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The preservative effects of chitosan film incorporated with thinned young apple polyphenols on the quality of grass carp (*Ctenopharyngodon idellus*) fillets during cold storage: Correlation between the preservative effects and the active properties of the film

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ABSTRACT

This study aims to investigate the preservative effects of chitosan film incorporated with thinned young apple polyphenols (YAP) on grass carp fillets (GCF) quality during cold storage. The results showed that the chitosan-YAP film wrapping effectively retarded the microbial proliferation, lipid and protein oxidation, changes in pH and color values and decrease in water holding capacity of GCF during cold storage. Besides, the wrapping with chitosan-YAP film could significantly delay the decrease in the content and functional properties of soluble myofibrillar protein, the loss of the texture and amino acids, and the adverse changes in the surface morphology and sensory values of GCF. Through correlation analyses, the preservative effects of the film may be attributed to its water retention capacity, antioxidant and antibacterial activities. Therefore, the chitosan-YAP film has a potential as a packaging material to extend shelf life of freshwater fish fillets during cold storage.

1. Introduction

In recent years, the design and development of biodegradable and edible packaging materials have been obtained an increasing interest throughout the world because of the limited natural resources, food safety and environmental pollution. Some polysaccharides, including starch, cellulose, pectin and chitosan have been explored as food packaging materials due to their good mechanical, film-forming and food safety properties (Shankar, Wang, & Rhim, 2017). To enhance the antioxidant and antibacterial activities of the carbohydrates-based films, some natural antioxidant extracts are often incorporated, like polyphenols and essential oils (Ma et al., 2016; Siripatrawan & Harte, 2010).

In order to increase apple production output and to improve apple quality, it is important and necessary to thin flowers and fruits in one month after blossom. Therefore, there are plenty of thinned young apples produced each year in China (Sun et al., 2017c,d), and these apples are usually discarded in grove. This is a waste of agricultural and food resource, because of the relatively high content of polyphenols in young apples (Sun, Guo, Fu, Li, & Li, 2013). Therefore, it is reasonable and valuable to collect the thinned young apples and to extract phenolic compounds for development of bioactive functions of young apple polyphenols (YAP). In our previous study, a food packaging chitosan (90% deacetylation) film incorporated with YAP has been developed (Sun et al., 2017a) (Fig. S1). Through H^1 NMR and FTIR analyses, YAP was successfully incorporated into the chitosan film through non-covalent binding interactions. In addition, the water retention capacity, antioxidant and antibacterial properties of the chitosan film were enhanced by the introduction of YAP (Sun et al., 2017a).

Grass carp (*Ctenopharyngodon idellus*) is one of the favorite freshwater fishes in China because of its high nutritional values, delicious flavor and aquacultural suitability. For convenience of production, processing and transportation, cold and chilled storage with the corresponding temperature of 4 and −18 °C are often required for freshwater fish. However, because of oxidation of lipids (mainly polyunsaturated fatty acids) and proteins (mainly sulfydryl-rich proteins), microbial reproduction and enzymic degradation, deterioration or decomposition may happen in fish fillets (Sun, Sun, Thavaraj, Yang, & Guo, 2017b). Therefore, some natural preservatives (like polyphenol-rich phytochemicals) have been applied directly or incorporated with edible chitosan film in fish preservation (Li et al., 2012).

To evaluate the preservative effects of a chitosan-phytochemicals film on fish, lipid oxidation indexes (including peroxide value (PV) and thiobarbituric acid reactive substance (TBARs)), protein oxidation

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indexes (including soluble myofibrillar protein (SMP) content, sulfhydryl groups content and adenosine triphosphatase activity), sensory evaluation and color values during cold storage have been investigated in previous studies (Li et al., 2012; Ojagh, Rezaei, Razavi, & Hosseini, 2010). However, there are limited literatures available to shed light on the preservative effects of chitosan-polyphenol film on the functional properties (including emulsifying activity, emulsifying stability and surface hydrophobicity) of SMP, the detailed textural properties and the water holding capacity (WHC) of fish fillets during cold storage, even though these properties are considered to be important indexes indicating the strength of gel network in fish (Utsumi, Matsumura, & Mori, 1997). Then, the effects of chitosan-polyphenol film on the loss of amino acids in fish during cold storage should also be determined, as amino acids are highly related with nutrition and flavor values of fish. In addition, the correlation between the preservative effects of chitosan-polyphenol film and the phytochemical properties of the film need to be further elucidated to explore the reasons of the preservative effects of the film.

Hence, the aim of this study is to investigate the application of chitosan-YAP film in preservation of grass carp fillets (GCF) during cold storage regarding the effects of the film on microbiological proliferation, chemical properties (PV, TBARs and TVB-N), physical properties (pH, color, water holding capacity), SMP functional properties, micromorphology, textural properties, amino acids, and sensory evaluation of GCF. Besides, the correlations between the preservative activities of the chitosan-YAP film and the active properties (water retention capacity, antioxidant and antibacterial activities) of the film were also established.

2. Materials and methods

2.1. Materials and reagents

The chitosan-YAP films with different YAP contents (0, 0.25%, 0.5%, 0.75 and 1.0%, w/w, YAP/chitosan) (defined as chitosan-0.25% YAP, chitosan-0.5%YAP, etc.) were produced according to the method established in our previous study (Sun et al., 2017a), and the film products were shown in Fig. S2 16 amino acids standards, including Asp, Thr, Ser, Glu, Gly, Ala, Val, Ile, Met, Leu, Try, Phe, Lys, His, Arg and Pro, ninhydrin, HPLC grade ethanol and reaction buffer (P/N 855–3407) were purchased from Wako Pure Chemical Co., Ltd. (Tokyo, Japan). Other chemicals were of analytical grade.

2.2. Preparation of GCF samples

The fresh grass carps were sliced into fillets (50 × 25 × 10 mm) and wrapped around with the chitosan and chitosan-YAP films (0.25%, 0.50%, 0.75% and 1.0% YAP, w/w, YAP/chitosan) (Fig. S2). The samples without film wrapping were used as blank. Each sample was placed into a petri dish (10 cm diameter) and covered with lid. All the samples were stored at 4 °C.

2.3. Microbiological analysis

5 g of each GCF sample treated with blank, chitosan and chitosan-YAP films at each sampling interval was transferred to a tube containing 30 mL of 0.1% (w/v) sterile peptone solution, and then homogenized for 5 min using an IKA® homogenizer (ULTRA-TURRAX, Germany). Then, the mixture was 10-fold diluted using 0.1% peptone solution, and 0.1 mL of the dilution was plated onto standard plate count agar. All the plates were incubated at 37 °C for 48 h. The microbial counts were expressed as the logarithm of the colony forming units per gram of GCF sample (Log_{10}CFU/g).

2.4. Determination of chemical properties of GCF

The chemical properties of GCF, including PV, TBARs and TVB-N values, were determined according to the methods established in our previous study (Sun et al., 2017b).

2.5. Determination of physical properties of GCF

2.5.1. pH value

The pH value of GCF during cold storage was determined according to one previous study (Li et al., 2012). Specifically, 5 g of each GCF sample treated with blank, chitosan and chitosan-YAP films at each sampling interval was mixed with 50 mL of distilled water thoroughly for 30 min and then filtered. The pH value of the filtrate was determined using a TPS® pH meter (AQUA, Australia).

2.5.2. Color values

The color values of each GCF sample treated with blank, chitosan and chitosan-YAP films at each sampling interval were determined using a Konica® colorimeter (CR-300, Japan), characterized as lightness (L^*), red/green (a^*) and yellow/blue (b^*) values. Total difference of color (ΔE^*) was calculated as follows (Vega-Gálvez et al., 2009):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where, ΔL^*, Δa^*,Δb^* are the differences between the color values of the samples and the color values of the GCF blank at day 0.

2.5.3. WHC

The WHC of each GCF sample treated with blank, chitosan and chitosan-YAP films at each sampling interval was determined according to the method established in our pervious study (Sun et al., 2017b) and expressed as the ratio of water (%) that the GCF sample held.

2.6. Determination of properties SMP in GCF

The content of SMP, the emulsifying activity index (EAI) of SMP solution with the blend oil volume fraction (v/v) of 0.4, the emulsifying stability index (ESI) of SMP-oil-water system, as well as the surface hydrophobicity of SMP solution were determined based on the methods established in our previous study (Sun et al., 2017b).

2.7. Scanning electron microscope (SEM) profiles of GCF

The GCF sample during cold storage was collected and sliced into a piece of cuboid (10 × 10 × 5 mm) and then soaked with glutaraldehyde before storage at 4 °C for 24 h. After that, the soaked sample was rinsed with phosphate buffered saline thoroughly, followed by soaking with tertiary butanol for 2 h. Then, the sample was lyophilized to remove water and then mounted on carbon adhesive discs attached on aluminum stubs, followed by coating with a fine layer of gold film (15 nm). The sample was observed at 1200-fold magnification operated with an accelerating voltage of 20 kV by use of a Hitachi® scanning electron microscope (S4800, Japan) and photographed by use of a Hitachi® camera (S570, Japan).

2.8. Determination of textural properties of GCS

The textural properties of each GCF sample treated with blank, chitosan and chitosan-YAP films at each sampling interval were measured through the texture profile analysis (TPA) of two consecutive compression cycles (Guerrero, O'Sullivan, Kerry, & de la Caba, 2015) using a TA® texture analyzer (TA. XT. Plus, England) equipped with a P/36R probe. The initial distance was 2 cm and the testing force was 5 g with the speed of 2 mm/s.