

Physicochemical and Sensory Properties of Nanopowdered Chitosan-Added Maribo Cheese during Ripening

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Abstract

Nanopowdered chitosan (NPC) has high biological activities, such as blood cholesterol lowering effect and antidiabetic activity. This study is carried out to determine the effects of nano-powdered chitosan-added Maribo cheese (NMC) for the physicochemical properties and sensory analysis during its ripening at 14°C for 6 mon. From the results, the moisture and fat levels are not significantly influenced from the addition of chitosan ($p>0.05$), but ash contents increased with increasing chitosan concentrations and the protein contents decreased with increasing chitosan concentrations. In the short-chain fatty acids analysis during the ripening, the total production is initially 13.79 ppm in 0.2% NMC and 13.81 ppm in control, and their levels have steadily increased to 59.94 and 53.11 ppm, respectively. For the color levels, the L^* values decreased, while the a^* and b^* values significantly increased during ripening for all samples ($p<0.05$). In texture analysis, the hardness and gumminess of NMC significantly decreased as compared to the control during ripening ($p<0.05$), while the cohesiveness, springiness and chewiness were not significantly different among the treatments ($p>0.05$). In sensory analysis, the butyric off-flavor and bitterness increased slightly with increasing concentrations of NMC during ripening. The overall acceptability of 0.2% NMC held the highest score amongst the samples during the ripening. From the results obtained, the 0.2% NMC was preferred during the ripening and observed the possibility of functional cheese.

Key words: maribo cheese, nanopowdered chitosan, ripening

Introduction

Chitosan, the main derivatives of chitin, is a linear aminopolysaccharide composed primarily of repeating unit of β -(14)-linked-N-acetylglucosamine. Various functions of chitosan, such as cholesterol lowering (Gallaher *et al.* 2000), antitumor (Qin *et al.* 2002), immuno-enhancing, antidiabetic (Hayashi and Ito, 2001), antihypertensive, wound healing, antifungal, and antimicrobial activity, have been reported in numerous studies. However, when chitosan is applied in the food industry, there has been difficulty in dissolving it in water. To increase solubility of chitosan, it has to be modified by chemical techniques. However, chemical modification can cause losing original physicochemical and biochemical properties (Sugimoto *et al.* 1998). Therefore, nanotechnology may be one of the solutions for this problem.

Nano-sized substances could increase solubility and bioavailability due to the extremely increased surface area. (Chaudhry *et al.* 2008, Park *et al.* 2007). To find the effect of hypoglycemic, nanopowdered chitosan (562 nm) was studied in which 2% NPC-treated group had 79% higher secretion in insulin than that of commercial powdered chitosan (Seo *et al.*, 2010). In addition, chitosan nanopowder had better hypolipidemic effect than ordinary chitosan in rats (Zhang *et al.*, 2013). Total cholesterol lowering effect from nanochitosan in rat was conducted in which NPC was reduced by 46.6% and PC by 18.6% (Park *et al.*, 2010). NPC as a functional ingredient was applied in milk that could disperse during storage period using by ascorbic acid as a stabilizer and fortifier of vitamin C (Seo *et al.*, 2011). To explore antibacteriocidal action, a nanochitosan study was also conducted to observe this action using lactic acid bacteria in yogurt. The result of this study showed that NPC-added yogurt contained lactic acid bacteria from 4.75×10^8 to 9.70×10^8 CFU/mL during storage at 4°C for 20 d, which was not significantly different from control ($p>0.05$) (Seo *et al.*, 2009).

Maribo cheese is one of the representative semi-hard

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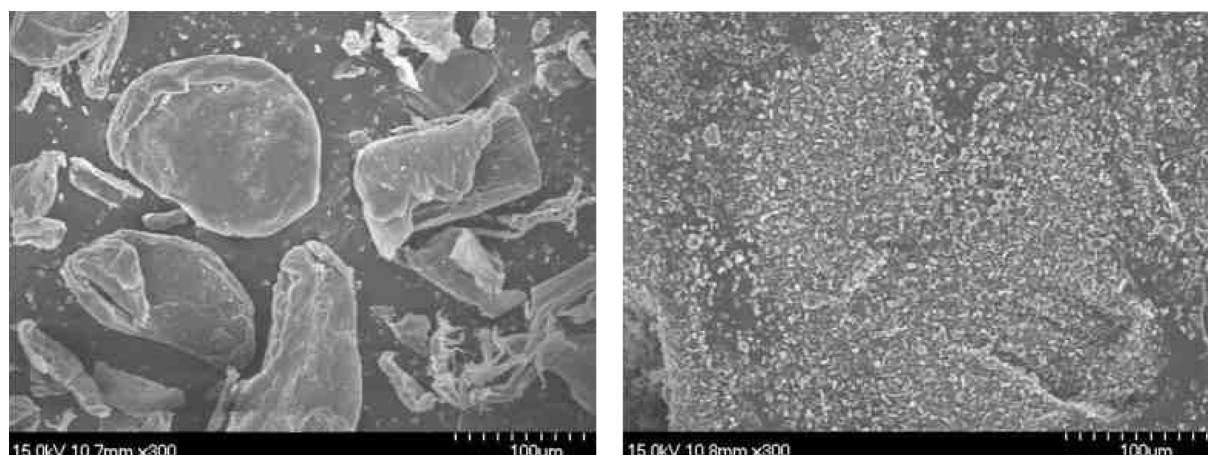


Fig. 1. Scanning electron microscope images for powdered chitosan and nanopowdered chitosan.

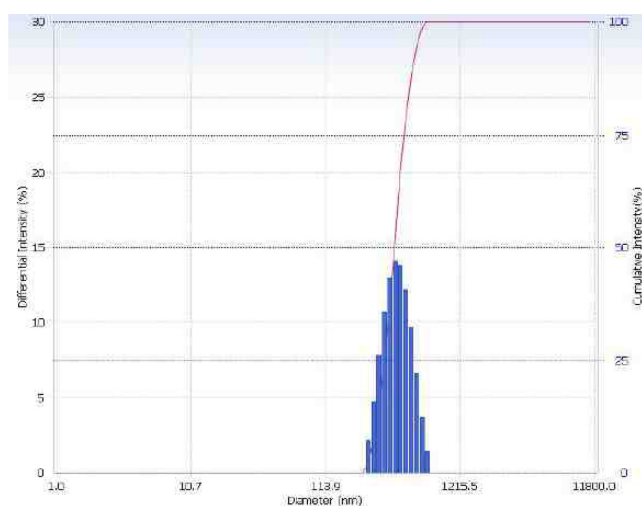


Fig. 2. Particle size of nanopowdered chitosan.

cheeses which has been made mainly in Denmark, and it resembles the various Latin America cheeses, such as chanco and paipa in Colombia and prato in Brazil (Brito *et al.*, 2000). Since nanotechnology is quite new area in food science, there are almost no reports in the literature on the production of NPC-added Maribo cheese. Therefore, the objective of this study was focused on the physicochemical and sensory properties of cheese added with NPC during ripening.

Materials and Methods

Materials

Raw milk to manufacture Maribo cheese was obtained from an animal farm affiliated with Sunchon National University (Sunchon, Korea). Powdered chitosan was obtained from Samsung Chitopia (Seoul, Korea) and milled

to NPC by dry milling method in Apexel Co. (Pohang, Korea). The average particle sizes of PC 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 (Fig. 1 and 2) (Seo *et al.*, 2009).

Manufacture of NCMC

Maribo cheese was prepared as described previously (Robinson, 1995) with some modification. Different concentrations of NPC (0.2 and 0.5%) were added at the curd after second whey drainage. After manufacturing, pressed cheese were dried for 2 d and ripened at 14°C with a relative humidity of 90-95% for 6 mon.

Analysis of chemical composition and yield of cheese

Cheese was analyzed for moisture, fat, and protein using methods of Association of Official Analytical Chemists (AOAC, 2000). Cheese yield was determined as wt. cheese ×100/wt. milk.

Analysis of short-chain fatty acids

Cheese samples (1 g) were taken periodically, stored for 0, 1, 2, 3, 4, 5, and 6 mon, extracted with diethyl ether and hexane for 2 h, and eluted through a 10 mm inner diameter glass column containing neutral alumina as described by Kwak *et al.* (1990). A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector was used. The quantitation of short-chain fatty acid (SCFA) was achieved using a 15 m × 0.53 mm inner diameter Nukol fused-silica capillary column (Supleco Inc., Bellefonte, PA, USA). The GC was operated with nitrogen carrier gas at 30 mL/min, hydrogen gas 30 mL/min, and air at 300 mL/min. The column oven was programmed at the

initial holding for 1min at 60°C and the first level holding to 170°C at 5°C/min for 10 min and holding for 20 min. Both temperatures for injector and detector were 220°C. Each FFA was identified by the retention time of standard.

Color measurements

Color values of control and NCMC were measured using a colorimeter (Minolta CT-310, Tokyo, Japan) after calibrating its original value with a standard plate. Measured L*, a*, and b* values were used as indicators of lightness, redness, and yellowness, respectively. All samples were measured ten times.

Texture profile analysis

Texture profile was measured using a TMS-Pro Texture Analyzer (Food Technology Co., VA, USA) with a cross-head of 5 cm/min and a chart recorder speed of 10 cm/min. Cube-shaped samples (1.5 cm³) were prepared. The basic characteristics, such as hardness (N), cohesiveness (J³), gumminess (N), springiness (m×10⁻³), and chewiness (J×10⁻³), were obtained.

Sensory attribute

Sensory evaluation was conducted by ten-trained sensory panelists. Flavor, taste, and texture were investigated on a 7-point scale (1 = very weak, 4 = moderate and 7 = very strong). Overall acceptability was also evaluated on a 7-point scale (1 = dislike very much, 4 = neither like nor dislike and 7 = like very much). Samples were presented in randomly coded glass plates.

Statistical analysis

All statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., USA). Analysis of variance 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

Chemical composition

The composition of NCMC is shown in Table 1. Moisture contents were not significantly different among all treatments with 36.95% in control cheese ($p>0.05$). However, protein decreased significantly with increasing added chitosans from 24.25% in control to 22.91% in 0.5% NCMC ($p<0.05$). Fat contents were not significantly affected from the addition of chitosan ($p>0.05$). Ash contents increased with increasing chitosan concentrations of PCMC and NCMC. 0.5% NCMC had the highest ash contents among the samples (4.00%). Addition of nanochitosan may contribute to decreasing of nitrogen contents and increasing of ash contents. Cichoscki *et al.* (2002) reported that chemical composition, such as protein, fat, and ash of Prato cheese which is one of Brazilian semi-hard cheeses wasn't significantly different during 60 d ripening.

SCFA

The production of SCFAs (C₄ through C₁₀) is important in cheese ripening (Lin and Jeon, 1987). They contribute indirectly to cheese flavor by acting as precursors for the production of volatile flavor compounds (Mcsweeney, 2003). The production of SCFA in NCMC during 6 mon ripening at 14°C was shown in Table 2. SCFAs in NCMC and PCMC were increased during ripening. Release of butyric acid (C₄) contributed to the increase in total amount of SCFAs until 1 mon. After that, capric acid (C₁₀) influenced the increase of total SCFAs. C₄ of all treatments was increased slightly during 3 mon ripening with no significant difference ($p>0.05$). At 4 mon, C₄ of all the samples was dramatically increased by almost 2 times. After 4 mon, C₄ of NCMC and PCMC was observed to have significantly more production than that of control ($p<0.05$). After the ripening, 0.2% NCMC and

Table 1. Composition of nanopowdered chitosan-added Maribo cheese

Treatment (% w/w)	Moisture	Protein	Fat	Ash
Control	36.95±0.6 ^A	24.25±0.3 ^{AB}	35.27±0.3 ^{BC}	3.33±0.04 ^D
0.2 PCMC	36.22±1.1 ^A	24.25±0.5 ^A	36.88±0.6 ^A	3.57±0.02 ^C
0.5PCMC	36.01±0.3 ^A	23.03±0.3 ^{BC}	33.97±0.5 ^C	3.60±0.00 ^C
0.2NCMC	36.21±0.6 ^A	23.31±0.5 ^{ABC}	35.62±0.5 ^{AB}	3.74±0.04 ^B
0.5NCMC	36.88±0.4 ^A	22.91±0.2 ^C	35.34±0.3 ^{BC}	4.00±0.00 ^A

Means within a column with different letters are significantly different at $p<0.05$

Control: untreated Maribo cheese.

PCMC: powdered chitosan-added Maribo cheese.

NCMC: nanopowdered chitosan-added Maribo cheese.

Table 2. Production of short-chain fatty acid in nanopowdered chitosan-added Maribo cheese during ripening at 14°C for 6 mon

Ripening period (mon)	Treatment (% w/w)	SCFA concentration (ppm)				Total
		C ₄	C ₆	C ₈	C ₁₀	
0	Control	5.36±0.12 ^A	2.36±0.18 ^A	2.85±0.01 ^A	3.24±0.25 ^A	13.81
	0.2 PCMC	5.55±0.11 ^A	2.40±0.23 ^A	2.81±0.03 ^A	3.31±0.19 ^A	14.07
	0.5 PCMC	5.39±0.04 ^A	2.49±0.14 ^A	2.82±0.06 ^A	3.28±0.34 ^A	13.98
	0.2 NCMC	5.27±0.13 ^A	2.74±0.13 ^A	2.43±0.35 ^A	3.35±0.36 ^A	13.79
	0.5 NCMC	5.48±0.34 ^A	2.56±0.14 ^A	2.76±0.11 ^A	3.30±0.13 ^A	14.10
1	Control	5.76±0.09 ^A	2.70±0.02 ^A	3.02±0.05 ^B	4.28±0.04 ^A	15.76
	0.2 PCMC	5.80±0.08 ^A	2.74±0.16 ^A	3.15±0.05 ^A	4.58±0.04 ^A	16.27
	0.5 PCMC	5.84±0.05 ^A	2.78±0.18 ^A	3.10±0.01 ^{AB}	4.61±0.16 ^A	16.33
	0.2 NCMC	5.70±0.03 ^A	2.77±0.09 ^A	3.03±0.05 ^{AB}	4.52±0.23 ^A	16.02
	0.5 NCMC	5.81±0.05 ^A	2.73±0.20 ^A	3.13±0.04 ^{AB}	4.56±0.09 ^A	16.23
2	Control	6.01±0.08 ^A	3.11±0.08 ^A	3.69±0.09 ^A	10.57±0.08 ^B	23.38
	0.2 PCMC	5.87±0.06 ^A	3.16±0.06 ^A	3.71±0.17 ^A	12.56±0.20 ^A	25.30
	0.5 PCMC	5.91±0.04 ^A	3.15±0.03 ^A	3.65±0.13 ^A	12.72±0.23 ^A	25.43
	0.2 NCMC	5.94±0.06 ^A	3.15±0.09 ^A	3.74±0.08 ^A	12.65±0.13 ^A	25.48
	0.5 NCMC	5.93±0.05 ^A	3.17±0.03 ^A	3.76±0.13 ^A	12.68±0.11 ^A	25.54
3	Control	6.69±0.64 ^{AB}	3.30±0.08 ^A	5.70±0.25 ^A	16.49±1.31 ^A	32.18
	0.2 PCMC	5.83±0.37 ^B	3.25±0.08 ^A	5.61±0.34 ^A	17.70±1.23 ^A	32.39
	0.5 PCMC	6.26±0.06 ^{AB}	3.55±0.10 ^A	5.70±0.13 ^A	17.57±0.68 ^A	33.08
	0.2 NCMC	7.16±0.47 ^{AB}	3.39±0.16 ^A	5.82±0.25 ^A	17.83±1.18 ^A	34.20
	0.5 NCMC	7.99±1.29 ^A	3.57±0.23 ^A	5.80±0.13 ^A	17.19±1.05 ^A	34.55
4	Control	11.09±1.24 ^B	4.22±0.08 ^C	5.89±0.04 ^A	18.14±0.11 ^C	44.98
	0.2 PCMC	13.79±0.07 ^A	4.56±0.08 ^A	5.74±0.06 ^B	18.21±0.06 ^C	42.30
	0.5 PCMC	14.52±0.65 ^A	4.49±0.10 ^{AB}	5.86±0.06 ^{AB}	18.94±0.35 ^{AB}	43.81
	0.2 NCMC	13.38±0.37 ^A	4.28±0.16 ^{BC}	5.95±0.03 ^A	18.40±0.31 ^{BC}	42.01
	0.5 NCMC	13.86±1.12 ^A	4.50±0.02 ^{AB}	5.92±0.04 ^A	19.27±0.08 ^A	43.55
5	Control	13.48±0.30 ^B	4.88±0.10 ^A	6.11±0.08 ^A	20.51±0.04 ^A	44.98
	0.2 PCMC	16.61±0.97 ^{AB}	4.92±0.06 ^A	6.23±0.06 ^A	21.62±0.11 ^A	49.38
	0.5 PCMC	17.19±0.67 ^A	5.00±0.02 ^A	6.32±0.35 ^A	21.38±0.74 ^A	49.89
	0.2 NCMC	16.16±0.67 ^A	4.93±0.09 ^A	6.10±0.01 ^A	20.68±0.04 ^A	47.87
	0.5 NCMC	16.52±1.55 ^A	5.00±0.16 ^A	6.23±0.11 ^A	21.15±0.78 ^A	48.90
6	Control	15.18±1.01 ^B	6.88±0.11 ^A	7.08±0.23 ^A	25.13±0.25 ^A	53.11
	0.2 PCMC	18.09±0.76 ^{AB}	6.95±0.13 ^A	7.14±0.06 ^A	25.15±2.35 ^A	57.33
	0.5 PCMC	21.97±0.92 ^A	7.08±0.13 ^A	7.17±0.04 ^A	27.38±2.64 ^A	62.78
	0.2 NCMC	19.34±2.61 ^{AB}	7.09±0.30 ^A	7.16±0.01 ^A	27.85±2.76 ^A	59.94
	0.5 NCMC	20.32±2.39 ^A	7.21±0.27 ^A	7.20±0.02 ^A	26.96±2.78 ^A	60.69

Means within a column with different letters are significantly different at $p < 0.05$

Control: untreated Maribo cheese.

PCMC: powdered chitosan-added Maribo cheese.

NCMC: nanopowdered chitosan-added Maribo cheese.

control revealed 19.34 and 15.18 ppm in C₄, respectively. C₁₀ was also increased during the ripening periods. In 0 and 1 mon, C₁₀ did not show difference among the samples. At 2 mon, C₁₀ of all the samples was considerably increased by more than 2 times. NCMC and PCMC had more C₁₀ contents compared to control, while the production of all samples was not significantly different ($p > 0.05$). However, addition of chitosan was not significantly influential in increasing caproic acid (C₆) and caprylic acid (C₈) during ripening period ($p > 0.05$). Both NCMC and PCMC had more C₆ and C₈ contents than those of

control. For total SCFAs, there was no big difference among the samples at 0 mon, however, 0.2% NCMC was increased from 13.79 to 59.94 ppm and control was increased from 13.81 to 53.11 ppm during the ripening. Compared to initial, NCMC had higher SCFA concentration than control during the ripening.

Color

Color of NCMC ripened at 14°C for 6 mon was shown in Table 3. L* value was significantly decreased in all samples during ripening ($p < 0.05$). 0.5% NCMC had the

Table 3. Changes in color of nanopowdered chitosan-added Maribo cheese during ripening at 14°C for 6 mon

Color value	Treatment (% w/w)	Ripening period (mon)						
		0	1	2	3	4	5	6
L*	Control	85.74±0.95 ^{Aa}	82.95±0.60 ^{Ab}	81.87±0.65 ^{Ac}	81.40±0.94 ^{Ac}	80.04±1.36 ^{Bd}	79.25±1.80 ^{Ad}	77.20±0.93 ^{Bc}
	0.2PCMC	85.86±0.38 ^{Aa}	82.81±0.60 ^{Ab}	82.30±0.60 ^{Ab}	81.49±1.07 ^{Ac}	81.26±0.89 ^{Ac}	79.98±1.05 ^{Ad}	78.47±0.73 ^{Ae}
	0.5 PCMC	85.95±0.53 ^{Aa}	82.97±0.73 ^{Ab}	82.06±0.97 ^{Ac}	81.46±0.49 ^{Ac}	80.92±1.06 ^{ABd}	79.91±1.79 ^{Ae}	77.31±0.49 ^{Bf}
	0.2 NCMC	86.06±0.67 ^{Aa}	82.98±0.55 ^{Ab}	82.25±0.41 ^{Abc}	81.41±1.71 ^{Ac}	81.04±0.84 ^{ABd}	79.65±0.85 ^{Ae}	77.49±1.14 ^{Bf}
	0.5 NCMC	85.92±0.48 ^{Aa}	82.78±0.54 ^{Ab}	80.76±0.56 ^{Bc}	80.64±0.79 ^{Ac}	80.31±1.28 ^{ABc}	78.25±0.50 ^{Ad}	75.86±0.57 ^{Ce}
a*	Control	2.99±0.17 ^{Ae}	3.07±0.02 ^{Ad}	3.12±0.02 ^{ABcd}	3.19±0.04 ^{Abc}	3.19±0.04 ^{Abc}	3.22±0.10 ^{Ab}	3.58±0.05 ^{Aa}
	0.2PCMC	2.93±0.10 ^{ABd}	3.09±0.05 ^{Ac}	3.14±0.07 ^{ABbc}	3.14±0.10 ^{Abc}	3.15±0.02 ^{ABbc}	3.20±0.14 ^{Ab}	3.41±0.09 ^{Ba}
	0.5 PCMC	2.92±0.09 ^{ABd}	3.61±0.05 ^{Ac}	3.15±0.06 ^{Abc}	3.14±0.05 ^{Ac}	3.15±0.15 ^{ABbc}	3.25±0.21 ^{Ab}	3.39±0.04 ^{BCa}
	0.2 NCMC	2.86±0.04 ^{Bc}	3.00±0.10 ^{Bb}	3.07±0.09 ^{BCb}	3.09±0.04 ^{Bb}	3.10±0.02 ^{Bb}	3.21±0.12 ^{Aa}	3.27±0.21 ^{BCa}
	0.5 NCMC	2.57±0.13 ^{Ce}	2.84±0.03 ^{Cd}	3.03±0.11 ^{Cc}	3.05±0.02 ^{Bc}	3.09±0.08 ^{Bbc}	3.19±0.15 ^{AAb}	3.26±0.24 ^{Ca}
b*	Control	10.93±0.33 ^{Ad}	12.06±0.11 ^{ABc}	12.62±0.22 ^{Bb}	12.69±0.23 ^{Bab}	12.85±0.53 ^{Bab}	12.86±0.05 ^{Cab}	12.93±0.12 ^{Ba}
	0.2PCMC	10.91±0.27 ^{Af}	11.89±0.32 ^{Be}	12.46±0.17 ^{Bd}	12.55±0.26 ^{Bcd}	12.71±0.22 ^{Bc}	12.93±0.16 ^{Cb}	13.25±0.14 ^{Aa}
	0.5 PCMC	11.05±0.39 ^{Ad}	12.30±0.35 ^{ABc}	12.94±0.58 ^{Ab}	13.07±0.20 ^{Aab}	13.14±0.20 ^{Aab}	13.17±0.24 ^{ABab}	13.42±0.53 ^{Aa}
	0.2 NCMC	11.11±0.23 ^{Ad}	12.15±0.29 ^{ABc}	12.33±0.24 ^{Bc}	12.67±0.08 ^{Bb}	12.98±0.19 ^{ABa}	13.01±0.35 ^{BCa}	13.20±0.18 ^{Aa}
	0.5 NCMC	10.87±0.10 ^{Af}	12.06±0.19 ^{ABe}	13.01±0.20 ^{Ad}	13.13±0.15 ^{Ac}	13.17±0.05 ^{Abc}	13.28±0.09 ^{Aab}	13.39±0.17 ^{Aa}

Means with different superscripts in a row (a-f) and column (A-C) are significant at $p < 0.05$.

Control: untreated Maribo cheese.

PC: powdered chitosan-added Maribo cheese.

NPC: nanopowdered chitosan-added Maribo cheese.

Table 4. Texture properties of nanopowdered chitosan-added Maribo cheese during ripening at 14°C for 6 mon

Texture description	Treatment (% w/w)	Ripening period (mon)						
		0	1	2	3	4	5	6
Hardness (N ²)	Control	14.97±0.61 ^{Aa}	9.76±1.21 ^{Ab}	9.47±0.94 ^{Ab}	7.41±0.47 ^{Bc}	7.32±0.80 ^{Bc}	7.19±1.22 ^{Bc}	7.15±0.66 ^{Bc}
	0.2 PCMC	14.99±0.62 ^{Aa}	9.92±0.90 ^{Ab}	9.87±1.09 ^{Ab}	9.33±1.02 ^{Ab}	9.34±0.48 ^{Ab}	9.27±0.71 ^{Ab}	9.14±0.09 ^{Ab}
	0.5 PCMC	15.08±0.51 ^{Aa}	10.26±0.82 ^{Ab}	10.05±1.41 ^{Ab}	9.71±0.60 ^{Ab}	9.51±0.74 ^{Ab}	9.42±0.51 ^{Ab}	9.23±1.48 ^{Ab}
	0.2 NCMC	14.98±0.30 ^{Aa}	9.48±0.80 ^{Ab}	9.18±0.62 ^{Abc}	8.89±0.98 ^{Abc}	8.43±0.81 ^{ABc}	8.38±0.62 ^{Ac}	8.19±0.49 ^{ABc}
	0.5 NCMC	15.04±1.16 ^{Aa}	9.96±1.00 ^{Ab}	9.64±0.99 ^{Ab}	9.30±1.11 ^{Ab}	9.26±1.42 ^{Ab}	9.23±0.86 ^{Ab}	9.20±0.12 ^{Ab}
Cohesiveness (J ³)	Control	0.78±0.02 ^{Aa}	0.73±0.02 ^{Ab}	0.69±0.01 ^{Ad}	0.71±0.01 ^{Ac}	0.70±0.01 ^{Ac}	0.68±0.01 ^{Ade}	0.67±0.01 ^{Ac}
	0.2 PCMC	0.78±0.01 ^{Aa}	0.73±0.02 ^{Ab}	0.68±0.02 ^{Ac}	0.68±0.04 ^{Bcd}	0.66±0.02 ^{Bcd}	0.66±0.02 ^{Bd}	0.65±0.01 ^{Ad}
	0.5 PCMC	0.78±0.02 ^{Aa}	0.74±0.02 ^{Ab}	0.68±0.02 ^{Ac}	0.69±0.01 ^{ABc}	0.69±0.02 ^{Ac}	0.67±0.01 ^{ABcd}	0.64±0.03 ^{Ad}
	0.2 NCMC	0.79±0.02 ^{Aa}	0.73±0.01 ^{Ab}	0.69±0.02 ^{Ac}	0.67±0.01 ^{Bcd}	0.69±0.01 ^{Ac}	0.68±0.02 ^{Ac}	0.66±0.01 ^{Ad}
	0.5 NCMC	0.79±0.02 ^{Aa}	0.74±0.01 ^{Ab}	0.70±0.02 ^{Ac}	0.69±0.01 ^{ABcd}	0.69±0.01 ^{Ac}	0.67±0.02 ^{ABde}	0.67±0.02 ^{Ae}
Gumminess (N)	Control	11.39±0.32 ^{Aa}	7.78±0.49 ^{Ab}	7.46±0.31 ^{Ab}	5.28±0.31 ^{Bc}	5.26±1.08 ^{Bc}	5.12±1.21 ^{Bc}	5.05±0.16 ^{Bc}
	0.2 PCMC	11.50±0.44 ^{Aa}	7.19±0.52 ^{Ab}	7.01±0.32 ^{Ab}	6.97±0.67 ^{Ab}	6.87±0.43 ^{ABc}	6.57±0.46 ^{ABc}	6.26±0.52 ^{Ac}
	0.5 PCMC	11.52±0.46 ^{Aa}	7.68±0.80 ^{Ab}	7.22±0.51 ^{ABc}	6.99±0.22 ^{ABc}	6.80±0.76 ^{Ac}	6.81±0.10 ^{Ac}	6.53±0.65 ^{Ac}
	0.2 NCMC	11.44±0.35 ^{Aa}	7.08±0.72 ^{Ab}	7.06±0.61 ^{Ab}	6.95±0.55 ^{Ab}	7.01±1.25 ^{Ab}	6.92±0.29 ^{Ab}	6.67±0.45 ^{Ab}
	0.5 NCMC	11.46±0.75 ^{Aa}	7.51±1.11 ^{Ab}	7.37±0.52 ^{Ab}	7.00±0.36 ^{Ab}	6.96±0.69 ^{Ab}	6.82±0.57 ^{Ab}	6.70±0.69 ^{Ab}
Springiness (m×10 ⁻³)	Control	0.79±0.01 ^{Aa}	0.75±0.03 ^{Aa}	0.75±0.08 ^{Aa}	0.70±0.01 ^{Ab}	0.70±0.02 ^{Ab}	0.68±0.02 ^{Ab}	0.68±0.03 ^{Ab}
	0.2 PCMC	0.80±0.11 ^{Aa}	0.73±0.02 ^{Aab}	0.73±0.07 ^{Aab}	0.71±0.02 ^{Ab}	0.70±0.02 ^{Ab}	0.69±0.04 ^{Ab}	0.66±0.03 ^{Ab}
	0.5 PCMC	0.85±0.12 ^{Aa}	0.77±0.12 ^{Aab}	0.76±0.11 ^{Aabc}	0.72±0.03 ^{ABc}	0.69±0.02 ^{ABc}	0.65±0.03 ^{Ac}	0.65±0.03 ^{Ac}
	0.2 NCMC	0.79±0.14 ^{Aa}	0.74±0.02 ^{Aab}	0.73±0.04 ^{ABab}	0.71±0.01 ^{Aab}	0.69±0.02 ^{Ab}	0.67±0.02 ^{Ab}	0.67±0.03 ^{Ab}
	0.5 NCMC	0.83±0.10 ^{Aa}	0.78±0.10 ^{Aab}	0.78±0.11 ^{Aab}	0.72±0.04 ^{ABc}	0.69±0.03 ^{ABc}	0.66±0.04 ^{Ac}	0.66±0.02 ^{Ac}
Chewiness (J×10 ⁻³)	Control	9.42±0.38 ^{Aa}	5.13±0.58 ^{Ab}	5.08±0.39 ^{Ab}	5.04±0.91 ^{Ab}	4.97±0.21 ^{Ab}	4.91±0.29 ^{Ab}	4.85±0.42 ^{Ab}
	0.2 PCMC	9.64±0.97 ^{Aa}	5.28±0.52 ^{Ab}	5.15±0.26 ^{Ab}	5.11±0.74 ^{Ab}	5.06±0.30 ^{Ab}	4.98±0.40 ^{Ab}	4.87±0.34 ^{Ab}
	0.5 PCMC	9.82±1.10 ^{Aa}	5.53±0.74 ^{Ab}	5.31±0.59 ^{Ab}	5.19±0.74 ^{Ab}	5.04±0.25 ^{Ab}	4.97±0.57 ^{Ab}	4.88±0.14 ^{Ab}
	0.2 NCMC	9.52±1.81 ^{Aa}	5.15±0.84 ^{Ab}	5.04±0.37 ^{Ab}	5.01±0.43 ^{Ab}	4.82±0.48 ^{Ab}	4.78±0.21 ^{Ab}	4.80±0.39 ^{Ab}
	0.5 NCMC	9.71±0.38 ^{Aa}	5.38±0.35 ^{Ab}	5.28±0.47 ^{ABc}	5.22±0.20 ^{ABc}	5.04±0.12 ^{ABc}	4.91±0.29 ^{ABc}	4.85±0.48 ^{Ac}

Means with different superscripts in a row (a-e) and column (A-B) are significant at $p < 0.05$.

Control = Maribo cheese.

PC: powdered chitosan-added Maribo cheese.

NPC: nanopowdered chitosan-added Maribo cheese.

lowest L* value after ripening (75.86). 0.2% NCMC was decreased from 86.06 to 77.49 and this decreasing trend was similar to the control. Concentration of chitosan affected decreasing of lightness. However, a* and b* values of all samples were increased. a* value of chitosan-added cheese was lower than that of control, whereas b* value of chitosan-added cheese was higher than that of control. a* value was decreased with increasing chitosan concentrations. Under the same concentration of chitosan-added cheese, PCMC had significantly high b* values than NCMC. b* values of 0.2% NCMC and 0.5% NCMC were 13.20 and 13.39, respectively. As chitosan concentration increased, b* value increased both in NCMC and PCMC.

Rohm *et al.* (1996) reported that Emmental cheese also had similar trends in specificity that L* value decreased but a* and b* values increased during 120 d ripening. According to Seo *et al.* (2011), nanochitosan solution-added milk samples significantly increased b* value but the addition of NPC did not significantly affect L* value of nanochitosan-added milk.

Texture analysis

Textural properties of NCMC during 6 mon ripening stored at 14°C are presented in Table 4. Hardness dec-

reased significantly in all samples during the ripening particularly at 1 mon ($p < 0.05$). After 3 mon, NCMC and PCMC had significantly higher hardness compared to control ($p < 0.05$). During the ripening, 0.5% NCMC and control decreased from 15.04 to 9.20 N² and from 14.97 to 7.15 N², respectively. PCMC was harder than NCMC at each ripening period. Hardness of cheese was decreased because proteolytic breakdown of α_{s1} -casein into peptides causes cheese to soften and lose structural integrity with time (Fox, 1989). Cohesiveness and gumminess were also decreased during the ripening in all samples. Cohesiveness was not significantly different among the samples ($p < 0.05$); however, cohesiveness of NCMC was higher than that of PCMC. After 3 mon, gumminess of control had significantly lower values than that of NCMC and PCMC ($p < 0.05$). NCMC was observed to have more gumminess than PCMC after 4 mon. Springiness and chewiness did not show any significant change by addition of chitosan in cheese during 6 mon ripening ($p > 0.05$). Springiness was slightly decreased during ripening and chewiness was decreased by almost 2 times at 1 mon in all samples. Tunick and Van Hekken (2000) reported that most of the cheeses became less cohesive with increasing age and the values for chewiness usually decreased with stor-

Table 5. Sensory properties of nanopowdered chitosan-added Maribo cheese during ripening at 14°C for 6 mon

Sensory description	Treatment (% w/w)	Ripening period (mon)						
		0	1	2	3	4	5	6
Butyric	Control	4.00±0.00 ^{Aa}	4.00±0.00 ^{Aa}	4.00±0.00 ^{Aa}	4.00±0.00 ^{Ba}	4.17±0.41 ^{Aa}	4.17±0.41 ^{Aa}	4.33±0.52 ^{Aa}
	0.2 PCMC	4.17±0.41 ^{Ab}	4.17±0.41 ^{Ab}	4.83±0.75 ^{Ab}	4.83±0.41 ^{Ab}	4.83±0.75 ^{Ab}	5.00±1.10 ^{Ab}	5.17±0.75 ^{Aa}
	0.5 PCMC	4.17±0.75 ^{Ab}	4.67±0.82 ^{Ab}	4.83±0.75 ^{Ab}	5.00±0.63 ^{Ab}	5.00±0.89 ^{Ab}	5.17±0.75 ^{Ab}	5.33±1.21 ^{Aa}
	0.2 NCMC	4.17±0.75 ^{Ab}	4.33±0.52 ^{Ab}	4.83±0.75 ^{Ab}	4.67±0.82 ^{ABab}	4.83±0.41 ^{Ab}	4.83±0.98 ^{Ab}	5.17±0.75 ^{Aa}
	0.5 NCMC	4.17±0.75 ^{Ab}	4.67±0.82 ^{Ab}	4.83±0.75 ^{Ab}	4.83±0.75 ^{Ab}	4.83±0.75 ^{Ab}	5.00±0.63 ^{Ab}	5.17±0.75 ^{Aa}
Bitterness	Control	4.00±0.00 ^{Ac}	4.50±0.55 ^{Ab}	5.00±0.41 ^{Aa}	4.50±0.55 ^{Ab}	4.33±0.52 ^{Abc}	4.00±0.41 ^{Ac}	4.00±0.41 ^{Ac}
	0.2 PCMC	4.00±0.00 ^{Ab}	4.83±0.98 ^{Ab}	5.33±0.82 ^{Aa}	4.67±0.82 ^{Ab}	4.67±1.21 ^{Abc}	4.33±0.82 ^{Ab}	4.33±0.52 ^{Ab}
	0.5 PCMC	4.17±0.75 ^{Ac}	5.00±0.89 ^{Abc}	5.67±1.03 ^{Aa}	5.17±0.75 ^{Ab}	5.00±0.41 ^{Ab}	4.83±0.41 ^{Abc}	4.50±0.55 ^{Abc}
	0.2 NCMC	4.00±0.63 ^{Ab}	4.33±0.52 ^{Ab}	5.17±0.75 ^{Aa}	4.67±0.82 ^{Ab}	4.50±1.05 ^{Ab}	4.33±0.52 ^{Ab}	4.17±0.75 ^{Ab}
	0.5 NCMC	4.17±0.41 ^{Ab}	4.83±0.98 ^{Ab}	5.33±0.82 ^{Aa}	5.00±0.89 ^{Ab}	4.83±0.41 ^{Ab}	4.50±1.05 ^{Ab}	4.33±0.52 ^{Ab}
Hardness	Control	4.50±0.55 ^{Aa}	3.83±0.41 ^{Ab}	3.83±0.41 ^{Ab}	3.50±0.84 ^{Bc}	3.33±0.52 ^{Abc}	3.17±0.41 ^{Bc}	3.00±0.41 ^{Bc}
	0.2 PCMC	4.50±0.55 ^{Aa}	4.17±0.98 ^{Aa}	4.00±0.89 ^{Aa}	4.17±1.17 ^{Aa}	4.00±0.63 ^{Aa}	3.83±0.41 ^{Aa}	3.67±0.52 ^{ABa}
	0.5 PCMC	4.83±0.41 ^{Aa}	4.67±0.82 ^{Ab}	4.33±0.52 ^{Ab}	4.00±0.63 ^{Ab}	4.17±1.17 ^{Ab}	4.00±0.41 ^{Ab}	3.83±0.41 ^{Ab}
	0.2 NCMC	4.50±0.84 ^{Aa}	4.17±1.17 ^{Aa}	4.17±0.41 ^{Aa}	4.17±0.75 ^{Aa}	3.83±0.98 ^{Aa}	3.67±0.52 ^{Aa}	3.50±0.55 ^{ABa}
	0.5 NCMC	4.50±0.55 ^{Aa}	4.50±0.84 ^{Aa}	4.33±0.52 ^{Aa}	4.33±0.82 ^{Aa}	4.00±0.89 ^{Aa}	3.83±0.41 ^{Aa}	3.67±0.82 ^{ABa}
Overall acceptability	Control	4.50±0.55 ^{Aa}	4.67±1.03 ^{Aa}	4.50±0.55 ^{Aa}	4.83±0.75 ^{Aa}	5.00±0.63 ^{Aa}	5.00±0.63 ^{Aa}	5.17±1.17 ^{Aa}
	0.2 PCMC	4.67±0.82 ^{Aa}	5.17±1.17 ^{Aa}	4.83±0.98 ^{Aa}	5.17±0.75 ^{Aa}	5.67±1.03 ^{Aa}	5.83±1.17 ^{Aa}	5.83±0.98 ^{Aa}
	0.5 PCMC	4.50±1.05 ^{Aa}	4.83±0.75 ^{Aa}	4.67±0.82 ^{Aa}	5.00±0.89 ^{Aa}	5.50±1.05 ^{Aa}	5.67±1.21 ^{Aa}	5.67±0.82 ^{Aa}
	0.2 NCMC	4.50±1.38 ^{Ab}	5.00±0.89 ^{Ab}	5.33±0.82 ^{Ab}	5.83±0.75 ^{Aa}	6.00±0.63 ^{Aa}	6.17±0.75 ^{Aa}	6.17±0.75 ^{Aa}
	0.5 NCMC	4.67±1.21 ^{Ab}	4.83±0.75 ^{Ab}	5.17±0.75 ^{Ab}	5.83±0.75 ^{Ab}	5.83±1.17 ^{Ab}	6.00±1.10 ^{Aa}	6.00±0.89 ^{Aa}

Means with different superscripts in a row (a-c) and column (A-B) are significant at $p < 0.05$.

Control = Maribo cheese.

PC: powdered chitosan-added Maribo cheese.

NPC: nanopowdered chitosan-added Maribo cheese.

age.

Sensory attribute

Sensory attributes of NCMC during ripening at 14°C for 6 mon are shown in Table 5. In butyric off-flavor, 0.2% NCMC and control were increased from 4.17 to 5.17 and from 4.00 to 4.33 during 6 mon ripening, respectively. Bitterness increased until 2 mon, thereafter it was decreased in all samples. In the process of protein degraded to amino acid, dipeptide could cause bitterness of cheese. In addition, cheeses added-chitosan powder had a slightly bitter taste than control because of the original bitter taste of nanopowdered and powdered chitosans. Hardness of control was rapidly decreased at 1 mon. Chitosan-added cheeses were significantly harder than control at 5 and 6 mon ($p < 0.05$). Hardness was 4.50 at initial and after ripening it was decreased to 3.50 in 0.2% NCMC and 3.00 in control. Hardness of chitosan-added samples was increased with increasing chitosan concentration. Overall acceptability was also affected by addition of nanopowdered and powdered chitosans in cheese samples, but there were not significantly different among the samples. After the ripening fore 2 mon, 0.2% NCMC had the best overall acceptability among the samples (6.17). Altieri *et al.* (2005) reported that sensory analysis between 0.075% chitosan-added Mozzarella cheese and chitosan-free cheeses were not different. According to Seo *et al.* (2011), 1-9% nano-chitosan-added milks did not significantly influenced bitter taste and overall scores ($p > 0.05$).

Conclusion

The current study was designed to develop on NPC-added Maribo cheeses and to evaluate the effect of adding NPC in the chemical, texture, and sensory properties during ripening. The data on the SCFA, color, texture, and sensory analysis obtained from the current study indicated that 0.2% concentration of NPC could be applicable in NPC-added functional cheese development. Therefore, the present study may provide the possibility of NPC-added Maribo cheese manufacture.

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