

QUANTITATIVE EVALUATION OF *LISTERIA MONOCYTOGENES* IN FRESH AND PROCESSED SURUBIM FISH (*PSEUDOPLATYSTOMA* SP)

Souza, V.M. de¹; Alves, V.F.¹; Destro, M.T.²; De Martinis, E.C.P.^{1*}

¹Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil; ²Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brasil.

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ABSTRACT

L. monocytogenes is a foodborne psychrotrophic bacterial pathogen of special importance for minimally processed foods. In this work, it was enumerated in samples of surubim fish by MPN technique. The population of *L. monocytogenes* was estimated as <0.012 MPN/cm² in fresh and <0.03 MPN/g in minimally processed fish.

Key-words: *Listeria monocytogenes*, MPN, fish, surubim

There is an increasing demand for fresh refrigerated ready-to-eat (RTE) foods and the microbiological criteria for international trading have been very strict. *L. monocytogenes* is an important psychrotrophic foodborne pathogen, which causes listeriosis, a disease with low incidence rate (0.1 to 11.3 cases per million of population), but responsible for the majority of deaths due to bacterial pathogens in the US (28%). It was implicated in several recalls of products last year (FSIS, 2007) and despite increased awareness on the risks of the disease, Koch and Stark (2006) reported that in Germany the number of listeriosis cases raised from 217 cases in 2001 to 519 cases in 2005 and stated that reasons for this increase remained unclear.

The disease caused by *Listeria monocytogenes* can manifest with mild flu-like symptoms, gastroenteritis or even life-threatening infection, depending on the number of bacterial cells, the host susceptibility and the virulence of the strain. Although no outbreaks reported have been linked to consumption of fish, this kind of food was already incriminated in sporadic cases of listeriosis (Destro, 2000). According to Lyytikäinen *et al.* (2006), at least one quarter of cases of listeriosis occurred in Finland during 1995-2004 were caused by certain sero-genotype or closely related genotypes which had also been found in vacuum-packed cold smoked or cold-salted fish products.

Minimally processed fish may support listerial growth even when stored under proper conditions. The aim of this work was

to determine the population of *L. monocytogenes* in surubim, a Brazilian fish which may be consumed after minimal processing and cold storage.

L. monocytogenes was enumerated in 33 samples of surubim fish (*Pseudoplatystoma* sp), collected between August 2004 and June 2005. Nineteen samples of fresh eviscerated surubim fish and 14 samples of vacuum-packed smoked sliced surubim fish (7 within 24 h of processing and 7 after 35 days of storage at 5°C) were analyzed. The method of Pagotto *et al.* (2002) was used combined with the most probable number technique, MPN (Peeler, 1992).

For that, 50 g of each sample of sliced fish was mixed with 450 ml of *Listeria* Enrichment Broth (LEB; UVM2, CM0863, and SR 0142, Oxoid, Basingstoke, UK) and transferred to series of three tubes to contain 10, 1, 0.1, and 0.01 g. For whole eviscerated fishes, samples were obtained by swabbing 5 different parts of each fish (5 times 25 cm²): three swabs from external and two swabs from inner surfaces. The swabs were placed in 50 ml saline (0.85% sodium chloride, w/v) and the suspension was mixed with 450 ml LEB. This broth was used to prepare series of three tubes for MPN: 100 ml (25 cm²), 10 ml (0.25 cm²), 1 ml (0.025 cm²), and 0.1 ml (0.0025 cm²). Inoculated LEB was incubated at 30°C for 24 h, when 0.1 ml of each tube was transferred to secondary enrichment in Fraser broth (CM 895 and SR 156, Oxoid) and incubated at 35°C for 48 h. From each blackened

*Corresponding Author. Mailing address: Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Av. do Café s/n, Ribeirão Preto, SP, Brasil, ZIP 14040-903. Tel.: +55 16 36024267, Fax: +55 16 36024725. E-mail: edemarti@usp.br

Fraser tube, an aliquot was surface plated on Oxford (CM 0856 and SR 0206, Oxoid) and PALCAM (CM 0877 and SR 0150, Oxoid) agar plates for isolation of colonies. Up to three colonies were selected and submitted to classical methods for identification of *Listeria* species (Pagotto *et al.*, 2002), combined with API *Listeria* (bioMérieux, Marcy l'Etoile, France).

The MPN method used in this work could detect populations of *L. monocytogenes* as low as 0.03 MPN/g of processed fish and 0.012 MPN/cm² of raw whole fish. Although laborious and time consuming, the MPN technique is still currently the method of choice for quantification of levels of *L. monocytogenes* below 100 CFU per gram (De Martinis *et al.*, 2007). A method for enumeration of *L. monocytogenes* that is at the same time easy to perform, sensitive, specific, rapid and not expensive is not available and the isolation of *L. monocytogenes* from highly contaminated matrices depends on the capability of the method to promote growth of low number of cells potentially injured and to minimize growth of accompanying microbiota (Ingianni *et al.*, 2001, Ryser; Donnelly, 2001).

In this work, *Listeria* sp was not detected in any sample analyzed and the population of *L. monocytogenes* was estimated to be less than 0.012 MPN/cm² in the fresh fish and less than 0.03 MPN/g in processed surubim. This data indicate the low risk of smoked sliced surubim in disseminating *L. monocytogenes* to consumers.

For risk analysis, quantitative data on foodborne pathogens is essential and our results contribute with quantification of *L. monocytogenes* in RTE fish from Brazil.

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RESUMO

Avaliação quantitativa de *Listeria monocytogenes* em surubim (*Pseudoplatystoma* sp) fresco e processado

L. monocytogenes é um patógeno psicrotrófico transmitido por alimentos, de importância especial para alimentos

minimamente processados. Neste trabalho, a bactéria foi enumerada em amostras de peixe surubim utilizando-se a técnica do NMP. A população de *L. monocytogenes* foi estimada como < 0.012 NMP/cm² do peixe fresco e < 0.03 NMP/g do peixe minimamente processado.

Palavras-chaves: *Listeria monocytogenes*, NMP, peixe, surubim

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