



ORIGINAL ARTICLE

Preparation Smart pH Biodegradable Film based on Potato starch/*Coleus scutellarioides* Anthocyanin Extract /Nano-Zinc Oxide for Monitoring Chicken Fillets

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KEYWORDS

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ABSTRACT: The purpose of the presentation investigation was to fabricate an intelligent edible film based on potato starch/*Coleus scutellarioides* anthocyanin extract (CSAE)/nano-ZnO. Then the edible film was investigated to monitor the chicken fillets. The active film was made by casting the potato starch/(CSAE)/nano-ZnO with glycerol/sorbitol (40 w/w%) on casting plates. CSAE was added into edible films (7, 15 and, 30 ml), and the films based on potato starch/(CSAE)/nano-ZnO characteristics, such as mechanical, color, and barrier behaviors were investigated. The active films with different concentrations of 7, 15 and, 30 ml of CSAE represented significantly ($p < 0.05$) lower water vapor permeability (WVP) than the pure biofilm with respective values of 2.66 2.4 and $1.43 \times 10^{11} (\text{gm}^{-1}\text{s}^{-1}\text{Pa}^{-1})$. The tensile strength of the intelligent films significantly decreased ($p < 0.05$) from 192.51 to 151.33 MPa by the adding 30 ml of CSAE into the film. Based on the results, the L^* index was significantly higher ($p < 0.05$) in the film containing 30 ml of CSAE. The lowest a^* factor was obtained in the film without extract. At the end of the preservation time, the TVB-N value of chicken fillets reached 38.25 mg N/100, and the mentioned index significantly increased ($p < 0.05$), the color characteristics of the intelligent edible films also changed. The color of the active films based on potato starch/nano-ZnO/CSAE was purple, but the a^* index significantly decreased. Therefore, the biodegradable film based on potato starch/nano-ZnO/CSAE can be utilized as a biocompatible indicator of chicken fillets.

INTRODUCTION

The increased need for healthier foods in recent years has led to a shift in food packaging science, with an

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emphasis on innovative smart packaging. [1]. Smart film packaging can represent the microbial and chemical attributes of foods[2]. Particularly, sensors based on pigments have been applied in bioindicators due to biological safety, antioxidant properties, and low cost [3]. Utilizing intelligent bioindicators, consumers can evaluate the food's quality in the chemical and microbial section at different times [2].

Due to its high nutritional value and low price chicken meat is the most consumed meat in the world. Hence foods with high value of protein are vulnerable to microbial growth. They can degrade before consumption when favorable preservation conditions are not provided [4]. An upward trend in spoilage influences chemical and physical properties of chicken, and spoilage including putrefaction, stickiness, and lightning/purple color occurs [5].

Additionally, the presence of volatile nitrogenous chemical compounds in chicken meat results in an unpleasant taste, making the product unattractive for the consumer[6]. Therefore, the freshness of chicken meats is an important index to select. In current scientific projects, intelligent indicators have been investigated to monitor the microbial and chemical spoilage of chicken fillets using biopolymer and anthocyanin pigments [7].

The most prevalent natural colorants found in plant resources are anthocyanin colorants, which are a subclass of flavonoid compounds[8]. Anthocyanin pigments are vulnerable to a variety of conditions, including pH, temperature, and light. Under adverse conditions, the color of the anthocyanin shifts from yellow to colorless [9]. A new approach to food packaging is suggested by the multifunctional behavior of anthocyanin pigment, which contains water-soluble colorants and a range of hues at different pH levels [10].

Coleus scutellarioides (belongs to *Lamiaceae* category) is a herb that cultivates mainly in Malaysia and Southeast Asia [11]. *Coleus scutellarioides* is non-toxic, and a fine resource for anthocyanin colorants. This plant has also represented significant antioxidant and antimicrobial characteristics. The leaves of *Coleus scutellarioides* have different applications in medicine, such as digestive diarrhea, disorders pain, scorpion stings, etc [12]. Different active compounds, including flavonoids, alkaloids, volatile essential oils, saponins, and,

polyphenols have been observed in the leaves of *Coleus scutellarioides* [13].

A biocompatible material, such as starch biopolymer can be created from agriculture and food industry by-products[14]. Nonsynthetic macromolecule's hydrophilic packaging characteristics limit their use in biodegradable film. Recent studies on the use of active substances and nanoparticles have shown that usage of fillers can reduce hydrophilic characteristics of edible film[15].

Nano-inorganic particles have bold physicochemical and electromagnetism behavior and exhibit antimicrobial and antifungal properties [16]. The addition of nanoparticle-ZnO in the biocompatible film as a filler could enhance tensile properties, decrease gas transfer, and also make other positive characteristics in edible film including decreasing hydrophilic properties [17].

In the presentation study zinc oxide nanorods as a nanoadditive and *Coleus scutellarioides* anthocyanin extract (CSAE) as a good resource of anthocyanin colorant were applied to make intelligent indicators to detection of the spoilage of chicken fillets.

MATERIALS AND METHODS

Coleus scutellarioides anthocyanin extract

Extraction of anthocyanin pigment extract from *Coleus scutellarioides* was performed based on Luchese, et al. [18]. The plant leaves were immersed and dried at 25°C. After that, the leaves of *Coleus scutellarioides* were freeze-dried for 72 h. Then, *Coleus scutellarioides* powder (200-mesh) was used for the extraction of pigments. About 5 g of obtained powder was added to 100 ml of ethanol 50% and stirred for 1 h at 600 rpm. At last, the suspension was separated through filter paper (Whatman No. 4), and the obtained anthocyanin extract of *Coleus scutellarioides* was stored in the dark cabinet at 4°C.

Intelligent film fabrication

Control potato starch/nano-ZnO and treatment biofilm with *Coleus scutellarioides* anthocyanin extract (CSAE) were fabricated by casting procedure. Nano-ZnO at levels of 0.5% (w/w, based on potato starch) was added into water (100 mL) and the dispersion was stirred

magnetically for 2 h at room temperature, and the suspension was exposed to ultrasound wavelength. About 4g of potato starch powder [19], and sorbitol/glycerol (40 w/w%, based on starch) [20] were added to the mixture. All the biomixture were heated at 87°C for 50 min. During cooling time of the suspension, various

amounts of CSAE (7, 15, 30 ml of extraction) were added to the bionanocomposite mixture. At last, control and treatment suspensions were decanted into casting plates, and dried at 25°C to make intelligent films. ESEM indicates that nano-ZnO has a dimension in nanometer (Figure 1).

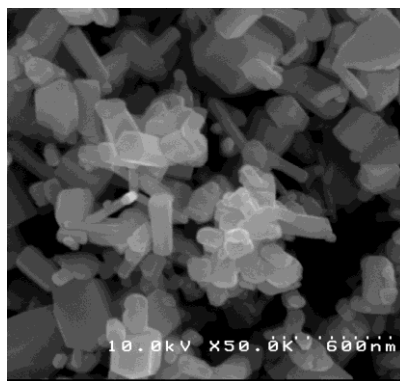


Figure 1. ESEM of nano-ZnO.

Mechanical properties

The different factors of mechanical properties such as tensile strength (TS) young's modulus (YM) and elongation at break (EAB) of the smart film were measured based on ASTM (2018) [21] standard method D882-18 by using the Texture Analyzer model of TA-XT2TM (Stable Micro System). Before evaluation of TS, YM, and EAB, the rectangular shape of biofilms (length =10 × width =2 cm) were conditioned for 48h 53% RH and at 25°C. Six replicates were performed for active films.

Water vapor permeability

The water vapor permeability of the smart film was evaluated according to ASTM E 96-05 [22]. In brief, specimens were cut from the active film according to the cup's opening diameter. The mouths of the cups were masked and sealed using an active film and parafilm, respectively. All cups were placed in a desiccator with silica gel. Variety in the weight of cups was detected after a fixed period time.

Color properties

CIELAB color indices such as L*, a*, and b* factors were measured utilizing a colorimeter model CM-3500D (Minolta, Osaka, Japan). L* value represents the

difference between dark and light. The a* index displays the difference between green (-a*) and red (+a*) and the b* factor illustrates the difference between blue (-b*) and yellow (+b*).

The use of the active films as a biocompatible indicator for chicken fillet

The chicken fillet samples were prepared from the sacrificed chickens in local market and transferred to the university laboratory at refrigerator temperature. About 200 g of chicken fillets were packaged in a propylene pack. A 16 cm² piece of the intelligent indicator biofilm was put inside the packaging. Next, the all packs were kept in an incubator for 16 h at 25 °C and 75% RH. During the preservation time, the total volatile basic nitrogen of chicken fillets and the a* value of the intelligent indicator were evaluated to measure the relationship between the chemical quality of fillets and biofilm color. The total volatile basic nitrogen (TVB-N) value of the fillet samples was assessed over conservation time according to steam distillation measurement [23]. Approximately 10 g of samples were distilled and collected in a flask containing methyl red and boric acid (2%). Then, the obtained specimen was titrated using hydrochloric acid (0.1 N).

Statistical analysis

Barrier, color, and mechanical, TVB-N tests were done with 6 replicates and analyzed using of the SPSS 27.0.1 and one way ANOVA test. The comparison of the average of all tests was performed by Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Mechanical properties of intelligent film

Generally, the tensile strength (TS) of films is dependent on biofilm constituents, preparation method, and concentration of fillers [24]. Active films with low tensile properties have higher values of elongation at break (EB) [25].

The TS, EB, and young's modulus (YM) of the intelligent edible films are illustrated in Figure 2 a, b, and

c respectively. TS and YM of CSAE 30 and neat film, were 151.33 MPa and, 2.61 MPa and, 192.51 MPa and 5.24 MPa, respectively (Figure 2a,c). Also, EB values of the intelligent films increased significantly ($p < 0.05$) as the level of extract added to the active film increased (Figure 2b).

The authors stated that TS decrease observed for the films based on gelatin with higher CSAE levels was due to the presence of anthocyanin pigments can decrease intermolecular interactions [26]. Another investigation proves that the decrease in TS value of film based on corn starch containing black bean anthocyanins was attributed to the pigments extract incorporated [27]. Also, the incorporation of anthocyanin extract of red cabbage into biocompatible film significantly decreased the TS of the biofilms which may be attributed to the plasticizer role of phenolic compounds of red cabbage [28].

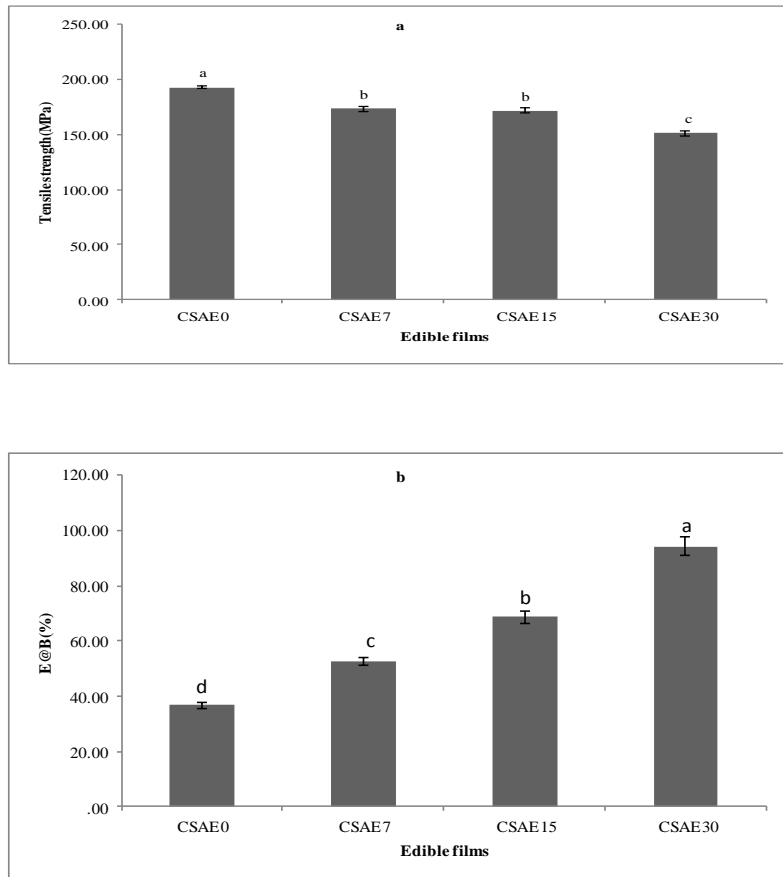


Figure 2. TS (a) EB (b) and YM (c) of control and smart films The bars represent average \pm standard deviation (SD). Different letters on the bars display the significant difference ($p < 0.05$).

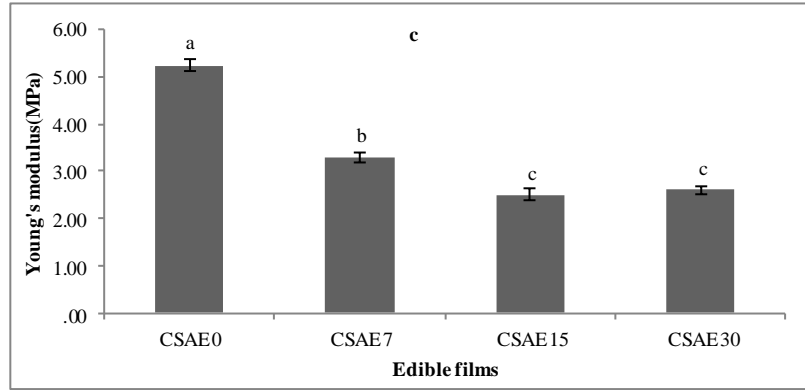


Figure 2. Continued.

Water vapor permeability of intelligent film

The water vapor permeability of potato starch/nano-ZnO with CSAE film was lower than neat film that displays the addition of CSAE greatly decreased the water vapor transfer of the active film (Table 1). The WVP of CSAE 7 showed insignificant ($p > 0.05$) decrease as the extract was incorporated into the film.

The mentioned factor is affected by the mobility, integrity, and free volume of the biomacromolecules the and amorphous- crystalline and, hydrophobic-hydrophilic ratios of the active films [29]. It has been proven that barrier characteristics of intelligent edible

films are significantly increased with an enhancement in the concentration of CSAE, which was related to high level of the hydroxyl group in the active compounds such as the phenolic category, possibly interacting with the biomacromolecules, subsequently the lower content of free hydroxyl groups[30]. Decreased WVP of edible films due to the addition of different extracts has also been found by other authors [31, 32].

Table 1. WVP of control and smart films

Film samples	WVP $\times 10^{11}(\text{gm}^{-1}\text{s}^{-1}\text{Pa}^{-1})$
CSAE0	2.87 \pm 0.19a
CSAE 7	2.66 \pm 0.11ab
CSAE 15	2.4 \pm 2.08b
CSAE 30	1.43 \pm 2.09c

Average \pm standard deviation (SD) values (n=6) of intelligent biofilm followed by varied letters are significantly different ($p < 0.05$).

Color properties

Color is a bold factor of packs affecting consumer satisfaction. Values of L, a and, b are influenced by the kind of fillers, preparation conditions, and main substance of film packaging [33].

The color properties of neat and potato starch/nano-ZnO films containing CSAE are shown in Table 2. The addition of extract has increased the a* value significantly ($p < 0.05$). Also, the values of b* for smart film CSAE30 are the highest compared with pure films. Table 2 exhibited an increase in L* value of the intelligent film's color. The L* value of the neat film was

82.77, and the value of potato starch/nano-ZnO/CASE30 biofilms was 96.3. As the level of CSAE incorporated increased, the values of a* and L* increased significantly ($p < 0.05$), showing the tendency of the biofilm to display red and light compared to the pure film.

Another study stated that the gelatin film with *Coleus scutellarioides* extract possessed higher L* and a* values as compared to pure samples [30]. Koosha and Hamedi [34] stated that the chitosan/PVA films with black carrot anthocyanins led to the change of a* value of biofilms from green to red.

Table 2. Color properties of control and smart films.

Film samples	L*	a*	b*
CSAE0	82.77±0.22d	20.58±0.14d	13.51±0.13d
CSAE 7	88.5±0.14c	63.78±0.15c	19.31±0.22c
CSAE 15	91.99±0.15b	84.37±0.23b	21.37±0.1b
CSAE 30	96.3±0.14a	95.95±0.16a	24.04±0.17a

Average ± standard deviation(SD) values (n=6) of intelligent biofilm followed by varied letters are significantly different (p<0.05).

Application of the biocompatible films as a bioindicator

for chicken fillet

To assay the relationship between the intelligent film's color and the value of TVB-N, the CSAE30 active film we selected. The application of the CSAE30 edible film as a natural sensor of chicken fillets during a preservation period for 16 h at 25°C was studied.

Protein compounds of meat are appropriate substances for bacteria and fungi activity. During meat's spoilage, ammonia and amine compounds were produced [7, 35].

The value of TVB-N and the a* color value of the intelligent edible film are exhibited in Table 3. It can be observed that the value of TVB-N increased during the preservation time. The increased value of TVB-N was attributed to protein degradation by enzymes secreted of bacteria and fungi and the production of amines. The initial value of TVB-N of fillets was 8.3 mg N/100 g and at the acceptable amount. Value of TVB-N reached 38.25 mg N/100 g, (T=16h) which was higher than the

allowable concentration (25 mg N/100 g) for the chicken fillets. Over preservation and with the upward trend value of TVB-N, the indicator's color also changed. First, the indicator's color was purple, but the value of a* gradually decreased. According to the results, a negative relationship was observed between the value of TVB-N and the indicator's color.

Consistent with the TVB-N value of the presentation investigation, the fabricated biosensor nano-fiber based on blueberry's anthocyanin/poly-L-lactic acid could exhibit microbial, fungal, and chemical spoilage of mutton during the preservation. With the upward trend in meat spoilage, the indicator's color changed [36].

Similar findings between the value of TVB-N in pork meat and the b*color value of the bioindicator affected by anthocyanin colorants incorporated starch/PVA were also explored [37].

Table 3. Indicator's color and value of TVB-N change during preservation time of fillets

Time(h)	TVB-N (mg N/100 g)	a*
0	8.3±0.12d	20.58±0.14a
8	11.93±0.15c	14.26±0.1b
12	25.53±0.18b	10.05±0.16c
16	38.25±0.23a	4.01±0.07d

Average ± standard deviation(SD) values (n=6) of intelligent biofilm followed by varied letters are significantly different (p<0.05).

CONCLUSIONS

In the presentation investigation, *Coleus scutellarioides* anthocyanin extract (CSAE) was applied to make intelligent active films based on potato starch/nano-ZnO. The results exhibited that the tensile strength of films based on potato starch/nano-ZnO was significantly

decreased by the incorporation of CSAE. Results represented that CSAE could change active film's color indices such as L* and a* values. With the progress of the chicken fillet's chemical spoilage during preservation, as the value of TVB-N enhanced, the CSAE30 films

represented an obvious color change from purple to colorless. In summary, the intelligent active films based on potato starch/nano-ZnO with anthocyanin extract can be utilized as a biosensor for chicken meat.

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Conflict of interests

The authors declare that there is no conflict of interest.

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