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ASPECTS OF VIETNAMESE SUTCHI CATFISH (*PANGASIUS HYPOPHTHALMUS*) FROZEN FILLET QUALITY: MICROBIOLOGICAL PROFILE AND CHEMICAL RESIDUES.

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ABSTRACT

Pangasius hypophthalmus fillets, classified according to different frozen technologies, were subjected to microbiological (Psychrophilic Total Aerobic Count = PTAC, *Enterobacteriacea*, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*) and chemical analyses (Hg, Pb, Cd and six "indicator" congeners of polychlorinated biphenyls=PCB_6). Mean count of psychrophilic aerobic total bacteria, *Enterobacteriaceae* and *S. aureus* in all samples examined was 4.44 log CFU/g, 2.16 log CFU/g and 1.07 log CFU/g, respectively. Any sample showed the presence of *Salmonella,* while *L. monocytogenes* was encountered in 2.1% of samples tested. The microbiological counts were different among fillet categories, but with values always lower than the limit of ICMSF. Also the chemical quality of processed products is satisfactory being the concentrations of all metals and six "indicator" PCBs below safety levels for human consumption. This study has contributed to addressing the current paucity in literature on the microbial and chemical status of frozen *P. hypophthalmus* fillets.

PRACTICAL APPLICATIONS

The present research reveals a good chemical and microbiological quality of the frozen Pangasius fillets, although the samples examined show a low incidence of *Listeria monocytogenes* and *Staphylococcus aureus*. Thus, it would be desirable a greater attention to the operator's personal hygiene practices during the processing procedures, appropriate preservation techniques and sanitation programs in the processing plants to avoid cross-contamination. The data contained in this study may also be useful for national and international food safety regulatory authorities.

INTRODUCTION

Pangasius hypophthalmus is a freshwater fish present not only in the main basins of South-East Asia, like the Mekong and Chao Phraya rivers (Belton *et al.* 2011), but also into other Asiatic rivers and ponds for aquaculture (Orban *et al.* 2008). Vietnam, in particular, have seen a considerable increase of aquaculture going from a rural activity to an intensive fish-farming system. This Vietnamese pangasius production is mainly destined to export market, considering that in 2008 the export of pangasius has equaled or exceeded other products of economic importance for Vietnam as rice, coffee and shrimps (Belton *et al.* 2011). In 2012, the volume of exports from Vietnam to the Europe was around 143,200 tons (FAO Globefish 2013) and Italy, in particular, annually imports approximately 12,780.00 tons of pangasius (Istituto Nazionale di Statistica 2013), available on the market as frozen or thawed fillets. The absence of the typical "fishy odor," spines, small bones and skin associated with the delicate flavor and the meat firm texture when cooked, gets this fish particularly appreciable by consumers. These characteristics together with availability on the market in standard size make this food especially suitable to the demand of the food service industry and restaurants (Orban *et al.* 2008). However, despite the active demand of this product on the European markets, Pangasius has generally a bad reputation in food safety and quality (Noseda *et al.* 2013). The Rapid Alert for Food and Feed (RASFF 2011) database reported several notifications of frozen fish originating from Vietnam contaminated with pathogenic bacteria. For frozen *P. hypophthalmus* fillets, *Listeria monocytogenes* was the bacteria more frequently isolated (RASFF 2011). Nevertheless, very little data can be found in worldwide literature about the microbiological and chemical quality of *Pangasius* (Minh *et al.* 2006; Orban *et al.* 2008; Tong Thi *et al.* 2013; Kulawik *et al.* 2016). In this context, the purpose of the present study was to evaluate the food safety of the Vietnamese sutchi catfish (*P. hypophthalmus*) fillets in terms of microbiological status (total count of aerobic psychrophilic bacteria, *Enterobacteriaceae*, *Staphylococcus aureus*, *Salmonella* spp. and *L. monocytogenes*) and some chemical contaminant content (mercury, cadmium, lead and six indicator congeners of polychlorinated biphenyls).

MATERIALS AND METHODS

Vietnamese sutchi catfish (*P. hypophthalmus*) fillets of different size (170–300 g) were obtained from an Italian trade import services company. After thawing at temperature ranging from 18 to 27C (for 1 h and not more than 3 h) a total of 210 samples, classified according to different frozen technologies (Individual Quick Frozen (IQF) = 70 samples; Deep Frozen Block (DFB) = 66 samples; Block Frozen (BF) = 74 samples) were subjected to microbiological analyses and chemical analysis. Before chemical analysis each fillet was homogenized and weighed sample portions were analyzed. For microbiological analyses, the samples were subjected to count of psychrophilic total aerobic bacteria (PTAC) according to the microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique (ISO 4833, 2003), the enumeration of *Enterobacteriaceae* (ET) agreeing to ISO 21528-2:2004 and *S. aureus* according to EN ISO 6888-2:2004. Further, the isolation of *Salmonella* spp. (EN ISO 6579:2008) and *L. monocytogenes* (EN ISO 11290-1:2005) were carried out.

For chemical analyses, the extractive analytical procedures and the instrumental conditions to determine metal and polychlorinated biphenyls (PCB) concentrations have been described in detail elsewhere (Storelli 2008). Briefly, aliquots of the homogenized samples were digested with a mixture of H_2SO_4-HNO_3 for Hg and HNO_3-$HClO_4$ for Pb and Cd. The content of metals was determined by atomic absorption spectrophotometer (AAS) (Perkin Elmer Analyst 800). Cd and Pb was analyzed by graphite furnace technique (THGA-800 P.E.) and Hg by a hydride system (FIMS 100) after reduction by $SnCl_2$. For six PCB indicator congeners (PCB_6: 28, 52, 101, 138, 153, 180), aliquots of the homogenized samples were grinded with Na_2SO_4, spiked with 20 ng of 2,2′,3,4,5,6′-H_6CB (PCB 143) used as internal standard and extracted with petroleum ether. Subsamples were taken in order to determine the tissue fat content by gravimetry. The extracts were cleaned by passing through 8 g of acid silica (H_2SO_4, 44% wet weight), using 50 mL of a mixture of hexane/dichloromethane (1/1, vol/vol) for elution of the analytes. For the analysis of PCBs, a Thermo Trace GC connected with a Thermo PolarisQ MS operated in electron impact ionization (EI) mode was equipped with a Rtx 200 capillary column (Thermo, Austin, TX). The MS was used in the SIM mode with two ions monitored for each PCB homologue group in specific windows. The cleaned extract was injected in splitless mode (injector temperature 90 C then to 300 C with 70 C/min). Quality control was performed through the analyses of procedural blanks, a duplicate sample and standard reference materials (metals: Tort-2 Lobster Hepatopancreas, Ontario, Canada; PCBs: CRM349, cod liver oils-BCR, Brussels). The limits of detection (LODs) and the standard of quantification (LOQ_s) for metals are the following: LODs = Hg: 5; Cd: 10; Pb: 10 ng/g wet weight; LOQs = Hg: 13; Cd: 0.38; Pb: 40 ng/g wet weight LODs and the LOQs for PCBs ranged from 0.02 to 0.50 ng/g on a lipid weight basis and from 1 to 2 ng/g on a lipid weight basis, respectively.

Statistical Analysis

Kruskal–Wallis test was used to test hypothesis about differences in the levels of contaminant accumulation and to determine whether there were differences in the contamination by pathogens as a function of the different frozen technologies. The level of significance was set as $P < 0.05$.

RESULTS AND DISCUSSION

Independently by different frozen treatments, the mean count total of psychrophilic aerobic total bacteria in samples was 4.44 log CFU/g. Psychrotrophic Gram-negative bacteria belonging to the genera *Acinetobacter*, *Flavobacterium*, *Moraxella*, *Shewanella* and *Pseudomonas* as well as Gram-positive microorganisms such as *Bacillus*, *Micrococcus*, *Clostridium* and *Lactobacillus* are widely distributed in the environment and consequently fish might have acquired them from various pathways including water, harvesting, transportation, storage, etc. However, the number of CFU/g obtained in this research, although moderately high, was not over the acceptable limit of total bacterial load (5.5–7.0 log CFU/g) set by International Commission on Microbiological Specification for Food (1986). The *Enterobacteriaceae* count, considered as another index of fish quality for samples tested was 2.16 log CFU/g. Concerning *Salmonella* spp. no colony was isolated from our samples, whereas microbial analyses detected *L. monocytogenes* in 12 of the 210 samples tested (1.8%). Recent reports from EFSA on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks (EFSA 2010, 2013) indicate that *Salmonella* is one of most common etiologic agents of foodborne diseases, responsible in Europe for 26.6% of all reported outbreaks, with 4.2% linked to consumption of fish and fishery products. On the contrary, the outbreaks caused by *L. monocytogenes* are not common when compared with those caused by *Salmonella*. Small-scale outbreaks and sporadic cases of listeriosis have been linked to cold-smoked salmon, smoked mussels and trout (Brett 1998; Laciar and de Centorbi 2002). The general prevalence of this bacteria in seafood ranges from 0 to more than 50% depending on the production site (Midelet-Bourdin *et al.* 2007), but the level of contamination remains relatively low, despite the fact that *L. monocytogenes* can grow well in diverse environmental conditions (Rocourt and Bille 1997). Studies on *L. monocytogenes* indicated that fish contamination occurred mainly along the processing line rather than the raw material (Parisi *et al.* 2013; Sudheesh *et al.* 2013). Also in our samples, the very low incidence of this bacterium seem be linked to level of hygiene that the operator puts in place during the handling of the product rather than from external contamination. Also the level of *S. aureus,* estimated to be 1.07 log CFU/g, although low, suggests a relation between product contamination and standard of hygiene. The adoption of good manufacturing practices during processing or food-service operations prevents, in fact, the presence of *S. aureus* which being a common resident of human skin and mucous membranes (Franklin and Lowy 1998; Dambrosio *et al.* 2013) is often present in handled foods. However, the occurrence of this pathogen on food does not imply necessarily a risk for the consumer, because it can produce enterotoxin when present in high numbers above 5 log CFU/g, value of about five times higher than that encountered in the samples under investigation. Nevertheless, the results obtained should not be underestimated because these could constitute a potentially food safety risk. A previous study has in fact noted the presence of this pathogen on the hands of the food operators during processing, particularly in the packaging area, marking the poor personal hygiene practices (Tong Thi *et al.* 2014). The applied preservation techniques to fresh fish like freezing, drying through smoking, salting, canning, etc. can

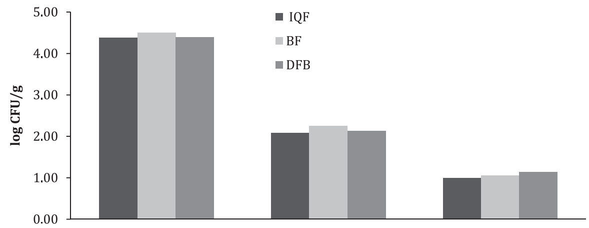

FIG. 1. MEAN MICROBIOLOGICAL COUNT IN *PANGASIUS* FILLET SAMPLES PROCESSED ACCORDING TO THE DIFFERENT FROZEN TECHNOLOGIES

also widely contribute to the microbiological quality of the product. For example, frozen storage of fishery products might result in a reduction of some microbiological counts (Noseda *et al.* 2012). Gram-negative bacteria die more rapidly during frozen storage than Gram-positives. In this context, because our samples are processed with different frozen treatments it is of concern the evaluation of the bacterial community. As shown in Fig. 1, the comparison revealed statistically significant differences among the three fillet categories having DFB and BF fillets a higher load than IQF samples ($P < 0.05$) (Kruskal–Wallis test). In detail, the mean values of PTAC for DFB and BF samples were 4.49 log CFU/g and 4.51 log CFU/g, respectively, while IQF fillets showed average values of 4.24 log CFU/g ($P < 0.05$). Similarly, the results of *Enterobacteriaceae* and *S. aureus* count showed statistically significant differences being higher in DFB (*Enterobacteriaceae*: 2.20 log CFU/g; *S. aureus*: 1.14 log CFU/g) and BF (*Enterobacteriaceae*: 2.29 log CFU/g; *S. aureus*: 1.05 log CFU/g) fillets than IQF (*Enterobacteriaceae*: 2.00 log CFU/g; *S. aureus*: 1.03 log CFU/g) samples ($P < 0.05$).

The concentrations of chemicals tested in Vietnamese sutchi catfish fillets are summarized in Table 1. The concentrations of Hg, Cd, Pb as well as those of six "indicator" PCBs were found not to vary depending on different frozen technologies ($P > 0.05$) and, therefore, the results were grouped and discussed all together. All fish fillets contained

TABLE 1. CONCENTRATIONS OF Hg, Cd, Pb (μg/g wet weight) AND SIX "INDICATOR" PCBs (PCB_6) (ng/g wet weight) IN PANGASIUS FILLET SAMPLES

	Min–max	Mean ± St. Dev.	European Community permissible levels
Hg	0.02–0.07	0.05 ± 0.01	0.50*
Cd	0.01–0.03	0.02 ± 0.01	0.05*
Pb	0.07–0.16	0.09 ± 0.02	0.30*
% Lipid	0.1–0.4	0.3 ± 0.1	–
PCB 101	0.03–0.10	0.05 ± 0.01	–
PCB 138	0.01–0.10	0.06 ± 0.02	–
PCB 153	0.04–0.15	0.09 ± 0.02	–
ΣPCBs	0.04–0.25	0.15 ± 0.06	125†

*Official Journal of the European Union 2011.
†Official Journal of the European Union 2006.

detectable levels of Hg and Pb, while Cd showed measurable levels only in 45.9% of samples analyzed. Pb showed the highest concentrations with values ranging from 0.07 to 0.16 μg/g wet weight (mean: 0.09 μg/g wet weight). However, none of the analyzed samples had values above 0.30 μg/g wet weight, limit established by European Union legislation (Official Journal of the European Union 2006). Hg levels between 0.02 and 0.07 μg/g wet weight (mean: 0.05 μg/g wet weight), were below the maximum allowable limit of 0.50 μg/g wet weight set by the European Commission (Official Journal of the European Union 2006). Also for Cd, the concentrations between 0.01 and 0.03 μg/g wet weight (mean: 0.02 μg/g wet weight) were well below the legal limit (Official Journal of the European Union 2006). Concerning PCBs, the analysis was limited to the so-called six "indicator" PCBs (PCB_6) recommended by the European Union as image of contamination because generally they represent approximately half of the total non-dioxin-like PCBs existing in food. In fact, the European Food Safety Authority (EFSA) Scientific Panel regarding Contaminants in the Food Chain chooses to use the sum of these six congeners because of appropriate marker for a human risk assessment of non-dioxin-like PCBs. In this study, the six congeners were not detected in most samples. In particular, the congeners PCB 28, 52 and 180 were below the limit of quantification in all sutchi catfish fillets, while PCBs 101, 138 and 153 were detected in 31.4%, 35.7% and 54.1% of examined samples, respectively. However, as can be seen in Table 1 the levels of these chemicals were very low and their sum, ranging from 0.04 to 0.25 ng/g wet weight (mean: 0.15 ng/g wet weight) was considerably below the limit of 125 ng/g wet weight, recently set by the regulation No 1259/2011 of the European Union (Official Journal of the European Union 2011).

The increasing global diffusion of fish products for human consumption requires accurate controls to ensure their quality and safety. The chemical aspect of processed products is satisfactory reflecting a quality-controlled diet of the farmed species associated to good environmental conditions of farming areas. Concerning the microbiological quality, the hygiene and sanitation procedures in the production area should be revised, as food worker proper training and sanitation programs can have a great impact on reducing the presence of some pathogens.

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