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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 beneficial 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-op. (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,