Physicochemical, microbial, and sensory properties of nanopowdered eggshell-supplemented yogurt during storage

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ABSTRACT

This study was carried out to investigate the possibility of adding nanopowdered eggshell (NPES) into yogurt to improve the functionality of yogurt and the effects of adding NPES on the physicochemical, microbial, and sensory properties of the products during storage. The pH and mean lactic acid bacteria counts of NPES-added (0.15–0.45%, wt/vol) yogurt ranged from 4.31 to 4.66 and from 6.56×10^8 to 8.56×10^8 cfu/mL, respectively, whereas these values ranged from 4.13 to 4.44 and 8.46×10^8 to 1.39×10^9 , respectively, for the control samples during storage at 5°C for 16 d, which indicates a prolonged shelf-life with NPES-supplemented yogurt. Color analysis showed that the lightness (L^*) and position between red and green (a^*) values were not significantly influenced by the addition of NPES. However, the position between yellow and blue (b^*) value significantly increased with the addition of the concentration (0.45%, wt/vol) of NPES at d 16 of storage. Sensory evaluation revealed that NPES-added yogurts showed a notably less sourness score and a higher astringency score than the control. An earthy flavor was higher in 0.45% NPES-supplemented yogurt compared with the control. Based on the results obtained from the current study, the concentration (0.15 to 0.30%, wt/vol) of NPES can be used to formulate NPES-supplemented yogurt without any significant adverse effects on the physicochemical, microbial, and sensory properties.

Key words: nanopowdered eggshell, functional yogurt, shelf-life

INTRODUCTION

Eggshell contains about 39% elemental Ca and the bioavailability of eggshell Ca is as high as that from CaCO_3 (Schaafsma and Beelen, 1999). As it is widely available, it can be a cheap and effective solution for a large number of Ca-deficient populations. The benefi-

cial effects of eggshell on bone mineral density of the skeletal system have been demonstrated in postmenopausal women (Makai and Chudáček, 1991; Schaafsma and Pakan, 1999). Eggshell also has some other nutrients, such as Mg, P, glycoproteins, and proteoglycans (Hincke, 1995; Hincke et al., 1999). The protein matrices that are present in eggshell are regarded to be involved in the mineralization process through their calcium-binding properties (Daengprok et al., 2003). It is of interest to know that chicken eggshell powder also contains Sr (380 mg/g, on average) and this micronutrient may have an anabolic effect on bone metabolism (Reginster et al., 1999). Surprisingly, this precious Ca source is currently considered as a waste product after breaking operations in egg-processing plants. A huge amount of eggshell is disposed of as landfill, giving rise to environmental pollution. However, upon the discovery of the positive effects of eggshell on human health, great demand exists for comprehensive research on improving the edibility and bioavailability of eggshell to be used in food products. In this regard, nanotechnology can be an effective and viable solution, as it has numerous applications in enhancing the biocompatibility of several food ingredients.

Nanotechnology is a new and emerging technique to be used in the food and pharmaceutical industries for promoting physical and biological properties, including solubility and stability. It has the potential to improve the food system in various ways; particularly, it is believed that nanosizing increases the bioavailability of the micro- and micronutrients present in food products. The enhancement of bioavailability of Ca through nanosizing has been reported recently by several studies (Park et al., 2008; Seo et al., 2009; Hilty et al., 2011). An ever-increasing trend exists in the food industry of developing food products supplemented with nanosized nutritional elements. Yogurt is one of the few potential food products in the market that are widely used as vehicles to supplement functional ingredients for mass population.

Yogurt is a dairy product manufactured through the lactic acid fermentation of milk and is popularly consumed throughout the world. It is recognized as a healthy food for containing a large amount of nutri-

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tional elements, including calcium. A well-known fact about calcium is that this mineral is an essential constituent of the human body and a large daily intake is necessary. According to the Institute of Medicine, an individual has to take 1,000 to 1,200 mg of calcium on a daily basis. It is well accepted that dietary calcium intake has never been sufficient and, for that reason, dietary intervention through the supplementation of calcium in foods is suggested to meet the requirement. Generally, the calcium content in commercial yogurt is limited to 332 mg per serving (175 mL). As a consequence, efforts have been made to supplement purified CaCO_3 in yogurt (Ramasubramanian et al., 2008). But the applicability of purified Ca in yogurt is still limited due to having undesirable side effects and low bioavailability. It is recommended that Ca should be taken from biological sources. In this regard, eggshell can be a cheap, easily obtainable but plentiful source of Ca for food applications. However, no report exists in the literature on the production of a nanopowdered eggshell (NPES)-added yogurt. Therefore, this study was carried out to develop health-promoting yogurt with added nanopowdered eggshell and to examine the effects of this addition on the physicochemical and sensory properties of yogurt.

MATERIALS AND METHODS

Materials

Commercial milk was purchased from Seoul Dairy Co. (Seoul, Korea). Nanopowdered and micropowdered eggshells were procured from Apexel Co. (Pohang, Korea). Agar powder was obtained from Showa Chemical Co. (Tokyo, Japan). Difco lactobacilli de Man, Rogosa, and Sharpe (MRS) broth was bought from Becton, Dickinson and Co. (Detroit, MI). All chemicals used in this experiment were of analytical grade.

Particle Size Analysis

Size and morphological characteristics of NPES and powdered eggshell (PES) were observed using field-emission scanning electron microscopy (SEM; S-4300; Hitachi Ltd., Tokyo, Japan). Each sample was spread on the surface of a stub with double-stick carbon tape. After sputtering with white gold for 120, the samples were examined by SEM operated at an accelerating voltage of 15.0 kV. The crystal shapes and sizes of NPES were determined using a transmission electron microscope (JEM-2010; JEOL Ltd., Tokyo, Japan). For transmission electron microscopy study, the sample was prepared on Formvar film-coated grids and was measured with an electron microscope operating at 100 kV accelerating voltage.

For analyzing the particle size distribution, 0.1 g of NPES was dispersed into 10 mL of ethanol. The suspension was then treated in a Branson 3210 ultrasonic system (Triad Scientific Inc., Manasquan, NJ) for 30 min and kept at room temperature for 10 min. After pretreatment, the suspension was poured into a cuvette and the particle size was measured by using a particle size analyzer (DelsaNano C; Beckman Coulter Inc., Fullerton, CA) under specified conditions of 25°C and 160° scattering angle. The particle size distribution of PES was determined by another instrument (Mastersizer 2000; Malvern Instruments Ltd., Worcestershire, UK) at 25°C and 90° scattering angle. Each treatment was done in triplicate.

Preparation of NPES- and PES-Supplemented Yogurts

Yogurts were manufactured following the modified procedure of Seo et al. (2009). Milk was first standardized adding nonfat milk powder (3.7%, wt/vol) and pectin (0.2%, wt/vol; Kanto Chemical, Tokyo, Japan). Three different concentrations (0.15, 0.3, and 0.45%, wt/vol) of nano- and micropowdered eggshells were added to the milk. The milk was then homogenized at 1,200 rpm for 10 min with the laboratory-scale blender (MS3040 electronic overhead stirrer; Tops Misung Scientific Co., Seoul, Korea). The homogenized milk was heat treated at 90°C for 10 min and cooled down to approximately 42 to 43°C. Commercial starter culture (0.002% wt/vol; Sacco srl, Cadorago, Italy) in freeze-dried direct-to-vat set form containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* was added immediately and incubated at 43°C for approximately 6 h. Yogurt samples were kept at 10°C for 24 h for stabilizing. The samples were then stored at 5°C in a refrigerator to evaluate the physicochemical and sensorial properties on d 0, 4, 8, 12, and 16 of storage. Each batch of yogurt making was done in triplicate.

pH

The pH value of each sample was measured with a waterproof digital pen-type pH meter (pH-222; Lutron Electronics Co. Inc., Coopersburg, PA). All samples were measured in triplicate and the results were calculated as the mean value.

Viscosity

The viscosities of the samples (50 mL) were measured at room temperature using a Brookfield viscometer (model LVDV-I+, version 3.0; Brookfield, Stonington,

MA) equipped with spindle no. 3, mixing at 60 rpm for 5 min. The viscosity was recorded every minute and all samples were measured in triplicate.

Lactic Acid Bacteria

The agar media was prepared by combining the powder MRS broth and 0.004% bromophenol blue (Sigma Aldrich, Germany) for lactic acid bacteria (LAB) counting. One milliliter of each yogurt sample was diluted with 9 mL of sterile 0.8% NaCl. Subsequent dilutions of each sample were plated in triplicate and incubated at 37°C for 48 h.

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 ($X = 97.83$, $Y = 81.58$, and $Z = 91.51$). Measured L^* , a^* , and b^* values were used as indicators of lightness, redness, and yellowness, respectively. All samples were measured in triplicate.

Sensory Analysis

The sensory evaluation of control and NPES-supplemented yogurt was performed with 10 judges selected from graduate students of the Department of Food Science and Technology at Sejong University (Seoul, Korea). Three 1-h training sessions were held. At the end of the session, sensory attributes were established: 2 appearance attributes, 5 flavor and taste attributes, and 2 texture attributes. Standards used to define these flavor and taste descriptors were present during training and sensory evaluation.

Before each session, commercial plain yogurt was presented to familiarize the assessors with the sensory

properties of the yogurt. The judges scored each attribute on a scale of 0 to 7, in which 7 was the highest intensity and 0 was none. Samples were stored at 7°C before assessment. In each session, the order of assessment of the sample was randomized. The scores of each sample were averaged over all assessors. The sensory evaluation was performed in duplicate.

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 the significant differences among the samples. Means were compared by using the Fisher least significant difference procedure. Significance was defined at the 5% level.

RESULTS AND DISCUSSION

Particle Size Analysis

The morphology of NPES and PES was observed by SEM as shown in Figure 1. The SEM images demonstrated that the particle size of PES apparently decreased during the manufacture of NPES. The average particle sizes of PES and NPES measured were about 20 to 200 μm by SEM and about 100 to 200 nm in diameter by the particle size analyzer, respectively (Figures 1 and 2).

pH

The changes in pH of yogurts supplemented with various concentrations of NPES are presented in Figure 3. All yogurt samples went through gradual decreases in pH during the storage period. Eggshell-supplemented yogurts exhibited higher pH values than the control.

Figure 1. Scanning electron microscopy micrographs of (A) nanopowdered and (B) powdered eggshells.

Figure 2. Particle size distributions of (A) nanopowdered and (B) powdered eggshells. Color version available in the online PDF.

The pH values in NPES- and PES-supplemented yogurts ranged from 4.31 to 4.66 and 4.23 to 4.53, respectively, whereas in the control yogurt it ranged from 4.15 to 4.44 throughout the storage time. However, pH in NPES-supplemented yogurts remained higher than both PES-supplemented and control yogurts throughout the period. Increasing the concentrations (0.15, 0.3, and 0.45%, wt/vol) of NPES and PES in yogurts resulted in an increase in the pH value. In the study of Pirkul et al. (1997), different concentrations of Ca salts were added to yogurt and it was found that the

Ca-supplemented yogurts had significantly higher pH values than the control yogurt. Furthermore, the higher concentrations of Ca salt in the yogurt resulted in a higher pH value. It turns out that the buffering activity of the Ca salts present in the eggshell probably contributed to the higher values of the PES-supplemented yogurts. Relatively greater pH values in the NPES-supplemented yogurts than the PES-supplemented ones can be attributed to the higher buffering capacity of Ca salts present in NPES obtained from nanosizing. Based on the analysis of the pH values, it can be speculated

Table 1. Changes in lactic acid bacteria¹ (cfu/mL) in nanopowdered eggshell (NPES)- or powdered eggshell (PES)-supplemented yogurt stored at 5°C for 16 d

Concentration of sample (% wt/vol)	Storage period (d)				
	0	4	8	12	16
Control	8.46×10^8 ^a	8.94×10^8 ^a	9.16×10^8 ^a	1.19×10^9 ^a	1.39×10^9 ^a
PES (0.15)	7.30×10^8 ^{bc}	7.80×10^8 ^b	8.14×10^8 ^b	8.82×10^8 ^b	9.12×10^8 ^b
PES (0.30)	7.52×10^8 ^b	7.60×10^8 ^c	7.88×10^8 ^c	8.26×10^8 ^{bc}	8.70×10^8 ^c
PES (0.45)	6.70×10^8 ^d	7.08×10^8 ^d	7.38×10^8 ^d	7.72×10^8 ^c	8.20×10^8 ^{de}
NPES (0.15)	7.46×10^8 ^b	7.48×10^8 ^c	7.78×10^8 ^c	8.10×10^8 ^{bc}	8.56×10^8 ^{cd}
NPES (0.30)	7.20×10^8 ^c	7.54×10^8 ^c	7.82×10^8 ^c	8.00×10^8 ^c	8.20×10^8 ^{de}
NPES (0.45)	6.56×10^8 ^d	6.52×10^8 ^e	7.06×10^8 ^e	7.54×10^8 ^c	8.02×10^8 ^e

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

¹A mixture of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*.

that adding NPES has a substantial effect in increasing the shelf-life of yogurt.

LAB

The changes in *L. bulgaricus*, *S. thermophilus*, and *B. bifidum* counts in NPES- or PES-supplemented yogurt samples stored at 5°C for 16 d are presented in Table 1. On the initial storage day, the mean LAB count of the control was significantly higher ($P < 0.05$) than those of NPES- or PES-supplemented yogurt samples. Moreover, increasing the concentrations of NPES and PES (0.15–0.45%, wt/vol) on d 0 of storage resulted in a reduction in the mean LAB population from 7.30×10^8 to 6.70×10^8 cfu/mL and from 7.46×10^8 to 6.56×10^8 cfu/mL, respectively. This finding can be explained by the fact that eggshell powder, being enriched with a high level of CaCO_3 , had antimicrobial properties. The LAB content in the NPES-supplemented yogurt was a little lower than that of PES-supplemented yogurt. However, on d 16 of storage, the LAB count in all yogurt samples, including the control, was increased. Comparatively lower pH at the later part of the storage could be explained by promoted growth of LAB in the yogurts. Increase in LAB populations in *Dioscorea opposita* Thumb.-supplemented yogurts at the later part of the storage period was also reported by Kim et al. (2011). A recent study revealed that nano-calcium had negative effects on bacterial growth (Ataee et al., 2011). The antimicrobial effects of NPES supplementation were found to restrict the growth of LAB in yogurts in the current study. However, LAB populations were not noticeably diminished in the NPES-supplemented yogurts. Therefore, it can be concluded that NPES-supplemented yogurt could have a prolonged shelf-life beyond 16 d.

Viscosity

The effect of NPES addition on the viscosity of yogurt during 16 d of storage is shown in Figure 4. On the

initial day, the viscosities of the yogurts were increased when the concentration of NPES was increased. The PES-supplemented yogurts also had the same trend but the viscosities of the NPES-supplemented yogurts were relatively higher than their counterparts. On d 4 of storage, viscosities in all yogurt samples were slightly increased. Sahan et al. (2008) reported that due to the rearrangement of the protein molecules, the viscosity of yogurt might increase during storage. However, on the final day of storage, the viscosities of all samples, including the control, had decreased considerably. The decrease in viscosity might have been caused by the whey separation with increasing storage time. Noticeably, throughout the entire storage time, the viscosities in the NPES-supplemented yogurts remained higher than the yogurts supplemented with PES.

The additions of NPES or PES caused the enrichment of CaCO_3 in yogurts, and higher concentrations of these 2 substances resulted in higher proportions of CaCO_3 in the yogurt samples. The increased CaCO_3 content increased the formation of calcium phosphate crosslinks within casein micelles. Singh and Muthukumarappan (2008) reported that Ca-enriched fruit yogurts were shown to have stronger structures. The firmness of Ca-enriched yogurts has been attributed to the colloidal calcium phosphate crosslinking between casein micelles caused by the increased Ca content. The result of the current study is in accordance with the findings of Singh and Muthukumarappan (2008). Higher viscosities in the NPES-supplemented yogurts can be explained by the fact that the finer particles of NPES allowed it to disperse well in yogurt and led to more calcium phosphate crosslinking in casein micelles to elevate the viscosities.

Color Analysis

The changes in color in the NPES- and PES-supplemented yogurts stored at 5°C for 16 d are presented in Table 2. The L^* values were not significantly affected

Figure 3. Changes in the pH of yogurts supplemented with nanopowdered and powdered eggshells during 16 d of storage at 5°C. At d 0, the yogurts had been stabilized for 24 h. PES = powdered eggshells; NPES = nanopowdered eggshells.

by the addition of NPES and PES on the initial day ($P < 0.05$). Only the yogurt sample containing PES (0.15%, wt/vol) had an exceptionally lower L^* value than the others. The L^* values in yogurt samples, including the NPES-supplemented ones, were considerably increased during the 16 d of storage. The greatest change was noticed in PES (0.15%, wt/vol), where the L^* value increased from 86.90 to 89.03. No noticeable changes were observed in the a^* and b^* values of

Figure 4. Changes in the viscosities of the yogurts supplemented with nanopowdered eggshells during 16 d of storage at 5°C. PES = powdered eggshells; NPES = nanopowdered eggshells.

NPES-supplemented and other yogurt samples, except in PES (0.15%, wt/vol)-supplemented yogurt, where it was found that the a^* and b^* values increased from 7.20 to 7.66 and 9.64 to 10.66, respectively.

Sensory Evaluation

The sensory properties of NPES- and PES-supplemented yogurts during 16 d of storage are shown in Table 3. Appearance, taste, flavor, and texture were

Table 2. Changes of color in nanopowdered eggshell (NPES)- or powdered eggshell (PES)-supplemented yogurt stored at 5°C for 16 d

Color ¹	Concentration of sample (% wt/vol)	Storage period (d)				
		0	4	8	12	16
L^*	Control	87.92 ^b	87.90 ^b	89.09 ^c	90.42 ^a	89.41 ^b
	PES(0.15)	86.90 ^d	87.91 ^b	88.97 ^c	88.82 ^b	89.03 ^c
	PES (0.30)	88.49 ^a	88.83 ^a	89.80 ^a	89.07 ^c	89.86 ^a
	PES (0.45)	87.89 ^b	88.02 ^b	89.35 ^b	89.33 ^b	89.66 ^{ab}
	NPES(0.15)	88.02 ^b	88.71 ^a	89.44 ^b	89.05 ^b	89.72 ^a
	NPES (0.30)	87.67 ^c	88.03 ^b	88.76 ^d	89.92 ^a	89.76 ^a
	NPES (0.45)	87.93 ^b	88.76 ^a	89.04 ^c	90.36 ^a	89.63 ^{ab}
a^*	Control	7.76 ^a	7.57 ^a	7.63 ^{ab}	7.47 ^a	7.72 ^{ab}
	PES (0.15)	7.20 ^d	7.56 ^a	7.68 ^a	7.73 ^b	7.66 ^{ab}
	PES (0.30)	7.50 ^b	7.54 ^{ab}	7.49 ^c	7.53 ^b	7.65 ^{ab}
	PES (0.45)	7.49 ^b	7.43 ^c	7.54 ^{bc}	7.58 ^b	7.68 ^{ab}
	NPES (0.15)	7.44 ^b	7.58 ^a	7.57 ^{bc}	7.47 ^b	7.73 ^a
	NPES (0.30)	7.31 ^c	7.47 ^{bc}	7.37 ^d	7.46 ^b	7.68 ^{ab}
	NPES (0.45)	7.41 ^b	7.62 ^a	7.26 ^e	7.44 ^b	7.62 ^b
b^*	Control	10.99 ^a	10.29 ^{cd}	10.83 ^{bc}	10.21 ^b	10.80 ^{bc}
	PES (0.15)	9.64 ^c	10.69 ^{bcd}	11.19 ^a	11.68 ^a	10.66 ^c
	PES (0.30)	11.08 ^a	11.00 ^{abc}	11.08 ^{ab}	10.46 ^b	11.27 ^a
	PES (0.45)	10.72 ^{ab}	10.45 ^{bcd}	11.08 ^{ab}	10.39 ^b	11.12 ^{ab}
	NPES (0.15)	10.84 ^a	11.45 ^{ab}	11.34 ^a	10.88 ^b	11.42 ^a
	NPES (0.30)	10.41 ^b	9.91 ^d	10.53 ^c	10.60 ^b	11.49 ^a
	NPES (0.45)	10.72 ^{ab}	11.87 ^a	10.53 ^c	10.69 ^b	11.35 ^a

^{a-e}Values with different superscript letters within a column are significantly different ($P < 0.05$).
¹ L^* , a^* , and b^* values are indicators of lightness, redness, and yellowness, respectively.

Table 3. Sensory characteristics¹ for nanopowdered eggshell (NPES)- or powdered eggshell (PES)-supplemented yogurt stored at 5°C for 16 d

Concentration of sample (% wt/vol)	Appearance		Flavor			Taste		Texture	
	Whey-off	Color	Earthy	Rancid	Sourness	Astringency	Chalky	Graininess	Firmness
0-d storage period									
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.0 ^b	4.0 ^{ab}	4.0 ^c	1.0 ^c	4.0 ^b	4.0 ^b
PES (0.15)	4.1 ^a	4.1 ^a	1.3 ^{ab}	1.1 ^{ab}	4.1 ^a	4.2 ^{bc}	1.1 ^c	4.3 ^{ab}	4.2 ^{ab}
PES (0.30)	4.4 ^a	3.6 ^{ab}	1.4 ^{ab}	1.0 ^b	4.0 ^{ab}	4.3 ^{ab}	2.0 ^b	4.4 ^a	4.3 ^a
PES (0.45)	4.5 ^a	3.6 ^{ab}	1.6 ^{ab}	1.2 ^{ab}	3.6 ^{bc}	4.4 ^{ab}	2.4 ^a	4.2 ^{ab}	4.3 ^a
NPES(0.15)	4.3 ^a	3.6 ^{ab}	1.4 ^{ab}	1.4 ^a	3.7 ^{bc}	4.2 ^{bc}	1.0 ^c	4.4 ^a	4.1 ^{ab}
NPES (0.30)	4.6 ^a	3.5 ^{ab}	1.4 ^{ab}	1.3 ^{ab}	3.5 ^c	4.2 ^{bc}	1.1 ^c	4.4 ^a	4.2 ^{ab}
NPES (0.45)	4.6 ^a	3.4 ^b	1.8 ^a	1.0 ^b	3.4 ^c	4.5 ^c	1.2 ^c	4.5 ^a	4.2 ^{ab}
4-d storage period									
Control	4.0 ^a	4.0 ^a	1.0 ^b	1.0 ^b	4.0 ^a	4.0 ^c	1.0 ^c	4.0 ^c	4.0 ^d
PES (0.15)	4.1 ^a	3.9 ^{ab}	1.1 ^{ab}	1.2 ^{ab}	3.9 ^{ab}	4.2 ^{bc}	1.2 ^c	4.2 ^{bc}	4.2 ^{cd}
PES (0.30)	4.3 ^a	3.7 ^{abc}	1.3 ^{ab}	1.3 ^a	3.9 ^{ab}	4.3 ^{ab}	2.1 ^b	4.2 ^{bc}	4.3 ^{cd}
PES (0.45)	4.4 ^a	3.6 ^{bc}	1.4 ^{ab}	1.4 ^a	3.8 ^{ab}	4.5 ^{ab}	2.6 ^a	4.2 ^{bc}	4.4 ^{bc}
NPES (0.15)	4.4 ^a	3.7 ^{abc}	1.2 ^{ab}	1.2 ^{ab}	3.7 ^{ab}	4.3 ^{abc}	1.0 ^c	4.4 ^{ab}	4.6 ^{ab}
NPES (0.30)	4.4 ^a	3.5 ^c	1.2 ^{ab}	1.2 ^{ab}	3.7 ^{ab}	4.3 ^{ab}	1.2 ^c	4.5 ^{ab}	4.6 ^{ab}
NPES (0.45)	4.5 ^a	3.5 ^c	1.6 ^b	1.4 ^{ab}	3.6 ^b	4.6 ^a	1.3 ^c	4.6 ^a	4.7 ^a
8-d storage period									
Control	4.0 ^a	4.0 ^{ab}	1.0 ^b	1.0 ^b	4.0 ^a	4.0 ^c	1.0 ^c	4.0 ^b	4.0 ^c
PES (0.15)	4.3 ^a	3.7 ^{bc}	1.2 ^{ab}	1.3 ^a	3.8 ^{ab}	4.3 ^{bc}	1.3 ^c	4.3 ^{ab}	4.3 ^{bc}
PES (0.30)	4.6 ^a	4.2 ^a	1.3 ^{ab}	1.3 ^a	3.7 ^{abc}	4.5 ^{ab}	1.9 ^b	4.3 ^{ab}	4.6 ^{ab}
PES (0.45)	4.4 ^a	3.6 ^{cd}	1.3 ^{ab}	1.3 ^a	3.6 ^{bcd}	4.6 ^a	2.3 ^a	4.4 ^a	4.7 ^a
NPES (0.15)	4.1 ^a	3.6 ^{cd}	1.4 ^{ab}	1.2 ^{ab}	3.6 ^{bcd}	4.2 ^{bc}	1.0 ^c	4.4 ^a	4.4 ^{ab}
NPES (0.30)	4.2 ^a	3.5 ^{cd}	1.2 ^{ab}	1.2 ^{ab}	3.4 ^{cd}	4.3 ^{bc}	1.3 ^c	4.5 ^a	4.5 ^{ab}
NPES (0.45)	4.4 ^a	3.4 ^d	1.3 ^a	1.2 ^{ab}	3.3 ^d	4.4 ^{ab}	1.4 ^c	4.4 ^a	4.5 ^{ab}
12-d storage period									
Control	4.0 ^a	4.0 ^a	1.0 ^b	1.0 ^b	4.2 ^a	3.8 ^d	1.0 ^c	3.8 ^a	3.8 ^c
PES (0.15)	4.1 ^a	3.7 ^{abc}	1.2 ^{ab}	1.3 ^{ab}	3.8 ^{ab}	4.2 ^c	1.3 ^c	4.1 ^b	4.1 ^b
PES (0.3)	4.4 ^a	3.7 ^{ab}	1.3 ^{ab}	1.4 ^{ab}	3.7 ^b	4.6 ^{ab}	1.7 ^b	4.1 ^b	4.3 ^{ab}
PES (0.45)	4.4 ^a	3.5 ^{bc}	1.4 ^a	1.5 ^a	3.6 ^b	4.7 ^a	2.1 ^a	4.1 ^b	4.5 ^a
NPES (0.15)	4.0 ^a	3.5 ^{bc}	1.3 ^a	1.3 ^{ab}	3.5 ^b	4.2 ^{bc}	1.0 ^c	4.3 ^b	4.3 ^{ab}
NPES (0.3)	4.3 ^a	3.6 ^{bc}	1.1 ^{ab}	1.3 ^{ab}	3.4 ^b	4.3 ^{abc}	1.2 ^c	4.3 ^b	4.4 ^{ab}
NPES (0.45)	4.3 ^a	3.3 ^c	1.2 ^{ab}	1.5 ^{ab}	3.3 ^b	4.5 ^{abc}	1.2 ^c	4.4 ^b	4.3 ^{ab}
16-d storage period									
Control	4.2 ^a	4.0 ^a	1.0 ^b	1.0 ^b	4.1 ^a	4.1 ^b	1.0 ^c	4.0 ^b	3.8 ^b
PES (0.15)	4.4 ^a	3.8 ^{ab}	1.3 ^{ab}	1.3 ^{ab}	3.8 ^{ab}	4.4 ^{ab}	1.2 ^c	4.3 ^{ab}	4.1 ^a
PES (0.3)	4.5 ^a	3.6 ^{abc}	1.3 ^{ab}	1.3 ^{ab}	3.8 ^{ab}	4.6 ^a	1.8 ^b	4.3 ^{ab}	4.2 ^a
PES (0.45)	4.6 ^a	3.5 ^{bc}	1.3 ^{ab}	1.5 ^a	3.7 ^{bc}	4.7 ^a	2.2 ^a	4.3 ^{ab}	4.3 ^a
NPES (0.15)	4.3 ^a	3.4 ^{bc}	1.4 ^a	1.2 ^{ab}	3.6 ^{bc}	4.3 ^{ab}	1.0 ^c	4.5 ^a	4.3 ^a
NPES (0.3)	4.2 ^a	3.3 ^c	1.2 ^{ab}	1.2 ^{ab}	3.5 ^{bc}	4.3 ^{ab}	1.2 ^c	4.5 ^a	4.3 ^a
NPES (0.45)	4.2 ^a	3.2 ^c	1.3 ^{ab}	1.3 ^{ab}	3.4 ^c	4.5 ^{ab}	1.3 ^c	4.6 ^a	4.4 ^a

^{a-d}Values with different superscript letters within a column are significantly different ($P < 0.05$).

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

analyzed during the storage period. The whey-off score did not significantly change during the storage time ($P > 0.05$). On the initial day, the b* score decreased with the increasing concentration of NPES and PES (0.15, 0.3, and 0.45%, wt/vol), due to the dark white color of the eggshell powder. Rancidity was not increased in the NPES- and PES-supplemented yogurts; however, the earthy flavor only was significantly increased in the yogurts supplemented with NPES and PES ($P < 0.05$). The high amount of Ca in the eggshell powder might have added the earthy flavor in the yogurts. Sourness was decreased by the addition of NPES and PES. The astringency score was increased in both NPES- and PES-supplemented yogurts. The most distinguishable attribute of the eggshell powder was chalkiness, which

was found considerably higher in the yogurts supplemented with PES. The chalkiness in the NPES-supplemented yogurts on the other hand was not significant ($P > 0.05$), mostly because of the fine granular sizes of the NPES. The ultra-fine granules of NPES in the yogurts were not strongly felt by the panelists, except for a little chalky taste (0.45% NPES). Both graininess and firmness were found to increase in the NPES- and PES-supplemented yogurts. It was assumed that NPES and PES, being solid particles, were responsible for giving the yogurts grainier and firmer texture. Singh and Muthukumarappan (2008) reported that the fruit yogurts enriched with Ca were not different from the control in terms of appearance, flavor, texture, and overall acceptability. Based on the data obtained from in the

current study, we suggest that NPES (0.15 and 0.3%, wt/vol) can be added to produce NPES-supplemented yogurt.

CONCLUSIONS

This study was undertaken to develop NPES-supplemented yogurt and to see the changes in the physicochemical, microbial, and sensory properties of yogurts upon the addition of NPES during storage at 5°C for 16 d. The resulting data on pH, viscosity, LAB, color, and sensory evaluation from this study suggested that the supplementation of NPES did not show any adverse effect on the yogurt quality. Therefore, we conclude that NPES (0.15 and 0.3%, wt/vol) could be applicable to the manufacture of functional yogurt.

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