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Effect of cinnamon essential oil on bacterial diversity and shelf-life in vacuum-packaged common carp (*Cyprinus carpio*) during refrigerated storage

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ABSTRACT

The present study investigated the effect of cinnamon essential oil on the quality of vacuum-packaged common carp (*Cyprinus carpio*) fillets stored at 4 ± 1 °C in terms of sensory scores, physicochemical characteristics (total volatile basic nitrogen (TVB-N), biogenic amines, and color), and presence of spoilage microbiota. A total of 290,753 bacterial sequences and 162 different genera belonging to 14 phyla were observed by a high-throughput sequencing technique targeting the V3–V4 region of 16S rDNA, which showed a more comprehensive estimate of microbial diversity in carp samples compared with microbial enumeration. Before storage, *Macrococcus* and *Aeromonas* were the prevalent populations in the control samples, but cinnamon essential oil decreased the relative abundance of *Macrococcus* in the treated samples. Variability in the predominant microbiota in different samples during chilled storage was observed. *Aeromonas* followed by *Lactococcus* were the major contaminants in the spoiled control samples. Microbial enumeration also observed relatively higher counts of *Aeromonas* than other spoilage microorganisms. Compared with the control samples, cinnamon essential oil inhibited the growth of *Aeromonas* and *Lactococcus* were the predominant components in the treated samples on day 10; plate counts also revealed a relatively high level of lactic acid bacteria during refrigerated storage. However, there were no significant differences ($P > 0.05$) in the composition of dominant microbiota between these two treatments at the end of the shelf-life. Furthermore, cinnamon essential oil treatment was more effective in inhibiting the increase of TVB-N and the accumulation of biogenic amines (especially for putrescine and cadaverine levels). Based primarily on sensory analysis, the use of cinnamon essential oil extended the shelf-life of vacuum-packaged common carp fillets by about 2 days.

1. Introduction

Common carp (*Cyprinus carpio*) is a popular freshwater fish species for many consumers and producers due to its abundance, high economic value and nutritional benefits. The global aquaculture production for common carp was approximately 3,791,913 tons in 2012 and it ranked third highest among freshwater fish species (FAO, 2012). Vacuum packaging is an effective packaging technology (Genç et al., 2013), which makes transport easier with a small packaging volume. Vacuum-packaged aquatic products have been increasing in markets. However, decomposition tends to occur rapidly with the growth and metabolism of microorganisms (Gram and Huss, 1996). These microorganisms are present in low numbers in fresh aquatic products, but they can contribute eventually to spoilage of vacuum-packaged aquatic products. *Photobacterium*, *Lactococcus*, *Lactobacillus* and *Aeromonas* are the predominant microorganisms of vacuum-packaged fish (Gui et al., 2014; Mace et al., 2012; Olofsson et al., 2007).

Given that the shelf-life of vacuum-packaged aquatic products is relatively short because of the activity of spoilage microorganisms, research on new preservation methods that can inhibit the growth of these microorganisms is required. Essential oils (EOs) are aromatic oily liquids that are extracted from some plant material, such as oregano, cinnamon, clove, thyme and rosemary. Some of these EOs have been used widely to preserve food products due to their antimicrobial and antioxidative properties (Patel, 2015; Uçak et al., 2011). Cinnamon (*Cinnamomum cassia* Presl) (family *Lauraceae*) usually grows in South Asia or South-East Asia, is a popular natural spice in China. Cinnamon EO has been listed as "Generally Recognized as Safe-GRAS" by the Food and Drug Administration in 21 e-CFR (electronic Code of Federal

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Regulation) part 182.20. Earlier studies have focused on the effect of cinnamon EO on extension of shelf-life of aquatic products in terms of their sensory scores, chemical analysis, and microbiological enumeration (Lu et al., 2010; Ojagh et al., 2010). However, there is limited information on how cinnamon EO affects the bacterial diversity of vacuum-packaged aquatic products, although this knowledge is likely to contribute to the development of cinnamon EO as a preservative. High-throughput sequencing allows for the rapid and in-depth sequencing analysis of microorganisms, especially for those that are present in low abundance, thus making it possible to monitor a more comprehensive bacterial diversity. Recent applications of this emerging method have proved useful in exploring the bacterial communities in intestines of fish and meat products (Zhao et al., 2015; Li et al., 2015; Xiao et al., 2013). To our knowledge, there are few studies on the changes in bacterial diversity and spoilage bacteria in vacuum-packaged aquatic products using high-throughput sequencing.

In the current study, we aimed to assess the effect of cinnamon EO on the bacterial communities and shelf-life of vacuum-packaged carp fillets stored at 4 ± 1 °C in terms of sensory scores, total volatile basic nitrogen (TVB-N), biogenic amines, color, and presence of spoilage microbiota. A combination of high-throughput sequencing and microbial enumeration methods was used to characterize the changes in bacterial communities and the dominant microbiota in carp samples during refrigerated storage.

2. Materials and methods

2.1. Sampling and packaging

Fresh common carp (n = 32, weight 1010.0 ± 91.0 g, length 39.0 ± 1.0 cm) were obtained from an aquatic products market in Beijing, China and were transferred to the laboratory alive in July 2015. Subsequently, these carp were killed, scaled, gutted and filleted, followed by washing with cold 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 (VP) and treated group (VC) were immersed in sterile water and 0.1% food grade cinnamon EO (about 48 fillets in 1 L of the solution) solution for 10 min (4 ± 1 °C), respectively. The concentration of 0.1% oil was optimized for the treatment based on our preliminary study. Carp fillets were immersed in food grade cinnamon EO solutions (0.06, 0.1, 0.2, 0.4 and 0.8%, w/v) for 10 min. Then all fillets were packaged under vacuum 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). A preliminary analysis suggested that the use of 0.1% cinnamon EO led to microbial shelf life increase and a minimum sensorial impact on carp fillets. And therefore, it was selected for the dip treatment.

Cinnamon EO that contained cinnamaldehyde (major component, accounting for 75%), cinene, and β-elemene, as stated by the manufacturer, was obtained from Zhengzhou Xomolon Food Flavor Co., Ltd. (Zhengzhou, Henan province, China). 0.1% cinnamon EO was prepared with 1 g of cinnamon EO in 1 L of sterile water and then 0.2 g of Tween 80 was added to this solution to help distribute and incorporate the cinnamon EO completely. After all fillets were packaged under vacuum, these samples were stored at 4 ± 1 °C for subsequent quality and bacterial analyses. Three fillets samples of each group were randomly selected and then samples of white dorsal muscle were taken for analyzing microbiological, physiochemical, and sensorial quality at 2-day intervals. However, bacterial communities were identified on days 0, 8, and 12 for VP samples and on days 0, 10, and 14 for VC samples.

2.2. Sensory assessment

The sensory analysis of each fillet was evaluated as described by Hong et al. (2012). Seven trained panelists from the laboratory staff evaluated the appearance, odor, texture, and morphology of raw fish white muscle, and scored each on a scale from 1 to 5 points. A total score of 20.0 points was considered fresh and the scores decreased according to spoilage to 9.0, which was the lowest acceptable limit.

2.3. TVB-N and color measurement

TVB-N value was determined according to the semi-micro steam distillation method (Hong et al., 2012) and the color measurement was performed with a fully-automatic colorimeter (ADCI-60-C, Beijing Chentaike Instrument Technology Co., Ltd., Beijing, China), including the assessment of L*, a*, and b* values. Three locations in the white muscle of each fillet were analyzed to obtain a mean value.

2.4. Measurement of biogenic amines

Extraction and derivatization of biogenic amines (BAs) in each fillet were performed according to the method of Shi et al. (2012). Afterwards, the quantification of BAs was carried out using HPLC (Shimadzu LC-10A; Shimadzu, Kyoto, Japan) that was equipped with a COSMOSIL 5C18-PAQ (4.6 × 250 mm) column and a SPD-10A (V) detector. The gradient elution program of ammonium acetate (0.1 M; solvent A) and acetonitrile (solvent B) as mobile phases was as follows: 0 min, 50% B; 25 min, 90% B; 35 min, 90% B; and 45 min, 50% B. The analysis was performed at 254 nm with a flow rate of 0.8 mL/min and an injection volume of 50 μL.

2.5. Enumeration of bacterial communities

At each sampling date, three fish samples of each group were randomly selected and evaluated for microbial enumeration. From each packet, a sample (25 g) of fish white muscle was aseptically weighed and then was transferred to a stomacher bag with 225 mL sterile 0.85% NaCl water. Then the mixture was homogenized for 30s in using a Stomacher (Masticator Basic L, S.A. Spain). Samples (0.1 mL) of triplicate serial dilutions (1:10, diluent, 0.85% NaCl water) were spread on the surface of different agar plates as described by Wang et al. (2014) and duplicates spread plates were prepared for enumeration. Total viable counts (TVC) were enumerated on plate count agar (PCA, Hai Bo Biological Technology Co. Ltd., Qingdao, China) and incubated at 30 ± 1 °C for 72 h. Iron agar medium (IA) and MRS agar (Hai Bo Biological Technology Co. Ltd., Qingdao, China) were used for the enumeration of H_2S-producing bacteria and lactic acid bacteria (LAB), respectively. For the former (IA), incubation was performed at 20 °C for 4 days and black colonies produced on IA were enumerated. MRS agar plates were incubated at 30 °C for 48 h. *Pseudomonas* sp. were determined on *Pseudomonas* agar (CFC, Oxoid code CM0559, U.K.) with *Pseudomonas* CFC supplement (SR 0103E), followed by incubation for 48 h at 20 °C. *Aeromonas* sp. were enumerated on *Aeromonas* Medium Base (AMB, Oxoid code CM0833, U.K., supplemented with SR 0136E) and they were incubated at 30 °C for 2 days. All units that formed colonies were recorded as log CFU/g.

2.6. DNA extraction

Bacterial DNA was extracted as described by Xiao et al. (2013) with some modifications. Fish white meat samples (10 g) were homogenized with 20 mL of sterile 0.85% NaCl water for 15 min in a stomacher blender. Subsequently, this mixture was centrifuged at a low speed of 200 × *g* for 5 min. The supernatants were removed and collected, followed by the next centrifugation at a high speed of 10,000 × *g* for 10 min. Then, the pellets were resuspended in 1 mL sterile water and transferred into a centrifuge tube. 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. Three parallel DNA samples from the same group were mixed together, and then DNA concentration was measured using 1.0% agarose gel electrophoresis.