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ORIGINAL ARTICLE

Tapioca Starch-Based Bionanocomposite Film with pH Sensing for Monitoring Rainbow Trout Fillets Freshness

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KEYWORDS

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ABSTRACT: Combining bioactive compounds such as anthocyanin extract directly into a biodegradable film containing nanofiller is a novel form of smart film. The purpose of this investigation was to prepare a smart film using tapioca starch/zinc oxide nanorod (ZnO-N) incorporated with the anthocyanin pigments from the dragon fruit peel. The films based on tapioca starch/ZnO-N/dragon fruit peel extract (DFPE) were fabricated using the casting technique, which involved incorporating different levels of extract (0, 4, 9, 14 v/v%). The hydrophilic behavior, such as moisture content, water vapor permeability (WVP), color, and thickness of films, was studied. Edible Films based on tapioca starch/ZnO-N, having the highest DFPE level, showed the highest moisture content (45.2%) and a^* value, compared to the other biocompatible films. Incorporating extract significantly ($P<0.05$) decreased the WVP from 4.35×10^{-7} to 3.4×10^{-7} gr $m^{-1}Pa^{-1}h^{-1}$. The thickness of the tapioca starch/ZnO-N films was increased by increasing the extract contents. While evaluating the freshness of rainbow trout fillets for 12 h, DFPE14% exhibited color changes. Therefore, tapioca starch/ZnO-N film containing DFPE has fine potential to use as a smart pH edible film in evaluating the freshness of rainbow trout fillets.

INTRODUCTION

In the past decade, smart film packaging has developed to solve global concerns about food quality and safety [1]. They are the most important investigations in the packaging field because the packaging components can monitor perishable food [2]. The smart indicators, including gas indicators and pH indicators or time and temperature indicators, can be added to film polymers or attached to the interior of a container [3]. This film packaging conducts smart functions, for example: detecting, identifying,

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tracking, and convey information [4]. Smart packaging simplifies decision-making to improve the quality of food, extend shelf life, and increase food safety [5].

Sea products are perishable and are susceptible to enzymatic and microbial spoilage across all stages of production. The compounds of these spoilage reactions are volatile substances including, dimethylamine (DMA), ammonia, and trimethylamine (TMA)[6]. These compounds are collectively known as total volatile basic nitrogen. (TVB-N)[7]. Evaluation of the TVB-N value is a standard chemical value employed to assay the quality of fish. Although these techniques provide fine results, they are mostly time-consuming and destructive [8].

Anthocyanin pigment possesses antioxidant and antimicrobial characteristics and a notable color-changing ability at different pH levels [9]. Anthocyanin colorants are compatible with various polymer molecules because they can detect color changes from red to yellow [10]. Therefore, the characteristics of anthocyanin pigments can notably benefit intelligent films in helping end users choose the quality of food easily by showing color changes, which represent the poultry meat freshness or dairy products [11].

Dragon fruit peel extract is a good choice for intelligent films because of it's pH-sensitivity, antioxidant characteristics, and high anthocyanin pigment content, allowing it to significantly show spoilage[12]. It also contributes to sustainability by transforming agricultural waste into valuable resources while remaining economically viable. These properties make it a functional and biocompatible option for intelligent packaging [13].

Starch is commonly employed as a film biomaterial since it is cheap, biocompatible, and abundant. It contains hydrocolloid compounds, which can be utilized to design an edible film[14]. Starch biopolymer granules are not soluble, while in the presence of heat and water, they can swell[15]. Films based on starch have several weaknesses in chemical, mechanical, and physical characteristics [16, 17]. The current technology used to improve physicochemical properties by the incorporation of nanoadditives into the biofilm. The formation of the bionanocomposite film is affected by the interaction between the nanofiller, plasticizer, and starch matrix[18]. The plasticizer likely plays a role in the interaction between starch and the nanofiller, influencing the resulting moisture barrier, mechanical, and physical characteristics[19].

The current investigation aimed to fabricate an intelligent film. Dragon fruit peel extract (DFPE) was used as a smart agent for monitoring the freshness of rainbow trout fish fillets. Also, in the physicochemical properties of film section, the impact of employing DFPE on the barrier, hydrophilic properties, and color parameters was investigated.

MATERIALS AND METHODS

Materials

Tapioca starch, sorbitol and glycerol, ethanol (80%), boric acid, and HCl (0.1N) were provided from Merck Germany. Zinc oxide nanorod was obtained from Nano Pooyeshyekta, Iran. Dragon fruit was purchased from the local market (Tehran, Iran)

Dragon fruit peel anthocyanin extract

Anthocyanin pigments were extracted from fruit peel according to the method of Wu, et al. [20], with a slight modification. The fruit was thoroughly washed and peeled, after which its peel was sliced into small pieces. Then, dragon fruit was stored at -2°C for 12h. The fruit peel was freeze-dried for 72h, milled, to produce the final powder. The 200-mesh powder obtained was provided for the extraction of anthocyanin pigment. Anthocyanin colorants were extracted by adding 5g of powder to 100 cc of ethanol (80% v/v). The suspension was stirred at 500 rpm for 1 h. At last, the solution was filtered (Whatman filter paper No. 5), and the anthocyanin extract was kept at 4°C in a dark bottle.

Intelligent film fabrication

pH-sensitive films based on tapioca starch/ZnO-N were fabricated using the technique stated Marvizadeh, et al. [21] with slight modifications. ZnO-N was incorporated into deionized water at 0.5% (w/w) and homogenized in an ultrasonic bath for 35 min. Exactly 4 g of tapioca starch was dispersed in nano-solution, followed by 1.6 g glycerol and sorbitol as a plasticizer[22]. The film dispersion was heated for 45 min at 88 ˚C and stirred using a magnetic stirrer. 4, 9, and 14 v/v% of DFPE were added to the film solution when the suspension cooled down to 39 ˚C. The dispersion was stirred for 35 min for homogeneous mixing. About 92 mL of the dispersion was poured on glass casting. Biofilms without extract served as neat sample. The biofilms were dried at 25ºC for 3d. At last, the films were peeled off and kept at 25ºC and 50% RH in a desiccator for analysis. A manual micrometer was utilized for the thickness of all films.

Moisture content

The film samples (3 cm × 3 cm) were dried at 105 ˚C in an oven model 658PR305225G (Thermo Scientific CO. USA) until stable weights were obtained. The final weight of biofilms was then evaluated, and the moisture content of films was computed using the following formula.

$$\text{Moisture content}\%=((M_t-M_i)/M_i)\times 100$$

Where the M_t is the initial weight of biofilms, and M_i is the final weight of the sample.

Water vapor permeability (WVP)

The WVP experiment was conducted based on the wet-cup technique (ASTM E96). The glass cups were filled with water 10 mm below the biofilm surface. The film's thickness was measured before the test. The biofilms were fixed using parafilm. All the cups were placed in the desiccator containing silica gels at 25 ºC. The cups were weighed for 12 h at 120- minute intervals. WVP of films was computed from the weight changes of samples plotted versus time to obtain the water vapour transmission rate (WVTR).

Color

The color changes of smart biofilms were evaluated using a colorimeter (Minolta CM-3500D; Osaka, Japan). The results of color test were measured in reflectance mode and stated in terms of greenness-redness (a*), lightness (L*), and blueness-yellowness (b*).

Application of the smart films as an indicator for fish fillet

Starch/ZnO-N/DFPE biofilm was used as the bioindicator of fillets to analyze the freshness of fillets (obtained from a local aquaculture farm (Dibaj city, Semnan province, Iran)) during preservation. About 55 g of samples were packed in silicon, and an indicator film (5 × 5 cm^2) was placed inside the silicon packs and stored for 12 days at 4°C. Value of volatile basic nitrogen (TVB-N) of the fillet was assayed over preservation according to the stream distillation measurement [23]. Exactly 10 g of fillets were distilled, and the resulting distillate was collected in a receiving flask containing boric acid (2%) solution with methyl red. The resulting solution was then titrated with 0.1 N HCl. The TVB-N (Total Volatile Basic Nitrogen) content of the fillets was expressed as mg N per 100 g of sample."

Statistical analysis

Moisture content, thickness, water vapor permeability, TVB-N, and color evaluation were performed with 6 replicates. One-way ANOVA was employed, and Duncan's test assessed the significance of the difference between averages ($p<0.05$). The SPSS version 27.0.1 software was utilized for this purpose.

RESULTS AND DISCUSSION

Moisture content and thickness

The moisture content and thickness are displayed in Figure 1 (a and b) respectively. Thickness and moisture content of intelligent film with DFPE14% and film without DFPE were 0.09 mm, 45.2%, and 0.07 mm, 39.04%, respectively. Also, both thickness and moisture content increased as the amount of DFPE employed in the biodegradable film increased.

The film containing DFPE14% demonstrated the highest moisture among biofilms due to the highest level of anthocyanin pigment incorporated during the film fabrication. Incorporating anthocyanin pigment disrupts the film matrix's network bonds, leading to an increase in moisture content [24]. In a recent study, an increase in the moisture content of gelatin film was observed after the incorporation of DFPE15% [12]. Another investigation also proved that the addition of red dragon fruit peel could notably develop the moisture content of chitosan[25].

The thickness of biocompatible film can insignificantly affect mechanical attributes, transparency, and water vapor permeability and O_2 of films [10, 26].

The biofilms revealed a rising trend in thickness. Control, DFPE4%, and DFPE9% had no significant difference (p> 0.05) in thickness.

The different works represented that the increase in thickness of biodegradable films with high level of extract was due to the high amount of solid content [27, 28]. Putra et al. (2019) stated that biofiller-DFPE had developed the total solids of the film's solution, enhancing the thickness of the smart film [29]. Another paper showed that the increase in thickness of gelatin film containing DFPE was related to the extract embedded [12].

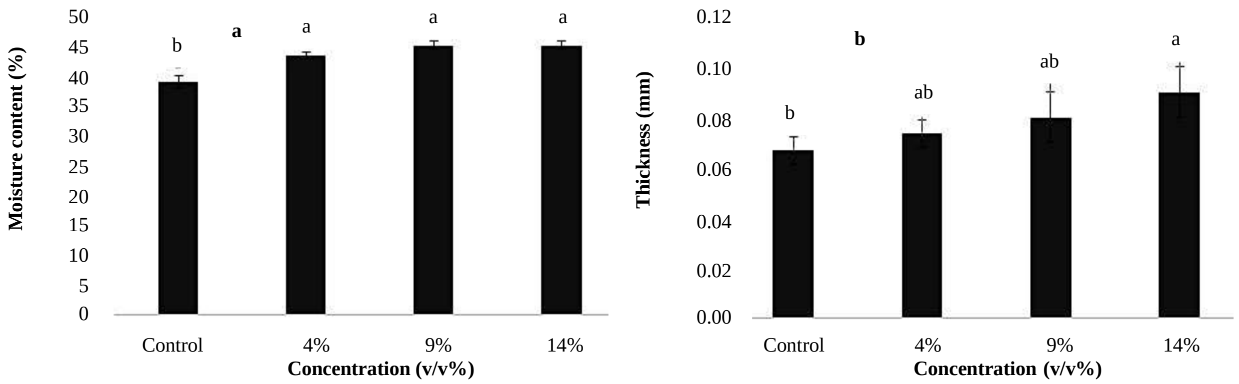

Figure 1. Moisture content (a) and thickness (b) of pure and DFPE films
The bars reveal average ± standard deviation (SD). Different letters on the bars display the significant difference ($p<0.05$).

Water vapor permeability

Water vapor permeability is one of the key properties in evaluating the application of edible films. Water vapor permeability shows the barrier characteristics of film to decrease the moisture travel between the surroundings and food to maintain food safety and quality[10].

The WVP of neat and intelligent films is illustrated in Figure 2. The addition of DFPE has decreased the WVP significantly ($p < 0.05$). Moreover, the WVP in the smart film with DFPE14% is the lowest compared to the film without extract and other treatments. The WVP of the tapioca starch/ZnO-N was 4.35×10^{-7} gr/mPah, and the biofilms with DFPE14% were 3.4×10^{-7} gr/mPah.

This may be related to decline in water affinity of the biofilm as the betacyanin and polyphenol compounds permeated the inner space of the biofilms, consequently creating a limited path for water vapor molecules [30]. Moreover, the extract may have created H bond with the biopolymer, and the free water trapped inner space of the biomatrix. These interactions could explain the observed reduction in inter-chain spacing between the polymers. Additionally, the mobility of water molecules is reduced, potentially leading to decreased water migration within the film matrix [31].

The mentioned trend was consistent with the results stated in the investigation of the addition of dragon fruit peel powder into sodium alginate films [32]. A similar trend was stated by Azlim, et al. [12]. They demonstrated that the WVP of the film based on gelatin with DFPE gradually decreased from 1.8804 g.mm/m^2.day.kPa in the control film to 1.4670 g.mm/m^2.day. kPa in the smart film with DFPE15%.

During the investigation of bionanocomposite films, a study indicated that the addition of nano-TiO_2 had exhibited a declining trend of water vapor permeability [33]. It could be due to the tortuous path in the matrix for the water molecules to pass through. This trend might be attributed to tortuous pathways within the matrix that hinder the transfer of water vapor molecules [34].

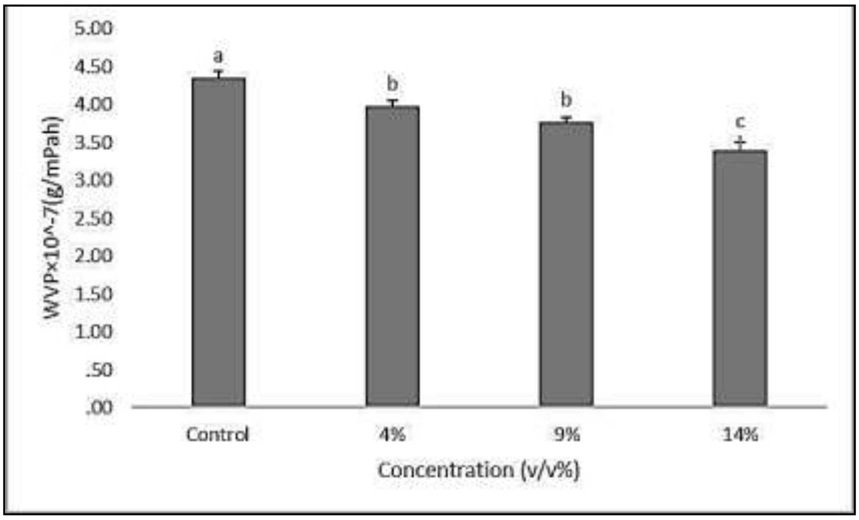

Figure 2. WVP of pure and DFPE films

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

Color

Table 1 indicates the color parameters of tapioca starch/ZnO-N biocompatible film embedded with various levels of DFPE. The findings represented significant decrease (p<0.05) in the L^* of the films with developing DFPE content in the intelligent film. The a* and b* of starch/ZnO-N film were 1.35 and 13.2, and the values were shifted to 2.22 and -11.88, in the smart film containing DFPE14%, respectively. The value of a* resulted in an increment trend, which indicated that the color shifted to red. Also, the decline trend can be observed in the value of b*, which changes the color of the films to bluish. The highest value of a* obtained for DFPE14% intelligent film was due to the highest incorporation of anthocyanin pigments into the film. Consistent with the color characteristics of the current paper, the fabrication of gelatin film DFPE could increase a* value of intelligent film [12]. Moreover, another work found that sago film with *Bauhinia Kockiana* extract developed the a^* value of the film. The authors stated that the sago films with *Bauhinia Kockiana* led to the a decrease in L^* value of biofilms [35].

Table 1. Color characteristics of tapioca starch/ZnO-N and smart films

Film sample	L^*	a^*	b^*
Control	84.2.±0.81a	1.35.±0.03c	13.2.±0.21a
DFPE4%	65.67±0.61b	1.47±0.04c	12.1±0.31b
DFPE9%	71.64±0.59c	1.82±0.08b	-3.96±0.35c
DFPE14%	52.2±0.88d	2.22±0.09a	-11.88±0.43d

Mean ± standard deviation values (n=6) of smart film followed by varied letters are significantly different ($p<0.05$).

Application of indicator film for fillets

The rainbow trout fillets illustrated an increase in TVB-N value from 8.26 to 35.94 at the end of 12 d of conservation time (Table 2). Film containing DFPE15%, with the highest a^*, was selected as a bioindicator to prove the relationship between the TVB-N value of fillets and the color changes in intelligent film. Throughout the 12 days of controlling, the biofilm was observed to be associated with a color change with the decreased a* value from 2.22 to -23.01. The alteration in color results from modifications in the chemical structure of active compounds present in the extract, particularly anthocyanins.

Consistent with current findings, Moradi, et al. [36] indicated that smart film made with potato starch/potato starch nanocrystals and Dutch rose extract can be employed as a bioindicator of the freshness of rainbow trout fillets with a color shift from light green to dark green. Similar findings have been stated in other investigations about the color shift of anthocyanin pigments extracted from plants and fruits due to increase in pH caused by fillet spoilage [37, 38].

Hence, there was a fine correlation between the TVB-N and the color of the starch/ZnO-N/ DFPE14% film. Therefore, DFPE possesses notable potential to monitor the rainbow trout fillets as a bioindicator.

Table 2. a^* value and TVB-N value change during preservation time of fillets

Day	TVB-N (mg N/100 g)	a^*
0	8.26±0.5c	2.22±0.09a
6	25.18±0.95b	-10.12±1.12b
12	35.94±1.05a	-23.01±1.4c

Mean ± standard deviation values (n=6) of smart film and fish fillet followed by varied letters are significantly different ($p<0.05$)

CONCLUSIONS

In this investigation, a new smart film based on tapioca starch/ZnO-N/dragon fruit peel anthocyanin extract was successfully fabricated. The incorporation of extract into bionanocomposite films affected the films' hydrophilic behavior, which increased moisture content. The smart film with DFPE14% indicated the lowest water vapor permeability. With the increase in the level of DFPE, the L^* value of the intelligent films decreased while their thickness increased. DFPE14% also controlled the freshness of rainbow trout fillets with decreased a* index and showed a significant color change to the naked eye.

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Conflict of interests

The authors declare that there is no conflict of interest.

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