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ORIGINAL ARTICLE

Fabrication Smart pH Bionanocomposite Film Based on Biocompatible Polymer and Roslle Calyx Extract/Nano-Zinc Oxide for Monitoring Rainbow Trout Fillets

Naeimeh Sheykholeslami[1], Saghar Sadat Zavareh Tabatabaee[2], Shahriyar Sheykhi[3], Anna Etemadi Razlighi[*4], Neda Fallah[*5], Maria Elizabeth[6]

[1]*Department of Food Hygiene, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran*

[2]*Department of Food Sciences and Technology, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*

[3]*Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz P.O. Box 51666-16471, Iran*

[4]*Departmant of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran*

[5]*Department of Food Science and Technology, Najafabad Branch, Islamic Azad University, Najafabad, Isfahan, Iran*

[6]*Research Group for Nano-Bio Science, Division of Food Technology, National Food Institute, Technical University of Denmark, Lyngby, Denmark*

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KEYWORDS

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ABSTRACT: This work aimed to make a smart biofilm based on tapioca starch/bovine gelatin and roselle calyx extract (RCE)/nanofiller-ZnO. Then the utilization of the biofilm was studied to monitor the rainbow trout fillets. The biofilms were fabricated by casting the starch/gelatin solution with sorbitol /glycerol (3.2g) on casting plates and were dried at 25°C. RCE was added into biofilms (0.5 g for TP/RCE/ZnO-N and 2g for TP/RCE), and the biofilm characteristics, such as thickness, color, and antioxidant properties were investigated. The films containing nanofiller-ZnO/RCE and RCE showed significantly ($p<0.05$) more antioxidant activity than the neat film which respective values of 18.31 11.42 and 3.1. The thickness of the smart films significantly increased ($p<0.05$) from 0.08 to 0.13 by the addition of RCE/nanofiller ZnO into the solution. According to the results, a∗ value was significantly higher ($p<0.05$) in smart film with nanofiller-ZnO/RCE and RCE. The lowest L* value was obtained in film with nanofiller-ZnO/RCE. At the end of the conservation, the TVB-N value reached 35.7 mg N/100 and with the TVB-N significantly increase ($p<0.05$), the color properties of the smart films also changed. The color of the biofilm was purple, but the a∗ value significantly decreased. Therefore, the film based tapioca starch/bovine gelatin/nanofiller ZnO/RCE can be applied as a natural indicator of rainbow trout fillets.

INTRODUCTION

In the current years, the enhanced demand for safer foods has changed the food packaging technology to focus on novel smart packaging [1]. Intelligent packaging can show the chemical and microbial quality of products[2].

*Corresponding author: anna.etemadi@yahoo.com; neda.fallah.tie@gmail.com (A. Etemadi Razlighi; N. Fallah)
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Particularly, biosensors have been used in smart packaging due to their convenience for utilization and low cost [3]. The intelligent pH bionanocomposite film is the most common bioindicator, and it announces changes in chemical properties of food based on the activity of bacteria and molds [2].

Seafood products are susceptible to putrefaction because enzyme activity strongly influences its chemical reactions, and microbial growth[4]. Also, the presence of volatile nitrogenous compounds in seafood, an unfavorable taste and aroma are created, and the food becomes undesirable to the end user [5]. Hence, the freshness of meat products is a key factor to select. In recent research, smart packaging has been studied to monitor the chemical and microbial quality of seafood using biopolymer and natural colorants [6].

Anthocyanin colorants belong to a category of flavonoid compounds and are major natural colorants and most abundant in plant resources [6]. Anthocyanin pigments are sensitive to different factors such as light, temperature, and, pH. The anthocyanin's color exchanges from yellow to colorless under undesirable conditions [7]. Anthocyanin pigment's multifunctional behavior includes water-soluble colorants, and varied colors at different pHs, which proposes a new technology for food packaging [8].

Roselle hibiscus is non-toxic, biocompatible, and a rich resource for anthocyanin pigments [6]. The anthocyanin colorant of roselle can perceive and represent pH changes through different colors. Roselle hibiscus is a good source of antioxidant compounds for example flavonoids, anthocyanin, and polyphenols [9]. The major ingredient found in roselle calyx is carbohydrates [10]. Anthocyanin pigment of roselle has been applied as a natural dye in various foods [11].

Starch biopolymer is biocompatible and can be fabricated from by-products of food industries and agriculture. The positive functional characteristics of protein macromolecules such as plasticity and elasticity are fine properties in creating film biodegradable packaging. Hence, bovine gelatin can be utilized as a natural substance for biofilm [12]. The limitation of nonsynthetic macromolecule utilization in biodegradable film is their hydrophilic behavior in packaging. Up-to-date investigations on nanofillers and active agent utilization represented that the application of fillers can decrease the hydrophilic properties of biofilm [13].

Nano-inorganic compounds have unique physicochemical characteristics and indicate antimicrobial properties because of the big surface area to volume ratio [14]. The addition of nano-ZnO in biopolymers matrix as nanoadditive could enhance tensile strength, and barrier behavior and also make other applications in film packaging including increasing hydrophobic properties [15].

In this work, nano-ZnO as a nanofiller and roselle calyx extract (RCE) as a fine resource of anthocyanin pigments was applied to create intelligent pH bionanocomposite film to detection of the freshness of fish sample.

MATERIALS AND METHODS

Roselle calyx extract

Hibiscus extract was performed according to a modified technique proposed by Giusti, et al. [16]. Approximately 1 g of RC powder was mixed with ethanol (15 mL) and the pH of the suspension was adjusted to 2 with HCl. The suspension was heated for 60 min at 50°C to get the last extract, followed by centrifugation for 5 min. The solvent was removed at 50°C using a Hei-VAP model rotary evaporator.

Smart biofilm fabrication

Nanofiller-ZnO and RCE-containing treatment biofilms as well as pure film-tapioca starch/bovine gelatin film devoid of nanofiller-ZnO and RCE were created using the casting procedure. After utilizing nanofiller at a level of %2 (w/w, based on starch powder) to 100 mL of distilled water, the mixture underwent magnetic stirring for 60 minutes at 25°C before it was subjected to an ultrasonic wavelength. The biosolution was supplemented with 0.4, 4 [17], and 3.2 g [18] of gelatin powder, starch, and plasticizer (glycerol/sorbitol mixture) respectively. All of the solutions were heated to 85°C for one hour. While the biodegradable solution was cooling (to 40°C), about 2g of roselle calyx extract for starch/ gelatin/RCE and 0.5g of extract for starch/

gelatin/RCE /nanofiller-ZnO were blended into the suspension, and homogenized for 1 hour. In order to create biodegradable films, the solutions were finally permitted to dry at 25°C using glass plates (17 x 17 cm^2). A micrometer was applied to evaluate the thickness of smart biofilms

Antioxidant properties

The antioxidant characteristics of the biofilms were studied by the 2,2- diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging evaluation [19]. In order to, 50 mg specimen was added to 10 mL of ethanol (90%) at 25 °C for 1 d. Then, 1 mL biofilm extract was added to 0.1 mM DPPH, and the reaction chemical occurred for 30 min at 25°C in the dark condition. At last, the biosolution absorbance was determined at 517 nm. In the control sample, the biofilm extract was substituted with 1 mL of ethanol. The DPPH% of the biofilms was computed using the following equation:

$$DPPH\% = ((Ac-At)/Ac) \times 100$$

Where: A_c is the absorbance value of the control specimen, and At is the absorbance value of the film sample.

Color properties

CIELAB color factors (L*, a*, and b* values) were evaluated utilizing a colorimeter (Minolta CM-3500D; Osaka, Japan). L^* value shows the difference between light and dark. The a^*value shows the difference between red (+a*) and green (−a*) and, the b^* value indicates the difference between yellow (+b*) and blue (−b*).

Application of the biofilms as a natural indicator for rainbow trout fillet

Tapioca starch/bovine gelatin/nanofiller ZnO/RCE biofilms were utilized for application as the bioindicator of rainbow trout fillets to assay the freshness of rainbow trout fish (purchased from a local aquaculture farm located at Dibaj city (Damghan, Semnan, Iran)) during conservation. About 50 g of fish fillets were packed in polyethylene, and a smart indicator (4 × 4 cm^2) was put inside the polyethylene packs and kept at 4°C for 12 days at Islamic Azad university Shahrood branch. The total volatile basic nitrogen (TVB-N) value of the fish sample was measured over conservation period by stream distillation evaluation [20]. About 10 g of fillets were distilled and collected in a flask with 2% boric acid and methyl red. Then, the obtained solution of the specimen was titrated using HCL (0.1 N). TVB-N value of fillets was illustrated as mg N/100 g of sample.

Statistical analysis

Each test was performed with 4 replicates and analyzed using the Minitab 21.4.2 and ANOVA test. The comparison of the obtained mean of each experiment was conducted by Tukey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Thickness and antioxidant properties of biodegradable film

Thickness is a bold factor of films affecting oxygen and water vapor permeability and mechanical properties. The mentioned index is influenced by biofilm fabrication techniques and drying conditions [21].

The thickness and antioxidant properties of the biofilms are shown in Figure 1a. The thickness of tapioca starch/bovine gelatin film, tapioca starch/bovine gelatin/RCE film, tapioca starch/bovine gelatin/RCE/nanofiller-ZnO film were 0.08 ± 0.005 mm, 0.1 ± 0.005 mm and, 0.13 ± 0.01 mm, respectively (Figure 1a).

The authors illustrated that thickness increase observed for the smart films with higher RCE concentrations was due to the enhancement level of solid content [22]. Another research showed that the increase in thickness of starch film containing RCE was associated with the extract incorporated [23]. Also, incorporation of roselle extract into alginate smart film significantly increased the thickness of the films which may be related to the conformational changes of biopolymer chains by the incorporation of roselle extract [24].

Free radicals are active compounds that can damage production and reduce their chemical and sensory quality. Antioxidant compounds are functional agents that can inhibit oxidation reactions in foods [25]. Therefore, films containing extract with an antioxidant characteristic play a bold role in increasing the shelf life and maintenance of chemical and nutritional value during the conservation, by reducing the oxidation rancidity [26].

The antioxidant of tapioca starch/bovine gelatin/RCE/nanofiller ZnO bionanocomposite was more than tapioca starch/bovine gelatin /RCE film indicating that the incorporation of filler greatly increased the antioxidant properties of the smart film (Figure 1b).

It has been illustrated that antioxidant properties are significantly increased with an enhancement in the level of nanofiller-ZnO and RCE, which was mainly related to flavonoid compounds in RCE which indicated strong antioxidant activity [27]. Similar changes in the antioxidant properties of film with nanofillers have been represented by Liu, et al. [28]. Anthocyanin pigments are polyphenol compounds, which contain high levels of phenolic hydroxyl. Phenolic hydroxyl removes free radical agents by creating phenoxy groups [29].

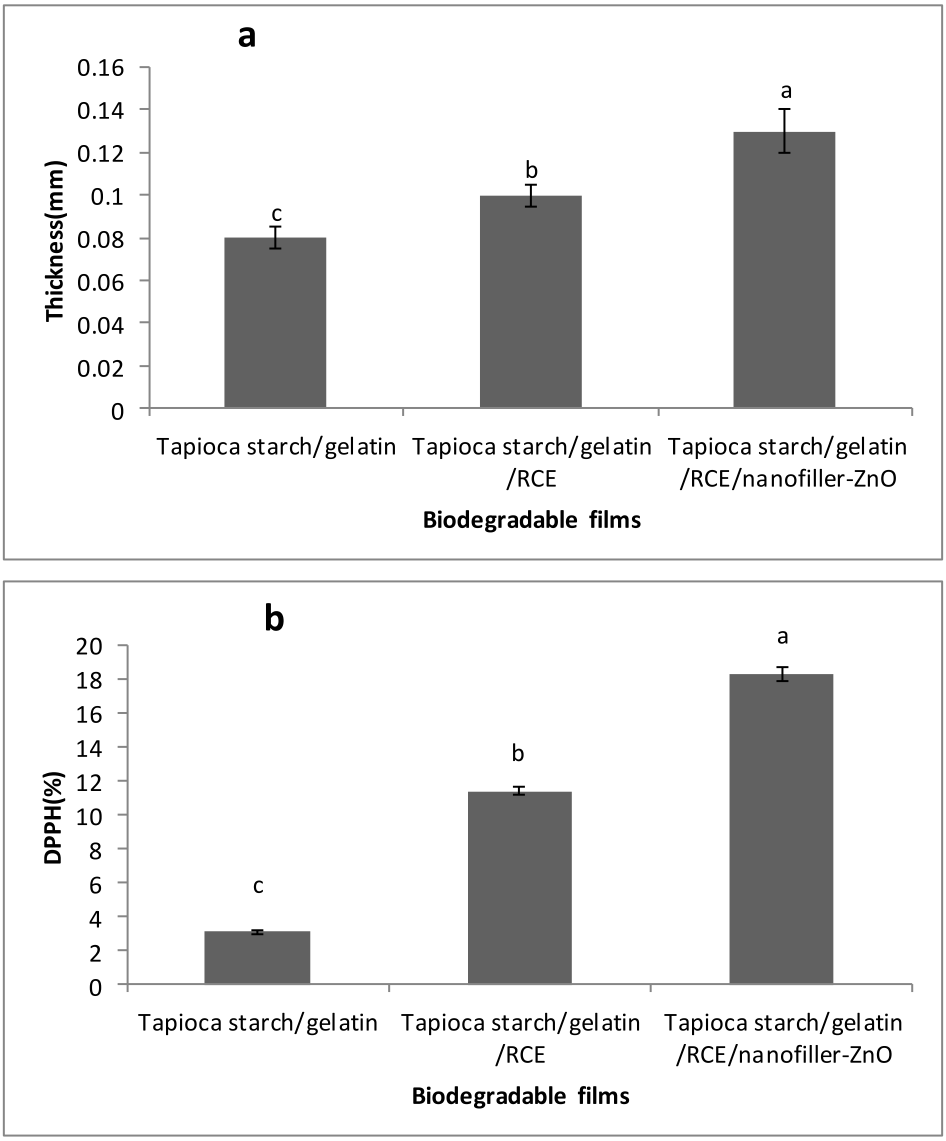

Figure 1. Thickness (a) and antioxidant properties (b) of tapioca starch/gelatin, tapioca starch/RCE, tapioca starch/RCE/nanofiller-ZnO
The bars display mean ± standard deviation. Different letters on the bars exhibite the significant difference at 5% level of probability.

Color properties

Color is a major index of packaging affecting consumer acceptance. Color index is influenced by fillers, fabrication conditions, polymers, and biopolymers of film packaging [30].

The color properties of tapioca starch/bovine gelatin, tapioca starch/bovine gelatin/ RCE, and tapioca starch/bovine gelatin /RCE/nanofiller-ZnO films are represented in Table 1.

Table 1 indicated an increase in a∗ and b∗ values of tapioca starch/bovine gelatin /RCE film (3.63;15.91) and tapioca starch/bovine gelatin/RCE/nanofiller-ZnO film (13.28;21.2). The L^* value of the tapioca starch/bovine gelatin film was 95.71, the value of tapioca starch/bovine gelatin/RCE and tapioca starch/bovine gelatin / RCE / nanofiller-ZnO biofilms was 92.23 and 81.34, respectively. It was reported that the tapioca starch/bovine gelatin/RCE/nanofiller-ZnO smart film possessed lower L^* value and higher a∗ as compared to pure samples (p< 0.05).

According to Marvizadeh, et al. [31], the tapioca starch/bovine gelatin film represents the 'L*' value of 95.76±0.01. Similar trends have been represented by the paper regarding increased a∗ and b∗ in smart film incorporated with RCE/nanofiller ZnO [27].

Table 1. Color properties of tapioca starch/gelatin, tapioca starch/RCE, tapioca starch/RCE/nanofiller-ZnO.

Film sample	L^*	a^*	b^*
Tapioca starch/gelatin	95.71.±0.7a	1.21.±0.04c	1.53.±0.08c
Tapioca starch/gelatin /RCE	92.23±0.49b	3.63±0.05b	15.91±0.21b
Tapioca starch/gelatin /RCE/nanofiller-ZnO	81.34±2.13c	13.28±0.05a	21.2±0.23a

Mean ± standard deviation values (n=4) of smart film followed by varied letters are significantly different (p<0.05).

Application of the biofilms as a natural indicator for rainbow trout fillet

To measure the relationship between the color of smart films and TVB-N content of fish samples, the tapioca starch/bovine gelatin biofilm containing nanofiller-ZnO/RCE we selected. The use of the mentioned film as a biosensor of fish samples during a conservation period of 12 days at 4°C was studied.

Protein contents in meat and meat product ingredients are susceptible to fungi and bacteria. During spoilage of meat, different volatile nitrogenous compounds, for example, amine and ammonia compounds were produced [32].

The TVB-N content of fillets and the a∗ value of the smart films are represented in Table 2. It can be found that the TVB-N value of the fish sample increased during the conservation time. The obtained findings were associated with compound degradation by enzymes secreted by bacteria and mold, chemical change of proteins, and fabrication of amines. Therefore, the initial TVB-N value of fish samples was appropriate and at the acceptable concentration (8.23 mg N/100 g). TVB-N value reached 35.7 mg N/100 g, which was higher than the acceptable level for the fish sample. Over conservation period and with the increasing TVB-N value of fish fillets, the color of smart biofilms also changed. Initially, the film's color was red, but the a* value gradually decreased. At last, a green color was seen. Based on the obtained findings, a relationship was found between the TVB-N content of the fish sample and the color of the smart biofilm.

Consistent with the findings of the current work, the fabricated smart nanofibre based on anthocyanin of blueberry/poly-L-lactic acid could indicate the chemical and microbial change of mutton during the conservation period. With the increase in meat spoilage, the biofilm's color changed [33].

Similar tendencies between the TVB-N values of pork meat and the color of the smart films affected by pigments such as anthocyanin were also explored in indicator biofilms, including starch/PVA- incorporated anthocyanins and betacyanins [34].

Table 2. Film's color and TVB-N value change during conservation time of fish samples.

Day	TVB-N (mg N/100 g)	a*
0	8.23±0.17c	13.28±0.05a
6	25.14±0.21b	1.12±0.08b
12	35.7±0.28a	-12.01±0.09c

Mean ± standard deviation values (n=4) of smart film and fish fillet followed by varied letters are significantly different (p<0.05).

CONCLUSIONS

In the current study, roselle calyx extract (RCE) was applied to fabricate intelligent biofilms based on tapioca starch/bovine gelatin. The results indicated that the antioxidant properties of films based tapioca starch/bovine gelatin were significantly increased by the incorporation of fillers. Results display that nanofiller and RCE could change biocomposite film's color factor such as a^* and b^* values. With the progress of fish sample chemical spoilage during conservation, as the TVB-N content enhanced, the smart films indicated a clear color shift from red to green. In summary, the smart biofilms based on tapioca/bovine gelatin/nanofiller-ZnO/RCE can be applied as a meat indicator and improve the chemical properties of fish fillets.

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Conflict of interests

The authors declare that there is no conflict of interest.

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