나노키토산 논문 SCI급 Top 10.

Journal of Dairy Science

December 2009 Volume 92, Number 12

CONTENTS

PHYSIOLOGY AND MANAGEMENT:

NUTRITION, FEEDING, AND CALVES:

GENETICS AND BREEDING:

OUR INDUSTRY TODAY:

ASSOCIATION AFFAIRS:

NOTICE: Page Charges for Publication

The page charge per printed page is $85 for professional members and $140 for nonmembers for papers published in *Journal of Dairy Science*. Open access (OA) publication is available upon payment of a $1000 OA fee in addition to standard page charges.

Future Annual Meetings

2010–July 11–15, Denver, CO

2011–July 11–14, New Orleans, LA

J. Dairy Sci. 92:5907–5916
doi:10.3168/jds.2009-2520

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

M. H. Seo, S. Y. Lee, Y. H. Chang, and H. S. Kwak[1]
Department of Food Science and Technology, Sejong University, Seoul 143-747, Korea

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

Received June 29, 2009.
Accepted September 1, 2009.
[1]Corresponding author: kwakhs@sejong.ac.kr

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-palatium (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

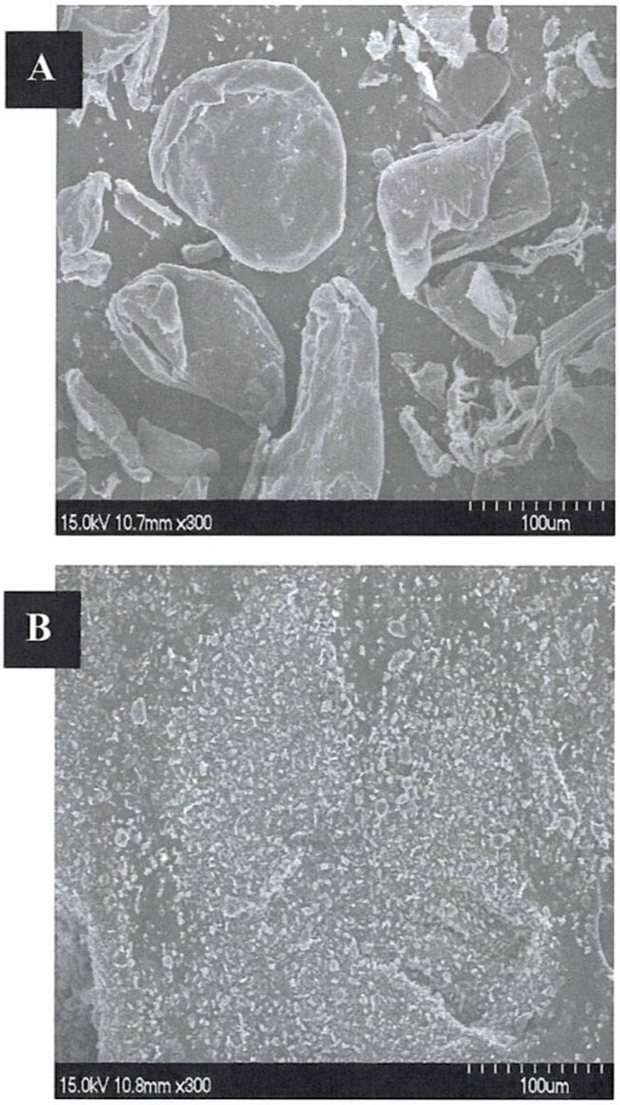


Figure 1. Scanning electron microscope images for commercially powdered chitosan (A) and nanopowdered chitosan (B).

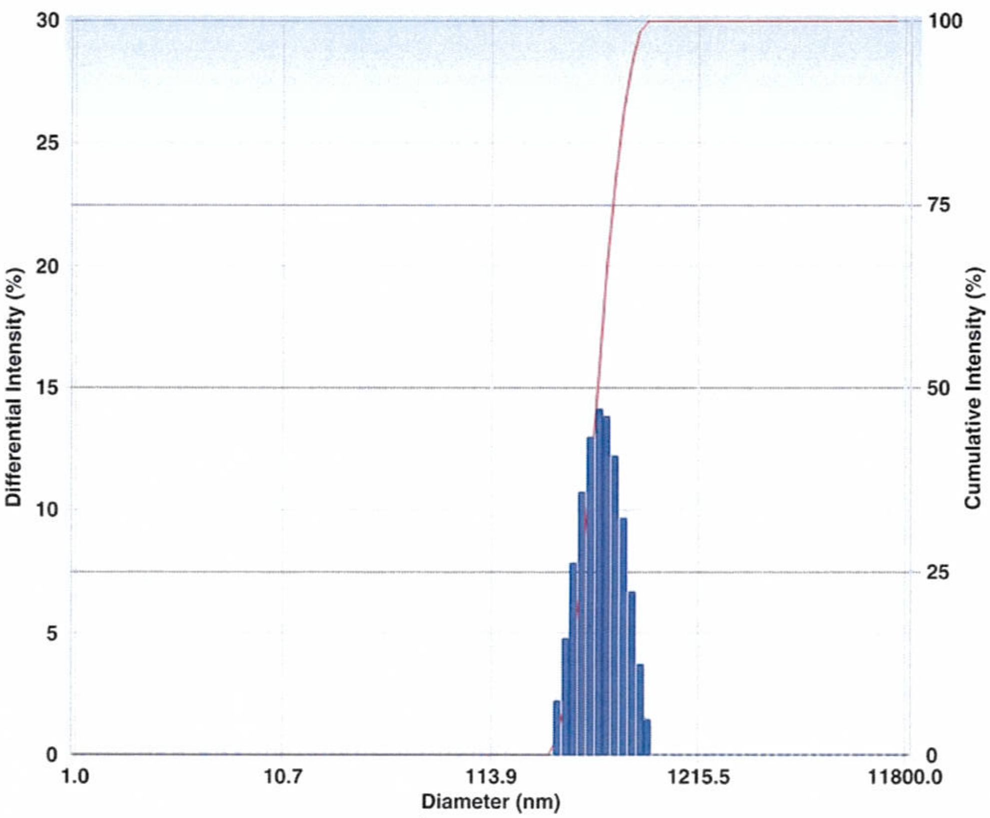


Figure 2. Particle size analysis of nanopowdered chitosan. Color version available in the online PDF.

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 × *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 × 180 mm), and 1 mL of 5α-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 × 0.32 mm i.d. × 0.25 μm 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

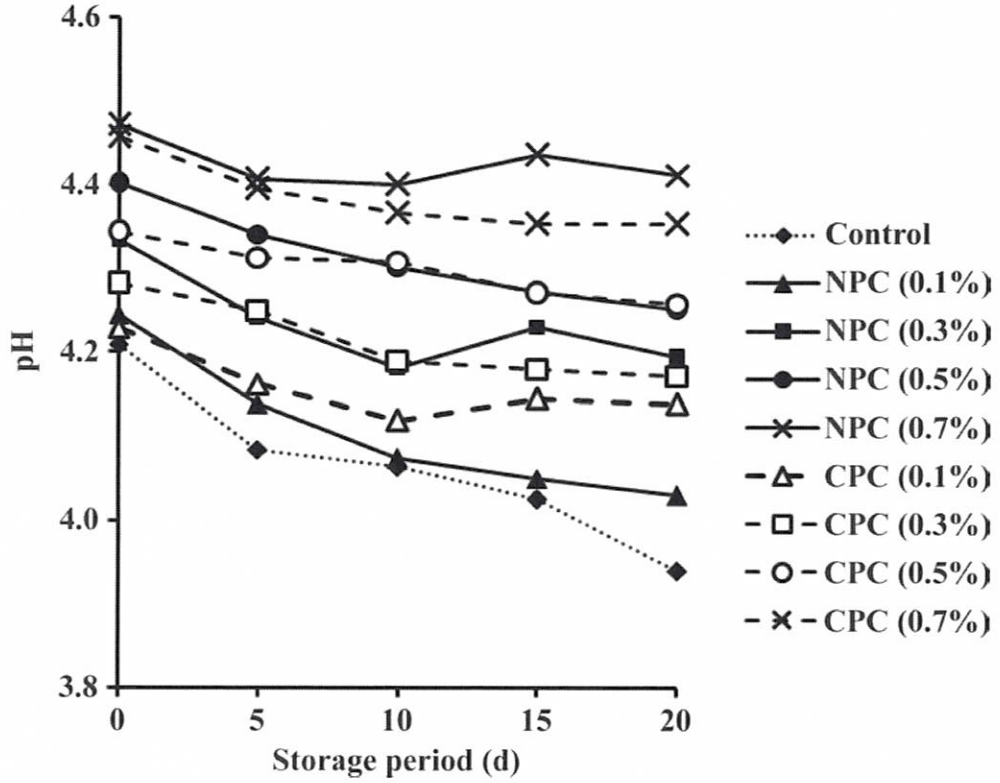


Figure 3. Changes in pH in nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt stored at 4°C for 20 d. Milk used was treated with 1% cross-linked β-cyclodextrin for all samples. At 0 d. yogurt had been stabilized for 24 h.

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 − (amount of cholesterol in β-CD-treated yogurt × 100/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

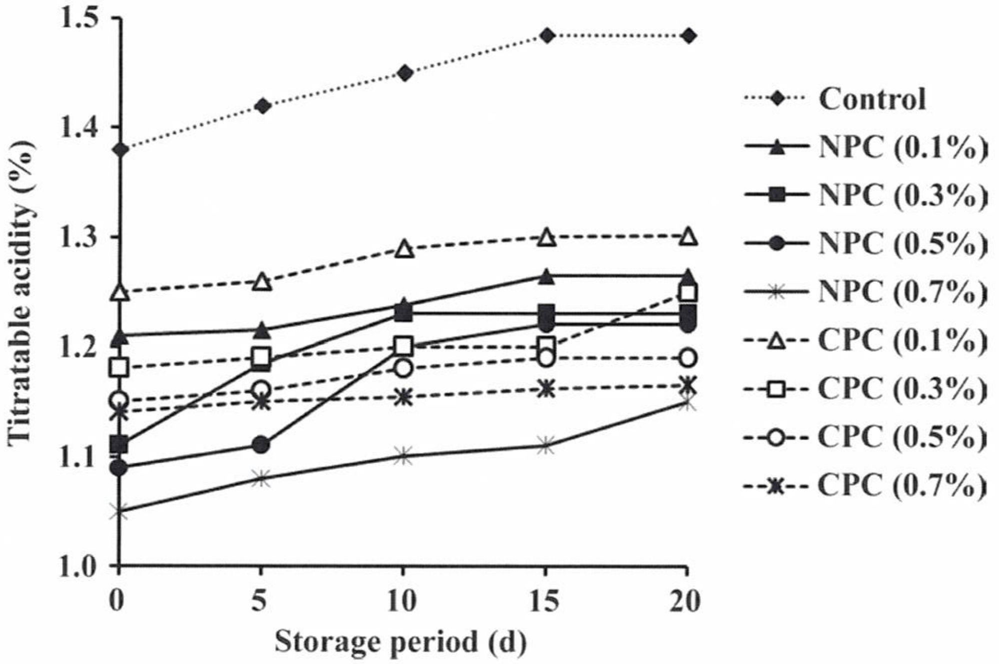


Figure 4. Changes in titratable acidity in nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt stored at 4°C for 20 d. Milk used was treated with 1% cross-linked β-cyclodextrin for all samples. At 0 d, yogurt had been stabilized for 24 h.

(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 bacteria[1] (cfu/mL) in nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt[2] 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^{8e}	8.75×10^{8c}	5.25×10^{8d}	4.05×10^{8d}
CPC (0.7)	1.08×10^{10d}	6.50×10^{8e}	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 ($P < 0.05$).
[1]The mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.
[2]Milk 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.

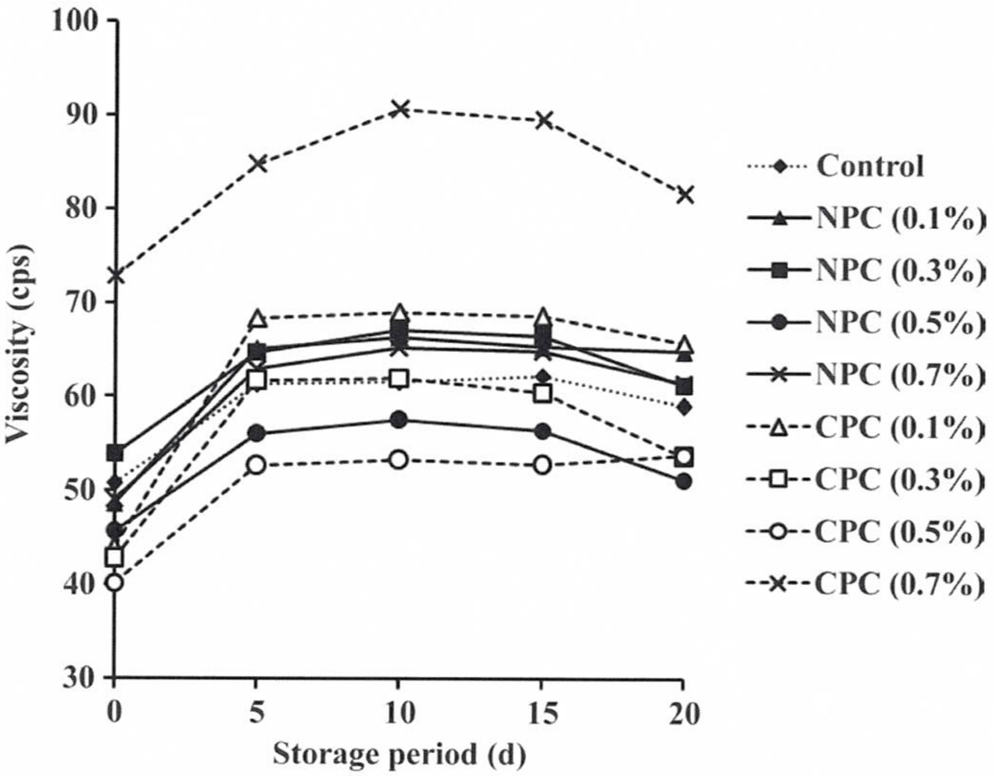


Figure 5. Changes in viscosity for nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt stored at 4°C for 20 d. Milk used was treated with 1% cross-linked β-cyclodextrin for all samples. At 0 d, yogurt had been stabilized for 24 h.

Table 2. Changes of color for nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt[1] 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^{c}	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^{c}	2.94^{cd}	7.43^{a}

[a–f]Values with different superscripts within the same column differ significantly ($P < 0.05$).
[1]Milk 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 characteristics[1] for nanopowdered chitosan (NPC)- or commercially powdered chitosan (CPC)-added and cholesterol-reduced yogurt[2] stored at 4°C for 20 d

Concentration of sample (%, wt/vol)	Appearance		Flavor		Taste			Texture		
	Whey-off	Color	Fishiness	Rancid	Sourness	Bitterness	Astringency	Grainy	Weak	Overall
0-d storage period										
Control	4.0^{a}	4.0^{ab}	1.0^{b}	1.0^{c}	4.0^{ab}	4.0^{bcde}	4.0^{fghij}	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^{bcde}	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^{abc}	$5.0^{bcdefgh}$	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^{bcde}	4.0^{fghij}	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^{bcde}	$4.5^{cdefghij}$	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^{bcde}	$4.8^{bcdefghi}$	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^{abc}	$4.8^{bcdefghi}$	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}	$4.4^{cdefghij}$	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}	$4.3^{cdefghij}$	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^{bcde}	$5.0^{bcdefgh}$	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^{abcd}
NPC (0.7)	4.1^{a}	3.6^{ab}	1.8^{a}	1.3^{bc}	3.5^{abcd}	4.8^{abc}	4.0^{fghij}	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}	$4.4^{cdefghij}$	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^{bcde}	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^{bcde}	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^{bcde}	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^{bcde}	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^{bcde}	5.1^{bcdefg}	4.0^{a}	4.6^{abcd}	4.1^{abc}
CPC (0.5)	4.1^{a}	2.9^{bcd}	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^{bcde}	3.7^{ij}	4.1^{a}	4.0^{abcd}	4.1^{abc}
NPC (0.3)	4.1^{a}	3.0^{abce}	1.2^{ab}	1.4^{abc}	3.2^{abcd}	3.7^{bcde}	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^{bcde}	$4.9^{bcdefghi}$	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^{bcde}
CPC (0.1)	4.1^{a}	4.3^{a}	1.1^{ab}	1.3^{bc}	2.8^{bcd}	3.1^{cde}	$4.4^{cdefghij}$	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^{bcd}	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 ($P < 0.05$).

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

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

al., 2008) and nonfat plain yogurt (Isleten and Karagul-Yuceer, 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.

ACKNOWLEDGMENTS

This study was supported by the Ministry for Food, Agriculture, Forestry and Fisheries Project in Seoul, Republic of Korea.

REFERENCES

Abu-Jdayil, B., and H. Mohameed. 2002. Experimental and modeling studies of the flow properties of concentrated yogurt as affected by storage time. J. Food Eng. 52:359–365.

Adams, M. L., D. M. Sullivan, R. L. Smith, and E. F. Richer. 1986. Evaluation of direct saponification method in determination of cholesterol in meats. J. Assoc. Anal. Chem. 69:844–846.

Gee, V. L., T. Vasanthan, and F. Temelli. 2007. Viscosity of model yogurt systems enriched with barley β-glucan as influenced by starter cultures. Int. Dairy J. 17:1083–1088.

Han, E. M., S. H. Kim, J. Aha, and H. S. Kwak. 2007. Optimizing cholesterol removal from cream using β-cyclodextrin cross-linked with adipic acid. Int. J. Dairy Technol. 60:31–36.

Han, E. M., S. H. Kim, J. Ahn, and H. S. Kwak. 2005. Cholesterol removal from homogenized milk with crosslinked β-cyclodextrin by adipic acid. Asian-australas. J. Anim. Sci. 18:1794–1799.

Hayashi, K., and M. Ito. 2002. Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KK-A^y mice. Biol. Pharm. Bull. 25:188–192.

Isleten, M., and Y. Karagul-Yuceer. 2006. Effects of dried dairy ingredients on physical and sensory properties of nonfat yogurt. J. Dairy Sci. 89:2865–2872.

Jaziri, I., M. Ben Slama, H. Mhadhbi, M. C. Urdaci, and M. Hamdi. 2009. Effect of green and black teas (*Camellia sinensis* L.) on the characteristic microflora of yogurt during fermentation and refrigerated storage. Food Chem. 112:614–620.

Kendra, D. F., and L. A. Hadwiger. 1984. Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Exp. Mycol. 8:276–281.

Kim, H. Y., H. Y. Bae, and H. S. Kwak. 2008. Development of cholesterol-reduced Blue cheese made by crosslinked β-cyclodextrin. Milchwissenschaft. 63:53–56.

Kim, J. J., T. H. Jung, J. Ahn, and H. S. Kwak. 2006. Properties of cholesterol-reduced butter made with β-cyclodextrin and added evening primrose oil and phytosterols. J. Dairy Sci. 89:4503–4510.

Kim, S. H., E. M. Han, J. Ahn, and H. S. Kwak. 2005. Effect of crosslinked β-cyclodextrin on quality of cholesterol-reduced cream cheese. Asian-australas. J. Anim. Sci. 18:584–589.

Kondo, Y., A. Nakatani, K. Hayash, and M. Ito. 2000. Low molecular weight chitosan prevents the progression of low dose streptozotocin-induced slowly progressive diabetes mellitus in mice. Biol. Pharm. Bull. 23:1458–1464.

Kroger, M. 1976. Quality of yogurt. J. Dairy Sci. 59:344–350.

Kumar, S. G., M. H. Rahman, S. H. Lee, H. S. Hwang, H. A. Kim, and J. W. Yun. 2009. Plasma proteome analysis for anti-obesity and anti-diabetic potentials of chitosan oligosaccharides in ob/ob mice. Proteomics 9:2149–2162.

Lee, H. W., Y. S. Park, J. W. Choi, S. Y. Yi, and W. S. Shin. 2003. Antidiabetic effects of chitosan oligosaccharides in neonatal streptozotocin-induced noninsulin-dependent diabetes mellitus in rats. Biol. Pharm. Bull. 26:1100–1103.

Lee, S. J., J. H. Hwang, S. Lee, J. Ahn, and H. S. Kwak. 2007. Property changes and cholesterol-lowering effects in evening primrose oil-

1. SEM (scanning electron microscope)으로 분석한 결과 일반 키토산 분말은 입자가 불균일한 크기로 흩어져 있는 반면, 키토산 분말을 분쇄하여 나노화 시킨 나노키토산 분말은 비교적 균일한 크기로 잘 분산되어 있었다 (Figure 1).

2. 일반키토산 분말은 직경이 평균 150μm (Figure1) 였고, 나노키토산 분말은 평균 560 nm 정도로 나타나 키토산 분말의 크기가 마이크론 크기에서 나노크기로 감소한 것을 알 수 있었다. Particle analyzer 으로 분석한 나노키토산은 약 560nm 크기로 분포되어 있는 것을 알 수 있었다 (Figure 2).

3. 나노키토산 분말의 일반성분을 분석한 결과 수분(8.82%), 회분(0.25%), 질소함량(6.66%), 탈아세틸화도(92.71%)로 키토산 분말과 유사하였으나, 분자량은 나노크기로 분쇄함에 따라 90,900 에서 16,500 으로 분자량이 1/5 로 감소함을 알 수 있었다 (Table 1).

4. 나노키토산 분말의 흡착력을 분석한 결과 수분흡착력(565.4g/100g), 지방흡착력(776.3g/100g)으로 키토산 분말의 흡착력보다 각각 16.0%, 9.4% 높았고, 색소흡착력은 나노키토산 분말이 2.9mg/g 으로 키토산에 비해 약 60%정도 낮은 값을 보였다 (Table 2).

5. 나노키토산 분말의 항균효과 결과 *S. aureus* 는 약 9 배 증가하였고, *E. coli* O157:H7 은 약 5 배, *B. subtilis* 는 약 2 배, *L. bulgaricus* 는 약 3 배, *C. albicans* 는 약 4 배, *S. cerevisiae* 는 약 27 배로 더 높게 나타났다. 따라서 항균효과는 미생물의 종류에 따라 다르고, 나노키토산 분말의 항균효과가 키토산 분말에 비해 현저히 높다는 것을 알 수 있었다.

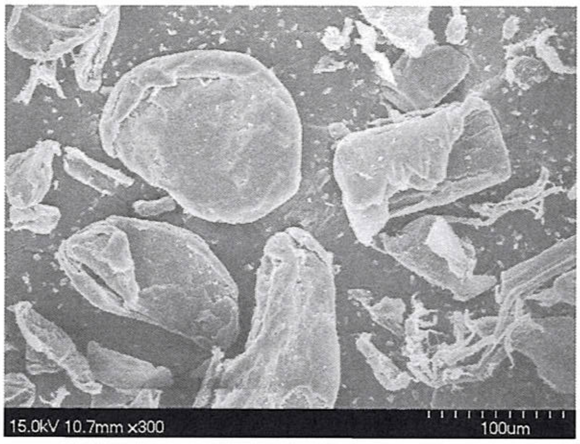
6. 나노키토산 분말 섭취군의 혈 중 포도당 농도는 555 mg/dℓ에서 242 mg/dℓ로 약 56% 감소하였고, 키토산 분말 섭취군은 533 mg/dℓ에서 383 mg/dℓ로 약 28%로 감소하였다. 키토산 섭취군에 비해 나노키토산 분말 섭취군에서 약 2 배이상 높은

감소율을 보였다. 또한 혈 중 총콜레스테롤의 농도에서 당뇨대조군은 209.8 mg/dℓ, 나노키토산 분말 섭취군은 143.5 mg/dℓ으로 약 31.6% 감소하였고, 키토산 분말 섭취군은 171.7 mg/dℓ으로 약 18.2% 감소되었다. 키토산 섭취군에 비해 나노키토산 분말 섭취군에서 약 1.7 배이상 높은 감소율을 보였다. 중성지질의 함량에서 당뇨대조군은 107.3 mg/dℓ, 나노키토산 분말 섭취군은 71.2 mg/dℓ으로 약 33.6% 감소하였고, 키토산 분말 섭취군은 83.1 mg/dℓ으로 약 22.5% 감소를 보였다. 혈 중 인슐린 농도에서 당뇨대조군은 306.6 μU/ml, 나노키토산 분말 섭취군은 131.4 μU/ml 으로 약 57.1 μU/ml 감소하였고, 키토산 분말 섭취군은 175.5 μU/ml 으로 약 42.8% 감소되었다. 췌장조직의 형태변화를 분석한 결과 나노키토산 분말 섭취군에서 정상의 췌장 소도세포와 유사한 형태를 나타낸 것을 알 수 있었다. 따라서 나노키토산 분말의 항당뇨 효과가 키토산 분말에 비해 현저히 높음을 알 수 있었다.

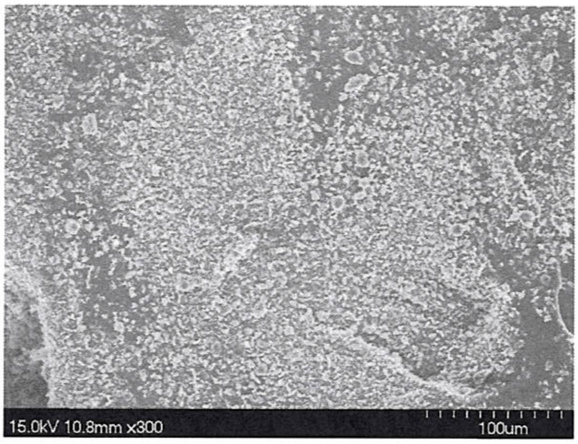
7. 콜레스테롤을 93.1% 제거한 요구르트에 나노키토산 분말을 첨가했을 때 pH 는 첨가량이 증가함에 따라 높게 나타났고(4.2~4.5), 저장기간에 따라 pH 는 감소하였고, 산도는 나노키토산 분말의 첨가량이 증가함에 따라 반대의 결과를 보였다. 유산균수는 나노키토산 분말의 첨가비율이 높을수록 유산균의 수가 적게 나타남을 관찰하였다. 저장 20 일 동안 유산균수는 1.20×10^9 CFU/ml 에서 1.17×10^6 CFU/ml 으로 감소함을 알 수 있었다. 점도의 변화에서 나노키토산 분말의 첨가량에 비례하여 점도가 감소함을 알 수 있었다. 색의 변화에서 L-값은 나노키토산 분말의 첨가량에 따라 낮은 값을 보였고, a-값은 대조군과 유의적인 차이를 보이지 않았으며, b-값은 나노키토산의 첨가량이 증가함에 따라 증가하는 경향을 나타냈다. 관능평가에서 appearance 는 대조군, 나노키토산 분말 첨가군에서 유의적 차이가 나타나지 않았고, taste 에서 실험군의 첨가비율이 높을수록 유의적($P<0.05$) 차이가 나타났다.

8. 수용성 나노키토산은 나노키토산 분말을 팽윤시킨 후 PGMS(polyglycerol monostearate)를 사용하여 중성의 상태로 제조하였다. 수용성 나노키토산을 첨가한 우유의 pH 는 첨가량이 증가함에 따라 대조군 보다 높았으며, 저장 15 일 동안 신선한 우유 품질을 유지하였고, 비타민 C 의 함량은 첨가비율이 높을수록 높았으며, 저장기간에 따라 감소함을 알 수 있었다. 색의 변화에서 수용성 나노키토산을 첨가하였을 때 저장 15 일동안 L, a-값은 유의적 차이가 없었으며, 저장기간중 수용성 나노키토산을 첨가한 우유의 b-값은 유의적으로 증가하였다. 관능평가 결과 color, rancidity, bitterness, astringency, overall 에서 대조군과 수용성 나노키토산 첨가군간에 유의적($P<0.05$)차이가 없음을 알 수 있었다.

9. 본 연구의 종합적인 결과에 의하면, 나노키토산은 기존의 키토산에 비해 항균, 항당뇨 효과가 더욱더 극대화 되었고, 혈중 콜레스테롤을 감소하는 기능성 요구르트 및 비타민 C 의 함량이 높은 우유의 개발이 가능할 것으로 기대된다. 따라서 고기능성 물질인 나노키토산을 식품에 접목시키면 그 가치가 향상될 것이다.



Powdered chitosan



Nanopowdered chitosan

Figure 1. Scanning electron microscope image for powdered chitosan and nanopowdered chitosan.

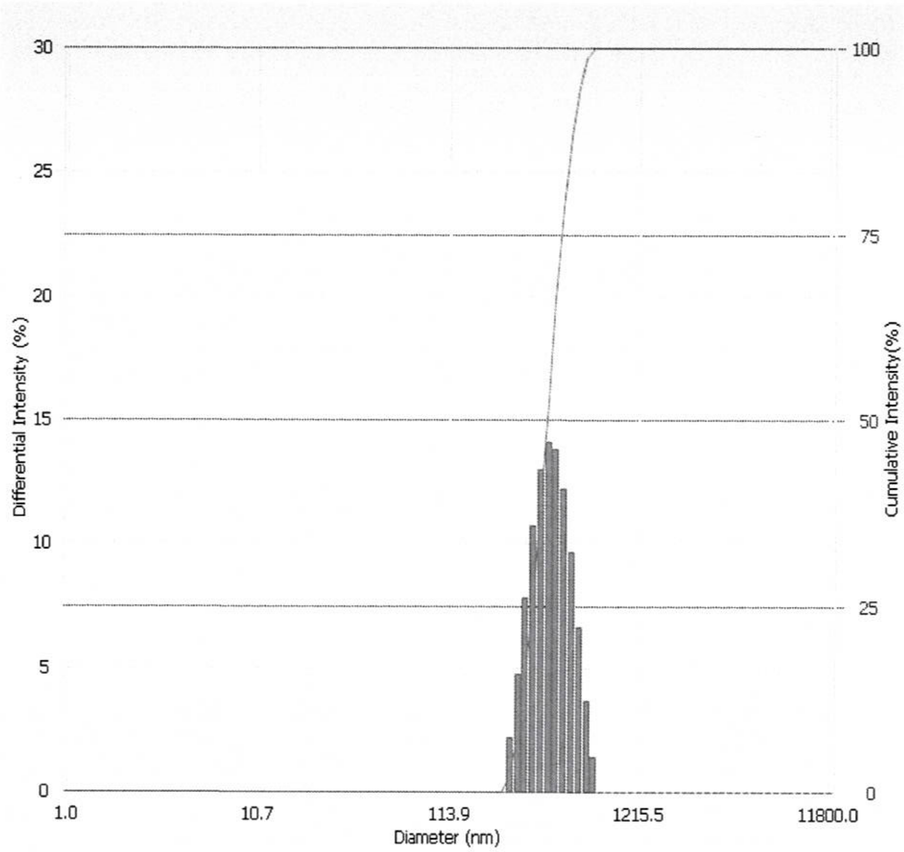


Fig. 2. Particle size analysis of nanopowdered chitosan.

Table 1. Composition of nanopowdered chitosan and powdered chitosan

Composition	Powdered chitosan[1)]	Nanopowdered chitosan[2)]
Moisture(%)	6.44 ± 0.09^{b}	$8.82 \pm 0.05^{a4)}$
Ash(%)	0.29 ± 0.02^{a}	0.25 ± 0.02^{b}
Nitrogen(%)	6.75 ± 0.08^{a}	6.66 ± 0.01^{b}
Molecular weight	$90{,}900 \pm 33.23^{a}$	$16{,}500 \pm 12.70^{b}$
DDA[3)] (%)	93.00 ± 0.17^{a}	92.71 ± 0.28^{a}

[1)] Commercial powdered chitosan with size 150 ㎛

[2)] 500 ~ 600nm

[3)] Degree of deacetylation

[4)] Values within the same column with different superscripts are significantly different at $p<0.05$ by Duncan's multiple-test

Table 2. Absorption capacity of nanopowdered chitosan and powdered chitosan

	Powdered chitosan[1)]	Nanopowdered chitosan[2)]
Water absorption(g)	473.2±0.6[b]	565.4±26.4[a3)]
Oil absorption(g)	703.0±28.1[b]	776.3±35.9[a]
Dye absorption(mg)	9.6±0.4[a]	2.9±0.1[b]

1) Commercial powdered chitosan with size 150 ㎛

2) 500 ~ 600nm

3) Value within the same column with different superscripts are significantly different at $p<0.05$ by Duncan's multiple-test