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Catfish Special Edition: Microbial Quality of Catfish Nuggets

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Abstract

Catfish nuggets the pieces of muscle tissue are produced by trimming filets during processing and cannot be sold as whole catfish fillets. There is little information regarding the microbial quality of raw nuggets. Catfish nuggets, purchased either fresh or frozen from local retailers in the northeast United States (NJ, NY, PA, and DE), were tested for aerobic plate count (APC) at 22 and 37°C, *Enterobacteriaceae*, and *Escherichia coli*/coliform using Petrifilms™. The BAX® polymerase chain reaction system was used to determine the presence of *Salmonella*, *Staphylococcus aureus*, *Listeria spp.*, and O157:H7. The overall average for APC at 22 and 37°C was 6.0 and 5.4 $\log_{10}$ CFU/g, respectively, which is within the finfish standard recommended by the International Commission on Microbiological Specifications for Food (ICMSF). No *E. coli* or *E. coli* O157:H7 was detected. Of the 150 nuggets tested, three were positive for *Salmonella* spp. and two were positive for enterotoxin negative *S. aureus*. *Listeria* spp. was detected, which is consistent with the findings of previous reports. The results obtained in this study were consistent with those obtained in other studies which assessed the microbial quality of finfish products.

Keywords: Fresh or Frozen Catfish nuggets; Catfish fillets; *E. coli; Staphylococcus; Listeria*

Introduction

In 2006 the U. S. Center for Disease Control classified food vehicles implicated in illness outbreaks into 17 food commodities [1] and in their 2007 report determined that finfish was associated with 41 outbreaks [2]. Obviously the safety and quality of our seafood supply is of critical importance. In the United States, the annual per capita consumption of fresh and frozen seafood is about 12 kg/person [3]. Approximately 7% of the total finfish marketed annually and consumed are *Siluriformes* - Basa, Swai or catfish [4]. Silva and Dean estimated that nuggets, the belly flap of catfish, averaged about 6.2% of the salable catfish product or about 3300 lbs salable catfish product/53000 lbs processed catfish [5].

The reported aerobic bacteria counts of finfish fillet products varies, depending if the product was purchased fresh or frozen at local retail establishment, or ordered from the Internet [6-9]. The International Commission on Microbiological Specifications for Foods (ICMSF) standard for both fresh and frozen finfish is maximum (M) of 10^7 CFU/g [10]. Chytiri et al. [11] reported that aquaculture raised fresh-water whole un-gutted and fillet trout can have an initial mesophilic of 2.5 log CFU/cm^2 and 3.8 log CFU/cm^2, respectively which exceeded the ICMSF limits after 18 d storage at 2 ± 0.5°C. The mean bacteria counts for retail fresh and frozen channel catfish was reported as ranging from 10^3 to 10^8 and 10^4 to 10^8 CFU/g, respectively with 93% (fresh) and 94% (frozen) being < 10^7 CFU/g [6]. Pao et al. [9] obtained a variety of raw aquacultured fish fillets (catfish, salmon, tilapia and trout) via the Internet and at local markets and reported a mean aerobic count of 5.7 $\log_{10}$ CFU/g and a psychrotrophic count of 6.3 $\log_{10}$ CFU/g.

In their review, Amagliani et al. [12] reported that *Salmonella*-contaminated fish and fish products are responsible for 1.4% of the foodborne outbreaks in the EU. However, only one catfish-related outbreak in the U. S. was reported in 1991 and was attributed to *Salmonella* Hadar [1,13]. Andrews et al. [6] surveyed retail fresh and frozen channel catfish (*Ictalurus punctatus*) for *Salmonella* and reported that the number of positive samples from the farm-raised catfish was seasonal with a 0.9% incidence for January - March versus 5.7% for July - September. McCoy et al. suggested that *Salmonella* may be the foodborne pathogen most likely associated with catfish [13].

Vibrio spp. had the same positive correlation between warm temperature and positive samples as seen with *Salmonella* [14,15]. *Staphylococcus aureus* and *Escherichia coli* were isolated from raw sushi [7] and from fresh aquacultured catfish fillets [16]. Atyah [17] reported the isolation of *S. aureus* from tilapia and Schärer et al. [18] isolated *Vibrio* spp. from freshwater fish fillets collected at a Swiss market.

In their report McCoy et al. stated that *Listeria monocytogenes* could be a contaminant on raw fish and cooking would eliminate this pathogen [13]. However, they also stated that the faster growth rate of *L. monocytogenes* on seafood would be a concern due to the difference in muscle tissue pH compared to the growth rate on beef and chicken [13]. Catfish fillets, collected directly from processing plants, were determined to be positive for *Listeria* spp. with 37% prevalence of *L. monocytogenes* [19]. *Listeria* spp. was also isolated from fish fillets, including catfish, purchased from local retail markets and via the Internet [9] and from raw fish at a sushi bar [7].

In addition to fillets, catfish nuggets (belly muscle) are available at retail markets in the U. S. Catfish nuggets can be purchased fresh, usually co-mingled or as a frozen product either in sealed packages produced by the processor or shipped frozen in bulk and packaged by the retailer. There is limited information regarding the microbiological background level or pathogen contamination on fresh or frozen catfish nuggets.

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Materials and Methods

Sample collection

Catfish nuggets, product of the United States, were purchased either fresh (non-frozen) or frozen from local retailers in the northeast (New Jersey, New York, Pennsylvania, and Delaware) (Table 1). The fresh nuggets were obtained either pre-weighed in a retailer's plastic wrapped tray or hand removed and weighed directly into a container (Figure 1a). The fresh nuggets were transferred to the laboratory under cold conditions (4°C) and were processed for microbiological analysis (background and pathogens) within 24 h of purchase.

Frozen samples were purchased either in 2 lb. processor-packaged sealed bags or by the pound in retailer's wrapped trays (Figure 1b); both products were in the freezer case. The samples were transferred frozen to the laboratory and were maintained frozen (-20°C) until used. When the fresh samples could not be processed within 24 h, the unopened package containing the nuggets were frozen (-20°C) and remained frozen until used. On the test day, the frozen samples (either purchased frozen or fresh then frozen) were thawed at room temperature and were processed for microbiological analysis (background and pathogens).

Sample preparation

Twenty samples per purchase were weighed, and placed in whirl pack stomacher bags (Nasco, Fort Atkinson, WI). Five samples for *Vibrio* determination, labeled A - E, were diluted 1:10 in alkaline peptone water (Becton, Dickson and Co.) for overnight enrichment and incubation at 37°C, followed by selective plating.

The BAX® analysis enrichment protocol was followed according to manufacturer directions (3M, St. Paul, MN). Five samples for *Listeria* detection, labeled A - E, were diluted 1:10 with UVM broth (Becton, Dickinson and Co., Sparks, MD), five samples for *Escherichia coli* O157:H7 detection, labeled A - E, were diluted 1:10 with mTSB+N broth (Becton, Dickinson and Co.). These samples were stomached (Steward Stomacher® 400 Circulator, Steward Ltd., West Sussex, UK) for 2 min and incubated at the protocol temperatures. The remaining five samples were diluted 1:10 in buffered peptone water (BPW, Becton, Dickinson and Co.) and stomached for 2 min. An aliquot was removed for microbial background counts from each bag (A-E) and placed in separate test tubes. The remaining samples in the stomacher bags were incubated for the *Salmonella*/*S. aureus* BAX® analysis.

Background analysis

Each BPW aliquot (A - E) was further diluted in peptone water (PW, Becton, Dickinson and Co.) and the manufacturer's procedure for Petrifilm™ was followed. Petrifilm™ Aerobic Plate Count (APC, 3M, St. Paul, MN), were prepared and incubated at 22 ± 2°C for 48 h to obtain psychrotrophic plate counts (PPC) and a second set prepared and incubated at 37 ± 2°C for 24 h for APC. *Enterobacteriaceae* and *E. coli*/coliform Petrifilm™ (3M) were inoculated and incubated according to the manufacturer's direction. The colonies on the PPC and APC films were counted by hand or counted using the 3M electronic reader. Statistical analysis showed no difference between the hand versus electronic reader counts ($p>0.05$).

Pathogen analysis

Pathogen PCR screening analysis was conducted using the DuPont Qualicon BAX® System for *Salmonella* (Standard Assay), *S. aureus* (Real-Time Assay), Genus *Listeria* (24E Assay) and *E. coli* O157:H7 (Real-Time Assay) (DuPont, Willington, DE). The enriched samples used for the BAX® analysis were refrigerated (4°C) in the event a positive result occurred and used to obtain a viable culture for confirmation.

Isolation and confirmation

The positive samples, as identified from the BAX® system, were used for viable cell isolation and confirmation. Isolation was done by plating the incubated sample, which were refrigerated, on selective agars and looked for characteristic colony morphology: XLT-4 agar (Becton, Dickinson and Co.) for *Salmonella*, PALCAM agar (Becton, Dickinson and Co.) for *L. monocytogenes*, and Baird-Parker agar (B-P, Becton, Dickinson and Co.) for *S. aureus*. Confirmation of presumptive-positive *Salmonella* isolates was done using the API® 20E test strips (bioMerieux) and Difco Antigen Agglutination test kit (Becton, Dickinson and Co.). The confirmed *Salmonella* isolates were sent to the USDA, APHIS, National Veterinary Services Laboratories, Ames, IA for serotyping.

From the B-P plates, black colonies with halos were selected for *S. aureus* and were confirmed using BBL Coagulase Plasma Rabbit test (Becton, Dickinson and Co.) and *Staphylococcal* Enterotoxin test kit (Oxoid, UK). Coagulase-positive samples were sent to the FDA Laboratory, Washington, DC, for confirmation of enterotoxin production.

Characteristic colonies from PALCAM were used for *Listeria* identification. Colonies were confirmed using Listeria API® test strips (bioMerieux).

The enriched *Vibrio* samples were streaked onto TCBS (Becton, Dickinson and Co.) and chromID™ *Vibrio* (bioMerieux® SA) agars and were incubated at 37°C. Persumptive-positive *Vibrio* isolates were re-plated for purity before identification by API® 20 E test strips (bioMerieux).

Log cfu/g						
	Aerobic *		Psychrotrophic		*Enterobacteriaceae*	
	Average	Range	**Average**	Range	**Average**	Range
Fresh n = 20	**7.2**	6.0-8.5	**8.0**	7.2-8.8	**6.6**	6.2-6.9
Fresh/frozen n=65	**5.8**	3.3-8.2	**6.4**	3.0-8.3	**4.7**	3.0-7.3
Frozen n=65	**3.3**	2.1-5.2	**3.7**	2.5-5.0	**2.3**	0-4.1

* ICMSF standard for aerobic counts: m = 5.0 x 10^5 (maximum - USA) and M = 10^7 cfu/g (Maximum - EU)

Table 1: Results of aerobic, psychrotrophic and Enterobacteriacea counts from catfish nuggets purchased at local retail stores located in *NJ, NY, PA* and *DE*.

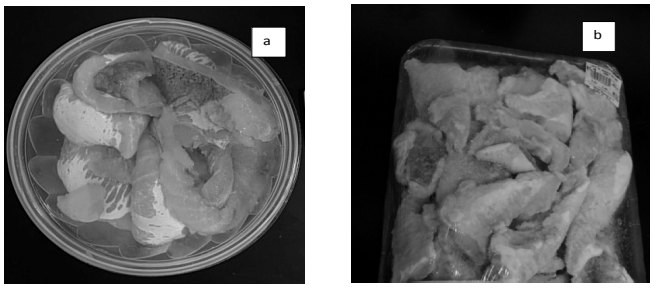

Figure 1: a: Fresh catfish nuggets packaged at the retail level. **b:** Frozen catfish nuggets in plastic retail container.

Statistical analysis

Analysis of Variance (ANOVA) was carried out using SAS 9.1 (SAS Institute, Inc., Cary, NC) [20].

Results and Discussion

The plate counts, determined after incubation at 22°C (psychrotrophic counts) and at 37°C (mesophilic counts) were compared and statistical analysis showed that the psychrotrophic counts were significantly higher ($p<0.05$) (Table 1). Silva et al. [21] also reported that the psychrotrophic counts than mesophilic counts in these products, and were 6.0 log CFU/g and 5.4 log CFU/g, respectively. Chytit et al. [11] reported an increase of mesophilic counts after 9 d of storage on ice (3.8 log CFU/cm^2 to > 6 log CFU/cm^2). Silva, et al. [21] reported that at 1 d the psychrotrophic level was 4 log CFU/g, and after 5 d refrigerated storage the level was > 5 log CFU/g. In their study, Fernandes et al. [22] reported that inoculated psychrotrophic pathogens grew at the refrigerated temperature equally as well as the indigenous microbes on aquacultured rainbow trout and channel catfish. Broekaert et al. [23] also reported that psychrotrophic counts increased when the finfish were stored on ice.

In their study on fish fillets, González-Rodríguez et al. [8] used the *Enterobacteriacea* counts as an indicator and stated that when the count exceeded 6 log cfu/g the fish quality was unacceptable. In their study Chytiri et al. [11] found that *Enterobacteriaceae* was a part of the spoilage microflora of filleted trout and reached a level of 5.5 log CFU/cm^2 when stored on ice. In this study, the *Enterobacteriaceae* counts for the fresh nuggets (Table 1) were > 6 log CFU/g which would indicate a need for improved handling.

The BAX® results from the 150 nuggets analyzed for *Salmonella* and *S. aureus* are listed in Table 2. Three isolates of *Salmonella* were retrieved from the samples after on XTL-4 plates. Characteristic colonies were identified, purified and the API® 20E confirmations done. The isolates were serotyped as *Salmonella* 4, 12: i:- and Newport. Heinitz et al. [24] reported the identified *S.* Newport on imported and domestic seafood products. Wyatt et al. [25] stated that when *Salmonella* was isolated from finfish, the incidence most likely occurred from cross-contamination due to improper processing or handling. Andrews et al. [6] stated that the occurrence of *Salmonella* on catfish fillets is dependent on seasonal variation with a prevalence ranging from 0.9% (Jan - March) to 5.7% (July - Sept).

	Salmonella[1]	*S. aureus*[2]
Fresh	1/20[a]	Neg
Fresh/frozen	1/65[b]	2/65
Frozen	1/65[b]	neg
Total	3/150	2/150

[1]Serotyped as: [a] *Salmonella* 4,12: i:-
[b]*Salmonella* newport
[2] Confirmed by API® Staph, coagulase positive and negative for enterotoxin type (A - E) production

Table 2: Viable pathogens recovered after positive BAX® results of catfish nuggets purchased at local retail stores.

	Percent Positive	
Species	**Fresh**	**Frozen**
L. innocua	22.0%	10.8%
L. welshimeri	25.4%	30.8%
L. monocytogenes	18.6%	35.4%
L . seeligeri	ND*	1.5%
L. grayi	ND*	1.5%

*ND = not detected

Table 3: Percent Listeria species identified from catfish nuggets by API® Listeria.

Two nuggets were positive for *S. aureus* (Table 2) by the BAX® analysis. The enriched samples were streaked on Baird-Parker agar. Characteristic positive colonies (black with halo) were confirmed to be *S. aureus* by the API® *Staph* and were coagulase positive. Neither isolate produced the classical food poisoning enterotoxin types A, B, C, D or E. In their report surveying 24 lots of freshwater fish, González-Rodríguez et al. [8] reported 4 confirmed isolates of *S. aureus*. They did not determine if these isolates were enterotoxin producers, but did determine that most of the isolates were coagulase- and thermonuclease negative. *Staphylococcus* spp. was isolated from tilapia, but they were considered non-toxin producers [26].

Even though isolation of *S. aureus* was reported from fish processing factory workers [27], and from both raw and frozen fish products [7,27], McCoy et al. [13] stated that most cases of *S. aureus* enterotoxin producers were the result abuse by the consumer or food service personnel. The presence of *S. aureus* in processing plants was also reported to be seasonal [13,16]. Since *S. aureus* does not compete well with the background microbial flora present on fish, it is not considered a problem [16,28] and no toxin would be produced. However, its presence in the processing plant could indicate contamination by workers [29].

The presence of *Listeria* spp. on the retail catfish nuggets confirmed the data previously report on *Listeria* spp. presence on raw fish products [7,9,19,28,29]. The species identification was done on the isolates obtained after plating on PALCAM and the percent prevalence of each species is listed in Table 3, which are similar to previously reported values [19]. *L. monocytogenes, innocua* and *welshimeri* were the most prevalent (Table 3). When these results were compared to those reported by Chow et al. [19] for the percentage of *Listeria* spp. found on fresh catfish fillets, the percentage was lower for each species identified in this study. Fernandes et al. [22] reported that when *L. monocytogenes* was inoculated onto trout and catfish, the microbe grew during refrigerated storage. The presence of *Listeria* spp. on finfish could be problematic, if there is cross contamination to ready-to-eat products [22]. McCoy et al. [19] stated that *L. monocytogenes* was not linked to any catfish fillet associated outbreaks.

Pao et al. [9] reported the finding of *E. coli* in 13.7% of the catfish fillets purchased from local retail markets and from Internet purchases. Neither *E. coli* O157:H7 nor *E. coli* were isolated from the retail catfish nuggets in this study. At a non-detection level, the results of this study were below the ICMSF minimum standard of 11 CFU/g [10].

Although there were reports that *Vibrio* spp. may be present on fresh water aquacultured finfish fillets [15,18], no study reported the presence of *Vibrio* spp. on catfish [13], which confirms the results obtained in this study where *Vibrio* spp. was not isolated from the retail catfish nuggets tested.

Conclusion

In this study pathogenic *E. coli* O157:H7, *S. aureus* and *Vibrio* were not detected on the nuggets and McCoy et al. [13] suggested that these pathogens would not be problematic for catfish fillets. *Salmonella* was isolated from the catfish nuggets, but *Salmonella* has a much higher prevalence on other meat products [30].

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