

Physicochemical, microbial, and sensory properties of yogurt supplemented with nanopowdered chitosan during storage

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ABSTRACT

This study was carried out to determine the possibility of adding nanopowdered chitosan (NPC) into cholesterol-reduced yogurt to improve the functionality of yogurt and the effects of adding NPC on the physicochemical, microbial, and sensory properties of the products during storage. The pH values and mean lactic acid bacteria counts of NPC-added (0.3 to ~0.7%, wt/vol) and cholesterol-reduced yogurt ranged from 4.19 to 4.41 and from 4.75×10^8 to 9.70×10^8 cfu/mL, respectively, when stored at 4°C for 20 d, thereby indicating a possibility of prolonging the shelf life of yogurt. In color, the a^* and b^* values for cholesterol-reduced yogurt were not significantly influenced by the addition of NPC (0.1 to ~0.7%, wt/vol); however, the L^* values significantly decreased with the addition of the greatest concentration (0.7%, wt/vol) of NPC at 0-d storage. The sensory test revealed that the astringency scores significantly increased at 0-d storage when the greatest concentration (0.7%, wt/vol) of NPC was added into cholesterol-reduced yogurt. Based on the data obtained from the current study, it is concluded that concentrations (0.3 to ~0.5%, vol/vol) of NPC could be used to produce an NPC-added and cholesterol-reduced yogurt without significantly adverse effects on the physicochemical, microbial, and sensory properties.

Key words: cholesterol-reduced yogurt, nanopowdered chitosan, cross-linked β -cyclodextrin, shelf life

INTRODUCTION

Chitosan, the main derivative of chitin, is a linear aminopolysaccharide composed primarily of repeating units of β -(1→4)-2-amino-2-deoxy-D-glucose (D-glucosamine) (Pillai et al., 2009; Shu et al., 2009). The hypoglycemic effects of chitosan have been reported in previous studies (Kondo et al., 2000; Hayashi and Ito, 2002; Lee et al., 2003; Yao et al., 2008; Kumar et al., 2009). According to Kumar et al. (2009), the control

group (no supplement of chitosan) had elevated blood glucose levels, whereas the levels of blood glucose were decreased in ob/ob mice groups fed chitosan (20 mg/kg per day) for 28 d. Lee et al. (2003) studied the antidiabetic effect of chitosan oligosaccharide in neonatal streptozotocin-induced non-insulin-dependent diabetes mellitus rats and found that the plasma glucose level was decreased by about 19% in diabetic rats after treatment with 0.3% chitosan oligosaccharide. Lee et al. (2003) also reported that chitosan oligosaccharide can be used as an antidiabetic agent because it elevates glucose tolerance and insulin secretion and reduces triglyceride levels.

Nanosizing is an emerging technique used for enhancing physical and biological properties including solubility and stability (Rasenack and Muller, 2004; Park et al., 2007). According to Park et al. (2007), nanocalcium supplementation in milk might be an effective way to improve bone calcium metabolism for ovariectomized rats. In our animal study investigating the cholesterol-lowering effect of nanopowdered chitosan (NPC) in rats, it was shown that NPC reduced total cholesterol by 46.6%, as compared with the commercially powdered chitosan (CPC), which reduced total cholesterol by 18.6% (J. H. Park; unpublished data).

In recent years, many different food ingredients, including evening primrose oil (Lee et al., 2007), β -glucan (Gee et al., 2007; Sahan et al., 2008), and green and black teas (Jaziri et al., 2009) have been included in yogurt formulations to improve the nutritional value. Moreover, reducing cholesterol in yogurt can be another great way to enhance the health benefits. Lee et al. (2007) reported that the cholesterol from milk (the major ingredient for the manufacture of yogurt) can be effectively removed by β -cyclodextrin (β -CD), and they employed the cholesterol-reduced milk for the production of cholesterol-reduced yogurt. They noted that the physicochemical and sensory properties of cholesterol-reduced yogurt were not remarkably different from those of the control (without the removal of cholesterol).

So-called healthy foods, especially those with nutraceutical properties, are in great demand in our health-conscious society. Nutraceutical yogurt could be a good

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vehicle in this respect if nutraceutical ingredients, such as NPC, were used to fortify yogurt and the cholesterol was removed from yogurt. However, there is no report in the literature on the production of an NPC-added and cholesterol-reduced yogurt. Therefore, the objectives of the current study were to investigate (1) the possibility of adding NPC into cholesterol-reduced yogurt and (2) the effects of adding NPC on the physicochemical, microbial, and sensory properties of the products during storage.

MATERIALS AND METHODS

Materials

Commercial milk (3.6% milk fat) was purchased from Seoul Dairy Co-op (Seoul, Korea). Commercially powdered chitosan was obtained from Samsung Chitopia (Seoul, Korea) and ground to NPC by the dry milling method in Apexel Co. (Pohang, Korea) at room temperature. Commercial β -CD (purity 99.1%) was purchased from Nihon Shokuhin Cako Co. Ltd. (Osaka, Japan). Cholesterol and 5α -cholestane were purchased from Sigma Chemical Co. (St. Louis, MO), and all solvents were of gas-chromatographic grade.

Particle Size Analysis

Commercially powdered chitosan or NPC was mounted on a brass stub (10 mm in diameter) using 2-sided adhesive tape. The stub surface was gently blown to remove unattached chitosan powders using a hand-held blower. The specimens were then made electrically conductive by coating under an argon atmosphere with a thin layer (approximately 30 nm in thickness) of platinum-palladium (8:2). The specimens were examined using a scanning electron microscope (Hitachi S-4700, Tokyo, Japan) operated at an accelerating voltage of 15 kV. The particle size of NPC was determined by Delsa Nano particle size analyzer (Beckman Coulter, Fullerton, CA).

Preparation of Cross-linked β -Cyclodextrin

A 100-g sample of β -CD was dissolved in 80 mL of distilled water and placed in a stirrer at room temperature with constant agitation for 2 h. Adipic acid (2 g) was then incorporated into the β -CD solution, and the pH was adjusted to 10 with 1 N NaOH. The β -CD solution was stirred at room temperature for 90 min and then readjusted to pH 5 with 0.5% acetic acid. The β -CD was recovered by filtering through Whatman No. 2 filter paper and washing 3 times with 150 mL of distilled water. The product was dried at 60°C in a

Lab-Line mechanical convection oven (O-Sung Scientific Co., Seoul, Korea) for 20 h and passed through a 100-mesh sieve (Han et al., 2005).

Manufacture of CPC- or NPC-Added and Cholesterol-Reduced Yogurt

To manufacture CPC- or NPC-added and cholesterol-reduced yogurt, cholesterol was first removed as follows: 500 mL of milk was placed in a 1,000-mL beaker, and 1.0% (wt/vol) β -CD was added. The mixture was stirred at 800 rpm with a blender (Tops, Misung

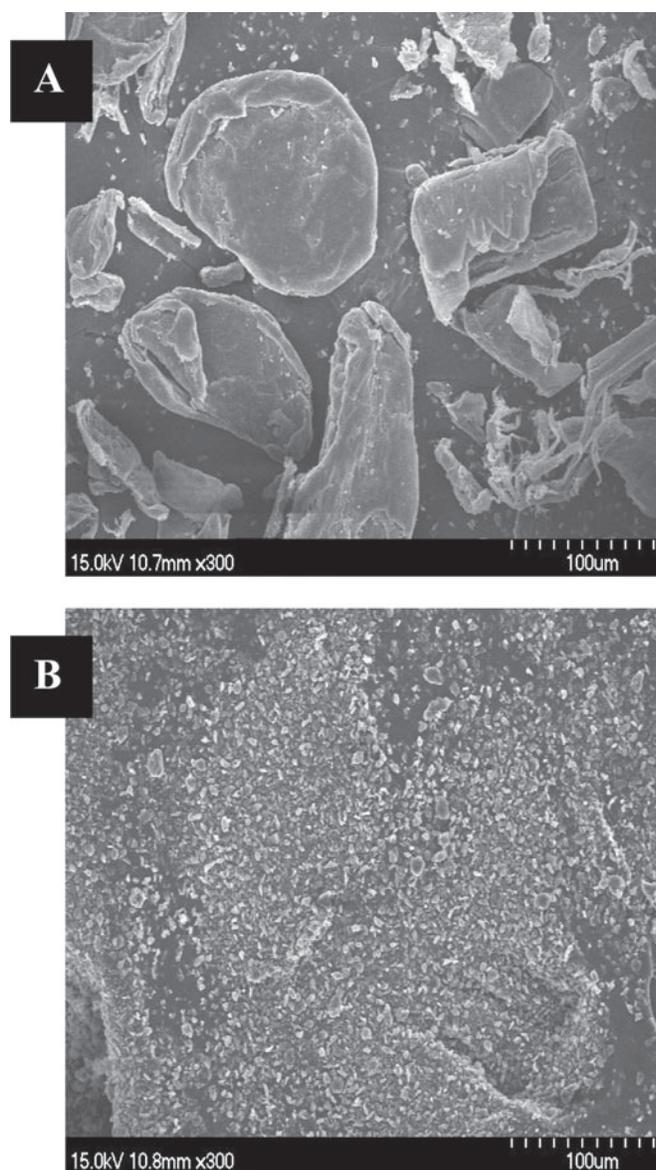


Figure 1. Scanning electron microscope images for commercially powdered chitosan (A) and nanopowdered chitosan (B).

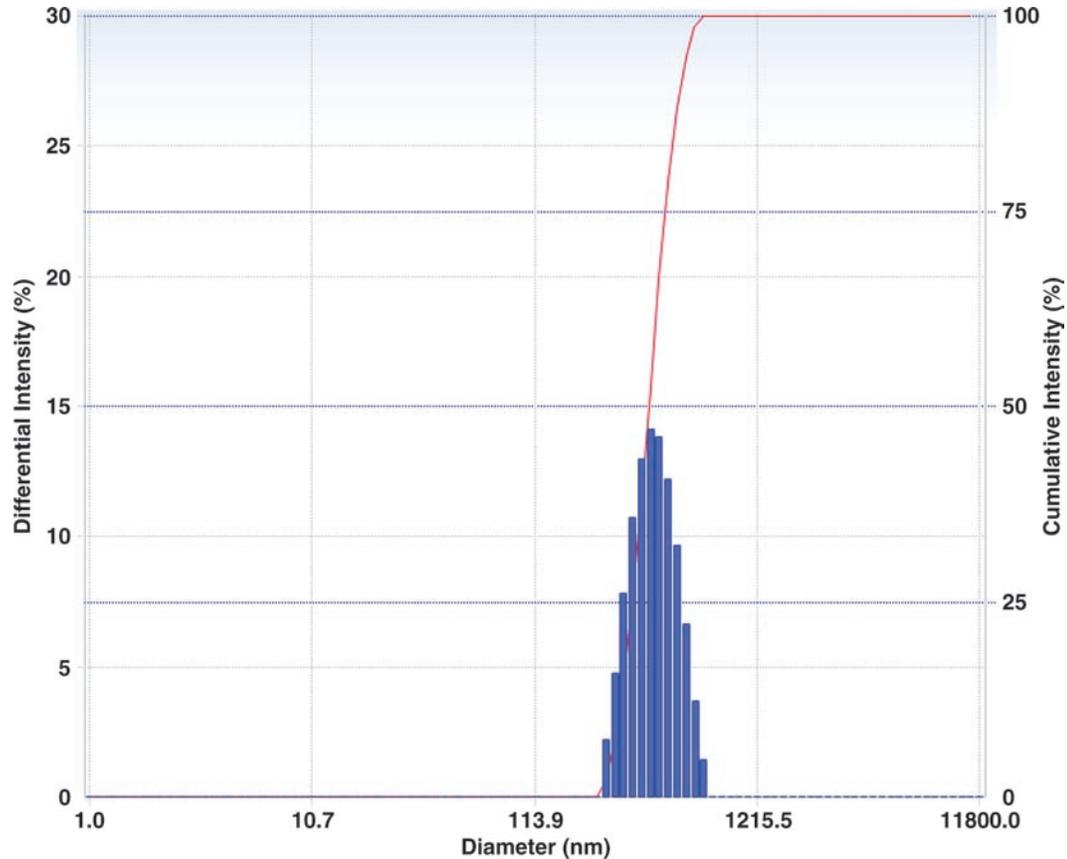


Figure 2. Particle size analysis of nanopowdered chitosan. Color version available in the online PDF.

Co., Seoul, Korea) in a temperature-controlled water bath at 10°C for 10 min. The mixture was centrifuged (HMR-220IV, Hanil Industrial Co., Seoul, Korea) at room temperature at $166 \times g$ for 10 min, and the supernatant, the cholesterol-removed milk, was collected for yogurt manufacture.

Nonfat dry milk (3.7%, wt/vol) and pectin (0.2%, wt/vol, Kanto Chemical, Tokyo, Japan) were added into the cholesterol-reduced milk and then homogenized at 50°C under 1,000 psi in a single-stage homogenizer (HC 5000, Micro Fluidics Corp., Newton, MA). The homogenized milk was heated at 90°C for 10 min and cooled to approximately 42 to 43°C. A 0.004% (wt/vol) commercial starter culture (Chr. Hansen Pty. Ltd., Bayswater, Australia) in freeze-dried direct-to-vat set form containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was added and fermented at 43°C for 6 h. The cholesterol-reduced yogurt samples were combined with different concentrations (0.1, 0.3, 0.5, and 0.7%, wt/vol) of CPC or NPC and stabilized at 10°C for 24 h. After stabilizing, each yogurt sample was stored for 0, 5, 10, 15, and 20 d at 4°C in a refrigerator

to evaluate the physicochemical and sensory properties. Each batch of yogurt making was done in triplicate.

Extraction and Determination of Cholesterol

For the extraction of cholesterol from yogurt, 1 g of a yogurt sample was placed in a screw-capped glass tube (15 mm \times 180 mm), and 1 mL of 5 α -cholestane (1 mg/mL) was added as an internal standard. The sample was saponified at 60°C for 30 min with 5 mL of 2 M ethanolic potassium hydroxide solution (Adams et al., 1986). The process was repeated 4 times. The hexane layers were transferred to a round-bottomed flask and dried under vacuum. The extract was redissolved in 1 mL of hexane and stored at -20°C until analysis.

The cholesterol was determined on a silica fused capillary column (HP-5, 30 m \times 0.32 mm i.d. \times 0.25 μ m thickness) using a Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA) equipped with a flame-ionization detector. The injector and detector temperatures were 270 and 300°C, respectively. The oven temperatures were programmed from 200 to 300°C

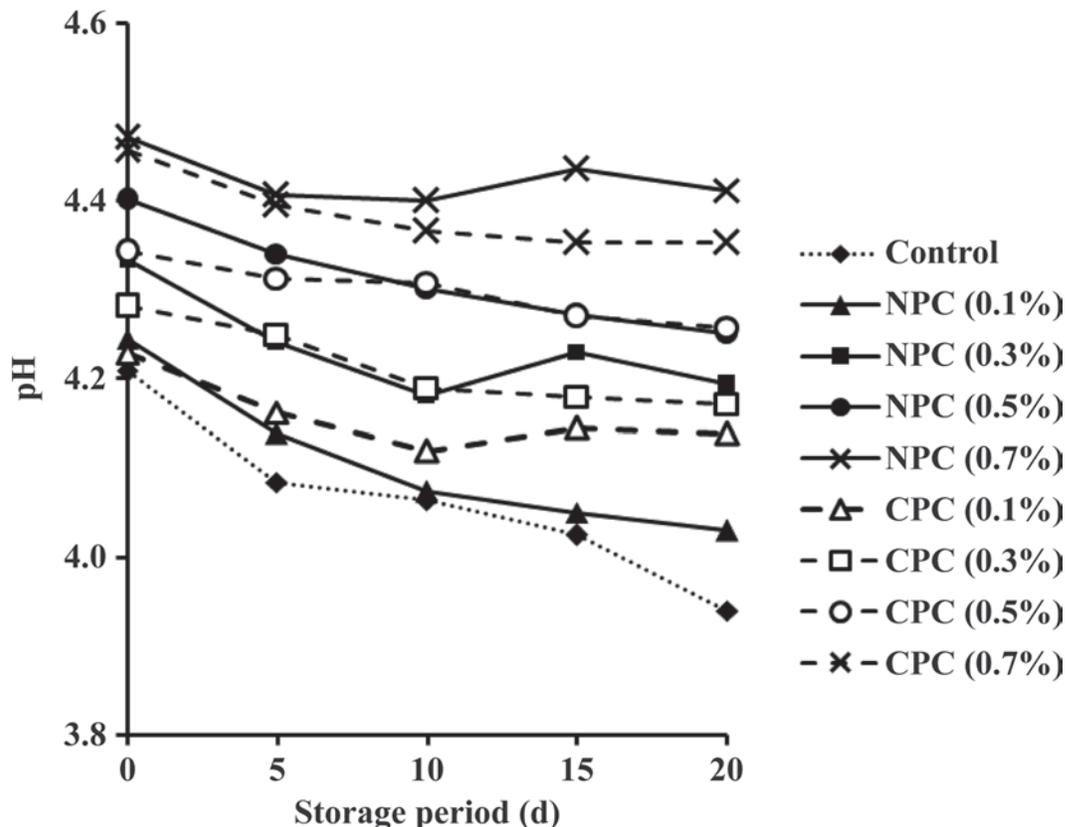


Figure 3. Changes in pH in nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt stored at 4°C for 20 d. Milk used was treated with 1% cross-linked β -cyclodextrin for all samples. At 0 d, yogurt had been stabilized for 24 h.

at 10°C/min and held for 20 min. Nitrogen was used as a carrier gas at a flow rate of 2 mL/min with a split ratio of 1:50. Quantification of cholesterol was done by comparing the peak areas with the response of an internal standard.

The percentage of cholesterol reduction was calculated as follows: cholesterol reduction (%) = $100 - (\text{amount of cholesterol in } \beta\text{-CD-treated yogurt} \times 100 / \text{amount of cholesterol in the control})$. Cholesterol determination for the control was averaged with each batch of treatments.

Chemical Analyses

The pH values of each yogurt sample were measured using a pH meter (Orion 900A, Boston, MA). The titratable acidity values of each yogurt sample were determined after mixing the yogurt sample with 10 mL of hot distilled water (90°C) and titrating with 0.1 N NaOH containing 0.5% phenolphthalein as an indicator to an end point of faint pink color. All samples were measured in triplicate.

Viscosity

The viscosity of yogurt samples (100 mL) was measured after mixing of the sample for 5 min at room temperature using a Brookfield Viscometer (Model LVDV I+, Version 3.0, Stonington, MA) with a spindle No. 2 at 60 rpm. All samples were measured in triplicate.

Color

Color values of each yogurt sample were investigated using a colorimeter (CR210, Minolta, Tokyo, Japan) after calibrating its original value with a standard plate ($X = 97.83$, $Y = 81.58$, $Z = 91.51$). Measured L^* , a^* , and b^* values were used as indicators of lightness, redness, and yellowness, respectively. All samples were measured in triplicate.

Lactic Acid Bacteria

de Man, Rogosa, Sharpe agar (Difco Laboratories, Detroit, MI) combined with 0.004% bromophenol blue

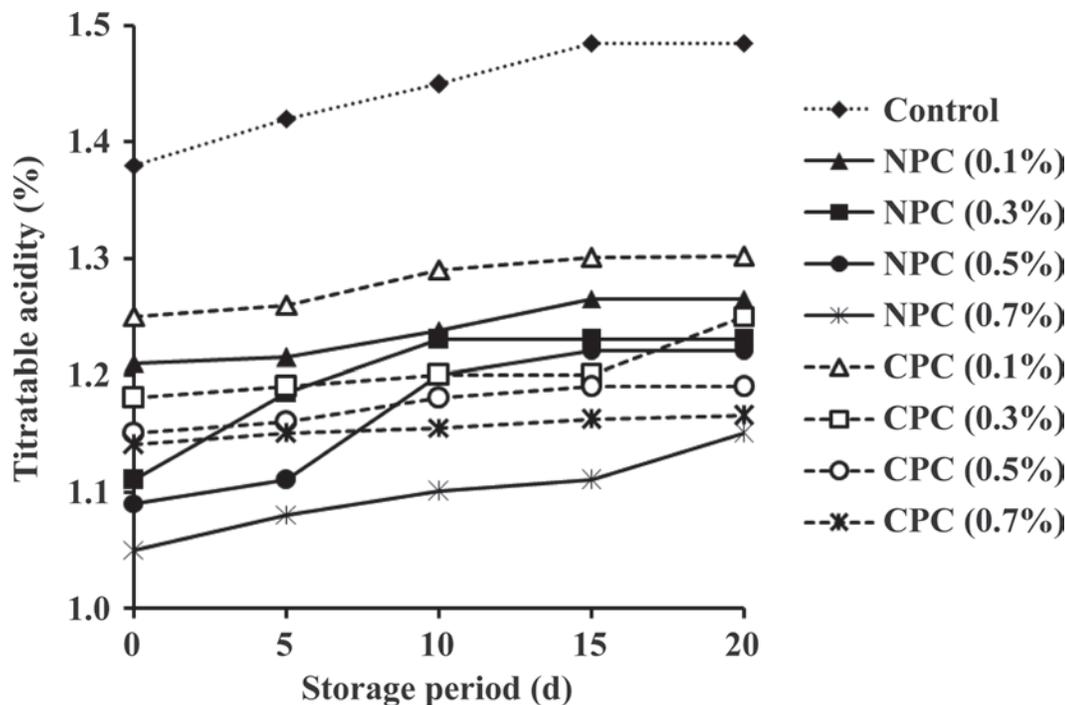


Figure 4. Changes in titratable acidity in nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt stored at 4°C for 20 d. Milk used was treated with 1% cross-linked β -cyclodextrin for all samples. At 0 d, yogurt had been stabilized for 24 h.

(Sigma Chemical Co.) was used for *L. bulgaricus* and *Strep. thermophilus* counting. One milliliter of yogurt samples was diluted with 9 mL of sterile peptone and water diluents. Subsequent dilutions of each sample were plated in triplicate and incubated at 37°C for 48 h.

Sensory Analysis

Eight trained sensory panelists evaluated randomly coded yogurt samples. The appearance, flavor, taste, texture, and overall acceptability were investigated on a 7-point scale (1 = very weak, 4 = moderate, 7 = very strong).

Statistical Analysis

All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC). An ANOVA was performed using the general linear models procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference procedure. Significance was defined at the 5% level.

RESULTS AND DISCUSSION

Particle Size Analysis

The morphology of CPC and NPC was observed by scanning electron microscope, as shown in Figure 1. The scanning electron microscope images demonstrated that the particle size of CPC apparently decreased during the manufacture of NPC. The average particle sizes of CPC and NPC measured were about 150 μm (as measured by scanning electron microscope) and about 562 nm in diameter (as measured by the particle size analyzer), respectively (Figures 1 and 2).

Cholesterol Removal

The cholesterol content of the control yogurt (without the supplementation of CPC or NPC) was 13.5 mg/100 g, and the cholesterol reduction reached 93.1% with 1% β -CD treatment (data not shown). This finding was in agreement with our previous study using powdered β -CD in which we reached a 93.5% cholesterol reduction in yogurt (Lee et al., 2007). Furthermore, the efficient removal of more than 90% of cholesterol by using cross-

Table 1. Changes of lactic acid bacteria¹ (cfu/mL) in nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt² stored at 4°C for 20 d

Concentration of sample (% wt/vol)	Storage period (d)				
	0	5	10	15	20
Control	9.15×10^{10a}	1.45×10^{10a}	4.40×10^{9a}	1.68×10^{9a}	1.70×10^{9a}
NPC (0.1)	7.00×10^{10b}	2.45×10^{9c}	1.29×10^{9b}	1.02×10^{9b}	9.90×10^{8b}
NPC (0.3)	2.49×10^{10c}	2.16×10^{9c}	1.85×10^{9b}	9.75×10^{8c}	9.70×10^{8b}
NPC (0.5)	2.45×10^{10c}	1.90×10^{9d}	1.09×10^{9b}	6.95×10^{8c}	6.25×10^{8c}
NPC (0.7)	1.85×10^{10d}	1.50×10^{9d}	9.20×10^{8c}	4.85×10^{8d}	4.75×10^{8d}
CPC (0.1)	6.34×10^{10b}	5.25×10^{9b}	1.38×10^{9b}	1.19×10^{9b}	9.00×10^{8b}
CPC (0.3)	2.25×10^{10c}	2.08×10^{9c}	1.31×10^{9b}	8.05×10^{8c}	7.45×10^{8c}
CPC (0.5)	1.18×10^{10d}	7.40×10^{8c}	8.75×10^{8c}	5.25×10^{8d}	4.05×10^{8d}
CPC (0.7)	1.08×10^{10d}	6.50×10^{8c}	3.00×10^{8d}	1.35×10^{8e}	2.50×10^{8e}

^{a-e}Values with different superscript letters within the same column differ significantly ($P < 0.05$).

¹The mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

²Milk used was treated with 1% cross-linked β -cyclodextrin for all samples.

linked β -CD has been found in other dairy products (Kim et al., 2005, 2006, 2008; Han et al., 2007).

Changes in pH and Titratable Acidity

Figure 3 shows the changes of pH values in CPC- or NPC-added and cholesterol-reduced yogurt stored at 4°C for 20 d. The pH values increased when CPC

or NPC (0.3 to ~0.7%, wt/vol) was incorporated into the cholesterol-reduced yogurt samples during storage. It was also found that at 0-d storage, elevating the concentrations of both NPC and CPC in the cholesterol-reduced yogurt samples from 0.3 to 0.7% (wt/vol) resulted in an increase in the pH values from 4.33 to 4.47 and from 4.28 to 4.46, respectively.

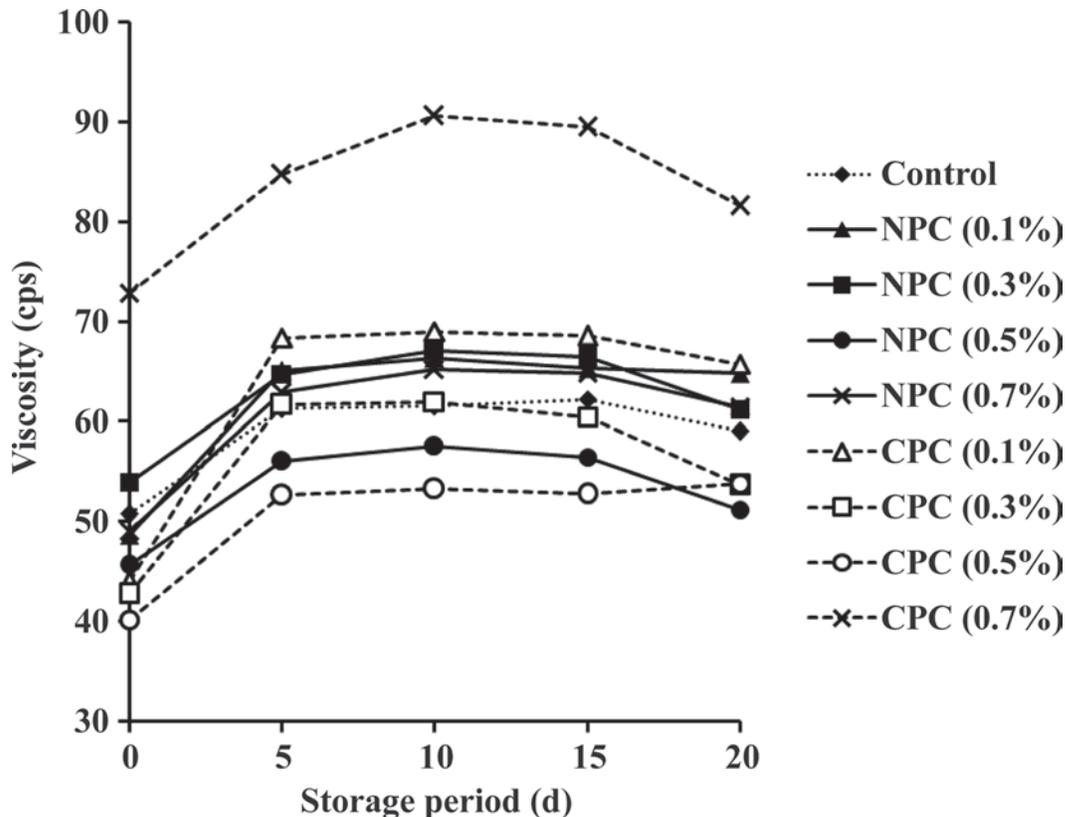


Figure 5. Changes in viscosity for nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt stored at 4°C for 20 d. Milk used was treated with 1% cross-linked β -cyclodextrin for all samples. At 0 d, yogurt had been stabilized for 24 h.

Table 2. Changes of color for nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt¹ stored at 4°C for 20 d

Concentration of sample (% wt/vol)	Storage period (d)														
	0			5			10			15			20		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control	88.96 ^a	3.04 ^a	5.81 ^a	89.00 ^a	3.05 ^{ab}	6.01 ^a	88.92 ^a	3.07 ^{abc}	6.14 ^b	89.88 ^a	3.20 ^a	6.84 ^b	88.88 ^a	3.27 ^{ab}	7.06 ^b
NPC (0.1)	88.46 ^a	2.88 ^{ab}	6.20 ^a	87.95 ^a	3.08 ^a	6.29 ^a	88.62 ^{ab}	3.02 ^{abc}	6.34 ^{ab}	89.52 ^{ab}	3.19 ^a	6.37 ^c	87.51 ^d	3.29 ^a	6.41 ^c
NPC (0.3)	88.86 ^a	2.81 ^{ab}	6.29 ^a	87.40 ^b	3.05 ^{ab}	6.33 ^a	88.82 ^{ab}	3.08 ^{abc}	6.40 ^{ab}	89.11 ^{ab}	3.20 ^a	6.42 ^c	86.58 ^e	3.23 ^{ab}	6.46 ^c
NPC (0.5)	87.70 ^a	2.79 ^{ab}	6.38 ^a	86.26 ^c	2.88 ^{ab}	6.39 ^a	87.75 ^c	3.15 ^{bc}	6.41 ^{ab}	88.91 ^b	3.10 ^a	6.45 ^c	85.86 ^e	3.19 ^{abc}	6.49 ^c
NPC (0.7)	86.00 ^b	2.71 ^b	6.47 ^a	85.21 ^d	2.73 ^b	6.40 ^a	86.30 ^d	3.18 ^a	6.43 ^{ab}	89.57 ^b	3.02 ^a	6.49 ^c	84.11 ^f	3.08 ^{abcd}	6.53 ^c
CPC (0.1)	88.94 ^a	2.88 ^{ab}	6.04 ^a	88.66 ^a	2.87 ^{ab}	6.12 ^a	88.67 ^{ab}	2.85 ^{ab}	6.39 ^{ab}	87.77 ^c	2.91 ^a	6.89 ^{ab}	88.15 ^{bc}	3.02 ^{bcd}	7.30 ^{ab}
CPC (0.3)	88.92 ^a	2.81 ^{ab}	6.20 ^a	88.64 ^a	2.82 ^{ab}	6.28 ^a	88.59 ^b	2.88 ^{abc}	6.43 ^{ab}	87.86 ^c	2.94 ^a	6.91 ^{ab}	88.24 ^b	2.91 ^d	7.33 ^{ab}
CPC (0.5)	88.77 ^a	2.74 ^b	6.34 ^a	88.70 ^a	2.75 ^{ab}	6.44 ^a	88.67 ^{ab}	2.88 ^{abc}	6.67 ^a	87.11 ^c	2.92 ^a	6.95 ^{ab}	87.85 ^c	2.93 ^{cd}	7.35 ^{ab}
CPC (0.7)	88.70 ^a	2.65 ^b	6.48 ^a	88.56 ^a	2.74 ^{ab}	6.52 ^a	88.61 ^{ab}	2.79 ^c	6.70 ^a	85.21 ^d	2.86 ^a	7.13 ^a	86.58 ^e	2.94 ^{cd}	7.43 ^a

^{a-f}Values with different superscripts within the same column differ significantly ($P < 0.05$).

¹Milk used was treated with 1% cross-linked β-cyclodextrin for all samples.

The normal pH of commercial yogurt products ranged from 4.0 to 4.4 (Kroger, 1976; Sahan et al., 2008). In the current study, increasing the storage period from 0 to 20 d considerably decreased the pH values for the control from 4.21 to 3.94, indicating that the yogurt quality remarkably decreased after 20 d of storage. However, it was observed that the pH values of CPC- or NPC-added and cholesterol-reduced yogurt samples were not dramatically changed during storage for 20 d, except for the 0.1% NPC-added and cholesterol-reduced yogurt sample, which exhibited the reduction of pH values from 4.25 to 4.03 during 20-d storage, demonstrating that the quality of cholesterol-reduced yogurt samples including NPC (0.3 to ~0.7%, wt/vol) was not remarkably varied during 20-d storage. Based on the results (regarding the pH changes of NPC-added and cholesterol-reduced yogurt) obtained from the current study, it was speculated that adding NPC (0.3 to ~0.7%, wt/vol) into the cholesterol-reduced yogurt could extend the shelf life.

Adding CPC or NPC (0.1 to ~0.7%, wt/vol) into the cholesterol-reduced yogurt samples decreased the values of titratable acidity (Figure 4). The values of titratable acidity for all the samples studied were slightly increased when stored at 4°C for 20 d. The findings (regarding the decrease in the pH values and the increase in the titratable acidity values for yogurt samples during 20-d storage at 4°C) obtained from the current study were consistent with Lee et al. (2007), who showed that pH decreased and titratable acidity increased when evening primrose oil-enriched and cholesterol-reduced yogurt samples were stored at 4°C for 15 d.

Lactic Acid Bacteria

The changes of *L. bulgaricus* and *Strep. thermophilus* in CPC- or NPC-added and cholesterol-reduced yogurt

samples stored at 4°C for 20 d are shown in Table 1. At 0-d storage, the mean microbial counts of the control were greater than those of CPC- or NPC-added (0.1 to ~0.7%, wt/vol) and cholesterol-reduced yogurt samples. Moreover, increasing the concentrations of CPC and NPC from 0.1 to 0.7% (wt/vol) at 0-d storage resulted in a reduction of the mean microbial counts from 6.34×10^{10} to 1.08×10^{10} cfu/mL and from 7.00×10^{10} to 1.85×10^{10} cfu/mL, respectively. The findings could be explained by the fact that chitosan has antimicrobial effects (Kendra and Hadwiger, 1984; Sudarshan et al., 1992; No et al., 2002; Qi et al., 2004). No et al. (2002) noted that chitosan markedly inhibited the growth of gram-positive bacteria such as *Staphylococcus aureus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum* and *Lactobacillus brevis*. Qi et al. (2004) showed that the antimicrobial activity of chitosan nanoparticles (mean diameter = 40 nm) was significantly greater than that of non-nanopowdered chitosan. In our preliminary test, the reduction of lactic acid bacteria in NPC- or CPC-added and cholesterol-reduced yogurt samples was found because of the antimicrobial activity of chitosan; therefore, we incorporated the greater concentration (0.004%, wt/vol) of starter culture into the cholesterol-reduced yogurt to maintain the quality of yogurt during storage, instead of the concentration 0.002% (wt/vol), which is recommended by the manufacturer of the starter culture.

Viscosity and Color

The viscosity values of all the samples studied increased sharply during 5-d storage and were almost constant until 15-d storage. After 20-d storage, the viscosity values were slightly decreased (Figure 5). Increasing values of viscosity were also observed in concentrated (Abu-Jdayil and Mohameed, 2002; Sahan et

Table 3. Sensory characteristics¹ for nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt² stored at 4°C for 20 d

Concentration of sample (% wt/vol)	Appearance		Flavor		Taste			Texture		Overall
	Whey-off	Color	Fishiness	Rancid	Sourness	Bitterness	Astringency	Grainy	Weak	
0-d storage period										
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.0 ^c	4.0 ^{ab}	4.0 ^{bcd}	4.0 ^{fg hij}	4.0 ^a	4.0 ^{abcd}	4.0 ^{abc}
NPC (0.1)	4.0 ^a	4.0 ^{ab}	1.3 ^{ab}	1.1 ^{bc}	3.3 ^{abcd}	3.9 ^{bcd}	4.1 ^{efghij}	4.3 ^a	3.4 ^{bcd}	3.4 ^{abcde}
NPC (0.3)	4.2 ^a	4.0 ^{ab}	1.3 ^{ab}	1.0 ^c	2.9 ^{bcd}	4.5 ^{abcd}	5.1 ^{bcdefg}	4.5 ^a	3.4 ^{bcd}	3.3 ^{abcde}
NPC (0.5)	3.8 ^a	3.6 ^{ab}	1.3 ^{ab}	1.0 ^c	2.6 ^{bcd}	4.8 ^{abcd}	5.0 ^{bcdefgh}	4.2 ^a	3.1 ^d	3.1 ^{abcde}
NPC (0.7)	4.1 ^a	3.6 ^{ab}	1.3 ^{ab}	1.3 ^{bc}	2.3 ^{cd}	5.0 ^{ab}	5.8 ^{ab}	4.6 ^a	3.9 ^{abcd}	3.1 ^{abcde}
CPC (0.1)	4.3 ^a	3.6 ^{ab}	1.3 ^{ab}	1.3 ^{bc}	2.8 ^{bcd}	4.5 ^{abcd}	4.8 ^{bcdefghi}	4.1 ^a	3.3 ^{cd}	3.8 ^{abc}
CPC (0.3)	4.3 ^a	3.1 ^{abcd}	1.3 ^{ab}	1.3 ^{bc}	3.0 ^{bcd}	4.5 ^{abcd}	5.1 ^{bcdefg}	4.3 ^a	3.1 ^d	3.3 ^{abcde}
CPC (0.5)	4.1 ^a	2.9 ^{abcd}	1.5 ^{ab}	1.1 ^{bc}	3.1 ^{bcd}	5.1 ^{ab}	5.3 ^{bcdefg}	4.3 ^a	4.1 ^{abcd}	3.1 ^{abcde}
CPC (0.7)	4.0 ^a	2.1 ^{cde}	1.8 ^a	1.0 ^c	2.1 ^d	5.1 ^{ab}	5.9 ^{ab}	4.4 ^a	4.1 ^{abcd}	3.1 ^{abcde}
5-d storage period										
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.0 ^c	4.0 ^{ab}	4.0 ^{bcd}	4.0 ^{fg hij}	4.0 ^a	4.0 ^{abcd}	4.0 ^{abc}
NPC (0.1)	4.0 ^a	4.0 ^{ab}	1.3 ^{ab}	1.1 ^{bc}	2.9 ^{bcd}	4.1 ^{bcd}	4.5 ^{bcdefghij}	4.2 ^a	4.1 ^{abcd}	4.2 ^{abc}
NPC (0.3)	4.2 ^a	4.0 ^{ab}	1.3 ^{ab}	1.0 ^c	3.1 ^{bcd}	4.6 ^{bcd}	4.8 ^{bcdefghi}	4.5 ^a	4.4 ^{abcd}	3.3 ^{abcde}
NPC (0.5)	3.8 ^a	3.6 ^{ab}	1.3 ^{ab}	1.0 ^c	3.4 ^{abcd}	4.7 ^{abcd}	4.8 ^{bcdefghi}	4.4 ^a	4.8 ^{ab}	3.1 ^{abcde}
NPC (0.7)	4.1 ^a	3.6 ^{ab}	1.8 ^a	1.3 ^{bc}	3.3 ^{abcd}	4.7 ^{abc}	5.3 ^{bcde}	4.6 ^a	5.0 ^a	3.1 ^{abcde}
CPC (0.1)	4.4 ^a	3.6 ^{ab}	1.3 ^{ab}	1.3 ^{bc}	3.6 ^{abcd}	3.4 ^{cde}	4.4 ^{bcdefghij}	4.1 ^a	4.6 ^{abcd}	4.1 ^{abc}
CPC (0.3)	4.3 ^a	3.0 ^{abcd}	1.3 ^{ab}	1.3 ^{bc}	2.7 ^{bcd}	3.4 ^{cde}	4.3 ^{bcdefghij}	4.3 ^a	4.0 ^{abcd}	3.3 ^{abcde}
CPC (0.5)	4.1 ^a	2.9 ^{abcd}	1.5 ^{ab}	1.1 ^{bc}	2.6 ^{bcd}	5.1 ^{ab}	5.4 ^{bcdefg}	4.3 ^a	4.0 ^{abcd}	3.1 ^{abcde}
CPC (0.7)	4.0 ^a	2.2 ^{cde}	1.5 ^{ab}	1.0 ^c	2.6 ^{bcd}	5.1 ^{ab}	5.6 ^{bc}	4.4 ^a	4.8 ^{ab}	3.1 ^{abcde}
10-d storage period										
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.0 ^c	4.0 ^{ab}	3.6 ^{cde}	3.7 ^{ij}	3.7 ^a	4.0 ^{abcd}	4.1 ^{abc}
NPC (0.1)	4.0 ^a	4.0 ^{ab}	1.3 ^{ab}	1.1 ^{bc}	4.0 ^{ab}	3.6 ^{cde}	4.2 ^{efghij}	3.9 ^a	4.2 ^{abcd}	4.4 ^{ab}
NPC (0.3)	4.2 ^a	4.0 ^{ab}	1.3 ^{ab}	1.0 ^c	3.5 ^{abcd}	4.0 ^{bcd}	5.0 ^{bcdefgh}	4.1 ^a	4.5 ^{abcd}	3.8 ^{abc}
NPC (0.5)	3.8 ^a	3.6 ^{ab}	1.3 ^{ab}	1.0 ^c	3.5 ^{abcd}	4.4 ^{abcd}	5.2 ^{bcde}	4.4 ^a	4.5 ^{abcd}	3.5 ^{abcde}
NPC (0.7)	4.1 ^a	3.6 ^{ab}	1.8 ^a	1.3 ^{bc}	3.5 ^{abcd}	4.8 ^{abc}	4.0 ^{fg hij}	4.4 ^a	4.7 ^{abc}	3.1 ^{abcde}
CPC (0.1)	4.4 ^a	4.0 ^{ab}	1.3 ^{ab}	1.3 ^{bc}	3.4 ^{abcd}	3.4 ^{cde}	4.4 ^{bcdefghij}	4.1 ^a	4.2 ^{abcd}	4.1 ^{abc}
CPC (0.3)	4.3 ^a	3.0 ^{abcd}	1.3 ^{ab}	1.3 ^{bc}	3.6 ^{abcd}	3.1 ^{cde}	5.4 ^{bcdefg}	4.3 ^a	4.5 ^{abcd}	3.3 ^{abcde}
CPC (0.5)	4.1 ^a	2.9 ^{abcd}	1.5 ^{ab}	1.1 ^{bc}	3.6 ^{abcd}	4.0 ^{bcd}	5.6 ^{bc}	4.3 ^a	4.5 ^{abcd}	3.1 ^{abcde}
CPC (0.7)	4.0 ^a	2.1 ^{cde}	1.5 ^{ab}	1.0 ^c	3.1 ^{bcd}	4.8 ^{abc}	6.3 ^a	4.4 ^a	4.7 ^{abc}	3.1 ^{abcde}
15-d storage period										
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.0 ^c	4.7 ^a	3.3 ^{cde}	3.4 ^j	4.0 ^a	4.1 ^{abcd}	4.9 ^a
NPC (0.1)	4.0 ^a	4.3 ^a	1.1 ^{ab}	1.3 ^{bc}	3.7 ^{abc}	4.1 ^{bcd}	4.1 ^{efghij}	4.1 ^a	4.2 ^{abcd}	4.3 ^{abc}
NPC (0.3)	4.2 ^a	3.0 ^{abcd}	1.2 ^{ab}	1.3 ^{bc}	3.6 ^{abcd}	3.7 ^{bcd}	3.9 ^{ghij}	4.1 ^a	4.3 ^{abcd}	4.0 ^{abc}
NPC (0.5)	3.9 ^a	3.2 ^{abc}	1.6 ^{ab}	1.6 ^{abc}	3.3 ^{bcd}	4.5 ^{bcd}	5.2 ^{bcdefg}	4.3 ^a	4.1 ^{abcd}	3.0 ^{abcde}
NPC (0.7)	4.1 ^a	4.0 ^{ab}	1.3 ^{ab}	2.0 ^a	2.7 ^{bcd}	5.1 ^{ab}	6.7 ^a	4.3 ^a	4.3 ^{abcd}	2.4 ^{cde}
CPC (0.1)	4.4 ^a	4.3 ^a	1.0 ^a	1.0 ^c	3.2 ^{bcd}	2.7 ^e	3.5 ^j	4.0 ^a	4.0 ^{abcd}	4.8 ^a
CPC (0.3)	4.2 ^a	3.6 ^{ab}	1.1 ^{ab}	1.4 ^{abc}	2.9 ^{bcd}	4.3 ^{bcd}	5.1 ^{bcdefg}	4.0 ^a	4.6 ^{abcd}	4.1 ^{abc}
CPC (0.5)	4.1 ^a	2.9 ^{abcd}	1.1 ^{ab}	1.7 ^{ab}	2.7 ^{bcd}	4.4 ^{abcd}	5.0 ^{bcdefghi}	4.3 ^a	4.1 ^{abcd}	4.3 ^{abc}
CPC (0.7)	4.0 ^a	1.6 ^e	1.7 ^{ab}	1.4 ^{abc}	2.6 ^{bcd}	5.9 ^a	6.0 ^{ab}	4.3 ^a	3.9 ^{abcd}	1.9 ^{de}
20-d storage period										
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.2 ^{bc}	4.1 ^{ab}	3.3 ^{cde}	3.7 ^{ij}	4.0 ^a	3.9 ^{abcd}	4.7 ^{ab}
NPC (0.1)	4.1 ^a	4.3 ^a	1.1 ^{ab}	1.6 ^{abc}	3.5 ^{abcd}	4.1 ^{bcd}	3.7 ^{ij}	4.1 ^a	4.0 ^{abcd}	4.1 ^{abc}
NPC (0.3)	4.1 ^a	3.0 ^{abcde}	1.2 ^{ab}	1.4 ^{abc}	3.2 ^{abcd}	3.7 ^{bcd}	3.9 ^{ghij}	4.1 ^a	4.1 ^{abcd}	3.8 ^{abc}
NPC (0.5)	4.1 ^a	3.2 ^{abc}	1.6 ^{ab}	1.6 ^{abc}	3.1 ^{bcd}	4.5 ^{bcd}	4.9 ^{bcdefghi}	4.3 ^a	3.8 ^{abcd}	3.8 ^{abc}
NPC (0.7)	4.1 ^a	4.0 ^{ab}	1.3 ^{ab}	2.0 ^a	3.1 ^{bcd}	5.1 ^{ab}	5.0 ^{bcdefghi}	4.3 ^a	4.1 ^{abcd}	2.8 ^{bcd}
CPC (0.1)	4.1 ^a	4.3 ^a	1.1 ^{ab}	1.3 ^{bc}	2.8 ^{bcd}	3.1 ^{cde}	4.4 ^{bcdefghij}	4.1 ^a	3.8 ^{abcd}	4.7 ^{ab}
CPC (0.3)	4.1 ^a	3.6 ^{ab}	1.1 ^{ab}	1.4 ^{abc}	2.9 ^{bcd}	4.3 ^{abcd}	4.9 ^{bcdefghi}	4.0 ^a	4.4 ^{abcd}	4.0 ^{abc}
CPC (0.5)	4.1 ^a	2.9 ^{abcd}	1.1 ^{ab}	1.7 ^{ab}	2.7 ^{bcd}	4.4 ^{abcd}	4.9 ^{bcdefghi}	4.3 ^a	4.1 ^{abcd}	3.7 ^{abc}
CPC (0.7)	4.1 ^a	1.9 ^{de}	1.3 ^{ab}	1.2 ^{bc}	2.6 ^{bcd}	5.9 ^a	5.9 ^{ab}	4.3 ^a	3.9 ^{abcd}	1.7 ^e

^{a-j}Values with different superscripts within the same column differ significantly ($P < 0.05$).

¹The scale of appearance, flavor, taste, texture, and color scores: 1 = very weak, 4 = moderate, 7 = very strong.

²Milk used was treated with 1% cross-linked β -cyclodextrin for all samples.

al., 2008) and nonfat plain yogurt (Isleten and Karagulyceer, 2006). According to Sahan et al. (2008), the increase in viscosity values for nonfat yogurt during 15 d of storage can be associated with the rearrangement of protein molecules.

The changes of color for CPC- or NPC-added and cholesterol-reduced yogurt samples stored at 4°C for 20

d are presented in Table 2. The L* values for all the samples studied were not considerably changed during storage. However, the a* and b* values for the control sample increased from 3.04 to 3.27 and from 5.81 to 7.06, respectively, when the storage period increased from 0 to 20 d. The 0.7% (wt/vol) NPC sample was the only sample that had a significantly lower L* value

at 0-d storage as compared with the control. The a^* values of CPC (0.5 and 0.7%, wt/vol) and NPC (0.7%, wt/vol) at 0-d storage were significantly decreased as compared with the control. The b^* values for the yogurt samples at 0-d storage were not significantly affected by the addition of CPC or NPC.

Sensory Evaluation

The sensory properties of CPC- or NPC-added and cholesterol-reduced yogurt samples stored at 4°C for 20 d are listed in Table 3. The whey-off scores for the cholesterol-reduced yogurt samples were not significantly influenced by prolonged storage (20 d) or the addition of CPC or NPC. The color score at 0-d storage was significantly decreased when the greatest concentration (0.7%, wt/vol) of CPC was added into the cholesterol-reduced yogurt samples, probably because of the original yellow color of chitosan. Only the cholesterol-reduced yogurt sample including CPC (0.7%, wt/vol) exhibited a significantly greater fishiness score at 0-d storage, as compared with the control. The rancid scores for the yogurt samples at 0-d storage were not significantly affected by the addition of CPC or NPC. In the taste test, it was revealed that adding CPC or NPC (0.7%, wt/vol) into cholesterol-reduced yogurt samples caused a significant decrease in the sourness scores and a significant increase in the astringency scores at 0-d storage. The greater astringency scores for yogurt samples that include the chitosan powders (0.7%, wt/vol) were probably the result of the original astringent flavor of the original chitosan powder. According to the texture test, the grainy and weak scores for the cholesterol-reduced yogurt samples at 0-d storage were not significantly affected by the addition of CPC or NPC. Finally, adding CPC or NPC into the cholesterol-reduced yogurt samples did not significantly influence the overall scores at d 0, 5, and 10.

Based on all the sensory data obtained from the current study, it is suggested that concentrations (0.1 to ~0.5%, wt/vol) of NPC could be used for the production of NPC-added and cholesterol-reduced yogurt without the deterioration of sensory properties.

CONCLUSIONS

The current study was designed to develop an NPC-added and cholesterol-reduced yogurt and to evaluate the effects of adding NPC on the physicochemical, microbial, and sensory properties of the final products during storage. The data on the pH, titratable acidity, microbial, color, and sensory analysis obtained from the current study indicated that concentrations (0.3 to 0.5%) of NPC could be applicable in NPC-added and

cholesterol-reduced yogurt development. The production of yogurt that incorporates NPC can broaden the utilization of chitosan, and the products can be regarded as possible health-promoting nutraceutical foods.

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