RESEARCH ARTICLE

Quince seed mucilage supplemented with titanium dioxide and Silicon dioxide nanoparticles

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ABSTRACT

Objective(s): Quince seed mucilage (QSM) serves as a new source of hydrocolloid which extracted from outer pericarp of *Cydonia oblonga* seeds upon wetting. It has been traditionally used for the treatment of diseases such as pharyngeal disorder, common cold, colic ulcer, and diarrhea. The aim of the present study was to evaluate the physico-mechanical and antimicrobial properties of quince seed mucilage supplemented with titanium dioxide (TiO_2) and Silicon dioxide (SiO_2) nanoparticles.

Methods: The antimicrobial property of designated QSM against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7 was determined using agar disk diffusion and broth micro-dilution assays. Thickness, tensile strength (TS), puncture force (PF), puncture deformation (PD), swelling index (SI), and color of active QSMs were evaluated using analytical instruments.

Results: The films containing TiO_2 and SiO_2 nanoparticles exhibited good antimicrobial effects against *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 ranged 0.82-6.88 mm and -2.78 - -0.28 log differences in population (DP) regarding agar disk diffusion and broth micro-dilution assays, respectively. The presented values, including TS, PF, and PD of QSM films, were in the ranges of 22.45-35.81 MPa, 10.42-15.49 N, and 15.53-18.45 mm, respectively.

Conclusions: Application of TiO_2 and SiO_2 nanoparticles greatly improved the antimicrobial and physico-mechanical properties of the prepared films.

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INTRODUCTION

Outbreaks of food-borne pathogens with high morbidity and mortality rates have been increased in recent decades and considered as a big concern in public attention and food safety in socio-economic development countries [1]. The Center for Disease Control and Prevention (CDCP) indicates that about 48-50 million cases of food-borne diseases occur in the United States, and there are 127,839 hospitalizations and 3,037 deaths related to food-borne diseases each year [2]. *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7 are the most commonly recognized food-borne microorganisms [3]. Several outbreaks of food-borne illnesses have been occurred due to the consumption of fresh food products such as fish and fishery products, meat and meat products, raw milk, and vegetable salads [2]. For this reason, there is a permanent status to discover effective and excellent antimicrobial compounds to ensure food safety and increase their shelf-life.

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During the last decade, increasing researches have been performed to fabricate active food packaging materials owing to its potential to provide food safety and control undesirable changes of product quality including chemical, microbial, and sensory properties [4]. Production of biodegradable/edible biopolymers from natural renewable resources including carbohydrates, proteins, and lipids is one of the main demand in the food industry fields owing to increasing concern about application of plastic packaging materials on our health and ecosystems [5]. Quince belongs to the *Rosaceae* family is considered as one of the most common used fruits in winter season with high nutritional compounds containing antimicrobial and antioxidant properties [6]. The quince seeds in Iranian folk medicine was used for the treatment of cold, pharyngeal disorder and also as an emulsifying agent in the preparation of hair-fixing lotions [7]. Previous studies suggested that quince seed mucilage (QSM) can be used as an edible film/coating material for protecting and extending the shelf life of fresh foods [6-9]. Biodegradable films/coatings might be used to carry antimicrobial and antioxidant compounds that provide an extra stress factor against foods' oxidative, sensorial, and microbial deterioration [10]. In recent years, the use of inorganic antimicrobial agents in food applications has attracted much interest in the control of food-borne and spoilage microorganisms [11-13]. Titanium dioxide (TiO_2) is a naturally occurring oxide of the element titanium, also referred to as titania. This substance occurs naturally as three mineral compounds known as anatase, rutile, and brookite [14]. There are a number of industrial applications for this mineral because of its very high refraction properties. In fact, TiO_2, known as "titanium white", is one of the whitest materials in the metal world [15]. Silicon dioxide (SiO_2) also exits in the nature in form of silica or silicate due to its strong affinity with oxygen. It is the second most abundant element on the earth crust while yet not considered as an essential element for plants [16]. The potential application of antimicrobial compounds in nano-size scale such as clay [11, 17], magnesium oxide [13], and zinc oxide [12] in food models and *in-vitro* condition have been extensively evaluated. Based on our knowledge, there was no published study regarding the antimicrobial activity of QSM supplemented with nanometals in food models and *in vitro* conditions. Therefore, the aims of the present study were to evaluate the physico-mechanical and antimicrobial properties of QSM supplemented with TiO_2 and SiO_2 nanoparticles.

MATERIALS AND METHODS

Materials

TiO_2 and SiO_2 nanoparticles (20-30 nm diameter and purity > 97-99%) were purchased from the Iranian Nanomaterials Pioneers, Mashhad, Khorasan Razavi, Iran. Glycerol, Brain Heart Infusion (BHI) broth, Brain Heart Infusion (BHI) agar, and buffered peptone water were obtained from Merck, Taufkirchen, Germany. Quince seeds were obtained from a local market, Kermanshah, Iran. *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 11774), *L. monocytogenes* (ATCC 19118), *S. typhimurium* (ATCC 14028) and *E. coli* O157:H7 (ATCC 10536) were purchased from the culture collection of the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.

Extraction of quince seed mucilage

Quince seeds were identified by a taxonomist at Razi University, Kermanshah, Iran. For this reason, the quince seeds were manually cleaned, dipped in ethanol to remove all foreign impurities, and dried by an oven at 45 ± 1 °C for 8 h. Then, the seeds were submerged in doubled distilled water at a ratio of 1:35 w/v and stirred on a hot plate-magnetic stirrer (IKA, Germany) based on the previously published methods [7, 9]. Then, the swelled seeds were stirred with a rod paddle blender at 12000 rpm at 40 ± 1 °C for 10 min to scrape the mucilage layer of the seed surface. The solutions were then filtered with cheesecloth and the obtained mucilage was dried by an oven at 45 ± 1 °C for 12 h. The dried purified QSM was kept in a dry place until the further experiment.

Preparation of active films

Preparation of nanocompostie films was conducted using a casting method according to the previous method reported by Khazaei et al., (2014) with some minor modifications [6]. The film-forming solution based on QSM was prepared by incorporating 5 g QSM powder into 100 ml distilled water and stirred on a magnetic stirrer/hot plate (IKA, Germany) at room temperature until completely dissolved. Glycerol as a plasticizer was added at 4 ml/g (concerning the amount of QSM) into the solution and stirred for 10 min at room temperature. TiO_2 and SiO_2 nanoparticles at concentrations of 0, 0.5 and

1%, alone and in combination, were incorporated in the solutions and continuously mixed using a homogenizer (HG-15D, Wise Tis, Korea) at 10000 × g for 10 min. All designated films were prepared by casting 50 ml of the mixtures on 12 cm glass plates and drying at ambient conditions for 48 h.

Preparation of bacterial strains

Lyophilize bacterial strains were activated in BHI broth at 37 ± 1 °C for 24 h. The second sub-culture of each microorganism were adjusted at appropriate optical densities using spectrometer as an inoculum dose [10].

Antimicrobial properties of designated films

The antimicrobial property of designated films against *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium,* and *E. coli* O157:H7 was determined using agar disk diffusion and broth micro-dilution assays [18].

In the agar disk diffusion assay, 15-20 ml melted BHI agar was poured into the sterile plastic petri dishes (diameter = 90 cm). After that, an amount of 0.1 ml of each bacterial strain suspension (8 log CFU/ml) was completely spread on the agar plate. After drying of the surface plate, the designated films containing different concentrations of 0, 0.5 and 1% with 12 mm diameter were put onto the inoculated plates and then the plates were incubated at 37 ± 1 °C for 24 h.

In the broth micro-dilution assay, the designated films as a film-forming solution form was used before drying at room temperature. Firstly, 180 µl of designated film-forming solutions and 20 µl BHI broth containing 8 log CFU/ml of bacterial suspension were added into the 96-well sterile micro-dilution plates with U-bottom wells. Two wells with and without bacterial pathogens as positive and negative controls were considered in the experiment and incubated as described above. The plates were packed with sterile plate sealers. All micro-plates were completely stirred on a plate shaker at 200 rpm for 1 min at room temperature. After incubation, from 10 µl of each well was diluted using ten-fold serial dilution, cultured on the BHI agar and incubated at 37 ± 1 °C for 24 h. Thereafter, the number of bacterial colonies was counted, and the results were expressed in terms of differences in the population (DP) according to the equation:

$$\text{Log DP} = \log\left(\frac{N}{N_0}\right) = \log N - \log N_0$$

Where N and N_0 are the bacterial populations (CFU/ml) at time t and zero, respectively.

Physico-mechanical properties of active films

Thickness, tensile strength (TS), puncture force (PS), puncture deformation (PD), and swelling index (SI) of active films were evaluated based on the previous methods [18, 19].

The color of film samples in terms of lightness (*L*), redness (*a*), and yellowness (*b*) were determined using Minolta Chroma Meter Model CR-400 (Minolta, Japan). Therefore, the film pieces (30 mm in diameter) were placed over the standard white plate (L^* = 94.24, a^* = -0.52, and b^* = 4.19). Total color differences (ΔE) were calculated by the following equation [20]:

$$\Delta E = \sqrt{(\Delta L)2 + (\Delta a)2 + (\Delta b)2}$$

Where ΔL (lightness difference) = $L^* - L$, Δa (red-green chromaticity difference) = $a^* - a$, and Δb (yellow-blue chromaticity difference) = $b^* - b$. L^*, a^* and b^* are the color parameter values of the standard and *L*, *a* and *b* are the color parameter values of the sample.

Statistical analysis

All experiments were performed in triplicate. The analysis was performed using SPSS 25 for Windows (SPSS, Chicago, IL, USA) software package. All data were subjected to one-way analysis of variance to determine the differences of samples. Significance level was considered $P < 0.05$ in all experimental data.

RESULTS AND DISCUSSION

The results of *in vitro* antibacterial activities of designated QSM based films with or without TiO_2 and SiO_2 nanoparticles are indicated in Table 1. In the agar disk diffusion assay, control film had not any clear inhibition zone against all microorganisms. When the designated films were removed from the agar surface plate, the bacterial growth was not observed. The similar findings were observed in the study of Jouki et al., (2014) [7]. Accordingly, the control QSM did not show antibacterial effects against *S. aureus*, *B. cereus*, *L. monocytogenes*, *Yersinia enterocolitica*, and *E. coli* O157:H7 in the agar disk diffusion assay. Wang et al., (2018) reported that QSM contained polyphenol compounds with high antioxidant and antimicrobial properties [21]. Our preliminary study showed that the antioxidant property of QSM in terms of DPPH radical scavenging activity was found to be 25.34% (experiment method was not shown). The absence of inhibition zone in control film is owing to the inability of films for diffusion via agar medium when it is in a solid form [20]. The lack of a clear inhibition zone in other straight bio-polymeric materials such as chitosan [20, 22] and gelatin [18] was also found. Moreover, the films containing TiO_2 and SiO_2 nanoparticles exhibited good antimicrobial effects against *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 ranged 0.82-6.88 mm and -2.78 - -0.28 log DP regarding agar disk diffusion and broth micro-dilution assays, respectively. Besinis et al., (2014) reported that TiO_2 and SiO_2 nanoparticles had good antimicrobial effects against *Streptococcus mutans* as an oral pathogen [23]. In the study of Santhoshkumar et al., (2014), TiO_2 nanoparticles displayed antimicrobial activity against *Proteus mirabilis*, *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* in the range of 17-25 mm [24]. Our findings are also in agreement with those reported for the effects of nano-metals against *S. aureus* [25], *L. monocytogenes* [26], and *S. typhimurium* [27].

The antibacterial activity of SiO_2 nanoparticles might be probably related to their oxidative attack on microbial surfaces, cell differentiation, adhesion, and spreading [23, 24]. TiO_2 nanoparticles produced reactive oxygen species (ROS) via oxidative stress, oxidized lipid oxidation of membrane fluidity, and also disrupt the cell integrity [23]. Based on the results of Fellahi et al., (2013), SiO_2 nanowires had strong antimicrobial activity against *E. coli* O157:H7 [28]. As can be seen in Table 1, *S. aureus* was the most susceptible bacteria followed by *B. subtilis*, and *B. cereus*. In general, Gram-negative bacteria composited of lipopolysaccharide and subsequently, more resistance to the Gram-positive bacteria against metal nanoparticles [3].

The mechanical properties of active QSM films including, TS, PF, and PD are presented in Fig. 1. The presented values including, TS, PF, and PD of QSM films were 22.45-35.81 MPa, 10.42-15.49 N, and 15.53-18.45 mm, respectively, and agreement with those reported by other authors [6, 7]. The mechanical properties of films containing a combination of TiO_2 and SiO_2 nanoparticles at concentrations of 0.5 and 1% were significantly higher than straight films ($P < 0.05$). Previous studies reviewed that mechanical characterization of biodegradable films is probably closed with microstructure and the intermolecular interactions [17, 29]. The higher mechanical properties of films containing nanoparticles might be related to the extremely interactions (hydrogenic and

Table 1. Antibacterial activity of quince seed mucilage supplemented with titanium dioxide (TiO_2) and Silicon dioxide (SiO_2) nanoparticles

Film formulation	*Staphylococcus aureus*		*Bacillus subtilis*		*Bacillus cereus*	
	Inhibition zone (mm)	Log DP**	Inhibition zone (mm)	Log DP	Inhibition zone (mm)	Log DP
Control	ND*	-0.38 ± 0.05	ND	-0.22 ± 0.03	ND	-0.15 ± 0.09
TiO_2 0.5%	5.22 ± 0.22	-1.35 ± 0.18	4.85 ± 0.45	-1.08 ± 0.33	4.55 ± 0.08	-1.04 ± 0.05
TiO_2 1%	5.55 ± 0.33	-1.87 ± 0.21	4.99 ± 0.18	-1.15 ± 0.45	4.76 ± 0.67	-1.13 ± 0.09
SiO_2 0.5%	4.76 ± 0.09	-1.06 ± 0.08	4.41 ± 0.09	-0.92 ± 0.11	4.22 ± 0.02	-0.82 ± 0.14
SiO_2 1%	4.99 ± 0.12	-1.25 ± 0.12	4.62 ± 0.03	-1.12 ± 0.11	4.45 ± 0.77	-1.12 ± 0.07
TiO_2 0.5% + SiO_2 0.5%	6.43 ± 0.08	-2.01 ± 0.45	6.12 ± 0.34	-1.28 ± 0.56	5.38 ± 0.18	-1.19 ± 0.08
TiO_2 0.5% + SiO_2 1%	6.67 ± 0.12	-2.14 ± 0.05	6.25 ± 0.13	-1.35 ± 0.02	5.56 ± 0.42	-1.27 ± 0.19
TiO_2 1% + SiO_2 0.5%	6.81 ± 0.67	-2.62 ± 0.34	6.59 ± 0.08	-1.44 ± 0.06	5.61 ± 0.56	-1.75 ± 0.36
TiO_2 1% + SiO_2 1%	6.88 ± 0.12	-2.78 ± 0.42	6.73 ± 0.29	-1.62 ± 0.06	5.75 ± 0.68	-1.95 ± 0.45
	Listeria monocytogenes		*Escherichia coli* O157:H7		*Salmonella typhimurium*	
Film formulation	Inhibition zone (mm)	Log DP**	Inhibition zone (mm)	Log DP	Inhibition zone (mm)	Log DP
Control	ND*	0.19 ± 0.05	ND	0.15 ± 0.08	ND	0.17 ± 0.04
TiO_2 0.5%	1.23 ± 0.51	-0.87 ± 0.09	1.13 ± 0.43	-0.75 ± 0.32	0.82 ± 0.44	-0.62 ± 0.14
TiO_2 1%	1.75 ± 0.33	-0.95 ± 0.62	1.35 ± 0.06	-0.85 ± 0.09	1.12 ± 0.02	-0.71 ± 0.09
SiO_2 0.5%	ND	-0.38 ± 0.03	ND	-0.41 ± 0.22	ND	-0.28 ± 0.01
SiO_2 1%	1.00 ± 0.00	-0.75 ± 0.12	ND	-0.67 ± 0.43	ND	-0.55 ± 0.55
TiO_2 0.5% + SiO_2 0.5%	2.97 ± 0.18	-1.05 ± 0.44	2.67 ± 0.46	-0.98 ± 0.24	2.37 ± 0.12	-0.75 ± 0.33
TiO_2 0.5% + SiO_2 1%	3.14 ± 0.25	-1.08 ± 0.62	2.76 ± 0.45	-1.01 ± 0.14	2.45 ± 0.44	-0.92 ± 0.15
TiO_2 1% + SiO_2 0.5%	3.33 ± 0.09	-1.11 ± 0.35	2.88 ± 0.12	-1.09 ± 0.05	2.52 ± 0.45	-1.01 ± 0.04
TiO_2 1% + SiO_2 1%	3.45 ± 0.32	-1.32 ± 0.42	3.12 ± 0.05	-1.23 ± 0.09	2.93 ± 0.08	-1.08 ± 0.05

*ND: Not detected.

**Log DP= log (N/N_0) = log N – log N_0; where N and N_0 are the bacterial population (CFU/ml) at time t and zero, respectively.

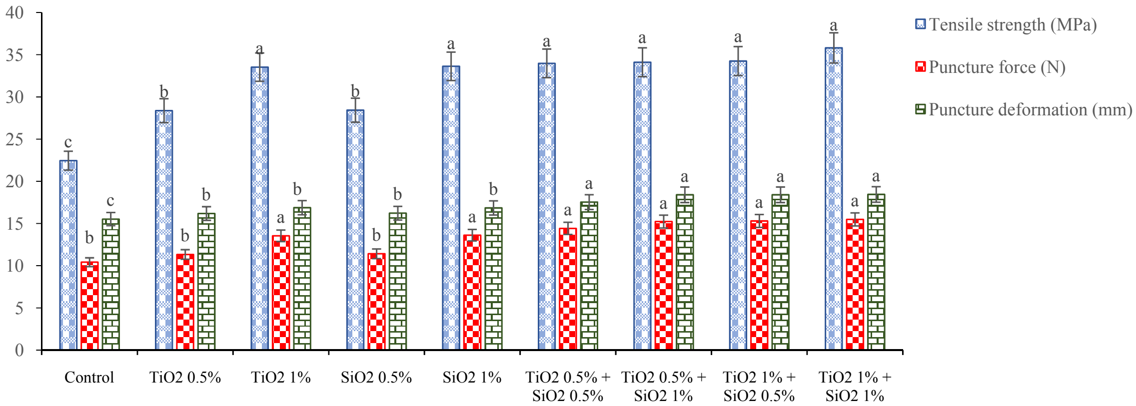

Fig. 1. Mechanical properties of quince seed mucilage supplemented with titanium dioxide (TiO_2) and Silicon dioxide (SiO_2) nanoparticles. [a-c] Different lower case letter among groups indicates significant differences (P < 0.05).

Table 2. Physical properties of quince seed mucilage supplemented with titanium dioxide (TiO_2) and Silicon dioxide (SiO_2) nanoparticles.

	Thickness (mm)	Swelling index (%)
Control	0.055 ± 0.001	75.45 ± 1.45^a
TiO_2 0.5%	0.056 ± 0.002	74.23 ± 2.88^a
TiO_2 1%	0.055 ± 0.001	71.87 ± 2.60^a
SiO_2 0.5%	0.056 ± 0.000	73.78 ± 4.58^a
SiO_2 1%	0.056 ± 0.002	72.45 ± 4.10^a
TiO_2 0.5% + SiO_2 0.5%	0.055 ± 0.001	60.73 ± 0.96^b
TiO_2 0.5% + SiO_2 1%	0.055 ± 0.001	62.45 ± 0.51^b
TiO_2 1% + SiO_2 0.5%	0.056 ± 0.000	62.76 ± 1.34^b
TiO_2 1% + SiO_2 1%	0.057 ± 0.002	64.34 ± 1.88^b

[a-c] Different lower case letter in same column indicates significant differences (P < 0.05).

Table 3. Color properties of quince seed mucilage supplemented with titanium dioxide (TiO_2) and Silicon dioxide (SiO_2) nanoparticles

	$L*$	$a*$	$b*$	ΔE
Control	87.45 ± 2.45^c	-1.34 ± 0.04^c	15.67 ± 1.54^c	13.28
TiO_2 0.5%	88.32 ± 0.99^b	-1.56 ± 0.09^b	15.93 ± 1.34^c	13.18
TiO_2 1%	88.98 ± 1.76^b	-1.67 ± 0.12^b	16.11 ± 2.65^b	13.07
SiO_2 0.5%	88.12 ± 4.23^b	-1.65 ± 0.05^b	16.34 ± 2.78^b	13.65
SiO_2 1%	88.32 ± 2.42^b	-1.63 ± 0.13^b	16.45 ± 2.49^b	13.65
TiO_2 0.5% + SiO_2 0.5%	89.47 ± 2.35^b	-1.85 ± 0.00^b	17.67 ± 3.14^a	14.35
TiO_2 0.5% + SiO_2 1%	92.16 ± 7.11^a	-2.34 ± 0.09^a	17.45 ± 3.33^a	13.54
TiO_2 1% + SiO_2 0.5%	92.23 ± 5.60^a	-2.34 ± 0.02^a	17.45 ± 5.15^a	13.53
TiO_2 1% + SiO_2 1%	92.51 ± 4.32^a	-2.34 ± 0.04^a	17.88 ± 2.06^a	13.91

[a-c] Different lower case letter in same column indicates significant differences (P < 0.05).

hydrophobic interactions) between compounds and film materials, which increased the integrity of the film network and finally increased mechanical properties of films [30]. Abdollahi et al., (2012) also reported that incorporating nanoclay increased the mechanical properties of chitosan films [17], which is in general agreement with our findings. Vejdan et al., (2016) found that TiO_2 nanoparticles affected the physic-mechanical properties (TS and elongation at break) of fish gelatin/agar bilayer film [30]. Based on our findings (Table 2), the film thickness was not significantly affected, which is probably due to the small diameter of these nanoparticles. The intrinsic SI of straight QSM film as presented in Table 2, was probably attributed to the hydrophilic groups such as carboxylic groups in its structure that interacted with water [31]. Our findings showed the SI of active films were significantly lower than pure films (P < 0.05), which is due to intermolecular cross-linking interactions among compounds [32]. Pure QSM films had an appearance with L^*, a^* and b^* color values of 87.45, -1.34, and 15.67, respectively (Table 3). Based on our findings, L^* and b^* of films containing TiO_2 and SiO_2 nanoparticles were higher than the control group. Similar results were reported by adding natural compounds such as plant extract/essential oil [18, 29] and metal nanoparticles [17, 27, 30].

CONCLUSION

Application of TiO_2 and SiO_2 nanoparticles greatly improved the antimicrobial and physico-mechanical properties of the prepared films. Further study should be conducted regarding the use of designated films in food models for improving the safety and quality control of fresh foods.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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