Food Microbiology 52 (2015) 197–204

Contents lists available at ScienceDirect

Food Microbiology

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

Changes in the microbial communities of air-packaged and vacuum-packaged common carp (*Cyprinus carpio*) stored at 4 °C

Yuemei Zhang, Qian Li, Dongping Li, Xiaochang Liu, Yongkang Luo*

College of Food Science and Nutritional Engineering, China Agricultural University, Beijing Higher Institution Engineering Research Center of Animal Product, Beijing 100083, PR China

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form
3 August 2015
Accepted 5 August 2015
Available online 7 August 2015

Chemical compounds studied in this article:
Phenethylamine (PubChem CID: 1001)
Putrescine (PubChem CID: 1045)
Cadaverine (PubChem CID: 273)
Histamine (PubChem CID: 774)
Tyramine (PubChem CID: 5610)
Spermidine (PubChem CID: 1102)
Spermine (PubChem CID: 1103)

Keywords:
Common carp
Vacuum packaging
Microbial communities
Spoilage
Biogenic amines

ABSTRACT

The dominant microbiota of air-packaged (AP) and vacuum-packaged (VP) common carp fillets during storage were systematically identified. Culture-dependent methods were used for microbial enumeration and 16S rRNA genes of the isolated pure strains were sequenced and analyzed. Different packaging conditions affected the growth of microbiota and the shelf life of carp. Shelf-life of AP and VP fillets was 8 and 12 days, respectively. Vacuum packaging delayed the increase of biogenic amines levels compared to air packaging, especially for cadaverine and tyramine levels. In the present study, a total of 13 different genera comprised the microbial communities of fresh carp fillets and *Acinetobacter* dominated the indigenous flora of carp. However, variability in bacterial community composition was observed in these two packaging conditions. *Pseudomonas* were the only microbiota found in the spoiled AP carp, whereas *Carnobacterium* followed by *Aeromonas* were found mainly in VP samples. Other genera *Shewanella*, *Lactococcus*, and *Pseudomonas* were also found in low numbers at the end of the VP fillets' shelf life. Additional microbial enumeration observed the highest *Pseudomonas* counts (8.77 log CFU/g on day 8) in AP samples and a relatively high level of lactic acid bacteria (7.74 log CFU/g on day 12) in VP samples.

1. Introduction

Common carp (*Cyprinus carpio*) is currently recognized as a freshwater fish species with high economic value and is often grown in aquaculture facilities in many countries (Winker et al., 2010). Total aquaculture production of common carp in China increased to approximately 3,022,494 tons in 2013 (Bureau of Fisheries of the Ministry of Agriculture, 2014). Due to its abundance, reasonable domestic price, and delicious taste, carp has become a popular species for many producers and consumers. It is usually distributed as the fresh whole fish in markets. However, there is a growing tendency among consumers who prefer fresh or thawed fillets because of their convenience. Given that the shelf-life of air-packaged (AP) carp fillets is relatively short, research on new packaging methods for fillets is required. Vacuum packaging is an effective packaging technology that offers a way of prolonging the shelf life of perishable fillets by excluding oxygen and inhibiting the growth of microorganisms (Noseda et al., 2012). Additionally, vacuum-packaged (VP) fillets have a small package volume, making international transport easier. Therefore, storage under vacuum packaging has been given increasing attention recently.

The undesirable growth and metabolism of microorganisms are the primary causes for spoilage of fish (Gram and Huss, 1996). However, not all microorganisms are responsible for fish spoilage. In general, fish is always contaminated with a small quantity of microorganisms that are designated as specific spoilage organisms (SSOs) when they are considered inedible (Dalgaard, 1995). These SSOs are present in low numbers in fresh fish and can eventually become dominant in spoilage microorganisms (Pennacchia et al., 2011). Therefore, it is meaningful to identify the SSOs at the time of spoilage and to analyze the relationship between SSOs and the shelf-life of products that supply fresh products to consumers.

* Corresponding author.
E-mail addresses: luoyongkang@263.net, luoyongkang@cau.edu.cn (Y. Luo).

http://dx.doi.org/10.1016/j.fm.2015.08.003
0740-0020/

Under chilled, aerobic storage conditions, several Gram-negative genera, particularly *Pseudomonas*, *Aeromonas*, *Shewanella*, Enterobacteriaceae, dominate the spoilage microorganisms of freshwater and marine fish (Noseda et al., 2012; Wang et al., 2014). However, vacuum packaging can inhibit the growth of aerobic bacteria commonly present on fish, resulting in an increase in Gram-positive bacteria that can respire better than Gram-negative bacteria in this type of packaging (Lyhs et al., 2002; Mace et al., 2012; Noseda et al., 2012).

With regard to common carp, Mahmoud et al. (2004) analyzed the microbiota that cause spoilage in skin, gills and intestines. Their analysis depended mostly on traditional microbiological methods, such as plate viable counts, isolation, and biochemical identification. However, the objective of this study was to characterize and monitor the changes in the microbial communities of AP and VP common carp fillets stored at 4 °C using a combination of culture-based and 16S rRNA gene analysis methods. We also hoped to gain more knowledge on the dominant microorganisms at the species level so that we could develop suitable control methods.

2. Materials and methods

2.1. Sampling and packaging

Twenty common carp (weight 1150 ± 180 g, length 43 ± 1 cm) were obtained from an aquatic products market in Beijing, China and were transported to the laboratory alive in September 2014. Subsequently, carp were stunned, deheaded, scaled, gutted, filleted, and washed immediately with cold sterile water. The fillets were then drained at 4 °C for 3 min and prepared for packaging. The fillets (weight 220 ± 35 g, about 12 × 10 × 2 cm^3) were randomly divided into two portions. Then they were packaged in air (n = 18) in well-sealed polyvinyl chloride bags (about 250x200 mm) and under vacuum (n = 21) in pouches of polyethylene/polyamide film (about 250 × 200 mm, having an oxygen permeability of 40–50 cm^3/m^2 per 24 h/atm at 85% relative humidity, 23 °C), respectively. All pouches were stored at 4 ± 1 °C. Samples of white dorsal muscle from three fillets were taken randomly for analyzing sensory scores, pH value, total volatile basic nitrogen (TVB-N), microbiological enumeration, and biogenic amines every 2 days. However, microbial communities were identified on days 0, 4, and 8 for AP samples and on days 6 and 12 for VP samples.

2.2. Sensory analyses

The sensory characteristic of each fillet (fresh and cooked) was evaluated using the method of Hong et al. (2012). Cooked carp fillets were prepared by steaming for 10 min at 100 °C. Seven members were trained to evaluate the color, odor, elasticity, and the morphology of raw fish muscle, as well as the flavor, odor, and broth turbidity of cooked carp fillets, scoring each on a scale from 1 to 5 points. A total score of 35.0 points was considered fresh, while 15.0 was regarded as the lowest acceptable limit.

2.3. Determination of TVB-N and pH

TVB-N value was measured by the semi-micro steam distillation method (Hong et al., 2012). The pH value was detected using a digital pH meter (Mettler Toledo FE20/EL20, Shanghai, China).

2.4. Determination of biogenic amines

Extraction and derivatization of biogenic amines (BAs) were carried out as described by Shi et al. (2012). Subsequently, BAs were determined and quantified by using HPLC (Shimadzu LC-10A; Shimadzu, Kyoto, Japan) equipped with a COSMOSIL 5C18-PAQ (4.6 × 250 mm) column and an SPD-10A (V) detector. Ammonium acetate (0.1 M; solvent A) and acetonitrile (solvent B) were used as mobile phases. The gradient elution program was as follows: 0 min, 50% B; 25 min, 90% B; 35 min, 90% B; 45 min, 50% B. The samples were detected at 254 nm with a flow rate of 0.8 mL/min and an injection volume of 50 μL. The column temperature was 30 °C.

2.5. Enumeration of microbial communities

Plate dilution gradient methods were carried out on samples to enumerate different microbial communities using the method of Wang et al. (2014). For all microbial enumeration, samples of serial dilutions (100 μL) were spread on the surface of dry media. Total viable counts (TVC) were determined in plate count agar (PCA) and they were incubated at 30 ± 1 °C for 72 h. Lactic acid bacteria (LAB) were enumerated in overlaid pour-plates of MRS agar and incubated at 30 °C for 48 h. *Pseudomonas* sp. were determined on *Pseudomonas* CFC Selective Agar (CFC) at 20 °C for 48 h. H_2S-producing bacteria were evaluated on iron agar medium (IA); black colonies produced on IA were enumerated after incubating at 20 °C for 4 days. All colony forming units were recorded as log CFU/g. All of the culture media were supplied by Hai Bo Biological Technology Co., Ltd. (Qingdao, China).

2.6. Isolation and identification of microorganisms

2.6.1. Isolation and purification

Bacterial DNA were isolated and purified in accordance with the method of Wang et al. (2014). After TVC enumeration, bacteria were isolated on PCA for identification of the dominant microbiota from fresh carp fillets (53 isolates), AP fillets stored on days 4 and 8 (46 and 55 isolates, respectively), and from VP fillets stored on days 6 and 12 (40 and 48 isolates, respectively), with the goal of purifying these strains. All of the colonies were selected from the highest dilution PCA spread plates which usually contained 30–100 isolates, using an inoculation loop, and sub-cultured at 30 ± 1 °C for 24–48 h in 5 mL of tryptic soy broth (TSB) (Aoboxing Universeen Bio-Tech Co., Ltd., Beijing, China). After being cultured, by repeated plate streaking, Gram staining and microscopy, the isolates from each sample were purified for identification by 16S rRNA gene sequence analysis. A single purified colony was sub-cultured in 5 mL of TSB at 30 ± 1 °C for 24–48 h.

2.6.2. Extraction and identification of DNA by 16S rRNA gene analysis

The 2 mL sample of TSB culture was transferred, each containing a single purified colony, to a centrifuge tube, where cells were collected by centrifugation. DNA was extracted from the centrifugal sediment using the bacterial DNA extraction kit (Bomad Biological Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. 1.0% agarose gel electrophoresis was used to determine quality of these DNA extracts. These extracts, which showed the clear, bright ladder in the agarose gel, were picked as the DNA templates for PCR (TC-512, Techne, UK).

Bacterial 16S rRNA was amplified by PCR using the universal bacterial forward primer 27f (5′-GAGATTTGATCCTGGCTCAG-3′) and the reverse primer 1495r (5′-CTACGGCTACCTTGTTACGA-3′) (Wang et al., 2014). All the oligonucleotide PCR primers used in this study were received from Bomad Biological Technology Co., Ltd. (Beijing, China; BBT). The PCR system was comprised of 12.5 μL 2 × Taq PCR Master Mix (containing thermostable DNA polymerase, $MgCl_2$, dNTPs, buffer), 1 μL of template DNA, 10.5 μL double distilled water, and 0.5 μL of each primer at a concentration of 10 μM with a total reaction volume of 25 μL. Amplification was performed under