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ARTICLE

Effect of Palm or Coconut Solid Lipid Nanoparticles (SLNs) on Growth of *Lactobacillus plantarum* in Milk

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Abstract

This study was performed to investigate the effect of palm or coconut solid lipid nanoparticles (PO-SLNs or CO-SLNs) on growth of *Lactobacillus plantarum* (*L. plantarum*) in milk during storage period. The PO or CO (0.1% or 1.0%) was dispersed both in distilled water (DW) and ultra high temperature milk (UHTM), and subsequently emulsified with Tween® 80 by ultrasonication (30% power, 2 min). Increase in particle size and encapsulation efficiency (EE%) in DW was observed with an increase in oil concentration, whereas a decrease in ζ-potential of SLNs was noted with an increment in oil concentration. Moreover, the CO-SLNs exhibited relatively smaller particle size and higher EE% than PO-SLNs. The CO-SLNs were found to be more stable than PO-SLNs. Higher lipid oxidation of PO or CO-SLNs in UHTM was observed during the storage test, when compared to PO or CO-SLNs in DW. However, there was no remarkable difference in lipid oxidation during storage period ($p>0.05$). In the growth test, the viability of *L. plantarum* in control (without PO or CO-SLNs in DW) exhibited a dramatic decrease with increasing storage period. In addition, viability of *L. plantarum* of PO or CO-SLNs in UHTM was higher than that of SLNs in DW. Based on the present study, production of SLNs containing PO or CO in UHTM is proposed, which can be used in lactobacilli fortified beverages in food industry.

Key words: solid lipid nanoparticles, *Lactobacillus*, growth, palm oil, coconut oil

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Introduction

In recent decades, there has been much attention on encapsulation of functional ingredients to entrap volatile compounds, protection of vulnerable active compounds, and inhibition of the growth of bacteria in beverage products. (Anton *et al.*, 2008; McClements *et al.*, 2007; Rao and McClements, 2011). Among the various encapsulation systems, solid lipid nanoparticles (SLNs) can easily be fabricated from food-grade ingredients using emulsifying techniques, such as hot or cold homogenization and ultrasonication. Basically, SLNs are particles consisting of a matrix made of solid lipid shell. Compared to nanoemulsions and liposomes, the SLNs have some distinct advantages such as high encapsulation efficiency of lipophilic compounds, possibility of large-scale production, slower degradation rate, and bioactive release for prolonged time (Fathi *et al.*, 2012; Miiller *et al.*, 2000). Especially, SLNs have been employed applied in food industry to extend shelf-life of food because of their ability to protect the incorporated bioactive ingredients against chemical reactions such as oxidation for prolonged times (Fathi *et al.*, 2012; Miiller *et al.*, 2000). The SLNs or nanoemulsions containing vegetable oils (Buranasuksombat *et al.*, 2011) and essential oils (cinnamon oil, eugenol, and carvacrol) have been applied in beverage industry to inhibit the growth of food borne bacterial pathogens, such as *Staphylococcus aureus*, and *Escherichia coli* (Devi *et al.*, 2010; Jo *et al.*, 2015; Thomsen *et al.*, 2013).

The vegetable oils play important functional and sensory roles, act as carriers of fat-soluble vitamins (A, D, E, and K), and provide energy and essential fatty acids such as linoleic, linolenic, and arachidonic acids (Dauqan *et al.*, 2011; Fasina *et al.*, 2006). Palm oil (PO) and coconut oil (CO) are representative vegetable oils in food ingredients for fabrication of biscuits, chocolates, cereals, etc. In general, PO is composed of 48.4% saturated fatty acids, 40.5% monounsaturated fatty acids, and 10.3% polyunsaturated fatty acids; whereas, CO consists of 78.5% sat-

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urated fatty acids, 6.9% monounsaturated fatty acids, and 2.2% polyunsaturated fatty acids (Satyanarayana and Muraleedharan, 2011). Moreover, melting point of PO (35°C) is higher than CO (25°C), because the former has higher saturated fatty acid content. Based on the thermal properties of PO and CO, the SLNs can be formed below the melting point, and transformed into nanoemulsions above the melting point of vegetable oils. Initially, the nanoemulsion of PO or CO was fabricated using ultrasonication homogenizing process at temperature higher than the respective vegetable oil's melting point. Then, the nanoemulsions were transformed into SLNs at below the melting point of PO or CO. In our study, the SLNs were added into milk containing *Lactobacillus* strains.

Lactobacillus strains have been utilized as dairy starters and play imperative role in both probiotic and bioprotective cultures as well as fermenting agents in fermented products. To improve the survival rate of probiotics and to extend their shelf-life, numerous studies have demonstrated the encapsulation system of lactic acid bacteria (Dolly *et al.*, 2011; Lee *et al.*, 2014). Attempts have been made to encapsulate probiotics using various coating materials such as starch, monoglycerin, gum and whey proteins (Burgain *et al.*, 2013; Dolly *et al.*, 2011; Lee *et al.*, 2014; Shi *et al.*, 2013). Consequently, the objective of our experiments was to study the effect of palm or coconut solid lipid nanoparticles (PO-SLNs or CO-SLNs) on growth of lactic acid bacteria in milk.

Materials and Methods

Materials

For the formulation of SLNs, PO and CO (for use in dispersed phase) were purchased from Lavender Soap Company (Malaysia) and Junsei (Japan), respectively. Polyoxyethylene (20) sorbitan monooleate (Tween®80) used as emulsifier was provided by Samchen Chemicals Co. Ltd. (Korea). Ultra high temperature milk was purchased from the milk market (Seoul Dairy Cooperative, Korea). All other chemicals used were of analytical grade.

For the microbial activation tests, *L. plantraum* 10hk2 strain was provided by Milae Resources ML Research Institute (Korea). The strains were cultured on lactobacilli MRS broth (Difco™, USA) for 12-18 h under incubation at 30°C.

Preparation of SLNs

The palm or coconut solid lipid nanoparticles (PO-SLNs or CO-SLNs) were prepared in different concentrations (0.1% PO and 1.0 %CO) in different continuous phases like distilled water (DW) and ultra high temperature milk (UHTM). Emulsifier (Tween®80, 0.1% and 1.0%) was added to continuous phase with same oil concentration. Initially, the PO-SLNs or CO-SLNs were produced by a two-stage homogenization procedure to obtain fine droplets of emulsion. The pre-emulsions were obtained by high-speed homogenization at 8,000 rpm for 3 min using an Ultra Turrax®T25 (IKA, Germany). Subsequently, the pre-emulsions were passed twice through a probe ultrasonicator (Model HD-2200, BANDELIN electronic GmbH & Co. KG, Germary) at 60 W power for 3 min. The experiment was carried out to investigate the storage stability for 30 d. During storage period, samples were kept at 7°C. Consequently, every sample was transformed from liquid droplet into solid particles upon cooling and termed as solid lipid nanoparticles (SLNs).

Particle characterization

The average particle size, size distribution, and ζ-potential of SLNs were determined by dynamic light scattering using a Zetasizer®Nano-ZS90 (Malvern Instruments, UK). The samples were diluted approximately 10 times with distilled water prior to each measurement to avoid multiple light scattering effects. The particle size was described by the mean (average) diameter, and the size distribution was indicated by the polydispersity index (PDI). Since ζ-potential is directly related to the electrophoretic mobility of the particles, the analyzer calculates ζ-potential from the measured velocity. All measurements were taken in triplicate.

Encapsulation efficiency

The encapsulation efficiency (EE%) of PO and CO was determined using UV/VIS spectrophotometer (OPTIZEN, Mecasys Co., Korea). To extract free oil, *n*-hexane and SLNs were mixed in 1:1 (v:v) ratio, and centrifuged at 4,000 rpm for 10 min. The extracted free oil was determined by measuring absorbance at 212 nm (PO) or 205 nm (CO). The EE% was indirectly calculated using a calibration curve constructed from a series of PO or CO based on *n*-hexane with standard concentrations. The EE% was then obtained as a percentage from the following equation;

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total use amount of oil (g)} - \text{Free oil amount (g)}}{\text{Total use amount of oil (g)}} \times 100$$

pH measurement

The pH of SLNs was determined during storage period using a pH meter (Model S220, Mettler Toledo GmbH, Switzerland). The average three determinations taken at room temperature were used for further studies.

Determination of thiobarbituric acid reactive substances (TBARS)

The TBARS of SLNs was determined by following the method of Chun *et al.* (2013). Typically, 1 mL of sample was mixed with 1 mL of 0.25 N HCl solution containing 15% trichloroacetic acid (TCA) and 0.375% thiobarbituric acid (TBA). Individual samples were heated to 95°C in a water bath for 15 min, and cooled to room temperature for 10 min. After cooling, the samples were centrifuged at 4,000 rpm for 10 min, and the absorbance of the clear supernatant liquid was measured at 535 nm in a UV/VIS spectrophotometer. The results were calculated as mg of malonaldehyde per kg of sample.

Bacterial growth analysis

For the inoculation of PO-SLNs or CO-SLNs with bacteria, 3 mL of *L. plantraum* was inoculated into 27 mL of SLNs. A 1 mL aliquot of the *L. plantraum* culture was properly diluted using sterilized 0.85% NaCl and plated on MRS agar (Difco™, USA). After 30°C incubation for 2 d, plates with visual colonies of 30-300 were counted. Results are expressed as the logarithm of the number of colony-forming units (Log CFU/mL).

Statistical analysis

One-way analysis of variance (ANOVA) was conducted using a SAS statistical program, ver. 9.2 (SAS Institute, NC) and the means were separated by Duncan's multiple range test ($p<0.05$). The correlations between independent variables and measured values were calculated as Pearson's correlation coefficients. All measurements were performed on at least three samples and are reported as the means and standard deviations.

Results and Discussions

Particle properties of PO-SLNs and CO-SLNs

The particle properties of PO-SLNs and CO-SLNs such as particle size, ζ-potential, and encapsulation efficiency (EE%) are presented in Table 1. The PO-SLNs and CO-SLNs were prepared by ultrasonication (30% power, 2 times), which led to a mean particle size between 200 to 500 nm, as well as a PDI between 0.2-0.6 (data not shown),

Table 1. Particle size, ζ-potential and encapsulation efficiency of solid lipid nanoparticles (SLNs) prepared with different types of oils and concentrations

Oil type[1)]	Concentration (wt%)	Continuous phase[2)]	
		DW	UHTM
PO	Particle size (nm)		
	0.1	388±28.22$^{Ba,3)}$	285±2.70^{Ab}
	1	586±14.34^{Aa}	294±1.91^{Ab}
	[-] ζ-Potential		
	0.1	19.9±0.72^{Ab}	30.3±0.75^{Aa}
	1	16.1±0.85^{Ba}	28.8±0.84^{Ba}
	EE (%)		
	0.1	78.1±2.75^{Ba}	60.0±7.69^{Bb}
	1	96.0±2.22^{Aa}	96.7±0.63^{Aa}
CO	Particle size (nm)		
	0.1	283±1.37^{Ab}	295±2.55^{Ab}
	1	446±10.87^{Ba}	298±1.13^{Ab}
	[-] ζ -Potential		
	0.1	25.8±0.55^{Ab}	31.3±0.85^{Aa}
	1	11.9±0.26^{Bb}	27.6±0.74^{Ba}
	EE (%)		
	0.1	78.7±5.53^{B}	N.D.*
	1	98.2±1.30^{Aa}	92.6±1.04^{a}

[1)]PO, Palm oil; CO, Coconut oil.
[2)]Solid lipid nanoparticles were prepared using different continuous phases such as distilled water (DW) and ultra high temperature milk (UHTM).
[3)] Individual data are expressed as the mean±SD of three separate experiments. Values with different superscripts indicate significant difference ($p<0.05$) by Duncan's multiple range test. ^{A,B}Means with different superscripts within the same column are significantly different ($p<0.05$). ^{a,b}Means with different superscripts within the same row are significantly different ($p<0.05$).
*N.D.; Not detected.

indicating wide distribution of particle size. A significant increase in particle size of PO and CO-SLNs prepared in DW (PO and CO-SLNs/DW) was observed with an increase in oil concentration from 228 nm to 586 nm. The particle size of SLNs/UHTM was lower than that of SLNs/DW, except for 0.1% CO-SLNs/DW, because milk contains various emulsifying ingredient such as whey protein and casein (McClements *et al.,* 2007). Moreover, PO-SLNs exhibited larger particle size than CO-SLNs, when SLNs were prepared in DW. However, no significant difference in the particle size of PO and CO-SLNs prepared in UHTM (PO and CO-SLNs/UHTM) was noted irrespective of oil type and concentration ($p>0.05$).

A decrease in the ζ-potential value of all SLNs was observed with an increase in oil concentration, and the SLNs/DW showed lower ζ-potential value as compared to SLNs/UHTM, regardless of oil type and concentration. In general, ζ-potential value indicates the moderately stable behaviour of SLNs or dispersion when they are over

±30 mV (McClements, 2005). From our study, the ζ-potential value of SLNs/UHTM was found to be -30 mV, which led us to confirm the presence of more stable droplets in SLNs/UHTM than SLNs/DW. In EE% measurements of SLNs, the PO or CO-SLNs in the presence of 0.1% oil showed higher EE% than in the presence of 1% oil, generally an increase in the EE% of SLNs was observed with an increment in oil concentration. Moreover, the EE% of PO-SLNs/DW was higher than that of PO-SLNs/UHTM at 0.1% PO concentration, whereas the EE% of PO-SLNs at 1% PO concentration exhibited no change with respect to continuous phase. According to Das *et al.* (2012), the particle size of clotrimazole-loaded SLNs prepared using emulsification-ultrasonication technique exhibited an increasing trend with an increase in lipid concentration, whereas ζ-potential value tended to decrease with increasing lipid concentration. However, no significant change in EE% was observed irrespective of lipid concentration.

Changes in particle size, ζ-potential, and EE%

The changes in particle size and ζ-potential of SLNs during storage period are shown in Fig. 1. The particle size and ζ-potential value of all SLNs did not change over the storage period, and there was no significant difference with respect to the oil type and concentration. As per the study conducted by Chun *et al.* (2013), the particle size of cinnamon oil milk was increased significantly as storage period of progressed for all cinnamon oil milk. In terms of EE% measurements, all SLNs samples exhibited no significant difference during storage period (Fig. 2). Silva *et al.* (2011) reported use of hot high pressure homogenization and ultrasound technique as suitable production methods for SLNs. The reported particle sizes were in the nanometer range for all prepared SLN formulations and the ζ-potential absolute values were high, predicting good long-term stability.

Change in pH

Fig. 3 presents the change in pH of PO or CO-SLNs in

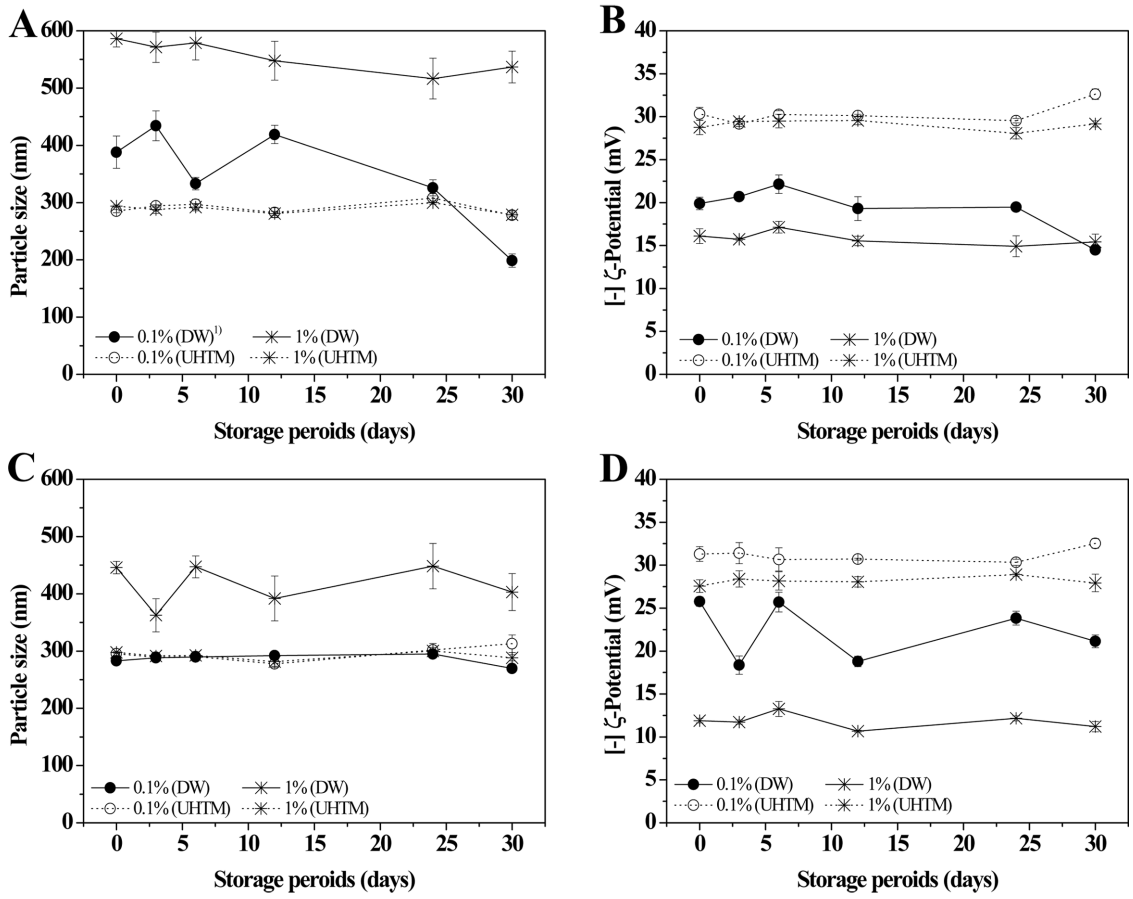

Fig. 1. Changes in (A, C) particle size and (B, D) ζ-potential of solid lipid nanoparticles prepared with (A, B) PO or (C, D) CO.
[1)]Solid lipid nanoparticles were prepared using different oil types (PO; Palm oil, CO; Coconut oil) and concentration (0.1% and 1.0%) in different continuous phases such as distilled water (DW) and ultra high temperature milk (UHTM).

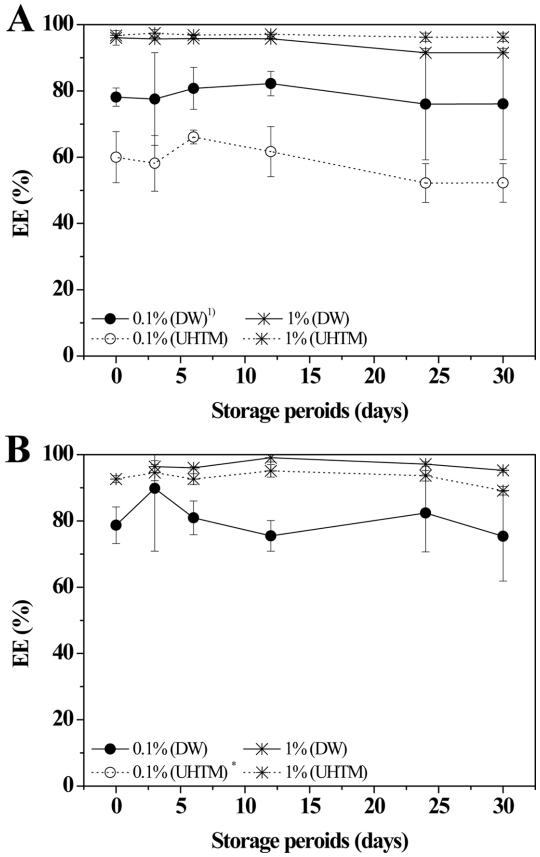

Fig. 2. Changes in encapsulation efficiency (EE%) of solid lipid nanoparticles prepared with (A) PO or (B) CO. [1)]Solid lipid nanoparticles were prepared using different oil types (PO, Palm oil; CO, Coconut oil) and concentration (0.1% and 1.0%) in different continuous phases such as distilled water (DW) and ultra high temperature milk (UHTM). *Not detected.

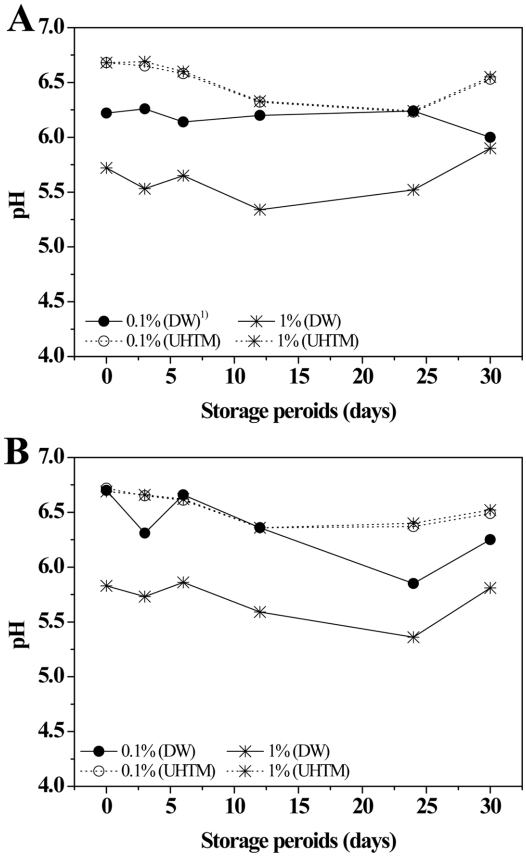

Fig. 3. Changes in pH of solid lipid nanoparticles prepared with (A) PO or (B) CO. [1)]Solid lipid nanoparticles were prepared using different oil types (PO, Palm oil; CO, Coconut oil) and concentrations (0.1% and 1.0%) in different continuous phases such as distilled water (DW) and ultra high temperature milk (UHTM).

DW or UHTM during storage period. The pH of SLNs/DW in the presence of 0.1% oil was higher than in the presence of 1% oil. In general, pH of Tween®80 has been reported as 5.7 at 3% (w/w) and 5.5 a 9% (w/w), respectively (Hsu and Nacu, 2003). In our experiments, the SLNs/DW in the presence of 1.0% oil was found to contain high concentration (1%) of Tween®80 when compared to SLNs/DW in the presence of 0.1% oil. Based on the results, we confirmed that concentration of emulsifier (Tween®80) was affected more than oil concentration, when PO or CO-SLNs were prepared in DW. Typically, pH of SLNs decreased with an increase in Tween®80 concentration. However, no significant difference in the pH of SLNs/UHTM was noted with respect to oil type and concentration. A constant decrease in the pH of all SLNs from 6.72 to 6.00 ($p<0.05$) was observed during storage period. A study by Chun *et al.* (2013) reported slight increase in the pH of cinnamon oil milk with increasing cinnamon oil concentration; however, no significant difference was reported during storage. According to Lee *et al.* (2013), peanut sprout extract was microencapsulated using water in oil in water (W/O/W) system by spray dryer to apply in milk. They reported that pH values of milk with added powdered peanut sprout extract microcapsules ranged from 6.8 to 6.6 during the storage.

Lipid oxidation

Fig. 4 shows the change in lipid oxidation of PO or CO-SLNs in DW or UHTM during storage period. The malonaldehyde value of SLNs/DW was found to be lower than that of SLNs/UHTM. In addition, 1.0% SLNs was more oxidative than 0.1% -SLNs in both DW and UHTM. During storage period, all PO or CO-SLNs/DW showed similar malonaldehyde value and there was no significant difference regardless of oil type ($p>0.05$). However, a slight increase in the malonaldehyde value of CO-SLNs/UHTM

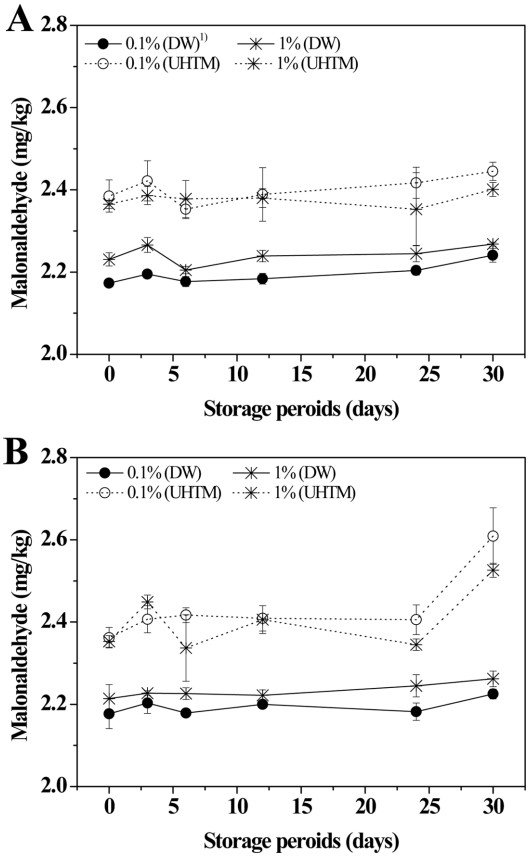

Fig. 4. Oxidation stability of solid lipid nanoparticles prepared with (A) PO or (B) CO. [1)]Solid lipid nanoparticles were prepared using different oil types (PO, Palm oil; CO, Coconut oil) and concentrations (0.1% and 1.0%) in different continuous phases such as distilled water (DW) and ultra high temperature milk (UHTM).

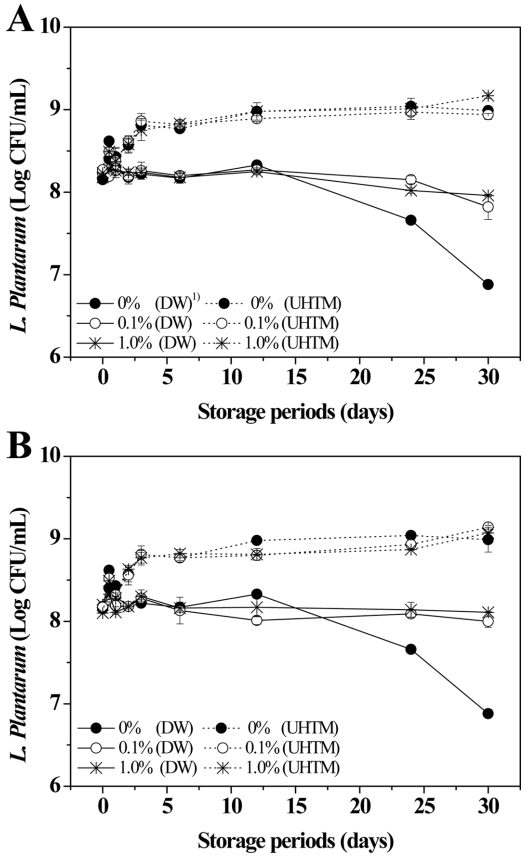

Fig. 5. Viability of *L. plantarum* in the presence of solid lipid nanoparticles prepared with (A) PO or (B) CO. [1)]Solid lipid nanoparticles were prepared using different oil types (PO, Palm oil; CO, Coconut oil) and concentrations (0%, 0.1% and 1.0%) in different continuous phases such as distilled water (DW) and ultra high temperature milk (UHTM).

was noted; but without any significant difference (p>0.05). Furthermore, relevance between lipid oxidation and encapsulation efficiency was hypothesized. The change in lipid oxidation of SLNs/DW or UHTM was not apparent, because there was no change in encapsulation efficiency of SLNs/DW or UHTM during storage period (Fig. 2). Contrary to our results, Lee *et al.* (2013) reported proportional increase in TBARS value with increasing concentrations of powdered peanut sprout extract microcapsules-supplemented milk during storage.

Viability of *L. plantarum* in emulsion system

The data on viability of *L. plantarum* with PO or CO-SLNs in different continuous phase during storage at 7°C temperature are presented in Fig. 5. The PO or CO-SLNs was produced by the addition of various concentration (0, 0.1, and 1.0%) of oil, and different continuous phases (DW and UHTM), and kept at 7°C for 30 d (Fig. 5). The SLNs/DW or UHTM were inoculated with *L. plantarum* and its growth was observed to confirm the growth effect of SLNs. For the initial few days, the growth of *L. plantarum* was observed to be 10^8 CFU g/mL in all samples. By second day, the growth of *L. plantarum* in UHTM was higher than in DW regardless of SLNs addition. The amount of *L. plantarum* in UHTM was increased by second day to 10^9 CFU/mL with or without PO or CO-SLNs. However, in DW without SLNs, the viability of *L. plantarum* was significantly decreased to 10^7 CFU/mL. Interestingly, the viability of *L. plantarum* was constant in the DW with PO or CO-SLNs at concentrations of 0.1, and 1.0%. In UHTM, there was no significant difference of SLNs addition on the growth of *L. plantarum.* In other words, PO or CO-SLNs maintained viable about *L. plantarum* about 10^8-10^9 log CFU/mL in milk. It was hypothesized that the nutrients in milk such as lactose, sugar, protein, and vitamin might aid in the growth of *L. plan-*

tarum. Moreover, there was no remarkable difference in viability of *L. plantarum* depending on different oil types. Hence, we could conclude that PO or CO-SLNs were effective in maintain the growth of *L. plantarum* in DW during the storage period. In a study by Yang *et al.* (2014), the effects of blackberry juice on bacteria including pathogens and probiotic bacteria in addition to nutritional value were investigated. The results demonstrated growth inhibitory effects of blackberry juice on food borne pathogens and growth promontory effect on *Lactobacillus*. In contrast, recently, immense studies have been carried out on the antimicrobial activities of essential oil nanoemulsions in beverage industry or in food packaging system (Abdollahi *et al.*, 2012; Cava *et al.*, 2007; Jo *et al.*, 2015; Severino *et al.*, 2015). A study by Cava *et al.* (2007) reports about the antimicrobial activity of essential oils of cinnamon bark, cinnamon leaf, and clove against *Listeria monocytogenes* Scott A in semi-skimmed milk.

Conclusion

This study was carried out in order to investigate the effect of PO or CO on the physico-chemical and microbial properties in milk. The PO or CO was found to be physically stable in both DW and UHTM. Our study clearly demonstrates that PO or CO-SLNs can significantly promote the growth of *L. plantarum* in DW. However, no effects of PO or CO on the growth of *L. plantarum* in UHTM during refrigeration storage were observed. Therefore, it is appropriate to propose the possibility of production of PO or CO-SLNs and their application in development of SLNs beverages fortified with *Lactobacillus*. Further research on SLNs needs to be conducted to elucidate the inhibitory and growth promoting effect of PO or CO-SLNs on food borne pathogens and *Lactobacillus*, together in milk.

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