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Stability of Chitosan Powder during Long-Term Storage at Room Temperature

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Physicochemical and functional properties of two chitosan products [low viscosity (LV) with 16.8 mPa s; high viscosity (HV) with 369.4 mPa s] were evaluated during 9-month storage at room temperature. As storage time increased, increased moisture content and DPPH radical scavenging activity and decreased viscosity and water binding capacity (WBC) were observed. The extent (%) of decreased viscosity and WBC and of increased DPPH radical scavenging activity during storage were more pronounced with the HV chitosan. Significant correlations were observed between WBC and viscosity ($r = 0.88$ for LV; $r = 0.96$ for HV) and between DPPH radical scavenging activity and viscosity ($r = -0.79$ for LV; $r = -0.87$ for HV). Although significant differences in $L^*a^*b^*$ values were observed during storage, color differences were not easily discerned visually. Antibacterial activity of the HV chitosan against two Gram-negative and two Gram-positive bacteria significantly increased with increasing storage time.

KEYWORDS: Chitosan powder; storage stability; binding capacity; DPPH radical scavenging activity; antibacterial activity

INTRODUCTION

Chitosan is a modified, natural nontoxic biopolymer derived by deacetylation of chitin [poly-β-(1→ 4)-*N*-acetyl-D-glucosamine], a major component of the shells of crustacea such as crab, shrimp, and crawfish (*1*). Recently, chitosan has attracted notable interest due to its biological activities, including antimicrobial (*2*), antitumor (*3*), antioxidative (*4*), and hypocholesterolemic functions (*5*). Chitosan has also been documented to possess several distinctive properties for use in water and fat uptake, emulsification (*6*), and dye binding (*7*).

Chitosan is now widely produced commercially from crab and shrimp shell wastes with different deacetylation grades and molecular weights/viscosities (*1*, *8*), and stored as a powder form at room temperature until used. During storage, however, specific characteristics of chitosan, that is, viscosity or molecular weight, may be altered, and thus influence its functional properties (*1*, *8*). Earlier studies (*9*−*11*) with chitosan solutions have revealed that the viscosity decreased with increased storage time. After storage, the chitosan solution generally exhibited lower antibacterial activity (*11*) and coagulating ability for recovery of proteinaceous solids from tofu wastewater (*12*). Therefore, it is realized that changes in the physicochemical and functional properties of chitosan powder during long-term storage should be monitored to effectively utilize chitosan products for a particular usage. To date, however, there is little available information on the storage stability of chitosan powder at room temperature.

The objectives of the present research were to evaluate stability of chitosan powder during 9-month storage at room temperature by monitoring changes in selected physicochemical properties and to compare selected functional properties of chitosan powder before and after 3-, 6-, and 9-month storage.

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MATERIALS AND METHODS

Materials. Two chitosans (acid-soluble and white-colored powder), prepared from crab leg shell immediately before the experiment, were provided by Keumho Chemical (Seoul, Korea). One chitosan had a viscosity of 16.8 (designated as LV), and the other had a viscosity of 369.4 mPa s (designated as HV) at 0.5% (w/v) concentration in 1% (v/v) acetic acid.

The dye used for evaluation of binding capacity was FD&C Red No. 40 {disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid}. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Co. (St. Louis, MO).

Storage Test. Each chitosan sample (50 g) was placed in three separate polyethylene zipper bags (18 cm × 20 cm; Clean Wrap Co., Gimhae city, Gyungsangnam-do, Korea). All bags were placed in a cardboard paper box and stored at room temperature for 9 months (December to September; temperature range of 13.0 to 29.3 °C). Chitosan samples were drawn at 3-month intervals for determination of physicochemical and functional properties. Triplicate experiments were conducted.

Measurement of Moisture, Color and Viscosity. Moisture content (%) was determined using a halogen moisture analyzer (HG53, Mettler Toledo, Greifensee, Switzerland). Color was measured with a portable Minolta chroma meter CR-200 (Minolta Camera Co. Ltd., Osaka, Japan) using illuminant C, and reported as L^* (lightness), a^* (redness) and b^* (yellowness). Three measurements were made at different locations on each sample. In addition, the whiteness index of chitosan powders was calculated as $100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$. Viscosity of chitosan solution was determined with a Brookfield viscometer, model LVDV-II+ (Brookfield Engineering Laboratories., Stoughton, MA). Chitosan solution was prepared in 1% (v/v) acetic acid at a 0.5% (w/v) concentration on a moisture-free basis. Measurements were made using a small sample adapter in the solution (8 mL) at 25 ± 0.3 °C, and reported in mPa s.

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Table 1. Changes in Moisture Content, Color and Viscosity Values of Chitosan Powders during 9-Month Storage at Room Temperature

		storage period (month)			
property[a]	chitosan[c]	0	3	6	9
moisture (%)	LV	9.2 ± 0.1 a	9.5 ± 0.0 b	10.1 ± 0.1 c	11.0 ± 0.3 d
	HV	9.4 ± 0.3 a	9.8 ± 0.1 b	10.3 ± 0.1 c	11.1 ± 0.2 d
color					
*L**	LV	78.1 ± 0.7 a	77.0 ± 1.2 a	77.3 ± 1.0 a	77.0 ± 0.1 a
	HV	80.4 ± 0.7 a	81.6 ± 0.6 ab	82.4 ± 1.0 b	83.1 ± 0.1 b
*a**	LV	0.45 ± 0.06 b	0.53 ± 0.01 b	0.51 ± 0.02 b	0.36 ± 0.02 a
	HV	0.22 ± 0.02 c	0.17 ± 0.02 b	0.15 ± 0.00 b	0.06 ± 0.01 a
*b**	LV	12.6 ± 0.2 a	13.1 ± 0.5 a	12.9 ± 0.2 a	14.0 ± 0.1 b
	HV	11.5 ± 0.4 a	11.5 ± 0.2 a	11.4 ± 0.4 a	11.7 ± 0.1 a
whiteness index	LV	74.7 ± 0.7 a	73.6 ± 1.2 a	73.9 ± 0.9 a	73.1 ± 0.1 a
	HV	77.2 ± 0.8 a	78.3 ± 0.5 ab	79.0 ± 1.0 b	79.4 ± 0.1 b
viscosity (mPa s)[b]	LV	16.8 ± 0.6 c	16.5 ± 0.2 c	13.2 ± 0.1 b	12.4 ± 0.1 a
	HV	369.4 ± 4.1 d	309.4 ± 4.4 c	249.2 ± 0.6 b	213.5 ± 2.6 a

[a] Mean ± standard deviation of triplicate determinations. Means with different lowercase letters within each row indicate significant differences ($P < 0.05$). [b] At 0.5% (w/v) chitosan concentration in 1% (v/v) acetic acid, on a moisture-free basis. [c] Chitosan with a viscosity of 16.8 (LV) and 369.4 mPa s (HV).

Spectroscopic Study. The IR spectra of the chitosan samples before and after 9-month storage were obtained with a JASCO FTIR spectrometer (300E, Tokyo, Japan). Chitosan samples (particle size less than 100 mesh) were mixed with KBr to form a homogeneous mixture for the FTIR measurements (*13*).

Water-Binding Capacity. Water-binding capacity (WBC) of chitosan was measured using a method of Cho et al. (*8*). Water absorption was initially carried out by weighing a centrifuge tube (50 mL) containing 0.5 g of sample, adding 10 mL of water, and mixing on a vortex mixer for 1 min to disperse the sample. The contents were left at ambient temperature for 30 min with shaking for 5 s every 10 min and centrifuged at 3200 rpm for 25 min. After the supernatant was decanted, the tube was weighed again. WBC was calculated as follows: WBC (%) = [water bound (g)/sample weight (g)] × 100.

Dye-Binding Capacity. Dye solution was prepared by dissolving dye in deionized water at a concentration of 250 mg/L. For the standard curve determination, the maximum absorbance of the aqueous dye solutions containing 2.5−20 mg of dye/L was measured with a spectrophotometer (Shimadzu UV-160A, Shimadzu Co., Tokyo, Japan) using deionized water as a blank.

Dyeing of chitosan was achieved by shaking 0.2 g of chitosan and 10 mL of aqueous dye solution (containing 2.5 mg of dye) in horizontally positioned screw-capped polypropylene conical tubes at 20 °C for 1 h using a shaker (100 rpm; MMS-3010, Tokyo Rikakikai Co., Japan). After settling of the dyed chitosan particles, the supernatant was withdrawn with a pipet and filtered through a glass filtering Gooch crucible (2G-3) using a glass microfiber filter paper (Whatman, 47 mm). The dyed chitosan was then repeatedly washed with deionized water and filtered until the filtrate was clear. The dye concentration of the combined filtrate was determined spectrophotometrically. The amount of dye bound to chitosan was determined by calculating differences in concentrations between the initial dye solution and the combined filtrate. Dye-binding capacity (DBC) was expressed as % adsorption (*8*).

DPPH Radical Scavenging Activity. DPPH radical scavenging activity of chitosan was determined by the method of Blois (*14*) with some modifications. 0.4 mL of chitosan solution [1.0% (w/v) in 1.0% (v/v) acetic acid] was added to 3 mL of 0.1 mM DPPH radical ethanolic solution. The reaction mixture was shaken vigorously and stored in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm using a spectrophotometer (Ultraspec 1000, Pharmacia Biotech Co., Cambridge, England). The free radical scavenging activity was calculated by the following equation:

$$\text{scavenging activity (\%)} = [1 - (\text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}})] \times 100$$

Assays for Antibacterial Activity. Four bacteria were tested for antibacterial activity of the HV chitosan (viscosity of 369.4 mPa s) before and after 3-, 6-, and 9-month storage. These included two Gram-positive bacteria (*Listeria monocytogenes* ATCC 19115 and *Staphylococcus aureus* KCCM 12255) and two Gram-negative bacteria (*Salmonella* Enteritidis ATCC 13076 and *Escherichia coli* ATCC 11775).

Antibacterial activity of chitosan was assayed as follows: Chitosan solutions were prepared in 1% (v/v) acetic acid at concentrations of 0.0 (control) and 1.0% (w/v). Each chitosan solution was then added to the Mueller Hinton broth (MHB, Merck, Darmstadt, Germany) to give final chitosan concentrations of 0.0 and 0.1%. These concentrations were selected based on our previous studies (*2*, *11*). The pH of MHB was adjusted to 5.9 with 1 N HCl and/or 1 N NaOH. The 0.05 mL of each bacterium (10^8−10^9 CFU/mL), subcultured in tryptic soy broth (Difco Laboratories, Detroit, MI) at 37 °C for 24 h, was inoculated into 10 mL of MHB and incubated at 37 °C for 24 h with constant shaking at 100 rpm. Viable cell counts (log CFU/mL) were enumerated on tryptic soy agar (Difco) by pour plating 1 mL of serial dilutions of MHB followed by incubation at 37 °C for 48 h.

Statistical Analysis. All experiments were carried out in triplicate, and means ± standard deviations or average values were reported. Means of the main effects were separated by Analysis of Variance followed by Duncan's multiple-range test using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL) software package. The Pearson correlation coefficients among viscosity, WBC, DBC, and DPPH radical scavenging activity were calculated as well. Statistical significance was concluded at $P < 0.05$.

RESULTS AND DISCUSSION

Changes in moisture content, color and viscosity values of LV and HV chitosan powders during 9-month storage at room temperature are shown in **Table 1**.

Moisture Content. The moisture content of LV and HV chitosans steadily increased with increased storage time (**Table 1**). The extent (%) of increased moisture content was comparable for both chitosans and independent of the chitosan viscosity. After 9-month storage at room temperature, the moisture content of LV and HV chitosans increased by 20% (from 9.2 to 11.1%) and 18% (9.4 to 11.1%) of the initial value, respectively. The reported moisture content of the commercial chitosan product was less than 10% (*15*). Youn et al. (*16*) also reported that the moisture content of chitosans dried by sun drying for 4 h was less than 8%. According to KFDA (*17*), the moisture content of chitosan powder should be below 10%. After 6 months of storage, the moisture content of both LV and HV chitosans was greater than 10%. For long-term storage at room temperature, chitosan powder should therefore be packed in a container that is impermeable to moisture to prevent moisture absorption from the storage room.

Color. The whiteness index of the LV chitosan did not change throughout the 9-month storage period while that of the HV

chitosan slightly increased with increasing storage time. The whiteness index was 74.7, 73.6, 73.9, and 73.1 for the LV chitosan, and 77.2, 78.3, 79.0, and 79.4 for the HV chitosan, respectively, after 0, 3, 6, and 9 months of storage (**Table 1**). A similar trend was noted for the L^* values. Although some significant differences in color L^*, a^* or b^* values were observed before and after 9-month storage by the instrumental measurement, actual color differences were not easily discerned by visual observation. Similarly, Xiuzhen et al. (*18*) also observed no differences in visual color of chitosan before and after storage for 36 months at room temperature.

Viscosity. The viscosity of both LV and HV chitosans decreased with increased storage time (**Table 1**). The viscosity decreased by 1.8%, 21.4% and 26.2% for the LV chitosan, and 16.2%, 32.5% and 42.2% for the HV chitosan from the initial value (16.8 and 369.4 mPa s, respectively) after 3, 6, and 9 months of storage at room temperature. The decrease in viscosity observed over time is probably due to partial degradation of stored chitosan.

There is little published information on the effect of storage time on the viscosity of chitosan powder at room temperature. Xiuzhen et al. (*18*) reported that there were no differences in viscosity of chitosan powder before and after 36-month storage at room temperature. Li et al. (*19*) also reported that there was no change in the viscosity (0.343 mPa s) of a solution prepared from stored chitosan acetate (water-soluble) after 90 days of storage at 25 °C. In this study, the extent (%) of decreased viscosity with increased storage time was more pronounced for the HV chitosan compared with the LV chitosan. In a separate experiment in our laboratory, we observed that the viscosity of chitosan oligomer powder (water-soluble), stored under the same conditions as in this study, decreased by 1.5% from the initial value of 1.31 to 1.29 mPa s after 9 months of storage at room temperature. From **Table 1**, we hypothesized that the lesser extent (%) of decreased viscosity of a solution was due to the initial low viscosity of stored chitosan, which was highly related to its low molecular weight.

Data from **Table 1** indicate that, under the present experimental conditions, the viscosity of chitosan decreased with increasing storage time. Therefore, it is anticipated that functional properties of chitosans before and after 3, 6, and 9 months of storage may differ since the physicochemical characteristics of chitosan influence its final functional properties (*1*). However, the IR absorption spectra (**Figure 1**) of the LV and HV chitosans before and after 9-month storage showed that the position and absorption intensity of the bands did not change during 9-month storage and were comparable and typical of chitosan as also reported by Youn et al. (*13*).

Water- and Dye-Binding Capacities. Changes in water-binding capacity (WBC) and dye-binding capacity (DBC) of LV and HV chitosans during 9-month storage at room temperature are shown in **Table 2**. As storage time increased, WBC of both LV and HV chitosans significantly decreased from the initial value of 568.5 to 519.0 (8.7%) for the LV chitosan and from 646.0 to 555.7 (14.0%) for the HV chitosan after 9 months of storage. On the other hand, there was no change in DBC of both LV and HV chitosans during 6 months of storage. Further increase in storage time from 6 to 9 months, however, resulted in slightly increased DBC, which was likely due to increased accessibility of dye ions into the amino groups of chitosan with decreased molecular weight (or viscosity) (*7*).

WBC and DBC (for FD&C Red No. 40) of commercial chitosans differ with individual products. Cho et al. (*8*) reported WBC and DBC of five commercial chitosan products ranging from 458 to 805% and 35.2 to 85.6%, respectively. According to

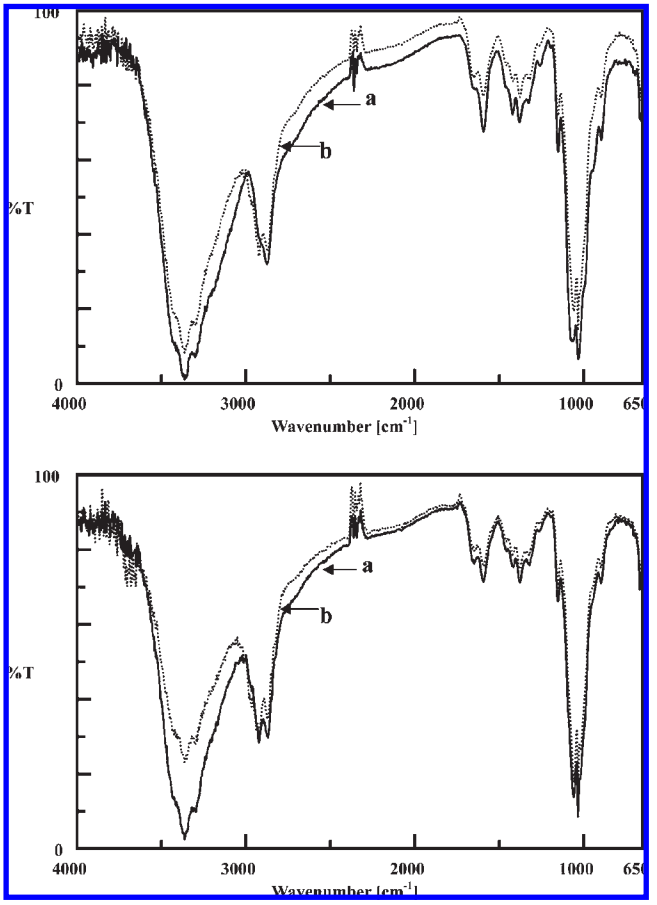

Figure 1. The FTIR spectra of LV (above) and HV (below) chitosans [chitosan with a viscosity of 16.8 (LV) and 369.4 mPa s (HV)] (a) before and (b) after 9-month storage at room temperature.

No et al. (*20*), WBC and DBC of six commercial chitosan products were in the range of 355−611% and 21.3−100%, respectively. The initial WBC (568.5 and 646.0%) and DBC (30.0 and 49.9%) values of the LV and HV chitosans observed in our study were comparable to those of commercial chitosans reported by these workers.

The viscosity (**Table 1**) and WBC (**Table 2**) of both LV and HV chitosans decreased with increasing storage time. This may imply that WBC of stored chitosan decreased when its viscosity decreased. As seen in **Table 3**, WBC was significantly correlated positively with viscosity ($r = 0.88$, $P = 0.0002$ for LV; $r = 0.96$, $P < 0.0001$ for HV). However, no direct correlation between WBC and viscosity of chitosans was observed by Cho et al. (*8*) and No et al. (*20*). No strong correlation between viscosity and DBC was observed for both chitosans in our study (**Table 3**) which is in agreement with the result reported by Cho et al. (*8*) and No et al. (*20*).

DPPH Radical Scavenging Activity. There was an observable trend that (1) the DPPH radical scavenging activity of LV and HV chitosans increased with increasing storage time, with more pronounced effect observed for the HV sample (**Table 2**) and (2) the viscosity of LV and HV chitosans decreased with increasing storage time (**Table 1**). Therefore, a significant negative correlation was observed between DPPH radical scavenging activity and viscosity ($r = -0.79$, $P = 0.002$ for LV chitosan; $r = -0.87$, $P = 0.0003$ for HV chitosan) (**Table 3**). In addition, a significant negative correlation was also observed between WBC and DPPH radical scavenging activity ($r = -0.62$, $P = 0.03$ for LV chitosan; $r = -0.79$, $P = 0.002$ for HV chitosan) (**Table 3**). The increase in

Table 2. Water- (WBC) and Dye-Binding Capacity (DBC), and DPPH Radical Scavenging Activity of Chitosan Powders during 9-Month Storage at Room Temperature

property[a]	chitosan[d]	storage period (month) 0	3	6	9
WBC (%)	LV	568.5 ± 6.0 c	543.0 ± 2.6 b	518.3 ± 3.2 a	519.0 ± 6.2 a
	HV	646.0 ± 4.0 d	592.7 ± 7.6 c	575.0 ± 3.6 b	555.7 ± 5.5 a
DBC (%)[b]	LV	30.0 ± 1.8 a	30.9 ± 0.7 a	29.4 ± 0.7 a	34.7 ± 0.7 b
	HV	49.9 ± 1.1 a	50.3 ± 1.5 a	50.1 ± 0.4 a	52.4 ± 0.9 b
DPPH radical scavenging activity (%)[c]	LV	19.4 ± 0.6 a	19.3 ± 0.4 a	21.5 ± 1.5 b	21.6 ± 1.1 b
	HV	10.9 ± 0.2 a	11.4 ± 1.0 a	12.9 ± 1.1 b	15.6 ± 0.2 c

[a] Mean ± standard deviation of triplicate determinations, on a dry basis. Means with different lowercase letters within each row indicate significant differences ($P < 0.05$). [b] At 2.5 mg of dye concentration/0.2 g of sample. [c] At 1% (w/v) chitosan concentration in 1% (v/v) acetic acid. [d] Chitosan with a viscosity of 16.8 (LV) and 369.4 mPa s (HV).

Table 3. Correlation (r) among Viscosity, Water- (WBC) and Dye-Binding Capacity (DBC), and DPPH Radical Scavenging Activity (DPPH) of Chitosan Powders

property	chitosan[a]	viscosity (mPa s)	WBC[b] (%)	DBC (%)	DPPH[b] (%)
viscosity (mPa s)	LV	1.00	0.88***	−0.45	−0.79**
	HV	1.00	0.96***	−0.58	−0.87***
WBC (%)	LV		1.00	−0.40	−0.62*
	HV		1.00	−0.56	−0.79**
DBC (%)	LV			1.00	0.31
	HV			1.00	0.56
DPPH (%)	LV				1.00
	HV				1.00

[a] Chitosan with a viscosity of 16.8 (LV) and 369.4 mPa s (HV). [b] *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

DPPH radical scavenging activity of chitosan with decreasing molecular weight (MW) has been observed by previous investigators (*21*−*23*). According to Kim and Thomas (*23*), the higher MW chitosan (120 kDa) would have lower mobility than the lower MW chitosan (30 kDa). Consequently, this would increase the possibility of inter- and intramolecular bonding of the high MW chitosan molecules, and, thus, the chance of exposure of their amine groups might be restricted. This may explain our findings in that the scavenging activity of chitosan increased with decreased viscosity over time (**Table 2**). Similarly, the LV chitosan having lower viscosity (**Table 1**) exhibited higher DPPH radical scavenging activity than did the HV chitosan having higher viscosity (**Table 2**). The scavenging activity of chitosan may be due to the reaction between free radicals and protonated amino groups (*24*, *25*).

Antibacterial Activity. The antibacterial activity of the HV chitosan powders during 9-month storage at room temperature was examined at concentrations of 0.0 (control) and 0.1% (w/v) for two Gram-negative and two Gram-positive bacteria incubated for 24 h at 37 °C. In this study, only the HV chitosan was tested because it exhibited a wide range of viscosity compared with LV chitosan during 9-month storage (**Table 1**).

As seen in **Table 4**, chitosans markedly inhibited the growth of bacteria tested; however, the inhibitory effects differed with the storage period of the chitosan and the type of bacterium. Antibacterial activity of chitosan against four bacteria increased with increased storage period of chitosan. In **Table 1**, viscosity of chitosan decreased from the initial value of 369.4 to 213.5 mPa s after 9-month storage. This indicates that chitosan with lower viscosity shows higher antibacterial activity under the present experimental conditions. Increase in antibacterial activity of chitosan with decreased viscosity was also reported by several investigators. For example, Uchida et al. (*26*) found that chitosan hydrolysate, slightly hydrolyzed with chitosanase, was more active as an antibacterial agent than was native chitosan. Cho et al. (*27*) reported that the antibacterial activity of chitosan against *Escherichia coli* and *Bacillus* spp. increased with decreasing viscosity from 1000 to 10 cP.

Table 4. Antibacterial Activity[a] of Chitosan Powders (HV Chitosan with 369.4 mPa s) during 9-Month Storage at Room Temperature

	bacteria	initial	control[a]	storage period (month) 0	3	6	9
Gram (−)	*Salmonella* Enteritidis	8.53	8.90 e	4.67 d	3.80 c	3.73 b	2.48 a
	Escherichia coli	9.19	9.35 d	4.68 c	3.65 b	3.64 b	2.32 a
Gram (+)	*Listeria monocytogenes*	9.70	9.82 d	5.80 c	4.90 b	4.91 b	3.58 a
	Staphylococcus aureus	9.51	9.88 e	5.76 d	4.69 c	4.52 b	4.28 a

[a] Viable cells (log CFU/mL) after incubation without (control) and with 0.1% (w/v) chitosan for 24 h at 37 °C. Values are an average of triplicate determinations. Means with different lowercase letters within each row indicate significant difference ($P < 0.05$).

Allan et al. (*28*) reported that *Staphylococcus aureus* was negligibly inhibited by chitosan at a concentration of 0.1% and that *E. coli* was only slightly affected at as high as 1.0%. Elsewhere, Seo et al. (*29*) found no antibacterial activity against *Listeria monocytogenes* at a 0.1% chitosan concentration, regardless of molecular weight (104−1333 kDa); however, in the present study, *S. aureus*, *E. coli*, and *L. monocytogenes* was inhibited by 4−5, 5−7, and 4−6 log cycles, respectively, by 0.1% (w/v) chitosan treatment depending on the storage period of chitosan.

In conclusion, this study demonstrated that both physicochemical and functional properties of chitosan powders were influenced by storage time. The viscosity decreased by 26.2% (LV chitosan) and 42.2% (HV chitosan) from the initial value after 9 months of storage at room temperature. These results indicate the instability of chitosan powders under the present storage conditions. Changes in viscosity of chitosan powder during storage influenced its functional properties. WBC decreased while DPPH radical scavenging activity increased with increased storage time. The extent of decreased viscosity and WBC, and of increased DPPH radical scavenging activity of stored chitosan powders during 9-month storage were more noticeable for the HV chitosan compared with the LV chitosan. Antibacterial activity of chitosan against two Gram-negative and two Gram-positive bacteria increased with increased storage time. Storage of chitosan powders at room temperature for up to 9 months could be advantageous if a chitosan product with higher dye-binding capacity, DPPH radical scavenging activity and antibacterial activity are desirable. Finally, to effectively utilize chitosan as a functional ingredient, the functional properties of chitosan products should be constantly monitored during long-term storage.

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