FOOD PACKAGING AND SHELF LIFE 2 (2014) 7–16

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/fpsl

Properties and antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation

Jiulin Wu, Shangying Ge, Hui Liu, Shuang Wang, Shanfei Chen, Jianhua Wang, Jianhua Li, Qiqing Zhang**

Institute of Biomedical and Pharmaceutical Technology & College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350002, China

ARTICLE INFO

Article history:
Received 10 January 2014
Received in revised form
18 April 2014
Accepted 30 April 2014
Available online 14 May 2014

Keywords:
Fish gelatin
Oregano essential oil
Antimicrobial film
Physical properties
Fish muscle preservation

ABSTRACT

Silver carp skin gelatin-chitosan films incorporated with different concentrations of oregano essential oil (OEO) were prepared and characterized. Results showed that OEO had the best antimicrobial activity among oregano, cinnamon and anise essential oil against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus enteritidis* and *Shigabacillus*. The incorporation of OEO into gelatin-chitosan film clearly improved antimicrobial activity. As regard to the film properties, the addition of OEO into gelatin-chitosan film significantly reduced mechanical property and increased light barrier, water vapor barrier. Film solubility of gelatin-chitosan film significantly decreased when containing 4% OEO ($p \leq 0.05$). The SEM result revealed gelatin-chitosan-OEO film had rough cross-section. FTIR spectra indicated that no new bonds were formed in gelatin-chitosan-OEO film. For fish preservation, total aerobic plate count and total volatile basic nitrogen values of fish muscle samples which packaged with gelatin-chitosan film incorporated with 4% OEO were significantly lower than those samples packaged with parafilm and control. These results indicated that the gelatin-chitosan film incorporated with OEO has the potential to extend the shelf-life of fish muscle.

1. Introduction

The antimicrobial packaging is a rapidly developing technology, which not only provides a barrier property but also performs the function of inhibiting the growth of pathogenic and spoilage foodborne microorganisms (Bastarrachea, Dhawan, & Sablani, 2011). Many studies have demonstrated that packaging films incorporated with antimicrobial agents as antimicrobial packaging could be effective for reducing levels of foodborne organisms (Cutter, 2006; Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010). In recent years, consumers have an increasing trend toward 'green' consumerism, demand for more natural, minimally processed products, fewer synthetic food additives, potentially biodegradable, recyclable and environmental friendly food packaging materials (Burt, 2004; Gübilmez, Yemenicioğlu, & Arslanoğlu, 2007). To satisfy these requirements, biodegradable edible packaging materials

* *Corresponding authors at*: Institute of Biomedical and Pharmaceutical Technology & College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350002, China. Tel.: +86 591 83725260; fax: +86 591 83725260.
E-mail addresses: wujiul590@126.com (J. Wu), zhangqiq@126.com (Q. Zhang).
http://dx.doi.org/10.1016/j.fpsl.2014.04.004

from natural polymers have been developed in order to achieve a partial alternative to plastic packaging. In this sense, natural plant products have been receiving more and more attentions.

Biodegradable edible films can be obtained from several natural polymers, such as polysaccharides, lipids and proteins (Campos, Gerschenson, & Flores, 2011; Tharanathan, 2003). Gelatin, obtained by partial hydrolysis of collagen, is a soluble protein compound which has been widely used in the manufacture of edible films for its excellent film forming ability, abundance and biodegradability (Gómez-Guillén et al., 2009; Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006). In general, gelatin is mainly prepared from the skins or hides, cartilages, tendons and bones of pig and cow. However, recent outbreaks of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) have resulted in anxieties of cattle gelatin. Besides, porcine gelatin is taboo in some religions such as Judaism and Islam. In recent years, fish gelatin has been received considerable attention because it is unlikely to be related to BSE and FMD and not forbidden for religious reasons. Therefore, fish skin, the major by-product of the fish-processing industry, can provide a valuable source of gelatin (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009). Many gelatin films produced from fish skin have been studied, such as blue shark (Limpisophon et al., 2009), cuttlefish (Hoque, Benjakul, & Prodpran, 2011), bigeye snapper and brownstripe red snapper (Jongjareonrak et al., 2006). However, fish gelatin film has poor mechanical property and water resistance, which are the main drawbacks for its applications (Gómez-Guillén et al., 2009). Natural polysaccharide incorporated into gelatin film is an effective way to improve film properties, such as chitosan (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011).

Currently, natural antimicrobial compounds are receiving more and more attentions. Essential oils (EOs) are plant extracts, whose antimicrobial activity have been studied. Researches find that spoilage microorganisms, foodborne and postharvest pathogens are sensitive to EOs (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Burt, 2004). Thus, EOs can be applied in the food products as natural antimicrobials. However, high concentrations of EOs are generally needed in direct food applications to achieve effective antimicrobial activity, which might have inappropriate flavors and odors in the product (Gutierrez, Barry-Ryan, & Bourke, 2009; Seydim & Sarikus, 2006). Therefore, researches focus on incorporation of EOs into edible films as an application in food packaging (Seydim & Sarikus, 2006). Many studies showed that the incorporation EOs into edible films to develop antimicrobial activity obtained good results (Ahmad, Benjakul, Prodpran, & Agustini, 2012; Emiroğlu et al., 2010; Sánchez-González, Cháfer, Hernández, Chiralt, & González-Martínez, 2011). Fresh and minimally processed fish is one of the most perishable food products, mainly due to fast microbial growth of microorganisms naturally present in fish or from contamination, which can occasionally result in either economic or health-related problems (Iturriaga, Olabarrieta, & De Marañón, 2012). Therefore, the edible film incorporated with EOs as antimicrobial film can be used to preserve fish muscle.

Silver carp (*Hypophthalmichthys molitrix*) is a major freshwater aquaculture species in China. Huge numbers of silver carp skins were generated annually and considered as by-products, resulting in environmental pollution and resources waste (Zhang, Duan, Tian, & Konno, 2009). The objective of the present study was to extract gelatin from silver carp skins, to analyze the antibacterial activity and properties of gelatin-chitosan films incorporated with OEO and further to determine whether the shelf-life of fresh grass carp muscle preservation was extended by the use of gelatin-chitosan-OEO film.

2. Materials and methods

2.1. Chemicals and microorganisms

Cultured grass carp (*Ctenopharyngodon idellus*) (1500–2000 g in body weight, October and November 2012) was purchased alive from a fish market of Yiyuan, Fuzhou, China. Instantly the fish was sacrificed, decapitated, eviscerated and skinned. The fish muscle was immediately used for experiment.

Silver carp skins were purchased from a fish market of Yusheng, Fuzhou, China. Food grade oregano essential oil (OEO), cinnamon essential oil (CEO), and anise essential oil (AEO) were purchased from Zhongxiang natural plant (Ji'an, Jiangxi). Chitosan was purchased from Roche (Mannheim, Germany). Other chemicals and reagents were analytical grade.

Escherichia coli, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enteritidis*, and *Shigabacillus* were obtained from the College of Biological Science and Technology, Fuzhou University, Fuzhou, China.

2.2. Preparation of gelatin from silver carp skin

Gelatin was extracted from silver carp skins according to the procedure of Jongjareonrak et al. (2006) with a slight modification. Residual meat of silver carp skins was manually removed. The treated skins were washed thoroughly with distilled water and cut into small pieces. Skin pieces were soaked in 0.01 M NaOH containing 1% H_2O_2 with a skin/solution ratio of 1/20 (w/v) at 4 °C with a gentle stirring. The solution was changed every 8 h for 3 times to remove noncollageneous proteins and pigments. Alkaline treated skins were washed with distilled water to neutral pH. The sample was defatted with 10% isopropanol with a skin/solution ratio of 1/20 (w/v) for 4 h at 4 °C with a gentle stirring, then washed thoroughly with distilled water. The defatted skins were soaked in 0.05 M acetic acid with a skin/solution ratio of 1/20 (w/v) for 4 h at 4 °C with a gentle stirring to swell the collagen in fish skin matrix. The acid treated skins were washed with distilled water to neutral pH. The swollen fish skins were soaked in distilled water with a skin/water ratio of 1/10 (w/v) at 45 °C for 12 h with a continuous stirring to extract gelatin from the skin matter. The mixture was centrifuged at 18,000 × *g* for 20 min at 10 °C. The supernatant was collected and freeze-dried using a freeze-dryer (Boyikang, Beijing, China). The dry matter was referred to as "gelatin".

2.3. Preparation of gelatin-chitosan-OEO films

For the complex gelatin-chitosan film forming solution (FFS), 3 g of gelatin plus 1 g of chitosan per 100 ml FFS were used.