Journal of Food Protection, Vol. 63, No. 9, 2000, Pages 1231–1239

Reduction of Endogenous Bacteria Associated with Catfish Fillets Using the Grovac Process†

M. RAMOS AND W. J. LYON*

Louisiana Department of Agriculture and Forestry Rapid Microbial Detection Facility, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803, USA

MS 99-370: Received 9 December 1999/Accepted 14 March 2000

ABSTRACT

Fresh catfish (*Ictalurus punctatus*) fillets are known to be contaminated with a large number of spoilage and pathogenic bacteria. The Grovac method, a new patented (U.S. 5,543,163) process, was evaluated for its efficacy in reducing the number of pathogens and spoilage microorganisms associated with food. This process involves using a processing solution containing ascorbic acid (AA) and sodium chloride (NaCl), vacuum, and tumbling. A total of 51 bacterial isolates were isolated and identified from whole catfish and catfish fillets using both selective and nonselective media, phenotypic tests, and the Vitek identification system. Psychrotrophic foodborne pathogens included: *Aeromonas hydrophila, Escherichia coli, Listeria* sp., *Plesiomonas shigelloides, Proteus* sp., *Staphylococcus aureus,* and *Vibrio parahaemolyticus.* High aerobic plate counts (2.6 $\times$ 10^7 CFU/g) for catfish fillets indicated that fillets were heavily contaminated during processing of catfish. The Grovac process showed that various treatment combinations of AA and NaCl resulted in a 1.2 to 2.3 CFU/g log reduction of microbial counts associated with catfish fillets. The effectiveness of the process may be related to the synergistic effect of tumbling, AA, NaCl, and vacuum. These results suggested that the Grovac process could be used as an alternative processing procedure to reduce microbial populations associated with catfish fillets and may be useful to improve the shelf-life and food safety of the product. Microbiological data from this study will be used for the development of a hazard analysis for the implementation of the hazard analysis critical control point program for processed catfish fillets.

The major catfish-producing states are Alabama, Arkansas, Louisiana, and Mississippi *(4)*. Commercial production of catfish in the United States has increased at a phenomenal rate in the past several years. Farm-raised catfish processed during 1998 totaled 564 million pounds, a new record that is 8% above the previous record of 525 million pounds processed in 1997. Catfish growers had sales of 469 million dollars in 1998, and the average price paid to producer was 74.3 cents per pound *(4, 39)*. Aquacultured channel catfish (*Ictalurus punctatus*) fillets are marketed as a fresh (ice-packed) product.

During processing of catfish (e.g., deheading, skinning, eviscerating), the microorganisms in the skin, gills, and gut can be spread onto the processing equipment, the workers, and the flesh of the fillet. Hence, the normal sterile flesh can be inoculated with millions of bacteria. Therefore, the microbial flora on catfish fillets leaving the processing plant may be different from that of whole catfish entering the plant *(6, 12, 15)*.

Traditionally, the growth of spoilage bacteria on catfish fillets has been prevented by using refrigeration temperatures *(14, 34, 35)*. This preservation method normally results in a short shelf life of 5 to 10 days. After this period of time, catfish fillets deteriorate primarily through microbiological spoilage. However, concern for the microbiological safety of chilled catfish fillets has grown in the last few years. Fernandez et al. *(8)* and Leung et al. *(23)* reported that psychrotrophic pathogens (e.g., *Listeria monocytogenes, Aeromonas hydrophila*) could survive and grow at refrigeration temperatures *(21, 24)*. Thus, the use of refrigeration can no longer be deemed sufficient to keep catfish fillets safe from bacterial hazards. However, catfish have not been implicated in any foodborne outbreak probably due to the fact that the meat is thoroughly cooked. Food scientists have evaluated various methods, including modified atmosphere packaging and antimicrobial preservatives, to improve the shelf life of refrigerated catfish products *(8, 18–20, 28, 32, 37, 38, 40)*.

The efficacy of the Grovac process *(13)* to reduce the growth of pathogens and spoilage microorganisms in fresh catfish fillets has not been thoroughly investigated. Baseline studies for the absence and presence of foodborne pathogens are required to evaluate the effectiveness of the Grovac process on reduction of endogenous pathogens associated with fresh catfish fillets. The microbiological data obtained in this study will be used in the development of hazard analysis and critical control point plans for processed catfish.

There is also concern that some psychrotrophic pathogens associated with catfish fillets can grow at refrigeration temperatures and that the pathogens may multiply to an undesirable level on refrigerated fresh catfish during a normal storage period. It is also possible that the Grovac process may affect the growth rate of endogenous micro-

---

* Author for correspondence. Tel: 225-388-2755; Fax: 225-342-0027; E-mail: wlyon@agctr.lsu.edu.

† Manuscript paper number 99-21-0627 of the Louisiana Agricultural Experiment Station, Baton Rouge, project S-263.

flora of catfish fillets. Consequently, the specific objectives of this study were as follows: (i) to evaluate baseline endogenous microflora associated with fresh whole catfish (*I. punctatus*) and fresh catfish fillets; (ii) to evaluate the reduction of endogenous microflora of fresh catfish fillets using the Grovac processing method; and (iii) to evaluate microbiological shelf life of refrigerated fresh catfish fillets at 4°C treated with the Grovac process.

MATERIALS AND METHODS

Fish source. Whole catfish and catfish fillets were obtained from Tony's Live Catfish & Seafood (Baton Rouge, La.) during the spring and the summer of 1999, respectively. Whole catfish are defined as unfilleted catfish with the skin and head intact. Live catfish arrived at the processing plant alive in aerated tanks via hauling trucks. The live farm-pond-raised catfish were held in tanks with aeration until processed. Catfish were electrically shocked, stunning them before they were manually beheaded, gutted, skinned, and filleted. Catfish fillets were not washed before they were packaged. Freshly killed whole catfish or catfish fillets were bagged in polyethylene bags, transported on ice to the Louisiana Department of Agriculture and Forestry/Louisiana State University Rapid Microbial Detection Laboratory, stored at refrigeration temperature (4°C), and subjected to microbiological analysis within 4 to 8 h.

Media preparation. Selective and nonselective culture media were used to isolate bacteria from both whole catfish and catfish fillets. Nonselective media tryptic soy agar (TSA; Difco, Detroit, Mich.) was used to isolate endogenous bacterial flora as recommended by Austin and Austin *(3)* and Inglis et al. *(15)*. TSA was prepared as described by the manufacturer for the isolation and cultivation of aerobic and facultative anaerobic bacteria from samples.

The isolation of pathogenic bacteria from whole catfish and catfish fillets in the presence of a high number of nonpathogenic microflora required the use of selective, differential or both selective and differential media such as cefsulodin irgasan novobiocin agar (BBL, Cockeysville, Md.), mannitol yolk polymyxin agar (Difco), modified cellobiose polymyxin colistin agar (Difco), modified Oxford agar (Oxoid Ltd., Columbia, Md.), *Pseudomonas* F agar (Acumedia, Baltimore, Md.), starch ampicillin agar (Difco), sulfite polymyxin sulfadiazin agar (Difco), thiosulfate citrate bile salt sucrose agar (Difco), tryptose sulfite cycloserine agar (Oxoid), violet red bile agar (Difco), and xylose lysine deoxycholate agar (Difco) *(41)*. Total coliform and *Escherichia coli* SimPlate (Idexx Laboratories, Inc., Westbrook, Maine) was used for the detection and quantification of total coliform and *E. coli* in the samples.

Catfish sample preparation. A representative 25-g sample of whole catfish was obtained by using the quadrant diminutive sampling method *(25)*. This method involves aseptically excising with a sterile knife three 25-g subsamples from the lateral line (gill, center, and posterior) of one side and two 25-g subsamples from the other side (lateral anterior and posterior). In the case of catfish fillets, a 25-g sample was obtained from the lateral line of both extremes and the center of the fillet. Then the sample of either whole catfish or catfish fillets, was placed in a sterile stomacher bag (VWR, West Chester, Pa.) with 225 ml of buffered peptone water (Difco) and homogenized with a stomacher (Tekmar, Cincinnati, Ohio, model 400) for 2 min at room temperature.

The homogenized sample dilution (10^{-1}) was serially diluted using 9.0 ml buffered peptone water (Difco) dilution blanks. Each dilution was plated onto the appropriate media in triplicate using the spread plate technique. Dilutions plated onto TSA (Difco) were incubated at 37°C for 24 h under normal aerobic conditions. Incubation temperatures and gaseous atmospheres were selected based on the oxygen requirements of specific bacteria.

Enumeration. Aerobic plate count (APC) provided an assessment of the microbial quality of whole catfish and catfish fillets. APC was determined by counting the CFU after incubation of plates. APC (CFU/g) was calculated as described by Peeler and Maturin *(31)*. Coliform and *E. coli* were enumerated as described by the manufacturer (SimPlate; Idexx Laboratories).

Identification of bacterial isolates: preliminary identification. Preliminary identification of bacterial isolates from whole catfish and catfish fillets was based on phenotypic tests such as colony characteristics, gram-stain reaction, oxidase test BR64 (Oxoid), catalase production, and reaction with coagulase (Staphylase Test Kit DR595; Oxoid) *(20, 23, 37)*. Colony characteristics such as form, elevation, margin, density, color, and size were noted after incubation of plates on both nonselective and selective media. Colonies from both nonselective and selective media were streaked onto TSA and incubated at 37°C for 18 h. Individual isolates were further identified by a using rapid microbial identification as described below.

Final identification. Preliminary identification of bacterial isolates from whole catfish and catfish fillets was followed by further identification by using the Vitek Automated Identification System (bioMérieux Vitek, Inc., Hazelwood, Mo.) as described by the manufacturer.

Statistical analyses. All data were analyzed by a Statisical Analysis System (SAS) computer program *(36)*. Statistical methods included paired dependent *t* test, analysis of variance (ANOVA), general linear model procedure, and Tukey's Studentized range test. Significance was based on a probability level of 0.05 ($P < 0.05$).

Evaluation of the Grovac processing method. A series of experiments were conducted to evaluate the reduction of endogenous microflora of catfish fillets using the Grovac processing method. This process involved the use of equipment composed of a vacuum tumbler (Lyco, Janesville, Wis., model LT-40) fitted with a vacuum pump (Ritchie, Bloomington, Minn., model no. 93000) and a cylindrical drum with three perforated paddles (8 by 27 cm).

The first experiment was conducted to evaluate the individual and combined effects of ascorbic acid (AA) plus NaCl and vacuum on the reduction of microbial load in catfish fillets using the Grovac process. Duplicate experimental trials consisted of the following treatments: (A) water + a slight vacuum to keep the lid closed (4-in Hg gauge reading); (B) water + 0.4% AA + 0.4% NaCl + a slight vacuum to keep the lid closed (4 in. Hg gauge reading); (C) water + high vacuum (28 in. Hg gauge reading); and (D) water + 0.4% AA + 0.4% NaCl + high vacuum (28 in. Hg gauge reading). The processing solution for treatment B or D was prepared by dissolving AA (0.4% wt/vol) and NaCl (0.4% wt/vol) in 2 liters of autoclaved tap water at ambient temperature. Two liters of autoclaved tap water at ambient temperature were used for treatments A and C.

A total of 40 catfish fillets were obtained less than 1 h postmortem, bagged in polyethylene bags, transported to the Louisiana Department of Agriculture and Forestry/Louisiana State University Rapid Microbial Detection Laboratory on ice, stored in a cold room at 4°C, and processed within 4 to 8 h. Ten fillets were randomly assigned to each treatment. Before processing, each fillet was split lengthwise and marked with small cuts for identification purposes. One half of the fish fillet was considered the control while the other one was subjected to a given treatment. Control samples were packaged in sterile bags (Whirl-Pak; Nasco, Madison, Wis.) and stored at 4°C until microbiological analyses were done.

Five half catfish fillets were used per treatment replication. The catfish fillets were placed in the vacuum tumbler at ambient temperature. Catfish fillets and processing solution were set up for all treatments by using 400 g of catfish to 2 liters of processing solution. Either sterile water or the sterile processing solution was added to the tumbler. Air from the tumbler was withdrawn to create a vacuum. The tumbler was rotated at 8 rpm for 8 min to expose the catfish fillets either to water or the processing solution. During tumbling, catfish fillets were held out of water or processing solution by the paddles. It was during this time that the fillets were subjected to vacuum pressure. Treated samples were aseptically removed from the tumbler, drained for 2 min, and placed in sterile bags (Whirl-Pak). The pH of water or processing solution was measured before and after each treatment by using a pH meter (model 410A; Orion, Boston, Mass.).

After processing, three half fillets out of five were taken randomly for microbiological analyses as described earlier. Log reduction in CFU/g for each fillet was calculated as log initial (control) minus log final (treated). The significance of the mean reduction for each treatment was determined by a paired dependent *t* test. In addition, log reduction means of all treatments were analyzed by ANOVA.

The second experiment was conducted to evaluate the effectiveness of three levels of AA (0.4, 0.8, and 1.2%) and three levels of NaCl (0.2, 0.4, and 0.6%) on the reduction of microbial loads in catfish fillets using the Grovac process. A 3 by 3 factorial design with two replications was used. Therefore, there were a total of nine possible treatment combinations of AA and NaCl. Duplicate experimental trials consisted of the following treatments: (A) 0.4% AA + 0.2% NaCl; (B) 0.4% AA + 0.4% NaCl; (C) 0.4% AA + 0.6% NaCl; (D) 0.8% AA + 0.2% NaCl; (E) 0.8% AA + 0.4% NaCl; (F) 0.8% AA + 0.6% NaCl; (G) 1.2% AA + 0.2% NaCl; (H) 1.2% AA + 0.4% NaCl; (I) 1.2% AA + 0.6% NaCl.

Six fillets at random were assigned to each treatment. Before processing, each fillet was split lengthwise and marked with small cuts for identification purposes. One half of the fish fillet was considered the control while the other one was subjected to a given treatment. Control samples were packaged in sterile bags (Whirl-Pak) and stored at 4°C until microbiological analyses were done. Three half catfish fillets were used per treatment replication. The catfish fillets were placed in the vacuum tumbler at ambient temperature. Grovac experimental conditions were as described earlier.

RESULTS AND DISCUSSION

Isolation and identification of bacteria from whole catfish and catfish fillets: number and types of bacteria. A total of 51 bacterial isolates were isolated and identified from whole catfish and catfish fillets using selective and nonselective culture media, and the Vitek Identification System (bioMérieux, Vitek; Table 1). From the 51 isolates, only 20 isolates were found to be associated with whole catfish. The low number of bacterial isolates associated with whole catfish could be due to the selectivity of the skin against some bacteria *(4)*. The microflora isolated from whole catfish is representative of bacteria normally found in fresh and sea water (e.g., *Acinetobacter* sp., *A. hydro-*

TABLE 1. *Types of bacteria isolated from whole catfish and catfish fillets*

Type of bacteria	Isolated from whole catfish	Isolated from catfish fillets
Acinetobacter baumannii	−[a]	+[b]
Acinetobacter lwoffii	+	+
Actinobacillus ureae	−	+
Aeromonas hydrophila	+	+
Aeromonas veroni biovar sobria	+	+
Bordetella bronchiseptica	−	+
Cedecea lapagei	−	+
Chromobacterium violaceum	+	−
Chryseobacterium (Flavobacterium) indologenes	+	+
Citrobacter freundii	+	+
Comamonas acidovorans	−	+
Corynebacterium xerosis	−	+
Enterobacter cloacae	−	+
Enterococcus avium (group D)	−	+
Enterococcus durans (group D)	−	+
Enterococcus faecalis (group D)	−	+
Enterococcus faecium (group D)	−	+
Enterococcus hirae (group D)	−	+
Escherichia coli	+	+
Hafnia alvei	+	−
Klebsiella pneumoniae	−	+
Listeria species	−	+
Morganella (Proteus) morganii	+	+
Nonfermenting gram-negative *Bacillus* (saccharolytic)	−	+
Nonfermenting gram-negative *Bacillus* (asaccharolytic)	−	+
Pasteurella haemolytica	+	−
Plesiomonas shigelloides	−	+
Proteus vulgaricus	−	+
Providencia (Proteus) alcalifaciens	+	+
Providencia (Proteus) rettgeri	−	+
Serratia liquefaciens	−	+
Serratia odorifera	+	−
Shewanella putrefaciens	−	+
Staphylococcus aureus	−	+
Staphylococcus auricularis	+	+
Staphylococcus capitis	−	+
Staphylococcus cohnii	−	+
Staphylococcus haemolyticus	−	+
Staphylococcus hominis	−	+
Staphylococcus hyicus	−	+
Staphylococcus lentus	+	−
Staphylococcus saprophyticus	−	+
Staphylococcus sciuri	+	+
Staphylococcus xylosus	+	−
Stenotrophomonas (Xanthomonas) maltophilia	−	+
Streptococcus agalactiae	+	−
Streptococcus anginosus	−	+
Vibrio alginolyticus	+	+
Vibrio cholerae	−	+
Vibrio fluviales	+	+
Vibrio parahemolyticus	+	−

[a] −, the isolate was not present.

[b] +, the isolate was present.

TABLE 2. *Aerobic and anaerobic counts (CFU/g) of whole catfish and catfish fillets on selective and nonselective media*

Microbial test	Whole catfish (CFU/g)	Catfish fillets (CFU/g)
APC (aerobic)	8.4×10^6	2.6×10^7
APC (anaerobic)	1.3×10^6	1.3×10^7
Coliforms	2.6×10^2	5.4×10^2
E. coli	0.7×10^1	0.1×10^1

phila, Flavobacterium, E. coli, Vibrio sp.); surfaces of freshwater and marine fishes (e.g., *Acinetobacter* sp., *Aeromonas* sp.); and intestinal microflora in normal channel catfish (e.g., *Acinetobacter* sp., *Aeromonas* sp., *Bacillus, Chryseobacterium indologenes, Citrobacter freundii, E. coli, Hafnia alvei, Klebsiella pneumoniae, Plesiomonas shigelloides, Proteus* sp., *Serratia* sp., *Shewanella putrefaciens, Streptococcus* sp., *Vibrio* sp.) *(4, 15, 22, 26, 27).*

In general, the number and types of bacteria isolated from whole catfish could also reflect: (i) the physiochemical conditions of the aquatic environment, e.g., temperature, pH, salinity; (ii) interactions between microorganisms and catfish, e.g., location of bacteria, resident microflora, transient residents; (iii) harvesting practices, e.g., catching, evaluating for off-flavor; (iv) handling, e.g., transportation, packaging; (v) the methods employed for isolation, e.g., types of media used, temperature and duration of incubation, the nature of the gaseous environment in the incubator; and (vi) the method of identification, e.g., the Vitek system.

The majority of bacterial isolates (43) were isolated and identified from catfish fillets. When comparing the number of bacterial isolates of whole catfish and catfish fillets (Table 1), it appears that the higher number of bacteria isolated from catfish fillets is not only determined by the natural microflora of whole catfish but also from other potential sources associated with catfish processing such as workers, processing equipment, surfaces, utensils, packaging materials, sellers, and air quality in the plant *(11).* One needs to take into consideration that the flesh of live fish is bacteriologically sterile; however, fillets are usually contaminated during processing, particularly in the gutting and filleting operations *(11, 29).* In addition, catfish fillets are an excellent nutrient-rich substrate for growth of psychrotrophic pathogens *(9).*

Selective and nonselective culture media used for isolation and enumeration. The most frequently isolated organism on either selective or nonselective culture media was *Aeromonas* sp. Similar results were reported by MacMillan *(26)* who consistently isolated *Aeromonas* sp. from the catfish viscera throughout an entire year of catfish production.

Total coliform and *E. coli* SimPlate (Idexx Laboratories) was used to detect and quantify the total coliform and *E. coli* from whole catfish and catfish fillets (Table 2). Coliform counts and the presence of *E. coli* may indicate contamination from fecal contaminated water or from the evisceration process. The fact that the whole catfish are contaminated with *E. coli* may indicate that *E. coli* was introduced in the pond via fecal contamination or introduced during plant processing. Andrews et al. *(2)* surveyed 41 processors to determine the bacteriological quality of fresh catfish. They observed that the APC ranged from 6.9×10^3 to 1.9×10^8 CFU/g, total coliforms from <3 to 9.3×10^3 CFU/g, and fecal coliforms from <3 to 4.6×10^2 CFU/g. Coliform and *E. coli* counts associated with one lot of catfish were in excess of 10^5 and 10^3 CFU/g, respectively (data not shown). The heavily contaminated lot was eliminated from this study based on the fact that the APC counts (8.2×10^9) were not representative of live catfish obtained from noncontaminated ponds.

It is important to consider that the high number of microorganisms in whole catfish and catfish fillets along with the presence of psychrotrophic bacteria equate to the possibility of a high rate of spoilage and thus a shorter shelf life of chilled catfish products.

Aerobic and anaerobic counts (CFU/g) of whole catfish and catfish fillets on selective and nonselective media are shown in Table 2. APC counts from whole catfish and catfish fillets showed that catfish samples were highly contaminated. The high APC value indicated that both whole catfish and catfish fillets are highly contaminated from several potential sources, including the aquatic environment, holding tanks, and processing.

TSA agar was used as a nonselective agar to isolate *Acinetobacter lwoffii, A. hydrophila, Aeromonas sobria, Staphylococcus auricularis,* and *Vibrio fluviales* from whole catfish and catfish fillets, and *Bordetella bronchiseptica, Enterobacter cloacae,* nonfermentative *Bacillus* (asaccharolytic), *S. putrefaciens, Staphylococcus capitis,* and *Staphylococcus cohnii* from catfish fillets. *A. hydrophila, A. sobria, E. cloacae,* and nonfermentative *Bacillus* (asaccharolytic) were also isolated on TSA under anaerobic conditions. *Vibrio* isolates were obtained sporadically, and it is assumed that they were cross contaminants from the plant's holding tanks. There have been no reports of *Vibrio* isolated from fresh catfish *(16).* A shortcoming in the identification of bacterial colonies using TSA was the similar appearance of bacteria on the plate; however, randomly selecting isolates (10/plate) from several plates should help eliminate bias.

Gram-positive and gram-negative bacteria. Twenty bacterial isolates from whole catfish and catfish fillets, approximately 40% of all isolates, were gram-positive bacteria. Thirty-one gram-negative bacteria accounted for the remaining 60% of all isolates (Table 3). Frerichs *(10)* reported that the majority of fish pathogens are gram-negative rods. These bacteria are causative agents responsible for acute septicemias with few symptoms and high mortalities, chronic conditions and low mortalities, or asymptomatic latent infections *(4).* We isolated several gram-negative pathogens associated with both catfish and catfish fillets, including strains of *A. hydrophila, E. coli, K. pneumoniae, Listeria* sp., and *P. shigelloides* (Table 3). According to Bean and Griffin *(5)* some human pathogens (e.g., *A. hydrophila, E. coli, P. shigelloides, Proteus* sp., *S. aureus, V.*

TABLE 3. *Classification of bacteria into groups and families from whole catfish and catfish fillets*

Group	Family	Bacteria
Gram-negative aerobic rods	*Pseudomonadaceae*	*C. acidovorans, S. putrefaciens,* and *S. maltophilia*
	Other genera	*B. bronchiseptica*
Gram-negative facultatively anaerobic rods	*Enterobacteriaceae*	*C. freundii, E. cloacae, E. coli, H. alvei, K. pneumoniae, M. morganii, P. vulgaricus, P. alcalifaciens, P. rettgeri, S. liquefaciens,* and *S. odorifera*
	Vibrionaceae	*A. hydrophila, A. sobria, P. shigelloides, V. alginolyticus, V. cholerae, V. fluviales,* and *V. parahemolyticus*
	Other genera	*A. ureae, C. lapagei, C. violaceum, C. indologenes,* nonferm. *Bacillus* (saccharolytic and asaccharolytic), and *P. haemolytica*
Gram-negative cocci	*Neisseriaceae*	*A. baumannii* and *A. lwoffii*
Gram-positive facultatively anaerobic cocci	*Micrococcaceae*	*S. aureus, S. auricularis, S. capitis, S. cohnii, S. haemolyticus, S. hominis, S. hyicus, S. lentus, S. saprophyticus, S. sciuri,* and *S. xylosus*
	Streptococcaceae	*E. avium, E. durans, E. faecalis, E. faecium, E. hirae, S. agalactiae,* and *S. anginosus*
Gram-positive rods	Coryneform bacteria	*C. xerosis*
	Other genera	*Listeria* sp.

fluviales, Vibrio cholerae) have been causative agents of foodborne outbreaks in the U.S. between 1973 and 1987. They also reported that fish was a major food vehicle for foodborne illness. Improper storage or holding temperature was the factor most often reported for outbreaks caused by fish. In addition, some food pathogens can grow at refrigeration temperatures such as *A. hydrophila, E. coli, Listeria* sp., *P. shigelloides, Proteus* sp., and *S. aureus (11, 33).*

S. aureus was the only disease-causing gram-positive bacteria isolated from catfish fillets (Table 3). The presence of pathogenic bacteria may have been caused by cross contamination from workers' hands and skin. The relatively high tolerance of most gram-positive bacteria to limiting factors such as a reduced water activity, refrigeration temperatures, and reduced pH may allow for a higher survival rate and longer persistence as compared to most gram-negative bacteria *(2, 3, 33).*

In addition, growth of gram-negative bacteria in refrigerated catfish fillets is the primary factor that determines spoilage of fresh fillets *(19).* Table 3 shows the genera of spoilage bacteria isolated from whole catfish and catfish fillets. Spoilage bacteria isolated from whole catfish and catfish fillets do not represent a health hazard to humans; however, the microflora can contribute to the elimination of some pathogens. The spoilage of catfish products is caused by numerous bacteria including strains of *Acinetobacter* sp., *Aeromonas* sp., *C. indologenes, C. freundii, H. alvei, Proteus* sp., *S. putrefaciens,* and *Vibrio* sp. *(3, 7, 29).* Other microorganisms, predominantly gram-positive bacteria such as *Bacillus, Enterococcus,* and *Staphylococcus* are artificially introduced to catfish and reflect the microflora of diets used to feed catfish.

The Grovac process. The Grovac process has been developed for enhancing the flavor and shelf life of fresh catfish fillets. This process uses vacuum on catfish fillets submerged in a processing solution of AA and NaCl. The use of vacuum in combination with the processing solution has a profound effect on the growth and viability of microbial cells. For example, AA acts as a pH-reducing solute when it is added to the solution. When the pH is reduced below the lower limit for growth of a microbial species, not only do the cells stop growing, but they also lose viability, the rate of which depends upon the extent of pH reduction *(33).* This is more apparent when a weak organic acid such as AA is used because of its high pK value (4.0) *(16).* A high pK value means more of the molecules are in the undissociated form that are subsequently capable to entering the cell, where they dissociate to generate H^+ in the cytoplasm. The high H^+ concentration has an adverse effect on the proton gradient between the inside and the outside of the cells. To overcome this problem, the cells pump out the protons by expending energy (ATP). This represents a large amount of energy that cells are not able to replenish. In addition, the low pH can act on the cellular enzyme and adversely affect their structural (by interfering with the ionic bonds) and functional integrity *(33).* In addition to its antimicrobial properties, AA is an antioxidant reducing the potential for flavor and color changes. The antioxidative role of AA includes free radical scavenging and oxygen scavenging. Their acidic and reducing properties are contributed by the 2,3-enediol moiety.

Salt in the Grovac process is not employed as a preservative because of the low concentrations ($<$1% wt/vol) used. However, it seems to be unique in that it has the ability to enhance desirable flavor by suppressing unpalatable flavors such as bitterness and sourness.

According to Groves *(13),* the hypotonic solution of AA and NaCl also enhances osmosis of compounds within the cellular structure that contributes significantly to the dilution and extraction of the geosmin content and off odors. Besides the processing solution, vacuum tumbling also contributes to lysis of bacteria associated with catfish fillets. In foods under vacuum, the low oxygen tension inside the system inhibits both chemical oxidation and microbial (proteolytic or lipolytic) activity *(16).*

TABLE 4. *Log CFU/g reduction of the APC for catfish fillets treated individually or combined with various process parameters*[a]

Treatment and process parameters[b]	Log initial	Log final	Log reduction[c]
A: Water	8.35 C (0.18)	7.93 CD (0.31)	0.42 D (0.20)
B: Water + 0.4% AA + 0.4% NaCl	8.43 C (0.10)	7.20 CD (0.28)	1.43 D (0.23)
C: Water + vacuum (28 in. Hg)	8.33 C (0.15)	7.53 C (0.19)	1.28 D (0.17)
D: Water + 0.4% AA + 0.4% NaCl + vacuum (28 in. Hg)	8.45 C (0.06)	6.40 D (0.42)	2.05 C (0.37)

[a] Mean values in a column not followed by the same letter are significantly different ($P < 0.05$).

[b] Variable process parameters include water, AA (0.4% wt/vol), and NaCl (0.4% wt/vol), and vacuum (28 in. Hg). Variables that were held constant were tumbling time (8 min) and drum rotation speed (8 rpm). pH initial (A), 9.03; pH final (A), 7.99; pH initial (B), 3.38; pH final (B), 3.54; pH initial (C), 9.13; pH final (C), 8.06; pH initial (D), 3.42; pH final (D), 3.68. All pH values are the average of three trials and the pH varied ± 0.2 between trials.

[c] Mean log values measured in CFU/g. Log reduction = log initial (control) − log final (treated). Numbers in parentheses refer to standard deviation of six measurements.

There is evidence that some control methods, individually or in combinations, discussed earlier can extend the shelf life of refrigerated catfish fillets. However, bacterial hazards associated with any process must be addressed. One must consider the overall safety of each product and evaluate whether the process increases or decreases pathogens associated with this product.

Experiment 1. The individual and combined effects of 0.4% AA plus 0.4% NaCl and vacuum (28 in. Hg) on the reduction of microbial load in catfish fillets are shown in Table 4. The data revealed that various combinations of the processing parameters were effective in reducing the microbial load in catfish fillets using the Grovac process (Table 4). The highest log reduction (2 log CFU/g) was achieved by combining 0.4% AA plus 0.4% NaCl and vacuum (28 in. Hg) (Table 4, treatment D). The bacterial reduction was apparently due to the acidic conditions (pH 3.4) of the process solution under vacuum. Ray *(33)* reported that the bactericidal effect is due to disruption of both the cell membrane integrity and the proton pump.

The paired *t* test showed that the mean log reduction of CFU/g (mean log initial of the control group minus mean log final of treated group) on catfish fillets was significant ($P < 0.05$) for all four treatments. ANOVA showed that the mean log reduction in CFU/g on catfish fillets from treatment D (Table 4) was significantly higher ($P < 0.05$) than the log reduction means (range from 1.3 to 1.4 CFU/g) of catfish fillets treated with 0.4% AA plus 0.4% NaCl, or vacuum (28 in. Hg) alone. However, there was no difference in reduction ($P > 0.05$) between catfish fillets among treatments A, B, and C when compared to that on catfish fillets treated with water. These collective data indicate that 0.4% AA plus 0.4% NaCl, or vacuum (28 in. Hg) alone does not effectively reduce log CFU/g of catfish fillets compared to the log CFU/g of treatment with water. Therefore, the bacterial reduction is most likely due to physical removal of bacteria by the water or the processing solution. The initial pH (before tumbling the catfish fillets) for treatment A, B, C, and D was 9.1, 3.4, 9.1, and 3.4, respectively.

It should be noted that during tumbling, the fillets were partially washed with either water or the processing solution while the tumbler was rotated at 8 rpm for 8 min. Thus, the increased reduction of CFU/g used in treatment D (Table 4) by an additional 0.7 to 0.8 log CFU/g with respect to the other treatments may be related to the synergistic effect of tumbling, AA, NaCl, and vacuum. This means that the microbial quality of catfish fillets was improved by the tumbling effect as well as the lethal and sublethal effect of the processing solution and vacuum on the endogenous microflora of catfish fillets.

In conclusion, experiment 1 showed that the overall catfish fillets microbial quality could be improved by combining 0.4% AA plus 0.4% NaCl and vacuum (28 in. Hg). Because the APC on catfish fillets from treatment D was below 7.0 log CFU/g (when spoilage of catfish occurred), the final microbial quality of the product was considered acceptable.

Experiment 2. The effectiveness of three levels of AA (0.4, 0.8, and 1.2%) and three levels of NaCl (0.2, 0.4, and 0.6%) on the reduction of microbial loads on catfish fillets using the Grovac process is shown in Table 5. ANOVA showed that levels of AA and NaCl were effective in lowering ($P < 0.02$) the initial APC counts in catfish fillets by a mean range of 1.2 to 2.3 log reduction (CFU/g). Treatment I with 1.2% AA and 0.6% NaCl achieved the highest mean log reduction of 2.3 log CFU/g compared to those from other treatments. However, mean log reduction of treatment I (Table 5) was not significantly different ($P > 0.05$) from other treatments, except treatments A and C. Comparisons among the average log reductions at different levels of AA and NaCl are shown in Table 5. ANOVA showed that there was a significant AA effect ($P = 0.01$), but no significant NaCl effect ($P = 0.22$) with respect to log reduction on catfish fillets. The highest mean log reduction on catfish fillets was achieved by treatments using 1.2% AA; however, it was not significantly different ($P > 0.05$) from the treatment using 0.8% AA.

Log reduction means of treatments at different NaCl levels were similar ($P > 0.05$). In addition, there was no interaction ($P > 0.09$) between AA and NaCl levels with respect to log reductions on catfish fillets. This meant that factors AA and NaCl were independent or the effects of AA did not significantly depend on the level of NaCl ($P < 0.05$).

In general, the degree of reduction varied with the concentration of AA. This could mean that the reduction in bacteria on the catfish fillets increased with increasing AA

TABLE 5. *Log initial, log final, and the average log reduction, and APC reduction of catfish fillets treated with different levels of AA and NaCl*[a]

Treatment	Initial pH	Final pH	Log initial	Log final	Log reduction[b]	APC reduction[c]
A: 0.4% AA + 0.2% NaCl	3.3	3.6	6.78 FE (0.44)	5.60 CD (0.30)	1.18 D (0.65)	93.4
B: 0.4% AA + 0.4% NaCl	3.3	3.7	7.58 CD (0.51)	5.95 C (0.29)	1.63 CD (0.58)	97.7
C: 0.4% AA + 0.6% NaCl	3.4	3.8	6.80 FE (0.32)	5.52 CD (0.40)	1.28 D (0.52)	94.8
D: 0.8% AA + 0.2% NaCl	3.0	3.3	7.73 C (0.50)	5.87 C (0.89)	1.87 CD (0.57)	98.6
E: 0.8% AA + 0.4% NaCl	3.0	3.3	6.72 FE (0.45)	5.22 CD (0.47)	1.50 CD (0.42)	96.8
F: 0.8% AA + 0.6% NaCl	3.0	3.3	7.25 CDE (0.26)	5.28 CD (0.74)	1.97 CD (0.70)	98.9
G: 1.2% AA + 0.2% NaCl	2.9	3.1	6.45 F (0.34)	4.63 E (0.44)	1.82 CD (0.58)	98.5
H: 1.2% AA + 0.4% NaCl	2.9	3.1	6.92 DEF (0.45)	5.42 CD (0.52)	1.50 CD (0.37)	96.8
I: 1.2% AA + 0.6% NaCl	2.9	3.1	7.28 CDE (0.28)	4.98 CD (0.52)	2.30 C (0.35)	99.5

[a] Mean values in a column not followed by the same letter are significantly different ($P < 0.05$).

[b] Mean log values measured in CFU/g. Log reduction = log initial (control) − log final (treated). Numbers in parentheses refer to standard deviation of six measurements. All pH values are the average of three trials.

[c] APC reduction (%) = (initial CFU/g − final CFU/g) (100)/initial CFU/g.

concentration. Mean log reductions on catfish fillets using treatments A and C with 0.4% AA were significantly lower ($P < 0.05$) than those from the other treatments. However, the similar mean log reduction on catfish fillets treated with 1.2 and 0.8% AA may have been related to the similar pH of processing solutions. Initial pH of solutions (before processing of catfish fillets) at 0.4, 0.8, and 1.2% AA was 3.3, 3.0, and 2.9, respectively.

TABLE 6. *Log reduction means on catfish fillets treated with different levels of AA and NaCl*

	Log reduction[a]
Level of AA (%)	
0.4	1.37 C (0.59)
0.8	1.78 BC (0.58)
1.2	1.87 B (0.54)
Level of NaCl (%)	
0.2	1.62 B (0.67)
0.4	1.54 B (0.44)
0.6	1.85 B (0.67)

[a] Mean log values measured in CFU/g. Log reduction = log initial (control) − log final (treated). Numbers in parentheses refer to standard deviation of 18 measurements. All pH values are the average of three trials and the pH varied ±0.04 between trials. Mean values in a column not followed by the same letter are significantly different ($P < 0.05$).

In conclusion, treatment combinations of AA and NaCl using the Grovac process decreased initial counts on catfish fillets from 93.4 to 99.5% (Table 6). The Grovac process seemed to be more effective in reducing the microbial counts on catfish fillets compared to other treatments using organic acids, phosphates, spray washing, either individual or combined *(8, 18–20, 28, 29)*. High concentrations of AA (>0.8%) caused the texture of the catfish meat become soft (data not shown).

Microbiological data from this study allowed us to evaluate the Grovac process on the reduction of microbial counts associated with catfish fillets at refrigerated temperatures. These data will allow us to evaluate biological hazards associated with processed catfish fillets and write effective hazard analysis and critical control point plans that will reduce pathogens in the product during processing. In addition, the efficiency of the Grovac process suggests that it can be used as an alternative processing procedure to reduce microbial populations on catfish fillets and be useful to improve the shelf life and food safety of the product.

An additional factor that must be considered and addressed is whether the reduction of spoilage bacteria will have an effect on the overall safety of the processed catfish. The relative low incidence of outbreaks of foodborne disease, despite severe contamination and colonization of foods with pathogens, is that many spoilage bacteria will compete with pathogens for limited nutrients that will result in competition exclusion of pathogens through production of antagonist metabolites *(1)*. Spoilage bacteria also produce significant amounts of organoleptic metabolites that are detected by unpleasant odors or tastes before or just as pathogens reach dangerous colonization levels. The consumer can detect these odors and are adequately warned that the food may be unsafe to eat *(17)*. Therefore, using any process that eliminates spoilage bacteria or normal microflora who are known to be natural antagonists to pathogens in food must be critically evaluated for potential growth of pathogens *(1, 30)*. Such occurrences could constitute a biological hazard in foods that are processed to eliminate biological load. The process system will need to be evaluated further for possible outgrowth of pathogens introduced during processing and determine if biological hazards increase during cold storage.

The Grovac process has been shown to remove muddy off flavors associated with catfish (data not shown); however, the process overall value in reduction of microbial flora associated with catfish fillets is marginal. The process is inexpensive and has merits for increasing quality and flavor (i.e., elimination of off flavors); however, the effects of the process on proliferation of contaminating pathogens still needs to be addressed in further studies. Further research to assess the changes in physiochemical and sensory quality of catfish fillets treated with the Grovac process are also needed.

ACKNOWLEDGMENTS

This research was funded in part by the Department of Agriculture and Forestry under the direction of Commissioner Bob Odom. The authors thank Tony's Seafood of Baton Rouge, Louisiana for providing the catfish used in these experiments.

REFERENCES

1. Ali, F. S., and F. O. Vanduyne. 1981. Microbial quality of ground beef after stimulated freezer failure. J. Food Prot. 14:62–65.
2. Andrews, W. H., C. R. Wilson, P. L. Poelma, and P. Romeo. 1977. Bacteriological survey of the channel catfish. J. Food Sci. 42:359–362.
3. Austin, B., and D. A. Austin. 1989. General introduction, p. 19–24. *In* B. Austin and D. A. Austin (ed.), Methods for the microbiological examination of fish and shellfish. Ellis Horwood Limited, New York.
4. Austin, B., and D. A. Austin. 1993. Bacterial fish pathogens: disease in farmed and wild fish, 12th ed. Ellis Horwood Limited, New York.
5. Bean, N. H., and P. M. Griffin. 1990. Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles, and trends. J. Food Prot. 53:804–817.
6. Bonnell, A. D. 1994. Quality assurance in seafood processing: a practical guide. Chapman & Hall, New York.
7. Davies, A. R. 1997. Modified-atmosphere packaging of fish and fish products, p. 200–223. *In* G. M. Hall (ed.), Fish processing technology, 2nd ed. Chapman & Hall, New York.
8. Fernandez, C. F., G. J. Flick, J. Cohen, and T. B. Thomas. 1998. Role of organic acids during processing to improve quality of channel catfish fillets. J. Food Prot. 61:495–498.
9. Fernandez, C. F., G. J. Flick, and T. B. Thomas. 1998. Growth of inoculated psychrotrophic pathogens on refrigerated fillets of aquacultured rainbow trout and channel catfish. J. Food Prot. 61:313–317.
10. Frerichs, G. N. 1993. Isolation and identification of fish bacterial pathogens, chapter 16. *In* V. Inglis, R. J. Roberts, and N. R. Bromage (ed.), Bacterial diseases of fish. Halsted Press, New York.
11. Garbutt, J. 1997. Essentials of food microbiology. Oxford Press, Inc., Oxford, England.
12. Garthwaite, G. A. 1997. Chilling and freezing of fish, p. 93–119. *In* G. M. Hall (ed.), Fish processing technology. VCH Publishers, New York.
13. Groves, B. M. August 1996. U.S. Patent 5,543,163.
14. Huang, Y. W., R. T. Lovell, and R. A. Dunham. 1994. Carcass characteristics of channel and hybrid catfish, and quality changes during refrigerated storage. J. Food Sci. 59:64–66.
15. Inglis, V., R. H. Richards, and K. N. Woodward. 1993. Public health aspects of bacterial infections of fish, p. 284–303. *In* V. Inglis, R. Roberts, and N. Bromage (ed.), Bacterial diseases of fish. Halsted Press, New York.
16. Jay, J. M. 2000. Intrinsic and extrinsic parameters of foods that affect microbial growth, p. 38–40. *In* Modern food microbiology, 6th ed. Aspen Publishers, Gaithersburg, Md.
17. Kautter, D. A., R. K. Lynt, and T. Lilly. 1981. Evaluation of the botulinum hazard from nitrogen-packed sandwiches. J. Food Prot. 44:59–61.
18. Kim, C. R., and J. O. Hearnsberger. 1994. Gram-negative bacteria inhibition by lactic acid culture and food preservatives on catfish fillets during refrigerated storage. J. Food Sci. 59:513–516.
19. Kim, C. R., J. O. Hearnsberger, and J. B. Eun. 1995. Gram-negative bacteria in refrigerated catfish fillets treated with lactic culture and lactic acid. J. Food Prot. 58:639–643.
20. Kim, C. R., J. O. Hearnsberger, A. P. Vickery, C. H. White, and D. L. Marshall. 1995. Extending shelf life of refrigerated catfish fillets using sodium acetate and monopotassium phosphate. J. Food Prot. 58:644–647.
21. Kirov, S., J. Janda, S. L. Abbott, and A. M. Carnahan. 1995. *Aeromonas* and *Plesiomonas*, p. 477–482. *In* P. R. Murray, E. J. Baron, M. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
22. Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn. 1992. Diagnostic microbiology, 4th ed. J. B. Lippincott Company, Philadelphia, Pa.
23. Leung, C., Y. Huang, and M. A. Harrison. 1992. Fate of *Listeria monocytogenes* and *Aeromonas hydrophila* on packaged channel catfish fillets stored at 4°C. J. Food Prot. 55:728–730.
24. Leung, C., Y. Huang, and O. Pancorbo. 1992. Bacterial pathogens and indicators in catfish and pond environments. J. Food Prot. 55:424–427.
25. Lyon, W. J. 1998. Food microbiology 4162 (II). Louisiana State University Publishing, Baton Rouge, La.
26. MacMillan, J. R. 1985. Infectious diseases. *In* C. S. Tucker (ed.), p. 1–23. Channel catfish culture. Elsevier, New York.
27. Marshall, D. L., and V. Jindal. 1997. Microbiological quality of catfish frames treated with selected phosphates. J. Food Prot. 60:1081–1083.
28. Marshall, D. L., and C. R. Kim. 1996. Microbiological and sensory analysis of refrigerated catfish fillets treated with acetic and lactic acids. J. Food Qual. 19:317–329.
29. Nickelson, R., and G. Finne. 1992. Fish, crustaceans, and precooked seafoods, p. 875–918, chapter 47. *In* C. Vanderzant and D. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
30. Oblinger, J. L., and A. A. Kraft. 1970. Inhibitory effects of *Pseudomonas* on selected *Salmonella* and bacteria isolated from poultry. J. Food Sci. 35:30–32.
31. Peeler, J. T., and L. J. Maturin. 1992. Aerobic plate count, p. 17–26, chapter 3. *In* FDA bacteriological analytical manual, 7th ed. Association of Official Analytical Chemists International, Arlington, Va.
32. Przybylski, L. A., M. W. Finerty, R. M. Grodner, and D. L. Gerdes. 1989. Extension of shelf life of iced fresh channel catfish fillets using modified atmospheric packaging and low irradiation. J. Food Sci. 54:269–273.
33. Ray, B. 1996. Fundamental food microbiology, 1st ed., p. 409–416, chapter 34. CRC Press LLC, Boca Raton, Fla.
34. Reddy, N. R., M. G. Roman, M. Villanueva, H. M. Solomon, D. A. Kautter, and E. J. Rhodehmel. 1997. Shelf life and *Clostridium botulinum* toxin development during storage of modified atmosphere-packaged fresh catfish fillets. J. Food Sci. 62:878–884.

35. Reed, R. J., G. R. Ammerman, and T. C. Chen. 1983. Chillpack studies on farm-raised channel catfish. J. Food Sci. 48:311–312.
36. SAS Institute, Inc. 1996. Statistical analysis system: 6.12. Statistical Analysis System Institute, Inc., Cary, N.C.
37. Silva, J. L., E. Harkness, and T. D. White. 1993. Residual effect of CO_2 on bacterial counts and surface pH of channel catfish. J. Food Prot. 56:1051–1053.
38. Silva, J. L., and T. D. White. 1994. Bacteriological and color changes in modified atmosphere-packaged refrigerated channel catfish. J. Food Prot. 57:715–719.
39. United States Department of Agriculture. 1999. National Agricultural Statistics Service, Washington, D.C.
40. Williams, S. K., G. E. Rodrick, and R. L. West. 1995. Sodium lactate affects shelf life and consumer acceptance of fresh catfish (*Ictalurus nebulosos*, *marmoratus*) fillets under simulated retail conditions. J. Food Sci. 3:636–639.
41. Yu, P. W., and J. A. Washington. 1985. Identification of aerobic and facultatively anaerobic bacteria. *In* J. A. Washington (ed.), Laboratory procedures in clinical microbiology, 2nd ed. Spring-Verlag, New York.