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Effects of chitosan oligosaccharides on microbiota composition of silver carp (*Hypophthalmichthys molitrix*) determined by culture-dependent and independent methods during chilled storage

Shiliang Jia[a], Xiaochang Liu[a], Zhan Huang[a], Yan Li[a], Longteng Zhang[a], 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 Higher Institution Engineering Research Center of Animal Product, China Agricultural University, Beijing 100083, China

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Putrescine (PubChem CID: 1045)
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ABSTRACT

This study evaluated the effects of chitosan oligosaccharides (COS) on the changes in quality and microbiota of silver carp fillets stored at 4 °C. During storage, 1% (w/v) COS treated samples maintained good quality, as evidenced by retarding sensory deterioration, inhibiting microbial growth, attenuating the production of total volatile basic nitrogen, putrescine, cadaverine and hypoxanthine, and delaying degradation of inosine monophosphate and hypoxanthine ribonucleotide. Meanwhile, variability in the predominant microbiota in different samples was investigated by culture-dependent and -independent methods. Based on sensory analysis, shelf-life of silver carp fillets was 4 days for the control and 6 days for COS treated samples. Meanwhile, *Pseudomonas*, followed by *Aeromonas*, *Acinetobacter*, and *Shewanella* were dominated in the control samples at day 4 and contributed to fish spoilage at day 6. However, COS inhibited the growth of *Pseudomonas*, *Aeromonas*, and *Shewanella* significantly. Consequently, *Acinetobacter* followed by *Pseudomonas* became the predominant microbiota in COS treated samples at day 6. With the growth of *Pseudomonas*, COS treated samples were spoiled at day 8. Therefore, COS improved the quality of fillets and prolonged the shelf life of silver carp fillets by 2 days during chilled storage, which was mainly due to their modulating effects on microbiota.

1. Introduction

Silver carp (*Hypophthalmichthys molitrix*) is one of the primary harvestable, freshwater fish species, and 4,506,564 tons was harvested in China in 2016 (Ministry of Agriculture and Fisheries Bureau, 2017). Meanwhile, silver carp is an important species for commercial production due to its high nutritional value, rapid growth rate, high yield, low feed demand, and resistance to diseases (Kasankala et al., 2012). However, aquatic products are highly perishable because of microbial growth, which is due to high moisture content, abundant nutrients (Fan et al., 2014) and higher pH as compared to meat products. Previous studies demonstrated that composition of fish microbiota could affect the generation of TVB-N, the degradation of ATP-related compounds, and the production of biogenic amines during storage (Liu et al., 2017; D. Li et al., 2017; Wang et al., 2014). Therefore, characterizing the composition of microbiota and evaluating the relationship between changes in quality and composition of microbiota during fish storage are important for quality control.

Chitosan oligosaccharides (COS), which are low molecular weight, biodegradative products of chitosan, are a mixture of oligomers that are comprised of β-1,4-linked D-glucosamine residues (Jia et al., 2016). Lodhi et al. (2014) reported that chitosan, which have degrees of polymerization < 20 and an average molecular weight < 3900 Da, are called COS. Recently, there is an increasing interest in COS, because they are more suitable for some industrial applications due to their low molecular weights, low viscosity, solubility in water, and short chain lengths (Hamed et al., 2016; Harish Prashanth and Tharanathan, 2007). In addition, COS are also reported to exhibit biological activity that includes antioxidant, antitumor, neuroprotection, cholesterol reduction, and immuno-enhancing effects (Jia et al., 2016). Barikani et al. (2014) reported that chitosan and its derivatives have a wide range of biological activity that could be used in the food industry to protect

* Corresponding author at: Beijing Advanced Innovation Center for Food Nutrition and Human Health, 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).

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food from microbial deterioration and to improve food safety, quality, and shelf-life. Hamed et al. (2016) also reported that the addition of chitosan to food inhibited growth of microorganisms and reduced poor appearance, off-flavors, and economic losses. Recently, several explanations have been proposed for antimicrobial activity of COS: 1) positive charges in COS interact with the negative charges of bacterial cell walls that lead to leakage of the intracellular molecules of microorganisms; 2) preventing nutrients from entering cells; 3) entering cells to bind to DNA and, thus, inhibit RNA and protein synthesis (Harish Prashanth and Tharanathan, 2007). Chitosan-based preservatives are preferred over chemical preservatives because chitinous materials are safer, and their antimicrobial activity can protect food products against microbial invasion (Khoushab and Yamabhai, 2010). Chitosan and chitosan-based coating or film have been developed for this purpose (Fernández-Saiz et al., 2013; Ramezani et al., 2015).

Although COS have been the subject of increased attention because they are nontoxic, highly soluble, and have positive physiological effects, there is limited information about the role of COS in the preservation of aquatic products, especially the effect of COS on bacterial communities in food. Meanwhile, getting a clear and comprehensive view of the composition of microbiota is important to understand the process of fish spoilage and to screen effective preservatives. Therefore, in this study, we assessed the effects of COS on changes in quality and microbiota of silver carp fillets stored at 4 °C. To do this, we evaluated sensory scores, ATP-related compounds (IMP, HxR, Hx), TVB-N, biogenic amines, and presence of spoilage microbiota. A combination of culture-dependent and culture-independent sequencing methods was applied to characterize the composition of microbiota and to determine the dominant microbiota in silver carp samples during chilled storage.

2. Materials and methods

2.1. Sample preparation

Farmed silver carp (weight of 1245 ± 88 g, length of 49.3 ± 1.4 cm) were purchased from a local aquatic products market in Beijing, China in January 2017, and transported to the laboratory alive. Within 2 h of arrival, the fish were stunned, scaled, gutted, filleted, and washed with sterile water. Afterwards, these fillets were left to drain on sterile, stainless steel wire mesh for 3 min and then they were divided randomly into two groups: control group (no treatment) and COS group (fillets immersed in 1% food grade COS solution (w/v) for 30 min). All samples were packed individually in polyvinyl chloride bags and stored in a refrigerated room (4 °C). The polyvinyl chloride bags were bought from Cleanwrap Co., Ltd. (Korea) and the oxygen permeability of the pouch was 13,960 $cm^3/(m^2 \times d \times atm)$. Meanwhile, the fillets were packed individually in polyvinyl chloride bags with some air, which ensured they were under aerobic environment. COS (with an average molecular weight ≤ 1000 Da, degree of deacetylation of ≥ 90%, and degree of polymerization of 2–6) that were used in this experiment were food grade and obtained from Dalian GlycoBio Company, Ltd. (Dalian, Liaoning province, China). Three samples of each group were selected randomly and analyzed for microbiological, physiochemical, and sensorial quality at 2-day intervals for 8 days.

2.2. Sensory assessment

The sensory attributes of the samples were evaluated by a sensory panel of nine trained and experienced assessors (six females and three males). The panelists were asked to evaluate the quality parameters that included color, odor, texture, and overall acceptability during storage. The panelists scored the samples using a 9-point descriptive scale (Amerine et al., 1965). A score of 7.0–9.0 indicated good quality, 5.0–6.9 indicated acceptable quality, 3.0–4.9 indicated unacceptable quality, and 1.0–2.9 denoted as completely spoiled, which meant the fillet had dull color, strong stench, and flabby texture.

2.3. Determination of TVB-N

Five grams of minced sample was stirred in 50 mL of deionized water for 30 min, and then the mixture was filtered. The filtrate was used for the determination of TVB-N, according to the semi-micro, steam distillation method (Hong et al., 2012).

2.4. Determination of biogenic amines

Extraction and derivatization of biogenic amines in each fillet were performed according to the method of Liu et al. (2017). Five grams of minced sample was homogenized twice with 10 mL of perchloric acid (0.6 M) for 1 min and centrifuged at 10,000*g* at 4 °C for 5 min. The supernatants were combined and made up to 25 mL with 0.6 M perchloric acid and stored at −20 °C for further analysis. Afterwards, the quantification of biogenic amines was carried out using HPLC.

2.5. Determination of ATP-related compounds and K value

ATP-related compounds (ATP, ADP, AMP, IMP, HxR, and Hx) were extracted according to the method described by L. Zhang et al. (2017). The extracted ATP-related compounds were analyzed by high-performance liquid chromatography (HPLC), which was equipped with an SPD-10A (V) detector and a COSMOSIL $5C_{18}$-PAQ column (4.6ID × 250 mm). K-value was calculated by the following equation: K-value (%) = [(HxR + Hx) / (ATP + ADP + AMP + IMP + HxR + Hx)] × 100%.

2.6. Determination of total viable counts (TVC)

Microbial tests were carried out at 0, 2, 4, 6, and 8 days of storage. At each sampling date, three samples of each group were selected randomly and evaluated for microbial enumeration. From each packet, a sample (5 g) of fish was weighed aseptically and then it was transferred to a stomacher bag with 45 mL sterile 0.9% NaCl solution. The mixture was homogenized for 30s using a Stomacher. Samples (0.1 mL) of serial dilutions (1:10, sterile 0.9% saline) of homogenates were spread onto the surface of prepared plate count agar (PCA) plates and incubated at 30 ± 1 °C for 72 h to calculate total viable counts (TVC). All counts were expressed as lg CFU/g.

2.7. Characterization of the microbiota based on culture-dependent methods

Composition of microbiota was determined on days 0, 4 and 6 for the control samples and on days 0, 6 and 8 for COS samples. After TVC enumeration, all colonies were selected from PCA plates that contained 30–100 colonies, cultivated in tryptic soy broth (TSB) at 30 ± 1 °C for 24–36 h, and then streaked to purify on a PCA plate. The purified colony was cultivated in TSB for further proliferation, after which 2 mL of TSB culture was centrifuged to collect the cells. Bacterial DNA was extracted according to the procedure for the Bacterial Genomic DNA Extraction Kit (Biomed Biological Technology Co., Ltd., Beijing, China).

A fragment (about 1400 bp) of the 16S rRNA bacterial gene was amplified using forward primer 27f (50'-GAGATTTGATCCTGGCT CAG-30′) and reverse primer 1495r (50'-CTACGGCTACCTTGTTA CGA-30′). Details of the PCR reaction were described previously (Liu et al., 2017). PCR products were submitted to Biomed Biological Technology Co. Ltd. (Beijing, China) for sequencing. A tentative identification was carried out by a similarity search using the Eztaxon database (http://www.eztaxon.org/). Sequences with > 97% identity were recognized as the same species.