International Journal of Food Microbiology 293 (2019) 87–93

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Short communication

Characterization of the microbial composition and quality of lightly salted grass carp (*Ctenopharyngodon idellus*) fillets with vacuum or modified atmosphere packaging

Jingbin Zhang[a,c], Yan Li[a], Xiaochang Liu[a], Yutian Lei[a], Joe M. Regenstein[c], Yongkang Luo[a,b,*]

[a] *Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*
[b] *Beijing Laboratory for Food Quality and Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*
[c] *Department of Food Science, Cornell University, Ithaca, NY 14853-7201, USA*

ARTICLE INFO

Chemical compounds studied in this article:
Putrescine (PubChem CID: 1045)
Cadaverine (PubChem CID: 273)
Histamine (PubChem CID: 774)
Tyramine (PubChem CID: 5610)

Keywords:
Grass carp
High-throughput sequencing
Modified atmosphere packaging
Vacuum packaging
Lactococcus

ABSTRACT

The effect of air packaging (AP), vacuum packaging (VP) and modified atmosphere packaging (MAP, 75% CO_2/25% N_2) on the composition of the microbial community of lightly salted grass carp (*Ctenopharyngodon idellus*) fillets stored at 4 °C was studied. Physicochemical characteristics (total volatile basic nitrogen (TVB-N) value, pH value and biogenic amines (BA)) were also monitored. Based on sensory evaluation, the shelf life of fillets under AP, VP, and MAP was 8, 16 and 24 days, respectively. High-throughput sequencing showed that *Acinetobacter* and *Pseudomonas* dominated the microflora of fresh grass carp. At the sensory rejection time, *Pseudomonas* and *Psychrobacter* dominated the AP products. *Lactococcus* became the predominant genus of both VP and MAP fillets, and there was no obvious difference in the composition of the dominant microbiota between these two treatments at the end of the shelf life.

1. Introduction

Grass carp (*Ctenopharyngodon idellus*) is one of the most important commercial freshwater-cultured fish species worldwide: 6,000,000 t were produced in 2016 (FAO, 2018). Recently, lightly salted fish fillets have been gaining great popularity among consumers, due to their convenience (ready-to-use) and health benefits. Given the perishable nature of lightly salted fish fillets, improved preservation methods are needed to extend their shelf life. Vacuum packaging (VP) and modified atmosphere packaging (MAP) in combination with refrigeration are two common methods to extend the shelf life of fish products.

Fresh fish are highly perishable after death because biochemical reaction that is caused by microorganisms and endogenous enzymes. Temperature and packaging are two factors influencing the succession of the microbial community (Gram and Dalgaard, 2002). The metabolism of Gram-negative spoilage bacteria, particularly *Pseudomonas*, *Aeromonas* and *Shewanella*, are the major cause for the spoilage of aerobic stored fish (Wang et al., 2014; Zhang et al., 2015). VP and MAP (higher content of CO_2) can inhibit the growth of Gram-negative aerobic bacteria, resulting in an increase in Gram-positive bacteria (Noseda et al., 2012). Recently, high-throughput sequencing technology based on 16S rRNA gene amplification has been applied to analyze the microbial diversity of fish and fish products (Silbande et al., 2016; Zhang et al., 2017), and the bacterial phylotypes found by this method were more reliable than culture-dependent methods.

Thus, the present study was undertaken to fill a knowledge gap regarding the microbial population dynamics and predominant microbiota during chilled storage of grass carp fillets with different packages (AP, VP and MAP), in combination with sensory and chemical analyses.

2. Materials and methods

2.1. Sample preparation

A total of 30 grass carp (weight of 1390 ± 90 g, length of 50 ± 2 cm) were obtained from an aquatic products market in Beijing, China and transported to the laboratory alive during March 2017. The fish were stunned by a blow to the head, scaled, gutted, and filleted manually. Four skin-on fillet pieces (150 ± 25 g, 10.6 ± 0.3 cm) were obtained from each fish, rinsed with running tap water and then

---

[*] Corresponding author at: College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China.
E-mail addresses: luoyongkang@263.net, luoyongkang@cau.edu.cn (Y. Luo).

https://doi.org/10.1016/j.ijfoodmicro.2018.12.022
Received 24 April 2018; Received in revised form 13 December 2018; Accepted 22 December 2018
Available online 24 December 2018
0168-1605/

Table 1
Changes in sensory score of grass carp fillets stored at 4 °C with different packaging conditions.

Storage time (days)		Color	Odor	Texture	Overall acceptability
AP	0	8.9 ± 0.1^{a}	8.9 ± 0.1^{a}	8.9 ± 0.1^{a}	8.9 ± 0.1^{a}
	2	8.3 ± 0.1^{b}	8.4 ± 0.1^{b}	8.2 ± 0.1^{b}	8.3 ± 0.2^{b}
	4	6.9 ± 0.1^{c}	6.4 ± 0.1^{c}	6.7 ± 0.3^{c}	6.3 ± 0.1^{c}
	6	4.9 ± 0.2^{d}	4.2 ± 0.1^{d}	5.2 ± 0.1^{d}	4.3 ± 0.1^{d}
	8	3.6 ± 0.1^{e}	3.3 ± 0.1^{e}	4.1 ± 0.1^{e}	3.7 ± 0.1^{e}
VP	0	8.9 ± 0.1^{a}	8.9 ± 0.1^{a}	8.99 ± 0.1^{a}	8.9 ± 0.1^{a}
	2	8.4 ± 0.1^{b}	8.3 ± 0.1^{b}	8.4 ± 0.0^{b}	8.3 ± 0.1^{b}
	4	7.8 ± 0.1^{c}	7.5 ± 0.1^{c}	8.0 ± 0.1^{c}	7.6 ± 0.1^{c}
	6	7.3 ± 0.1^{d}	7.1 ± 0.1^{d}	7.7 ± 0.1^{d}	7.4 ± 0.1^{d}
	8	6.8 ± 0.1^{e}	6.5 ± 0.1^{e}	6.9 ± 0.1^{e}	6.7 ± 0.1^{e}
	10	6.1 ± 0.1^{f}	5.5 ± 0.2^{f}	6.5 ± 0.1^{f}	5.9 ± 0.1^{f}
	12	5.4 ± 0.2^{g}	4.5 ± 0.1^{g}	6.1 ± 0.1^{g}	5.1 ± 0.1^{g}
	14	5.1 ± 0.1^{h}	4.2 ± 0.2^{h}	5.5 ± 0.1^{h}	4.2 ± 0.1^{h}
	16	3.7 ± 0.2^{i}	3.5 ± 0.1^{i}	5.0 ± 0.1^{i}	3.7 ± 0.1^{i}
MAP	0	8.9 ± 0.1^{a}	8.87 ± 0.1^{a}	8.9 ± 0.1^{a}	8.93 ± 0.1^{a}
	4	8.3 ± 0.0^{b}	8.37 ± 0.1^{b}	8.4 ± 0.1^{b}	8.33 ± 0.1^{b}
	8	7.7 ± 0.1^{c}	6.93 ± 0.1^{c}	7.8 ± 0.3^{c}	7.43 ± 0.1^{c}
	12	6.7 ± 0.1^{d}	6.13 ± 0.1^{d}	7.0 ± 0.1^{d}	6.57 ± 0.1^{d}
	16	6.3 ± 0.1^{e}	5.13 ± 0.1^{e}	6.5 ± 0.1^{e}	5.57 ± 0.1^{e}
	20	6.1 ± 0.1^{e}	4.57 ± 0.1^{f}	6.2 ± 0.1^{f}	4.43 ± 0.1^{f}
	24	5.0 ± 0.1^{f}	3.67 ± 0.1^{g}	5.6 ± 0.2^{g}	3.54 ± 0.1^{g}

*Same lowercase letters in a column indicate no significant differences ($P > 0.05$).

AP- air packaging; VP-vacuum packaging; MAP- modified atmosphere packaging MAP: 75% CO_2/25% N_2.

drained for 3 min on stainless steel wire mesh.

Preliminary experiments with different salt concentrations, times and ratios of marinating were done to determine the most acceptable taste for consumers. The most desirable taste was obtained by immersing fillets in a brine that contained 100 g/L NaCl at 6 ± 1 °C for 100 min with a fish:brine ratio of 1:1 (w/v). After that, the fillets were divided into three groups of 40 fillets each for AP, VP, and MAP treatments (75% CO_2/25% N_2).

For AP, each fillet was packaged in one polyvinyl chloride bag (about 250 × 200 mm; Cleanwrap Co., Ltd., Gimhae City, Korea) with air. For VP, each fillet was packaged with vacuum (Model No. RS-400, Beijing Rishang Science & Trade Co., Ltd., Beijing, China) in one pouch of polyethylene/polyamide film (about 250 × 200 mm, with an oxygen permeability of 40–50 cm^3/m^2 per 24 h/atm at 85% relative humidity, 23 °C; Taizhou Mingke Plastic Co., Ltd., Taizhou, Zhejiang, China). For MAP, each fillet was packaged in one polystyrene tray (224 × 133 × 60 mm, with an oxygen permeability < 10 cm^3/m^2 per 24 h/atm at 0% relative humidity, 23 °C; water vapor permeability < 15 g/m^2 per 24 h/atm at 90% relative humidity, 38 °C; Model No. TQBC-1175, Sealed Air Corp., Charlotte, NC, USA). The loaded trays were filled with a gas mixture (75% CO_2/25% N_2, using a PBI-Dansensor model Amix 9000 gas mixer (Dansensor, Ringsted, Denmark)) and then thermos-sealed with a film (with an oxygen permeability < 20 cm^3/m^2 per 24 h/atm at 100% relative humidity, 4 °C; water vapor permeability < 15 g/m^2 per 24 h/atm at 100% relative humidity, 4 °C; Lid 1050, Sealed Air Corp.) using a MAP machine (Model No. HX-300H, Hengxian Tech Co., Ltd., Beijing, China).

After packaging, the samples were stored under refrigeration (4 ± 0.5 °C) for subsequent quality and bacterial analyses. Three fillets of each group were randomly selected and then samples of white dorsal muscle were taken for analyzing quality and microbiological enumeration at 2-day intervals for AP and VP, and 4-day intervals for MAP. Microbial communities were determined on days 0, 4, and 8 for AP samples, on days 0, 8, and 16 for VP samples, and on days 0, 12 and 24 for MAP samples.

2.2. Sensory assessment

Nine staffs (5 females and 4 males, 20–29 years old) belonging to the laboratory were trained according to Codex Alimentarius (1999) procedure. The color, odor, texture, and overall acceptability of raw fish muscle were evaluated by all panelists on each day of sampling, using a 9-point scale, where a score of 7.0–9.0 indicated "good quality", a score of 4.0–6.9 indicated "still suitable", a score of 1.0–3.9 indicated "inappropriate".

2.3. Determination of TVB-N and pH

Five grams of minced sample was stirred in 50 mL deionized water for 30 min and then the mixture was filtered. The filtrate was used for the determination of the TVB-N value, according to the semi-micro steam distillation method of Song et al. (2011). The pH of the filtrate was measured using a digital pH meter (Mettler Toledo FE20/EL20, Shanghai, China).

2.4. Determination of biogenic amines (BA)

The extraction, derivatization and quantification of the BA of grass carp fillets were carried out with HPLC according to the procedures of Hong et al. (2013) with minor modification. Briefly, 5 g of minced sample was homogenized twice with 10 mL cold perchloric acid (0.6 M) for 1 min and centrifuged at 10,000 ×g at 4 °C for 15 min. The supernatants were combined and adjusted to 25 mL with perchloric acid (0.6 M) and then stored at −20 °C for further analysis. The concentration of putrescine (PUT), cadaverine (CAD), histamine (HIM), and tyramine (TYM) were quantified using Sigma external standards (Sigma-Aldrich, Shanghai, China).

2.5. Enumeration of microorganism

Total viable counts (TVC) were determined on plate count agar (PCA) using the method of Li et al. (2016). Briefly, samples (0.1 mL) of serial dilutions (1:10, sterile 0.9% saline) of homogenates were spread on the surface of PCA. Plates were incubated at 30 °C for 72 h. All counts were done in duplicate and expressed as $\log_{10}$ cfu/g.

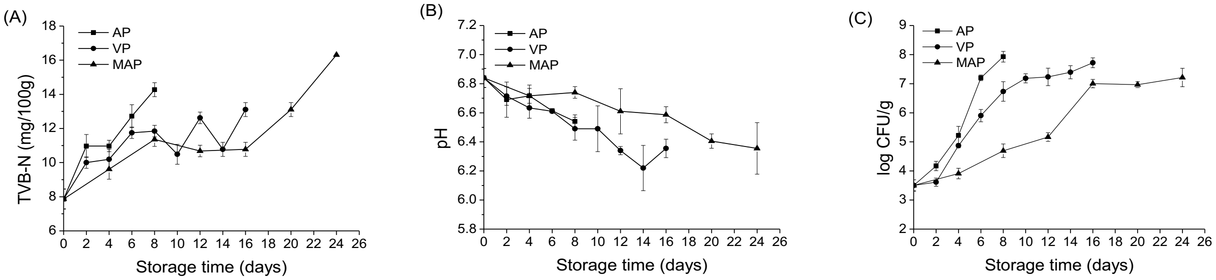

Fig. 1. Changes in (A) Total volatile basic nitrogen (TVB-N); (B) pH and (C) Total viable counts (TVC) for grass carp fillets stored at 4 °C with different packaging conditions. (AP- air packaging; VP-vacuum packaging; MAP- modified atmosphere packaging MAP: 75% CO_2/25% N_2).

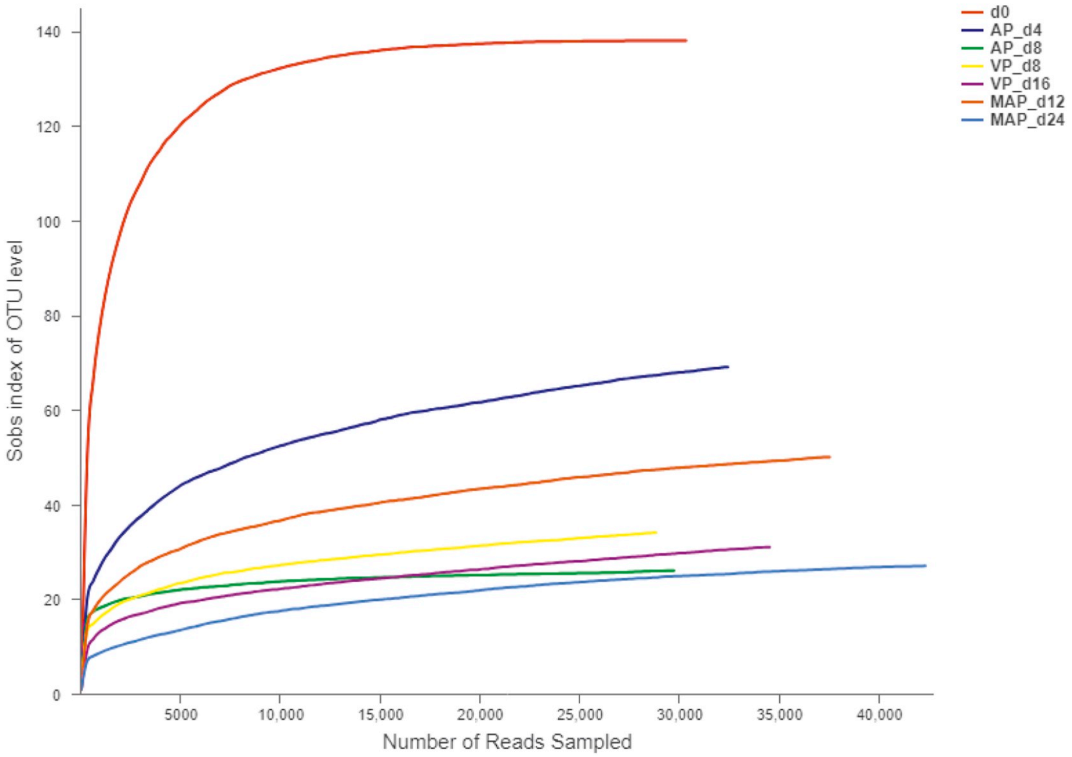

Fig. 2. Rarefaction curve of microbiota in grass carp fillets stored at 4 °C with different packaging conditions.

2.6. *High-throughput sequencing*

2.6.1. *DNA extraction*

Ten grams of fish white muscle was stirred with 20 mL sterile physiological saline (0.9% NaCl) for 15 min using the thermostatic oscillator, and then centrifuged at 200 × *g* for 5 min at 4 °C. The supernatant was centrifuged at 10000 × *g* for 10 min at 4 °C to obtain the sediments. DNA was extracted from the pellet using a bacterial DNA extraction assay kit (Biomed Biological Technology Co., Ltd., Beijing, China). DNA samples from three samples at each sampling point of certain group were mixed together and stored at −20 °C for a maximum of four weeks before high-throughput sequencing.

2.6.2. *Illumina MiSeq PE300 high-throughput sequencing*

The V3-V4 regions of the 16S rRNA gene were amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Jia et al., 2018) with different barcodes. After 2% agarose gel electrophoresis, PCR products were extracted from the gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and were quantified using a QuantiFluor™ -ST (Promega, Madison, WI, USA). The purified amplicons were multiplexed into a single pool using equal molecular weights, and they were paired-end (PE) sequenced (2 × 300 bp) by the Majorbio Co. (Shanghai, China) on an Illumina MiSeq PE300 platform. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP161637).

2.6.3. *Sequences data processing*

Raw fastq files were demultiplexed and quality-filtered using Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1, http://qiime.org/index.html). Operational taxonomic units (OTU) were clustered with 97% similarity cutoff using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (https://www.drive5.com/usearch/manual/uchime_algo.html). The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115) 16S rRNA database using a confidence threshold of 70%. Subsequently, ACE, Chao1, Shannon index, and Good's coverage were used to evaluate alpha diversity (bacterial community richness, sequencing depth, and diversity), using the Mothur program (v.1.30.1, https://www.mothur.org/). A heat-map was used to evaluate beta diversity, and to determine the influence of the time of storage and the three different types of packaging on the microbial communities, using R software (v2.15.3, https://www.r-project.org/).

2.7. *Statistical analysis*

All values were expressed as mean ± standard deviation of experiments done in triplicate. Differences between two groups were done using a *t*-test. Differences among storage time were done by analysis of variance (ANOVA) and Duncan's multiple range test using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software. The significance level was $P < 0.05$.

3. Results and discussion

3.1. *Sensory analysis*

Scores for color, odor and texture decreased significantly ($P < 0.05$) with storage time with all atmospheres (Table 1). The observed shelf life of grass carp was ~24 days for MAP, ~16 days for VP and ~8 days for AP. Thus, compared to AP, MAP and VP significantly ($P < 0.05$) improved the overall sensory scores, and extended the shelf life by 16 days and 8 days, respectively. The shelf life of lightly salted grass carp with MAP is longer than that of common carp and crispy grass carp stored under MAP (Hudecova et al., 2010; Babic et al., 2015; Pan et al., 2018), this may due to the addition of salt.

3.2. *Changes in TVC*

The initial TVC value was 3.50 log CFU/g, reflecting the high quality of the fish and processing hygiene (Fig. 1C). The TVC for AP,

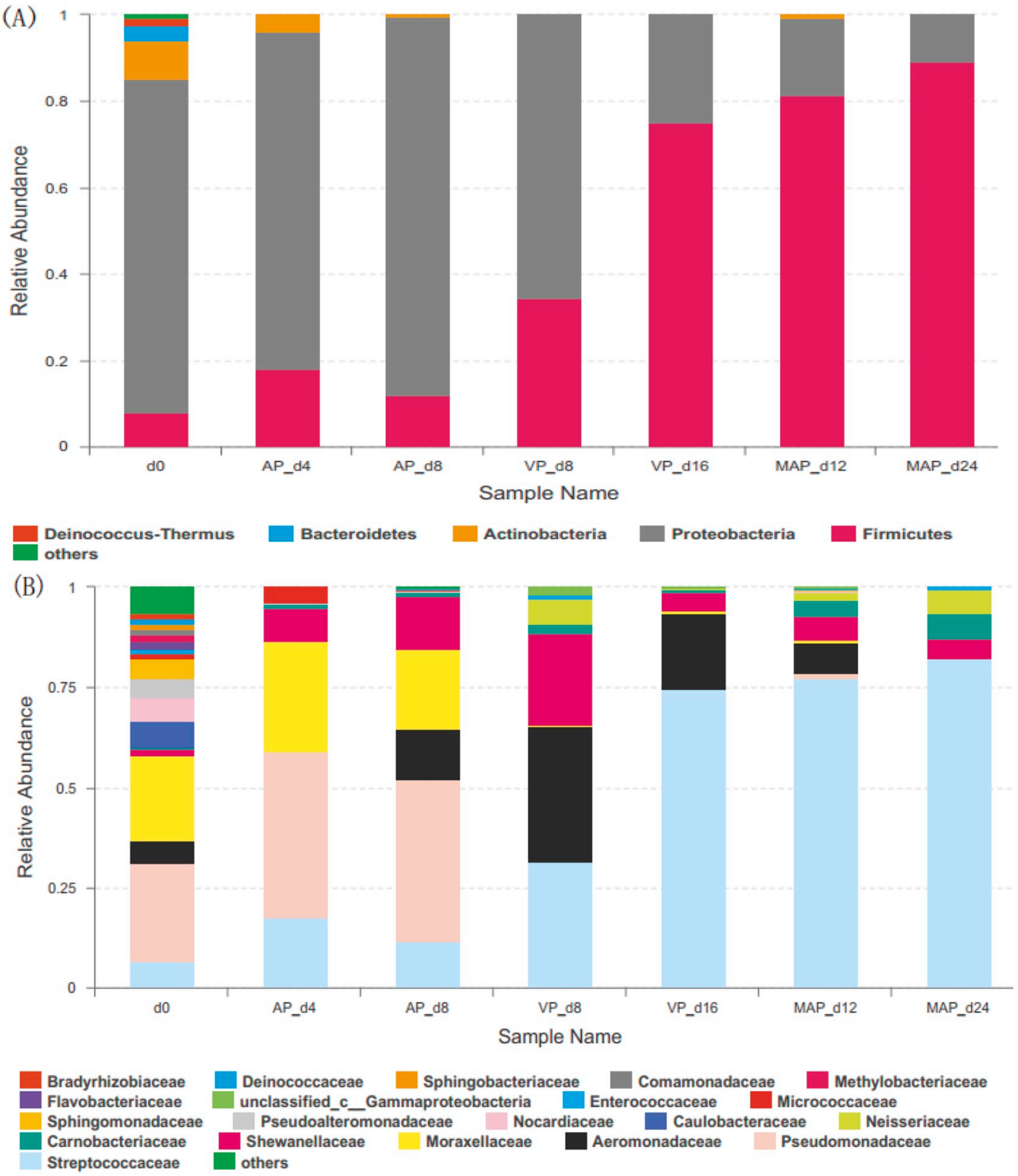

Fig. 3. Relative abundance of microbiota based on (A) the phylum level and (B) the family level in grass carp fillets stored at 4 °C with different packaging conditions.

VP, and MAP samples reached 7 log CFU/g on day 6, day 10 and day 16, respectively, which is the upper tolerable limit for freshwater species (ICMSF, 1986). The much lower TVC in MAP could be explained by the absence of O_2 and the presence of CO_2 (Stamatis and Arkoudelos, 2007).

3.3. Illumina sequencing analysis

Illumina sequencing of bacterial 16S rRNA genes generated a total of 275,325 effective sequences, with an average length of 449 bp. The sample of fresh grass carp fillets had the greatest number of OTU and the highest Shannon index (Table 3), which suggested that the bacterial species in fresh grass carp fillets were more diverse than those in stored grass carp fillets. Sampling completeness assessed by Good's coverage estimator gave values above 99.9% for all the samples, which suggested that the sequencing reads obtained from each sample were sufficient to analyze the bacterial diversity. The rarefaction curves of alpha diversity, based on the OTUs (Fig. 2), showed that all the samples tended to approach the saturation plateau at 4000 sequences. This indicated that the data size of the sequences tended to be more rational and can cover almost all the microbial communities.

3.4. Impact of packaging and storage time on microbial composition

The bacterial 16S rRNA gene sequences were classified at both the phylum and family levels to investigate the succession of microbial communities. Microorganisms present in grass carp fillets belonged to four different phyla (Fig. 3A). Proteobacteria and Firmicutes were predominant in all the samples, which was consistent with the phyla found in common carp (Zhang et al., 2017).

The family level analysis showed that the composition of bacterial communities changed dramatically during storage (Fig. 3B). At the beginning of the storage, the dominant family were Pseudomonadaceae (25%) and Moraxellacea (21%), which belong to the phylum Proteobacteria. At day 4, Pseudomonadaceae (41%), Moraxellaceae (27%) and Streptococcaceae (18%) constituted the dominant microbiota of AP grass carp fillets. Pseudomonadaceae are also the most important in this group at the end of storage (day 8). For grass carp fillets stored in VP,

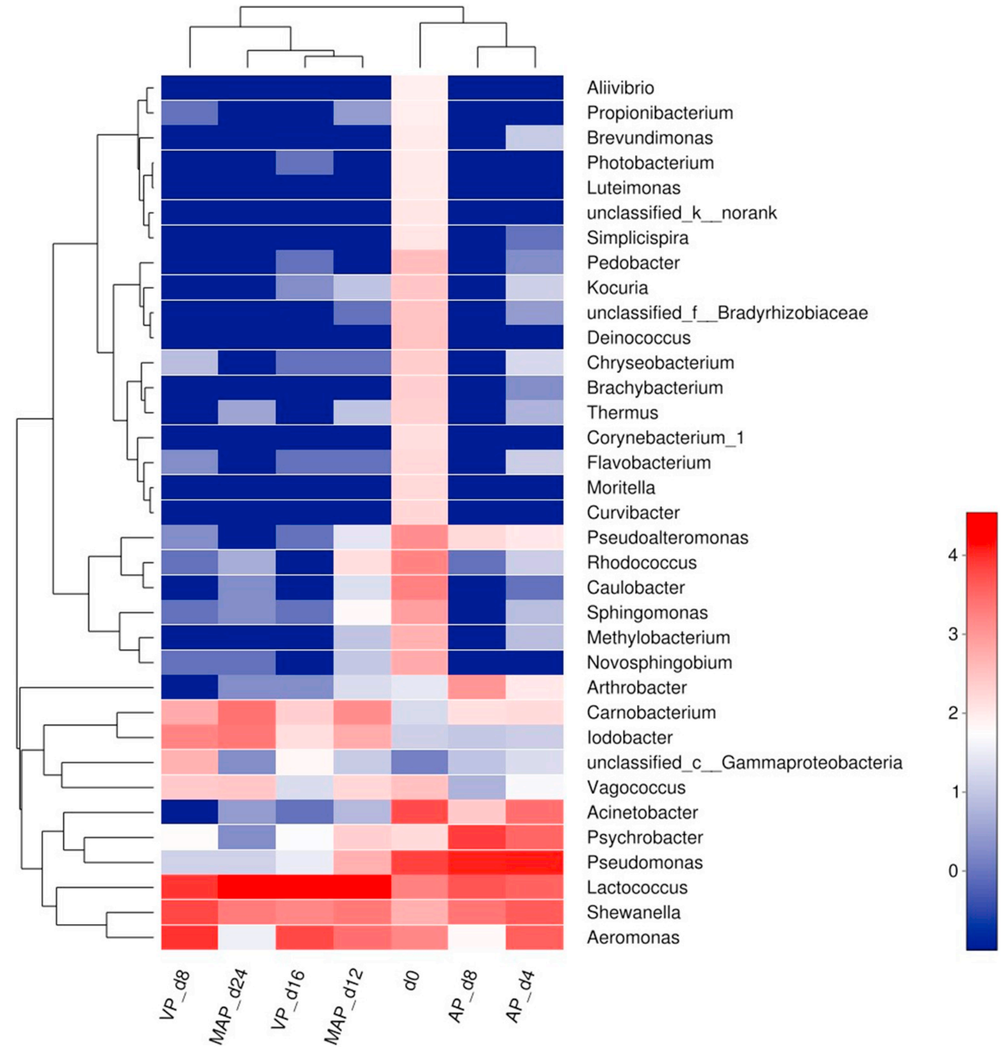

Fig. 4. Relative abundance of microbiota at the genus level. Rows in the heat-map represent different genus-level phylotypes, while columns represent different grass carp samples. The color intensity is proportional to the abundance of OTU in each row.

Streptococcaceae and Aeromonadaceae became the dominant families during storage. The grass carp stored in MAP was dominated by Streptococcaceae throughout the storage period, while Aeromonadaceae and Shewanellaceae represented in low percentages.

Based on the relative abundance of the top 35 abundant phylotypes from all the samples, a heat-map (Fig. 4) at the genus level was generated. The redder and the bluer color illustrated the higher and the lower relative abundances, respectively. All the samples were segregated into two clusters: one cluster was composed of the sample of fresh grass carp fillets and two AP samples, and the other cluster was formed by VP and MAP samples. At day 0, the predominant genera were *Pseudomonas* (25%) and *Acinetobacter* (20%). The psychrotrophic Gram-negative bacteria, *Acinetobacter*, *Shewanella* and *Pseudomonas*, predominated in the initial microbiota of temperate water fish (Gram and Huss, 1996; Parlapani et al., 2013). *Pseudomonas* had a higher relative abundance in AP fillets compared to other samples and it has been reported to be a spoilage inducer in various fish species from fresh and marine waters (Gram and Huss, 1996; Zhang et al., 2015) under aerobic chilled storage. Consistent with previous research (Powell and Tamplin, 2012; Zhang et al., 2017), almost no *Pseudomonas* was detected in VP and MAP samples since *Pseudomonas* is sensitive to both CO_2 and the removal of oxygen (Silbande et al., 2016). *Lactococcus* was detected in all samples, but had a higher relative abundance in VP and MAP samples. *Lactococcus* is sometimes associated with food spoilage, particularly in chilled VP or MAP meat and seafood (Saraoui et al., 2016). It may not produce an off-odor as Gram-negative bacteria usually do. However, it could generate weak buttery odors (Sade et al., 2017), which may lead to the deterioration of fish and fish products. The genus *Aeromonas* was observed in almost all samples, and it has the ability to grow well in an anaerobic environment (Gui et al., 2014). Zhang et al. (2015) identified *Aeromonas* as the main spoilage organism in VP common carp fillets and Liu et al. (2017) also found that *Aeromonas* could contribute to the spoilage of lightly salted bighead carp stored aerobically.

3.5. *Chemical analyses*

The initial value of TVB-N was 7.86 mg/100 g (Fig. 1A). During the first 8 days, TVB-N concentrations of AP fillets were significantly ($P < 0.05$) higher than the other two groups. After 8 days, the TVB-N values of VP and MAP samples fluctuated reaching a maximum at day 16 and day 24, respectively. The initial pH value of grass carp on day 0 was 6.84 (Fig. 1B). The pH of all the samples decreased throughout the storage period. However, pH was higher for AP fillets than for VP and MAP samples.

The concentration of HIM did not change much during the storage period with the three different conditions (Table 2), which may due to the low histidine content in white muscle fish (Prester, 2011). TYM was

Table 2
Changes in biogenic amines content of grass carp fillets stored at 4 °C with different packaging conditions.

Storage time(days)		Biogenic amines(mg/kg)			
		PUT	CAD	HIM	TYM
AP	0	5.2 ± 0.0^{a}	0.5 ± 0.0^{a}	4.2 ± 0.2^{a}	6.7 ± 0.6^{a}
	2	5.2 ± 0.1^{a}	0.9 ± 0.1^{b}	4.1 ± 0.1^{ab}	6.4 ± 0.0^{a}
	4	5.1 ± 0.0^{a}	1.4 ± 0.1^{c}	3.9 ± 0.2^{bc}	6.6 ± 0.6^{a}
	6	5.2 ± 0.0^{a}	1.7 ± 0.2^{d}	3.7 ± 0.2^{c}	4.6 ± 0.3^{b}
	8	6.5 ± 0.3^{b}	2.8 ± 0.2^{e}	3.7 ± 0.1^{c}	4.5 ± 0.1^{b}
VP	0	5.2 ± 0.0^{ab}	0.5 ± 0.0^{a}	4.2 ± 0.2^{a}	6.7 ± 0.6^{a}
	2	5.1 ± 0.0^{b}	0.9 ± 0.2^{b}	4.0 ± 0.1^{ab}	7.7 ± 0.6^{a}
	4	5.1 ± 0.0^{ab}	0.9 ± 0.1^{b}	3.9 ± 0.1^{bc}	9.1 ± 0.7^{a}
	6	5.3 ± 0.1^{ab}	1.0 ± 0.0^{b}	4.0 ± 0.1^{bc}	8.1 ± 0.5^{a}
	8	5.4 ± 0.1^{a}	0.6 ± 0.1^{a}	3.6 ± 0.1^{de}	66.8 ± 2.7^{b}
	10	6.2 ± 0.2^{c}	0.7 ± 0.1^{a}	3.8 ± 0.1^{cd}	52.5 ± 2.0^{c}
	12	5.6 ± 0.2^{d}	0.6 ± 0.1^{a}	3.8 ± 0.0^{cd}	60.5 ± 2.4^{d}
	14	6.3 ± 0.0^{c}	0.2 ± 0.0^{c}	3.5 ± 0.2^{e}	54.9 ± 1.5^{c}
	16	5.9 ± 0.3^{e}	0.3 ± 0.0^{c}	3.6 ± 0.1^{de}	59.4 ± 1.4^{d}
MAP	0	5.2 ± 0.0^{a}	0.5 ± 0.0^{a}	4.2 ± 0.2^{a}	6.7 ± 0.6^{a}
	4	5.2 ± 0.1^{a}	1.0 ± 0.1^{b}	3.8 ± 0.3^{a}	7.5 ± 0.5^{a}
	8	5.3 ± 0.2^{a}	0.9 ± 0.2^{b}	3.8 ± 0.1^{a}	71.8 ± 1.5^{c}
	12	5.8 ± 0.1^{b}	0.2 ± 0.0^{c}	3.5 ± 0.1^{a}	68.1 ± 1.9^{c}
	16	5.5 ± 0.3^{a}	0.3 ± 0.0^{c}	3.8 ± 0.3^{a}	49.2 ± 2.7^{d}
	20	6.2 ± 0.2^{c}	0.3 ± 0.1^{c}	3.8 ± 0.4^{a}	62.8 ± 2.5^{e}
	24	6.0 ± 0.2^{bc}	0.2 ± 0.0^{c}	3.7 ± 0.2^{a}	61.4 ± 3.9^{e}

*Same lowercase letters in a column indicate no significant differences ($P > 0.05$).

Table 3
Comparison of phylotype coverage and alpha diversity estimation of the 16S rRNA gene libraries by sequencing on an Illumina MiSeq PE300 platform for grass carp fillets stored at 4 °C with different packaging conditions.

Storage time(days)	Total tags	OTUs	Average length	Shannon	Chao1	ACE	Goods coverage
d0	43,376	138	444	3.36	138	138	1
AP_d4	35,918	69	449	2.26	84	85.5	0.999
AP_d8	41,196	26	449	2.10	29	28.1	0.999
VP_d8	34,207	34	449	1.78	52	54.3	0.999
VP_d16	37,013	31	449	0.86	42.3	69.0	0.999
MAP_d12	38,855	50	449	1.04	54	59.2	0.999
MAP_d24	44,760	27	449	0.73	28.3	30.1	0.999

the most prevalent amine in grass carp. It increased significantly ($P < 0.05$) over time with VP and MAP while its concentration in the AP samples decreased. Levels of PUT and CAD in AP samples increased progressively during the storage period, and stayed much higher than those in VP and MAP samples.

Pseudomonas was the dominant bacteria in AP samples. It has been reported to be a strong ammonium producer (Gram and Huss, 1996) and contributed to the formation of PUT and CAD (Rezaei et al., 2007). The higher concentration of TVB-N, PUT and CAD in AP samples was probably due to the high percentage of *Pseudomonas* in AP fillets.

Lactic acid bacteria (LAB) was the largest bacterial group in VP and MAP samples. LAB can produce organic acids (Leroi, 2010) instead of volatile ammonium compounds. The lower TVB-N and pH in VP and MAP samples was probably due to the metabolism of LAB and suppression of *Pseudomonas*. LAB may have been responsible for TYM production (Leisner et al., 1994) and accumulation in VP and MAP samples.

4. Conclusion

This study was done to show the differences in microbiota composition and physicochemical changes of grass carp fillets stored using different atmospheric conditions during refrigerated storage. Compared to AP, MAP and VP extended the shelf life of grass carp fillets by 16 days and 8 days, respectively. For AP fillets, *Pseudomonas* had the highest numbers at the end of storage. VP and MAP resulted in the overwhelming abundance of *Lactococcus*. Further work needs to be done to evaluate the active mechanisms of VP and MAP on bacteria in grass carp fillets.

Acknowledgement

This study was supported by National Key R&D Program of China (2018YFD0901001) and China Agriculture Research System (CARS-45). We would like to thank Thomas A. Gavin, Professor Emeritus, Cornell University, for help with editing the English of an early version of this paper. Thanks to the China Scholarship Council (CSC) for the visiting student program.

References

Babic, J., Milijasevic, M., Vranic, D., Veskovic-Moracanin, S., Djinovic-Stojanovic, J., 2015. Effect of modified atmosphere pakaging on the shelf-life of common carp (*Cyprinus carpio*) steaks. Procedia Food. Sci. 5, 2–5.
Codex Alimentarius, 1999. Codex Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories CAC-GL31–1999, Training of Assessors. Codex Alimentarius Commission, Rome, pp. 9–16.
FAO, 2018. FAO-Yearbook of Fishery Statistics: Aquaculture Production. Food and Agriculture Organization of the United Nations, Rome, pp. 30.
Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria - problems and solutions. Curr. Opin. Biotechnol. 13 (3), 262–266.
Gram, L., Huss, H.H., 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33 (1), 121–137.
Gui, M., Binzhao, Song, Y, J., Zhang, Z.C., Hui, P.Z., Li, P.L., 2014. Biogenic amines formation, nucleotide degradation and TVB-N accumulation of vacuum-packed minced sturgeon (*Acipenser schrencki*) stored at 4 °C and their relation to microbiological attributes. J. Sci. Food Agric. 94 (10), 2057–2063.
Hong, H., Luo, Y.K., Zhou, Z.Y., Bao, Y.L., Lu, H., Shen, H.X., 2013. Effects of different freezing treatments on the biogenic amine and quality changes of bighead carp (*Aristichthys nobilis*) heads during ice storage. Food Chem. 138 (2–3), 1476–1482.
Hudecova, K., Buchtova, H., Steinhauserova, I., 2010. The effects of Modifed atmosphere packaging on the microbiological properties of fresh common carp (*Cyprinus carpio* L.). Acta Vet. Bron. 79, 93–100.
ICMSF, 1986. Sampling plans for fish and shellfish. In: Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications. University of Toronto Press, Toronto, pp. 181–196.
Jia, S.L., Liu, X.C., Huang, Z., Li, Y., Zhang, L.T., Luo, Y.K., 2018. Effects of chitosan oligosaccharides on microbiota composition of silver carp (*Hypophthalmichthys molitrix*) determined by culture-dependent and independent methods during chilled storage. Int. J. Food Microbiol. 268, 81–91.
Leisner, J., Millan, J., Huss, H., Larsen, L., 1994. Production of histamine and tyramine by lactic acid bacteria isolated from vacuum-packed sugar-salted fish. J. Appl. Bacteriol. 76 (5), 417–423.
Leroi, F., 2010. Occurrence and role of lactic acid bacteria in seafood products. Food Microbiol. 27 (6), 698–709.
Li, Q., Li, D.P., Qin, N., Hong, H., Luo, Y.K., 2016. Comparative studies of quality changes in white and dark muscles from common carp (*Cyprinus carpio*) during refrigerated (4 °C) storage. Int. J. Food Sci. Technol. 51 (5), 1130–1139.
Liu, X.C., Zhang, Y.M., Li, D.P., Luo, Y.K., 2017. Characterization of the microbiota in lightly salted bighead carp (*Aristichthys nobilis*) fillets stored at 4 °C. Food Microbiol. 62, 106–111.
Noseda, B., Islam, M.T., Eriksson, M., Heyndrickx, M., De Reu, K., Van Langenhove, H., Devlieghere, F., 2012. Microbiological spoilage of vacuum and modified atmosphere packaged Vietnamese *Pangasius hypophthalmus* fillets. Food Microbiol. 30 (2), 408–419.
Pan, Z.Q., Li, L., Shen, Z.H., Chen, Y., Li, M., 2018. Characterization of the microbiota in air- or vacuum-packed crisp grass carp (*Ctenopharyngodon idella* C. et V.) fillets by 16S rRNA PCR–denaturing gradient gel electrophoresis and high-throughput sequencing. J. Food Prot. 81 (6), 1022–1029.
Parlapani, F.F., Meziti, A., Kormas, K.A., Boziaris, I.S., 2013. Indigenous and spoilage microbiota of farmed sea bream stored in ice identified by phenotypic and 16S rRNA gene analysis. Food Microbiol. 33 (1), 85–89.
Powell, S.M., Tamplin, M.L., 2012. Microbial communities on Australian modified atmosphere packaged Atlantic salmon. Food Microbiol. 30 (1), 226–232.
Prester, L., 2011. Biogenic amines in fish, fish products and shellfish: a review. Food Addit. Contam. 28 (11), 1547–1560.
Rezaei, M., Montazeri, N., Langrudi, H.E., Mokhayer, B., Parviz, M., Nazarinia, A., 2007. The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chem. 103 (1), 150–154.
Sade, E., Penttinen, K., Bjorkroth, J., Hultman, J., 2017. Exploring lot-to-lot variation in spoilage bacterial communities on commercial modified atmosphere packaged beef. Food Microbiol. 62, 147–152.
Saraoui, T., Leroi, F., Bjorkroth, J., Pilet, M.F., 2016. *Lactococcus piscium*: a psychrotrophic lactic acid bacterium with bioprotective or spoilage activity in food-a review.

J. Appl. Microbiol. 121 (4), 907–918.

Silbande, A., Adenet, S., Smith-Ravin, J., Joffraud, J.J., Rochefort, K., Leroi, F., 2016. Quality assessment of ice-stored tropical yellowfin tuna (*Thunnus albacares*) and influence of vacuum and modified atmosphere packaging. Food Microbiol. 60, 62–72.

Song, Y.L., Liu, L., Shen, H.X., You, J.A., Luo, Y.K., 2011. Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*). Food Control 22 (3–4), 608–615.

Stamatis, N., Arkoudelos, J.S., 2007. Effect of modified atmosphere and vacuum packaging on microbial, chemical and sensory quality indicators of fresh, filleted *Sardina pilchardus* at 3 °C. J. Sci. Food Agric. 87 (6), 1164–1171.

Wang, H., Luo, Y.K., Huang, H.P., Xu, Q., 2014. Microbial succession of grass carp (*Ctenopharyngodon idellus*) filets during storage at 4 °C and its contribution to biogenic amines' formation. Int. J. Food Microbiol. 190, 66–71.

Zhang, Y.M., Li, Q., Li, D.P., Liu, X.C., Luo, Y.K., 2015. Changes in the microbial communities of air-packaged and vacuum-packaged common carp (*Cyprinus carpio*) stored at 4 °C. Food Microbiol. 52, 197–204.

Zhang, Y., Li, D., Lv, J., Li, Q., Kong, C., Luo, Y., 2017. Effect of cinnamon essential oil on bacterial diversity and shelf-life in vacuum-packaged common carp (*Cyprinus carpio*) during refrigerated storage. Int. J. Food Microbiol. 249, 1–8.