a_w may be attributed to structural changes in proteins during storage at low temperatures, such as 4 °C. These changes can affect the water-holding capacity of the meat, potentially leading to an increase in free water content and, consequently, a higher a_w value (Li et al., 2018).

GABA production

The GABA contents of Nham samples during fermentation and subsequent storage are shown in Fig. 2. A significant interaction between probiotic culture and fermentation and storage time was observed ($p \leq 0.05$), indicating that GABA accumulation depended on both bacterial strain and processing stage.

After 3 days of fermentation, GABA contents varied markedly among treatments, with Starter V3 (T2) producing the highest level, followed by *L. plantarum* TISTR 543 (T3) whereas the control (T1) and *L. lactis* subsp. *lactis* TISTR 1520 (T4) exhibited lower values. These differences suggest that the capacity for GABA biosynthesis is strain dependent, which is primarily associated with variations in glutamate decarboxylase activity and acid tolerance among LAB strains (Dhakal, Bajpai, Baek & Kang, 2012; Cui, Miao, Niyaphorn & Qu, 2020).

During refrigerated storage, GABA contents significantly increased in all treatments, reaching the highest level in T2 after 27 days. This increase may be attributed to continued LAB metabolic activity under low temperature conditions as well as the release of intracellular GABA following cell lysis after fermentation, as previously reported in fermented food systems (Pannerchelvan et al., 2025). The increase observed in the control sample further indicates the contribution of naturally occurring GABA-producing LAB in Nham.

Overall, inoculation with selected probiotic cultures, particularly Starter V3, effectively enhanced GABA accumulation, highlighting the potential of probiotic starter selection as a strategy to improve the functional value of fermented meat products.

Change in color

A desirable Nham product is characterized by its natural pink hue. The color parameters L*, a*, and b*, along with the appearance of Nham across four treatments on days 1, 3, 4, and 27, are presented in Figs. 3 and 4, respectively. The L* values of the four Nham treatments throughout the fermentation and storage periods ranged from 51.36 to 57.50. This indicates that the Nham products in this study exhibited relatively low lightness values. On day 1 of fermentation, the Nham sample from treatment T3, which was inoculated with *L. plantarum* TISTR 543, exhibited the highest lightness value. In contrast, the control treatment (T1) had the lowest lightness value, which was significantly different from the other treatments.

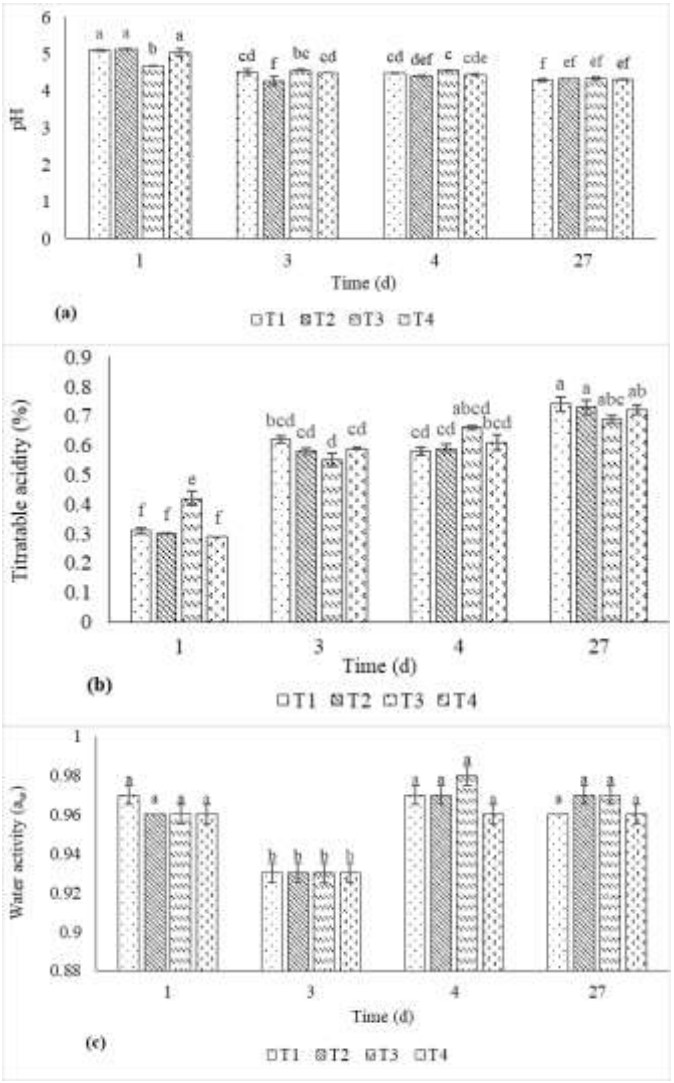

Figure 1. Changes in pH values (a), titratable acidity (b) and water activity (c) during fermentation and storage of Nham samples. T1 = control; T2 = inoculated with Starter V3; T3 = inoculated with *L. plantarum* TISTR 543; T4 = inoculated with *L. lactis* subsp. *lactis* TISTR 1520. Mean values with different letters indicate significant differences among treatment combinations (culture × time) ($p \leq 0.05$)

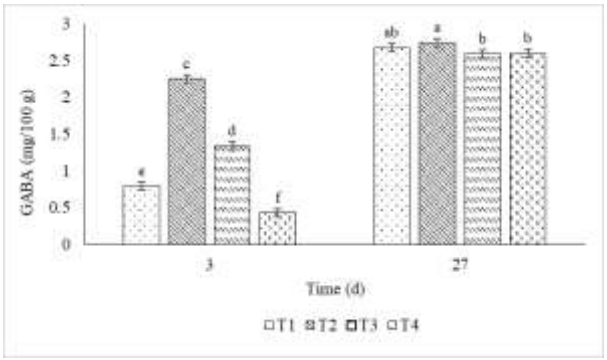

Figure 2. GABA production during fermentation and storage of Nham samples. T1 = control; T2 = inoculated with Starter V3; T3 = inoculated with *L. plantarum* TISTR 543; T4 = inoculated with *L. lactis* subsp. *lactis* TISTR 1520. Mean values with different letters indicate significant differences among treatment combinations (culture × time) ($p \leq 0.05$)

However, on day 3 of fermentation, no statistically significant differences in lightness values were observed among the treatments, a trend that remained consistent on days 4 and 27 of storage. This finding suggests that the inoculation of probiotic bacteria had a

significant effect on lightness only on the first day of fermentation, but not thereafter. These results align with those reported by Visessanguan et al. (2006), who compared three groups of Nham: a control, a group inoculated with *L. curvatus* at 10^4 CFU/g, and another at 10^6 CFU/g. Their study found no significant differences in lightness values among the three groups after three days of fermentation, with values ranging from 53.84 to 56.29. As fermentation progressed to day 3, the lightness value of the control treatment (T1) increased from that on day 1, whereas the other treatments showed no statistically significant changes in lightness compared to day 1. The observed change in lightness in T1 may be attributed to the production of lactic acid, acetic acid, and carbon dioxide during fermentation by lactic acid bacteria, which could influence the lightness of the product. On the fourth day of storage, a decrease in lightness was observed only in the T3 Nham sample compared to day 3. However, on day 27 of storage, there were no statistically significant differences in lightness among all treatments when compared to day 3.

The a* values of the four Nham treatments measured on days 1, 3, 4, and 27 indicated redness, ranging from 9.55 to 16.19. On day 1 of fermentation, Nham samples inoculated with probiotic bacteria (T2 and T3) exhibited significantly higher redness compared to the control treatment (T1). However, after 3 days of fermentation, no statistically significant differrences in redness were observed among all treatments. This trend remained consistent on days 4 and 27 of storage. These findings suggest that the addition of probiotic starter cultures influenced the redness of Nham on the first day of fermentation but had no significant effect on redness on days 3, 4, or 27. On day 3, redness values in all treatments had decreased from those observed on day 1, ranging between 10.18 and 10.68, indicating a characterristic reddish-pink appearance. This result is consistent with the findings of Tangwatcharin et al. (2020), who also reported a reduction in redness during a 3-day fermentation period in traditional Thai fermented meat. The decrease in redness values may be attributed to the addition of Super N, a curing agent containing sodium nitrite, in all four treatments. Sodium nitrite interacts with myoglobin, the pigment in pork muscle, forming nitrosohemochrome, which imparts a reddish-pink color to the product. However, as fermentation progresses, acid production and protein degradation by lactic acid bacteria may reduce the stability of nitrosohemochrome. Additionally, lipid oxidetion may further contribute to the decline in nitrosohemochrome content. This explanation aligns with the report by Panya, Riebroy, Assavanig, Benjakul and Visessanguan (2002), which indicated that Nham tends to lose its redness and appear pale when subjected to prolonged fermentation periods. Nonetheless, during the storage period on days 4 and 27, saw no significant differences in redness values observed when compared to day 3, suggesting that the reduction in redness stabilized after the initial fermentation stage. Based on these results, it can be concluded that Nham inoculated with *L. plantarum* TISTR 543 (T3) and *L. lactis* subsp. *lactis* TISTR 1520 (T2) exhibited levels of redness comparable to the sample inoculated with a commercial starter culture (T2). The commercial starter is known to enhance and stabilize the red color of Nham, helping maintain its natural reddish appearance throughout the storage period.

The b* values of the four Nham treatments on days 1, 3, 4, and 27 indicated the presence of yellowness, with values ranging from 11.30 to 14.80. On day 1 of fermentation, Nham samples inoculated with probiotic bacteria (T2, T3, and T4) exhibited significantly higher yellowness values than the control treatment (T1). However, on day 3, there were no statistically significant differences in yellowness values among all treatments. On day 4 of storage, the T2 sample showed a lower yellowness value compared to T4. Nevertheless, on day 27, no significant differences in yellowness were found among all treatments. These findings suggest that the addition of probiotic starter cultures resulted in higher yellowness in Nham on day 1 compared to the control, but had no significant effect on the yellowness value on days 3, 4, or 27. After three days of fermentation, the yellowness values of all probiotic-inoculated treatments (T2, T3, and T4) decreased compared to their initial values on day 1, while the control (T1) maintained a relatively stable yellowness value. This decline in yellowness in the probiotic-treated samples is likely due to biochemical changes occurring during fermentation, which may also affect other color attributes such as lightness and

redness (Singkhum & Kangkun, 2021). On day 4 of storage, the yellowness values remained statistically similar to those observed on day 3. However, on day 27, only the T3 sample exhibited a further decrease in yellowness value compared to day 3, while other treatments remained stable.

Change in TPA

Changes in TPA parameters of Nham during fermentation and refrigerated storage are presented in Table 2.

The results indicate that both fermentation time and the type of inoculum significantly influenced texture development, particularly hardness, gumminess, and chewiness, while other parameters exhibited more limited responses. Among the evaluated parameters, hardness showed the most pronounced response to inoculation.

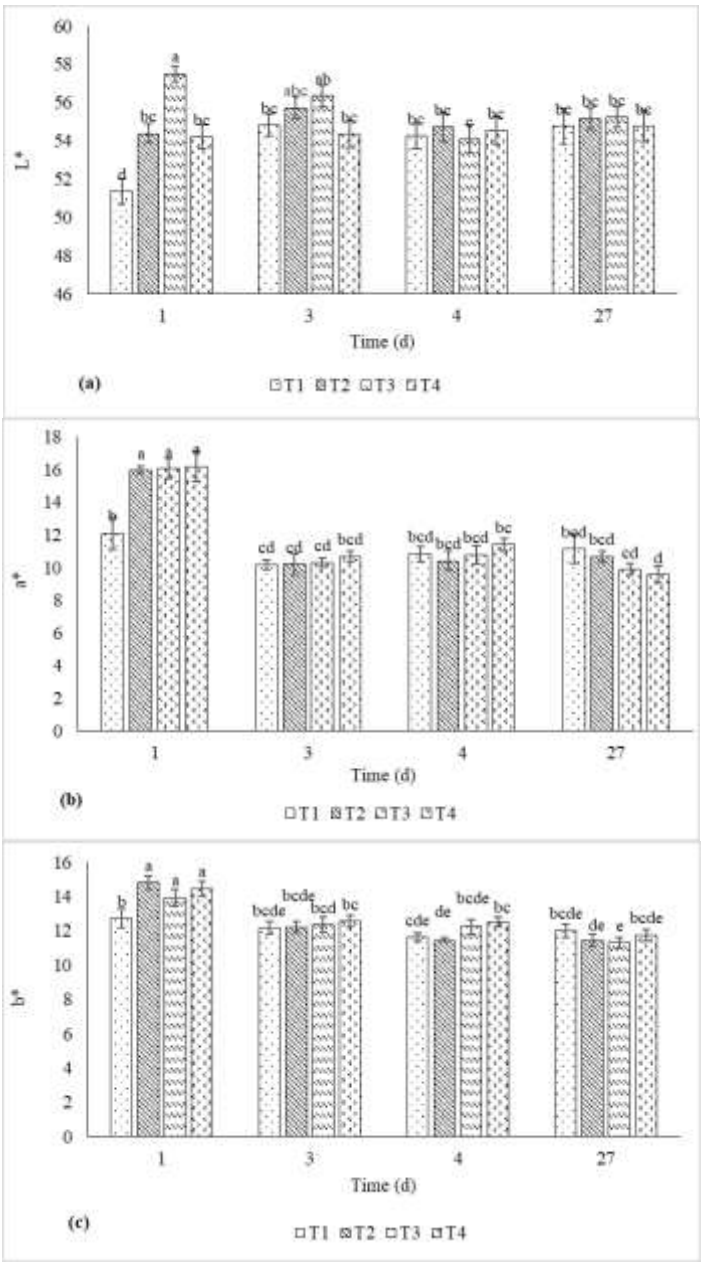

Figure 3. Changes in color values L* (a), a* (b), and b* (c) during fermentation and storage of Nham samples. T1 = control; T2 = inoculated with Starter V3; T3 = inoculated with *L. plantarum* TISTR 543; T4 = inoculated with *L. lactis* subsp. lactis TISTR 1520. Mean values with different letters indicate significant differences among treatment combinations (culture × time) ($p \leq 0.05$)

Figure 4. Appearance of Nham samples during fermentation and storage. T1 = control; T2 = inoculated with Starter V3; T3 = inoculated with *L. plantarum* TISTR 543; T4 = inoculated with *L. lactis* subsp. lactis TISTR 1520

All treatments exhibited a significant increase in hardness from day 1 to day 3 of fermentation; however, samples inoculated with *L. plantarum* TISTR 543 (T3) and *L. lac*tis subsp. *lactis* TISTR 1520 (T4) showed greater increases than the control (T1) and the commercial starter (T2). This indicates that these inocula accelerated the formation of a compact protein matrix, likely through enhanced acidification and protein denaturation. Similar effects of probiotic LAB on hardness development in fermented sausages have been reported, where rapid pH reduction promoted protein aggregation and firmer texture formation (Liu et al., 2024).

Gumminess and chewiness, which are strongly dependent on hardness, followed comparable trends. Both parameters increased during fermentation in inoculated treatments, particularly T3 and T4, indicating increased resistance to deformation as fermentation progressed. These results suggest that probiotic cultures contributed to a denser and more structured protein network, consisting with observations that probiotic LAB can enhance textural strength in fermented meat products through combined acidification and enzymatic activity (Gu, Qiao, Jin, He & Tian, 2023).

In contrast, adhesiveness showed no consistent differences among treatments during fermentation and exhibited greater variability during storage. This indicates that adhesiveness was more strongly associated with moisture migration and protein water interactions than with reinforcement of the protein network. Springiness and cohesiveness remained relatively stable across all treatments and sampling times, indicating that elastic recovery and internal bonding within the protein matrix were largely preserved despite progressive structural tightening during fermentation. These results indicate that the effects of starter cultures were more pronounced on firmness-related texture attributes than on elastic properties.

Resilience tended to decrease as fermentation progressed, particularly in T3 and T4. The reduction in resilience, together with increased hardness, reflects irreversible structural changes in the protein matrix as fermentation advanced. During refrigerated storage, hardness, gumminess, and chewiness generally stabilized, although a slight decrease was observed in T4 at day 27, indicating that the texture of Nham inoculated with *L. lactis* subsp. *lactis* TISTR 1520 may be more susceptible to structural modification during prolonged storage.

Overall, the results demonstrate that inoculum selection played a critical role in modulating texture development and stability in Nham. *L. plantarum* TISTR 543 promoted rapid firmness development during fermentation, while *L. lactis* subsp. *lactis* TISTR 1520 influenced both initial texture formation and textural changes during storage. The commercial starter culture exhibited intermediate behavior, whereas the control relied primarily on indigenous microflora for texture development.

Microbiology analysis

The enumeration of LAB in the four groups of Nham samples during fermentation at 30 °C for 1 and 3 days, followed by storage at 4 °C for 4 and 27 days, is presented in Table 3. On day 1 of fermentation, LAB counts in all treatments ranged from 6.85×10^{6} to 1.10×10^{8}

CFU/g, with no statistically significant differences observed among treatments. The control treatment (T1) exhibited LAB levels comparable to those of treatments supplemented with probiotic cultures. This similarity may be attributed to favorable conditions during the first day of fermentation, which supported the growth of indigenous LAB naturally present in the raw materials, resulting in LAB counts like those found in probiotic-supplemented treatments. On day 3, LAB counts had increased, ranging from 1.06×10^9 to 1.89×10^9 CFU/g. Probiotic-supplemented samples tended to exhibit higher LAB populations compared to the control, particularly in the treatment supplemented with *L. plantarum* TISTR 543 (T3). After 4 days of cold storage at 4 °C, LAB counts in all treatments ranged from 1.69×10^9 to 2.55×10^9 CFU/g. All probiotic-supplemented treatments (T2, T3, and T4) showed significantly higher LAB counts than the control (T1). This may be due to the addition of probiotic cultures during Nham production in treatments T2, T3, and T4, and the ability of these strains to survive and grow, albeit slowly, under refrigerated conditions. After 27 days of storage at 4 °C, LAB counts ranged from 7.30×10^8 to 1.83×10^9 CFU/g across treatments, with T3 maintaining the highest LAB population. These results indicate that *L. plantarum* TISTR 543 exhibited a strong tolerance to the acidic environment developed during Nham fermentation. As fermentation progressed to day 3, LAB counts in all treatments increased compared to day 1, corresponding with a decrease in pH and an increase in lactic acid content (Figure 1), reflecting active LAB growth during the early fermentation stage. On day 4 of refrigerated storage, LAB populations further increased in all treatments, particularly in T2 (commercial probiotic) and T4 (*L. lactis* subsp. *lactis* TISTR 1520), indicating continued LAB metabolic activity under low temperature conditions. In contrast to these findings, Visessanguan et al. (2006) reported a reduction in LAB populations after 3 days of

Table 2.
Change in texture profile analysis (TPA) of Nham samples

Items	Time (d)	T1	T2	T3	T4
Hardness (N)	1	30.73±1.15^{fg}	24.82±1.96^{g}	34.46±4.64^{cdef}	24.88±1.21^{g}
	3	33.20±3.39^{def}	35.57±3.54^{bcdef}	40.48±2.93^{abcde}	41.58±3.23^{abcd}
	4	42.49±3.86^{abc}	37.72±3.12^{abcdef}	43.80±1.75^{ab}	44.62±3.78^{a}
	27	44.42±2.01^{a}	40.02±2.73^{abcde}	42.50 ± 2.49^{abc}	32.19±2.90^{efg}
Adhesiveness (g.s)	1	-0.44±0.14^{a}	-0.67±0.03^{a}	-0.61±0.02^{a}	-0.28±0.00^{a}
	3	-1.06±0.30^{a}	-0.82±0.03^{a}	-0.13± 0.04^{a}	-0.92±0.02^{a}
	4	-3.25±0.75^{a}	-12.58±0.45^{abc}	-5.48±0.26^{ab}	-11.91±0.29^{abc}
	27	-7.91±0.62^{ab}	-16.64±0.45^{bc}	-23.02±0.22^{c}	-5.78±0.27^{ab}
Springiness (ratio) ^{ns}	1	0.87±0.03	0.87±0.04	0.87±0.02	0.90±0.03
	3	0.87±0.03	0.88±0.03	0.87±0.03	0.86±0.01
	4	0.89±0.02	0.88±0.02	0.85±0.02	0.84±0.03
	27	0.90±0.02	0.88±0.04	0.86±0.01	0.88±0.02
Cohesiveness (ratio)	1	0.70±0.01^{ab}	0.72±0.02^{a}	0.70±0.01^{ab}	0.69±0.01^{abc}
	3	0.66±0.02^{bcde}	0.72±0.03^{a}	0.66±0.02^{bcde}	0.67±0.01^{abcd}
	4	0.63±0.02^{def}	0.63±0.02^{cdef}	0.65±0.03^{bcdef}	0.63±0.02^{cdef}
	27	0.62±0.01^{ef}	0.63±0.04^{cdef}	0.60±0.02^{f}	0.60±0.02^{f}
Gumminess (g.s)	1	2202.27±105.31^{bcd}	1653.27±192.75^{d}	2438.94±313.25^{abc}	1740.62±92.20^{d}
	3	2225.93±199.20^{bcd}	2630.66±339.52^{ab}	2722.81±240.90^{ab}	2851.59±224.82^{ab}
	4	2723.37±209.81^{ab}	2435.20±195.95^{abc}	2888.54±100.90^{a}	2896.50±307.93^{a}
	27	2699.26±179.55^{ab}	2534.22±220.58^{abc}	2566.71±227.8^{abc}	1932.34±193.98^{cd}
Chewiness (g.s)	1	1933.52±148.80^{bcde}	1455.52±201.02^{e}	2102.74±225.55^{abcd}	1570.85±83.04^{de}
	3	1904.40±130.32^{bcde}	2323.67±329.50^{ab}	2359.76±191.6^{ab}	2466.41±199.49^{ab}
	4	2427.93±213.76^{ab}	2134.19±169.63^{abc}	2456.76±117.04^{ab}	2420.41±253.42^{ab}
	27	2610.03±51.46^{a}	2213.19±184.12^{abc}	2203.16±192.14^{abc}	1690.18±157.69^{cde}
Resilience (ratio)	1	0.32±0.01^{abc}	0.35±0.03^{a}	0.34±0.02^{a}	0.33±0.01^{ab}
	3	0.30±0.02^{bcd}	0.33±0.01^{ab}	0.27±0.02^{def}	0.29±0.01^{cde}
	4	0.26±0.01^{def}	0.27±0.01^{def}	0.26±0.02^{def}	0.25±0.01^{ef}
	27	0.24±0.01^{f}	0.25±0.02^{ef}	0.24±0.01^{f}	0.25±0.01^{ef}

T1 = control; T2 = inoculated with Starter V3; T3 = inoculated with L. plantarum TISTR 543; T4 = inoculated with L. lactis subsp. lactis TISTR 1520. Mean values with different letters indicate significant differences among treatment combinations (culture × time) ($p \leq 0.05$). ^{ns} Means values are not significantly different ($p > 0.05$)

fermentation in both control and *Lactobacillus curvatus*-inoculated Nham, with final counts of 10^7 to 10^8 CFU/g. The higher LAB counts observed in the present study may be attributed to differences in starter culture composition, inoculum level, and fermentation and storage conditions. After 27 days of storage, LAB counts in T1, T2, and T3 were not significantly different from those observed on day 3, whereas a significant decrease was detected in T4. This reduction may indicate that the rate of cell death exceeded cell proliferation in this treatment, potentially due to nutrient depletion and the accumulation of organic acids during prolonged storage, which have been reported to negatively affect LAB viability (Sionek, Szydłowska, Trząskowska & Kołożyn-Krajewska, 2024). Overall, probiotic-supplemented Nham treatments (T2, T3, and T4) main-tained LAB counts within the range of 10^8 to 10^9 CFU/g after fermentation and throughout both 4 and 27 days of refrigerated storage. These levels are commonly considered indicative of adequate probiotic viability, suggesting that Nham fortified with probiotic cultures has the potential to deliver health benefits.

The total viable counts of Nham samples from the four treatments after 3 days of fermentation and 4 days of storage are presented in Table 3. On day 3, the total viable counts ranged from 6.73×10^7 to 1.08×10^8 CFU/g, with no statistically significant differences observed among the treatments. On day 4, total viable counts ranged from 3.78×10^8 to 1.30×10^9 CFU/g.

Treatments T1, T2, and T4 exhibited no significant differences in total viable counts, but all showed significantly lower total viable counts compared to treatment T3. Storage for 4 days led to an overall increase in total viable counts across all treatments, with treatment T3, which was inoculated with *L. plantarum* TISTR 543, showing the highest increase in microbial load.

These findings indicate that the total viable counts in all four Nham treatments were relatively high, ranging from 10^7 to 10^9 CFU/g. This high microbial load is likely attributable to the presence of LAB, originating both from natural contamination of raw materials and from deliberate inoculation. Moreover, the similarity in values between total viable counts and LAB counts (Table 3) suggests that LAB may constitute the dominant microbial population in Nham. These observations are consistent with the findings of Visessanguan et al. (2006), who reported that the total viable counts in Nham during fermentation closely resembled the LAB counts.

The yeast and mold count of the Nham samples after 3 days of fermentation and 4 days of storage are also presented in Table 3. On day 3, yeast and mold counts ranged from 2.99×10^4 to 1.16×10^5 CFU/g. The treatment inoculated with *L. lactis* subsp. *lactis* TISTR 1520 (T4) exhibited the lowest yeast and mold counts, which were significantly lower than those of the control (T1) and the commercially inoculated treatment (T2). No statistically significant differences were observed among treatments T1, T2, and T3. The reduction in yeast and mold counts in T4 could be attributed to higher levels of organic acids generated during fermentation, which inhibit the growth of these fungi. Additionally, bacteriocins produced by *L. lactis* subsp. *lactis* TISTR 1520 may have contributed to antifungal activity. These observations align with the findings of Jetwanna, Pilasombut, Limsupavanich, Sawatvivat and Settakul (2011), who demonstrated that inoculation with bacteriocin-producing strains *L. lactis* subsp. *lactis* P2 and Sb2 during beef Nham fermentation resulted in a decrease in yeast and mold counts after 3 days of fermentation, coinciding with an increase in LAB counts. Moreover, Swetwiwathana and Visessanguan (2015) reported that *L. lactis* subsp. *lactis* N100 and N190, which were isolated from Nham, exhibited certain probiotic potentials and could produce the bacteriocin Nisin Z. On day 4 of storage, yeast and mold count across all treatments ranged from 7.73×10^3 to 1.44×10^4 CFU/g, with no significant differences observed among treatments. Notably, the yeast and mold count on day 4 was significantly lower than that on day 3, indicating statistically significant decline during storage. This reduction may result from limited LAB activity at 4 °C, which continues to generate and accumulate organic acids, particularly lactic acid that inhibits fungal growth. The results of the microbiological analysis for *S. aureus*, *Salmonella* spp., and *E. coli* in Nham samples are presented in Table 3. It was found that none of the pathogens was detected in any of the treatment groups during the fermentation process at 30 °C for 3 days and subsequent storage at 4 °C for 4 days.

Table 3.
Microbiology analysis of Nham samples

Items	Time (d)	T1	T2	T3	T4
Lactic acid bacteria (CFU/g)	1	$1.12\times10^{7}\pm7.07\times10^{4f}$	$6.85\times10^{6}\pm4.95\times10^{3f}$	$1.10\times10^{8}\pm1.13\times10^{5f}$	$1.41\times10^{7}\pm1.27\times10^{4f}$
	3	$1.06\times10^{9}\pm3.54\times10^{6de}$	$1.35\times10^{9}\pm1.91\times10^{6cde}$	$1.89\times10^{9}\pm1.41\times10^{6abc}$	$1.72\times10^{9}\pm4.03x\times10^{6bcd}$
	4	$1.69\times10^{9}\pm7.50\times10^{6bcd}$	$2.49\times10^{9}\pm1.34\times10^{6a}$	$2.55\times10^{9}\pm3.54\times10^{6a}$	$2.22\times10^{9}\pm5.52\times10^{6a}$
	27	$1.02\times10^{9}\pm5.52\times10^{6de}$	$7.35\times10^{8}\pm2.12\times10^{5ef}$	$1.83\times10^{9}\pm3.61\times10^{6abc}$	$7.30\times10^{8}\pm4.95\times10^{5ef}$
Total viable count(CFU/g)	3	$8.04\times10^{7}\pm9.33\times10^{4c}$	$7.70\times10^{7}\pm7.00\times10^{4c}$	$6.73\times10^{7}\pm6.86\times10^{4c}$	$1.08\times10^{8}\pm2.50\times10^{5c}$
	4	$3.78\times10^{8}\pm1.65\times10^{5b}$	$5.07\times10^{8}\pm5.34\times10^{5b}$	$1.30\times10^{9}\pm4.50\times10^{6a}$	$4.87\times10^{8}\pm6.29\times10^{5b}$
Yeast and Mold (CFU/g)	3	$1.16\times10^{5}\pm1.27\times10^{3a}$	$8.27\times10^{4}\pm4.43\times10^{2a}$	$7.07\times10^{4}\pm3.44\times10^{2ab}$	$2.99\times10^{4}\pm3.08\times10^{2bc}$
	4	$7.75\times10^{3}\pm6.79\times10^{c}$	$7.73\times10^{3}\pm8.13\times10^{c}$	$1.51\times10^{4}\pm3.18\times10^{2c}$	$1.44\times10^{4}\pm1.11\times10^{2c}$
Staphylococcus aureus (CFU/g)	3	Not detected	Not detected	Not detected	Not detected
	4	Not detected	Not detected	Not detected	Not detected
Salmonella spp. /25g	3	Not detected	Not detected	Not detected	Not detected
	4	Not detected	Not detected	Not detected	Not detected
Escherichia coli (MPN/g)	3	Not detected	Not detected	Not detected	Not detected
	4	Not detected	Not detected	Not detected	Not detected

T1 = Nham control; T2 = Nham inoculated with Starter V3; T3 = Nham inoculated with L. plantarum TISTR 543; T4 = Nham inoculated with L. lactis subsp. lactis TISTR 1520. Mean values with different letters indicate significant differences among treatment combinations (culture × time) ($p \leq 0.05$)

The absence of these pathogenic bacteria may be attributed to the increased lactic acid content observed during both fermentation and storage periods (Figure 1), which likely inhibited the growth of such microorganisms. Additionally, bacteriocins produced by LAB during Nham production may have contributed to the suppression of these pathogens. The use of hygienic raw materials and equipment throughout the production process likely further minimized microbial contamination. These findings are consistent with the report by Jetwanna et al. (2011), who also found no detectable levels of *Salmonella* spp., *S. aureus*, or *E. coli* in fermented beef Nham across all experimental groups on days 0 and 3 of fermentation. Similarly, Chokesajjawatee et al. (2009) reported a significant reduction in *S. aureus* counts when the pH of Nham dropped to ≤ 4.6, with continued reduction observed during refrigerated storage. At a pH of 4.55 during storage at 4 °C, *S. aureus* was undetectable. In the present study, all four Nham treatments met the microbiological safety standards set by TISI (2004).

CONCLUSIONS

This study showed that the use of pure and commercial probiotic starter cultures affected GABA formation and key quality attributes of Nham. The commercial culture Starter V3 produced the highest GABA levels, while *L. plantarum* TISTR 543 exhibited strong acidification and maintained the highest LAB viability during storage. All probiotic treatments achieved desirable pH, acidity, and a_w values, indicating successful and stable fermentation. Probiotic cultures enhanced textural development, particularly hardness and gumminess, whereas color characteristics remained generally comparable among treatments after the initial fermentation stage. Microbiological assessment confirmed high LAB counts and the absence of pathogenic bacteria in all samples. Overall, the application of probiotic LAB, especially Starter V3 and *L. plantarum* TISTR 543, supports the production of Nham with improved fermentation consistency, enhanced GABA content, and satisfactory safety and quality. These findings highlight the potential for developing Nham as a functional fermented meat product with added health-related benefits. Sensory evaluation was not included in the present study, and future research should incorporate sensory analysis to further assess consumer acceptance.

AUTHOR CONTRIBUTIONS

Conceptualization, P.P.; Methodology, P.P.; Investigation, formal analysis, validation, writing-original draft preparation, P.P.; Writing-review and editing, P.P.; Supervision, P.P.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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UTICAJI ČISTIH I KOMERCIJALNIH PROBIOTIČKIH KULTURA NA FORMIRANJE GAMA-AMINOBUTERNE KISELINE (GABA) I KVALITATIVNE KARAKTERISTIKE TAJLANDSKE FERMENTISANE SVINJSKE KOBASICE (NHAM)

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Sažetak: Ova studija je istraživala uticaje čistih i komercijalnih probiotičkih starter kultura na formiranje gama-aminobuterne kiseline (GABA) i ključne kvalitativne karakteristike tajlandske fermentisane svinjske kobasice (Nham). Pripremljena su četiri tretmana: kontrola bez inokulacije, komercijalna probiotička kultura (Starter V3), *Lactobacillus plantarum* TISTR 543 i *Lactococcus lactis subsp. lactis* TISTR 1520, pri čemu su sve kulture imobilisane na kuvanoj proklijanoj nerafinisanoj pirinčanoj podlozi. Tokom fermentacije na 30 °C i skladištenja na 4 °C, procenjivana su fizičko-hemijska svojstva, uključujući pH, ukupnu kiselost i aktivnost vode, kao i boju, teksturu, sadržaj GABA i mikrobiološki kvalitet. Rezultati su pokazali da su probiotičke starter kulture značajno povećale produkciju GABA, pri čemu je Starter V3 dao najviše vrednosti, a zatim *L. plantarum* TISTR 543. Inokulacija probioticima ubrzala je zakiseljavanje, što je rezultiralo konstantno nižim pH vrednostima i višom ukupnom kiselošću u poređenju sa kontrolom, ukazujući na efikasniju fermentaciju. Broj laktobacila se značajno povećao tokom fermentacije i ostao visok tokom skladištenja, pri čemu je *L. plantarum* TISTR 543 pokazao najbolju sposobnost preživljavanja. Teksturalne osobine, posebno tvrdoća i žvakljivost, poboljšane su u uzorcima sa dodatkom probiotika, što odražava pojačano formiranje gela povezano sa interakcijama proteina i kiselina. Iako su se parametri boje razlikovali među tretmanima prvog dana fermentacije, te razlike su kasnije nestale. Svi tretmani su ispunili mikrobiološke standarde bezbednosti tokom fermentacije i skladištenja, bez prisustva *Staphylococcus aureus, Salmonella* spp. ili *Escherichia coli*. Sveukupno, nalazi potvrđuju da probiotičke starter kulture, posebno Starter V3 i *L. plantarum* TISTR 543, mogu poboljšati performanse fermentacije, povećati akumulaciju GABA i doprineti razvoju bezbednog, visokokvalitetnog Nham proizvoda sa funkcionalnim potencijalom za zdravlje.

Ključne reči: *Nham, probiotik, starter V3, Lactobacillus plantarum TISTR 543, Lactococcus lactis* subsp. *lactis TISTR 1520*

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