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ORIGINAL ARTICLE

Biodegradable Composite Film Based on Tapioca Starch/Bovine Gelatin with Roselle Calyx Extract and Zinc Oxide Nanorod

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KEYWORDS

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ABSTRACT: Biodegradable films based on natural molecules are used for food production protection to extend shelf life. The current work aimed to fabricate and characterize bionanocomposite films. The biodegradable films were prepared from a mixture of tapioca starch/bovine gelatin (TB) with the loading of roselle calyx extract (RCE) and zinc oxide nanorod (ZnO-N). The incorporation of ZnO-N and RCE improved the mechanical and barrier properties of films. ZnO-N/RCE and RCE incorporated composite films indicated lower water vapor permeability (WVP) than neat film, 3.36×10^{-7}, 3.96×10^{-7}, and, 4.2×10^{-7} g/mPah respectively. Elongation at break, Young's modulus, and tensile strength of TB film, TB/RCE, and TB/RCE/ZnO-N film were measured as follows: 18.44%, 14.56%, and 10.69%; 0.79 MPa, 1.52 MPa, and 2.65 MPa; and 14.4 MPa, 21.85 MPa, and 28.22 MPa, respectively. The solubility of the active films decreased from 40.33% to 33.68% by the addition of RCE and ZnO-N into the composite matrix. This study indicated composite films based on starch/protein incorporated RCE and ZnO-N have the positive potential for applications in bionanocomposite packaging.

INTRODUCTION

In the current decade, there is extended demand for packaging based on biopolymers which replaces synthesis packaging in response to environmental pollution [1, 2]. Starch is a biodegradable material and can be produced from crude material of food industries and various by-products of agriculture. The good functional properties of protein molecules to create network, elasticity, and plasticity are beneficial in fabricating biopackaging. Therefore, bovine gelatin biopolymer can be applied as raw material for biodegradable films [3, 4]. The limitation of natural polymer application in film is the hydrophilic behavior of the biopolymers[5]. Current works on nanoparticle and extract utilization indicated that the application of nanoadditives and active substances can improve the hydrophilic properties of natural polymers [6].

Active packaging is a novel technology developed by incorporating functional agents including antibacterial, nanoadditive, antioxidants, extracts, and essential oils, into the packaging to increase or maintain food quality

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Roselle (*Hibiscus sabdariffa*) is a well-known tropical shrub [7]. Hibiscus sabdariffa is grown in Asia continent (Malaysia and India) and is native to Africa. Roselle known as Krachiap Daeng (Thailand), Jamaica flowers, Oiselle (French), roselle (English), and Sorrel [8]. *Hibiscus sabdariffa* is a rich resource in active agents including flavonoids, anthocyanin, polyphenols, antioxidant and antibacterial activity [9]. The main macromolecular component found in roselle ethanol extract is carbohydrates. The extract contained significant amounts of glucose and fructose, with concentrations of 5.6 and 6.4 g per dry weight, respectively. Research conducted suggests that these monosaccharides can lead to long-term performance benefits in sports. This is attributed to the effect of polysaccharides on muscle metabolism, which can improve performance [10, 11].

Nano-metal oxides have unique physicochemical characteristics, show strong functional properties due to their high surface area to volume ratio, and have good antimicrobial properties at low levels [12]. The addition of nano-ZnO in bio and synthesis polymers as nanoadditive could improve mechanical characteristics and also make other favorable effects in food packaging for example barrier properties [13]. Nano-metallic and nano-Metal oxide including Mg, Cu, Ag, CaO, ZnO, and TiO_2 are the most effective antimicrobial mineral compounds [14]. Antimicrobial activity, good photocatalytic, UV absorber, and barrier properties are only some benefits of nano-ZnO [15].

To the best of our knowledge, there is no study on roselle calyx extract/ZnO nanoparticle in a biocomposite film based on tapioca starch/bovine gelatin, so the presentation study aimed to estimate ZnO- N/roselle calyx extract effects on hydrophilic, mechanical, and barrier properties of biodegradable films.

MATERIALS AND METHODS

Materials

Tapioca starch was obtained from Avebe (Veendam Holland) and bovine gelatin was procured from CDH Co (Dehli, India). Liquid sorbitol and glycerol were purchased from Ajax Chemicals (Taren Point, Australia). Nano-ZnO was purchased from US-NANO (Houston, USA).

Roselle calyx extract

To obtain roselle extract Giusti, et al. [16] method with slight modification was used.

About 16 mL of 80% ethanol was mixed with 1 g of roselle calyx powder, and the pH of the solution was adjusted to 2 using HCl. To obtain the final extract, the specimens were centrifuged for 5 min at 3000 rpm after being heated for 60 min at 50°C. A rotary evaporator (Heidolph Hei-VAP, GER) was used to separate the solvent at 50°C.

Film preparation

Pure tapioca starch/bovine gelatin and treatment film containing RCE and ZnO-N were generated by casting technique. ZnO-N at levels of 2 (w/w%, based on starch) were added into distilled water (100 mL) and the mixture was stirred magnetically at 25°C for 1 h, and nano-dispersion was subjected to ultrasound wavelength. About 4g of tapioca starch, 0.4 g of bovine gelatin [17], and sorbitol/ glycerol (3.2 g) [18] were added to the nano-solution. All the solutions were heated at 84°C for 60 min. During cooling period of the bionanocomposite and composite solution (40°C), various amounts of roselle calyx extract (1.5 g for TP/RCE/ZnO-N and 2 g for TP/RCE) were incorporated into the mixture and homogenized for 1 h. At last, the TP, TP/ RCE, and TP/ RCE/ ZnO-N solutions were decanted into glass plates, dried at room temperature to form biodegradable films

Water vapor permeability (WVP)

The WVP of the biofilm was estimated based on the standard E96-05 method (ASTM, 2005). The biofilm, along with deionized water was placed in cups with area of 0.00196 m^2. After the glass cups were placed in a container with silica gel to generate a vapor pressure of 4362 Pa. At last, changes in the the weight of cup were evaluated using a weighing scale at 0, 2, 4, … , 10 h.

Solubility

Gravimetric assay was performed to evaluate solubility according to Marvizadeh, et al. [19]. The biofilm specimen strip was cut into a rectangular shape of 3 × 3 cm^2 and dried for 24h at 25°C. After that, the weight of the samples was taken, and the biofilms were immersed in deionized water (18 MΩ) for 1h at 25°C and the remaining specimen was separated through tissue Whatman (NO. 1) tissue and dried film at 60°C for 1d.

$$\text{Solubility}(\%)=\frac{\text{Initial film weight-Final film weight}}{\text{Initial film weight}}$$

The mentioned equation was applied to calculate the solubility of film

Mechanical properties

ASTM 882–10 (ASTM, 2002) method was performed to evaluate mechanical factors such as the elongation at break (EB), Young's modulus (YM), and tensile strength (TS), of biofilms with a texture analyzer (TA.XT2, Stable Micro System, Surrey, UK). The rectangular strips (100 × 20 mm) were cut from different biofilms. The testing speed of the pre-test, test, and post-test of the probe was 1, 5, and 10 mm/s respectively. The initial separation of 50 mm, and crossed speed of 30 mm/min, was applied and a load cell (20 g) was coupled to the probe.

Statistical analysis

ANOVA test was performed to compare data of WVP, solubility, and mechanical properties of films based on tapioca starch/bovine gelatin/roselle calyx extract/Zinc Oxide nanorod at 95% confidence level. Minitab software (17.3.1) was applied to estimate the experiment data.

RESULTS AND DISCUSSION

WVP of biodegradable film

One of the key factors in describing the transport of gas molecules from the environment into the package is water vapor permeability [20]. The WVP of the different biofilms are arranged in Table 1. The WVP of TB film, TB/RCE film, TB/RCE/ZnO-N film were 4.2×10^{-7} ± 0.08 g/mPah, 3.96×10^{-7} ± 0.05 g/mPah and, 3.36×10^{-7} ± 0.07 g/mPah, respectively (Table 1). The WVP of TB/RCE/ZnO-N bionanocomposite was less than TB/RCE film showing that the incorporation of ZnO-N greatly decreased the WVP of the biodegradable film.

The WVP improvement in TB/RCE film and TB/RCE/ZnO-N film might be related to changes in the interactions between additives and biopolymers [21].

The other work stated that the reduction in WVP of guar gum/chitosan film containing RCE/nano-ZnO was associated with additives added [12]. Similar improved barrier properties of the active film were found in previous studies [8, 22].

Table 1. WVP of TB, TB/ RCE, TB/RCE/ZnO-N

Film sample	WVP×10^{-7} (g/mPah)
TB	4.2±0.08a
TB/RCE	3.96±0.05b
TB/RCE/ZnO-N	3.36±0.07c

Mean ± SD values of film followed by various letters are significantly different ($p<0.05$)

Solubility

The solubility of the film may be a key index in defining utilization for films based on biopolymers [23]. The solubility of TB, TB/ RCE, and TB/RCE/ZnO-N films are shown in Table 2. The tapioca/bovine gelatin biofilm had (40.33% ± 0.05) solubility in water which is higher than TB/ RCE (36.89% ± 0.23) and TB/RCE/ZnO-N (33.68% ± 0.17) films. Incorporation of ZnO-N and RCE into tapioca starch/bovine gelatin biofilm significantly decreased the solubility in water of the composites which may be related to the bonding between the biopolymers and extract finally reducing the affinity toward H_2O molecules, which makes a hydrophobic system. Similar findings were stated in another study for biofilms fabricated from guar gum/chitosan containing RCE and nano-ZnO [12]. According to the mentioned study, the hydrophobic property is positive for obtaining active biofilm with improved characterizations. Aydin and Zorlu [24]stated that the solubility in water and water content of alginate biofilms incorporated with roselle extract were decreased with increasing level of the roselle extract.

Accordingly, Fallah, et al. [25] have stated that when the nanoadditive is added to biofilms, hydrogen bonds form between the nanoadditive and the biopolymer.

Table 2. Solubility of TB, TB/ RCE, TB/RCE/ZnO-N

Film sample	Solubility (%)
TB	40.33.±0.5a
TB/RCE	36.89±0.23b
TB/RCE/ZnO-N	33.68±0.17c

Mean ± SD values of film followed by various letters are significantly different (p<0.05)

Mechanical properties

The mechanical characteristics of the biofilms were evaluated by tensile strength (TS), elongations at break (EB), and Young's modulus (YM), which were represented in Table 3. As observed, TB film indicated poor mechanical properties as compared to TB/RCE film and TB/RCE/ZnO-N film. Table 3 showed an increase in TS and YM values of TB/RCE film (21.85 MPa; 1.52 MPa) and TB/RCE /ZnO-N film (28.22 MPa; 2.65 MPa). The EB value of the TB film was 18.44%, the value of TB/RCE and TB/RCE/ZnO-N biofilms were 14.56% and 10.69%, respectively. It was stated that the TB/RCE/ZnO-N biofilm possessed lower EB and higher TS as compared to neat samples (p< 0.05).

Nano-ZnO formed covalent and hydrogen bonds and improved the mechanical properties of the polymer matrix [26]. The improvement of the biofilms can be related to the interaction between biopolymer molecules and polyphenolic compounds of extract. Also, The obtained findings related to the incorporation of RCE into biofilms enhancing the hydrogen bond with biofilms structure, as polymer chains act more regularly and then the TS of biofilm increased [27].

Similar trends in mechanical characteristics as affected by phytochemicals such as polyphenols were also found in active films, including guar gum/chitosan incorporated roselle extract biofilm [12]. Other composite films containing bamboo [28] and black carrot [29] extract had similar findings.

Table 3. TS, EB and YM of TB, TB/ RCE, TB/RCE/ZnO-N

Film sample	*TS(Mpa)*	*EB (%)*	*YM*
TB	14.4±0.21c	18.44±0.31a	0.79±0.05c
TB/RCE	21.85±0.20b	14.56±0.25b	1.52±0.08b
TB/RCE/ZnO-N	28.22±0.31a	10.69±0.22c	2.65±0.09a

Mean ± SD values of film followed by various letters are significantly different (p<0.05)

CONCLUSIONS

In the current study, we incorporated roselle calyx extract and nano-ZnO into the tapioca starch/bovine gelatin composite matrix to fabricate an active film. Results represent that nanoadditives and roselle calyx extract could improve biocomposite film's characteristics such as solubility and water vapor permeability. Also, the TB edible films containing nanoadditives and extract had strong mechanical properties due to covalent and hydrogen bonds and the total phenolic compound of RCE.

The obtained results presented that active film based on tapioca starch/bovine gelatin/ RCE and tapioca starch/bovine gelatin/RCE/ZnO-N may have application potential in the non-food and food packaging industries.

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Conflict of interests

The authors declare that there is no conflict of interest.

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