



ORIGINAL ARTICLE

A Novel Biocompatible Film Incorporating Red Cabbage Anthocyanin Extract into Tapioca Starch/Bovine Gelatin/Zinc Oxide Nanorod

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ABSTRACT: The advances in the use of new biomaterials capable to improve the shelf life of foods may contribute to reduce the food waste problem. The presentation study, the effect of red cabbage anthocyanin extract (RCAE) at different amounts (1, 2.5, 5, and 10 v/v%) on the thickness, opacity, moisture content, and antioxidant properties of bionanocomposites film based on tapioca starch/bovine gelatin/0.5 zinc oxide nanorod (ZnO-N) was studied. The incorporation of RCAE up to 10% into the edible film increased the thickness of the film by about 40%, while the moisture content decreased by about 11.5% ($p < 0.05$). The antioxidant activity of the films increased from 4.74% to 92.28% after RCAE loading increased from 0 v/v% to 10 v/v%. With increasing in the RCAE content, the opacity of the biocompatible films increased. In summary, the functional characteristics presented that active film containing RCAE could be employed as a biodegradable packaging in food industry.

INTRODUCTION

Food packaging is an important part of food industrial because of its bold influence on the increment of quality of food, shelf life, and safety [1]. Plastics are commonly employed as packaging materials due to their low price and weight (compared to metals and glass), and processable characteristics, which can be shifted into various types of products including films, bottles, and containers [2]. Therefore, the many benefits make them attractive to food products. However, growing concerns are emerging regarding the negative impacts of plastics

on both human health and the environment [3, 4], thus, encouraging an interest in scholars to find alternatives for synthetic plastic. The possibility of biodegradable film fabrication from biopolymers such as polysaccharides, lipids, and proteins has been investigated by many scholars in recent years [5, 6].

The physicochemical properties of starch films show a wide variety depending on the additives and the processing method. Starch films have negative properties such as high hydrophilic behavior and poor mechanical

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properties compared to synthetic polymers [7, 8]. To produce starch-based packaging with desirable physicochemical properties, biopolymers must be modified by physical, mechanical, or chemical methods, or combined with nanoparticles, essential oils, and extracts [9]. Bionanocomposite film packaging is incorporating nanoparticles into food packaging as an antibacterial agent [10]. Nanobiocomposite films increase the shelf life and quality of food. In addition to being barriers to water vapor, and gases, these materials have good thermal and mechanical properties and can also serve as carriers for antioxidants and antimicrobial agents [11, 12].

Anthocyanin, which belongs to the flavonoid family, is the most commonly found natural colorant in plant sources [13]. These pigments are sensitive to various environmental factors such as pH levels, temperature fluctuations, and exposure to light. When exposed to unfavorable conditions, the color of anthocyanin pigments can change from yellow to colorless [14].

Red cabbage is highly consumed vegetable, easy to cultivate, and commercialized at low prices [15]. Red cabbage is known as one of the common sources of anthocyanin pigments, possessing fine nutritional value and favorable impacts on human health due to it has phytochemicals such as oligosaccharides, vitamins, minerals, and micronutrients and some bioactive compounds including high amounts of anthocyanin, glucosinolate, and flavonol [16]. Red cabbage contains anthocyanin pigment which is a soluble in water colorant that makes red, blue, and purple colors to plants [17]. Compared with anthocyanin pigments from other resources, anthocyanin of red cabbage have attracted much attention from scholars due to their abundance, availability, and low cost [18]. Thus, these characteristics make RCAEs suitable for employing in biocompatible films [19].

Due to the possibility of nanoparticles migrating into food packaging during fabrication and preservation, concerns have arisen regarding the safety and healthy of their use in packaging industry. This migration may negatively impact the sensory quality of food. Moreover, while certain nanoparticles possess safe properties, health issues might arise if the migration rate surpasses

the allowed and limiting values [20]. Based on EFSA study on the safety of ZnO nanoparticle in food contact materials, it is recommended that the Commission enforce a daily upper limit of 25 mg of Zn. The study also showed that the material does not migrate at the nanoscale, indicating that the safety assessment should focus on the migration of soluble Zn^{2+} [21].

Recently, biofilms have been supported with red cabbage rich in anthocyanin pigments to develop active packaging film. To the best of our knowledge, there is no study on physicochemical, and antioxidant properties of film with nanofiller/red cabbage anthocyanin extract so the presentation work purposed to evaluation red cabbage anthocyanin extract effects on functional properties of bionanocomposite film.

MATERIALS AND METHODS

Materials

Ethanol, plasticizer such as sorbitol and glycerol, bovine gelatin, and tapioca starch, were purchased from Sigma-Aldrich (Steinheim, Germany). Nano-ZnO was acquired from US-NANO (Oklahoma, USA). The red cabbage was provided from a local market in Semnan, Iran. Also, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Calcium chloride were obtained from Merck Co. (Germany).

Red cabbage anthocyanin extract

About 150g of chopped red cabbage was added to ethanol 70% (80mL) and pH of the mixture was controlled to 2. The solution was stored in a dark condition at 5°C for 1 day. After that, filtration and centrifugation for 25 min at 1008g were conducted and the suspension was separated. The anthocyanin extract of red cabbage was neutralized using NaOH (2.5 M) solution and employed for the film fabrication [22].

Film fabrication

To fabricate edible film, ZnO-N at level of 0.5 w/w% (based on starch powder) was mixed with 100 mL of water. The suspension was stirred for 3 h, at 25°C. After that, the nano-mixture was subjected to ultrasound wavelength. Approximately 4 g of tapioca starch powder,

0.4 g of bovine gelatin [23], and plasticizer (1.6 g of sorbitol/glycerol) [24] were combined with nano-mixture. All the biosuspensions were heated at 86°C for 45 min. During cooling period various levels of RCAE (2.5, 5, and 10 v/v%) were incorporated into the film solution. Finally, neat solutions and suspensions containing RCAE were decanted into casting glass plates and dried at 20°C to make biocompatible films.

Opacity

The use of UV-visible spectrophotometer model UV-1650PC (Shimadzu, Tokyo, Japan) at 600 nm was applied to the evaluate of the film's opacity (length= 5 cm × width= 1 cm) [25]. The experiment was conducted in four repetitions and opacity computed as follows.

$$\text{Opacity (\%)} = A_{600\text{nm}}/x$$

where $A_{600\text{nm}}$ is the absorbance of film at 600 nm
x is the thickness of the film (mm).

A micrometer was used to evaluate the thickness at five various places and the mean was taken as the film thickness.

Moisture content

Exactly, 3 g of film was weighed and placed in desiccator containing CaCl_2 before exposed to heat for 4 h at 105 °C. Moisture content evaluated as follows

$$\text{Moisture content\%} = ((m_1 - m_2)/m_2) \times 100$$

where;

m_1 = initial weight of the film (g) m_2 = weight of the dried film (g)

Antioxidant activity

The antioxidant activity of the biocompatible film was investigated by DPPH free-radical scavenging measurement [26].

To prepare the samples, 50 mg of the specimen was combined with 10 mL of 90% ethanol at 25 °C for 24 hours. After that 1 mL of the resulting biofilm extract was mixed with 0.1 mM DPPH solution, and the mixture was stored for 30 minutes at 25 °C in a dark environment. Finally, the absorbance of the resulting biosolution was measured at a wavelength of 517 nm. In the neat specimen, the biocompatible film extract was

replaced with ethanol (1 mL). The percentage of DPPH scavenging activity from the biofilms was calculated using the specified equation.

$$\text{DPPH\%} = ((A_c - A_t)/A_c) \times 100$$

where the A_t is the value of absorbance of the biocompatible film sample and A_c is the value of absorbance of the neat specimen.

Statistical analysis

Thickness, moisture content, opacity, and antioxidant activity were conducted with 4 replicates. Statistical analysis was done using graphpad software (version 10.4.0.621) and ANOVA test. To evaluate the significant difference between the means, Tukey's test method was used ($p < 0.05$).

RESULTS AND DISCUSSION

Opacity and thickness of film

Film thickness can significantly affect mechanical properties, transparency, and barrier properties of films[27]. The opacity of film has positive effect on film to decrease ultraviolet transmission and protect food against oxidation reactions [28].

The opacity and thickness of the biocompatible films are revealed in figure 1 a, and b, respectively. Opacity and thickness of 10% RCAE and control film, were 2.08%, 1.2% and, 0.098 mm, 0.07 mm, respectively. Also, two mentioned indices increased significantly ($p < 0.05$) as the amount of RCAE added to the biofilm increased. The increase in opacity may be affected by the original color of the extract.

Nadi, et al. [29] found increase in basil seed gum/chitosan biofilm opacity from 2.44 to 4.24 after the incorporation of 10% red cabbage extract. Another investigation also stated that the addition of RCAE could greatly increase the opacity of sodium alginate/arboxymethyl cellulose films [30].

The researchers showed that the thickness of film increase in the biofilms with higher extract amounts was due to the high concentration of solid content [29, 31]. Another work states that the increase in film thickness based on chitosan with 5% purple and black rice extract was attributed to the filler incorporated [32]. No

increases in the film thickness based on chitosan with different levels of RCAE were obtained in the investigation of Chen, et al. [19]. These findings are

probably attributed to the low concentration of extract in chitosan films.

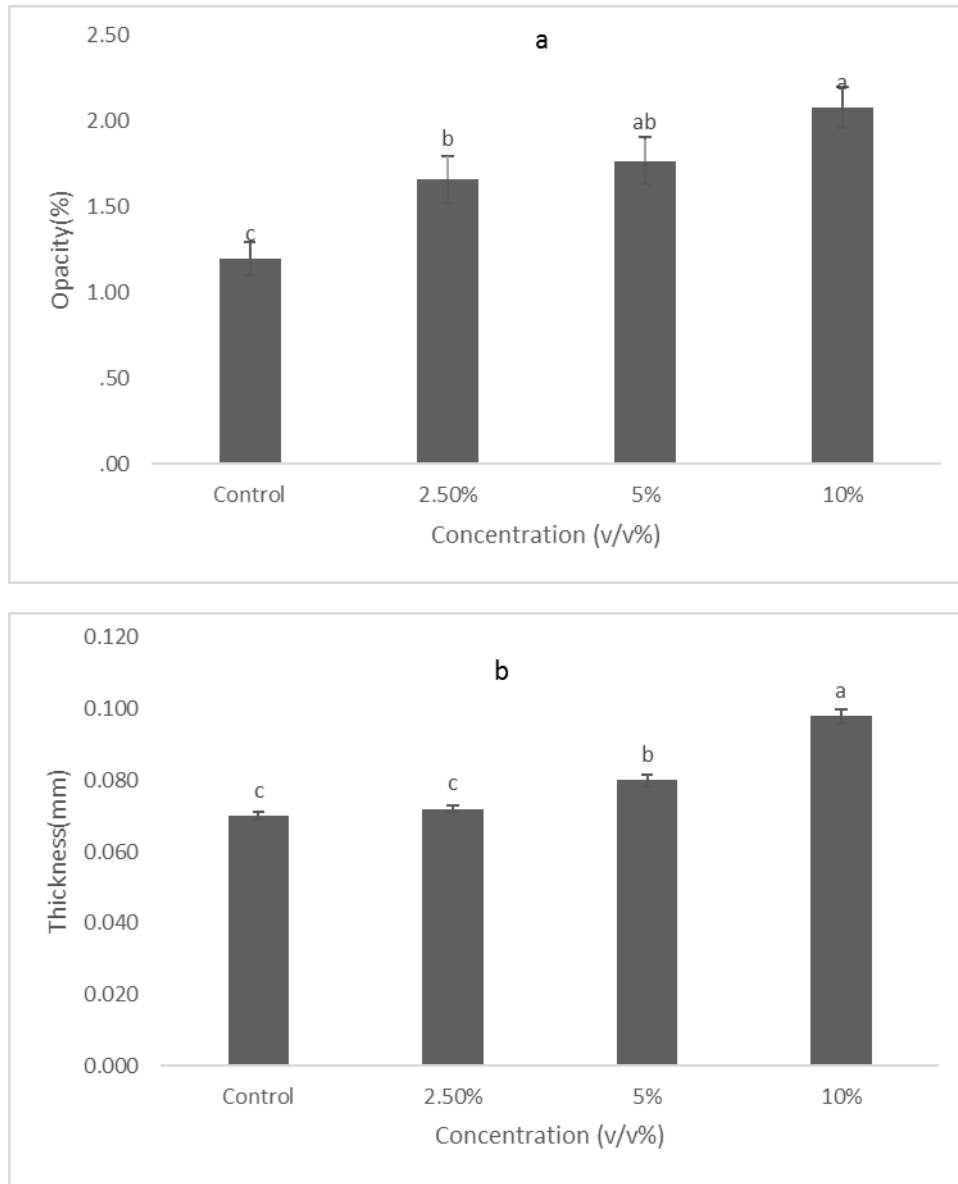


Figure 1. opacity (a) and thickness (b) of pure and RCAE films

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

Moisture content

The shelf-life of foods could be affected by their moisture content, high level of moisture content in food would lead to spoilage because it would contribute fungi and microbial growth [33]. The film food packaging is expected to be hydrophobic in order to protect food against spoilage[34]. Consequently, the low moisture content of the biofilm is beneficial for the packing material. One of the sections where there is a demand for improvement in film is the decrease of the moisture

content [25].

The moisture content of pure and treatment films containing RCAE are shown in figure 2. The addition of RCAE has decreased the moisture content significantly ($p < 0.05$). Moreover, the moisture content in 10% RCAE film is the lowest compared with other films. The moisture content of the starch/gelatin/ZnO-N was 40%, and the value of 10% RCAE biofilms was 35.45%. The moisture content of 2.5 and 5% RCAE represented

insignificant ($p>0.05$) decrease as the filler was incorporated into the biofilm.

Anthocyanin pigments contain many OH groups that form H bonds with biopolymer, which decreases their interaction with H_2O molecules [29]. Hence, increase in anthocyanin extract of the film could decrease the

moisture content of biofilm. A similar investigation was represented by Otálora González, et al. [35] They observed that the moisture content of the low methoxyle pectin film containing red cabbage extract gradually decreased from 44.9% in the neat film to 37% in the biofilm containing extract.

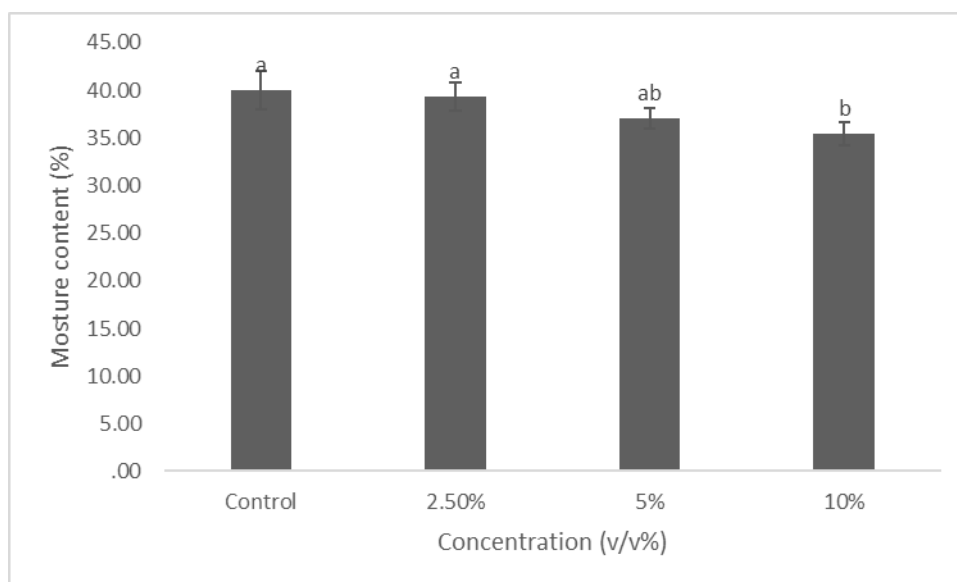


Figure 2. moisture content of pure and RCAE films

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

Antioxidant properties

Figure 3 shows the antioxidant activity of starch/gelatin/ZnO-N biofilm incorporated with various levels of RCAE. The findings showed significant increase in the antioxidant properties of the biofilms ($p<0.05$) with increasing RCAE content in the biocompatible film structure. The antioxidant properties of neat film were 4.74% and the value was improved to 92.28%, in 10% RCAE.

The antioxidant characteristics may be related to the polyphenol anthocyanin pigments, which have high levels of hydroxyl groups. Hence, OH groups neutralize free radicals by phenoxy groups [36].

These results show the high potential of biodegradable films with RCAE in antioxidant film and the very promising progression in functional film.

Cheng, et al. [37] observed that biofilms with RCAE improved the antioxidant activity of modified starch. They prove that the acetylated distarch phosphate films with RCAE led to the improvement of antioxidant properties of modified starch from 2.92% to 45.21%. Another investigation revealed that the psyllium seed gum film with mulberry pomace extract possessed higher antioxidant activity compared to neat samples[38]. Consistent with the antioxidant properties of the presentation scholar, the making sodium alginate/CMC film containing RCAE could improve antioxidant activity. With the incorporation of extract in film, DPPH% developed [30].

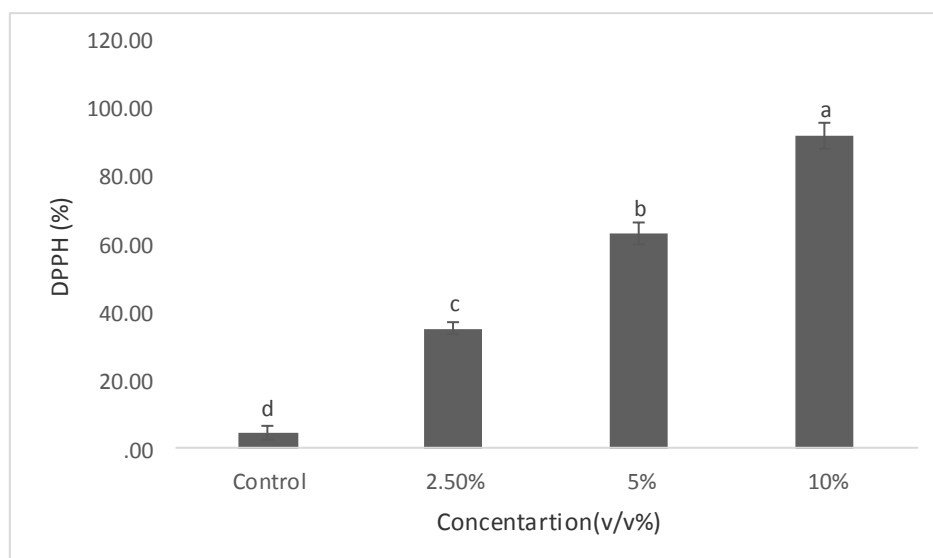


Figure 3. Antioxidant activity of pure and RCAE films

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

CONCLUSIONS

The tapioca starch, bovine gelatin, and zinc oxide nanorod film with the red cabbage anthocyanin extract (RCAE) decreases the moisture content compared to the neat biofilm. The film with 10% RCAE showed the greatest antioxidant activity. Also, the incorporation of 10% RCAE indicates high thickness of tapioca starch/bovine gelatin/zinc oxide nanorod films. On the other hand, the opacity was increased after the addition of RCAE. The starch/gelatin/ZnO-N films containing RCAE with improved moisture content and antioxidant activity as well as increased opacity could be used as biodegradable film food packaging in food science.

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Conflict of interests

The authors declare that there is no conflict of interest.

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