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Microbiological and Physico-Chemical Quality of Green Mussels *Perna viridis* (Linnaeus, 1758) along the Supply Chain in Bacoor City, Cavite, Philippines

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Abstract

Bacoor City, Cavite, is one of the Philippines' largest mussels producers. Thus, determining the changes in the microbiological and physico-chemical attributes of the mussels along its supply chain is essential to ensure the safety and quality of this commodity. Mussel samples were subjected to a time-distribution study to identify the presence of foodborne pathogens and to determine the changes in pH and drip loss. A high prevalence of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Vibrio* spp. was detected in the mussel culture sites. Microbiological counts and detection showed increased aerobic plate count (APC) along the supply chain, higher than the standard limits. The total coliform still conformed to the required range while E. coli levels increased along the supply chain, exceeding acceptable levels for raw consumption. For the presence of pathogenic bacteria in the different points of the supply chain, results revealed that enteric bacteria E. coli and *Salmonella* were present. Furthermore, pathogenic strains of *Vibrio* such as *V. parahaemolyticus*, *V. cholerae*, and *V. alginolyticus* were detected. The study emphasizes the need for improved post-harvest practices, including proper temperature control and packaging, to maintain the quality and safety of green mussels. Additionally, efforts to mitigate bacterial contamination in the culture areas and implement effective depuration processes are necessary to ensure consumer safety.

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Introduction

Green mussel (Perna viridis) is widely commercially cultivated in the Philippines.[1,2,3] The meat of *P. viridis* is a valuable food source for human consumption. It is an alternative source of cheap animal protein, containing 36.15% protein, 19.72% lipids, and 24.54% carbohydrates.[4]

Green mussels are known as filter feeders, which feed on suspended organic particles such as phytoplankton, zooplankton, and other organic materials by pumping water through a set of gill filaments and selectively discharging the inorganic particles like sediments through the excurrent siphon.[5,6] Therefore, these bivalve mollusks may also accumulate harmful microorganisms such as bacteria, viruses, and parasitic pathogens that could impact their quality and safety as food, and eventually, it may cause diseases in consumers.[7,8] Spoilage and potential safety issues are some of the implications of these contaminants which are also a result of pollution and improper handling in post-harvest and storage procedures.[8,9]

In the Philippines, Cavite is the top three-performing province that contributes to the bulk of shellfish harvest in the country.[10] Furthermore, Bacoor Bay is the most significant mussel-producing area in the Philippines, with approximately 1,350 hectares devoted to mussel farming and an estimated yearly production of 4,000 metric tons per hectare of live mussels.[11] The quality of mussels is highly influenced by the time between harvest and consumption, as they are highly perishable. Therefore, improved harvesting and post-harvesting processes are needed to ensure that mussels reach the market in optimal condition. To ensure the safety of mussel consumption, the current study aims to detect the level of pathogens and determine the physico-chemical changes at each point in the supply chain, specifically from producers to consumers.

Materials and Methods

Sampling Site

In the province of Cavite, green mussels (*P. viridis*), water, and soil sediment samples were collected within Canacao Bay's vicinities in April, the harvest month. Three (3) sampling areas (SA) were randomly selected between the coordinates 14˚ 29' 17.7" N to 14˚ 29' 37.98" N latitude and 120˚ 55' 39.6" E to 120˚ 55' 15.18" E longitude.

Survey Tool

The survey tool used to collect information about the structure and activities of the green mussel supply chain was based on Nuñal *et al*.[12] and Love *et al*.,[13] with some modifications. Respondents were chosen based on their direct participation in the supply chain, starting from the producers and identifying upstream and downstream customers. Respondents were interviewed based on the following inclusion criteria: i) the producer should be actively involved in mussel culture for more than five years; ii) the wholesaler should be actively involved in the direct buying and selling of the harvest for more than 5 years; iii) retailer should be actively involved in retailing to restaurants, food hub, and consumers for more than five years; and iv) consumers who are regular buyers for more than 5 years.

Simulation of the Supply Chain

Simulated time and distribution of green mussels along the supply chain in Bacoor City, Cavite, were identified through interviews and discussions with the respondents, specifically the producers, wholesalers, retailers, and consumers. The simulation was designed based on the results of surveys and interviews by following the actual handling practices, transit time, the temperature used, and packaging from mussel farmers to consumers. After harvest, four (4) sampling points within the supply chain of green mussels were identified for the simulation: at 0-3 hours, initial cleaning by the farmers after harvest; at 2-5 hours corresponding to the time upon receiving the mussels from producer to wholesalers; at 2-8 hours corresponding to the time when the supplies arrived at the market and sold by the retailers; and at 1-5 hours corresponding to the time when the products reach the consumers. All analyses conducted in this study were done at the Center for Lake and Sustainable Development (CLSD) of the Laguna State Polytechnic University, Los Baños, Laguna.

Physico-chemical Analysis of the Sampling Areas

The physico-chemical quality of water (temperature, dissolved oxygen, total dissolved solids, conductivity, pH, and salinity) in three (3) sampling areas was tested using a multi-parameter device (Laqua Horiba Multiparameter WQ330K). Surface water samples and sediments from the seabed were also collected.

Microbiological Analyses

Water and seabed sediment samples were collected at every sampling area using sterile 10 mL conical centrifuge tubes. Meanwhile, approximately 100 g of green mussel meat was shucked from the pooled samples and placed inside sterile tubes. All samples were packed individually in sterile plastic bags and preserved in ice, maintaining temperatures below 0°C until further microbiological analyses.

Aerobic Plate Count (APC)

Ten grams of samples were homogenized in 90 mL of sterile peptone water. Serial dilution was performed up to 10^{-5} and 0.1 mL sample was spread plated in plate count agar containing 0.5% sodium chloride (NaCl). After incubation at 37 °C for 24 h, colonies were counted and expressed in log10 CFU/g.[14]

Enumeration of Coliform, Fecal Coliform and *E. coli*

With some modifications, the Food and Drug Administration Bacteriological Analytical Manual protocols for quantitative enumeration of coliform and *E. coli* were used.[15] About 100 g of mussel meat was homogenized and added to 100 mL peptone water. Then, the Most Probable Number (MPN) was done using nine test tubes containing 9 mL of single-strength MacConkey broth purple with sterilized Durham's tube. In every 3 test tubes, 1 mL of sample from 10^{-1}, 10^{-2}, and 10^{-3} serially diluted tubes were inoculated. Tubes were incubated at 37ºC for 24 h and then examined for acid and gas production. After incubation, test tubes showing gas production were counted and recorded as positive for coliforms. For the confirmation test on the presence of fecal coliforms and *E. coli*, a loopful of suspension from positive tubes was inoculated to test tubes containing Escherichia coli (EC) broth, incubated at 44ºC for 24 h and were examined for gas production. To enumerate *E. coli*, the inoculum from the positive fecal coliform tubes was streaked onto eosin methylene blue agar (EMB) and incubated at 37 ºC for 24 h. Colonies exhibiting a metallic sheen with a dark center were inoculated into peptone water at 44ºC for 24 h. Test tubes were added drop-wise with an indole reagent and the formation of a reddish/pinkish top layer indicates the presence of *E. coli* in the sample.[16]

Detection of *Salmonella*

To detect *Salmonella*, a 25 g homogenized shellfish meat sample was added to 250 mL of lactose broth and incubated at 35 °C for 24 h for pre-enrichment. Afterward, 1 mL of the mixture was added to 10 mL of tetrathionate (TT) broth and vortexed, then incubated at 35 ± 2 °C for 24 h. A loopful from the TT broth was streaked on xylose lysine deoxycholate (XLD) agar plates, which were then incubated at 35 °C for 24 h. Presumptive *Salmonella* colonies were identified on the plates. For confirmation, suspected colonies were touched with a sterile inoculating needle and streaked on a triple sugar iron (TSI) slant, followed by a stab culture. The TSI cultures were incubated at 37ºC for 24 h.[17]

Detection and Enumeration of *Vibrio* spp.

To detect *Vibrio* species in green mussel samples, 100 g of homogenized sample was added to 100 mL of alkaline peptone water and serially diluted to 10^{-3} to determine the MPN value. The diluted samples were incubated overnight at 35 ± 0.5ºC and checked for turbidity. Then, loopfuls from these turbid tubes were streaked onto thiosulfate-citrate-bile salt sucrose (TCBS) agar and *Vibrio* Chromogenic (Condalab, Spain) culture media and incubated at 35 ± 0.5ºC for 24 h. Colonies were examined for the presence of different *Vibrio* species. Presumptive identification was based on colony color and growth characteristics in the culture media. When observed on TCBS agar, *V. parahaemolyticus* colonies exhibit a green coloration, while *V. cholerae* colonies manifest as flat yellow in appearance. On the other hand, when examining samples on *Vibrio* Chromogenic Agar, the presence of *V. cholerae* is denoted by colonies displaying a pink-rose color. *V. parahaemolyticus* is indicated by colonies with a greenish-blue hue, while *V. alginolyticus* is represented by colorless colonies.18

pH and Drip Loss of Mussel Meat

Collected green mussel meat samples were minced and weighed to determine the pH level. About 15 g of minced mussel meat was homogenized with 35 mL of distilled water (adjusted to pH 7) and kept in the solution for 30 min. Meanwhile, drip loss was determined by placing 100 g of green mussel meat in a conical-shaped filter paper and allowing it to

sit and exude the liquid. The percent drip loss was measured by comparing the differences in the initial and final weight after a determined period. The percentage drip loss was computed following Min *et al*.[19] Analyses were done in triplicates.

Statistical Analysis

Fisher's ANOVA and Tukey's post-hoc test was applied for parametric statistical evaluation. Each test was done using Jamovi® (Version 3.2)

Results

Post-Harvest Practices along the Supply Chain Point

The results of interviews along the supply chain points indicated that harvested green mussels from producers reaching the table of consumers were commonly packed live in saturated woven polypropylene (PP) sacks and transported without ice via closed van trucks, jeepneys, or open-top tricycles and exposed in natural weather condition making it susceptible to possible spoilage and contamination. In the culture areas situated in Canacao Bay, the harvesting of green mussels typically commences at 06:00 AM and concludes around 10:00 AM. The duration of the harvesting process is dependent on factors such as the number of green mussels ordered by wholesalers and the specific culture methods employed by the producers. Three culture methods are practiced in the area: stake, raft, and long-line method, wherein the latter is a modified raft method. A long-line fishing gear design was adapted instead of using bamboo rafts as floating materials where green mussel cultches are attached. Producers started the initial cleaning of shells immediately in the boat after harvest. They frequently rinsed them with seawater from the culture areas before transporting them to the landing site which has a distance of approximately 4 km. These activities take about 3 h before the shellfish reach the wholesalers. The wholesalers also do an additional 2-5 h post-harvest activities upon receiving the shellfish. Usually, the post-harvest activities were thorough washing and cleaning of shells, sorting by size, packing, and tagging in a woven PP sack, and transporting to the retailers in nearby municipalities and provinces. The most prolonged duration where green mussels as live products stayed between supply chain points is in the retailer's custody. Typically, it takes about 2-8 h before it reaches the consumers due to the demand for green mussels and competition amongst retailers in the market. Although the commodity stayed longer in this supply chain point, the commodity was still exposed in an open area without ice. Most retailers have no proper storage facilities or equipment (e.g., insulated containers) for storing their commodities for an extended period. Consumers typically have a maximum waiting time of 1-5 h to prepare the green mussels they purchase from the market before consuming them. Many shellfish consumers commonly employ the depuration method, which involves soaking mussels several hours before cooking to induce them to expel the contents of their stomach. This practice is adopted to ensure the safety of the mussels before consumption. Generally, common supply chain points of harvested green mussels in Bacoor City implemented simple post-harvest practices from producers to consumers, such as cleaning, washing, and frequent rinsing with seawater to delay spoilage. During transportation from point to point of the supply chain, no proper storage, equipment, or packaging was utilized to prolong the shelf-life of the commodity.

Physico-chemical and Microbial Characteristics of the Culture Site

The physico-chemical characteristics of water in three sampling areas within the vicinity of Canacao Bay, Cavite, where green mussels are cultivated are the following: temperature (31.38±0.22ºC); dissolved oxygen (6.54±0.82 mg/L); total dissolved oxygen (23,595±292.40 mg/L); conductivity (47.21±0.66 S/m); pH (7.41±0.22); and salinity (30.81±0.29 ppt).

Meanwhile, the microbial quality of water and sediment samples obtained from the green mussel culture environment in Bacoor City, Cavite, is presented in Table 1. The MPN values of total coliforms in sediments were found higher compared to the levels detected in the water samples taken from the surface. Additionally, high levels of fecal coliforms and *E. coli* were detected in the water samples. *Vibrio* spp. was also detected in water and sediment samples in all sampling areas. Table 2 displays the results of the confirmatory detection of *Vibrio* spp. and *Salmonella* spp. in the samples. The analysis indicates the presence of all four *Vibrio* species among sampling sites. Although some sites, such as SA2 for sediment, did not detect V. cholerae, its presence on other sampling sites still indicates its presence in Canacao Bay. Hence, the

same assumption can be made for the presence of *V. vulnificus*, although it was not detected in the water samples. Notably, *V. vulnificus*, a known virulent pathogen, was only detected in the sediment samples. Meanwhile, the presence of *Salmonella* was detected in both the water and sediment samples.

Table 1: Detection of bacterial pathogens in sediment and water samples collected from the culture areas of green mussels in Bacoor City, Cavite

Pathogens	Sediment*				Water*			
	SA1	SA2	SA3	Mean	SA1	SA2	SA3	Mean
Total Coliform	75	28	75	59.33	43	20	38	33.67
Fecal coliform and *E. coli*	150	460	460	356.67	1100	210	1100	803.33
Vibrio spp.	15	15	15	15	43	20	15	26

Values expressed in MPN/100g or mL.

Table 2: Confirmatory detection of bacterial pathogens in sediment and water samples collected from the culture areas of green mussels in Bacoor City, Cavite

Pathogens	Sediment*			Water*		
	SA1	SA2	SA3	SA1	SA2	SA3
V. parahaemolyticus	+	ND	+	+	+	ND
V. cholerae	+	ND	+	ND	ND	ND
V. alginolyticus	+	+	+	+	+	+
V. vulnificus	+	ND	+	ND	ND	ND
*Salmonella**	+	+	+	+	+	+

(+) - presence detected, (ND) - presence not detected, *detected at 25g

Microbial Counts of Mussels Along the Supply Chain

The microbial quality of green mussels harvested from Canacao Bay, Bacoor City, Cavite, was recorded during a simulated time and distribution across supply chain points. The APC in every supply chain point was the following: producer (log 7.40±0.58 CFU/g); wholesaler (log 7.63±0.40 CFU/g); retailer (log 8.31±0.92 CFU/g); consumer (8.23±0.98 CFU/g). Although an increased count was observed, results indicated no significant differences among sampling points.

The MPN values of the three microbiological parameters about the different points in the mussel supply chain are shown in Table 3. Results showed an increase in total coliforms (11-21 MPN/100g), fecal coliforms and *E. coli* (43-290 MPN/100g) along the supply chain. Meanwhile, *Vibrio* spp. increased in concentration from the producer to retailer (3.6–6.1 MPN/g) and decreased from retailer to consumer chain (6.1–3 MPN/g). Confirmatory tests of the presence of pathogens listed in Table 4 revealed that among the *Vibrio* species, *V. alginolyticus* was present in all sampling points. Earlier, *V. parahaemolyticus* was detected and reported to be present in the water and sediment samples, however, it was not detected in the mussel samples along the supply chain. Both *V. cholerae* and V. vulnificus were present in the wholesaler point only. The presence of *Salmonella* in the mussel samples along the supply chain was also confirmed.

Table 3: Detection of bacterial pathogens in green mussel samples collected from Bacoor City, Cavite during time and distribution study

Pathogens	Supplies Chain*				Standard
	Producer	Wholesaler	Retailer	Consumer	Range
Total Coliform	11	14	15	21	230 MPN/100g (FDA NSSP)
Fecal coliform and *E. coli*	43	93	150	290	230 MPN/100g (Codex Alimentarius)
Vibrio spp.	3.6	6.1	6.1	3	<30 MPN/g (FDA NSSP)

*Values expressed in MPN/100g.

Table 4: Confirmatory detection of bacterial pathogens in green mussel samples collected from Bacoor City, Cavite during time and distribution study

Pathogens	Supplies Chain			
	Producer	Wholesaler	Retailer	Consumer
V. parahaemolyticus	+	ND	+	+
V. cholerae	+	ND	+	ND
V. alginolyticus	+	+	+	+
V. vulnificus	+	ND	+	ND
Salmonella	+	+	+	+

Values expressed (+) - presence detected, (ND) - presence not detected, *detected at 25g sample

Table 5: Analyses of green mussel meat collected in Bacoor City, Cavite during time and distribution study

Analyses	Supplies Chain			
	Producer	Wholesaler	Retailer	Consumer
pH	6.16 ± 0.03^{a}	6.22 ± 0.16^{a}	06.07 ± 0.4^{a}	6.22 ± 0.57^{a}
Drip Loss (%)	$>1^{a}$	25.60 ± 4.73^{b}	3.45 ± 0.29^{a}	5.97 ± 2.66^{a}

*Values expressed as mean ± SD. Superscripts indicate significant differences at P<0.001, and P>0.05 across the sampling areas.

Physico-chemical Analyses of Green Mussel Meat

The average pH value and percentage of drip loss from green mussels during the simulated time and distribution study are shown in Table 5. The average pH value of samples between each supply chain point has no significant differences (p=0.227) wherein the pH value remained slightly acidic during the supply chain simulation (6.07-6.22). On the other hand, by the time the green mussels arrived at the wholesalers after 3 h, the average percentage drip loss significantly increased (p=<0.001) from 0

to 25.60±4.73% during the 2-5 h holding period. Afterward, the percentage drip loss slowed down to 3.45-5.97% in the retailer and consumer points.

Discussion

Post-Harvest Practices Along the Supply Chain Point

The post-harvest practices described in this research do not conform with WHO,[20] which states that vehicles for the transportation of live shellfish should have proper chilling equipment to maintain a temperature as close as possible to 0°C tolerable to the live commodity and to avoid contamination, exposure to extreme temperature, and drip loss due to the drying effect of the sun and wind. According to Boyd and Wilson[21] mussels transported at ambient and chilling temperatures have an expected shelf-life of four days and nine days, respectively. PNS-BAFS[22] recommended that the product should be packaged using suitable food-grade materials that are clean, free of contaminants, and any foreign objects. Also, bulk packaging of mixed species is not recommended. Proper packaging should be used since it enables food to be transported safely for long distances from its place of origin while maintaining its nutritional value.[23]

Physico-chemical and Microbial Characteristics of the Culture Site

The ideal values of water parameters suitable for culturing green mussels are 27 to 30ºC for water temperature; >5 mg/L for dissolved oxygen; 7.0 to 8.5 for pH and 27 to 35 ppt for salinity; 10,000-100,000 mg/L for TDS; 30-55 S/m for conductivity.[2,24,25] Based on these reference ideal values, it affirms that Canacao Bay passed the recommended parameter values and is ideal for the culture of green mussels.

In this study, the sampling was during summer which resulted in a significant increase in the densities of total coliforms. Atherholt *et al*.,[26] stated that the occurrence of high coliform bacteria counts is primarily observed during the summer season, which corresponds to a period of elevated water temperatures. Furthermore, the high *E. coli* counts in the water can be attributed to the association of the pathogen with particles present in the water.[27] Most of the studies indicated higher levels of bacterial load in the sediment than in the water samples of a lake or bay environment.[28,29] Also, the high level of fecal coliforms and *E. coli* and *Vibrio* spp. in the water sample was due to harvest activities in the sampling area which caused the mixing of sediments and water during the sampling. With the presence of *Vibrio* species in the culture sites, *V. alginolyticus* was present in both water and sediment samples since it is the most halotolerant among all species of *Vibrio*.[30] Notably, V. vulnificus, a known virulent pathogen, was detected in the sediment samples only because they tend to suspend in particulate matter.[31,32] According to Kaysner *et al*.,[18] *Vibrio* spp. are fragile to extreme heat and cold, hence, the varying results among samples could be due to the conditions during transport from the sampling site to the laboratory. Meanwhile, the presence of *Salmonella* in the samples was already expected since the sampling sites were near the residential areas, where some of the houses and stilts were built upon the water, and disposal of wastes was not properly observed. According to Liu *et al*.,[33] the presence of *Salmonella* in the environment indicates contamination by sources such as sewage, animal feces, or contaminated runoff. Meanwhile, the Philippine standard for bivalve culture sites should fall under the Class SA waters wherein the total coliform and *E. coli* count should be 70 MPN/100mL and ≤ 1.1 MPN/mL.[25] In the present study, the culture sites passed the standard for total coliforms but they failed the standard limit for *E. coli* count. Fecal coliforms, including *E. coli*, are employed as markers to evaluate the quality of shellfish and determine the classification of the areas where shellfish are cultivated and harvested.[34] The presence of an excessive amount of fecal contamination in seawater can have severe consequences because it can lead to outbreaks of waterborne diseases, affecting not only swimmers but also those who consume seafood from contaminated waters.[35]

Microbial Counts of Mussels Along the Supply Chain

The microbial quality of green mussels harvested from Bacoor City, Cavite was recorded during simulated time and distribution across supply chain points. The APC of mussel samples was higher than the standard limits set by PNS/BAFS,[36] which is log 5.70 CFU/g only. As mentioned previously, the mussel culture area failed the DENR[25] standards for the total coliform load. Since APC measures the total bacterial population in a sample, the recorded values have exceeded the required limit for fresh bivalves. It can be noted that APC increased along the supply

chain because icing or lowering the temperature during transit was not practiced. This increase can be attributed to the lack of proper practices such as icing or temperature control during transit since most bacteria associated with food are mesophilic and they multiply rapidly in warm temperatures which typically range from 20 to 45ºC.[37]

Meanwhile, the results for total coliforms conformed to the range required by the FDA NSSP which is 230 MPN/100g, while the fecal coliform and *E. coli* content increased to a level deemed to be unacceptable for raw consumption. As mentioned in this study, the fecal coliform content of the water samples did not pass the standards set by DENR25 for suitable culture sites. Furthermore, the immobile nature and filter-feeding mechanism of mussels make them capable of harboring and transmitting foodborne pathogens. This is attributed to their propensity to accumulate pathogenic bacteria and other biological particles within their tissues over an extended period.[38,39] The bacterial load in the retailer-to-consumer chain experienced an increase due to a holding period of 2-8 h. During this interval, mussels are stacked in the market or stored in sacks before being sold to customers. This period of storage can contribute to a further rise in the bacterial load of the mussels. Fecal coliforms, specifically *E. coli*, have a rapid doubling time of 20 min, hence, this long period before the consumers cook the mussels is already detrimental and can lead to an increased risk of foodborne diseases.[40] In the microbiological food safety standards for live bivalve mollusks, one of the criteria is the absence of *Salmonella* spp. which means that none of the samples should contain any detectable levels of this bacteria.[41] *Salmonella* is a common culprit behind foodborne illnesses in humans. Non-typhoidal salmonellosis is associated with a high incidence of gastroenteritis cases that are linked to the consumption of contaminated food while typhoidal strains have the potential to cause a serious systemic illness known as enteric fever. This bacterium is a major cause of illness worldwide and is associated with substantial morbidity and, in some cases, mortality.[33,42] Meanwhile, due to the filter-feeding mechanism of mussels, *Vibrio* spp. has also been detected in the samples. Different species of *Vibrio* cause illness in humans such as *V. cholerae* which causes cholera, an intense diarrheal ailment that can become life-threatening in the absence of treatment. This disease is typically disseminated through contaminated water sources and direct person-to-person contact. Conversely, non-cholera *Vibrio* species like *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* lead to *Vibrio*sis, a group of infections commonly acquired from exposure to seawater or the ingestion of inadequately cooked seafood that is contaminated.[43] Despite its presence, the concentration was within the acceptable limit of <30 MPN/g as stipulated in the FDA NSSP. The reduction in *Vibrio* load observed between the retailer and consumer stages can be attributed to the depuration process practiced by consumers. During this process, mussels are soaked in water for several minutes until a turbid solution is obtained. This soaking allows the mussels to expel the contents of their digestive glands.[44] Also, *V. parahaemolyticus* is halophilic and undergoes rapid lysis upon contact with freshwater, hence the reduction in their number.[45] The primary purpose of the depuration process is to mitigate the risks associated with the consumption of live shellfish, particularly mussels, by final consumers.[46] In other countries, mussels that are purchased alive and intended for cooked consumption need to undergo the depuration process.[47] This is because the mere commercialization of these shellfish does not guarantee the elimination of disease-causing agents, necessitating the implementation of depuration to ensure their safety.[48,49]

Physico-chemical Analyses of Green Mussel Meat

The results of this study were in agreement with the pH range of seafood which typically ranges from 6.2 to 6.5.[50] Azanza *et al*.[51] and Lin *et al*.[52] reported that the pH levels of fresh green mussels are near neutral. Also, bivalves such as clams and oysters have a pH of 6.26-6.48 and 6.28-6.42, respectively.[53] In this pH range, autolytic enzymes are active thereby causing the rapid autolysis of fish muscle. This process occurs more swiftly in seafood than in mammals and poultry.[50,54,55] In general, seafood is less stable and classified as highly perishable food products due to its high moisture content and the presence of nutrients which are more susceptible to microbial spoilage.[56,57] Furthermore, a low pH in mussels is an indicator of spoilage since this favors the growth of microorganisms such as enterococci, *lactobacilli*, and yeasts.[54] These spoilage bacteria generate odor and unpleasant flavors in seafood due to their metabolic activities.[56] Meanwhile,

throughout the simulated experiment, which lasted approximately 12 h, the samples lost about 35% fluid from their tissues. As a result, there is a loss of water, iron, and proteins during the transformation of muscle into meat.[58] The significant drip loss experienced during the initial 6-hour period from the producer to the wholesaler can be attributed to prolonged transportation in vehicles lacking proper cooling systems. According to Huff-Lonergan,[59] a high storage temperature can lead to an increased drip loss which can affect the quality of the product. The results of this study were in agreement with Otto *et al*.[60] wherein drip loss in case-ready meats exhibited a notable rise during the initial day, followed by diminishing increments in the latter half of the observation period during a 7-day experiment period. The low percent drip loss achieved in the later stages of the simulation can be attributed to the near-neutral pH of the samples. In seafood, a low pH level is associated with high drip loss.[61,62,63] In terms of the physico-chemical quality of the fresh mussels, the pH level of the samples did not lower to a point where it can promote increased drip loss which can subsequently affect the quality in terms of sensorial characteristics. Hence, maintaining the fluids in mollusks as live seafood commodities is essential for preserving their freshness, viability, and quality.[64]

Conclusion

Mussel samples were subjected to a time-distribution study to identify the presence of foodborne pathogens and to determine the changes in pH and drip loss. In terms of the physico-chemical properties of water, the parameters meet the requirements for a culture site. However, the results of the experiments revealed that the mussel culture site was high in fecal coliforms and *E. coli*, and the presence of *Vibrio* and *Salmonella* were detected. Results of the interview with the individuals involved in the supply chain revealed that post-harvest practices used were not compliant with recommended standards by WHO and FAO. Hence, the presence of the aforementioned pathogenic bacteria at the different points of the supply chains was detected. The APC of the samples also exceeded the microbial load required for fresh bivalves. The pH level of the fresh mussel meat remained constant while experiencing an increased drip loss during the initial period of the simulation. The presence of these pathogens emphasizes the importance of implementing stringent safety measures. These measures include closely monitoring the growing areas, enhancing sanitary practices, employing depuration techniques, and ensuring the thorough cooking of mussels. By implementing these measures, the aim is to effectively reduce or eliminate the microbial load present in mussels, comply with international and recommended standards, and ultimately ensure their safety before consumption.

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Conflict of Interest

The authors declare no conflict of interest.

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