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A visual pH sensing film using natural dyes from *Bauhinia blakeana Dunn*

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ABSTRACT

Natural dyes extracted from the flower of *Bauhinia blakeana Dunn* were immobilized in chitosan to prepare a colorimetric pH sensing film, which color changed from red to green in the pH range 2.2–9.0. The UV–vis spectra of the natural dyes in solution and in the sensing film were studied. The characteristics of the pH sensing film, including response time, reproducibility, reversibility, stability toward light and the storage time, were investigated. Since pH value of a pork or fish sample concerns its freshness, the pH sensing film is expected to provide a convenient, non-destructive and visual method to help the estimation of pork and fish spoilage in ambient conditions.

1. Introduction

In recent years, numerous efforts have been directed toward the development of optic or visual pH sensors because of their advantages such as small and compact size, safety, long distance transmission, sensitivity and low cost [1]. Generally, most optic or visual pH sensors are composed of a pH-sensitive dye and a solid support. In the fields of food safety, it has been reported that the microbial population and pH of foods increase over the storage period as result of pork deterioration [2] owing to the protein spoilage, revealing the relationship between the pH value of foods and their freshness. The pH sensing provides a useful approach to monitor or indicate the food freshness. Under the consideration, several scientists have reported their results on monitoring pH variations of chilled pork deterioration using chitosan as a immobilization matrix [3]. In general pH sensing, chemical reagents such as bromocresol purple [4], bromocresol green [5], bromophenol blue, chlorophenol red and cresol red [6] have been chosen as pH-sensitive dyes. There have been reported that a pH sensing approach for fish spoilage using a pH-sensitive dye, bromocresol green [7], but, in food applications, the synthetic chemical compounds are difficult to use owing to their possible toxicity and health threat to human beings. Recently, it has been reported that curcumin could be used for the real-time monitoring of shrimp spoilage, indicating that the application of natural dyes extracted from plants. Although the application has begun in food detection and become the trend, the color change of curcumin from yellow to orange is not very obvious. Compared with these chemical reagents, in general, natural dyes have obvious advantages because of their low toxicity and easy preparation, and they are renewable and pollution-free. Recently, natural dyes containing anthocyanin have revealed great potential for development as pH indictor dyes due to their color changing significantly at different pH values. In addition to the usage of curcumin [8] and purple cabbage pigment [9], anthocyanins extracted from grapes anthocyanin from grape and spinach extracts as pH indictor dyes have been reported by Golasz [2], Yoshida [3] and Veiga-Santos et al. [10].

For the immobilization of the pH sensing dyes, many solid matrixes, including glass beads, polymeric materials, hydrogel polymeric membranes, sol–gel matrices [11] and even cassava starch [2,10] have been evaluated and applied. Among the immobilization materials, chitosan is a type of natural polyaminosaccharide produced through the alkaline deacetylation of chitin from insect cuticles and crustacean shells, and has proved good biocompatibility, biodegradability and antibacterial properties. Chitosan membranes have been explored in many usages, such as in water–ethanol pervaporation, enzyme immobilization and cationic specimen transportation, protein separation and concentration, controlled ingredient-release and environmental applications [12] since it is good permeability and adsorption, and a good degree of mechanical properties [13]. Recently, chitosan

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has been used as immobilization matrixes to construct pH [3] and temperature [14] films.

In this study, two bio-based materials were used to develop a novel colormetric pH sensing film. *Bauhinia blakeana Dunn* is widely cultivated in China and has attracted people because of its long florescence period and gorgeous flower color. The flower has been used as a traditional Chinese medicine because of its medical functions [15] and non-toxity. In our study, natural dyes containing anthocyanin were extracted from *B. blakeana Dunn*, and then were immobilized in chitosan to be used as a pH sensing film. The color change of the pH sensing film is sensitivity and can be observed by the naked eye in the pH range 2.2–9.0. According to the previous report that pH values relate to pork or fish deterioration, the sensitive pH sensing film will provide a convenient, rapid and non-destructive method to help the estimation of pork and fish spoilage in ambient conditions.

2. Experimental

2.1. Materials and reagents

B. blakeana Dunn was collected from the campus of Xiamen University. The flowers were cleaned using water and dried in an oven at 45 °C, then powdered using a stirring mill (Philips, HR2006). The natural dyes containing anthocyanin were extracted from the flower powder using ethanol/water (40/60, v/v) for 30 min at 60 °C with sonication. The filtrates had the solvents removed at 50 °C using a rotary evaporator (Heidolph, Germany), and then, the concentrated solution of the natural dyes was kept in a refrigerator.

Chitosan was purchased from J&K (China; degree of deacetylation, 18–20%; molecular weight, 100,000–300,000). Buffer solutions were prepared with citric acid/disodium hydrogen phosphate ($C_6H_8O_7/Na_2HPO_4$) and their pH values were measured using a digital pH meter (CyberScan pH 510, EUTECH). Glutaraldehyde 25% solution in water was a biochemical reagent. All chemicals were used without further purification. All solutions were prepared using ultrapure water obtained from a Millipore purification system (Millipore, Ltd., USA). Fresh rib meat as pork samples were purchased from a supermarket in Xiamen. The meat was cut into slice and stored in a refrigerator until used. Alive fish samples of yellow cartfish (*Pelteobagrus fulvidraco*) purchased from a supermarket in Xiamen free market were selected in the experiment. The alive fish was bled, gutted and get rid of its head and tail. The other parts were cut into four pieces and stored in a refrigerator until used.

2.2. Preparation of the pH sensing film

Chitosan acetic acid solution was obtained by dissolving 1.0 g chitosan powder in 100 mL 1% (v/v) acetic acid solution stirred with a magnetic stirrer at 60 °C until the powder was completely dissolved. The solution was then cooled to ambient temperature, and 1% (wt %) glutaraldehyde (based on chitosan) [16] was added slowly into the solution and cross-linked for 30 min. 1.5 mL of the cross-linked chitosan solution was then dipped onto a glass slide (48 mm × 12.4 mm × 0.9 mm). The prepared glass slide was dried in ambient temperature for 24 h, then in an oven at 40 °C for another 24 h. After solvent evaporation, the film was immerged into the concentrated solution of the natural dyes for 24 h, then washed using ultrapure water and put in citric acid–Na_2HPO_4 buffer solution (pH 5.0) overnight. After these processes, the prepared sensing film was stored in the dark before use.

2.3. Apparatus

The UV–vis spectra of the natural dye and the sensing film in the different pH buffer solutions were recorded using a SHIMADZU UV-2550 UV-vis spectrophotometer in the range 400–800 nm. A Nicolet iS10 was used for the FTIR analysis of the film. The color images of the sensing film were captured using a Nikon D300 digital camera (Japan).

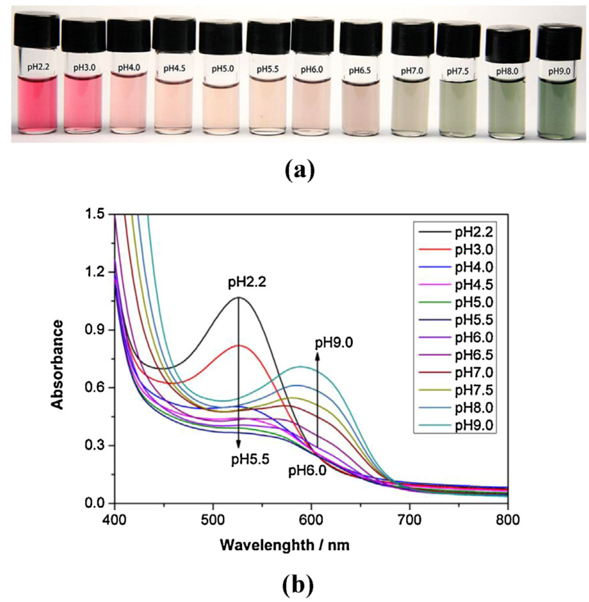

Fig. 1. Colors (a) and UV–vis spectra (b) of the natural dyes containing anthocyanin at different pH citric acid–Na_2HPO_4 buffer solution. (For interpretation of the references to color near the citation of this figure, the reader is referred to the web version of the article.)

2.4. Sample analysis

50 g fresh pork or fish sample was placed on a 60 mm culture dish. To ensure a closed space, the dish was wrapped with a fresh-keeping film conglutinating the pH sensing film. The sensing film was used to observe the pH change in the headspace of the sample, and its color change was recorded at intervals using a digital camera. All samples were stored at 25 °C during pH detection.

3. Results and discussion

3.1. UV–vis spectra of the natural dyes containing anthocyanin in solution and the sensing film

The color of the natural dyes containing anthocyanin changed obviously at different pH. As shown in Fig. 1a, when the solution pH was lower than 4.5, the dyes presented a red color. The solution color changed to pink around pH 5.0–5.5, and then from purple to green in the pH range from 6.5 to 9.0. The UV–vis spectra of these solutions over the same pH range were tested and, as shown in Fig. 1b, in the pH range 2.0–4.5, an obvious absorption peak around 528 nm could be obtained. The intensity decreased with the increase of pH, and the peak almost disappeared when the solution was around pH 5–5.5. In the higher pH solution, an absorption peak around 600 nm appeared and obviously increased from pH 7.0, corresponding with the color change from purple to green.

Chitosan film is homogenous, insoluble in water and alkaline solution, but soluble in acidic medium. To avoid the soluble limitation of chitosan film in lower pH, in our study, glutaraldehyde was used to cross link chitosan in order to decrease its insolubility and to improve its practicability in acidic conditions. Furthermore, the amine groups on the chitosan underwent the protonation process (forming protonated amine), by which the natural dyes containing anthocyanin could be absorbed through various types