Role of Organic Acids during Processing To Improve Quality of Channel Catfish Fillets†

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ABSTRACT

A microbial preparation derived from aquacultured channel catfish fillets (Ictalurus punctatus) was acidified with 0, 1, 2 and 4% (vol/vol) weak organic and held in an ice bath at 0°C to simulate the chilling process. Additionally, catfish fillets were sprayed under varying pressures at 15°C with organic acids to evaluate the efficacy of concentrations of organic acids and spray pressures to ameliorate the microbiological quality. To determine plate counts, the dilution fluid was neutralized to pH 7.2 with 1.0 M NaOH. The aerobic plate counts of microorganisms in the chilling water were monitored over a 20-min interval. Aerobic plate counts were found on the channel catfish fillets before and after spray washing with organic acids. Plates were incubated at 35°C for 48 h. The addition of organic acids to the microbial preparation used in simulating the chilling process significantly (P < 0.05) reduced the number of bacteria surviving. The number of surviving bacteria in the chilled water decreased with increasing concentration and time of exposure to organic acids. Propionic acid had the most detrimental effect on organisms present in the microbial preparation followed by acetic and lactic acids. Spray washing of catfish fillets with water did not significantly (P < 0.05) affect the microbial quality of fillets. However, catfish fillets sprayed with organic (lactic and propionic) acids significantly (P < 0.05) reduced the microbial counts by 10-fold. Lactic and propionic acids were not significantly (P > 0.05) different in influencing the aerobic counts of the catfish fillets.

Fresh muscle foods deteriorate primarily due to growth of aerobic microflora. The aerobic spoilage is due to inherent and opportunistic bacteria contaminating muscle tissue after slaughter. Hence, muscle foods must be preserved to sustain their overall intrinsic quality. Muscle foods have been stored under refrigeration conditions (2 to 4°C), in ice (0°C), and in modified-atmosphere packaging (8). The storage and packaging conditions extend shelf life by preventing proliferation of natural microflora on muscle foods. Additionally, muscle foods may be further subjected to biochemical, chemical, physical and/or electromagnetic processing which are usually nonthermal. These processes further extend the shelf life through the sublethal and lethal injuring of indigenous microorganisms (4). Nonthermal processing such as irradiation has also been attempted successfully (15), but irradiated products have limited acceptance because of inadequate consumer education. The shelf-life of irradiated muscle foods is extended chiefly due to reduction in the number of viable aerobic bacteria following the killing and injuring of bacterial cells. Several other nonthermal processing methods, both chemical and biochemical, that can improve the overall quality of muscle foods have been reported to increase the shelf life of muscle foods through the reduction of bacterial counts. These include dipping in organic acid solutions, spray washing using water or organic acid solutions and the use of enzymes (4).

Aquacultured channel catfish (Ictalurus punctatus) fillets are chiefly marketed as a fresh product (6, 9). The fish fillets have a shelf life of about 12 to 15 days following which they deteriorate primarily through microbiological spoilage. An extension of the product shelf-life would enable delivery to distant markets such as Canada and off-shore locations, particularly Europe. Various organic (e.g., lactic and acetic) acids have been utilized in an attempt to reduce the indigenous flora of channel catfish fillets (12, 13). Fernandes et al. (5) attempted a riboflavin-methionine photochemical system to extend the shelf life of channel catfish fillets during the chilling process. Spray washes with water have been used to reduce surface microbial populations in muscle foods (14, 16).

During processing, aquacultured channel catfish fillets are chilled to sustain their quality. Fish fillets are submerged in (batch processing) or conveyed (continuous processing) through iced water (0°C) for 20 to 30 min. in chilling tanks. The bacterial load of the chilling tank increases over time and is the predominant source for cross contamination. Additionally, the fillets are sprayed with water during various stages of processing, the principal function being to

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clean the tissue. However, it is unclear whether such spray washing reduced microbial load. This study attempts to explore the possibility of using weak organic acids independently and combined with varying pressure sprays to improve the overall catfish fillet microbial quality. Two independent unit processes that may improve catfish fillet quality were identified as submersion in chilling tanks and organic acid spray washing, both processes were attempted independently. The first attempt was to simulate the chilling process and observe the effect of adding weak organic acids to alter the microbial load in chilling tanks. Additionally, a separate attempt was made to spray fillets with weak organic acids under varying pressure to directly improve their microbial quality.

MATERIALS AND METHODS

Preparation of channel catfish microflora suspension. A phosphate buffer (15 mM, pH 7.2) was prepared and sterilized by autoclaving at 121°C for 20 min. A suspension of the catfish heterogenous microflora was prepared by massaging each of three aquacultured catfish fillets for 2.0 min. with sterile phosphate buffer (100 g/10 ml). Suspension preparations were pooled to achieve a uniform inoculum. Dimethyl sulfoxide (5.0%, vol/vol) (Sigma Chemical Co., St. Louis, MO) was added to the suspension. The microfloral suspension was distributed in 5.0-ml aliquots and subsequently stored at −80°C until used (5). Standard plate counts were determined on the microfloral preparation.

Suspension of catfish microflora in organic acids. The microfloral suspension was thawed and 2 ml of suspension was added to flasks containing 498 ml of each monoprotonic organic acid held in an ice bath (0 to 2°C). Weak organic acids used in this study were acetic (Fisher Scientific Co., Fair Lawn, NJ), dl-lactic (Fisher Scientific Co.) and propionic (Fisher Scientific Co.) acids. Organic acid concentrations evaluated were 0 (control), 1, 2, and 4% (vol/vol). About 10.0 ml of the mixture was withdrawn at 0, 5, 10, and 20 min. The reaction was terminated (neutralized) by adding a predetermined quantity of 1.0 M NaOH. The sample was held on ice until microbial analyses were performed and all analyses were completed within an hour. Standard plate counts were determined on the neutralized aliquots following appropriate dilution.

Organic spray washing of catfish fillets. A spray washer was designed to apply the weak organic acid solutions. The organic acid solutions were sprayed with a positive displacement pump; flow was monitored and regulated with a rotameter and flow control valve. The fillets were sprayed on a conveyor belt moving at about 30 cm/min.

Frozen channel catfish fillets were procured from a processor and held at −80°C. Two days prior to spray washing, the fillets were allowed to thaw in a refrigerator (2 to 4°C). Standard plate counts were determined from 10 fillets (about 100 g) prior to washing with either lactic acid or propionic acid. Concentrations of the organic acids sprayed were 0, 1, and 2% (vol/vol) and the pressure at the nozzle was 7.5 lb/in² (organic acid flow of 6.0 liters/min) and 5.0 lb/in² (organic acid flow of 5.0 liters/min). Three fillets were spray washed (15°C) for each treatment, placed in sterile stomacher bags, and held on ice until microbial analyses were performed.

Microbial analysis. Standard plate counts were determined on the liquid suspensions and catfish fillets following neutralization to pH of 7.2. Fillets were stomached with an equal quantity of cold 15 mM phosphate buffer. Microbial counts were determined on plate count agar (Difco Laboratories, Detroit, MI) after incubation at 35°C for 48 h (19).

Statistical design and analyses. The effect of organic acids on the microfloral suspension was analyzed as a split-plot with time treatment design with randomized complete blocks using SAS procedures (17). The day of experiment was a blocking (replicate) criterion and all experiments were replicated (on 3 different days). For each experiment, the treatments (whole plot, organic acid concentration) were randomized while the time of sampling (min) was the split-plot. The means for duplicate plate counts were averaged. The average counts were analyzed following log transformation of the data (18) and are reported as geometric means. The effect of spray washing was analyzed using randomized complete blocks (17) treatment design. The day of experimentation was used as a blocking criterion (replicate); the experiment was replicated two times.

RESULTS AND DISCUSSION

Organic acids effect on catfish microfloral suspension. The killing and injuring effects of organic acids on the channel catfish microfloral suspensions are shown in Figure 1. The organic acids reduced the geometric mean counts of the suspended microfloral preparation. Generally, the degree of reduction varies with the quality and quantity of organic acid. Additionally, suspension exposure time also influences...
injury. Most studies that have used organic acid treatments do not report the neutralization of their dilution buffer before the enumeration of bacterial counts. This is important because most enumerations are performed on nonselective standard methods agar. The standard plate count agars facilitate the growth of sublethally injured and viable cells. However, the presence of organic acid in the dilution medium would impair the growth of sublethally injured cells. Thus, the counts reported following acid treatments without neutralization would be lower than the estimated count following neutralization of the acidulants (5).

Acetic acid. The effect of 0 to 4% acetic acid (CH$_3$COOH) on catfish microflora during a 20-min suspension period is shown in Fig. 1a. There was a reduction in the geometric means of microorganisms during the observation period. The microbial counts at the end of 20 min were significantly lower ($P < 0.05$) at 2 and 4% levels but not significantly different at the 1% level. Following 20 min of exposure of the suspension in 0, 1, 2, and 4% acetic acid the counts decreased by 0, 0.5, 1.5 and 2.5 log CFU/ml, respectively. Killing and injuring of the microflora in the medium increased with increasing organic acid concentration and suspension time. Other researchers (12) have also observed that the application of acetic acid dips decreased bacterial counts on muscle foods, including catfish fillets. Watson et al. (20) also observed that acetic acid affected the growth of Yersinia enterocolitica in fermented dairy foods.

Lactic acid. Fig. 1b illustrates the effect on catfish microflora following suspension in 0 to 4% lactic acid (CH$_3$CH(OH)COOH). A decrease in the aerobic plate count geometric means was observed. The microbial counts at the end of 20 min were significantly lower ($P < 0.05$) at 4% acid but not significantly different at 1 and 2% acid. From an initial count of 10$^{6.3}$ CFU/ml, the aerobic counts decreased to 10$^{5.8}$, 10$^{5.2}$, and 10$^{4.7}$ CFU/ml for 1, 2, and 4% dl-lactic acid respectively. Several researchers (10-13) reported a reduction in microbial numbers following treatment of channel catfish fillets with lactic acid. Dorsa and Marshall (2) observed that lactic acid influenced the survival of Listeria monocytogenes in crawfish tail.

Propionic acid. The detrimental effect of 0 to 4% propionic acid (CH$_3$CH$_2$COOH) on catfish microfloral suspensions is demonstrated in Fig. 1c. The microbial counts at the end of 20 min were significantly lower ($P < 0.05$) at 2 and 4% acid but not significantly different at 1% acid. The geometric means of viable cells decreased with increasing concentrations of propionic acid, with 4% propionic acid having the most detrimental effect. A 4% solution of propionic acid reduced the geometric mean of bacterial counts from 6.0 log CFU/ml at 0 min to 3.5 log CFU/ml in 20 min. The 4% concentration was used as an upper limit to compare its effect with other organic acids at the same level. The decrease in aerobic plate counts following suspension in propionic acid was similar to the decrease in microbial counts of other monoprotic organic acids as observed in this and other studies (10). Watson et al. (20) observed that propionic acid was effective in reducing Y. enterolitica counts in simulated fermented dairy foods.

The killing and injuring following suspension of catfish microflora in weak monoprotic organic acids to simulate the chilling process is due to the undissociated organic acid in the extracellular environment, which is transported through the cell wall and subsequently dissociates in the intracellular environment (7). The intracellular dissociation of the organic acid reduces the intracellular pH and results in denaturation of enzymes which in combination with several other factors leads to injury (sublethal activity) and/or killing (lethal activity). Of the three organic acids, propionic acid was the most effective followed by acetic and lactic acid. Similar observations were made by Watson et al. (20) in simulated fermented dairy foods.

Besides organic acids, salts of organic acids have also been used to increase the shelf life of catfish fillets. Williams et al. (21) observed a shelf life extension of fresh catfish (Ictalurus nebulosus marmoratus) fillets with 1 to 2% sodium lactate. A decrease in aerobic plate count was observed with 2% sodium lactate; however, total and fecal coliform counts were not affected. In addition to antimicrobial properties, sodium lactate reportedly decreases water activity (1).

Spray washing of catfish fillets. Lactic acid. The effect of spray washing catfish fillets with lactic acid is illustrated in Fig. 2a. Spray washing with water reduced the geometric mean of the standard plate count by about 0.5 log CFU/g. There was no difference in the spray washing efficacy at high and low flow rates. Spray washing with lactic acid reduced the overall count from the control by about 1.0 log CFU/g. Since spray washing with organic acids reduced the geometric means by an additional 0.5 log CFU/g, it was concluded that spray washing improved the microbial quality of the fillet due to both the washing as well as the sublethal and lethal effect of dl-lactic acid on the indigenous microflora of channel catfish fillet.

![Figure 2. Effect of spraying washing (5 and 7.5 lb/in$^2$) with 0 to 2% monocarboxylic acids (lactic acid and propionic acid) on the aerobic counts of aquacultured channel catfish fillets.](http://meridian.allenpress.com/jfp/article-pdf/61/4/495/1660506/0362-028x-61_4_495.pdf)
Propionic acid. Fig. 2b illustrates the geometric means of standard plate counts of catfish fillets following spray washing with propionic acid. Propionic acid spray reduced the aerobic plate counts by half a log cycle. Both flow rates (high and low) and acid concentrations did not significantly differ in their killing and injuring effects. Spray washing with propionic acid provided effects similar to those of dl-lactic acid and reduced the overall microbial counts by 10-fold. Since spray washing with either organic acid reduced the geometric means by an additional 0.5 log CFU/g, it can be concluded that spray washing ameliorates the microbial quality of the fillet due to washing as well as the sublethal and lethal activities of the organic acid.

Both lactic and propionic acids used for spray washing were equally efficacious and there was no significant difference in channel catfish fillet microbial quality following spray washing. Further, both organic acids are monoprotic and both are naturally present in food systems. Hence, these organic acids along with other organic acids could be used to ameliorate the microbial quality of muscle foods.

Monocarboxylic (such as acetic, lactic, propionic) acids and tricarboxylic acids have been investigated for their bacteriocidal and/or bacteriostatic activity. Dorsa et al. (3) sprayed combinations of citric acid on crawfish tail and observed a bacteriocidal and/or bacteriostatic effect.

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