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Effect of pasteurization and Sa-tay marinade on quality changes in oyster (*Crassostrea belcheri*) meat

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ABSTRACT

The combination of pasteurization and Sa-tay marination represents a practical and applicable strategy for enhancing the microbial safety, sensory quality, and shelf life of oysters, thereby supporting both public health and the seafood industry. This study aimed to investigate the effects of pasteurization and Sa-tay marination on quality changes in oyster meat after inoculation with test pathogenic bacteria. Results showed that pasteurization at 80°C for 8 minutes completely eliminated an initial inoculum of pathogenic bacteria (10^8 CFU/g), including *Escherichia coli*, *Salmonella* Typhimurium, *Vibrio cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. The combination of pasteurization and Sa-tay marination effectively eliminated *E. coli*, *S. Typhimurium*, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* in oyster meat. However, the total volatile base (TVB) value slightly increased after 9 and 12 days of storage. In contrast, trimethylamine nitrogen (TMA-N) was not detected. In summary, pasteurization is a highly effective method for reducing pathogenic bacteria in oyster meat. Without inoculation with pathogenic bacteria, oyster meat marinated with Sa-tay showed improved safety, resulting in lower microbial counts. When combined with Sa-tay marination, this approach also supports extended shelf life. This technique offers a practical solution for seafood processors aiming to improve product safety and commercial viability.

Contribution/Originality: This research presents an approach to improving the safety and shelf life of oyster meat (*Crassostrea belcheri*) by combining pasteurization and Sa-tay marination a method not extensively explored in seafood preservation. This study fills a research gap in oyster meat preservation and offers a scientifically validated method for extending product shelf life in the seafood industry.

1. INTRODUCTION

Oysters are an important economic aquatic animal in Thailand because they are high in nutrients, have a delicious taste, and possess appealing characteristics. Generally, consumers prefer to eat raw or uncooked oysters. However, oysters are filter feeders, and therefore they filter plankton, microorganisms, and various suspended particles in seawater into their digestive systems, including pathogenic bacteria, biotoxins, and hazardous chemical substances that can accumulate in their meat. Coliforms, *Salmonella* spp., and *Vibrio parahaemolyticus* have been detected in oyster meat collected from Phang Nga, Thailand (Jeamsripong, Chuanchuen, & Atwill, 2018). Foodborne outbreaks of pathogenic bacteria have been related to the consumption of raw or partly cooked oysters (Bougeard et al., 2011; Odeyemi, 2016). Among the most concerning pathogens are Vibrio species, particularly *V. parahaemolyticus* and *V.*

vulnificus, which are gram-negative, halophilic bacteria commonly found in warm coastal and estuarine waters worldwide. These bacteria can cause severe gastroenteritis and primary septicemia (Silva, 2005). The spoilage microbiota of oysters, which includes *Vibrio* spp., *Pseudomonas* spp., *Aeromonas* spp., and Enterobacteriaceae, also plays a role in food safety and shelf life (Diner et al., 2023). These become a limitation due to safety issues related to oyster consumption. Moreover, there are other limitations, such as quality and storage period at the post-harvest stage, as there have been reports of rapid deterioration and decomposition of fresh oysters due to microorganism contamination and enzyme degradation.

When dining out, most consumers prefer fresh oysters, which pose a high risk of causing digestive issues. The consumption of raw or partially cooked oysters remains a major source of foodborne illnesses, highlighting the need for effective risk reduction strategies to safeguard public health and ensure food safety. Therefore, it is essential to study risk reduction methods for fresh oyster consumption, along with processing techniques to extend the shelf life of oyster products, for which limited existing information is available. Pasteurization in seafood involves applying an appropriate level of heat to eliminate or significantly reduce pathogenic microorganisms to safe levels, without compromising the product's sensory qualities, such as color, aroma, and texture. This process extends shelf life and minimizes the risk of foodborne illnesses caused by pathogens such as Vibrio spp. and Salmonella spp. The temperature and duration depend on the type of product and target microorganisms. Therefore, pasteurization is particularly suitable for processed fresh seafood, such as oysters and shrimp (Balasubramaniam, Martinez-Monteagudo, & Gupta, 2015).

Sa-tay is a well-known dish among Thai people, available in restaurants and street food stalls. Due to its good taste, creamy texture, and yellow to brown-yellow color, it is popular. The basic ingredients include turmeric rhizome, galangal rhizome, and garlic bulb, although the recipe may vary. Sa-tay marinade is a complex blend of ingredients that significantly influences the texture, flavor, and overall quality of the meat it is applied to. Key characteristics of sa-tay marinade include its ability to enhance weight gain, reduce cooking loss, and improve tenderness in various types of meat, such as beef and chicken. Studies have shown that marinating meat in sa-tay marinade for 180 minutes at 4°C results in significant weight gain and reduced cooking loss, with brine solution showing the highest increase in weight gain and reduction in cooking loss compared to tamarind juice and tamarind juice plus salt (Suwandojo, Handajani, & Annisa, 2023; Yunita, Radiati, & Rosyidi, 2023; Yusop, O'sullivan, Kerry, & Kerry, 2009). Some ingredients used in the Sa-tay mixture have been reported that may contain antimicrobial, antioxidant and medicinal value (Ankri & Mirelman, 1999). The oyster meat marinated with Sa-tay mixture received higher acceptable scores compared to un-marinated (Bunruk, Siripongvutikorn, & Sutthirak, 2013). Therefore, this study focused on the physicochemical and microbiological qualities of oyster meat and employed pasteurization and marination with a Sa-tay mixture as part of the processing method to extend its shelf life.

2. MATERIALS AND METHODS

2.1. Raw Material

Oysters (*Crassostrea belcheri*), with a size of 9-10 animals per kilogram, were purchased from farmers located at Ao Bandon, Kanchanadit District, Surat Thani Province, Thailand, and transported to the Scientific Laboratory and Equipment Center, Prince of Songkla University, Surat Thani Campus, as well as the Faculty of Agro-Industry, Prince of Songkla University, Hatyai Campus. The outer shells were cleaned by washing and brushing away soil and other physical contaminants prior to bringing them to the laboratory. The cleaned oysters were placed in a clean tray, where the shells were opened using a sterile knife. The oyster meat was removed and placed in a sterile dish. They were then ready for use in the next experiment.

2.2. Sa-Tay Mixture Preparation

The sa-tay mixture was purchased from the supermarket. This commercial mixture contains chili, lemongrass, caraway, mustard, coriander, onion, and kaffir lime leaves. To prepare the sa-tay marinade, a mixture was used comprising 50% commercial curry powder, 25% garlic, and 25% galangal.

2.3. Chemical and Culture Media

Chemicals used were of analytical grade. Culture media were Difco.

2.4. Preparation of Pathogenic Bacteria and Inoculation into Oyster Meat

The tested pathogenic bacteria were received from the Institution of National Science Research and Technology, Thailand. They were tested for biochemical properties along with PCR to confirm the species. Prior to conducting the experiment, all types of pathogenic bacteria were cultured in TSB and incubated at 35°C for 24 hours. The pasteurization was performed by injecting approximately 5 ml of culture suspension into 45 g of oyster meat with a concentration of 10^8 CFU/g. The injected oyster meat was heated at 65, 70, 75, and 80°C, with samples taken at 0, 1, 2, 5, and 8 minutes for bacterial determination. The samples were rapidly cooled to room temperature and plated onto plate count agar to enumerate the existing pathogenic bacteria using the standard plate count method (BAM, 2011).

2.5. Effect of Pasteurization on Oyster Meat Quality

Three oyster meats were placed into individual plastic bags. Subsequently, they were pasteurized at a temperature of 80°C for 8 minutes and rapidly cooled to room temperature. Quality analyses were conducted for both physicochemical and microbiological parameters. Physicochemical parameters included color, pH, total volatile base nitrogen (TVB), trimethylamine nitrogen (TMA-N), and lactic acid. Microbiological quality was evaluated by analyzing mesophile bacteria, fecal coliforms, *E. coli*, *Salmonella* spp., *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*.

2.6. Effect of Sa-Tay Marinating on Shelf-Life of Oyster Meat

Sa-tay marinade was prepared. The Sa-tay mixture included 50% commercial curry powder, 25% garlic, and 25% galangal. The fresh garlic and galangal were blended using a blender (Philips HR-2068 blender, Thailand). Then, they were mixed with the curry powder. The oyster meat was marinated with the mixture for 30 minutes. The marinated oyster meat was pasteurized by placing it in three plastic bags and heating at 80°C for 8 minutes. It was quickly cooled to room temperature. The marinade samples were stored at 4°C for 12 days. They were sampled every 3 days to analyze changes in quality.

2.7. Determination

2.7.1. Physicochemical Determination

The color of marinated oyster meat was evaluated using a colorimeter equipped with HunterLab Universal Software. Color values were expressed in terms of L, a*, and b*, where L indicates lightness (ranging from 0 to 100), a* represents the red-green axis (positive values indicate redness, while negative values indicate greenness), and b* represents the yellow-blue axis (positive values indicate yellowness, while negative values indicate blueness).

The pH of the oyster meat sample was determined by homogenizing it with sterilized distilled water at a ratio of 1:5 (sample:water). The mixture was left to stand for 2 minutes before measurement. The pH was then measured using a Mettler Toledo 350 pH meter (Singapore).

The total volatile base nitrogen (TVB-N) content was determined using the Conway micro-diffusion method, as described by Conway and Byrne (1936). A 2 g portion of oyster meat was homogenized with 8 ml of 4% trichloroacetic acid (TCA) at 6,500 rpm for 1 minute. The homogenate was filtered using Whatman No. 41 filter paper, and the filtrate was collected for analysis. One milliliter of the extract was placed in the outer ring of a Conway unit, while

the inner ring contained 1% boric acid with Conway indicator. To initiate the reaction, 1 ml of potassium carbonate (K_2CO_3) was added to the extract in the outer ring. The unit was sealed and incubated at room temperature for 90 minutes, allowing volatile bases to diffuse into the boric acid solution. The inner solution was then titrated with 0.02 N hydrochloric acid (HCl) until the color changed from green to pink. The TVB-N content was calculated based on the volume of HCl used.

Trimethylamine (TMA) was measured using the same extract prepared for the TVB-N analysis. In this procedure, 1 ml of 10% neutralized formaldehyde was added to the extract in the outer ring of the Conway unit. As in the TVB-N method, the inner ring contained 1% boric acid with Conway indicator, and the reaction was initiated with 1 ml of K_2CO_3. After sealing the unit and incubating it at room temperature for 90 minutes, the amount of TMA that had diffused into the inner solution was determined by titration with 0.02 N HCl, using the same color-change endpoint.

Lactic acid content was determined following the method of Simsek, Sagdic, and Ozcelik (2007). Five grams of oyster meat were blended and homogenized with 50 ml of distilled water. After mixing, five drops of 1% phenolphthalein indicator were added. The mixture was then titrated with 0.1 M sodium hydroxide (NaOH). The lactic acid content was calculated using the following formula:

$$\% \text{ Lactic Acid} = \frac{\text{Molarity of NaOH X molecular mass of lactic acid X 100}}{\text{Weight of the sample (g)}}$$

where, Molarity of NaOH = 0.1

Molecular mass of lactic acid = 90.08

2.7.2. Microbiological Determination

Total viable count (mesophile bacteria), *Salmonella* spp., fecal coliforms, *E. coli*, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* were determined according to the procedure of BAM (2002).

2.7.3. Data Analysis

Data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Duncan's New Multiple Range Test (DMRT).

3. RESULTS

3.1. Effect of Pasteurization of Oyster Meat on Pathogenic Bacteria

Pathogenic bacteria pose significant health risks, leading to foodborne illnesses and outbreaks. Pasteurization is a widely used heat treatment process that aims to eliminate or reduce microbial contamination while preserving the quality of food. The effectiveness of pasteurization depends on factors such as temperature, duration, and bacterial heat resistance. This study investigated how different pasteurization conditions affect the survival of pathogenic bacteria in oyster meat, with a focus on enhancing microbiological safety without compromising product quality.

3.1.1. E. Coli

The effect of pasteurization temperature of oyster meat on *E. coli* was shown in Figure 1. The initial amount of *E. coli* was 1.8×10^8 CFU/g. After heating at 65–80°C, the *E. coli* count decreased with prolonged incubation time. In particular, the count rapidly decreased from 10^8 CFU/g to 10^4 CFU/g within the first minutes of incubation. After that, the rate of reduction slowed. After 8 minutes, *E. coli* was completely eliminated at 70, 75, and 80 °C. However, at 65 °C, approximately 10^3 CFU/g remained.

3.1.2. S. Typhimurium

Figure 2 revealed the effect of pasteurization temperature on *S.* Typhimurium. Initially, the bacterial count was 2.8 x10^8 CFU/g. When heating at 65-80°C, the *S.* Typhimurium count significantly decreased. Notably, complete elimination was observed after 5 minutes at 80°C.

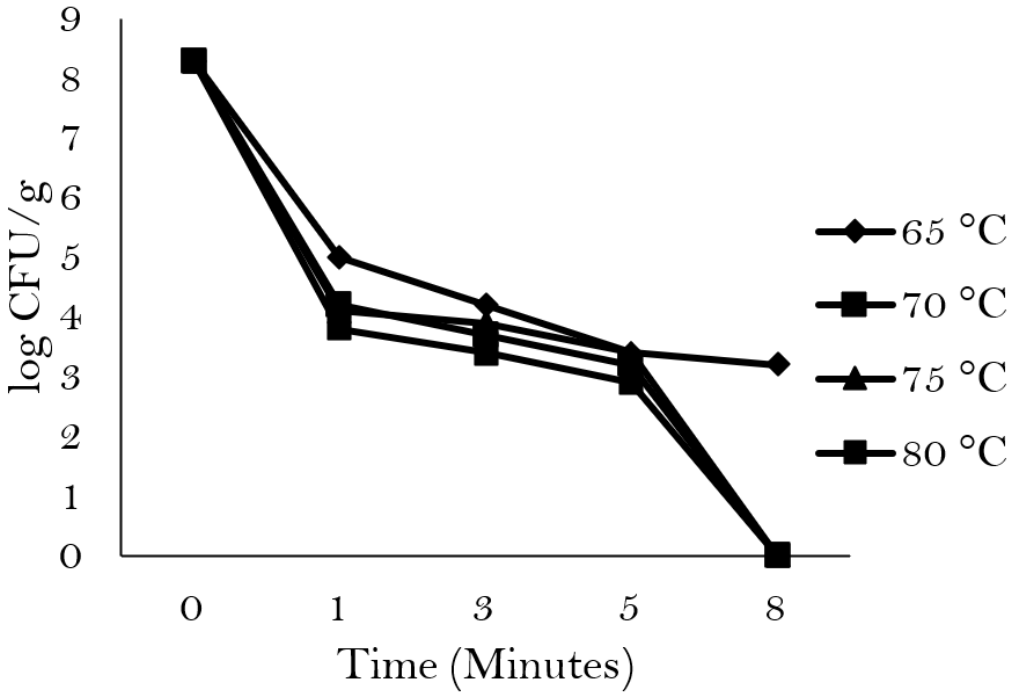

Figure 1. Survival of *E. coli* during pasteurization at various times and temperatures.

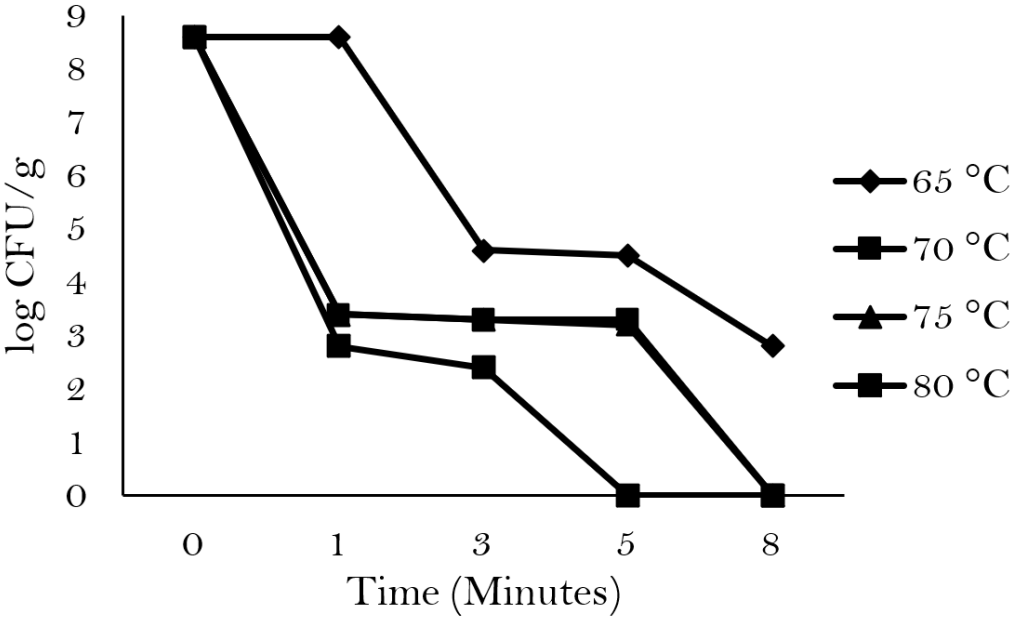

Figure 2. Survival of S. Typhimurium during pasteurization at various times and temperatures.

3.1.3. V. Cholerae

In this study, *V. cholerae* was inoculated into oyster meat at an initial concentration of 2.6 x 10^8 CFU/g. Complete elimination was observed after 5 minutes at 75°C and 80°C, and after 8 minutes at 65°C and 70°C. However, a rapid reduction in bacterial count was consistently observed within the first minute of heating across all treatments (Figure 3).

3.1.4. *V. parahaemolyticus*

The heat resistance of *V. parahaemolyticus* in oyster meat is shown in Figure 4. The initial count of *V. parahaemolyticus* was 1.9x10^8 CFU/g. Samples were heated at 65-80°C. As the incubation time increased, the *V. parahaemolyticus* count decreased, with complete elimination observed at 75°C and 80°C after 8 minutes. However, approximately 10^3 CFU/g remained at 65°C and 70°C after 8 minutes of heating.

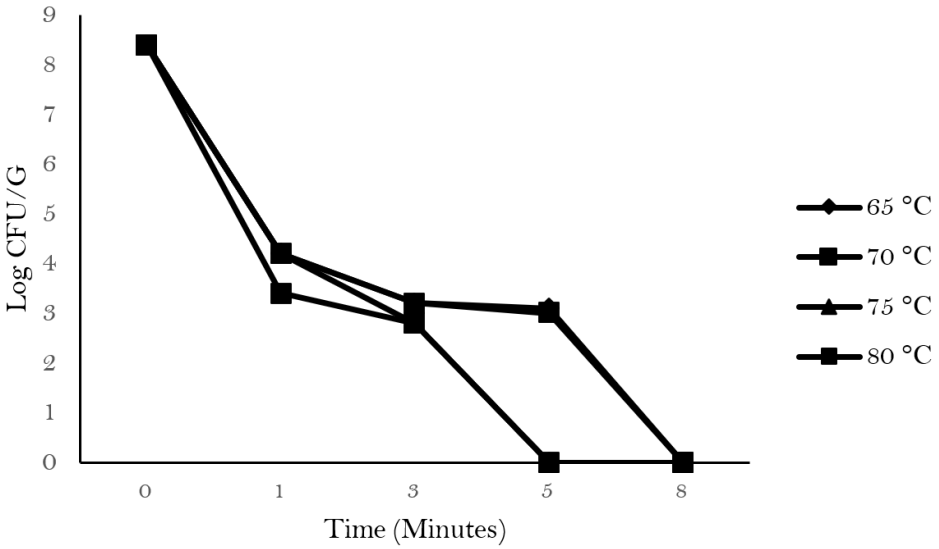

Figure 3. Survival of *V. cholerae* during pasteurization at various times and temperatures.

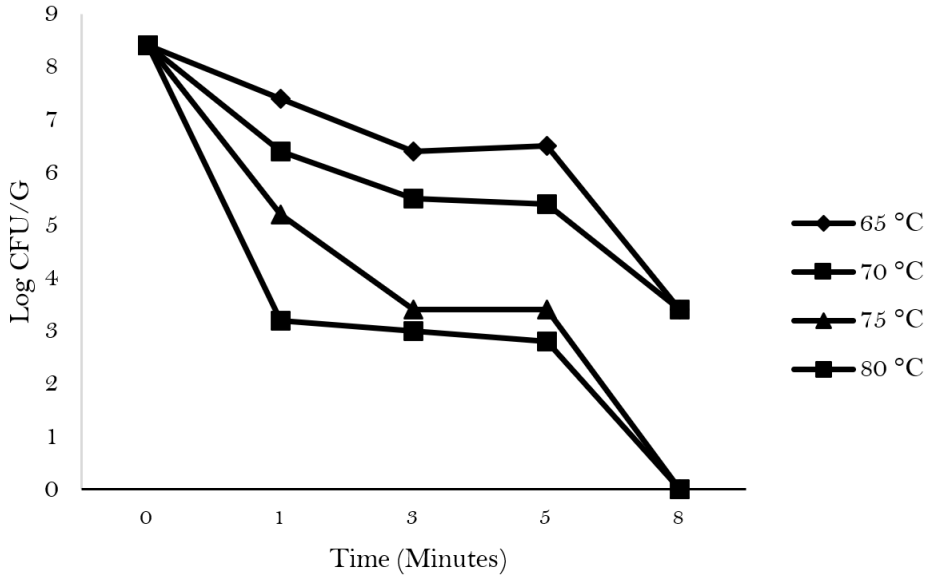

Figure 4. Survival of *V. parahaemolyticus* during pasteurization at various times and temperatures.

3.1.5. *V. Vulnificus*

The survival of *V. vulnificus* is shown in Figure 5. It was observed that the *V. vulnificus* count decreased significantly when samples were heated at 65-80°C. The count decreased from 2.7x10^8 CFU/g to undetectable levels within 5 and 8 minutes at 80°C and 75°C, respectively. *V. vulnificus* remained detectable at 65°C and 70°C, with approximately 10^3 CFU/g present after 8 minutes of heating.

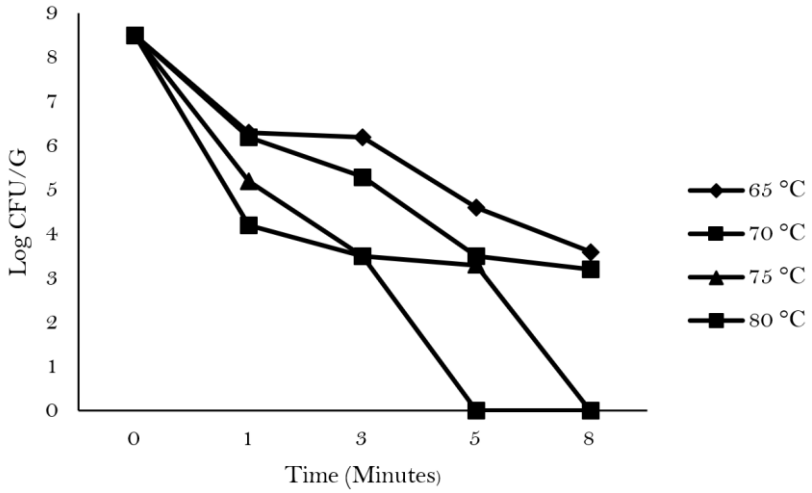

Figure 5. Survival of *V. vulnificus* during pasteurization at various times and temperatures.

3.2. Effect of Pasteurization of Oyster Meat Quality

Pasteurization is widely applied to seafood products to reduce microbial load while preserving sensory and nutritional properties. While the effectiveness of pasteurization in improving food safety is well documented, its impact on the physical, chemical, and microbiological quality of oyster meat requires further investigation. Factors such as temperature and heating duration can influence the color, pH, TVB, TMA-N, and microbial stability of pasteurized oysters.

3.2.1. Physical Quality

Table 1 shows that the unpasteurized oyster meat exhibited a brighter color compared to pasteurized samples. The L value decreased from 66.10±0.01 to 55.19±0.33, likely due to heat-induced protein denaturation and subsequent precipitation, which led to a darker appearance. Additionally, the cooking loss of pasteurized oyster meat was 26.07%.

Table 1. Physical qualities of pasteurized oyster meat.

Parameters	Experiment	
	Control	Pasteurized
Color L	66.10±0.01^{a}	55.19±0.33^{b}
a*	0.75±0.04^{ns}	0.67±0.12
b*	14.31±0.11^{a}	8.42±0.51^{b}
% Cooking loss	-	26±0.07

Note: Data are expressed as means ± the standard deviations (n = 3).
^{ns} = The mean values within row are not significantly different (P > 0.05).
Mean values within a row with different superscript letters (^{a,b}) are significantly different (P < 0.05).

3.2.2. Chemical Quality

Table 2 reveals that pasteurization had a minimal effect on pH. Similarly, pasteurization had a minimal effect on lactic acid levels. This corresponds to the slight change in pH observed after pasteurization. Neither pH nor lactic acid levels showed significant differences after pasteurization (p>0.05). The pH values of both control and pasteurized samples ranged from 5.80 to 5.99. TVB and TMA-N were not detected.

Table 2. Chemical qualities of pasteurized oyster meat.

Parameters	Experiment	
	Control	Pasteurization
pH	5.80±0.01	5.99±0.01
Lactic acid content (%)	0.09±0.01	0.1±0.01
TVB-N (mg/100 g)	Not detected	Not detected
TMA (mg/100 g)	Not detected	Not detected

3.2.3. Microbiological Quality

Pasteurization resulted in a reduction of microorganisms, particularly total viable count (TVC) and the coliform group. TVC decreased from 5.1×10^2 to 3.1×10^2 CFU/g. The coliform group was also reduced, as determined by the MPN method. Fecal coliforms and *E. coli* levels were reduced from 540 and 26 MPN/g, respectively, to <1.8 MPN/g. *V. cholerae* and *Salmonella* spp. were not detected in 25 g samples. Similarly, *V. parahaemolyticus* and *V. vulnificus* levels were <0.3 and <3.0 MPN/g, respectively. These results indicated no contamination from the four tested pathogenic microorganisms in the oyster meat samples (Table 3).

Table 3. Microbiological qualities of pasteurized oyster meat.

Parameters	Experiment	
	Control	Pasteurization
Total viable plate count (CFU/g)	5.1×10^2	3.1×10^2
Fecal Coliforms (MPN/g)	540	<1.8
Escherichia coli (MPN/g)	26	<1.8
V. vulnificus (MPN/g)	<3.0	<3.0
V. parahaemolyticus (MPN/g)	<0.3	<0.3
V. cholerae (/25g)	Not detected	Not detected
Salmonella spp. (/25g)	Not detected	Not detected

3.3. Effect of Sa-Tay Marination on Shelf-Life of Oyster Meat

3.3.1. Physical and Chemical Qualities

The pH of the marinated samples was found to be higher than that of the non-marinated samples, at approximately 6.0 and 5.5, respectively (Table 4). After storage at 4°C for 9 days, the pH gradually decreased in all sample sets. However, TVB levels appeared to slightly increase after 9 and 12 days of prolonged storage. The TVB value in all samples remained below 30 mg N/100 g. TMA-N was not detected in any of the sample sets. Marination with Sa-tay mixture was found to reduce the a* value. This marination also reduced the brightness (L value) of the pasteurized oyster meat.

3.3.2. Microbiological Quality

Table 5 presents the changes in the microbiological qualities of pasteurized oyster meat combined with Sa-tay marination. Pasteurization of oyster meat resulted in a lower bacterial count compared to non-pasteurized samples. Total viable count (TVC) tended to increase as storage time progressed. The results also revealed that the TVC of non-pasteurized and non-Sa-tay marinated samples exceeded 10^6 CFU/g from day 3 onward. Nevertheless, the TVC in Sa-tay-marinated samples was higher than in non-marinated ones. All pasteurized samples maintained TVC levels below 10^6 CFU/g throughout the entire experiment. Coliform bacteria, including fecal coliforms and *E. coli*, were undetectable (<1.8 MPN/g) in the oyster meat samples after pasteurization, as determined by the MPN method. It is assumed that bacterial levels may have exceeded 1600 MPN/g in the non-pasteurized samples by day 3. In contrast, both fecal coliforms and *E. coli* in the non-pasteurized but marinated samples exceeded 1600 MPN/g by day 12. Pathogenic bacteria, including *V. parahaemolyticus* and *V. vulnificus*, were found at <0.3 and <3.0 MPN/g, respectively, and their levels remained stable over time. Moreover, no contamination of *V. cholerae* or *Salmonella* spp. was detected in any 25 g oyster meat sample throughout the storage period.

Table 4. Changes in the physical and chemical qualities of pasteurized oyster meat combined with Sa-tay marination.

Storage time (Days)	Treatment	Chemical				Physical (Color)		
		pH	Lactic acid content (%)	TVB (mg N/100 g)	TMA (mg /100 g)	L	a*	b*
0	No pasteurize +No marinade	5.50±0.01	0.18±0.01	Not detected	Not detected	51.44±0.04	2.74±0.07	15.65±0.13
	No pasteurize +Marinade	6.02±0.03	0.10±0.01	Not detected	Not detected	52.58±0.02	1.11±0.1	14.88±0.13
	Pasteurize +No marinade	5.78±0.01	0.24±0.05	Not detected	Not detected	55.51±0.03	3.25±0.07	29.71±0.10
	Pasteurize +Marinade	6.15±0.04	0.22±0.02	Not detected	Not detected	47.49±0.02	1.15±0.06	27.83±0.23
3	No pasteurize +No marinade	4.79±0.02	0.39±0.01	Not detected	Not detected	56.52±0.08	2.60±0.06	16.19±4.88
	No pasteurize +Marinade	5.80±0.02	0.14±0.01	Not detected	Not detected	59.37±0.05	1.8±0.09	16.02±0.12
	Pasteurize +No marinade	4.59±0.03	0.46±0.01	Not detected	Not detected	54.89±0.13	3.12±0.22	25.54±0.40
	Pasteurize +Marinade	5.75±0.04	0.11±0.01	Not detected	Not detected	50.08±0.20	0.7±0.09	24.61±0.28
6	No pasteurize +No marinade	4.55±0.04	0.74±0.03	Not detected	Not detected	61.49±0.03	2.73±0.10	17.01±0.21
	No pasteurize +Marinade	6.21±0.05	0.23±0.02	Not detected	Not detected	55.03±0.02	1.66±0.10	14.34±0.16
	Pasteurize +No marinade	4.76±0.03	0.79±0.02	Not detected	Not detected	57.01±0.03	4.08±0.05	28.50±0.15
	Pasteurize +Marinade	6.24±0.02	0.26±0.02	Not detected	Not detected	49.40±0.32	1.29±0.05	27.61±0.37
9	No pasteurize +No marinade	4.34±0.04	0.55±0.01	1.00	Not detected	67.82±0.30	3.66±0.80	21.25±0.23
	No pasteurize +Marinade	5.53±0.03	0.26±0.01	Not detected	Not detected	59.61±0.07	1.32±0.05	15.32±0.09
	Pasteurize +No marinade	4.48±0.01	0.55±0.04	0.75	Not detected	53.84±16.28	3.44±0.05	34.54±0.08
	Pasteurize +Marinade	5.39±0.01	0.15±0.01	0.50	Not detected	54.22±0.03	1.14±0.06	28.98±0.15
12	No pasteurize +No marinade	4.50±0.60	0.71±0.04	1.50	Not detected	72.08±0.10	3.98±0.05	20.32±0.14
	No pasteurize +Marinade	6.37±0.01	0.11±0.01	0.75	Not detected	56.33±0.05	1.53±0.08	14.53±0.09
	Pasteurize +No marinade	4.60±0.02	0.55±0.26	Not detected	Not detected	66.94±0.05	2.90±0.29	29.78±0.72
	Pasteurize +Marinade	6.37±0.01	0.14±0.02	Not detected	Not detected	44.87±0.23	4.89±0.51	27.49±0.40

Table 5. Changes in the microbiological qualities of pasteurized oyster meat combined with Sa-tay marination.

Bacteria type	Treatment	Storage time(days)				
		0	3	6	9	12
Total viable plate count (CFU/g)	No pasteurize +No marinade	3.0×10^{4}	1.4×10^{6}	4.7×10^{7}	9.4×10^{7}	6.0×10^{7}
	No pasteurize +Marinade	3.0×10^{4}	1.2×10^{6}	3.8×10^{7}	1.1×10^{8}	5.4×10^{7}
	Pasteurize +No marinade	4.4×10^{2}	8.2×10^{2}	4.8×10^{4}	1.0×10^{5}	8.6×10^{4}
	Pasteurize +Marinade	<250 EAPC	9.5×10^{2}	7.4×10^{3}	4.4×0^{5}	1.0×10^{5}
Fecal coliforms (MPN/g)	No pasteurize +No marinade	920	>1,600	>1,600	>1,600	>1,600
	No pasteurize +Marinade	920	>1,600	>1,600	>1,600	>1,600
	Pasteurize +No marinade	<1.8	<1.8	<1.8	220	>1,600
	Pasteurize +Marinade	<1.8	<1.8	<1.8	46	<1.8
Escherichia coli (MPN/g)	No pasteurize +No marinade	<1.8	1,600	25	350	24
	No pasteurize +Marinade	21	48	48	47	24
	Pasteurize +No marinade	9.3	<1.8	130	>1,600	<1.8
	Pasteurize +Marinade	<1.8	<1.8	<1.8	46	<1.8
Vibrio parahaemolyticus (MPN/g)	No pasteurize +No marinade	<0.3	<0.3	<0.3	<0.3	<0.3
	No pasteurize +Marinade	<0.3	<0.3	<0.3	<0.3	<0.3
	Pasteurize +No marinade	<0.3	<0.3	<0.3	<0.3	<0.3
	Pasteurize +Marinade	<0.3	<0.3	<0.3	<0.3	<0.3
Vibrio vulnificus (MPN/g)	No pasteurize +No marinade	<3.0	<3.0	<3.0	<3.0	<3.0
	No pasteurize +Marinade	<3.0	<3.0	<3.0	<3.0	<3.0
	Pasteurize +No marinade	<3.0	<3.0	<3.0	<3.0	<3.0
	Pasteurize +Marinade	<3.0	<3.0	<3.0	<3.0	<3.0

Table 5. Microbiological qualities changes of pasteurized oyster meat combined with Sa-tay marinating (Continued).

Bacteria type	Treatment	Storage time(days)				
		0	3	6	9	12
Vibrio cholerae (/25 g)	No pasteurize +No marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	No pasteurize +Marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	Pasteurize +No marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	Pasteurize +Marinade	Not detected	Not detected	Not detected	Not detected	Not detected
Vibrio spp. (/25 g)	No pasteurize +No marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	No pasteurize +Marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	Pasteurize +No marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	Pasteurize +Marinade	Not detected	Not detected	Not detected	Not detected	Not detected
Salmonella spp. (/25g)	No pasteurize +No marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	No pasteurize +Marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	Pasteurize +No marinade	Not detected	Not detected	Not detected	Not detected	Not detected
	Pasteurize +Marinade	Not detected	Not detected	Not detected	Not detected	Not detected

4. DISCUSSION

4.1. Effect of Pasteurization on Pathogenic Bacteria in Oyster Meat

Pasteurization is typically conducted at temperatures below the boiling point of water, with the primary objective of extending shelf life by inactivating non-spore-forming pathogenic bacteria and most vegetative spoilage microorganisms, as well as by suppressing microbial and enzymatic activity. This method is often applied in combination with other preservation techniques, such as concentration, acidification, or chemical inhibition. There are two main types of pasteurization processes: slow and rapid. Slow pasteurization involves moderate heating over an extended period; common temperature-time combinations include 63–65°C for 30 minutes or 75°C for 8–10 minutes. In contrast, rapid pasteurization applies higher temperatures for shorter durations, typically heating food to 65–80°C for less than 10 minutes, followed by immediate cooling (He, Kong, Liu, Xia, & Wang, 2021). For instance, the pasteurization of marinated shrimp in green curry paste at 65–70°C for varying durations significantly reduced total viable bacterial counts, including *Listeria* spp., thereby ensuring product safety for up to 15 days under refrigerated storage at 0–3°C (Mohamed & Anuntagool, 2015).

E. coli is a common natural microbiota found in raw oysters due to the mollusk's nature as a filter feeder, which allows it to accumulate and concentrate pathogenic microorganisms (Plaza & Gabriel, 2008). It is considered an indicator of water quality, pollution, or fecal contamination. Heating effectively reduces *E. coli* levels, with a rapid initial decline followed by a slower reduction over time. Complete elimination occurs at higher temperatures, while lower temperatures may leave some bacteria remaining even after extended exposure. This is because pasteurization can significantly alter the structure and composition of *E. coli*, leading to increased release and changes in lipopolysaccharide (LPS) components, which are crucial for the bacterium's endotoxicity and immune evasion. For instance, pasteurization of *E. coli* results in changes in LPS composition, increased LPS release, and alterations in the distribution of specific components such as D-xylose, octadecanoic acid, and D-ribopyranose (Abraham, Venter, Lues, Ivanov, & de Smidt, 2012). These findings are consistent with those of Chung and colleagues, who reported that no *E. coli* was detected in Akoya oysters (*Pinctada fucata*) heated at 50°C for 5, 10, or 20 minutes, indicating that the oysters were likely safe for consumption with or without treatments within 14 days of storage (Chung, Howieson, & Chaklader, 2021). Moreover, pulsed electric field (PEF) technology, an alternative to traditional thermal pasteurization, has demonstrated complete elimination of *E. coli* in seafood, proving its efficacy in ensuring food safety on an industrial scale (Duvoisin et al., 2022).

Salmonella enterica is a leading cause of foodborne illness worldwide and is most commonly associated with contaminated foods of animal origin. The incidence of Salmonella infections has risen dramatically since the 1980s, with many cases caused by seafood, particularly due to the consumption of shellfish. Salmonellosis is characterized by fever, abdominal cramps, and diarrhea. Heating significantly reduces *S.* Typhimurium levels, with higher temperatures leading to complete elimination within a short duration. This is similar to *E. coli*, which also tends to decrease rapidly within the first minute of heating at 70, 75, and 80°C. The efficiency of bacterial destruction was low at 65°C compared to other temperatures. This may be because both *E. coli* and *S.* Typhimurium are Gram-negative bacteria, resulting in a similar pattern of heat destruction. However, the efficacy of pasteurization in controlling *Salmonella* spp. in seafood products highlights the importance of optimizing pasteurization parameters to maximize food safety without compromising product quality.

Vibrio bacteria normally inhabit coastal waters where oysters live. Because oysters feed by filtering water, *Vibrio* spp. can accumulate in their tissues. The consumption of raw or undercooked oysters can cause illness due to the presence of *Vibrio* spp. in the oyster tissues. *V. cholerae*, the type species of the genus Vibrio, is the causative agent of cholera outbreaks and epidemics. Cholera enterotoxin (CT) is the primary virulence factor responsible for the disease cholera. In our work, *V. cholerae* was introduced at the beginning and was completely eliminated after heating, with higher temperatures achieving faster reduction. The most significant decrease occurred within the first minute of heating. Chung and colleagues concluded that 10- and 20-minute heat treatments at 50°C were effective in

suppressing the growth of *Vibrio* spp. until days 7–14 (Chung et al., 2021). These findings were similar to the work of Plaza & Gabriel, (2008) who reported that total *Vibrio* spp. could be easily reduced with mild heat treatment. Hence, pasteurization, particularly at higher temperatures and for adequate durations, is a crucial step in reducing *V. cholerae* in oyster meat, thereby enhancing food safety and reducing the risk of bacterial infections.

Most of the existing cases of oyster-related gastroenteritis were caused by *V. parahaemolyticus*, resulting in mild illnesses, including diarrhea and vomiting. *V. parahaemolyticus* is pathogenic to humans. It is found in marine and estuarine environments and may cause gastrointestinal illness, either as isolated cases or outbreaks. The heat resistance of *V. parahaemolyticus* in oyster meat decreases with prolonged heating. Higher temperatures lead to complete elimination, while lower temperatures result in partial reduction after the same duration. Chung and his team reported that *V. parahaemolyticus* was detected in Akoya oysters heated at 50$^{\circ C}$ for 5, 10, or 20 minutes during 14 days of storage (Chung et al., 2021). Pasteurization is effective in significantly reducing *V. parahaemolyticus* in oyster meat; however, its efficacy can vary depending on the bacterial strain, initial contamination levels, and specific processing conditions, highlighting the need for integrated approaches to ensure seafood safety.

V. vulnificus is recognized as the leading cause of seafood-related mortality and is most commonly associated with the consumption of raw oysters harvested from the Gulf Coast. Epidemiological and clinical studies have demonstrated that *V. vulnificus* can cause severe septicemia and death following either ingestion of contaminated seafood or wound infections acquired from marine environments. Heat treatment has been shown to significantly reduce the survival of *V. vulnificus*, with higher temperatures achieving complete inactivation in shorter timeframes, whereas lower temperatures allow some bacterial survival even after extended exposure. Our results support existing evidence that thermal processing is effective in inactivating pathogenic *Vibrio* spp, including *V. cholerae* and *V. vulnificus* (Blake, Weaver, & Hollis, 1980; Joseph, Colwell, & Kaper, 1982). Research on low-temperature pasteurization has further demonstrated its efficacy in reducing *Vibrio* spp. to non-detectable levels. In one study, both artificially inoculated *V. vulnificus* and *V. parahaemolyticus*, as well as naturally occurring *V. vulnificus* in live oysters, were subjected to pasteurization at 50°C for up to 15 minutes. Microbiological analysis over a 14-day storage period revealed that low-temperature pasteurization effectively reduced pathogen levels from over 10^5 CFU/g to undetectable levels within 10 minutes of treatment (Andrews, Park, & Chen, 2000).

4.2. Effect of Pasteurization on Oyster Meat Quality

The oyster meat was pasteurized at 80°C for 8 minutes, which was identified as the optimal condition for eliminating foodborne pathogenic bacteria based on the pasteurization study. After rapid cooling to room temperature, quality analyses were conducted to assess physicochemical properties and microbiological quality. Pasteurization caused the oyster meat to become darker due to protein changes induced by heat. Additionally, heat treatment led to a noticeable loss of moisture during cooking. Solo-de-Zaldívar and co-workers found that pasteurization produced a significant decrease in water-binding capacity and cooking loss in fish mince gel. This was due to structural changes during pasteurization, which caused greater network damage (Solo-de-Zaldívar, Tovar, Borderías, & Herranz, 2015). Mozuriene and colleagues also reported reduced water-holding capacity and increased cooking loss in pork meat marinated with a lacto-fermented marinade (Mozuriene et al., 2016).

pH is influenced by the conversion of glycogen to lactic acid and by the degradation of muscle components (e.g., proteins and nucleotides) into amine compounds and bases during storage. This indicates that pasteurization had a minor chemical effect on pH. Pasteurization also had a minimal effect on lactic acid. This was attributed to a slight change in pH following pasteurization. Neither pH nor lactic acid showed a significant difference after pasteurization ($p>0.05$). In this study, the pH values of both control and pasteurized samples ranged between 5.80 and 5.99, indicating good quality with minimal presence of organic acids. These findings were consistent with previous studies. Previous studies also reported that freshly shucked oysters typically have a pH between 6.0 and 6.6, and that a lower pH can indicate quality degradation (Cruz-Romero, Kelly, & Kerry, 2007; Liu, Yang, Yuan, & Wu, 2010). Bunruk et

al. (2013) reported that the initial pH value of the oyster sample was 6.57, which decreased to 5.6 during storage. Similar pH trends were observed in oyster meat treated with 2 ml garlic, 2 ml garlic combined with 8% Sa-tay marinade, and 8% Sa-tay marinade alone.

TVB comprises the volatile amines, mainly dimethylamine, trimethylamine, and ammonia. TVB and TMA-N were not detected. This indicates that the oyster meat samples were very fresh. Chung and his team found that TVB-N was significantly influenced by pasteurization time and storage duration, with notable increases observed from day 10 onward in all heat-treated Akoya oyster samples, except for those treated for 5 minutes compared to the 0-minute control. TVB-N increased gradually from day 4 onward in oysters treated for 5 minutes (Chung et al., 2021).

Pasteurization caused a reduction in microorganisms, particularly in the TVC and coliform groups. It significantly reduced the total viable count and coliform bacteria, including fecal coliforms and *E. coli.* Additionally, *V. cholerae* and *Salmonella* spp. were not detected, while *V. parahaemolyticus* and *V. vulnificus* were present at levels of <0.3 and <3.0 MPN/g, respectively. It was evident that there was no contamination by these pathogenic microorganisms in the tested oyster meat samples. Chung and his team also reported that low-temperature pasteurization, especially the 10-minute heat treatment, resulted in the lowest average TVC count while still being effective in suppressing *Vibrio* spp. After 20 minutes of treatment, the major bacterial levels in oysters showed no presence of *E. coli* or *V. parahaemolyticus* (Chung et al., 2021).

4.3. Effect of Sa-Tay Marination on Shelf-Life of Oyster Meat

The effect of Sa-tay marination on the shelf life of oyster meat was investigated. It was found that the pH of the marinated sample was higher than that of the non-marinated sample. This could be due to components of the Sa-tay mixture containing alkaline substances that became apparent after marination. Moreover, pasteurization did not affect the pH value. After storage at 4°C for 9 days, the pH tended to decrease in all sample sets. This was related to the increase in lactic acid content over the prolonged storage period. The non-pasteurized marinated sample showed a higher pH compared to the pasteurized sample. This might be because the Sa-tay mixture supported the growth of microorganisms that produce lactic acid Bunruk et al. (2013) reported that the initial pH value of the oyster sample was 6.57, and oyster meat marinated with 8% Sa-tay (without garlic juice injection) appeared to have a higher pH, although the difference was not significant compared to other treatments. In contrast, Babikova, Hoeche, Boyd, and Noci (2020) reported that the marinated product from Irish sprat (*Sprattus sprattus*). Their results showed that a reduction in pH and water activity, along with increased salt content, could inhibit all microbial growth across all curing stages. However, the TVB value appeared to slightly increase after 9 and 12 days of storage. The TVB value of all detected samples was lower than 30 mg N/100 g, the threshold considered unfit for human consumption (El Marrakchi, Bennour, Bouchriti, Hamama, & Tagafait, 1990; Harpaz, Glatman, Drabkin, & Gelman, 2003). TMA-N was not detected in any of the sample sets. Marination with the Sa-tay mixture reduced the a* value, as one component yellow curry powder imparted a yellow shade to the product. This marination also reduced the brightness (L value) of the pasteurized oyster meat.

The pasteurization of oyster meat resulted in a lower bacterial count compared to non-pasteurized samples. Nevertheless, the TVC of the sa-tay marinated sample was higher than that of the non-marinated sample. This might be due to contamination introduced by the ingredients in the sa-tay mixture. Coliform group, including fecal coliform and *E. coli*, in the oyster meat samples, was not detected after pasteurization, as assessed by the MPN method. It is suspected that the levels in the non-pasteurized samples on day 3 may have exceeded 1,600 MPN/g. Conversely, both fecal coliform and *E. coli* in the non-pasteurized marinated sample exceeded 1,600 MPN/g on day 12. Pathogenic bacteria, namely *V. parahaemolyticus* and *V. vulnificus*, were not detected using the MPN method, and prolonged storage did not affect their presence. Furthermore, no contamination with *V. cholerae* and *Salmonella* spp. was detected in the oyster meat samples throughout the storage period. Marination liquids have been reported to reduce microflora in marinated products. This method has also been studied in rainbow trout using apple juice, black currant, rhubarb,

and tomato pomace as marinating solutions. The results showed that mesophilic aerobic microorganisms, including *Pseudomonas* spp., yeast and mold, remained at acceptable levels throughout the storage period at 6 ± 1°C for 15 days (Roasto et al., 2023). Sengun, Goztepe, and Ozturk (2019) reported that the marination liquids, including koruk juice, exhibited antibacterial activity against *S.* Typhimurium, a bacterium commonly found on contaminated poultry meat. Additionally, the koruk juice marination improved the quality characteristics of poultry meat by reducing background microflora counts. Bunruk et al. (2013) reported that mesophilic bacteria and lactic acid levels in the marinated oyster samples gradually increased with extended storage time. However, oyster meat injected with garlic juice but without Sa-tay marination showed the highest total bacterial count. Meanwhile, psychrophilic bacteria, coliforms, fecal coliforms, *E. coli*, *S. aureus*, *Salmonella* spp., and *Vibrio* spp. remained at low levels throughout the storage period.

5. CONCLUSION

It can be concluded that pasteurization was effective in eliminating five tested pathogenic bacteria species, which are *E. coli*, *S. Typhimurium*, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. This was observed following inoculation at 10^8 CFU/g and treatment at 80°C for 8 minutes. Under these pasteurization conditions, bacterial contamination in oyster meat was effectively eliminated. A decrease in bacterial counts was observed in both pasteurized and marinated oyster meat compared to fresh oyster meat. The Sa-tay marinade had a slight effect on the physical and chemical properties of oyster meat. Therefore, the combination of pasteurization and Sa-tay marination could effectively eliminate *E. coli*, *S. Typhimurium*, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* in oyster meat.

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