Food Hydrocolloids 69 (2017) 308–317

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring

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ARTICLE INFO

Article history:
Received 5 December 2016
Received in revised form
20 February 2017
Accepted 20 February 2017
Available online 23 February 2017

Keywords:
Colorimetric film
Fish freshness
Roselle anthocyanins
Starch
Polyvinyl alcohol
Intelligent packaging

ABSTRACT

Novel colorimetric films were developed for real-time monitoring of fish freshness based on starch/polyvinyl alcohol (SPVA) incorporated with roselle (*Hibiseus sabdariffa* L.) anthocyanins (RACNs). Firstly, RACNs were extracted from roselle dehydrated calyxes. Secondly, SPVA aqueous solution was obtained with a mass rate of 2:1 (starch/PVA). Thirdly, the colorimetric films were fabricated by immobilizing 30, 60 and 120 mg RACNs/100 g starch into SPVA matrix with casting/solvent evaporation method. FTIR spectra of the colorimetric films showed that RACNs were successfully immobilized into the SPVA matrix. X-ray diffraction spectra and SEM micrographs indicated that the crystallinity of PVA was reduced during the film-forming process and the compatibility between starch and PVA was improved, owing to the presence of RACNs. The incorporation of RACNs led to a decrease of water content and tensile strength and an increase of elongation at break of the colorimetric films compared with the SPVA film. The color stability test showed that the colorimetric films were stable at refrigeration temperature and room temperature up to 14 days with relative color changes below than 5%. The colorimetric films with lower content of RACNs were found more sensitive towards ammonia. An application trial was conducted to monitor the freshness of silver carp (*Hypophthalmichthys molitrix*) at refrigeration temperature. The colorimetric films presented visible color changes over time due to a variety of basic volatile amines known as total volatile basic nitrogen (TVB-N). Hence, these colorimetric films can be used to monitor the real-time fish freshness for intelligent packaging.

1. Introduction

Fish is highly perishable due to enzymatic reaction and microbial contamination (Zhang, Sun, Xiao, Liu, & Zheng, 2016). Considering the food quality and safety, it is essential to evaluate the fish freshness during the supply chain. TVB-N has been widely regarded as an useful indicator of the fish freshness for a long period (Olafsdóttir et al., 1997). It is comprised of ammonia (NH_3), trimethylamine (TMA) and dimethylamine (DMA) generated by the enzymatic decomposition of trimethylamine oxide (TMAO) (Byrne, Lau, & Diamond, 2002). A variety of approaches have been developed to determine the TVB-N level. Chemical methods such as Kjeldahl method can provide precise results, but they are generally time consuming and destructive to samples. Other rapid and non-destructive detection methods, such as FTIR spectroscopy, can also provide satisfactory results (Cai, Chen, Wan, & Zhao, 2011), whereas they need advanced instruments and highly skilled operators. Consequently, these methods are not suitable for consumers to evaluate the real-time freshness.

In the last decades, there was a rapidly growing interest in developing intelligent packaging systems for 'on-package' tracing the real-time food quality. Particularly, colorimetric indicators have received wide attentions because they can exhibit straightforward information by visible color changes. As regard to evaluating the fish freshness, Pacquit et al. (2007) developed a colorimetric indicator by spin-coating bromocresol green onto a PET substrate. The color of the indicator gradually changed from yellow to green in response to the increasing TVB-N level at room temperature. Similarly, polyaniline-based colorimetric indicator has also been demonstrated to detect the fish spoilage (Kuswandi, JayusRestyana,

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http://dx.doi.org/10.1016/j.foodhyd.2017.02.014
0268-005X/

Abdullah, Heng, & Ahmad, 2012). These colorimetric indicators fixed in the headspace of the packaged fish presented specific color changes upon reaction with the TVB-N in the form of gas sensors. In this way, these intelligent packaging systems had great potential to indicate the real-time fish freshness.

Recently, more researches have focused on the natural pigments as a source of color agents, because they are safer and more eco-friendly as compared to chemosynthetic dyes. Anthocyanins are natural water-soluble pigments that have wide response ranges to pH variation. Several kinds of anthocyanins have been utilized to fabricate the colorimetric indicators for sensing the food quality, such as anthocyanins extracted from red cabbage (Pereira, de Arruda, & Stefani, 2015), grape skin (Ma & Wang, 2016) and purple sweet potato (Choi, Lee, Lacroix, & Han, 2017). Zhang, Lu, and Chen (2014) developed a pH sensing film by incorporating anthocyanins extracted from *Bauhinia blakeana Dunn* with chitosan and the pH sensing film presented a distinguishable color change from purple to green due to the basic volatile gases generated from the fish, suggesting that the anthocyanins-based colorimetric films were good candidates of gas sensors for monitoring fish freshness. When a constant amount of fish samples was stored in a specific circumstance (e.g. temperature, packaging volume), the concentration of the TVB-N in the headspace was definite after a specific period of storage. Therefore, the extent of color change of the colorimetric film was related to the content of the anthocyanins. However, the effect of the anthocyanins content on color rendering properties of the colorimetric film has not been investigated yet.

Roselle (*Hibiseus sabdariffa* L.) is an herbaceous plant, cultivated largely in tropical and subtropical areas of both hemispheres (Sinela et al., 2017). Its calyxes contain high amounts of anthocyanins up to 1.5 g/100 g on dry weight basis (Degenhardt, Knapp, & Winterhalter, 2000). The biological activities of roselle anthocyanin (RACNs), such as antioxidant activity (Tsai, McIntosh, Pearce, Camden, & Jordan, 2002) and anti-hypertensive effect (Ajay, Chai, Mustafa, Gilani, & Mustafa, 2007) have been widely studied, while the potential use of RACNs for the development of colorimetric indicators has not been explored. In order to immobilize the anthocyanins, several natural polymers have been used for making different colorimetric films, including chitosan (Zhang et al., 2014), starch (Choi et al., 2017; Golasz, Silva, & Silva, 2013) and cellulose (Ma and Wang, 2016). Among them, starch has received greater attention because of its stability to heat, acid and base conditions. However, pure starch film generally lacks the strength and processability, which can be alternatively addressed by adding polyvinyl alcohol (PVA) (Sin, Rahman, Rahmat, & Mokhtar, 2011). Since 1980s, starch/polyvinyl alcohol (SPVA) films have been studied for packaging applications (Tang & Alavi, 2011; Tang, Zou, Xiong, & Tang, 2008). They have good transparency (Cano, Cháfer, Chiralt, & González-Martínez, 2015a), which is beneficial for the development of colorimetric films. Furthermore, starch and PVA are both non-toxic, renewable and biodegradable (Lu, Xiao, & Xu, 2009; Rezaei, Nasirpour, & Fathi, 2015), which can eliminate the public concerns over food safety and environmental problems.

Therefore, in this study, we aimed to develop new colorimetric films by incorporating various content of RACNs into SPVA matrix through casting/solvent evaporation method. The microstructure of colorimetric films was studied by using X-ray diffractometer and SEM. The effect of the RACNs content on mechanical properties, color stability and sensitivity toward ammonia of the colorimetric films was investigated. Finally, the colorimetric films were employed to monitor the freshness of silver carp (*Hypophthalmichthys molitrix*) at refrigeration temperature (4 °C).

2. Material and methods

2.1. Materials

Roselle dehydrated calyxes and live silver carp were obtained from the local market in Zhenjiang, China. Other materials including soluble starch, polyvinyl alcohol (MW: 1750 ± 50), ethyl alcohol (C_2H_6O), potassium chloride (KCl), sodium acetate ($CH_3COONa \cdot 3H_2O$), magnesium oxide (MgO), methyl red ($C_{15}H_{15}N_3O_2$), methylene blue ($C_{16}H_{18}ClN_3S$), boric acid (H_3BO_3), ammonia solution ($NH_3 \cdot H_2O$, 25%–27%), acetic acid (CH_3COOH) and hydrochloric acid (HCl) were all purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Plastic Petri dishes were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Extraction of anthocyanins from roselle dehydrated calyxes

The anthocyanins were extracted according to Chang et al. (2012) with a slight modification. The roselle dehydrated calyxes were crushed and blended with 75% ethanol aqueous solution at a solid–liquid ratio of 1:10 for 2 h at 25 °C. The extract was centrifuged at 8000 rpm for 20 min. The extraction procedure was repeated three times. Ethanol in filtrate was removed with a rotary evaporator at 35 °C in dark. Finally, the solution was freeze-dried under vacuum and the obtained anthocyanins extract powder was stored in sample vials at 4 °C in the nitrogen atmosphere.

2.3. Determination of total anthocyanins content in extract powder

The anthocyanins content in extract powder was measured by pH differential method (Wang, Li, Chen, Xin, & Yuan, 2013) using a UV–Vis spectrophotometry (Agilent CARY 100, Varian Corporation, USA). The anthocyanins extract powder (20 mg) was dissolved in 10 mL distilled water, and 1 mL anthocyanins solution was dissolved in 9 mL of 0.025 M potassium chloride buffer (pH 1.0) and 9 mL of 0.4 M sodium acetate buffer (pH 4.5) respectively in separate test tubes. Absorbance of sample was measured at 520 and 700 nm. The anthocyanins content was expressed in mg/g.

2.4. Preparation of the colorimetric films

Firstly, 100 mL aqueous dispersion containing 2 g starch and 1 g PVA was heated at 100 °C in a water bath and stirred with a magnetic stirrer until it was completely dissolved. Based on the calculated anthocyanins content (9.51 ± 0.41 mg/g) (refer to section 2.3), a certain amount of anthocyanins extract powder was then added to the cooled SPVA solution at 30, 60 and 120 mg RACNs/100 g starch, expressed as RACNs-30, RACNs-60 and RACNs-120, respectively. The mixtures were then homogenized (Ultra Turrax IKA T25 digital, Germany) at 8000 rpm for 5 min and degassed with a sonicator (Branson CPX5800H, USA) for 5 min at room temperature. Each film was prepared by casting 10 mL of the film-forming solution into a clean and smooth plastic Petri dish with a 53 mm diameter. The Petri dishes were placed on a level surface in an incubator at 35 °C with 50% RH for 36 h. After that, the films were peeled from the Petri dishes and stored at 4 °C with 75% RH for further use.

2.5. Spectral characteristic of RACNs

The color and spectra of RACNs solution at different pH (2–12) were recorded using a UV–Vis Spectrophotometer (Agilent CARY