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ORIGINAL ARTICLE

Antimicrobial and Hydrophilic Behavior of Soluble Soy Polysaccharide Starch/Cold Water Fish Gelatin Films Incorporated with Nano-Titanium Dioxide

Neda Fallah[1], Narmin Nabeghvatan[2], Tahereh Sadeghi[*3], Anna Etemadi Razlighi[*4], Mohammad Mehdi Marvizadeh[*5], Abdorreza Mohammadi Nafchi[6]

[1]*Department of Food Science and Technology, Qom Branch, Islamic Azad University, Qom, Iran*

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

[3]*Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

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

[5]*Young Researchers and Elite Club, Damghan Branch, Islamic Azad University, Damghan, Semnan, Iran*

[6]*Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Penang, Malaysia*

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KEYWORDS

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ABSTRACT: Edible films based on starch are applied for food protection to extend shelf-life of food products. The biodegradability of film compounds was significantly improved by the utilization of biopolymer molecules in comparison to petroleum packaging, and it could be a replacement for synthetic packaging. In the presentation work, the effects of nano titanium dioxide (TiO_2-N) were studied on the moisture content, water solubility, moisture uptake, and antimicrobial properties of soluble soy polysaccharide (SSPS) starch/cold water fish gelatin films. The nano titanium dioxide was incorporated into the SSPS starch/cold water fish gelatin dispersion at various levels (1, 2, 3, and 5 w/w% based on SSPS starch). The moisture content of the biofilms decreased from 14.12% to 12.28% and water solubility decreased from 93.18% to 71.46% by the incorporation of 5% TiO_2-N into the biopolymer matrix. Increasing TiO_2-N from 0 to 5% decreased the moisture uptake, a value of 9.83–6.81 g/g dried film. According to an increased inhibition zone of 1-5% TiO_2-N for *Staphylococcus aureus* and *Escherichia coli* from 32.44 to 62.27 mm^2 and from 22.15 to 45.17 mm^2 respectively, SSPS starch/cold water fish gelatin/TiO_2-N films showed antimicrobial characterizations against *Staphylococcus aureus* and *Escherichia coli*. In summary, TiO_2-N improves the functional properties of SSPS/cold water fish gelatin biofilms and can be applied in the food industry.

INTRODUCTION

For the past decade, the using of packaging based on synthesized material have been provided a major production of residues, which makes the opinion of applying biodegradable materials in film production from biodegradable resources[1, 2]. As renewable and biodegradable materials natural polymers have advantages compared to synthetic polymers. Food quality is maintained during the production and distribution cycles by biodegradable films that are inhibitory to volatile substances and oils.[3, 4]. There have been found some often occurring problems, however, that prevent their industrial application. First, because of their innately reduced stiffness and strength, in some cases, their mechanical qualities are quite inferior compared to synthetic polymers. Second, many materials, especially in wet conditions, are extremely

*Corresponding author: sadeghibahar2030@gmail.com; anna.etemadi@yahoo.com; engmmarvi@gmail.com (T. Sadeghi; A. Etemadi Razlighi; M.M. Marvizadeh)
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sensitive to water, with certain materials dissolving quickly or significantly losing mechanical strength when they absorb water. Third, their current low manufacturing level and high cost prevent them from being used in a broad range of applications.[5].

Recently, soluble soybean polysaccharide (SSPS), a new polysaccharide, was isolated from the cell wall component of soybean cotyledons. A homogalacturonan (α-1,4-galacturonan) and rhamnogalac-turonan (repeating units consisting of α-1,4-galacturonic acid and α-1,2-rhamunose) polysaccharide, branched by β-1,4-galactan and α-1,5 or α-1,3- arabinan chains, make up the SSPS-like structure of pectin[6]. The film's properties of SSPS have been measured in previous study [7]and findings indicate that SSPS starch can fabricate biofilms with excellent appearance characterization and good mechanical behavior. However, SSPS starch is sensitive to water vapor and inherently soluble in water [6].

Cephalopods and fish skins, an important by-product of the fisheries industry, can be used to extract fish gelatin. It could also be used as a natural polymer in film preparation because of it's capacity to form film[8]. Fish gelatin films have been the subject of numerous research recently in an effort to distinguish them from synthetic films, which are the main source of environmental contamination and can be applied to preserve food from oxidation and drying [9]. The gelatin biopolymer forms a three-dimensional structure with intermolecular microcrystalline connections, and the low water content of this network may provide brittle biofilms. Therefore, to prepare edible film using sorbitol or glyserol as a plasticizer, its plasticity should be increased, which may also the reduction of vitreous transition[10]. However, different challenges, like sensitivity to moisture and limited antioxidant activity, restrict the usage of fish gelatin films. In terms of water sensitivity, fish gelatin film, partly dissolves and decomposes when used in contact with extremely moisture food [11].

In the recent decade, nanotechnology has helped to solve these problems [12]. Molecules and atoms the physicochemical and biological characterizations of structures at the nanoscale differ significantly from their microscale systems, providing special functional applications [13].

Nanocomposite materials consisting of metal nanoparticles (gold, silver, copper, iron, etc.) have attracted much attention because of their distinct optical, electrical, magnetic, antibacterial, and catalytic properties [14]. Natural biopolymer bionanocomposites-based packaging materials have great potential for enhancing food quality, safety, and stability as an innovative packaging and processing technology [15]. These fabrications of biocomposites containing nanoadditive have more favorable film properties, like excellence in mechanical and hydrophobic behavior. Decreasing the water vapor permeability, improving efficiency in the application of biofilm as an antimicrobial biopackaging. [16]. Acyl lipid constituents, such as linolenic, linoleic, and, oleic acids, have one or more allyl groups within the fatty acid molecule and are readily oxidized to hydroperoxides [17]. Incorporating a nanoadditive into biofilms decreased the penetration to gas molecules and could also partially reduce hydroperoxide compounds[18].

There are some reports about the improvement of biopolymers by the incorporation of nanoparticles, such as nano-titanium dioxide[19].In the case of nanosized titanium dioxide, it has been extensively investigated in many thermoplastics (PVC, PP, PS, PE, etc.) for its robust and general reactivity, high photostability and, biocompatibility[20]. Nano titanium dioxide is used as a white pigment in paper and plastic because of its UV-resistant properties. TiO_2 can also be found in food itself, for example in sugar glazing in confectionery or instant beverages [21].

In this study, nano titanium dioxide was applied as a nanofiller to fabricate SSPS starch/cold water fish gelatin film. The films based on starch/protein were studied for their antimicrobial, moisture content, and water solubility behavior.

MATERIALS AND METHODS

Materials

SSPS starch was purchased from SIM Co (Penang, Malaysia), and cold water fish gelatin was purchased from Sigma Chemical Co (Louis, USA). Glycerol and sorbitol were obtained from Macklin Biochemical

(Shanghai, China). Nano titanium dioxide was purchased from Nano meghyas borhan (Mashhad, Iarn).

Film preparation

Neat SSPS starch/cold water fish gelatin and active film containing Nano-TiO_2 were fabricated by casting method. Nanoadditive at levels of 1, 2, 3, and 5(w/w%, based on SSPS starch) were added to 100 mL of distilled water and aqueous solutions were stirred magnetically for 60 min and solutions were exposed to ultrasound wavelength for 45min. Neat starch/gelatin biofilm suspension was fabricated by dispersing SSPS starch (8 g), cold water fish gelatin(0.8 g) [22, 23], and glycerol/sorbitol (3.2 g) [24] in 100mL of Nano-solution. All the suspensions were heated at 85°C for 1h followed by cooling at 37°C. At last, the suspensions were poured on casting plates, and dried at 25°C (RH = 50%) to form bionaocomposite films.

Characterization of starch/gelatin film

Moisture content

The moisture content of biofilms was measured by evaluating the weight difference of biofilms before and after drying in a desiccator containing $CaCl_2$ and placed in an oven at 40°C for 24 h. Five replications of each biofilm were applied for calculating the moisture content.

Solubility

Pieces of each biofilm were cut and placed in a glass container with P_2O_5 for 48h and weighed to measure the dry weight of the film. The solubility of the biofilms was evaluated by immersion test in 80 ml of deionized water with periodic agitation for 1 h at 25°C. At last, the remaining pieces of biofilms were filtered and dried at 60°C to achieve constant weight. The solubility (%) of films was calculated as follow:

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

Antimicrobial characterizations

Antimicrobial experiment on the biofilms was performed by applying the agar diffusion technique based on Maizura, et al. [25]. First 1 mL of inoculation containing 10^5–10^6 CFU/mL of gram-negative *E. coli* and gram-positive *S. aureus* were seeded onto Mueller Hinton agar (MHA) solid media. Film disks were then placed on MHA media. After that, the plates were then incubated for 1 day at 37°C. Following that, the plates had a "zone of inhibition" of the film disc examination. Computing the inhibition zone of gram-negative *E. coli* and gram-positive *S. aureus* on MHA media allowed researchers to determine the film's antimicrobial effects. The zone of inhibition of bactrial was determined by calculating the area of the entire zone and subtracting it from the film disk area.

Moisture uptake

Moisture uptake of active film was determined following the technique of Tajik, et al. [26]. Specimen film (2 cm × 2 cm) was cut and dried with P_2O_5 for 2 days and then weighed (w_1). The dried film was conditioned with potassium sulfate solution (RH = 97%) for 2 days. The active film was removed from control condition and weighed (W_2).

The moisture uptake was stated as following equation:

Moisture uptake = $(w_2-w_1)/w_1$

Statistical analysis

One-Way ANOVA was utilized to compare data of solubility, moisture content, and antimicrobial behaviors of films at 95% confidence level. Data from antimicrobial and chemical experiments were analyzed by Minitab 17.3.1 software.

RESULTS AND DISCUSSION

Moisture content

The moisture content of the nano titanium dioxide/SSPS starch/cold water fish gelatin films is presented in Table 1. The moisture content decreased as TiO_2-N concentration was enhanced. The interaction between the glycerol and sorbitol, biopolymer molecules, and nanofiller is probably to blame for the decreased availability of the hydroxyl group to interact with water, which results in a less hygroscopic matrix[27]. According to Hajizadeh, et al. [28] interactions between the titanium and the hydroxyl groups from the glycerol/sorbitol and water, and also between the titanium and the plasticizer of the ion, occur.

Table 1. Moisture content of SSPS starch/cold water fish gelatin/TiO_2 film.

TiO_2-N (%)	Moisture content (%)
0	14.12±0.08a
1	13.34±0.05b
2	12.95±0.06c
3	12.56±0.1d
5	12.28±0.06e

Data are mean and SD. Different letters in column show significant difference at $p<0.05$ among SSPS starch/cold water fish gelatin/TiO_2 films

Solubility

Solubility in water may be an important factor in defining applications for biopolymer composite films [29]. The solubility of the nano titanium dioxide/SSPS starch/cold water fish gelatin films is presented in Table 2. The incorporation of nano titanium dioxide into SSPS starch/cold water fish gelatin matrix significantly decreased the solubility of the biocomposites. These presentation findings are in agreement with other scholars on biocomposite film containing nanostructure [30, 31]. This result might be explained by interactions between the film-forming component of the biopolymer and TiO_2-N[32]. According to another study, more hydrogen bonds between the components of the matrix and TiO_2 are formed as the nanofiller concentration of film increases[33].

Table 2. Solubility of SSPS starch/cold water fish gelatin/TiO_2 film

TiO_2-N (%)	Solubility (%)
0	93.18.±2.11a
1	89.45±1.59a
2	83.85±1.63b
3	77.71±1.87c
5	71.46±1.75d

Data are mean and SD. Different letters in column show significant difference at $p<0.05$ among SSPS starch/cold water fish gelatin/TiO_2 films

Antimicrobial properties

Table 3 indicates the impacts of TiO_2-N level on the antimicrobial characterization of different biofilms against gram-negative *E. coli* and gram-positive *S. aureus*. The ncorporation of nano titanium dioxide into SSPS starch/cold water fish gelatin matrix significantly increased the antimicrobial activity of the biocomposites. These findings are consistent with other scholars on nanobiocomposite [34-36]. The physical and/or chemical contact between nanofiller and the microbial cell wall is one mechanism that relates the antimicrobial property of nanofiller to this phenomenon. The microorganism's cell wall might allow the Ti4+ to permeate and react with different components, which would ultimately influence the viability of the cells [37] and the formation of H_2O_2 due to presence of nanoadditive particles[38].

Table 3. Antimicroial properties of SSPS starch/cold water fish gelatin/TiO_2 film

TiO_2-N (%)	Inhibition zone(mm^2)	
	S. aureus	*E. coli*
0	1.13±0.05a	1.13±0.05a
1	32.14±0.46b	22.15±0.51b
2	37.18±0.73c	27.69±0.79c
3	42.23±1d	33.24±0.84d
5	62.27±0.88e	45.17±1e

Data are mean and SD. Different letters in columns show significant difference at $p<0.05$ among SSPS starch/cold water fish gelatin/TiO_2 films

Moisture uptake

The moisture uptake of pure SSPS/gelatin and SSPS/gelatin / TiO_2-N nanocomposite films was between 9.83 and 6.81 g/g dried film (Table 4). The findings indicated that neat biofilms had the highest moisture uptake. The incorporation of nanoadditive significantly ($p < 0.05$) decreased the moisture uptake of the biodegradable films depending on the level of TiO_2-N. The higher moisture uptake present in the neat film based on SSPS/gelatin may be due to hydroxyl groups in the biopolymer structure.

TiO_2-N may interact with the biopolymer molecules upon adding the nanoadditive into SSPS/gelatin matrix, which may reduce biopolymer-water interaction[39].

Similar findings have been stated by scholars regarding reduced moisture uptake in biofilms incorporated with TiO_2-N[23], and ZnO-nanorod [40].

Table 4. Moisture uptake of SSPS starch/cold water fish gelatin/TiO_2 film

TiO_2-N (%)	Moisture uptake (g/gdried film)
0	9.83.±0.08a
1	8.54±0.1b
2	8.09±0.11c
3	7.65±0.13d
5	6.81±0.08e

Data are mean and SD. Different letters in column show significant difference at $p<0.05$ among SSPS starch/cold water fish gelatin/TiO_2 films

CONCLUSIONS

In this study, we incorporated nano-TiO_2 into the SSPS starch/cold water fish gelatin matrix to fabricate bionanocomposites. Results obtained in this study indicate that nano titanium dioxide particles could improve bionanocomposites film properties such as moisture content, moisture uptake, and solubility. Nano-TiO_2 embedded in biofilms based on starch/protein represents good antimicrobial characterization against gram-negative *E. coli* and gram-positive *S. aureus*. The findings indicated that biodegradable film based on SSPS starch/cold water fish gelatin/nano-TiO_2 may have favorable potential in the food packaging industries.

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Conflict of interests

The authors declare that there is no conflict of interest.

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