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Properties of fish myofibrillar protein film incorporated with catechin-Kradon extract

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ABSTRACT

Properties of fish myofibrillar protein (FMP) films incorporated with catechin-Kradon extract (*Careya sphaerica* Roxb.) (CK) were investigated. The incorporation less than 9 mg/ml of CK improved tensile strength, but this slightly declined when increasing the concentration ($P < 0.05$). Significant decreases for elongation at break (51.38–132.76%), transparency (3.35–3.88), and water vapor permeability ($1.56 - 2.08 \times 10^{-9}$ g m^{-1} s^{-1} Pa^{-1}) were observed when the concentration of CK increased ($P < 0.05$). Nevertheless, film thickness (11.45–19.48 μm), solubility (18.82–38.30%), and antioxidant activity increased markedly as the level of CK increased ($P < 0.05$). FMP films with CK added possessed low L^* value but high a^* and b^* values, and they exhibited excellent UV light barrier properties. However, the only antimicrobial activity that was observed was against *Vibrio parahaemolyticus*. According to these findings, FMP films incorporated with 9 mg/ml of CK have potential for being used as active packaging.

1. Introduction

Quality and appearance of food is very important for consumers. Food quality changes easily and quickly due to microbial and chemical degradation and this can happen during handling, transportation, and storage (Saghir, Wagner, & Elmadfa, 2005). The oxidation reaction of lipid is the main cause of spoilage. Therefore, preventing microbiological and chemical deteriorations are critical challenges for the food industry. Several innovative packaging techniques have been produced with the goal of both maintaining the quality and prolonging the shelf life of foods. Packaging is used for protecting the food from the outside environmental and preventing contamination. In recent years, so-called active packaging has emerged as an alternative method for protecting food. Antimicrobial and/or antioxidant packaging are the main systems that effectively kill or suppress microbial growth and delay the oxidation of pigments and lipids present in food (Kaewprachu & Rawdkuen, 2016). This technology actually incorporates active agents into the packaging materials, which provides antimicrobial and/or antioxidant properties that do not exist in traditional packaging.

Recently, many studies have focused on active agents from natural sources. Non-natural synthetic chemical agents, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) are suspected of carcinogenic potential and toxicity, which is a clear concern for human health. Many studies found that TBHQ promoted carcinogenesis in animal model and cell culture. This is because its genotoxic and cytotoxic properties (Hirose, Yada, Hakoi, Takahashi, & Ito, 1993; Negar, Susan, & Ayman, 2007). So, its use is restricted as it is considered as a food additive. There are many natural possibilities such as plants or herb extracts. Many have been effectively incorporated into biodegradable films. Some include catechin-lysozyme (Rawdkuen, Suthiluk, Kamhangwong, & Benjakul, 2012), longan seed extract (Sai-Ut, Benjakul, & Rawdkuen, 2015), pomegranate peel extract (Emam-Djomeh, Moghaddam, & Ardakani, 2015), honeysuckle flower extract (Wang, Wang, Tong, & Zhou, 2017), coconut husk extract (Nagarajan, Benjakul, Prodpran, & Songtipya 2015), and basil leaf essential oil (Arfat, Benjakul, Prodpran, Sumpavapol, & Songtipya 2014).

Kradon (*Careya sphaerica* Roxb.) is very common in the North and Northeastern part of Thailand. Lupeol, taraxerol, β-sitosterol, and quercetin are the main phenolic compounds found in Kradon leaves (Maisuthisakul, 2012). Kradon extract has been noted for high antioxidant activity (Maisuthisakul & Pongsawatmanit, 2005; Sriket, 2014) and antimicrobial activity (Daduang, Vichitphan, Daduang, Hongsprabhas, & Boonsiri, 2011; Panomket, Wanram, & Srivoramas, 2011). The preparation and utilization of Kradon extract containing phenolic compounds could provide a high value-added potential for those leaves. Green tea extract has been utilized widely as an active

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agent for the preparation of active films (Rawdkuen et al., 2012; Siripatrawan & Harte, 2010). Catechins are the main phenolic compound that are mostly found in tea, and they have shown to inhibit a wide range of microorganisms as well as to have good antioxidant activity (Almajano, Carbó, Jiménez, & Gordon, 2008; Oh, Jo, Cho, Kim, & Han, 2013).

Traditional packaging is normally made up from petroleum-based polymeric materials. They have been continuously used because of their inexpensive, lightweight, durability, and functional advantages (such as thermosealability, microwavability, and optical properties) (Marsh- & Bugusu, 2007). On the other hand, these materials are not easy to degrade and generate much heat and exhaust gases when burned, thus posing a negative impact on environmental. In addition, a variety of by-product from agricultural or marine sources has been utilized frequently to manufacture biodegradable film. Protein is the most commonly used material for producing biodegradable based films due to film-forming ability, high nutritional value, and abundance. The myofibrillar proteins from fish muscle particularly have been used effectively as a starting material (Nie, Gong, Wang, & Meng, 2015). Biodegradable film developed from fish myofibrillar protein (FMP) showed to be slightly transparent and had colorless characteristics with an excellent UV light barrier when compared to a commercial wrap film (polyvinyl chloride) (Kaewprachu, Osako, Benjakul, & Rawdkuen, 2016a). Rostamzad, Paighambari, Shabanpour, Ojagh, and Mousavi (2016) suggested that FMP film had potential to be used for producing active packaging.

Phenolic compounds have been reported to interact with proteins via hydrophobic interactions, hydrogen bonding, ionic bonds, and covalent bonds (Hoque, Benjakul, & Prodpran, 2011; Mekoue Nguela, Poncet-Legrand, Sieczkowski, & Vernhet, 2016; Wu et al., 2013). The aromatics rings of polyphenol would combine with hydrophobic sites of proteins, such as pyrrolidine rings of prolyl residues, via hydrophobic interactions, while hydrogen bonding occurs between H-acceptor sites of the proteins and the hydroxyl groups of the polyphenol (Bourvellec & Renard, 2012; Ozdal, Capanoglu, & Altay, 2013). Moreover, under alkaline conditions with the presence of oxygen, oxidized polyphenols (quinone) could react with lysine, methionine, cysteine, and tryptophan residues in protein molecules (Liu, Ma, McClements, & Gao, 2017; Nie, Zhao, Wang, & Meng, 2017; Strauss & Gibson, 2004). This reaction can be induced the formation of covalent C—N and C—S bonds via cross-linking, which are more thermally stable and rigid than other interactions (Prodpran, Benjakul, & Phatcharat, 2012).

Adding catechin and Kradon extract to act as the active agents to inhibit microbial growth and retard the oxidation of lipids may affect the overall functional properties of the FMP film. Incorporating active agents at appropriate amounts can provide antimicrobial and antioxidant properties with only a small change to the functional properties of film. Therefore, the objective of this investigation was to study the properties of FMP films contained the combination of catechin-Kradon extract (CK) at different concentrations (0–12 mg/ml). Their properties were compared with a low density polyethylene (LDPE) wrap film.

2. Materials and methods

2.1. Chemicals and microbials

Catechin hydrate (C1251), 2,2-diphenyl-1-picryl hydrazyl (DPPH), and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Calibiochem (Darmstadt, Germany). Microbial transglutaminase (MTGase) (Activa TG-K: 100 activity units per gram) was supplied by Ajinomoto Co. Inc. (Tokyo, Japan). All other reagents used were of analytical grade.

Salmonella Typhimurium ATCC 13311, *Staphylococcus aureus* ATCC 12600 and *Listeria monocytogenes* CIP 107776 were obtained from Laboratory of Food Microbiology, Tokyo University of Marine Science and Technology, Tokyo, Japan. *Vibrio parahaemolyticus* was isolated from a patient and provided by Tokyo Metropolitan Institute of Public Health, Tokyo, Japan.

2.2. Preparation of Kradon extract

Kradon leaves (*Careya sphaerica* Roxb.) were obtained from a local market in Chiang Rai, Thailand. They were subjected to liquid nitrogen and then stored in a plastic bag at −20 °C for further extraction.

To prepare the Kradon extract, 1 g of frozen Kradon leaves containing 69.44% moisture content were combined with distilled water using a sample to water ratio of 1:32 (w/v). After stirring for 30 s, the mixture was then subjected to extraction using a household microwave (LG Thailand Co. Ltd., Bangkok, Thailand) at 500 W for 62 s (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). The extract was filtered and the supernatant was then collected. Finally, the supernatant was subjected to freeze drying. The extract was subsequently referred to as “Kradon extract”.

2.3. Preparation of fish myofibrillar protein (FMP)

Fresh tilapia (*Orcochromis niloticus*) (400–500 g/fish) was purchased from a local market in Chiang Rai, Thailand. It was washed, fileted, and minced uniformly. FMP was prepared as described in Kaewprachu et al. (2016a). The minced fish was added with five volumes of 50 mM NaCl. After homogenization (11,000 rpm for 2 min), the mixture was then centrifuged at 10,000 × *g* for 10 min at 4 °C and filtered through cheese cloth. The washed mince was collected and re-washed twice. After that, the FMP was dried in a freeze dyer, packed under vacuum conditions, and stored in freezer (−20 °C) for further analysis.

2.4. Preparation of fish myofibrillar protein film incorporated with catechin-Kradon extract

Firstly, a film-forming solution (FFS) was prepared as described in Kaewprachu et al. (2016a). FMP 1% (w/v) was added with the distilled water, followed by homogenization at 11,000 rpm for 1 min, and the pH was adjusted to 11 using 1 N NaOH. It was centrifuged at 3000 × *g* at room temperature for 10 min. The supernatant was then collected. Glycerol at 25% (w/w, based on protein content) was used as a plasticizer and stirred at room temperature for 30 min. After stirring, MTGase (2% w/w, based on protein content), for use as a cross-linker, was added into the mixture and stirred continuously for 30 min to obtain the FFS.

Prior to incorporating active agents, the combination of catechin-Kradon extract solution (CK) was prepared by dissolving catechin in 60% ethanol while the Kradon extract was dissolved in distilled water. The catechin solution was added to the Kradon extract solution in a ratio of 1:1. It was then incorporated into FFS in order to obtain final concentrations of 0, 3, 6, 9, and 12 mg/ml and was continuously stirred at room temperature for 1 h. The bubbles contained FFS were eliminated by a hybrid mixer (HM-500; Keyence Co., Tokyo, Japan) for 10 min. Finally, the de-aerated FFS (4 ± 0.01 g) was casted onto a rimmed silicone resin plate (50 × 50 mm) and dried for 24 h at 25 ± 0.5 °C and 50 ± 5% relative humidity (RH). The obtained dry films were manually peeled and conditioned at 50 ± 5% RH at 25 °C for 48 h, prior to be tested.

2.5. Film properties determinations

2.5.1. Film thickness

The film thickness was measured by using a dial-type thickness gauge (Series 7300; Mitsutoyo Co., Kanagawa, Japan). 6 random locations around each of the 10 film samples were used for determining thickness.