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RESEARCH ARTICLE

Destruction of Some Food Poisoning Bacteria and Shelf-Life Extension of Seafood

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Abstract

This study was carried out on *Caranx sexfasciatis* fillets and *Penaeus semisulcatus* which are widely caught and consumed in the Republic of Yemen. Microbial analysis was processed on Standard plate count (SPC), *Escherichia coli* and *Listeria monocytogenes.* Overall means of SPC in seafood types of local markets was 3.67 $\log_{10}$ cfu/g in *P. semisulcatus* and 4.11 $\log_{10}$ cfu/g in *C. sexfasciatis*, but *E. coli* was 1.50 $\log_{10}$ cfu/g in *P. semisulcatus* and was 1.13 $1\log_{10}$ cfu/g in *C. sexfasciatis*, while *L. monocytogenes* was not detected in both *C. sexfasciatis* and *P. semisulcatus*. Overall means of SPC in seafood types of seafood exporting companies was 1.33 $\log_{10}$ cfu/g in *C. sexfasciatis* and 2.13 $\log_{10}$ cfu/g in *P. semisulcatus*, while *E. coli* and *L. monocytogenes* were not detected in both *P. semisulcatus* and *C. sexfasciatis*. The present study evaluated the impact of some acetic, lactic, citric acids and sodium or ammonium bicarbonate as compared with tap water. Results obtained indicated that organic acids eliminated most of *L. monocytogenes, E. coli* and SPC immediately after treatment at time zero (25°C) and during storage at 5°C for 7 d and those increased the shelf-life of seafood during storage at 5°C compared with the tap water.

Keywords: *Caranx sexfasciatis, Penaeus semisulcatus*, microbial analysis, standard plate count, seafood.

Introduction

Yemen is blessed with a long coastline of about 2500 kms from the Saudi Arabia borders in the north on the Red Sea to the Aden Gulf and the Arabian Sea to the Omani borders. The Yemeni marine environment is rich in different kinds of fish and other seafood; the number of marine types, which is available in the Aden Gulf and Red Sea is about 600 kinds of fish and other seafood (Marine Science and Biological Research Authority, Aden, 2007). Aquatic environments of Yemen harbor various biological populations including food-borne pathogenic bacteria such as *Vibiro paraheamolyticus, Staphylococcus aureus* and *Escherichia coli* (Al-Zoreky *et al*., 2008). *Listeria monocytogenes* and other *Listeria* spp. were also isolated from a popular seafood (raw tuna) sample in Sana'a Secretariat (Al-Zoreky and Sandine, 1993). Fish and shellfish are excellent protein sources for human consumption; in addition, they have higher contents of hydrosoluble and liposoluble vitamins, minerals and polyunsaturated fatty acids (PUFAs). Interestingly, omega-3 fatty acids, found mainly in fat-rich fish such as salmon, mackerel, herring and sardines confer health benefits to humans not found in any other foods. Omega-3 fatty acids from fish can lower blood triglycerides, reduce abnormal heart rhythms, reduce blood pressure by small but significant amounts improve blood clotting regulation (Nettleton, 1995). The *Enterobacteriaceae* count is considered as another index of fish quality because it is related to storage in ice, washing and evisceration (Zawadzki, 1993).

Increasing demand for seafood products and consumer awareness on food safety have been calling for better quality fish products. The quality of fresh fish is a major concern to industry and consumers. Like marine fish, freshwater fish are extremely perishable food commodities. Deterioration of fish mainly occurs because of bacteriological activity leading to loss of quality and subsequent spoilage (Church, 1998). Moreover, freezing or refrigeration are too expensive and require complicated equipment. It is therefore, necessary to use safer antibacterial substances for fish preservation (Church, 1998). Considering the above facts, this study was aimed with the following objectives:

1. Microbiological quality of some common seafood exported as chilled product.
2. The effect of some widely used antibacterial, organic acids (lactic, acetic and citric acids) on the growth of some pathogens during chilled (5ºC) storage under vacuum packaged of two marine types *Caranx sexfasciatis* and *Panaeus semisulcates*.

Materials and methods

Collection of samples: This study was carried out on fish fillets *Caranx sexfasciatis* beheaded and peeled shrimps *Penaeus semisulcates* which are economically important. *Caranx sexfasciatis* fillets and Peeled shrimps *Panaeus semisulcates* were obtained from three exporting companies in Al-Hodeidah and local markets in Sana'a Secretariat, Yemen during June 2007 until May 2008.

Random frozen samples of seafood were also obtained from exporting companies (three in Al-Hodeidah). Meanwhile, freshly chilled caught samples (<16 h after catching) of seafood from local markets in Sana'a were also gathered and examined for microbiological quality. Samples were individually put in sterilized polyethylene bags and transported to the laboratory in insulated thermic boxes containing ice to maintain fish quality. The ice was packaged in plastic bags with direct ice contact thus, to prevent contamination of samples. When necessary, samples were kept refrigerated overnight prior to analysis. The frozen samples from exporting companies were stored at their original conditions in the walking freezer at (-12°C) prior to microbiological evaluation. Samples with visible deterioration, injury or disease were excluded from the study. Aseptic techniques were strictly followed during collection, transportation and analysis of samples. The standard methods of AOAC (2000) and International Commission on the Microbiological Specifications of Foods (ICMSF, 1978) were adopted for preparation and microbiological analysis of seafood samples.

Preparation of antimicrobial solutions: The following alimentary additive solutions were used for inhibition of pathogenic bacteria and extending the fish shelf life: 98 mL of tap water as control, 2% v/v lactic acid (85% certified) (Fluka, Germany-pH 2.7), 2% acetic acid (glacial) (v/v) (BDH, Germany-pH 2.3), 2% citric acid (monohydrate, 99.9%; (w/v) (BDH, Germany-pH 3), 2% of sodium or ammonium bicarbonate (99%); (w/v) (BDH, Germany) at 5ºC and carried out without filtration (Gram, 1992).

Preparation of bacterial inoculums: Cultures of *Listeria monocytogenes* (ATCC 7644) and *Escherichia coli* (ATCC 10536) was obtained from the American Type Culture collection (ATCC). Cultures were activated by transferring stock culture in 10 mL Broth Heart Infusion (BHI) (HiMedia, India) and incubated overnight at 35°C, streaked onto Broth Heart Infusion Agar (BHIA) (HiMedia, India) plates and incubated for 24 h at 35°C (Hsiao and Siebert, 1999). Working cultures were kept on BHIA slants at 5°C and subcultured every 2 weeks. Inoculums was prepared by transferring (0.1 mL) a loopful of culture from the slants to 10 mL BHI and incubated for 18 h at 35°C. The growth of cultures was measured by the optical density at 625 nm (1 cm disposable curette) and using the respective non-inoculated broth as blank.

Fish inoculation and treatment: Fresh chilled peeled shrimp or Bayad fish slices were surface spread with 0.1 mL of diluted active culture of *Listeria monocytogenes* (ATCC 7644) or *Eschrichia coli* (ATCC 10536) with a sterile glass rod. Two inoculation levels were $\log_{10}$ 3 or $\log_{10}$ 5 cfu/g of either *L. monocytogenes* and *E. coli* prior to spreading.

After inoculation, the fillets were divided into six groups each 6 slices dipping in cold solutions of organic acids, bicarbonates salts and tap water (control) for 30 min for attachment (ratio of 1:2). After dipping the slices, they were drained to remove excess solution and kept in plastic bags which were vacuumed packaging or under vacuum by packaging machine and stored at 5°C for 7 d or -12ºC for 21 d.

Samples preparation for microbiological analysis: The vacuum packed bags were opened according to AOAC (2000). About 6 representative samples (25 g in duplicates) of chilled fish or thawed frozen fish were separately homogenized in a wiring blender for 1 min with 225 mL of sterile peptone water (0.1%) under aseptic conditions to give 1/10 dilution of serial dilutions ($1/10^2$ to $1/10^4$) were further prepared in peptone water. The frozen samples were thawed overnight in the refrigerator prior to homogenization and dilution. Samples were subjected to microbiological analysis after treatments procedure (day 0) and after storage for 3, 5 and 7 d at 5°C.

Enumeration of Listeria monocytogenes: Presence of *L. monocytogenes* and other *Listeria* spp. was confirmed according to Vanderzant *et al.* (1971). One mL from each dilution of prepared samples was streaked on *Listeria* selective base agar (HiMedia, India) with the antibiotic supplement. The plates were incubated at 37°C for 48 h and analyzed for the presence of *Listeria* colonies by gram-staining. Colonies appearing on this agar were examined to select black colonies as presumptive *Listeria monocytogenes*.

Enumeration of Escherichia coli: Escherichia coli was enumerated on Eosin Methylene Blue Agar according to Gildberg (2004). One mL from each dilution of prepared samples was streaked on Eosin Methylene Blue Agar (Oxoid, U.K) and incubated at 37°C for 48 h and analyzed for *Escherichia* colonies. Colonies were examined by gram-staining and fluorescent green colonies appearing on this agar confirmed *E. coli.*

Enumeration of Standard Plate Count (SPC): SPC was determined by surface spreading homogenate dilutions (0.1 mL) on PCA (Difco, USA) (Gram, 2010). Inverted plates were incubated at 35°C for 24-48 h. Mean values of colony forming units (cfu) were calculated as the average of two dilutions.

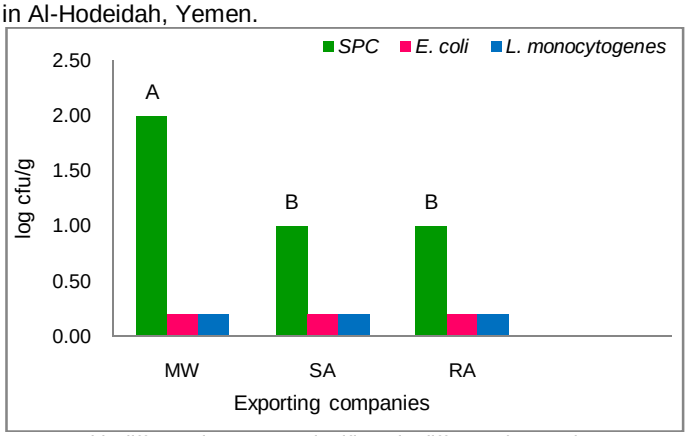
Statistical analysis: Statistical analysis of data obtained from this study were done using statistical analysis software (SAS version 6.12; SAS Institute, kc., cary, North Caroline, U.S.A., 1997).

Microbiological counts were converted to $\log_{10}$ values prior to statistical analysis and analysis of variance (ANOVA) was used to evaluate any difference among means with significance defined at $p<0.05$; the Duncan was used to separate means. The microbial quality of fish samples (Al-Hodeidah and local markets in Sana'a) was analyzed by paired t-test.

Results and discussion

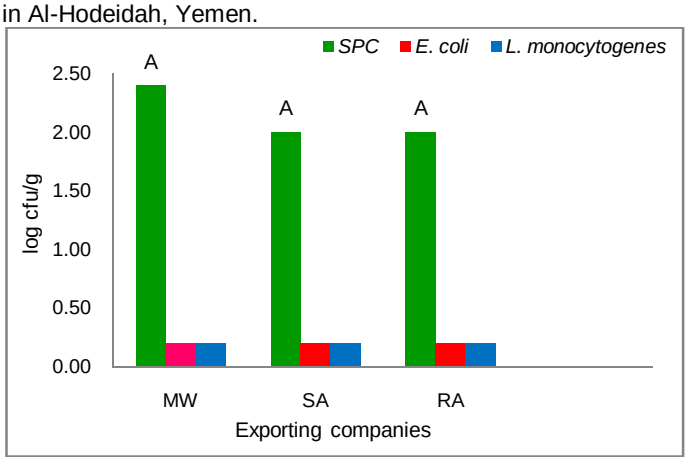
Data of microbial quality of *Caranx sexfasciatis* and *Penaeus semisulcatus* from exporting companies (MW, SA and RA) in AL-Hodeidah are shown in Fig. 1 and 2. Standard Plate Count was 2, 1 and 1 $\log_{10}$ cfu/g respectively. Depending on the source of fish, bacterial growth is primarily responsible for fish spoilage. Thus, SPC provides a good index of freshness quality as well as degree of contamination (Pedrosa-Menabrito and Regenstein, 1990). Recommended microbiological limits of SPC for fish and fish products range between 6 to 7 $\log_{10}$ cfu/g (ICMSF, 1987). Fish with SPC counts exceeding 7 $\log_{10}$ cfu/g starts to spoil, showing notable sensory and microbiological changes (Marshall and Kim, 1996). It was reported that such types in SPC could be due to variation of bacterial flora amongst fish in different seasons (Davis, 1993). *Escherichia coli* and *Listeria monocytogenes* were not detected (<1 $\log_{10}$ cfu/g) in Bayad fish and Shrimp of companies of MW, SA and RA as shown in Fig. 1 and 2. *Escherichia coli* are gram-negative rod bacteria, which produce acid and gas from lactose at 35ºC for 48 h, used as product safety indicators for fecal contamination or for sanitation during cultivation and processing of seafood (Huidobro *et al*., 2002). The recommended limits of fecal counts is not more than 2.6 $\log_{10}$ cfu/g and *E. coli* must not exceed 2 $\log_{10}$ cfu/g in fish and fish products (ICMSF, 1987). Among human pathogens, *Listeria monocytogenes* has received considerable attention as an emerging food-borne pathogen over the last 20 years. It is gram-positive, small rod, non-spore-forming bacteria and can grow between 0-44ºC with optimum growth at 30 to 37ºC (Bergry's Manual, 1977). *Listeria monocytogenes* has been isolated from fresh, frozen, smoked and dried salted seafood products **(**Bremer *et al.,* 2003). It was stated that growth of *L. monocytogenes* at -0.4°C is ubiquitous and can spread through fish farm environments by water birds, bedding and animal feed (Marshall and Kim, 1996). Data of microbial quality of shrimp for exporting companies in AL-Hodeidah are shown in Fig. 2. The SPC were 2.4, 2 and 2 $\log_{10}$ cfu/g, respectively of companies MW, SA and RA. Usually, SPC of high quality farm-raised fish were below 5 $\log_{10}$ cfu/g (Andrews *et al.,* 1977). Table 1 showed that citric, acetic and lactic acids had complete inhibitory effect on *E. coli* or *L. monocytogenes* (<1 $\log_{10}$ cfu/g) at 0 d even the end of storage (7 d) compared to the control (2.37 or 2.79 $\log_{10}$ cfu/g) respectively, at day 0 and the count of pathogens increased to 3.35 or 3.28 $\log_{10}$ cfu/g at the end of storage (7 d), respectively.

Fig. 1. Means of SPC, *E. coli* and *L. monocytogenes* counts of *C. sexfasciatis* from seafood exporting companies in Al-Hodeidah, Yemen.



Counts with different letters are significantly different ($p<0.05$).

Fig. 2. Means of SPC, *E. coli* and *L. monocytogenes* counts of *P. semisulcatus* from seafood exporting companies in Al-Hodeidah, Yemen.



The alkaline solutions ($NaHCO_3$ or NH_4HCO_3) had no effect on *L. monocytogenes* (ATCC 7644) or *E. coli* (ATCC 10536) inoculated into fillets from exporting companies. This experiment showed, however, stimulating growth of *L. monocytogenes* or *E. coli* by treating with bicarbonates (Table 2). Montville and Kaiser (1993) reported that sodium or ammonium bicarbonates present in the seafood have the potential to control fungal growth. Organic acids have a long history of being utilized as food additives and preservatives for preventing food deterioration and extending the shelf-life of perishable food (Ricke, 2003). The microbial load (SPC) of dipped and untreated *C. sexfasciatis* fillets during the course of storage is summarized in Table 2. Mean SPC for the control samples at day 0 was 1.52 $\log_{10}$ cfu/g. SPC of *C. sexfasciatis* fillets treated with tap water (control) and salts increased with length of storage at 5ºC. Significant differences ($p<0.05$) were observed in SPC of fish treated with water and alkaline salts (7 d). Acid treatments (excepting alkaline salts) were effective in reducing microbial populations (SPC) of fish immediately after treatments and during cold storage.

Table 1. Effect of some organic acids and alkaline salts on *L. monocytogenes* or *E. coli* in *C. sexfasciatis*.

Microorganism[b]	Storage days	Control	Citric acid	Acetic acid	Lactic acid	N_aHCO_3	NH_4HCO_3
L. monocytogenes	0	2.37[a]	<1[b]	<1[b]	<1[b]	2.62[a]	2. 33[a]
	3	2.97a	1.11[c]	1.00[c]	1.66[b]	3.00[a]	3.00[a]
	5	3.33[a]	<1[b]	<1[b]	<1[b]	3.20[a]	3.50[a]
	7	3.35[b]	<1[c]	<1[c]	<1[c]	4.51[a]	4.56[a]
E. coli	0	2.79[a]	<1[b]	<1[b]	<1[b]	2. 65[a]	2. 60[a]
	3	2.69[a]	<1[b]	<1[b]	<1[b]	3.19[a]	3.21[a]
	5	3.14[a]	<1[b]	<1[b]	<1[b]	3.30[a]	3.35[a]
	7	3.28[c]	<1[b]	<1[b]	<1[b]	4.41[a]	4.46[a]
SPC	0	1.52[a]	1.33[a]	1.40[a]	1.25[a]	1.70[a]	1.61[a]
	3	2.82[a]	2.00b	1.18[b]	2.03[b]	2.86[a]	2.76[a]
	5	4.72[b]	1.70[d]	1.08[c]	1.68[c]	5.66[a]	5.47[a]
	7	6.72[b]	1.62[c]	1.00d	1.58[c]	7.67[a]	7.55[a]

Counts with different letters are significantly different ($p<0.05$).

Table 2. Effect of organic acids on sensory properties of seafood stored at 5°C for 7 d.

Sample	Treatment	Sensory changes
C. sexfasciatis fillets	Citric acid	Very slight discoloration and no effect on odor
	Acetic acid	Very slight discoloration and no effect on odor
	Lactic acid	Very slight discoloration and no effect on odor
	$NaHCO_3$	Off-flavor, discoloration
	NH_4HCO_3	Off-flavor, discoloration
Control	-	Off-flavor, discoloration
P. semisulcatus	Citric acid	Very slight discoloration and no effect on odor
	Acetic acid	Very slight discoloration and no effect on odor
	Lactic acid	Very slight discoloration and no effect on odor

The chemicals used and the time of storage significantly ($p<0.05$) influenced SPC of fish as shown in Table 1. Those SPC were less than counts of the study by Palumbo and Williams (1994). The antimicrobial effectiveness of acids observed in the present study supports the observations of Metcalfe and Marshall (2004) who reported that the application of antimicrobials (trisodium phosphate, lactic acid) to poultry legs prolonged the lag phase and reduced the maximum numbers of most microbial groups that were reached at the end of the period of study, these authors found that the numbers of bacteria on control samples increased from the beginning of storage. An antimicrobial solution will have identical effects on the microflora of chilled raw meat (Genigeorgis, 1985).

Table 2 shows sensory evaluation (color, odor and overall acceptability) for *Penaeus semisulcatus* and *Caranx sexfasciatis* treated (dipping) with organic acids (citric, acetic, lactic and bicarbonate) at day 0 and during the course of storage (5°C) compared to tap water (control). The normal grayish white color were reported in *C. sexfasciatis* fillets and peeled shrimp *P. semisulcatus* before treatment procedure with organic acids, $NaHCO_3$, NH_4HCO_3 or tap water. The dipping in $NaHCO_3$ or NH_4HCO_3 produced whitening color (not acceptable) for 0 to 7 d and those were salts produced bad odor (off-flavor) at 5 to 7 d. Control had fish samples of normal grayish white color at 0 d changed to darker, brownish color and off-flavor after 7 d when compared to treated samples with organic acids at temperature of the refrigerating.

On the other hand, citric, acetic and lactic acid treatment caused slightly whiter color (acceptable) during 0 till 7 d. No off-flavor that could cause sensory rejection of chilled treated samples with organic acids was detected at any point during storage when opening the vacuum bags. Sensorial studies are important when an antimicrobial procedure is evaluated because, ideally, the use of any decontamination intervention should reduce microbial loads without reducing sensorial quality. The present study coincides with the reports from other researchers. Kilinc and Cakli (2004) observed that fish treated with an organic acid (lactic acid) had acceptable color, while those treated with tap water showed some skin discoloration. Embarek (1994) found that odor, taste and acceptability of poultry carcasses were not affected by treatments involving dipping for 6 min in 1% lactic acid. Numerous researchers have investigated the effect of organic acids on the sensorial quality of meat and poultry and most of them found that unacceptable color (as well as “off” odors) were brought about when using more than 5% of organic acids. Sensorial evaluation showed that the treatment with organic acids making fish much more acceptability than alkaline salts and untreated samples by the end of the period of storage (Table 2). Sodium bicarbonate was reported to be effective in improving the water-holding capacity and prevented oxidative of fresh meats, beef, pork and poultry (Kauffman *et al.,* 2000). Spoilage of fresh and lightly preserved fish is caused by the growth and activity of specific spoilage organisms (SSOs) which produce metabolites causing off-flavors or off-odors and consequently cause consumer food rejection (Gram and Dalgaard, 2002).

Table 3. Effect of some organic acids on *L. monocytogenes*, *E. coli* and SPC in Shrimp inoculated with approx. 3 $\log_{10}$ cfu/g and stored at 5°C.

Microorganism[b]	Storage days	Control	Citric acid	Acetic acid	Lactic acid
L. monocytogenes	0	2.11[a]	<1[c]	<1[c]	1.08[b]
	3	2.71[a]	<1[c]	<1[c]	<1[c]
	5	3.07[a]	<1[c]	<1[c]	1.08[b]
	7	3.09[a]	<1[c]	<1[c]	<1[c]
E. coli	0	2.70[a]	<1[b]	<1[b]	<1[b]
	3	2.40[a]	<1[b]	<1[b]	<1[b]
	5	2.82[a]	<1[b]	<1[b]	<1[b]
	7	2.96[a]	<1[b]	<1[b]	<1[b]
SPC	0	1.20[a]	1.00[a]	<1[b]	1.00[a]
	3	2.50[a]	1.50[b]	1.26[b]	1.63[b]
	5	4.40[a]	1.30[b]	1.00[b]	1.30[b]
	7	6.00[a]	1.18[b]	<1[c]	1.15[b]

Counts with different letters are significantly different ($p<0.05$).

Shrimp treated in organic acids for 30 min caused significant reductions ($P<0.05$) of *L. monocytogenes* counts from the beginning of the experiment (0 d) until the end of storage of the inoculated peeled shrimp *P. semisulcatus* as compared to control (Table 3). Complete inhibition ($P<0.05$) of *L. monocytogenes* growth was observed in *P. semisulcatus* treated with acetic or citric acid solutions during the storage period (7 d) comparing to the control (tap water) which recorded 3.09 $\log_{10}$ cfu/g (Table 3). Effect of washing with organic acids of acetic, lactic and citric acids combined with VP on *E. coli* in stored fresh peeled shrimp at 5°C is shown in Table 3. The results showed a significant difference ($P<0.05$) in counts of *E. coli* in peeled shrimp treated with acid solutions comparing with the control (tap water). *Escherichia coli* was not detected (<1 $\log_{10}$ cfu/g) in peeled shrimp treated with citric acid, acetic acid and lactic acid, whereas the count of the pathogen (*E. coli*) increased to 2.96 $\log_{10}$ cfu/g in control for 7 d (Table 3). Results obtained in this study are similar to those reported by Ahamad and Marth (1989). Using lactic acid was more effective in inhibiting *E. coli* compared to acetic acid at equal concentrations (Ahamad and Marth, 1989). The effect of dipping with acids on SPC compared to the control of shrimp is shown in Table 3. SPC was 1 $\log_{10}$ cfu/g in either citric or lactic acids while SPC was <1 $\log_{10}$ cfu/g in acetic acid and 1.20 $\log_{10}$ cfu/g in control at 0 d. Acetic acid treatment was reported to produce significant reduction in microbial populations and extend the shelf-life of various refrigerated fish such as channel catfish (*Ictalurus punctatus*) fillets (Zheng *et al.*, 2002). When the SPC reaches 6 $\log_{10}$ cfu/g per g or mL in a food product, it is assumed to be at or near spoilage. Einarsson and Lauzon (1995) mentioned that initial SPC of iced sardines was 3.16×10^2 $\log_{10}$ cfu/g, reaching the limit counts of 10^6–10^7 $\log_{10}$ cfu/ g at 9 d. In this study, the limit of acceptability (10^6 $\log_{10}$ cfu/g) in terms of SPC was 7 d for shrimp stored in VP. Clingman and Hooper (1986) found that fresh fish products stored under VP had an overall increase of shelf life of 7 d over aerobically stored fish. Vacuum-packed ice chilled cod fillets had 7.7 $\log_{10}$ cfu/g H_2S-producing bacteria after 10 d (Dalgaard, 1995).

Conclusion

Comparing the effects of the three treatments, lactic acid was found to be more effective against *E. coli* than acetic acid and citric acid on *P. semisulcatus* and *C. sexfasciatis* fillets. The reduction of aerobic total counts on *P. semisulcatus* and *C. sexfasciatis* fish treated with lactic acid was highest as compared to those obtained from citric acid and acetic acid treatments. The reduction of *L. monocytogenes* by citric acid was highest as compared to those obtained from acetic and lactic acids. However, the reduction of total counts by citric acid was higher than that of acetic acid and lactic acid. Sodium and ammonium bicarbonates were not effective against *E. coli.* Finally, the use of citric acid, lactic acid and acetic acid to wash or sanitize meat fish will not eliminate the pathogenic aerobic total count completely, but will reduce the number of most harmful pathogens and microbial loads on meat fish, which will increase the shelf-life and meat fish quality. The major benefit of the use of organic acid to wash meat fish is generally recognized as safe (GRAS) which also increased the shelf-life and meat fish quality.

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