Food Microbiology 30 (2012) 408–419

Contents lists available at SciVerse ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Microbiological spoilage of vacuum and modified atmosphere packaged Vietnamese *Pangasius hypophthalmus* fillets

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ARTICLE INFO

Article history:
Received 9 September 2011
Received in revised form
14 December 2011
Accepted 28 December 2011
Available online 3 January 2012

Keywords:
Pangasius hypophthalmus
Microbiological spoilage
MAP
SPME GC–MS
SIFT-MS

ABSTRACT

This study investigated the identity, growth and metabolite production of micro-organisms causing spoilage of *Pangasius hypophthalmus* fillets packaged in air, vacuum and modified atmospheres (MAP) (MAP 1: 50% CO_2–50%N_2 and MAP 2: 50%CO_2–50%O_2) during storage at 4 °C. Based on the time it took for psychrotrophic total colony counts to exceed 7 log cfu g^{-1}, the shelf life of the fillets packaged in air, vacuum, MAP 1 and MAP 2 was estimated to be 7, 10, 12 and 14 days respectively. The longest lag phases were observed in the samples packaged in MAP 2 (50%CO_2–50%O_2). In the fillets packaged in air and under vacuum, the dominant flora identified by partial 16S rDNA sequencing at the end of the shelf life generally consisted of Gram-negative bacteria mostly belonging to the genera *Serratia* and *Pseudomonas*. In contrast, lactic acid bacteria (*Carnobacterium maltaromaticum* and *Carnobacterium divergens*) and *Brochothrix thermosphacta* were identified as the dominant spoilage flora in the samples packaged under the two MAPs investigated. By means of solid-phase microextraction gas chromatography mass spectrometry (SPME GC–MS) analysis, volatile organic compounds in the headspace of the samples at the end of the shelf life were identified for each packaging condition. Based on these results, a selective ion flow tube mass spectrometry (SIFT-MS) method was developed to quantify the production of volatile metabolites during storage of the fillets. The results of these analyses indicated that several compounds contributed to the bacterial spoilage of *Pangasius* fillets e.g., ethanol, 2,3-butanediol, diacetyl, acetoin, ethyl acetate, acetic acid and sulfur compounds. It also emerged that the production of these compounds was dependent on the packaging condition applied.

1. Introduction

Pangasius hypophthalmus, also referred to as *Pangasianodon hypophthalmus*, sutchi catfish, striped catfish and 'tra', has become an economical valuable freshwater fishery product. This fish is mainly offered on the European market as skinned and boneless frozen fillets (Karl et al., 2010). However, recent tendency is that consumers' demand favors fresh or thawed fillets, usually packaged in a modified atmosphere. *Pangasius* fillets became an affordable 'white fish' substitute for cod and other white fleshed fishes on the Western market, and its acceptability and popularity is at the moment still increasing (Phan et al., 2009). This has led to the introduction of intensive farming programs and to the development of large scale aquaculture enterprises in Vietnam. Sutchi catfish farming in the Mekong Delta is the largest farming sector based on a single species in a single geographical area (Phan et al., 2009). Special efforts have been made to meet up to the quality and safety standards required by the importing countries (Orban et al., 2008; Rahman et al., 2006). Processing technologies and preservation techniques were standardized to assure the quality of the product. The production process for fillets intended for export is more or less similar in the different processing companies of the region.

Despite the high economic importance of this fish species, little or no information is available on the shelf life of these *Pangasius* fillets, the microbiological spoilage flora and their ability to produce volatile organic metabolites which may contribute to the spoilage process. As for any fishery product, the initial microbiological quality of the

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doi:10.1016/j.fm.2011.12.025

Pangasius fillets at the end of processing, is dependent on many factors in the production process. Factors like the microbiological quality of the water and the sediment of the culture ponds will affect the microbiological ecology present on the living fish and also in the end product (Orban et al., 2008). Additionally, the temperature, environment and (hygienic) handling during processing and distribution steps like slaughtering, gutting, filleting, skinning, trimming, washing, sizing, grading, weighing, freezing, packing, transport and storage will eventually contribute to the microbiological quality of the product as well. The initial microbiological flora on freshly harvested, properly handled pond reared fish products typically consists of a diverse mixture of Gram-negative and Gram-positive species from several genera including *Acinetobacter*, *Aeromonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Micrococcus*, *Moraxella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Vibrio* (ICMSF, 2005). Frozen storage of fishery products might result in a reduction of some microbiological counts, which makes the assessment of pre-freezing quality of the product more difficult. In general, Gram-negative bacteria die more rapidly during frozen storage than Gram-positives. Frozen thawed aquaculture products spoil about as fast as and similar to non frozen products. On extended refrigerated storage under aerobic conditions, Gram-negative genera, particularly *Pseudomonas* and *Acinetobacter*, are considered to be the dominating spoilage flora of tropical aquaculture fishery products (ICMSF, 2005). Mohan et al. (2008) reported a shelf life of 10 days for *Pangasius* fillets packaged in air and stored at a temperature between 0 and 2 °C. Storage of the same fillets in the presence of an O_2 scavenger extended the shelf life by an additional 10 days. The initial number of mesophilic and psychrotrophic bacteria of the final product was found to be 3.28 and 2.89 log cfu g^{-1}. As time of storage increased, psychrotrophic bacteria showed significantly higher growth than the mesophilic bacteria. Hossain et al. (2005) reported a shelf life of 20 days for *Pangasius* fillets stored under ice. This estimation was based on the sensorial quality and the total volatile basic nitrogen (TVB-N) content.

The aim of this study was to gain more knowledge on the microbiological flora dominating spoiled fillets and the microbiological metabolites (volatile and non-volatile) contributing to spoilage process. To achieve this goal, the effect of four packaging conditions [air packaged, vacuum packaged, MAP 1 (50%CO_2–50%N_2) and MAP 2 (50%CO_2–50%O_2)] was evaluated at a storage temperature of 4 °C.

2. Materials and methods

2.1. Raw material

A batch of frozen *P. hypophthalmus* fillets, with a fillet weight varying between 200 and 225 g, was produced for a Belgian trader by a Vietnamese company. At the explicit request of our laboratory, this batch was processed without the use of any chlorination steps nor the use of additives e.g., salts, preservatives, polyphosphates. Concerns have been raised regarding the use of chlorinated water by some processing companies to decontaminate the fillets during the washing steps. Also the use of polyphosphates, to improve the water retention by proteins of the fish, in the production process has been reported (Orban et al., 2008). The time between slaughtering and individual quick freezing (IQF) freezing was estimated by the company to be *ca.* 4 h. This batch remained for 3 days at the Vietnamese site stored at −18 °C and was subsequently transported and shipped at −18 °C from Vietnam to the Belgian trader. Fifty kg of this untreated batch was then transported at −18 °C from the Belgian trader to the Laboratory of Food Microbiology and Preservation (Ghent University) where it was stored at −21 °C until the start of the experimental setup. The shelf life when stored at −18 °C was labeled by the company on the cardboard boxes and was covering a time period of 24 months. All analyses performed occurred within 3 months after production.

2.2. Experimental setup

Initially, five randomly selected fillets were taken to measure the pH, a_w, water and salt content of the fillets. Before use 30 kg of fillets were thawed at 4.0 ± 0.7 °C over a 15 h period in a refrigerator with forced ventilation. These fillets were then portioned aseptically in 150.0 g ± 3.0 g pieces without the use of any disinfectants to avoid chemical contamination. The portions were packaged applying 4 packaging conditions: packaged in air, packaged under vacuum, MAP 1 (50% CO_2, 50%N_2) and MAP 2 (50%CO_2, 50%O_2). All samples, with the exception of the vacuum packaged fillets, were packaged in trays with a gas/product ratio of 4/1. The packaged fillets were stored in a refrigerator at 4.0 ± 0.7 °C for a period of 24 days. On a regular basis (days 0, 3, 5, 7, 10, 12, 14, 17, 19, 21 of storage) 3 samples of each packaging condition were randomly selected for the assessment of the microbiological quality, pH, headspace (%O_2, %CO_2) composition, levels of headspace volatile organic compounds with SIFT-MS and levels of lactic acid (by means of HPLC coupled to a RI-detector). The samples for SIFT-MS analysis were immediately frozen inside their packaging and subsequently thawed and analyzed on day 24. Samples for SPME GC–MS analyses were collected at the start of the experiment (day 0) and thereafter on days, 10, 14 and 21, when sensorial deviations were clearly noticeable.

2.3. pH, water activity, water/ash content and salt content

pH was measured with a pH-electrode (InLab® 427, Mettler Toledo GmbH, Schwerzenbach, Switzerland) connected to a pH-meter (SevenEasy, Mettler Toledo GmbH). The product temperature during pH measurments was 4.0 ± 2.0 °C. The water activity was measured with an automated aw-Kryometer (AWK-20, NAGY Messsysteme GmbH, Gäufelden, Germany). The water content was determined gravimetrically after drying an aliquot of the homogenate for 10 h at 105 °C. The homogenate was heated in aluminum dishes containing sea sand to avoid splashing. The chloride content was determined according to the Mohr method (ISO 9297:1989). The salts were extracted from the mixed *Pangasius* samples by cooking the fillets for 10 min in distilled water. The concentration of chloride ions was determined by means of a silver nitrate (Merck, Darmstadt, Germany) titration with a 5% (w/v) chromate indicator (Merck, Darmstadt, Germany).

2.4. Packaging and gas composition (%O_2, %CO_2) measurements

Vacuum packaging was performed with a Multivac A300/42 (Hagenmuller, Wolfert-schwenden, Germany) packaging unit. The trays were packaged using a Tray sealer MECA900 (DecaTechnic, Herentals, Belgium). Multilayer packaging materials were used: PA/PE/EVOH/PE for the packaging foil and PP/EVOH/PP for the trays, both having a low gas permeability. The barrier film is 90 micron thick and has an oxygen transmission rate of 2.0 cm^3/m^2.d.bar (23 °C, 85% R.H.). Analysis of the O_2 and CO_2 levels in the headspace of the packaged samples was performed with a Checkmate® 9900 O_2/CO_2 (PBI Dansensor A/S, Ringsted, Denmark).

2.5. Microbiological analysis

From each sample, 30 g was aseptically placed in a sterile stomacher bag and diluted 10 times in physiological saline peptone solution (PPS, 0.85% NaCl, 0.1% peptone). After homogenization for 1 min in a Stomacher 400 Lab Blender (LED Techno, Heusden-Zolder,