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Evaluation of Some Organic Acids as Potential Decontaminants of *Vibrio parahaemolyticus* in Fresh Shrimp

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Abstract: Citric acid and acetic acid were evaluated for their effects on the growth and survival of *Vibrio parahaemolyticus* artificially inoculated into fresh shrimp. Fresh shrimp samples were dipped in Tryptic Soy Broth (TSB) containing (~10^7 Colony Forming Units (CFU)/ml) (7 log CFU/g) of *Vibrio parahaemolyticus* and left for 30 min. at room temperature (25°C) to allow attachment. Initial counts of *Vibrio parahaemolyticus* in shrimp samples immediately after dipping in TSB broth were 10.91 log CFU/g. Inoculated shrimp samples (25°C) were dipped in citric acid 5% and 10% and acetic acid 4% and 8% for 5, 15, 30, 60 minutes and 24 hours. Initial counts of *Vibrio parahaemolyticus* in shrimp samples decreased following treatment with citric acid 5% and 10% for 5 min. by 5.68 log CFU/g (52.06%) and 7.91 log CFU/g (72.5%), respectively and following treatment with acetic acid 4% for 5 min. by 6.61 log CFU/g (60.59%). Growth of *Vibrio parahaemolyticus* in shrimp samples was completely inhibited after being dipped in acetic acid 8% for 5 min., acetic acid 4% for 15 min and citric acid 10% for 30 min. When compared to several other mild preservation procedures, treatment with citric and acetic acids is inexpensive and uncomplicated method. Results of the present study are envisaged to be useful for commercial applications for effective decontamination of shrimp.

Key words: Shrimp · *V. parahemolyticus* · Citric Acid · Acetic Acid

INTRODUCTION

Studies and dietary recommendations have suggested that increased consumption of seafood can contribute to a more healthful diet [1]. Additionally, shrimp, as a food component, has many characteristics like tenderness, easily digested, additive-free and minimally processed. These characteristics make them a product that almost completely fulfils the demands of consumers [2] and the market's demand in terms of consistent quality, off-season product availability and controlled sizes [3].

However, shrimp like any other seafood is a highly perishable product that provides favorable conditions for the growth and proliferation of various pathogenic and spoilage microorganisms [4]. It is a known fact that microbial contamination can lower the quality of shrimp, reduce shelf life [5] and lead to mass mortality, slow growth and deformity of shrimp causing major economic losses in shrimp aquaculture [6].

As food safety is a major global concern that affects the consumer and those in the food service sector [7], serious attention has to be given to the aquaculture industry as fish can act as a vector for human pathogenic bacteria [8]. The promotion of environmentally sound practices in all fields of shrimp production is a relevant point for the aquaculture industry if sustainability is to be achieved**.** Accordingly, to prevent food borne pathogen illness from shrimp, pathogens in shrimp must be eliminated or reduced to a safe level and the pathogen growth in shrimp must be controlled [9]. Extensive efforts have been pursued to assure a safe supply of shrimp, but disease and death due to naturally occurring bacteria have been observed. This might be a result of underestimated and under managed microbial contamination.

Among the indigenous microbiota of coastal environments, *Vibrio parahaemolyticus* is targeted as a causative agent of human disease due to the consumption of shrimp [10].

However, instances of food poisoning related to *V. parahaemolyticus* may be due to the habit of consuming raw or semi-cooked seafood and shellfish or to post-process contamination of foods with this organism where raw and cooked fish are handled in the same area or through cross contamination of cookware or

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utensils. The bacteria proliferate rapidly when contact surfaces are not cleaned properly or the seafood is not kept out of the temperature "danger zone" [11]. If such contamination occurs, there are chances that *V. parahaemolyticus* multiplies in the shrimp preparations despite the addition of spices [4] leading to the development of acute gastroenteritis.

The main symptoms of illness are diarrhea, headache, vomiting, nausea, abdominal cramps, chills and slight fever. Although the illness is self-limited, the infection may cause septicemia that is life-threatening to immunocompromised people such as those with HIV, cancer, liver disease, insulin-dependent diabetes, hemochromatosis (iron overload), stomach troubles, as well as prolonged steroid use [11].

Many methods have been developed to prolong the shelf life of fishery products, such as washing, storage at low temperatures, cold shock, freezing, ultraviolet irradiation, salt treatment and decontamination using chlorine, ozone and chloroform [12]. However, applying high thermal treatment to fish gives an unacceptable decrease in its sensory quality [13]. Additionally, chemical preservatives can control microbial growth, but consumers are always concerned about the use of artificial preservatives in food, which may have potentially undesirable effects on human health [14].

Nowadays consumer's demands have increased for the use of safe, non toxic, natural preservatives having less or no side effects and resistance in microbes against them[15], having high organoleptic qualities, extending the shelf life and improving the safety of seafood products[16] and therefore, diminishing health problems for consumers of seafood [17]. This has led to somewhat of a renaissance in research activity on the discovery and application of alternatives capable of either killing microorganisms outright or at the very least retarding growth sufficiently to limit their dissemination [18].

Acidifiers consisting of several organic acids and their salts present a promising alternative in aquaculture [6]. Organic acids are natural preservatives which are classified as "generally regarded as safe" (GRAS) by the USFDA [19] and could be used directly in the washing process to control microbial contamination and keeping the quality of fresh products. Use of these natural, food-grade antimicrobial ingredients will provide an additional "hurdle technology" protection beyond low temperature alone [20].

If we can sterilize *V. parahaemolyticus* not by heat treatment but by the addition of some acidic ingredients before eating, it would be one of the safest and most convenient ways to avoid infection with *V. parahaemolyticus* [21].

However, even though citric and acetic acids are reported to be the most widely accepted preservatives in various meat and poultry products, information available with respect to their inhibitory effects on the survival of *Vibrio parahaemolyticus* in seafood products, particularly in shrimp is scarce and not well characterized [22].

The beneficial effects of acid preserved products have attracted the attention of the scientific community to investigate the natural biocidal activities of citric and acetic acids (as a function of concentration and dipping time), in order to evaluate their decontamination efficacy and to explore their possible use as disinfectants and inhibitors for *Vibrio parahaemolyticus* artificially inoculated in fresh shrimp and therefore provide guidelines for preparing and serving shrimp safely.

MATERIALS AND METHODS

Bacterial Strains: Vibrio parahaemolyticus was obtained from the Food Microbiology Laboratory. *Vibrio parahaemolyticus* was maintained on trypticase soy agar slants (containing 3% NaCl) at 4°C. A loopful of *V. parahaemolyticus* was transferred aseptically into 10 ml sterile Alkaline Peptone Water (APW: Merck, Germany) plus 3% NaCl and followed by cultivating separately at 37°C for 24 hrs in shaker incubator. After incubation, *V. parahaemolyticus* was counted by using spread plate method [23] and then adjusted to ~10^7 CFU/ml [16] with tube dilution methods. The number of CFU/ml was considered as initial inoculums load to inoculate into fresh shrimp samples.

Shrimp Samples: A total of 5 groups of freshly caught shrimp samples (50 g each = total 250 g) were purchased directly from the local fishermen in Tanta, Egypt in July 2011. The shrimps were placed in ice before being taken to the laboratory. All samples were washed in sterile distilled water and disinfected with alcohol.

Artificial Contamination of Shrimp Samples With *Vibrio Parahaemolyticus*: Shrimp samples were dipped in 100 ml Tryptic Soy Broth (TSB, Merck, Darmstadt, Germany) containing an 24 hrs-old culture (with ~10^7 CFU/ml) [16] and left for 30 min. at room temperature (25°C) to allow attachment. The contaminated samples were stored in sterile glass beakers covered with glass lids at ambient temperature (30±2°C). *Vibrio parahaemolyticus* in the samples was enumerated to get the initial load before dipping treatments were performed [22, 24].

Decontamination with Citric and Acetic Acids: Every 50g of contaminated shrimp samples with known *V. parahaemolyticus* load were dipped into containers containing 100 ml of citric acid (5% and 10%) and acetic acid (4% and 8%) solutions at room temperature 25°C±1°C for 5, 15, 30, 60 min. and 24 hrs. This room temperature was mainly selected for dipping because of the fact that *V. parahaemolyticus* is more sensitive to higher temperature compared with lower or refrigeration temperature. Additionally, higher temperature is reported to yield the best antimicrobial effects with respect to organic acids [25]**.** The samples without citric acid and acetic acid dipping served as control and were dipped in 100 ml sterile distilled water (2% NaCl). All the containers were properly labeled. Solutions covered all surface of the whole shrimps (Head-on). After dipping in antimicrobial solutions, the samples were removed by sterile forceps and allowed to drain for 10 min on a presterilized metal net under laminar air flow. The samples were then analyzed for remaining populations of *V. parahaemolyticus* [22, 24].

Microbiological Analysis: Microbiological procedures for counting *V. parahaemolyticus* were determined following standard methods of ISO 8914 [26]. To determine *V. parahaemolyticus* count, 25g shrimp samples were transferred aseptically to a stomacher bag containing 225 ml of alkaline peptone water (APW; 1% tryptone peptone, 2% NaCl, pH 8.6) and homogenized for 2 min using a stomacher (Interscience-BagMixer 400, St. Mon., France). Further, the spread plate method was employed to enumerate *V. parahaemolyticus*. This method involves the spreading of 0.1 ml aliquots of 10-fold sterile serial dilutions (1:10, diluent, alkaline peptone water with 2% NaCl) of shrimp homogenates onto the surface of solidified thiosulfate citrate bile salt sucrose agar (TCBS, Merck, Darmstadt, Germany) and the TCBS plates were incubated at 37°C for 24 hrs. The formation of colonies that are round (2-3 mm diameter) and bluish green on TCBS was considered positive for *V. parahaemolyticus* and microbial counts were expressed as CFU/ml. All the experiments were conducted in triplicates.

Statistical Analysis: *Vibrio parahaemolyticus* counts on shrimp samples were converted into logarithms of the number of colony forming units per gram (log CFU/g) for statistical analysis. Analysis of Variance (ANOVA) was performed using SPSS Version 15.0 and comparison of means were made using Tukey's test at the 95% confidence level (significance level at $P \leq 0.05$).

RESULTS

The obtained results are represented in the following tables and figure:

DISCUSSION

Modern food processing technologies often rely on non thermal processes to provide microbiologically safe and stable food products. Some of the promising technologies include mild chemical decontamination treatments with organic acids due to increased consumer demands for fresh foods [27].

Table (1) showed *V. parahaemolyticus* counts on shrimp samples treated with different concentrations of citric and acetic acids. The initial count of *V. parahaemolyticus* in shrimp samples after inoculation was 10.91 log CFU/g. *Vibrio parahaemolyticus* counts in shrimp samples dipped in citric acid 5%, citric acid 10% for 5 min. and citric acid 5% for 30 min. were significantly lower ($p<0.05$). Moreover, the growth of *V. parahaemolyticus* in shrimp samples was completely inhibited after dipping in acetic acid 8% for 5 min., acetic acid 4% for 15 min. and citric acid 10% for 30 min. Table (2) and Fig. (1) showed the log reduction in numbers of *V. parahaemolyticus* in shrimp samples dipped in different concentrations of citric and acetic acids. *Vibrio parahaemolyticus* counts in treated shrimp samples declined from 10.91-5.23 log CFU/g (52.06%) and from 10.91-3 log CFU/g (72.5%), when treated with citric acid 5% and 10% for 5 min., respectively. Moreover, when treated with 4% acetic acid for 5 min. *V. parahaemolyticus* counts declined from 10.91-4.30 log CFU/g (60.59%) and inhibited completely when treated with acetic acid 8% for 5 min. the results indicate that the inhibition of *V. parahaemolyticus* is related to the concentration of citric and acetic acids and a dipping time period. We observe that *V. parahaemolyticus* counts declined and even inhibited completely, when increasing the concentration and also extended the dipping time of citric and acetic acids.

Our results agree with those of others who reported the antimicrobial effects of organic acids in meat [28]**,** poultry [29], catfish [30], mussel [24] and shrimp [31] and also with Smigic *et al*. [27] who stated that acetic acid is the most acceptable organic acid for decontamination of food products.

Organic acids have a long history of use in the food industry as food additives and preservatives for preventing food deterioration and extending shelf life of

Table 1: The effects of various concentrations of citric acid and acetic acid on counts of *Vibrio parahaemolyticus* (log CFU/g) in artificially inoculated shrimp samples

		Citric acid		Acetic acid	
Duration of decontamination (min.)	Control (Distilled water)	5%	10%	4%	8%
5	6.48±1.45[a]	5.23±0.64[ab]	3±0.30[b]	4.30±3.00[b]	ND
15	6.48±1.45[a]	6.30±0.30[a]	3.398±1.48[a]	ND	ND
30	5.76±0.31[b]	4.04±1.84[a]	ND	ND	ND
60	6.39±0.40[a]	6.19±0.33[a]	ND	ND	ND
24 hrs	ND	ND	ND	ND	ND

-ND: Not Detected -The values are represented as means±SD of three replicates.
-a and b are significantly different ($P < 0.05$).

Table 2: Log reduction & % in *Vibrio parahaemolyticus* artificially inoculated into shrimp samples dipped in different concentrations of citric acid and acetic acid

			Citric acid				Acetic acid			
	Control (Distilled water)		5%		10%		4%		8%	
Duration of decontamination (min.)	Log	%	Log	%	Log	%	Log	%	Log	%
5	4.43	40.6	5.68	52.06	7.91	72.5	6.61	60.59	10.91	100
15	4.43	40.6	4.61	42.25	7.512	68.85	10.91	100	10.91	100
30	5.15	47.20	6.87	62.97	10.91	100	10.91	100	10.91	100
60	4.52	41.43	4.72	43.26	10.91	100	10.91	100	10.91	100
24 hrs	10.91	100	10.91	100	10.91	100	10.91	100	10.91	100

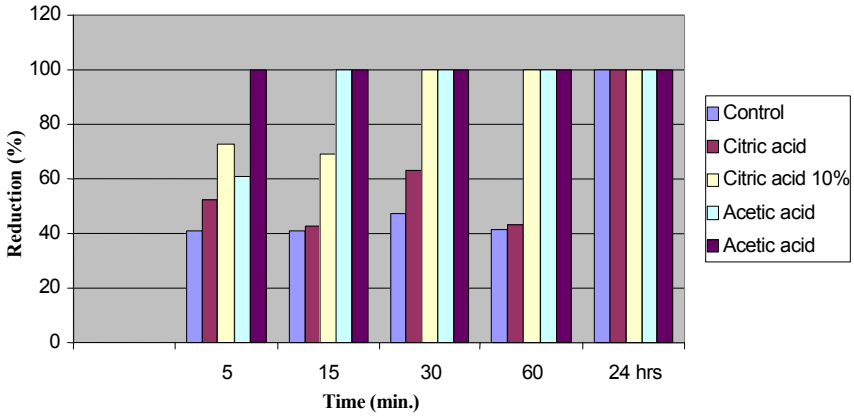

Fig. 1: Reduction & % in *Vibrio parahaemolyticus* artificially inoculated into shrimp samples dipped in different concentrations of citric acid and acetic acid

perishable food ingredients and have been demonstrated to be effective under a wide variety of processing conditions [18]. Specific organic acids have also been used to control microbial contamination and dissemination of food borne pathogens in food production and processing [32]. Citric acid is the primary organic acid in lemon juice which is a food ingredient used for flavoring and adding acidity and acetic acid (vinegar) is a food acidulant.

Corbo *et al*. [33] treated fish burgers with the combination of thymol, lemon and grape fruit seed extract and reported an extended shelf life that is about 40% similar to results of Cosansu *et al*. [34]. Similarly, [35] added vinegar to a beef product and reported the positive effect of this treatment on the microbiological stability and sensory quality.

The inhibiting effect of citric acid and acetic acid against *V. parahaemolyticus* was reported by Hasegawa *et al*. [36, 15, 24]. Likewise, lower degree of spoilage was reported among samples stored with citric acid [37]. Sengor *et al*. [38] reported an increased shelf life of dogfish fillets when treated with salt, ascorbic acid and citric acid.

Additionally, our results on the control samples (treated with distilled water) showed significant differences on comparison with the individual initial microbial load. However, no significant reduction ($P > 0.05$) in the microbial load was recorded on comparing these control samples with time of dipping. This might be directly attributed to the washing effects (dipping), wherein the adhering pathogens or microorganism residing on the surface of shrimps are removed up to a certain extent [22].

In general, it should be noted that the antibacterial efficacy of organic acids might depend on several factors such as the type of the acid used, pH of the medium, concentration and temperature of the acid solution, type of the food product, initial microbial load [39], the methods of application, dipping time[40] and the inherent resistance of the target microorganism to the acid used [41]. Moreover, the degree of undissociation of the acid is directly related to its antimicrobial activity; that is, more undissociation results in greater antimicrobial activity as the undissociated acid can penetrate the cell membrane and lower the internal pH of the cell [42]. Molecular weight of organic acids also plays a role, where lower molecular weight organic acids such as acetic acid are more effective than higher molecular weight organic acids such as citric acid[43], because they are lipophilic and can diffuse across the cell membranes of Gram-negative bacteria [44], in addition to, the length of carbon chain of organic acid used, where Gram-negative bacteria are able to uptake and metabolize long and medium-chained organic acids [45]. The effect of organic acids is mainly assigned to its ability to penetrate the cell membrane in its undissociated form, wherein more of the acid would be undissociated at lower pH than at neutral pH[20] and enter the cytoplasm of the cell, dissociate within the cell and therefore decreasing the intracellular pH, providing acid-binding capacity[46], increasing the turgor pressure within the cell due to increase in anions from the acids and expulsion of H^+ ions from the cells [47], disturbing transmembrane proton motive force, denaturating acid-sensitive proteins and DNA[41] and causing an inhibition of acid sensitive enzymes [48] and various essential metabolic and anabolic processes [49]. These actions weaken the cells and make them more susceptible to bacteriocins and other bactericidal compounds [50] leading to injury (sublethal activity) and/or killing (lethal activity). Other mechanisms that could inhibit the growth of undesirable bacteria such as competition for nutrients, available energy or adhesion sites [51] should be considered.

Other toxicity mechanisms have been proposed that attribute membrane uncoupling capabilities for organic acids. It has been speculated that organic acids interfere with cytoplasmic membrane structure and membrane proteins such that electron transport is uncoupled and subsequent ATP production is reduced or that organic acids serve as uncouplers that generally dissipate pH and electrical gradients across cell membranes [41].

Out of the above mentioned study, it can be concluded that citric and acetic acids are safe, economic and effective alternative preservatives for extending the shelf life of fresh shrimp, offering additional barrier "hurdle technology" to inhibit the growth of *V. parahaemolyticus* in shrimp. Where, the effectiveness of citric and acetic acids was uniform as the concentrations and dipping time increased and it can be arranged in descending order as acetic acid (8%) □acetic acid(4%) □citric acid(10%) □citric acid(5%).

This study offers a novel approach to use acetic and citric acids which provide a GRAS-type chemical source with the potential to develop a natural , excellent, attractive and effective antimicrobial strategy against *V. parahaemolyticus* .Therefore, one of the practical application of the present study is the use of citric acid (5% and 10%) and acetic acid (4% and 8%) as potential decontaminants in seafood processing industries, during washing and processing line, for improving the preservation methods and keeping quality of products by reducing the risks posed to consumers and inhibition of pathogenic bacteria particularly *V. parahaemolyticus* which can be found in marine products. Such approach can have wide implications for improvement of food safety. Evaluation of *V. parahaemolyticus* stress responses in food systems, particularly raw shrimp, is a critical area of future research.

REFERENCES

1. National Academy of Sciences "NAS" 2007. Institute of Medicine, Food and Nutrition Board, Seafood Choices: Balancing Benefits and Risks, 2007. The U.S. Dietary Guidelines for Americans, 2005, for example, cites limited evidence suggesting an association between consumption of fatty acids and reduced risks of mortality from cardiovascular disease for the general population. Accessed at http://www.health.gov/dietaryguidelines/ default.htm

2. Murchie, L.W., M. Cruz-Romero, J.P. Kerry, M. Linton, M.F. Patterson and Smiddy, 2005. High pressure processing of shellfish: a review of microbiological and other quality aspects. Innovative Food Science and Emerging Technologies, 6(3): 257-270.
3. Gillet, R., 2008. Global study of shrimp fisheries. FAO Fisheries Technical Paper. No. 475. Rome, FAO, pp: 331.
4. Venugopal, M.N., I. Karunasagar and M.C. Varadara, 2001. Growth and survival of Kanagawa positive *V. parahaemolyticus* in fish and prawn preparations held at ambient and elevated temperature. J. Asian Fisheries Sci., 14: 83-88.
5. Wan Norhana, M.N., S.E. Poole, H.C. Deeth and G.A. Dykes, 2010. Prevalence, persistence and control of Salmonella and Listeria in shrimp and shrimp products: a review. J. Food Control., 21: 343-361.
6. Mine, S. and R. Boopathy, 2011. Effect of organic acids on shrimp pathogen, V. harveyi. J. Curr. Microbiol., doi 10.1007/s00284-011-9962-2.
7. Jacxsens, L., J. Kasuga, P.A. Luning, M. Van der Spiegel, F. Devlieghere and M. Uyttendaele, 2009. A microbial assessment scheme to measure microbial performance of food safety management systems. Int. J. Food Microbiol., 134: 113-125.
8. Apun, K., M.Y. Asiah and K. Jugang, 1999. Distribution of bacteria in tropical freshwater fish and ponds. Int. J. Environmental Health Res., 9: 285-292.
9. Yang, J. and D. Lee, 2009. Lemon, pH and citric acid for Kelaguen safety without temperature control. Micronesica, 41: 19-31.
10. Normanno, G., A. Parisi, N. Addante, N.C. Quaglia, A. Dambrosio and C. Montagna, 2006. *V. parahemolyticus, V. vulnificus* and microorganisms of fecal origin in mussels (*Mytilus galloprovincialis*) sold in the Puglia region (Italy). Int. J. Food Microbiol., 106: 219-222.
11. Institute of Food and Agricultural Sciences "IFAS" 2009. Preventing food borne and non food borne illness: *V. parahemolyticus*. This document is FSHN09-01, one of a series of the Food Science and Human Nutrition Department, Florida Cooperative Extensio Service, Institute of Food and Agricultural Sciences, University of Florida. Original publication date: July 2009. Visit the EDIS Web site at http://edis.ifas.ufl.edu.
12. Masniyom, P. and O. Benjama, 2007. Effect of lactic acetic and citric acids on quality changes of refrigerated green mussel, *Perna virdis* (Linnaeus, 1758). Songklanakarin J. Sci. Technol., 29: 1123-1134.
13. NACMCF (National Advisory Committee on Microbiological Criteria for Foods) 1990. Recommendations for refrigerated foods containing cooked, uncured meat or poultry products that are packaged for extended, refrigerated shelf life and that are ready-to-eat, or prepared with little or no additional heat treatment. Washington, DC: NACMCF, USA.
14. Tassou, C.C., E.H. Drosino and G.J.E. Nychas, 1995. Inhibition of resident microbial flora and pathogen inocula on cold fresh fish fillets in olive oil, oregano and lemon juice under modified atmosphere or air. J. Food Protec., 59: 31-34.
15. Jayana, B.L., T. Prasai, A. Singh and K.D. Yami, 2010. Study of antimicrobial activity of lime juice against *V. cholera*. Scientific World, 8: 44-46.
16. Shirazinejad, A. and N. Ismail, 2010. Effect of lactate treatments on survival of food borne pathogens in frozen shrimp (*Penaeus merguiensis*). American J. Agricultural and Biological Sciences, 5: 242-246.
17. Chaiyakosa, S., W. Charernjiratragul, K. Umsakul and V. Vuddhakul, 2007. Comparing the efficiency of chitosan with chlorine for reducing *V. parahaemolyticus* in shrimp. J. Food Control, 18: 1031-1035.
18. Ricke, S.C., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. J. Poultry Sci., 82: 632-639.
19. Bogaert, J.C. and A.S. Naidu, 2000. Acid antimicrobials: Lactic acid. In: Natural Food Antimicrobial Systems, Naidu, A. S. (Ed.). CRC Press, New York.
20. Lin, Y.T., R.G. Labbe and K. Shetty, 2005. Inhibition of *V. parahaemolyticus* in seafood systems using oregano and cranberry phytochemical synergies and lactic acid. Innovative Food Science and Emerging Technologies, 6: 453-458.
21. Tomotake, H., T. Koga, M. Yamato, A. Kassu and F. Ota, 2006. Antibacterial activity of citrus fruit juices against Vibrio species. J. Nutr. Sci. Vitaminol., 52: 157-160.
22. Shirazinejad, A., N. Ismail and R. Bhat, 2010. Lactic acid as a potential decontaminant of selected food borne pathogenic bacteria in shrimp (*Penaeus merguiensis* de Man). J. Food borne Pathogens and Disease, 7: 1531-1536.

23. Food and Drug Administration "FDA" 2001. Methods for Specific Pathogens: *Escherichia coli*, Salmonella and Vibrio, 8th Ed. FDA Center for Food Safety and Applied Nutrition, Bacteriological Analytical Manual. Available at www.cfsan.fda.gov/~ebam/bam-toc.html, assessed May 15, 2005. (Online).
24. Terzi, G. and A. Gucukoglu, 2010. Effects of lactic acid and chitosan on the survival of *V. parahaemolyticus* in mussel samples. J. Animal and Veterinary Advances, 9: 990-994.
25. Smulders, F., 1995. Preservation by microbial decontamination; the surface treatment of meats by organic acids. In: New Methods of Food Preservation. Gould GW (Ed.). London: Blackie Academic and Professional.
26. International Organization for Standardization "ISO" 8914 1990. Microbiology-General Guidance on Methods for the detection of *V. parahemolyticus*. ISO, Geneva, Switzerland.
27. Smigic, N., A. Rajkovic, D. S. Nielsen, N. Arneborg, H. Siegumfeldt and F. Devlieghere, 2010. Survival of lactic acid and chlorine dioxide treated *Campylobacter jejuni* under suboptimal conditions of pH, temperature and modified atmosphere. Int. J. Food Microbiol., 141: S140-S146.
28. Quattara, B., R.E. Simard, R.A. Holley, G.J.P. Piette and A. Begin, 1997. Inhibitory effect of organic acid upon meat spoilage bacteria. J. Food Protec., 60: 246-253.
29. Kim, C.R. and D.L. Marshsall, 2000. Quality evaluation of refrigerated chicken wings treated with organic acids. J. Food Quality, 23: 327-335.
30. Bal'a, M.F.A. and D.L. Marshall, 1998. Organic acid dipping of catfish fillets: Effect on color microbial load and *Listeria monocytogenes*. J. Food Protec., 61: 1470-1474.
31. Al Dagal, M.M. and W.A. Bazaraa, 1999. Extension of shelf life of whole and peeled shrimp with organic acid salts and bifidobacteria. J. Food Protec., 62: 61-65.
32. Cherrington, C.A., M. Hinton and I. Chopra, 1991. Organic acids: Chemistry, antibacterial activity and practical applications. Adv. Microb. Physiol., 32: 87-108.
33. Corbo, M.R., B. Speranza and A. Filippone, 2008. Study on the synergic effect of natural compounds on the microbial quality decay of packed fish hamburger. Int. J. Food Microbiol., 127: 261-267.
34. Cosansu, S., S. Mol, D. Alakavuk and S. Ozturan, 2011. The effect of lemon juice on bonito (*Sarda sarda*, Bloch, 1793) preserved by sous vide packaging. Int. J. Food Sci. and Technol., 46: 395-401.
35. Jang, J.D., G.H. Seo, E.S. Lyu, K.L. Yam and D.S. Lee, 2006. Hurdle effects of vinegar and sake on Korean seasoned beef preserved by sous vide packaging. J. Food Control, 17: 171-175.
36. Hasegawa, J., Y. Hara-Kudo, T. Nishina, H. Konuma and S. Kumagi, 2002. Survival of *V. parahaemolyticus* serovar O3:K6 strains under acidic. Shokuhin Eiseigaku Zasshi, 43: 90-94.
37. Schirmer, B.C., R. Heiberg, T. Eie, *et al*. 2009. A novel packaging method with a dissolving CO headspace combined with organic acids prolongs the shelf life of fresh salmon. Int. J. Food Microbiol., 133: 154-160.
38. Sengor, G.F., S. Mol and D. Ucok, 2007. The effect of ascorbic acid, citric acid and salt on the quality of spiny dogfish (*Squalus acanthias*) fillet. J. Aquatic Food Product Technology, 16: 103-113.
39. Gomez-Lopez, V.M., A. Rajkovic, P. Ragaert, N. Smigic and F. Devlieghere, 2009. Chlorine dioxide for minimally processed produce preservation: a review. Trends in Food Science and Technology, 20: 17-26.
40. Smulders, F., 1995. Preservation by microbial decontamination; the surface treatment of meats by organic acids. In: New Methods of Food Preservation. Gould GW (Ed.). London: Blackie Academic and Professional.
41. Davidson, P.M., 2001. Chap. 29. Chemical preservatives and natural antimicrobial compounds. PP. 593-627 in Food Microbiology-Fundamentals and Frontiers. 2nd Ed. M.P. Doyle, L.R. Beuchat and T.J. Montville, Ed. American Society for Microbiology, Washington, DC.
42. Brudzinski, L. and M.A. Harrison, 1998. Influence of incubation conditions on survival and acid tolerance response of *Escherichia coli* O157:H7 and non O157:H7 isolated exposed to acetic acid. J. Food Protec., 61: 542-546.
43. Ryu, J.H., Y. Deng and L.R. Beuchat, 1999. Behavior of acid adapted and unadapted *Escherichia coli* O157:H7 when exposed to reduced pH achieved with various organic acids. J. Food Protec., 62: 451-455.
44. Lückstädt, C., 2008. The use of acidifiers in fish nutrition. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3, No. 044. Available at http:// www.cababstractsplus.org/cabreviews.

45. Russell, J.B. and F. Diez-Gonzalez, 1998. The effects of fermentation acids on bacterial growth. Advances in Microbial Physiology, 39: 205-234.
46. Roth, F.X. and M. Kirchgessner, 1995. The role of formic acid in animal nutrition. BASF AG Ludwigshafen, Fifth Forum Animal Nutrition, pp: 5-20.
47. Foster, J.W., 1999. When protons attack: microbial strategies of acid adaptation. Curr. Opin. Microbiol., 2: 170-174.
48. Davidson, P.M. and T.M. Taylor, 2007. Chemical preservatives and natural antimicrobial compounds. In: Doyle, M.P. and L.R. Beuchat, (Eds.), Food Microbiology: Fundamentals and frontiers. ASM Press, Washington DC, pp: 713-745.
49. Abee, T. and J.A. Wouters, 1999. Microbial stress response in minimal processing. Int. J. Food Microbiol., 50: 65-91.
50. Nykänen, A., S. Vesanen and H. Kallio, 1998. Synergistic antimicrobial effects of nisin whey permeate and lactic acid in microbes isolated from fish. Lett. Appl. Microbiol., 27: 480-486.
51. Verschuere, L., H. Heang, G.R. Criel, P. Sogerloos and W. Verestrate, 2000. Microbial control through preemptive colonization by selected bacterial strains. Appl. Environ. Microbiol., 65: 2527-2533.