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pH-sensitive films containing anthocyanins extracted from black bean seed coat and red cabbage

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ABSTRACT

The aim of this study was to develop pH-sensitive films based on corn starch and anthocyanins extracted from black bean seed coat and red cabbage. The pH-sensitive films were developed from solvent casting of polymer solutions containing corn starch, glycerol, and anthocyanin extract (from red cabbage or black bean) prepared at pH 5. The color of films changed from pink to purple and blue, as a function of the pH. The pH-sensitive films were evaluated by their morphological, chemical, physical, mechanical and thermal properties. In addition, the stability was evaluated during 28 days of storage (presence and absence of light; with and without cooling). The pH-sensitive films with red cabbage anthocyanins showed a higher stability than that with black bean anthocyanins when stored at room temperature and exposed to light. Both pH-sensitive films exhibited greater color stability when stored under refrigeration as compared to storage at room temperature.

1. Introduction

Many intelligent packaging systems are capable of providing consumers with quality information in real time for packaged food products (Rukchon, Nopwinyuwong, Trevanich, Jinkarn, & Suppakul, 2014). For example, colorimetric pH indicator has been exploited in intelligent packaging due to its easy to use, low cost, and well-characterize properties. It can be integrated into food packaging structures to monitor the changes in acidic and basic components in food products (e.g., CO_2, organic acids, amines, ammonia), allowing consumers to check the quality of the food as the indicator changes color (Silva-Pereira, Teixeira, Pereira-Júnior, & Stefani, 2015). The use of natural polymers for the development of packaging has been widely investigated due to their biodegradability and reduction of the accumulation of waste in the environment. Corn starch is an interesting alternative for the development of packages because it is a low cost polymer, widely distributed in nature, involves a simple process of obtaining, being biodegradable, besides being able to be transformed into a thermoplastic material in the presence of a plasticizer, with application of thermal and mechanical energy, forming thin, flexible, transparent films, besides allowing the incorporation of substances with specific properties such as anthocyanins.

Natural pigments such as anthocyanins can be added to biodegradable starch films to provide the desirable functional properties of a pH indicator. Anthocyanins are secondary metabolites widely distributed in fruits and vegetables (e.g., red cabbage, sweet potato, bean husk, grapes), making them a promising source of natural indicator that covered a broad color spectrum as a function of pH (Ananga, Georgiev, Ochieng, Phills, & Tsolova, 2013). Several studies investigated the use of biopolymers and anthocyanins for the production of pH indicators. For example, Veiga-Santos, Ditchfield, and Tadini (2011) developed biodegradable pH indicator films based on cassava starch plasticized with sucrose and invert sugar containing grape and spinach extracts as sources of anthocyanin and chlorophyll, respectively. They reported that indicators containing the anthocyanin extract exhibited greater color spectrum, and are more efficient for pH monitoring in comparison with those containing chlorophyll or a mixture thereof. Silva-Pereira et al. (2015) developed a pH indicator for monitoring the deterioration of fresh fish fillets, consisting of chitosan, corn starch, and red cabbage extract. The indicator films exhibited optical and

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morphological properties sensitive to the changes in pH as the product, suggesting that the indicator was potentially useful as a quality indicator.

Anthocyanins derived from various sources may exhibit different intensity and color stability due to their inherent differences in chemical structures (Ananga et al., 2013). Studies related to the evaluation of time, temperature, and light effects on the stability of pH indicators, especially those developed with anthocyanins, are limited. The aim of this study was to develop a pH-sensitive film based on corn starch and anthocyanins extracted from black bean seed coats and red cabbage leaves. The films were evaluated for chemical structure, and their morphological, physical, mechanical and thermal properties. The color stability of pH-sensitive films was evaluated during 28 days of storage at 4 °C and 25 °C under dark and under light.

2. Material and methods

2.1. Material

Commercial corn starch (amylose 31%; supplier *Unilever*) was used for the preparation of the films. Black beans and red cabbage, purchased in local market in the city of Pelotas, RS, Brazil, were used for the anthocyanin extraction. The seed coats of black beans were manually separated with aid of a scalpel. The red cabbage was cut manually with a knife. Subsequently the samples of black bean seed coat and purple cabbage, individually, were frozen with liquid nitrogen and milled in a ball mill (Marconi, MA 350, Brazil) to obtain a powder for subsequent extraction of the anthocyanins (Davis, Rodriguez-Saona, & Wrolstad, 2001). The pelargonidin standard was purchased from Sigma-Aldrich, with ≥90% purity. The eluent was filtered with a nylon filter of 0.45 μm.

2.2. Extraction of anthocyanins

The extraction and quantification of anthocyanins were carried out according to the method described by Francis (1982). In falcon tubes, 1 g of black bean seed coat sample or 10 g of red cabbage sample was added, 30 mL of acidified ethanol (85 mL of ethanol P.A. and 15 mL of 1.5 mol L^{-1} HCl) was added and submitted to constant agitation (Phoenix, AP-22, Brazil) for 1 h. The supernatant was separated manually and 20 mL of acidified ethanol was added again in the sample, being subjected to constant agitation. After the extraction, the solvent fractions were pooled, filtered and the spectrophotometer readings (Jenway, 6705, England) were run at 525 nm for quantification. The extracts of anthocyanins were diluted in acidified ethanol until reaching a concentration of 0.07 mg mL^{-1}, and then used to elaborate pH-sensitive films.

2.3. Identification and relative quantification of anthocyanins

Liquid chromatography-mass spectrometry (LC-MS) analysis was performed on a UFLC system (Ultra Fast Liquid Chromatograph Prominence, Shimadzu, Japan), consisting of a degasser, a binary pump, an autosampler, and a temperature-controlled column compartment. The mass spectrometer (Bruker micrOTOF Impacto HD, Bruker Daltonics, Bremen, Germany) has a dual electrospray ionization source, with positive ionization detection mode. Mobile phase A was consisted of 0.1% formic acid in water, while mobile phase B consisted of 0.1% formic acid in acetonitrile. Mobile phase gradient used was as follows: 0 min - 5% B; 4 min - 80% B; 6 min - 80% B; 7 min - 15% B; and 15 min - 15% B. The equilibration time between successive runs was 5 min. Other operating parameters were as followed: flow rate 0.4 mL min^{-1}; injection volume 1 μL; column temperature 35 °C.

MS analysis was carried out in positive ionization mode with spectra acquired over a mass range of *m/z* 50 to 1200. The operating parameters were: capillary voltage 4.0 kV; drying gas temperature 180 °C; drying gas flow rate 8.0 L min^{-1}; nebulizing gas pressure 2 bar; collision RF 150 Vpp; transfer time 70 μs; and pre-pulse storage 5 μs. Furthermore, automatic MS/MS experiments were performed by adjusting the collision energy values as follows: *m/z* 100, 15 eV; *m/z* 500, 35 eV; and *m/z* 1000, 50 eV, using nitrogen as the collision gas. The MS data was processed by Data Analysis Software 4.0 (Bruker Daltonics, Bremen, Germany), which provided a list of possible elemental formulas.

The anthocyanins of the black bean seed coat and red cabbage were characterized by their mass spectra UV/Vis fragmentation patterns MS^n UV/Vis (220–800 nm), and compared with data from the database (Metlin, MassBank, Kegg). The calibration curve was developed by adding pelargonidin standard into the sample matrix at different concentrations. Anthocyanins present in the samples were quantified using the peak area of pelargonidin and its concentration.

2.4. Preparation of the pH-sensitive films

The pH-sensitive films were prepared by a casting technique, according to the methodology described by Silva-Pereira et al. (2015), with some modifications. The film solution was prepared from 3 g of commercial corn starch, 0.9 g glycerol and 80 mL of distilled water. The film solution was subjected to heating in a water bath (Fisatam, 550, São Paulo, Brazil) at 85 °C for 15 min. After cooling to 40 °C 20 mL of anthocyanin extract at a concentration of 0.07 mg mL^{-1} (previously determined in spectrophotometer) was added, followed by adjusting the solution to pH 5 using a NaOH 1 mol L^{-1} solution. The extract concetration used in this work was defined according to preliminary test results. Subsequently the solution was homogenized in an Ultra Turrax (IKA, T18 Basic, Germany) at 14,000 rpm for 10 min. Next, 20 g of the film-forming solution was spread on a rimmed acrylic dish, 9 cm in diameter, and dried in a convective air oven (Ethik, 420TD, Goiânia, Brazil) at 30 °C for approximately 16 h. The films were then conditioned at room temperature (16 °C) and 60% RH for 48 h before testing. The control film was prepared under the same conditions, except that the anthocyanin extract was replaced by acidified ethanol.

2.5. Morphology

The morphologies of the surface and the cross section of the pH-sensitive films were evaluated by a scanning electron microscope (Jeol, JSM-6610LV, USA), under an accelerating voltage of 10 kV at 500× magnification.

2.6. Solubility, thickness and mechanical properties

The solubility of the pH-sensitive films was determined according to Gontard, Duchez, Cuq, and Guilbert (1994). The films were cut in a circle with a diameter of 2.5 cm and kept in an oven at 105 °C for 24 h. The samples were then immersed in 50 mL of distilled water in falcon tubes and subjected to constant stirring on a horizontal shaker table at 175 rpm and a temperature of 25 °C for a period of 24 h. After the resulting films were removed from the tubes, and dried at 105 °C until constant weight. The solubility was expressed in terms of solubilized mass, through the relation between the initial and final mass of the films. Film thickness (mm) for each film was determined by taking ten measurements using a digital micrometer. Mechanical properties of the film (tensile strength and percent elongation) were measured using a