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ORIGINAL ARTICLE

Preparation and Characterization of Biocompatible Films Based on Cassava Starch/Bovine Gelatin/Zinc Oxide Nanorod Supported with Saffron Anthocyanin

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KEYWORDS

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ABSTRACT: In the presentation study, cassava starch/bovine gelatin/zinc oxide nanorod (n-ZnO) active bionanocomposite films were fabricated by incorporating saffron anthocyanin (SA) using a solvent casting technique. Their physicochemical properties such as, antioxidant, total phenolic content (TPC), thickness, and antibacterial activity, were investigated. Incorporating SA significantly ($P<0.05$) increased the film thickness from 0.06 mm to 0.14 mm. The introduction of the 2.5% SA into the biofilms also increased their antioxidant properties (DPPH radical scavenging) by more than 5 times. TPC of the cassava/gelatin films was improved by increasing the SA contents. Starch/gelatin/n-ZnO/SA films represented excellent antimicrobial behavior against *S. aureus* and *E. coli*. These characteristics suggest that saffron anthocyanin has good potential as a biofiller in composite films based on cassava starch/bovine gelatin/n-ZnO for active films in the food and pharmaceutical sciences.

INTRODUCTION

To date, synthetic plastics have been commonly used in packaging because of their inexpensive manufacturing costs and strong mechanical properties[1]. Petrochemical plastics employed in different industries have environmental issues due to the fact they are based on synthetic polymers, are non-degradable, generate greenhouse gases, and present global concern [2]. Hence, it is important to fabricate novel biopackaging that is "green" and renewable with the same properties as petrochemical packaging[3].

In the category of polysaccharides, starch is the cheapest polysaccharide. Starch constitutes 60% of the composition of cereal grains. The ratio of amylose to amylopectin depends on the age of the starch, typically comprising 20%-25% amylose and 75%-80% amylopectin[4]. The biodegradability and recyclability of starch make it highly suitable for packaging [5]. As mentioned, starch is composed of amylose and a high amount of amylopectin, which has weak mechanical and physical properties. The phenomenon of recrystallization results in harder films with less stretchability. The addition of other polymers to the starch matrix with organic and inorganic fillers improves the mechanical and physicochemical properties of starch films [6].

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The advances in nanotechnology have led to the development of materials with new properties used as antimicrobial agents. Studies have shown that nanoparticles such as titanium, silver, chromium, and zinc and their oxides have high antibacterial properties, good mechanical characteristics, and hydrophobic behavior[7-9].

In the last two decades, antimicrobial packaging has attracted significant attention [10]. Edible coatings have been considered to delay or prevent the growth of microorganisms in many foods [11]. Antimicrobial packaging made from bionanocomposites represents a new generation of packaging with a nanostructure, produced by combining metal nanoparticles with biopolymers [12]. In active packaging, the release of antimicrobial agents from the polymer matrix to the food surface occurs slowly, maintaining a high concentration of the antimicrobial agent on the product surface [13].

Anthocyanin pigment has different physiological fine effects, including antioxidant, anticancer, anti-inflammatory, neuroprotective capabilities, and antibacterial [14].

Saffron (*Crocus Sativus* L.), commonly cultivated in Iran and devoted about 90% of saffron production worldwide, has violet-colored flowers with anthocyanin pigments as the major colorant [15]. The saffron petals composed a bold portion of the dry weight of plant, proposing that these may be valuable by product and a source of anthocyanin pigments [16]. The principal antioxidant chemicals in petals, such as flavonoids, anthocyanins, and flavonols, are responsible for these functional qualities [17]. Various saffron compounds contain kaempferol, helichrysosid, astragalin, kaempferol-3-glycopyranosyl (1-2)-6 acetylglucopyranoside, miricetin, kaempferol-3-glucopyranosyl(1-2)-glucopyranoside, quercetin, petunidin, and delphinidin [15].

Recently, films based on biopolymers have been reinforced with saffron extract rich in anthocyanin colorants to develop an active film. To the best of our knowledge, there is no scientific paper on total phenolic content, antioxidant properties, and antibacterial activity of film with n-ZnO/saffron anthocyanin, so the current study purposed to assay saffron anthocyanin effects on functional characteristics of the biocompatible film.

MATERIALS AND METHODS

Materials

Ethanol, bovine gelatin, plasticizer including sorbitol and glycerol, Folin-Ciocalteu, and cassava starch were obtained from Sigma-Aldrich, USA. Nanoparticle-ZnO was obtained from Nano Pooyeshyekta, Iran. Saffron petals were provided from Mashhad, Iran. Also, Mueller Hinton agar, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Na_2CO_3 were purchased from Merck Germany.

Saffron anthocyanin extract

Petals of saffron were dried at 25°C after the stigma was separated, and then dried saffron was sieved and stored in a dark condition at 4ºC. The anthocyanin pigment extraction was conducted using the method stated by Khazaei, et al. [18]. Powdered petals were mixed with ethanol: citric acid-acidified water (25:75 v/v), at a ratio of 20:1 (w/v), for 1day before being centrifuged for 15 min at 5000 g to separate any remaining solids. After that, the solvent was removed from the anthocyanin extract using a rotary evaporator (Heidolph Hei-VAP, GER), and the final filtrate was stored in a dark container at 4°C.

Film fabrication

To produce the active film, 0.5% n-ZnO (based on cassava powder w/w%) was combined with 100 mL of deionized water. The solution was stirred for 4 h. The deionized water samples with n-ZnO were exposed to ultrasound wavelength. Exactly 4 g of cassava powder, 0.4 g of gelatin powder [19], and sorbitol/glycerol (1.6 g) [20] were added to the final suspension. All the nano-suspensions were heated at 87°C for 40 min. During the cooling term, different levels of SA (2.5%, 5%, and 10% v/v) were employed in the biofilm solution. Finally, pure suspensions and solutions containing SA were decanted into casting plates and dried at 25°C to make biodegradable films.

Antioxidant activity and thickness

The antioxidant activity of active films incorporated with various amounts of SA was evaluated by DPPH (1,1-

diphenyl- 2-picrylhydrazyl) free radical measurement [21]. The samples (50 mg) were combined with the DPPH radical (0.1 mM) in 90% ethanol (10 mL). The color changes were assayed at 517 nm after 30 min of reaction in dark conditions. The pure solution was fabricated by combining ethanol (1mL) and DPPH radical. The %DPPH scavenging activity from the biocompatible films was computed using the following equation

$$DPPH\%=((Abs_c-Abs_t)/Abs_c)\times 100$$

Where the Abs_t is the absorbance of the biofilm sample and Abs_c is the absorbance of the pure sample.

To assay thickness, five various places of active films using a micrometer were measured the data average was reported as the thickness of the film.

Total phenolic content (TPC)

TPC of edible films was evaluated according to the Folin-Ciocalteu technique, as stated by Jridi, et al. [22], with slight modification. About 2 mL of 10%Folin-Ciocalteu reagent was combined with 0.5 mL of biofilm extract or SA in the dark room for 5 min. After that, 2.5 mL of Na_2CO_3 solution (7.5 %w/v) was incorporated into the solution and stored at 25 °C in the dark. At last, the absorbance of the specimens was measured at 765 nm. A calibration curve ranging from 0 to 100 µg/mL was established utilizing gallic acid as the standard reference.

Antibacterial activity

An antimicrobial behavior experiment on the biofilms was performed using the agar diffusion technique based on Maizura, et al. [23]. Plates containing Mueller Hinton agar (MHA) were seeded with inoculums (1 mL) containing about 10^5–10^6 CFU/mL of *S. aureus* and *E. coli*. Biofilm disks were put on the plates and incubated for 24 h at 37°C. Finally, the plates were evaluated for the "inhibition zone" of the biofilm discs. Antimicrobial impacts of the biocompatible films were measured by calculating zone of inhibition against *E. coli* and *S. aureus*. The inhibition zone is evaluated as follows:

$$\text{Inhibition zone}= A_w-A_d$$

where the A_w is the whole zone area of the biofilm sample and A_d is the area of the film disk

Statistical analysis

Antioxidant activity, thickness, total phenolic content, and antibacterial activity were performed with 5 replicates. Statistical analysis was carried out using SPSS software (version 27.0.1) and ANOVA test. To assay the significant difference among the averages, Tukey's test technique was applied ($p < 0.05$).

RESULTS AND DISCUSSION

Antioxidant activity and thickness

The antioxidant properties of film materials play an important role in food quality, as lipid oxidation can result in discoloration, nutrient loss, and off-flavors[24].

The antioxidant activity and film's thickness are represented in Figure 1 a, and b, respectively. Antioxidant activity and thickness of film with 10% SA and film without extract were 56.03%, 4.84%, 0.14 mm, and 0.06 mm, respectively. Also, both antioxidant activity and thickness increased as the level of SA employed in the biocompatible film increased. The increase in antioxidant activity may be attributed to the radical-scavenging activity of the hydroxyl groups of the phenolic compound on the anthocyanin pigment [25].

Ekrami, et al. [17] observed an increase in the antioxidant activity of salep mucilage from 0% to 42.62% after the addition of 10% SA. Another study also revealed that the incorporation of SA could notably increase the antioxidant properties of chitosan/pullulan films [26].

The thickness of the biofilm can notably affect mechanical behavior, transparency, and oxygen and water vapor permeability of films [27].

Another work demonstrated that the increase in thickness based on corn starch with saffron extract was related to the biofiller embedded [28]. The different authors revealed that the thickness increase in the biocompatible films with higher filler concentration based on the extract was due to the high level of solid content [29, 30]. No change in the thickness based on konjac glucomannan with 1-4% saffron petal extract was shown in the study of

Hashemi and Jafarpour [31]. These data are probably related to the low level of filler in films.

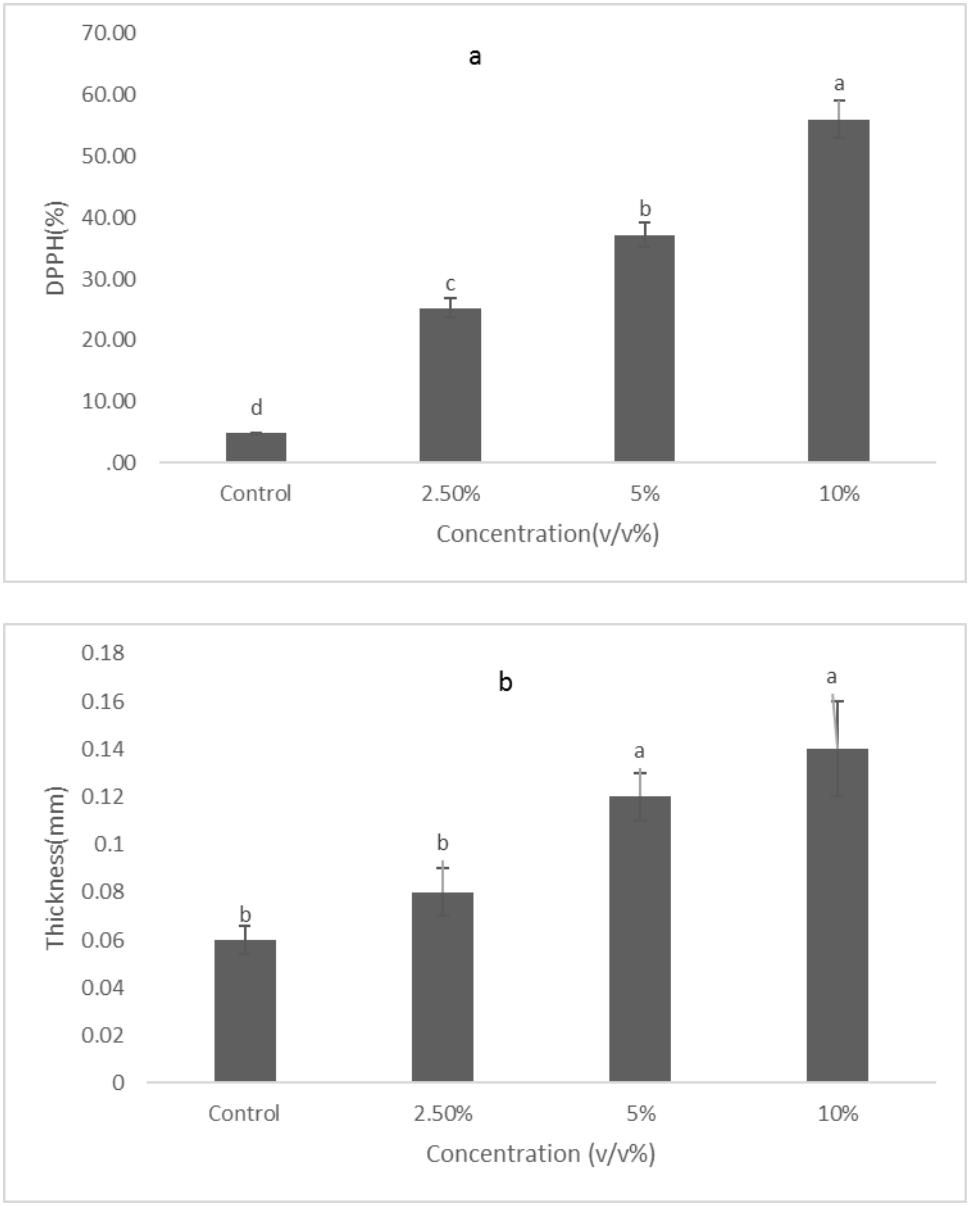

Figure 1. Antioxidant activity (a) and thickness (b) of neat and SA films

The bars reveal average ± standard deviation (SD). Different letters on the bars display the significant difference ($p<0.05$).

Total phenolic content

Phenolic compounds exhibit different biological activities, such as antibacterial and antioxidant effects [32]. Incorporating plant extracts containing phenolic compounds in biocompatible films contributes to the improvement of shelf life and prevents food spoilage[2].

The TPC of pure and films containing SA are indicated in Figure 2. The addition of SA has increased the TPC significantly ($p < 0.05$). Moreover, the TPC in film with 10% SA is the highest compared to the control and treatment films. The TPC of the cassava/gelation/n-ZnO was 0, and the biofilms containing 10% SA were 160.42 mgGAE/100g. The TPC of biofilm with 2.5 and 5% SA illustrated significant ($p<0.05$) increase as the anthocyanin pigment was added to the biodegradable film.

Carbohydrates, flavonoids, alkaloids, bibenzyl derivatives, glycosides, terpenoids, and alkaloids have all been detected as major compounds in saffron petals. Phenolic, flavonoid compounds, and anthocyanin pigments are likely the active components of the petals of

saffron [25]. Hence, when the SA content was increased from 0 to 10 v/v%, the TPC of the film developed.

A similar result was demonstrated by Ekrami, et al. [17]. They stated that the TPC of the salep mucilage film with SA gradually increased from 7.57 mgGAE/100g in the pure film to 158.99 mgGAE/100g in the biocompatible film with extract.

Similarly, Ebrahimi, et al. [33] reported that bovine gelatin-based films with purple basil leaf extract had a higher TPC than the neat sample film. According to these findings, they stated that the TPC of films was attributed to the level of SA added to the biofilms.

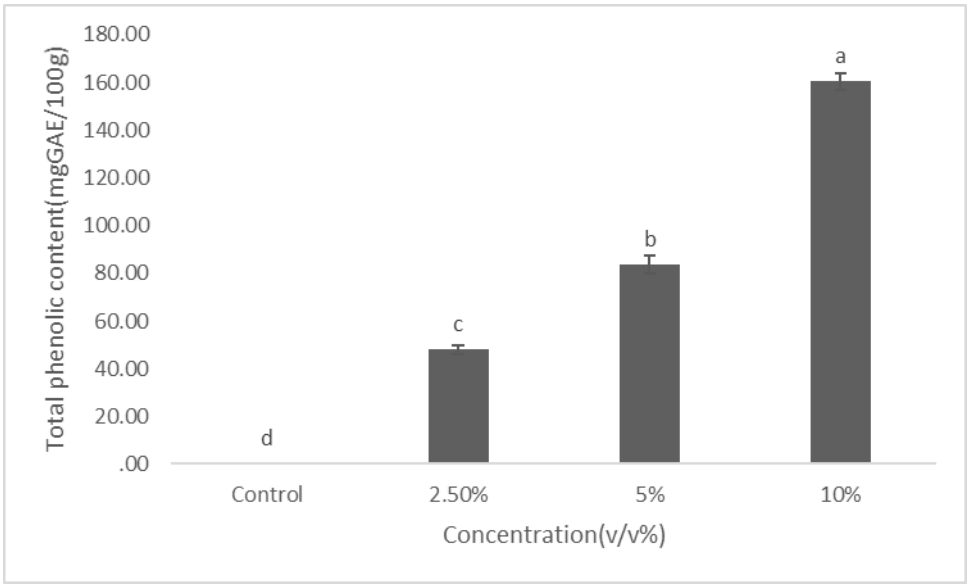

Figure 2. Total phenolic content of neat and SA films.

The bars reveal average ± standard deviation (SD). Different letters on the bars display the significant difference ($p<0.05$).

Antibacterial activity

Figure 3 displays the antibacterial activity of cassava/bovine/n-ZnO biodegradable film embedded with different levels of SA. The findings revealed significant increase ($p<0.05$) in the antibacterial activity of the biocompatible films with the development SA content in the bionanocomposite film structure. The inhibition zone of neat film against *E. coli* and *S. aureus* was 14.26, and 12.35 mm^2 and the value was changed to 35.34 and 40.96 mm^2, in film containing 10%SA, respectively.

The antimicrobial behavior of n-ZnO can be related to several mechanisms, such as the release of antibacterial ions [34]. Interaction of n-ZnO with *E. coli* and *S. aureus*, subsequently deteriorating the integrity of microorganism cell and the generation of ROS by the impact of light radiation [35]. In recently investigation reported that the antibacterial activity of n-ZnO may arise from the mechanical disruption of the bacterial cell membrane, which occurs due to the abrasive nature of the nanoparticles. This abrasiveness is attributed to surface imperfections, including corners and edges found on the ZnO nanoparticles[36].

Saffron anthocyanin extract seems to have antibacterial properties due to highly antibacterial compounds including, crocin and safranal [37]. The antimicrobial behavior of phenolic compounds, including anthocyanin pigments, has been suggested to be due to several mechanisms, such as their ability to develop cell membrane permeability, interfere with important metabolism pathways, and inhibit the absorption of vital compounds for cell growth [38].

Consistent with the antibacterial properties of the current investigation, the fabrication of salep mucilage film with SA could improve antibacterial activity against *E. coli* and *S. aureus* [33]. Also, another investigation observed that methyl cellulose film with saffron petal anthocyanin improved the antibacterial effects of biofilms. The authors reported that the methyl cellulose films with

saffron extract led to an increment of inhibition zone of biofilms against *E. coli* from 0 to 20.2 mm.

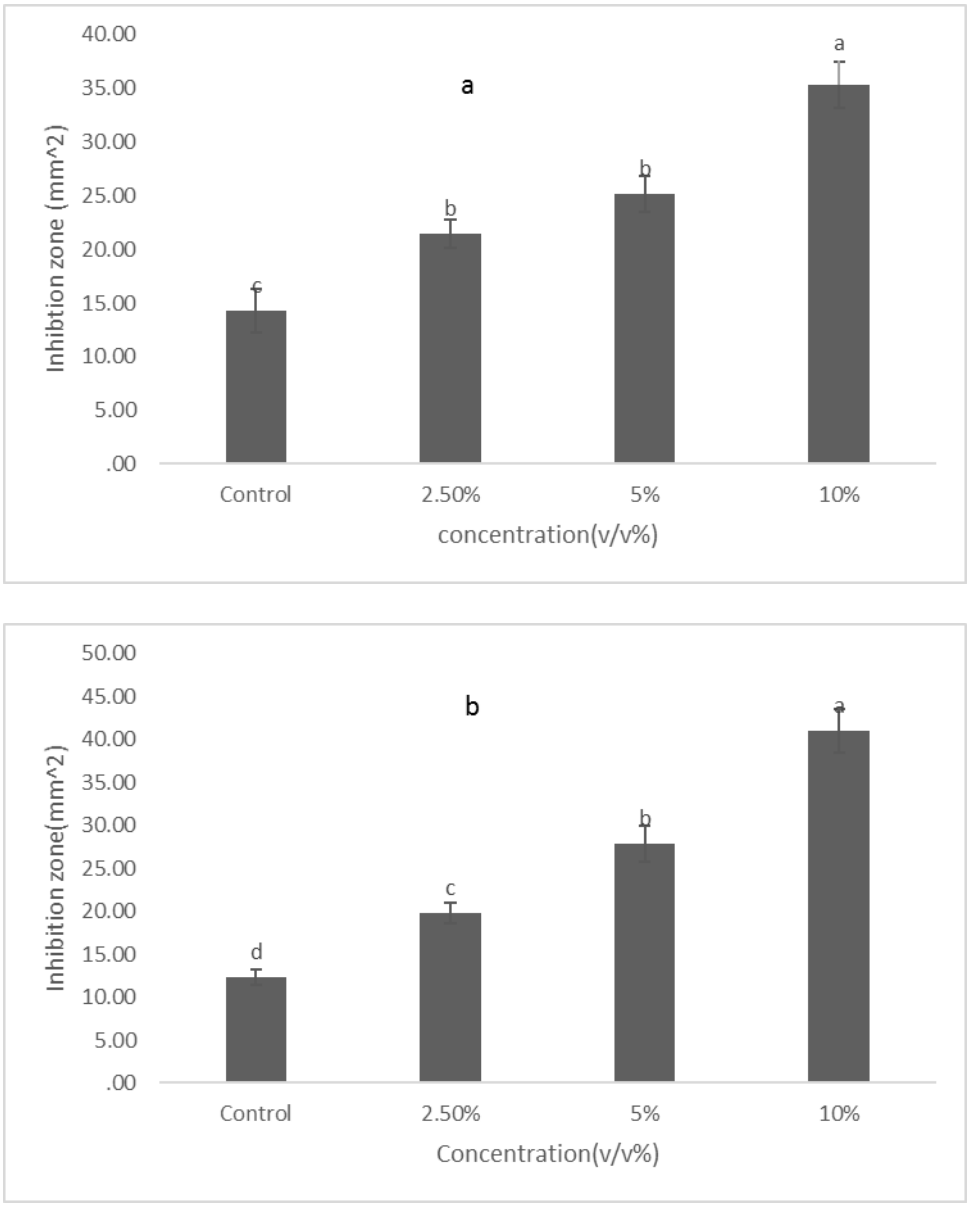

Figure 3. Inhibition zone of neat and SA films(a*) E. coli* and (b) *S. aureus*

The bars illustrate average ± standard deviation (SD). Different letters on the bars display the significant difference ($p<0.05$).

CONCLUSIONS

The active film based on cassava starch/gelatin/nanorod-zinc oxide containing saffron anthocyanin (SA) increased the thickness compared to the control biofilm. The active film with 10% SA indicated the greatest TPC. Also, the introduction of 10% SA represents high antioxidant activity of films. Furthermore, the antibacterial activity was developed after the addition of SA. The cassava starch/bovine gelatin/n-ZnO biofilms containing SA with increased antioxidant activity and TPC, as well as improved antibacterial activity could be employed as active, film food packaging.

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Conflict of interests

The authors declare that there is no conflict of interest.

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