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The effects of edible chitosan-based coatings on flavor quality of raw grass carp (*Ctenopharyngodon idellus*) fillets during refrigerated storage

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Adenosine monophosphate (PubChem CID: 6083)
Inosine monophosphate (PubChem CID: 8582)
Hypoxanthine ribonucleoside (PubChem CID: 6021)
Hypoxanthine (PubChem CID: 790)
Trimethylamine (PubChem CID: 1146)
Histidine (PubChem CID: 6274)

ABSTRACT

This study investigated the effects of chitosan-based coatings on flavor retention of refrigerated grass carp fillets by using various indicators: free amino acids (FAA), nucleotides, trimethylamine (TMA), volatile profile, sensory quality, and electronic nose analysis. The results indicated that chitosan-based coatings contributed to the significant reduction of off-flavor compounds, such as TMA, hypoxanthine (Hx) and histidine, and accumulation of inosine monophosphate (IMP) and umami-associated FAA. GC–MS analysis showed 23 volatile organic compounds, including many C_5–C_9 aldehydes and alcohols in the fresh fillets. The coating treatments, especially chitosan-clove bud essential oil composite coatings, sharply reduced the relative content of off-odor volatiles, such as hexanal, octanal and 1-octen-3-ol. According to the results of the sensory evaluation and electronic nose analyses, chitosan coating combined with glycerol monolaurate and clove bud essential oil was a promising method to improve the edibility of grass carp fillets by maintaining flavor quality during refrigerated storage.

1. Introduction

Freshwater fish are popular with consumers because of their good taste and nutritional values as well as low price. Traditionally, freshwater fish have been sold as live fish in China. This increases the cost because of transportation and holding issues. In addition, with the development of a workable cold-chain and more electronic commerce, the production of fresh fillets will grow as the younger generation in China recognizes its convenience for subsequent processing (Yu, Xu, Jiang, & Xia, 2017a). However, the disadvantage of fillets is their comparatively short shelf-life because of high water activity, abundant nutrients and neutral pH (Abdollahi, Rezaei, & Farzi, 2014). The post-mortem changes caused by biochemical reactions and microbial metabolism result in deterioration of texture and flavor, and eventually lose of edibility (Liu, Liang, Xia, Regenstein, & Zhou, 2013). Thus, research on how to improve quality and delay the deterioration of fresh fillets during refrigerated storage is potentially useful.

Currently, bio-based coatings and films have been widely studied and are perceived as an efficient and eco-friendly way to extend the shelf life of food products. Chitosan as the second most abundant polysaccharide after cellulose has been studied for fish preservation, due to its unique properties, such as biocompatibility, antimicrobial activity and film-forming properties (Günlü & Koyun, 2013; Mohan, Ravishankar, Lalitha, & Gopal, 2012). Many researchers have explored the effects of chitosan-based coatings on the quality and shelf life of refrigerated fish fillets, including seawater fish, such as sardine (*Sardinella longiceps*), yellow croaker (*Pseudosciaena crocea*) and sea bass (*Lateolabrax japonicus*) (Li et al., 2012; Mohan et al., 2012; Qiu, Chen, Liu, & Yang, 2014), and freshwater fish such as silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*) and rainbow trout (*Oncorhynchus mykiss*) (Raeisi, Sharifi-Rad, Quek, Shabanpour, & Sharifi-Rad, 2016; Ramezani, Zarei, & Raminnejad, 2015; Yu, Li, Xu, Jiang, & Xia, 2017). These results indicate the positive effectiveness of chitosan coatings in prolonging the shelf life of fillets by 4–8 days, based on common evaluation standards, such as total viable counts (TVC), spoilage bacteria counts, total volatile base nitrogen

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(TVB-N), K-value, etc., which are used to ensure food safety and edibility. However, an important reason that consumers are attracted to fish is its delicious flavor. The changes of taste compounds and flavor precursors including free amino acids, nucleotides, trimethylamine oxide (TMAO) and volatile organic compounds (VOC) may produce off-flavors, and cause organoleptic rejection (Feng, Ng, Mikš-Krajnik, & Yang, 2016; Shi et al., 2017). At present, although edible chitosan coatings used as a preservation technology have been studied, the changes in flavor and sensory quality of fillets treated with edible chitosan coatings have not been examined.

Grass carp is one of the most abundant cultured freshwater fish in China, with an annual harvest of 5.7 million tons in 2015, according to the 2016 China Fisheries Report (Anonymous, 2016). As an important economic fish, the changes in flavor and taste during refrigerated storage as well as potential protective measures have attracted researcher and commercial attention. Therefore, this study aimed to investigate the changes of flavor-related compounds (including ATP-related compounds, FAA and TMA) and the volatile profile of fillets to determine the potential effects of chitosan-based coatings on the flavor of refrigerated fillets.

2. Materials and methods

2.1. Preparation of coating solution

Chitosan powder from crabs with a deacetylation degree of 85% and average molecular weight of 400 kDa according to the manufacturer was purchased from Jinan Haidebei Marine Bioengineering Co. (Jinan, Shandong, China). Clove bud essential oil (EO) was purchased from Tiamay Aromatic Plant Co. (Shanghai, China) and glycerol monolaurate (GML) was supplied by Hangzhou Funchun Food Additive Co., Ltd. (Hangzhou, Zhejiang, China). Tween 20 and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The final concentrations of chitosan, GML and EO for the coating treatments are listed in Table 1. The coating solutions of C1 and C2 were prepared according to the method described by Yu, Xu, et al. (2017a). Briefly, C1 solution contained 2% chitosan (*w/v*), 1% acetic acid (*v/v*) and 0.5% glycerol (*w/v*), while C2 solution was prepared by adding GML solution (GML: ethanol: Tween 20 = 1:1:6) to C1 so the final GML concentration was 0.3% (*w/v*). For the microemulsions of C3, C4 and C5, different volumes of EO were added to GML solution and the mixtures were dispersed in the chitosan solutions to become microemulsions, using an Ultra Turrax T18 high shear mixer (IKA Werke GmbH & Co. KG, Staufen, Germany) at 25,000 rpm for 10 min. The microemulsions were degassed by ultrasound (QE5200; Ultrasonic Instruments Co., Ltd, Suzhou, Jiangsu, China) for 10 min and then used immediately for coating. The EO and GML levels were based on previous studies from this laboratory (Yu, Xu, et al., 2017a; Yu, Xu, Jiang, & Xia, 2017b).

2.2. Preparation and immersion treatment of fillets

Fresh live grass carp (*Ctenopharyngodon idellus*) (weight: 2.5 ± 0.2 kg; length: 54.3 ± 3.2; $n = 36$) were purchased from Vanguard Market (Wuxi, Jiangsu, China) in April and immediately killed using percussive stunning by trained store personnel. These carp, covered with crushed ice, were transported to Food Processing Technology Lab of Jiangnan University within 20 min. After decapitation and evisceration, the carp were cleaned with tap water and then randomly divided into six groups. Six fillets ($\sim 4 \times 3 \times 1.5$ cm^3) were obtained from each skin-off dorsal muscle and all fillets were rinsed with cold sterile distilled water. The above operations were done within 1 h after arrival. After draining for 20 min in a sterile biochemical incubator (Shanghai Yiheng Instruments Co., Ltd., Shanghai, China) with air flow (4 °C and 50% RH), five groups of cubes were immersed in respective coating solution for 5 min at 4 °C, with a fillet/solution ratio of nearly 1:5 (*w/v*), while the sixth group (CK) was treated with distilled water for an equal time. When immersion was done, fillets were removed and drained for 90 min on pre-sterilized metal nets in the sterile biochemical incubator. After that, each sample was individually placed in a sterile polyethylene bag and stored in a refrigerator at 4 ± 0.5 °C for subsequent assessments. The samples from each group were randomly taken out for analysis according to predetermined time intervals.

Table 1
The final concentrations of chitosan, GML and EO in coating solutions.

Codes for coating treatments	Chitosan (w/v)	GML (w/v)	EO (w/v)
CK	–	–	–
C1	2.0%	–	–
C2	2.0%	0.3%	–
C3	2.0%	0.3%	0.1%
C4	2.0%	0.3%	0.5%
C5	2.0%	0.3%	1.0%

GML: glycerol monolaurate; EO: clove bud essential oil.

2.3. Sensory evaluation

Sensory analysis of raw samples was carried out according to the published method of Yu, Xu, et al. (2017a). Briefly, 13 trained panelists (6 men and 7 women between 22 and 40 years old) from laboratory staff provided comprehensive scores from aspects of color, odor and overall acceptance, with 5 being the highest score in terms of freshness (fresh sample) and the scores decreased with the gradual deterioration of the fish. The indoor environment in the sensory laboratory was controlled at 20 ± 1 °C and 55 ± 2% humidity, and all panelists conducted independent evaluation without interaction. The fillets were given to the panelists with random three-digit numbers. A final mean score of 3 points from all panelists was viewed as the lowest acceptable quality.

2.4. Trimethylamine (TMA) analysis

Trimethylamine (TMA) was determined using AOAC method 971.14 (1980) with slight modifications. Samples of muscle (3 g) were homogenized (T10 shear mixer; IKA Werke GmbH & Co. KG) with 27 mL of 7.5% trichloroacetic acid. After centrifugation at 10,000*g* for 10 min (4K15 centrifuge; Sigma Laborzentrifugen GmbH, Osterode, Germany), 5 mL supernatant were mixed with 1 mL of formaldehyde (10%), 10 mL of anhydrous toluene and 3 mL of K_2CO_3 in a tube. After the 5-mL toluene layer was pipetted to another tube containing 5 mL picric acid solution (0.02%), the absorbance of the mixed solution was measured at 410 nm against a blank, using a UV–Vis spectrophotometer (UV-1000; Techcomp Co., Ltd., Shanghai, China). The TMA-N value was calculated by standard curve of TMA (purity > 98%; Sinopharm Chemical Reagent Co., Ltd) and expressed as mg/100 g sample.

2.5. ATP-related compounds analysis

ATP-related compounds were extracted with 6% cold perchloric acid solution. Samples (2 g) were homogenized with 7.5 mL extracting solutions and centrifuged at 10,000*g* for 5 min at 4 °C. The extraction process was repeated once and the combined supernatants were neutralized immediately to pH 6.5–6.8 with 10 M and 1 M KOH solutions. The precipitate in neutralized solution was removed by centrifugation (3000*g*, 5 min and 4 °C) and the supernatant was diluted to 25 mL with cold distilled water. The final solution was filtered through a nominal 0.22-μm membrane filter (Dongkang Technology Co., Ltd., Tianjin, China) and ATP-related compounds were determined using HPLC (Waters e2695; Milford, MA), equipped with a photo-diode array detector (Waters 2998) and a Waters C_{18} column (5 μm, 4.6 mm id × 250 mm). The mobile phase was 98% potassium phosphate buffer (0.05 M, pH 6.8) and 2% methanol, and the detection wavelength was