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Research article

Effect of beeswax edible film on preservation of Naem product quality during storage

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Abstract

Importance of the work: Beeswax (BW) could be a natural ingredient applied in edible packing film to effectively extend the shelf-life of Thai fermented sausage ('Naem').

Objective: To evaluate the capability of edible films made from beeswax in extending the shelf-life of Naem.

Materials & Methods: Naem samples were wrapped using one of four different film groups: polyvinyl chloride (PVC) or 0%, 1% or 3% BW. Changes were determined and compared in the pH, moisture content, color (L*, a* and b*), lipid oxidation and aerobic plate count (APC) and growth rates of lactic acid bacteria, *Escherichia coli*, *Enterobacteriaceae*, *Staphylococcus* spp. and *Salmonella* spp. in the Naem samples of the four groups during refrigerated storage.

Results: The 1% and 3% BW groups had significantly higher pH, L* and b* values after storage for 15 d than the PVC and 0% BW groups. The moisture content in the PVC group was significantly higher than in the other three groups. Naem in the 1% or 3% BW group had the lowest lipid oxidation level at 1.13 mg malondialdehyde/kg at an initial storage stage and still maintained a lower level after storage for 15 d. Additionally, their APC counts were significantly lower than for the PVC and 0% groups during storage. No *Enterobacteriaceae* were detected in the 3% BW group, while *E. coli*, *Staphylococcus* spp. and *Salmonella* spp. were not found in all groups after storage for 15 d.

Main finding: The edible film made from 3% BW significantly improved the shelf-life of Naem, with fewer changes in color, lipid oxidation, and microbial growth.

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Introduction

Thai fermented sausage ('Naem') is a type of semi-dry sausage that is made from ground raw meat, cooked rice, salt, sugar, garlic and seasonings and usually incubated within 3–5 d at 30–35°C and 50% humidity, depending on the growth rates of lactic acid bacteria and nitrate-reducing bacteria (Deesanam et al., 2019). Fermentable carbohydrates are used by these microorganisms to produce organic acids (mainly lactic acid) that contribute to a variety of flavors and textures. At the end of the process, Naem becomes lightly reddish in color with a sour taste that limits most pathogen growth. The ranges of pH and water activity of Naem are 4.6–5.3 and 0.90–0.95, respectively (Lücke, 2000). However, some studies have noted that several harmful microorganisms, such as *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*, can contaminate fermented sausages through cross-contamination from equipment or personnel during processing and in retail outlets, causing health hazards to consumers (Bremer et al., 2004; Colak et al., 2007; Holck et al., 2017). Usually, sodium nitrite is used as an antimicrobial agent in Naem (Sueprasarn et al., 2017). Some natural extracts that are rich in antioxidants could partially substitute for sodium nitrite to extend the shelf-life of Naem by inhibiting microbial growth as well. For example, *Hibiscus sabdariffa* extract in Naem products provided better conservation quality with stable and acceptable appearance (Tangkham et al., 2022).

Food packaging plays an important role in preserving or protecting food products against spoilage; furthermore, edible film packaging materials could help to extend quality and freshness of food products during distribution and storage. Edible film is defined as any material applied by wrapping or coating to food to extend the shelf-life and can be consumed together with food. The edible film materials are commonly made from natural and biodegradable agricultural products containing digestible cellulose acetate, soy protein, chitosan, and gelatin, among other items (Sánchez-Ortega et al., 2014). Beeswax is a natural glazing compound produced from honeybees and considered as a generally recognized as safe (GRAS) material (US Food and Drug Administration, 2021). Beeswax contains hydrocarbon (15%), esters (71%), free acids (8%) and other compounds (6%) according to Tinto et al. (2017). Trevisani et al. (2017) reported that using beeswax coating extended the shelf-life of Italian salami for 55 d. The use of beeswax in edible films resulted in enhanced color stability and deceleration of the oxidation process of meat myoglobin and browning of meat (Trevisani et al., 2017). Another potential advantage of using beeswax is its antimicrobial properties, which slow microbial growth and retard spoilage such as from *Salmonella typhimurium* and *Staphylococcus aureus* (Orsi et al., 2005; Ferreira et al., 2007; Antúnez et al., 2008; Zhang and Xiao, 2013; Wilson et al., 2015; Pinto et al., 2017). Thus, beeswax edible film could reduce harmful microorganisms and lipid oxidation and extend the shelf-life of Naem products as well. Wardhono et al. (2019) and Pérez-Vergara et al. (2020) stated that higher beeswax concentrations used in making edible film could make the films more fragile with the loss of good film-forming properties. Furthermore, high beeswax concentrations in the films would negatively impact the original sensory properties of the packed foods. Therefore, the current study evaluated the capability of edible film made with 1% or 3% of beeswax to extend the shelf-life of a Naem product. During the storage of the Naem product, changes were monitored in the pH, moisture content, color (L*, a* and b* values), lipid oxidation, aerobic plate count (APC) and growth rates of lactic acid bacteria, *E. coli*, *Enterobacteriaceae*, *Staphylococcus* spp. and *Salmonella* spp.

Materials and Methods

Preparation of beeswax edible film

Honeycomb was collected from the McNeese State University Farm (Lake Charles, LA, USA). The honeycomb was cleaned and diluted with drinking water at a ratio of 70:30 (weight per weight). The mixture was heated at 75–80°C for 10 min, immediately transferred to pasteurized jars and allowed to cool. All jars were covered with aluminum foil and stored in a refrigerator prior to making the beeswax (BW) edible films. The ingredients for different experimental groups are listed in Table 1. Baking soda (sodium bicarbonate) was used to assist in completely dissolving the beeswax.

Table 1 Film ingredients of four experimental groups

Ingredient	PVC	0% BW	1% BW	3% BW
Water	-	97.70	96.67	94.65
Beeswax	-	0.00	1.00	3.00
PVC	100.00	-	-	-
Gelatin	-	2.30	2.30	2.30
Baking soda	-	0.00	0.03	0.05

PVC = polyvinyl chloride; BW = beeswax

Except for the PVC group, the other groups were heated at 60°C for 30 min until they became clear-liquid solutions. The solution of each group (5 mL) was poured and spread uniformly in a plastic mold (15 cm × 15 cm). Five molds were used to prepare film for each group. Then, the molds were transferred to the dehydrator (set at 60°C) for 24 hr. After each mold had cooled, the cast film was removed and cut into two pieces (each 7.5 cm × 15 cm), producing 10 pieces of film in each group for use in wrapping the separate Naem samples.

Preparation of Naem sample

The Naem sample was prepared according to the ingredient formula described by Tangkham et al. (2022), consisting of 75.2% ground pork (20% fat), 14.8% cooked jasmine sticky rice, 4.4% ground garlic, 1.5% table sugar, 1.1% salt and 3% *Hibiscus sabdariffa* extract. After the ground raw meat had been mixed with the ingredients completely, 25 g of the mixture sample were weighed and wrapped in the PVC or 0%, 1% or 3% BW film. The wrapped samples were placed at 27°C for 72 hr to carry out fermentation. Then, after fermentation, each of the wrapped Naem samples was placed in a plastic bag and randomly stored in a refrigerator at 4°C. Two of the individually wrapped Naem samples in each group were removed on days 1, 5, 9, 12 and 15 of storage and grounded uniformly for the analyses described below.

Determinations of pH, moisture content and color

The Naem samples were analyzed for pH using an electro portable meter probe (Model 2000; VWR International, LLC; Radnor, PA, USA). Before use in the study, the pH meter was calibrated using pH 4.0 and pH 7.0 buffers.

The moisture content was analyzed according to the method of the Association of Official Analytical Chemists (2000). Each Naem sample (5 g) was weighed and dried in a hot-air oven at 60°C for 24 hr (Precision Thelco Model 26; Thelco; Englewood, CO, USA). Then, each Naem sample was reweighed, and the result was recorded. The percentage of moisture content was calculated using Equation 1:

$$\text{Moisture content (\%)} = (\text{Sample weight} - \text{Dried sample weight}) / \text{Sample weight} * 100 \quad (1)$$

The color values (L*, a* and b*) of each Naem sample were measured using a colorimeter (Model CR-10; Konica Minolta; Wayne, NJ, USA) that used reflectance spectrophotometry to calculate the color appearance parameters from the reflectance spectrum using an 8 mm aperture, a 10° observer angle and a D65 illuminant source.

Determination of lipid oxidation

The thiobarbituric acid-reactive substances (TBARS) method (Papastergiadis et al., 2012) was used to measure the lipid oxidation of each Naem sample. A Naem sample (15 g) was blended with 30 mL of trichloroacetic acid solution. The sample solution was passed through Whatman No. 1 filter paper. Then, 5 mL aliquot of the filtrate was transferred to separate test tubes and mixed with 5 mL of 0.02 M thiobarbituric acid. The mixture was vortexed and heated in a boiling water bath at 100°C for 40 min. Next, the reaction mixture was allowed to cool under running water, followed by the absorbance being determined at 530 nm using a spectrophotometer (Du-640; Beckman; Indianapolis, IN, USA). The TBARS value was calculated using a standard curve based on tetraethoxypropane (TEP) dilutions (1, 1, 3, 3, TEP) and expressed as milligrams of malondialdehyde (MDA) per kilogram of sample. A 1 µmole/mL TEP standard solution was prepared by dissolving 0.22 g of TEP in 1 L distilled water.

Determination of aerobic plate count and lactic acid bacteria

Peptone buffer (1 L) was prepared by diluting 10 g of peptone powder, 5 g of sodium chloride, 3.5 g of dipotassium phosphate and 1.5 g of mono-potassium phosphate. The buffer was autoclaved at 121°C for 15 min. Next, 1 g of each sample was mixed with 9 mL of peptone buffer and vortexed to release any bacteria.

Then, 1 mL of each sample solution was pipetted onto aerobic bacterial count film (3M Petrifilm; 3M; Angleton, TX, USA). The films were incubated at 37°C for 24 hr. The red colonies on film were manually counted and marked individually. The aerobic plate count (APC) was calculated and expressed as log CFU (colony forming unit) per gram of Naem sample.

For determination of lactic acid bacteria, 59.5 g of all-purpose tween (APT) agar were weighed and boiled with 1 L of distilled water until it had dissolved completely. The solution was then autoclaved at 121°C for 15 min. Next, 25 mL of the agar solution was poured into a sterilized plate to form the

solidified APT agar medium. Each sample solution (0.1 mL) was transferred and spread uniformly on the plate surface. Then, the plate was incubated at 37°C for 24 hr in an anaerobic container with added pack-CO_2 (AnaeroPack™; MGC Inc.; New York, NY, USA). White and mucoid colonies on the plate were counted and marked individually. The number of lactic acid bacteria was calculated and expressed as log CFU per gram of Naem sample.

Determination of E. coli, Enterobacteriaceae, Staphylococcus spp. and Salmonella spp.

The numbers of *E. coli.*, *Enterobacteriaceae*, *Staphylococcus* spp., or *Salmonella* spp. were determined using 3M Petrifilm (3M; Angleton, TX, USA). A sample (1 mL) of solution was pipetted onto the film and well spread on the film surface. The films were incubated in a horizontal position and clear side up at 37°C for 24 hr. The bacteria colonies appeared as different colors on different types of 3M Petrifilm, with *E. coli* as blue colonies, *Enterobacteriaceae* as red colonies, *Staphylococcus* spp. as red-violet colonies and *Salmonella* spp. as dark blue/black colonies. The total colonies on each type of bacteria were counted and marked individually. The number of each type of bacteria was calculated and expressed as log CFU per gram of Naem sample.

Statistical analysis

All the determinations were carried out in duplicate for individual samples. Data were analyzed using the SPSS software (version 27; SPSS Inc.; Chicago, IL, USA) and one-way analysis of variance. All data were presented as mean ± SD values. Significance was tested at the $p < 0.05$ level.

Results and Discussion

Changes in pH and moisture content of Naem samples wrapped with different films

The pH values of the Naem samples in the four groups after storage for 1 d following fermentation were in the range 4.99–5.29 (Fig. 1), which was within the acceptable pH range (4.8–5.4) for semi-dry fermented sausage (Food and Agriculture Organization of the United Nations, 2021). The Naem samples wrapped with the beeswax edible films had slightly higher pH values compared to the samples wrapped with the PVC and 0% BW film, which may have resulted from the very small amount (0.03–0.05%) of baking soda used in dissolution of beeswax for making the edible films. Lashgari et al. (2020) stated that the pH of sausage could fluctuate during storage because the sausage fermentation resulted in a series of dynamic reactions during storage for 60 d such as the production of amines, peptides, amino acids and lactic acid. Although over the storage period of 15 d, the pH values of all groups had not changed significantly compared to first day of storage, the pH of the Naem samples wrapped with the 1% or 3% BW edible films increased slightly from days 1–9 (Fig. 1). These results could have been due to the degradation of the organic acids in the beeswax as a substrate during respiration (Eshetu et al., 2019). Generally, the current study suggested that beeswax edible film had minimal impact on the pH of Naem sample during storage for 15 d.

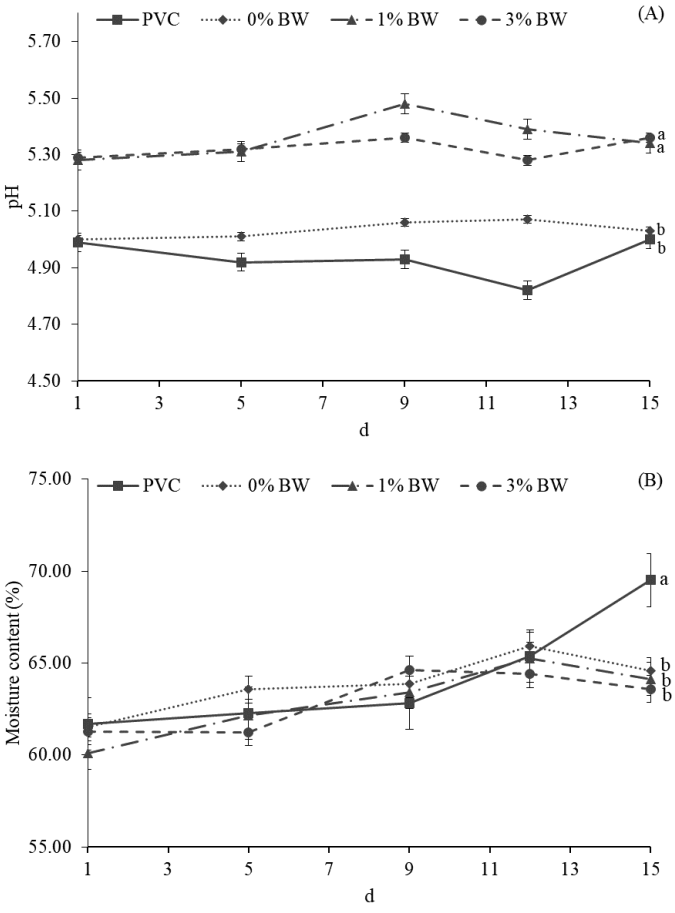

Fig. 1 Naem samples during storage for 15 d: (A) pH; (B) moisture content, where PVC = polyvinyl chloride and BW = beeswax; values are shown as mean ± SD; different lowercase letters on day 15 indicate significant ($p < 0.05$) differences between treatments.

During the meat fermentation process, microorganisms, such as yeast or bacteria, not only convert sugars and other organic compounds into various products, including alcohol, acids and gases, but also produce a certain amount of water (Voidarou et al., 2020). In the current study, the moisture content of the Naem samples slightly increased during storage and after 15 d, the moisture contents in the four groups were in the range 60.09–69.52% (Fig. 1). There was a significant difference in the moisture content between the Naem sample wrapped with the PVC film and the other three groups on day 15. The Naem sample wrapped with PVC had the highest moisture content (69.52%), while the 3% BW edible film group had the lowest moisture content (63.59%) on day 15, which could be explained by the PVC film being a synthetic plastic material with a firm texture that was relatively impermeable to water and gases, compared to the other films in the study. Thus, it formed a very effective barrier that prevented water vapor and gases escaping the Naem sample matrix. Although beeswax was reported to be hydrophobic (Bahrami et al., 2019), it consists of different types of organic compounds from small hydrophilic molecules, such as organic acids and sugars to large hydrophobic molecules, such as wax with long chain carbohydrates. The varieties and complexities of those compounds allowed the film prepared from beeswax to let some water vapor to escape over time and slowed down the diffusion of water vapor within the Naem sample. These properties could lead to a reduction in moisture within the sausage, especially in the area directly in contact with the film. The reduced moisture may have slowed down the growths of *E. coli*, *Staphylococcus* spp. and *Salmonella* spp. that depend on water for their proliferation and reproduction, which is also unfavorable for the growth of other spoilage microorganisms and pathogens (Holck et al., 2017; Qiu et al., 2022). Thus, the use of beeswax edible film might create an environment less conducive to microbial proliferation and extend the shelf-life of Naem, while also preventing the build up of undesirable contaminants during storage. Compared to 10 d, which is the usual shelf-life for Naem made with sodium nitrite and stored at 4°C (Sueprasarn et al., 2017), and the PVC group, using the beeswax edible film extended the Naem shelf-life to 15 d with an acceptable pH and moisture content.

Changes in color (L, a*, b*) of Naem samples wrapped with different films*

There were visual and color changes in the Naem samples over the storage period due to the different wrapping materials. The Naem samples wrapped with PVC and 0% BW exhibited a noticeable brown-gray coloration, while the Naem wrapped with 1% and 3% BW still retained a subtle red hue after storage for 15 d. The lightness (L* value) of the Naem samples wrapped with either the PVC or 0% BW films significantly decreased after storage for 15 d (Fig. 2). The L* values of the Naem samples wrapped with beeswax (1% or 3%) edible films after storage for 15 d were similar or slightly higher than on day 1 of storage (Fig. 2). The decrease in the L* value of the meat samples during storage was associated with the oxidation of myoglobin forming darker color in meat (Tangkham et al., 2022). The presence of a high moisture content in the PVC group accelerated the oxidation reaction and resulted in a rapid decrease in the L* value (Lashgari et al., 2020). This correlation emphasized the critical role of moisture content in influencing the oxidative processes affecting meat color stability during storage.

The redness (a* value) of the meat decreases during storage due to the oxidation of myoglobin, resulting in a shift towards a brown hue (Tangkham et al., 2022). However, *Hibiscus sabdariffa* extract (the additive used in making the Naem samples) consisted of natural red colorants which also contributed to the a* value of the Naem samples in the current study. The a* values increased across all groups during storage (Fig. 2). The PVC or 0% beeswax groups had a significantly lower increase compared to the 1% or 3% beeswax groups because of the high oxidation rate of myoglobin in the PVC or 0% BW groups, which overcame the increase in the a* value derived from the extract. The results were in agreement with Trevisani et al. (2017), who reported that beeswax edible film enhanced color stability and mitigated the oxidation of myoglobin to prevent the meat from turning brown.

Similar to the L* value, the yellowness (b* value) of the Naem samples wrapped with PVC, 0% or 1% BW decreased significantly during storage, while the Naem samples with 3% BW on day 15 of storage had a similar b* value to that on day 1 of storage. In addition, its b* value (14.75) was significantly higher than for the other three groups during storage for 15 d (Fig. 2). These results showed that the edible film with 3% beeswax had the capability to prevent color deterioration of the Naem sample during storage.

Overall, the results highlighted the importance of the wrapping materials, specifically beeswax, in maintaining the color stability of Naem during storage. The incorporation of *Hibiscus sabdariffa* extract with its natural red colorants enhanced the redness of the Naem samples. In addition, the use of beeswax edible film, particularly at 3% concentration, demonstrated potential as an effective means to mitigate color deterioration during the storage period.

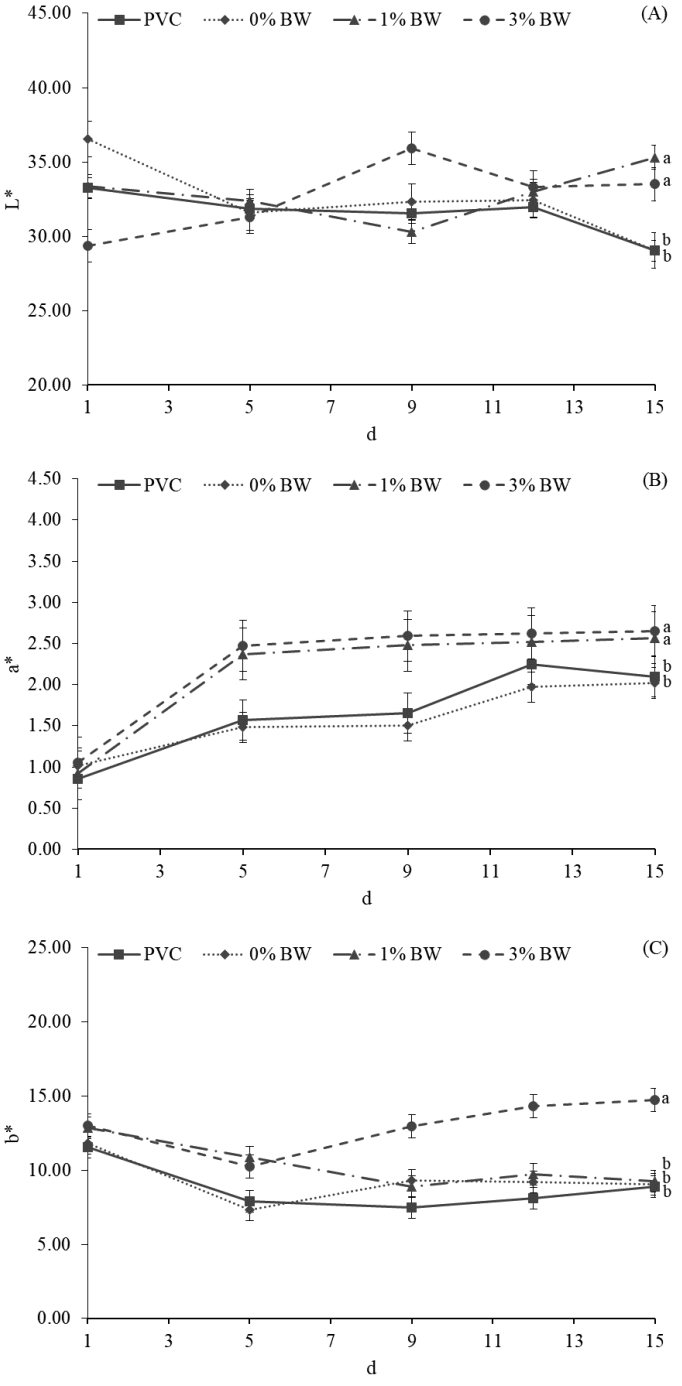

Fig. 2 Mean ± SD color values of Naem samples during storage for 15 d: (A) L*; (B) a*; (C) b*, where PVC = polyvinyl chloride and BW = beeswax; different lowercase letters on day 15 indicate significant ($p < 0.05$) differences between treatments.

Changes in lipid oxidation of Naem samples wrapped with different films

The TBARS value is a factor used to evaluate the degree of lipid oxidation. The high TBARS value after Naem fermentation could have been caused by high oxygen availability during the mechanical process of meat preparation (Trevisani et al., 2017). In the initial storage stage, the TBARS value in the Naem samples wrapped in either the 1% or 3% BW films were approximately two times lower than for the PVC or 0% BW groups (Fig. 3), indicating that BW film significantly inhibited lipid oxidation in the Naem sample at the beginning of storage. However, their lipid oxidation rate increased gradually and reached the same level as that of the PVC and 0% BW film groups (Fig. 3). In this study, *Hibiscus sabdariffa* extract was a rich source of antioxidants in all four groups and effectively lowered the lipid oxidation in the Naem samples. This was due to the extract being high in antioxidant vitamin C, carotenoids and anthocyanins (Hopkins et al., 2013). Generally, among the four groups, the level of lipid oxidation in the Naem sample wrapped with 3% BW edible film was the lowest after 15 d of storage. The TBARS value range of this study was within the acceptable range for fermented sausage of 0.51–2.8 mg MDA/kg sample (Marco et al., 2006) and similar to the TBARS range reported by Zhang et al. (2017) for dry fermented sausage incorporated with rose polyphenols. In addition, it has been reported that some antioxidant compounds in beeswax could significantly retard the lipid oxidation in sausage. For example, according to Trevisani et al. (2017), the use of beeswax coating on Italian salami can potentially reduce the development of lipid oxidation because of natural antioxidants such as phenolic contents. Notably, some degree of lipid oxidation is natural and preferable in fermented meat products. However, as with other fermented meat foods, excessive oxidation can negatively impact the sensory qualities, nutritional value and shelf-life of Naem (Terefe, 2016). Monitoring the TBARS values over time

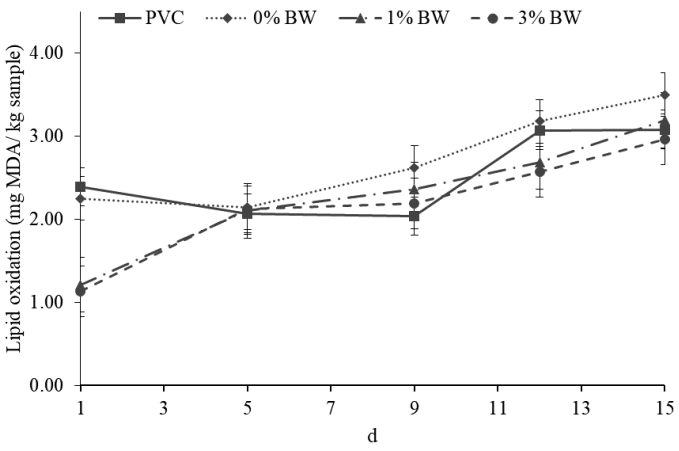

Fig. 3 Mean ± SD values for lipid oxidation of Naem samples during storage for 15 d, where PVC = polyvinyl chloride, BW = beeswax and MDA = malondialdehyde.

provided valuable information about the oxidative stability of Naem to ensure its quality. Food manufacturers may use strategies such as adding antioxidants, optimizing processing conditions and using appropriate packaging to control and reduce lipid oxidation in fermented meat products.

Changes in microorganism activity of Naem samples wrapped with different films

Pathogenic bacteria can contaminate Naem during the early phase of the fermentation process (Chokesajjawatee et al., 2009; Holck et al., 2017). The pH range of the four groups in this study was suitable for both Naem fermentation and the growth of microorganisms. It has been reported that pathogens may survive during fermentation because Naem is commonly consumed as a raw product without heating (Swetwiwathana and Visessanguan, 2015; Zhao et al., 2016). Therefore, food safety has always been an important issue with Naem products. Reduction of the growth of harmful pathogens has been reported by reducing the fat content or adding novel ingredients, or both, to inhibit bacteria during Naem preparation (Chao and Yin, 2009).

The changes in the APC in the Naem products stored for 15 d are shown in Fig. 4, with no significant differences among the four groups on days 1 and 5. The Naem products wrapped with BW film had significantly lower APC counts than the samples wrapped with PVC on days 9 and 12. Specifically, on day 15, the Naem wrapped with either 1% or 3% BW film had significantly lower APC counts than the other two groups. The aerobic bacterial counts were highest (2.90 log CFU/g) in the PVC group after storage for 15 d, which could have been associated with the higher moisture content in the Naem. According to Qiu et al. (2022), reducing humidity significantly decreased bacteria growth. In the current study, the group wrapped with 3% BW had the lowest APC count (1.62 log CFU/g) on day 15. This result was in agreement with the results of Tangkham and Lemieux (2016), who reported APC values in the range 3.21–3.45 log CFU/g in venison jerky, which were considered safe for human consumption. Furthermore, according to Pinto et al. (2017), wrap containing natural products, such as waxes and oil, could display antimicrobial activity and affect the growth of various groups of microorganisms. Szulc et al. (2020) indicated that beeswax had efficacy against both Gram-positive and Gram-negative bacteria, as well as against fungi. Therefore, using beeswax as an edible film could offer some level of protection against microbial contamination in Naem products.

Lactic acid bacteria are commonly used in the production of fermented sausages such as the Naem product in the current study. These bacteria contribute to the fermentation process by converting sugars present in the meat into lactic acid, lowering the pH of the sausage and creating an acidic environment that helps to preserve the sausage and to enhance its flavor and texture. The counts of lactic acid bacteria were in the range 5.55–5.96 log CFU/g during storage for 15 d (Fig. 4). On day 15, the group with 3% BW had a higher number of lactic acid bacteria than the PVC or 0% BW groups. According to Comi et al. (2020), *Lactobacillus sakei*, *Lactobacillus curvatus* and *Lactobacillus plantarum* are the main species of lactic acid bacteria involved in sausage fermentation. In another study, among the *Lactobacillus* species, *Lactobacillus hilgardii* showed preferable growth within a pH range of 4.5–5.5 (Schillinger et al., 2006). The pH values of the Naem products in the four groups in the current study were in the range 4.99–5.29, which was a suitable range for growing lactic acid bacteria.

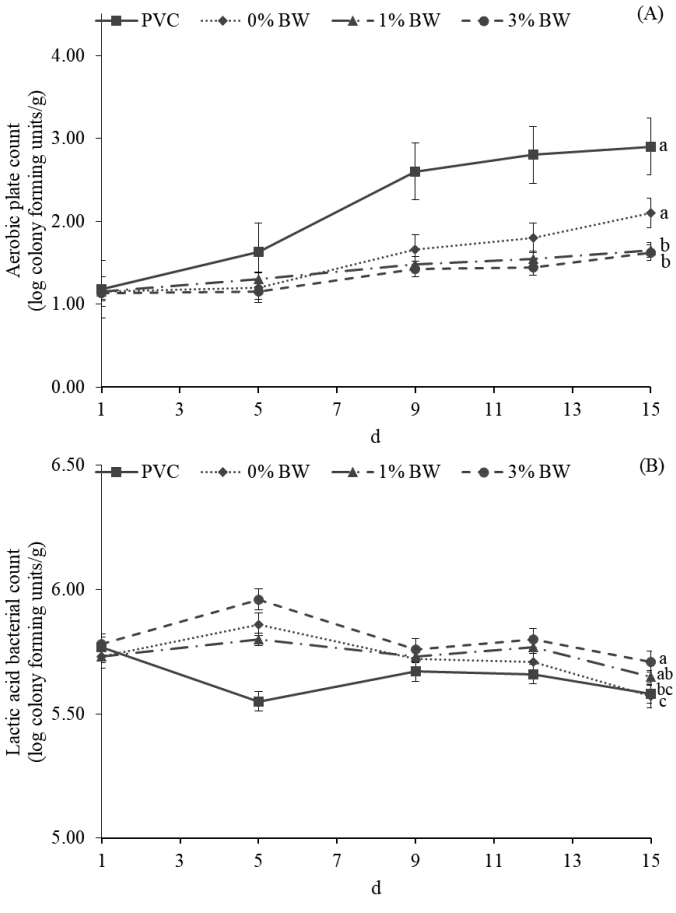

Fig. 4 Mean ± SD values for Naem samples during storage for 15 d: (A) aerobic plate counts; (B) lactic acid bacteria, where PVC = polyvinyl chloride and BW = beeswax; different lowercase letters on day 15 indicate significant ($p < 0.05$) differences between treatments.

In addition, Bartkiene et al. (2017) reported that *Lactobacillus sakei* strains had antimicrobial effects on *E. coli*, *Samonella typhimurium*, *Y. enterolitica*, *B. cereus* and *Staphylococcus aureus* strains. Furthermore, Orsi et al. (2005) reported that propolis, which is a resinous mixture of beeswax and other oils, had antimicrobial activity against *Salmonella enteritidis* isolated from food and *Salmonella typhimurium* isolated from human infections. In the current study, *E. coli*, *Staphylococcus* spp. and *Salmonella* spp. were not present in any of the four groups after storage for 15 d. The results of the current study were similar to other research in that the higher concentrations of BW in edible film and lactic acid bacteria in products, the lower the number of bacteria, such as *Enterobacteriacea*, *E. coli*, *Staphylococcus* spp. and *Salmonella* spp. in Naem samples (Orsi et al., 2005; Bartkiene et al., 2017).

The initial *Enterobacteriaceae* counts for the PVC, 0% and 1% BW treatments were in the range 1.00–1.10 log CFU/g (Table 2). No *Enterobacteriaceae* was found in the group with 3% BW after storage for 15 d (Table 2). Overall, the counts of *Enterobacteriaceae* in the Naem samples wrapped with 1% BW edible film were significantly lower (1.80 log CFU/g) than in the PVC and 0% BW treatments after storage for 15 d. This could be attributed to the combination of the added *Hibiscus sabdariffa* extract and the beeswax (Tangkham et al., 2022). Furthermore, one potential advantage of using beeswax as a part of the edible material is its antimicrobial properties that lower microbial growth and retard spoilage (Ferreira et al., 2007). Therefore, using beeswax edible film can be effective against bacterial contamination and extend the shelf-life of Naem products.

Traditional Naem products have a short shelf-life and there are concerns regarding pathogenic contamination because Naem is made from fermented raw meat and commonly consumed without heating. The current study investigated the use of beeswax edible film for packing Naem to prolong the shelf-life of the Naem while maintaining acceptable levels for the pH and moisture content after storage for 15 d. The beeswax film reduced the lipid oxidation of the Naem and retained meat pigments during storage. In particular, it inhibited harmful microbial growth in the Naem and enhanced the growth of lactic acid. Consequently, it could be used to reduce the use or the amount of traditional sodium nitrite preservative that is used in Thai fermented sausage Naem to extend shelf-life. The current results showed that beeswax can be used as an excellent ingredient in making edible food package film to effectively preserve food products.

Table 2 Mean ± SD *Enterobacteriaceae* counts (log colony forming units/g) of Naem samples during storage for 15 d

Days	PVC	0% BW	1% BW	3% BW
1	1.10±0.00^{a}	1.01±0.00^{a}	1.00±0.00^{a}	-
5	1.20±1.00^{a}	1.30±0.00^{a}	1.24±0.06^{a}	-
9	1.56±0.00^{a}	2.32±0.00^{b}	1.38±0.00^{a}	-
12	2.39±0.09^{a}	2.40±0.00^{a}	1.64±1.24^{b}	-
15	2.40±1.00^{a}	2.60±0.00^{a}	1.80±0.00^{b}	-

PVC = polyvinyl chloride; BW = beeswax

Mean ± SD in same row with different lowercase superscripts denote significant ($p < 0.05$) differences.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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