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Quality Changes of Chilled Green Mussel (*Perna viridis*) Pre-Treated with Organic Acids and Sodium Tripolyphosphate

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Abstract

This study assessed the potential of lactic acid, citric acid, and sodium tripolyphosphate (STPP) as pre-treatments to improve the quality and shelf life of vacuum-packed green mussel during chilled storage. Blanched mussel meat samples were dipped in 2% lactic acid (LA), 2% citric acid (CA), 10% sodium tripolyphosphate (STPP), 10% STPP + 2% lactic acid (STPP-LA), 10% STPP + 2% citric acid (STPP-CA) solutions and chilled at 3˚C. Vacuum-packed green mussel pre-treated with lactic acid have higher sensory scores and remained acceptable at 24 days of storage. Psychrophilic and lactic acid bacteria counts were significantly lower ($P<0.05$) than other treatments and remained within the safe limit throughout the storage period. Total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), pH and ammonia increased with storage in all samples but values for acid pre-treated samples remained within the safe limit. However, the weight of organic acid pre-treated samples significantly decreased ($P<0.05$) than the control (no pre-treatment). Results of the quality assessment indicated that lactic acid pre-treatment can significantly extend the shelf life of green mussel for 24 days at chilled storage compared to 6 days in the untreated sample.

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Keywords

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Lactic Acid;
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Shelf-Life.

Introduction

Green mussel (*Perna viridis*) is a commercially significant commodity in the Philippines.[1] Its latest production had amounted to 18,800 metric tons and considered as top-six among commercially aquaculture species in our country. The major mussel producing provinces include Cavite, Capiz, Samar, Bataan, and Negros Occidental.[2] The species is favored as part of the human diet due to its high levels of vitamins and minerals.[3,4] The mussel has served as an alternative cheap protein source for coastal communities.[5]

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Bivalves have a short shelf life after harvest due to their neutral pH, high amino acid and water activity that favors microbial spoilage. Its high initial microbial load due to their filter-feeding nature further contributes to its shorter shelf life.[6] The green mussel can be kept alive for 36-48 hours after harvest at ambient temperature.[7] This period is not enough to transport green mussels in distant mussel farms to markets.

The development of processing methods to increase the shelf life of mollusc is important.[8] Packaging can enhance the quality preservation of food products.[9] Appropriate packaging together with refrigeration plays an effective role in maintaining the quality of fish and other fishery products.[10,11] Pre-treatment process is also necessary to increase shelf life and quality of fishery products.[12] Dipping in organic acids are popular food treatments in the industry because of their natural occurrence in various vegetables and animal substrate.[13] Lactic and citric acids are generally recognized as safe (GRAS) and are beneficial in extending the shelf life of meat products subjected to cold storage.[14] Two percent lactic and acetic acids were found effective in reducing the occurrence of pathogens (*S. Typhimurium* and *E. coli* O157: H7) in food.[15] However, drawbacks for acid utilization include high exudates and cooking loss.[5] Sodium tripolyphosphate (STPP), another commonly used additive in the preservation of meat and fishery products helps address textural problems in cold storage.[16,17] This compound is legally utilized and permitted for improving the eating quality of many foods, particularly meat and fish.[18] It can effectively control drip loss and cooking loss of pre-treated food products,[19] inhibit bacterial growth in fishery products stored in ice and retard oxidation of unsaturated fatty acids in seafood.[20] A study on the utilization of combined citric acid and STPP had been utilized to increase product yield and improve texture quality.[21] The possible potential of organic acids and STPP together is seen to increase shelf life and improve the quality of chilled seafood products but there were no studies done specifically on the mussel. Thus, this study assessed the effects of pre-treatments using lactic acid, citric acid, STPP and their combinations on the quality of vacuum-packed green mussels during chilled storage. The percent concentration was used for a possible better-quality preservation and longer shelf life for the product.

Materials and Methods

Collection and Preparation of Mussel Samples

The green mussel was freshly harvested from Taytay, Palawan, Philippines. Samples were declustered at harvest area and packed in an insulated box added with ice following the 5:1 mussel/ice ratio (w/w).[22] The samples were transported to Iloilo, Philippines via airfreight and immediately brought to the University of the Philippines Visayas Fish Processing Laboratory with 7 hours of travel time from the harvest site. The mussels were washed with tapped water to remove adhering dirt. Samples were sorted to remove dead mussels that exhibit foul odor and gaping.

Green mussels were subjected to blanching at 85˚C for 2 mins using a 1:5 mussel/water ratio (w/v). Then, the blanched mussel was submerged in iced water for immediate cooling. Three kilos of mussel meat per group were utilized for pre-treatment. Organic acid concentrations used were 2% (w/v) with 5 minutes soaking time and sodium tripolyphosphate concentration was 10% (w/v) using 1-minute soaking time. The following were the different pretreatments used for this study and all were individually dissolved in distilled water: 2% lactic acid (LA), 2% citric acid (CA), 10% sodium tripolyphosphate (STPP), 10% STPP + 2% lactic acid (STPP-LA), 10% STPP + 2% citric acid (STPP-CA) solutions. The pretreatments with the combination also utilized distilled water for rinsing of samples with 5 minutes draining time. All samples were packed using Nylon PE bags (6in. x 10in. x 75 microns) and sealed. The storage condition of the pretreated green mussel was 3˚C±1˚C for 24 days and the analyses were performed every 3days (Figure 1).

Evaluation of Sensory Qualities

Samples were wrapped in aluminum foil and steamed for 18 minutes in boiling water. Sensory assessment of organic acid pretreated and control samples were done by a descriptive and acceptability test using a line-scale method.[23,24] Ten semi-trained panelists were asked to assess the odor (seaweedy and ammoniacal), flavor (naturally sweet and sour) and acceptability (odor, flavor, and general) of green mussels. The descriptive ratings of the

different attributes were as follows: seaweedy odor, 10=pronounced, 1=absent; ammoniacal odor, 10=pronounced, 1=absent; natural sweet flavor, 10=intense, 1=absent; sourness, 10=pronounced, 1=absent; and acceptability, 10=like extremely, 1=dislike extremely. Before evaluation, tests and parameters were explained to panelists. They were instructed not to eat the mussels during the sensory evaluation. Water and unsalted crackers were provided as taste neutralizer.

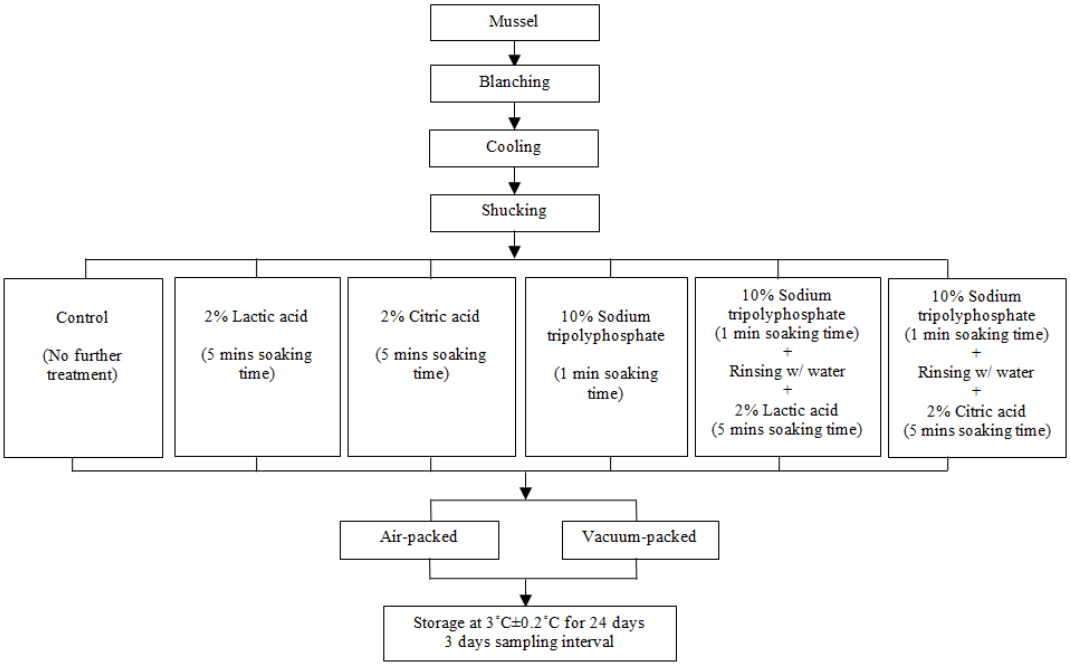

Fig. 1 :The process flow of the study

Microbiological Evaluation

The spread plating method was followed for the estimation of the microbial population in the green mussel samples. About 10 g of sample was weighed under sterile conditions and homogenized with 90 ml of 0.01% peptone water. After tenfold serial dilution, the diluted samples were spread onto agar. Lactic acid bacteria were determined on MRS agar, incubated for 48 h at 37˚C.[25] The psychrophilic bacteria were enumerated on plate count agar and incubated for 5 days at 8˚C.[26] The results were expressed in terms of log colony-forming unit (log CFU) per g of mussel sample.

Physico-chemical Analyses

Determination of pH

The pH value of green mussel with and without acid pre-treatment was prepared[27] and determined using pen-type pH meter.

Determination of Weight Loss

For weight loss analysis, green mussel was weighed per treatment, packed and subjected to chilled storage. The pre-weighed samples were withdrawn and weighed again every sampling interval. Separate packs of the sample were prepared for every sampling period. The weight loss was determined using the following formula[28]

Weight loss (%) = B/A x 100 (where: A were the initial weight and B as the final weight)

Determination of Volatile Compounds

The extraction and analysis of volatile compounds including total volatile basic nitrogen (TVBN) and trimethylamine nitrogen (TMAN) were carried out according to the Conway method.[29] The results for both volatile compounds were expressed as mg N/100g.

Determination of Ammonia

The ammonia content of pretreated and untreated green mussel was measured according to the Indophenol method.[30] The result was expressed as mg/kg muscle.

Statistical Analysis

The results were analyzed using one-way ANOVA and the mean was statistically evaluated using Duncan's multiple range test (DMRT) with the level of significance set at $P<0.05$. All the statistical analyses were carried out using the SPSS (version 20) software (SPSS Inc., Chicago, Illinois).

Results and Discussion

Sensory Attributes and Acceptability of Chilled Green Mussel

This study assessed the organoleptic changes in chilled mussel pre-treated with organic acids and sodium tripolyphosphate using a descriptive and acceptability test. Figure 2 shows the sensory attribute of green mussel pre-treated with the organic acid and STPP in vacuum-packed chilled for 24 days at 3˚±1˚C. The score for seaweedy odor declined with storage. Control in air-packed had the highest mean value of 7.79±1.32 for seaweedy odor on day 3. But both control samples in the air- and vacuum-packed together with STPP pre-treated mussel in vacuum-packed were rejected on the 9th day of storage. Lactic acid pre-treatment effectively maintained the seaweedy odor compared to other treatments.

Ammonia is an end product produced during the degradation of urea and other nitrogenous compounds by the action of microorganism.[31] The high score indicates the detection of ammoniacal odor. STPP-LA had the lowest score for ammoniacal odor at the start of storage compared with other samples but it increased with storage. STPP prevented the formation of ammoniacal odor on early days of storage but not in the later period which resulted in the product getting rejected after day 6. Lactic pre-treatment of green mussel in vacuum-packed slowed down the formation of distinct ammoniacal odor in day 9 until the end of the 24th day of chilled storage.

The natural sweet flavor of mussel was retained by the control sample in air-packed until the 2nd sampling days of chilled storage. A significant reduction in the natural sweet flavor was noticed in the acid treated samples, probably due to the masking effect of sour flavor on the samples. Overall, the natural sweet flavor of the samples declined with storage. In postharvest shellfish, the initial breakdown of ATP results in the accumulation of inosine monophosphate that contributes to pleasant flavor. But subsequent breakdown results in loss of flavor.[32]

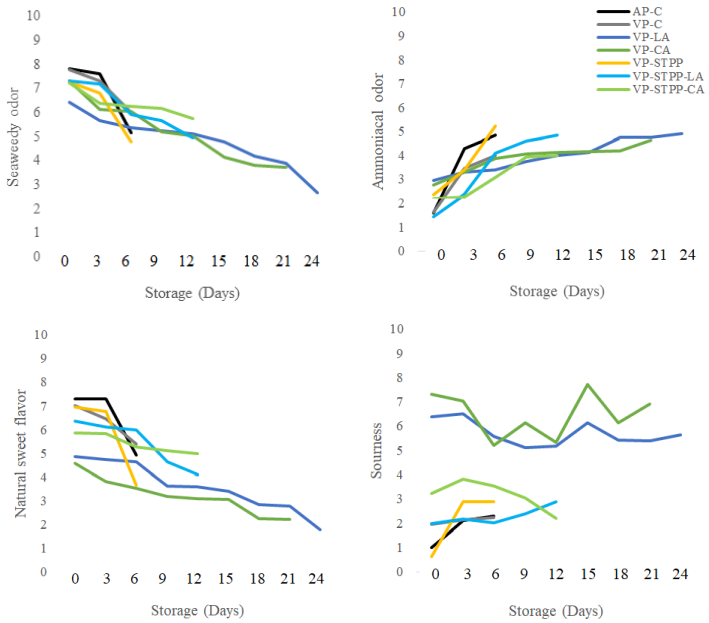

Fig. 2: The sensory attribute of organic acid and STPP pre-treated chilled green mussel

(C) control; (LA) lactic acid; (CA) citric acid; (STPP) sodium tripolyphosphate; (STPP-LA) sodium tripolyphosphate with lactic acid; (STPP-CA) sodium tripolyphosphate with citric acid. Values are presented as mean+standard deviation of three determinations; means with different letters in the same row are significantly ($p<0.05$) different from each other per sampling day.

Sourness attribute was included in the test to check whether acid pre-treatment will result in the significant souring of the product and to test the consumer's acceptance of the acid. The significantly high score indicates that acid pre-treatment contributes to a noticeable product souring. Significant differences were observed between control and pre-treated samples.

Figure 3 indicates the sensory acceptability of chilled green mussel pre-treated with organic acids and STPP. Control samples in the air- and vacuum-packed had a significant mean score (8.03) for odor at the start of the storage. Vacuum-packed CA, STPP-LA, and STPP-CA have a high score of acceptability until the 3rd sampling day of storage after which an abrupt decline in odor scores was observed. This indicates that vacuum-packaging can effectively preserve the desirable odor of mussel at the initial week of storage and decline its scores which is an indication of product spoilage and may be attributed to ammonia formation.

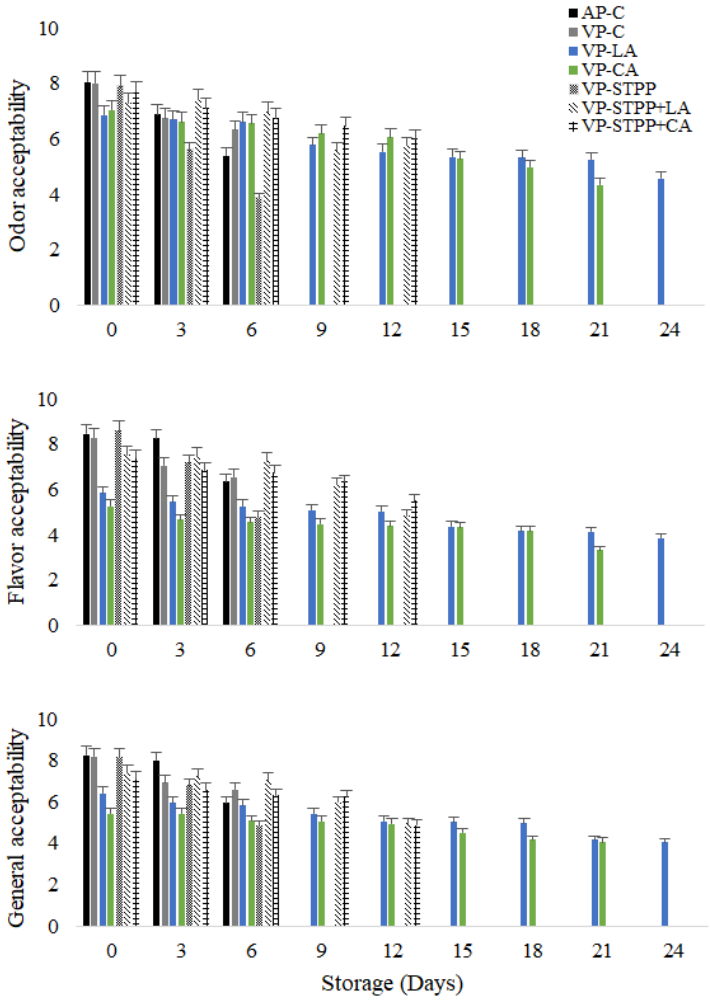

Fig. 3: Acceptability of chilled green mussels. Score: 10=like extremely, 1=dislike extremely

Control in an air- and vacuum-packed had significant high flavor acceptability on day 0 ($P<0.05$). STPP and its combination with citric acid and lactic acid had effectively preserved the attributes of mussel flavor, thus the score attained for STPP-acids were higher than control and acid alone.

In summary, a decrease in the quality and acceptability of the samples was observed with storage. STPP had the beneficial effect of maintaining and preserving the sensory attributes of chilled green mussels but was not effective for longer storage as shown by the early rejection of samples. Lactic acid pre-treatment enhanced the shelf life and was effective in retarding off-odor development until the 24th day of storage compared to the untreated sample with 6 days of shelf life. The off-odor (i.e., ammoniacal) development contributed largely to product rejection in the control and STPP-treated samples.

Microbiological Changes of Chilled Green Mussel

Microbiological analysis of spoilage organisms is necessary to determine the quality and safety of foods.[4] Microbial load determined in this study includes lactic acid bacteria count and psychrophilic bacteria count in chilled mussel stored for 24 days (Figure 4). The anoxic environment prevents microbial growth and delays spoilage due to the slow proliferation of bacteria capable of tolerating anaerobic conditions.[33] Lactic acid bacteria are the group of spoilage organisms known to dominate vacuum-packed foods due to their ability to grow rapidly under anaerobic conditions at low temperatures; they can tolerate carbon dioxide in the environment.[4] The acceptable limit for lactic acid bacteria used in most storage studies of the mussel is 7 log CFU/ g.[34] Results obtained from this study show that the lactic and citric acid-treated samples had lower initial bacterial count than other pre-treated samples. Organic acid pretreatment resulted in lower bacterial count throughout storage relative to STPP treated and control samples. Antimicrobial property of weak acids including lactic and citric acid is related to the concentration of their undissociated acids (pKa). Lactic acid with higher pKa value has higher antimicrobial property than acids with lower pKa like citric acid. Lactic acid can pass through the cell membrane and dissociate once inside the cell and acidify the internal pH and prevent the growth of microorganism.[35]

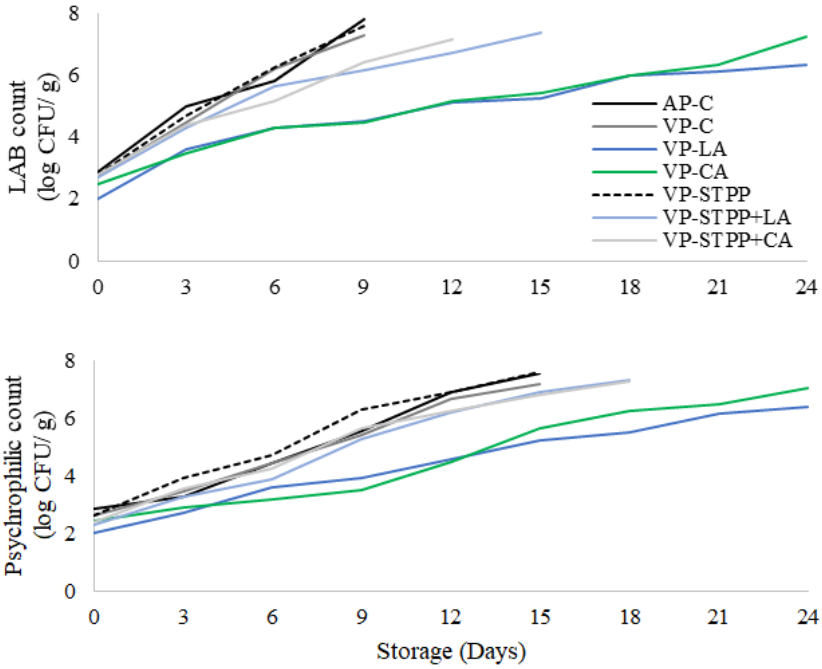

Fig. 4: Effect of pre-treatment on lactic acid bacteria count and psychrophilic bacteria count of chilled green mussel

Lactic and citric acid pre-treated meat effectively reduced its growth compared to control and STPP-treated samples. A safety limit of 6 log CFU/g[36] was reached at 15 days of storage in the control, STPP, and STPP-acid treated samples while CA and LA samples remained safe until 18 and 21 days of storage, effectively.

Studies suggested that mackerel and anchovy having a psychrophilic microbial of 6 log CFU/g was acceptable,[36] thus, it was used as the basis for this study. Psychrophilic bacteria count of organic acid treated samples was lower than STPP and untreated samples.

Physico-Chemical Changes of Chilled Green Mussel

pH values are frequently used as the physical quality control for fish, seafood and fish products and are always assessed together with TVB-N, TMA-N, and TBARS for seafood quality assessment.[37] It can be used as an indicator of postmortem change of glycogen to lactic acid and the degradation of muscle components during long storage.[38] Control samples had near-neutral pH value at the start of storage study and declined with storage. Pre-treatment using lactic and citric acids resulted in medium acidification (4.3-4.6) of the samples, with values significantly lower than the control ($P<0.05$) (Figure not included).

The loss of water directly explains the loss of mass in meats and substantial yield loss in the manufacturing process.[39] Weight loss in vacuum-packed control (Figure 5) was significantly lower compared to air-packed control and vacuum-packed acid pretreated samples ($P<0.05$).

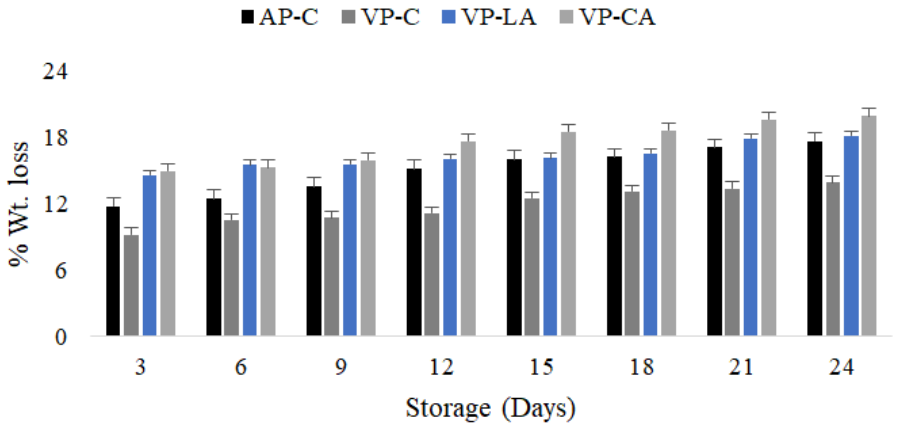

Fig. 5: Changes in a weight loss of chilled green mussel. Values are presented as the mean ± standard deviation of three determinations

Total volatile basic amines (TVB) are widely used to assess seafood quality and comprised the measurement of trimethylamine (by spoilage organism), dimethylamine (by autolytic enzyme in frozen storage), ammonia (by the deamination of amino acids and nucleotide catabolites) and other volatile basic nitrogenous compound present in fish and fishery products.[40,41] It is the product of bacterial spoilage commonly used in the assessment of the freshness index of seafood products.[5,42] Spoilage pattern in fresh seafood is generally depicted by the increase in TVB-N concentration that is related to bacterial proliferation.[43,44] Figure 6 shows the TVB-N content of chilled green mussel pre-treated with organic acids. Total volatile basic nitrogen of chilled green mussel was lower in organic acid pre-treated green mussel than control samples. The lactic acid in vacuum-packed has the lowest TVB-N values during the entire storage ($P<0.05$). The acceptable safety limit for TVB-N in fish muscle is 35 mg N/ 100g; beyond this value, fish is considered spoiled.[42,45] The control samples in the air- and vacuum-packed reached safety limit at days 15 and 18 of storage, respectively.

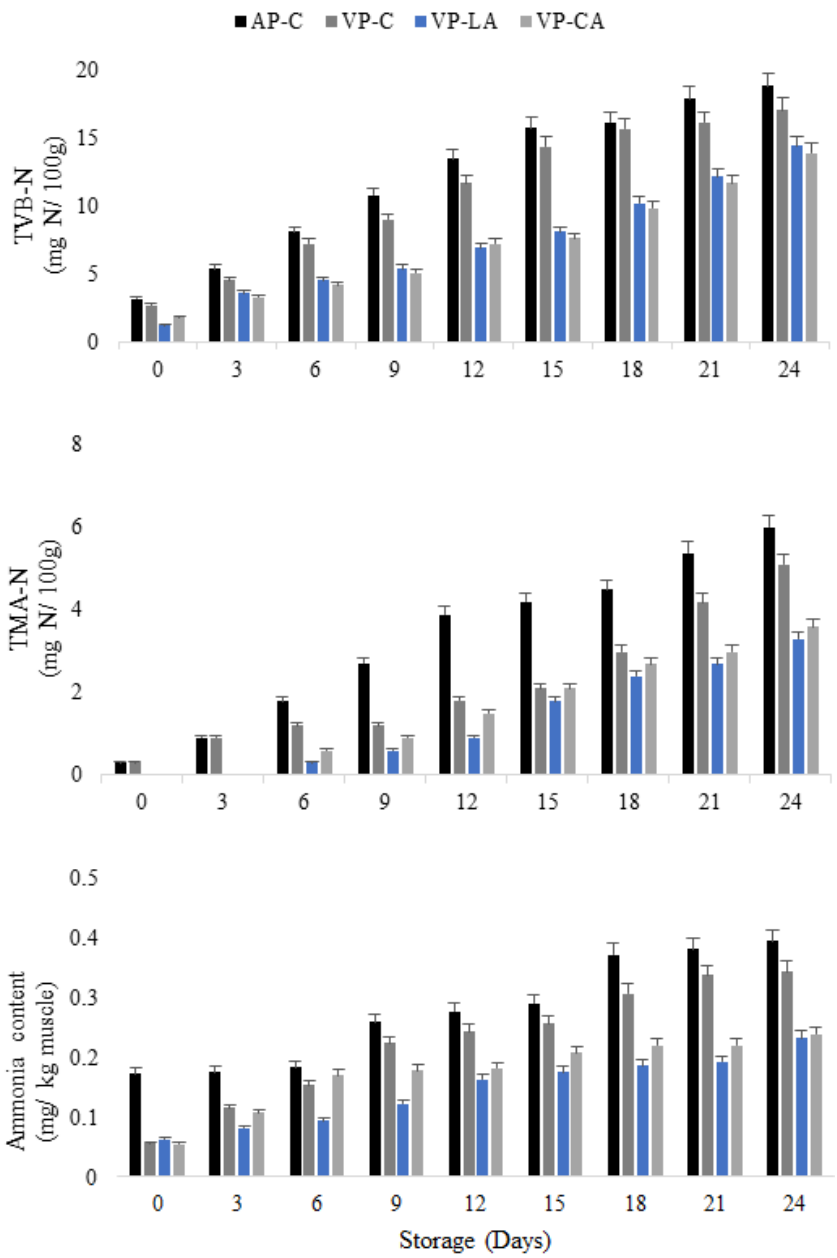

Fig. 6: Effect of organic acid pre-treatment in volatile compounds and ammonia content of chilled green mussel

Trimethylamine nitrogen (TMA-N) is another spoilage product commonly used in measuring freshness in marine fishes.[41,46] The results obtained were similar to TVB-N wherein acid treated green mussels attained the increase significantly lower values compared to the untreated samples. TMA-N value among fish species is around 10-15 mg TMA-N/ 100g fish which is then determined the acceptable limit for human consumption.[41] The suggested limit for TMA-N of the mussel is 3 mg N/ 100g.[47] Vacuum-packed green mussel pre-treated with lactic acid had attained significantly lower values than the untreated samples ($P<0.05$).

Ammonia present in post-mortem flesh is derived from the deamination of adenine nucleotides to inosine monophosphate that is known to occur rapidly during harvesting and death of the fish or within few hours after storage in ice. Its increasing level reflects the decomposition of muscle in food products.[48] Ammonia concentration of vacuum-packed chilled green mussel pre-treated with lactic acid attained significantly lower values compared to untreated samples ($P<0.05$). Both air- and vacuum-packed control samples reached the safety limit for ammonia after 9 days of storage while vacuum-packed lactic acid pre-treated samples attained the safety limit on day 24.

Conclusions

Lactic acid pre-treatment was the most effective method in preserving the quality of chilled mussel. The product can remain acceptable up to 24 days of chilled storage when done in combination with vacuum packaging. Citric acid pre-treatment in combination with vacuum packaging may also be used in the absence of lactic acid since it can extend product shelf life for up to 21 days compared to the non-treated samples which only lasted for 6 days. Although STPP effectively protected product textural integrity and prevented drying and weight loss, it was not effective in retarding microbial growth which may have contributed to faster product quality deterioration. The vacuum-packed STPP treated mussel had the shortest shelf life of only 6 days. The combination of treatments was not also effective in extending the quality and shelf life because they only lasted for 12 days of storage.

Thus, lactic acid can be effectively applied to the food industry particularly in primary processing of shellfish like green mussels to extend shelf life and preserve quality up to 24 days of chilled storage.

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Conflict of interest

The authors declare no conflict of interest.

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