Physicochemical and sensory properties of milk supplemented with lactase microcapsules coated with enteric coating materials

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ABSTRACT

In this paper, we report the physicochemical and sensory properties of milk supplemented with a powder of microencapsulated lactase. The core material was lactase (β-galactosidase), the primary coating material was medium-chain triglyceride (MCT), and the secondary (enteric) coating material was either hydroxypropyl methylcellulose phthalate (HPMCP) or shellac, comparing both against market milk as a control. The physicochemical properties of both types of microcapsules were analyzed, including the particle size, zeta potential, and in vitro release behavior. To survey the stability of the microcapsules in milk during storage, we studied the residual lactose content and pH. Furthermore, to determine the properties of milk supplemented with the microcapsules, changes in color and sensory properties were evaluated during storage. The particle sizes (volume-weighted mean; D[4,3]) of the microcapsules coated with HPMCP or shellac were 2,836 and 7,834 nm, respectively, and the zeta potential of the capsules coated with shellac was higher than the zeta potential of those coated with HPMCP. The pH levels of milk supplemented with the lactase microcapsules were similar to those of the control (unsupplemented market milk); however, for milk supplemented with HPMCP-coated microcapsules, the pH was slightly lower. The core material, lactase, was released from the microcapsules during 12-d storage, and 18.82 and 35.09% of lactose was hydrolyzed in the samples for HPMCP- and shellac-coated microcapsules, respectively. The sensory characteristics of milk containing microcapsules coated with HPMCP did not show a significant difference during storage. However, that containing shellac-coated microcapsules was somewhat higher in color values than others. In particular, it showed significance from 0 to 4 d storage in L* and C* values. In conclusion, a powder of lactase microcapsules coated with HPMCP can be suitable as a supplement for milk.

Key words: lactose intolerance, microencapsulation, HPMCP, enteric coating

INTRODUCTION

Milk is an excellent source of high-quality protein, calcium, and riboflavin. Nonetheless, more than two-thirds of the world’s population cannot digest lactose effectively because of lactose intolerance, which makes milk consumption problematic (Messia et al., 2007). Lactose, a disaccharide found in milk at concentrations of 4.8 to 5.2%, is absorbed in the form of glucose and galactose after hydrolysis by lactase (β-d-galactosidase, EC 3.2.1.23) secreted in the mucosal membrane of the small intestine. Lactose that is not hydrolyzed and absorbed in the small intestine is passed directly into the colon in a person who cannot digest lactose smoothly. The undigested lactose increases the osmolarity of the digesta, causing inflow of water into the colon. Finally, the lactose is metabolized with an increase in acidity and production of gas and toxins by coliform bacteria; these processes cause diarrhea, abdominal pain, flatulence, and bloating (Heyman, 2006; Choi et al., 2007).

To solve this problem, various methods, such as enzyme immobilization techniques (Baret and Dohan, 1983; Lartillot, 1993) and ultrafiltration and reverse osmosis systems (Lange, 2005), have been developed. According to Onwulata et al. (1989), milk depleted of lactose by lactase immobilization has a sweeter taste than that of unmodified milk, due to the generated glucose and galactose, which have a sweeter taste than lactose by approximately 4 fold. Many people who have lactose intolerance do not prefer lactose-hydrolyzed...
milk. The ultrafiltration and reverse osmosis technique can selectively remove lactose from milk, but approximately 5% product loss occurs. Furthermore, drinking lactose-filtered milk is known to be ineffective for the absorption and translocation of calcium into bone tissues (Kwak et al., 2012). Moreover, this technique leads to a loss of the authentic sweet taste of milk. In general, the sensory properties of milk have a direct relation with consumer acceptability, and they are important factors in adult milk consumption (Francis et al., 2005; Adhikari et al., 2010).

The lactase microencapsulation technique has some advantages over other techniques. First, this technique can maintain the original sweet taste of milk and aid in calcium absorption in the intestinal tract due to nonadjustment of milk composition, such as removal of lactose from the milk. Consequently, this technique avoids the inconvenience of taking lactase separately after ingestion of milk. The procedure of microencapsulation is simple and cost effective (Ahn et al., 2013). Additionally, according to Kwak et al. (2001), milk supplemented with lactase microcapsules coated with medium-chain triglyceride (MCT) and polyglycerol monostearate (PGMS) has sensory characteristics similar to market milk. Nonetheless, because microcapsules contain liquid, they cannot be dried into a powdered form for long-term storage because the outer layer consists of an oil phase. A powder of lactase microcapsules coated with enteric material might address this problem. In one study, it was demonstrated that enteric-coated lactase microcapsules have good physicochemical properties and stability (Ahn et al., 2018b). No toxicity of hydroxypropyl methylcellulose phthalate (HPMCP) has been observed in toxicological tests, and it has been widely used as an enteric coating material (Kitagawa et al., 1970; Ito and Toida, 1972; Rowe et al., 2009). Shellac is listed as “generally recognized as safe” by the US Food and Drug Administration (FDA), as it is nontoxic and does not cause physiological harm (Hernandez, 1994). Nevertheless, there are insufficient reports on the physicochemical and sensory properties of milk containing enteric-coated lactase microcapsules. Therefore, the main objective of this study was to confirm the physicochemical characteristics of milk containing enteric-coated lactase microcapsules and to evaluate its sensory properties.

**MATERIALS AND METHODS**

**Materials**

Food-grade commercial lactase (Godo YNL-2) used as the core material was acquired from Godo Shusei Co. Ltd. (Tokyo, Japan); its activity was 7,448 unit/g. This lactase is a liquid enzyme prepared from Kluyveromyces lactis. According to the product information (certificate analysis) from the manufacturer, it contained no lipase and traces of protease and invertase (23 pU and 1.7 pU, respectively). One unit was designated as 1.0 μmol of o-nitrophenyl β-D-galactopyranoside hydrolyzed into o-nitrophenol and D-galactose by the lactase within 1 min. For lactase activity measurements, o-nitrophenyl β-D-galactopyranoside was purchased from Sigma-Aldrich (St. Louis, MO). For the primary and secondary enteric coatings, MCT and shellac were purchased from Weliga Co. Ltd. (Seongnam, Korea) and Shellac Korea Co. Ltd. (Busan, Korea), respectively, and HPMC was provided by Samsung Fine Chemical Co. Ltd. (Seoul, Korea). Various hydrophilic-lipophilic balance (HLB)–rated emulsifiers, such as polyglycerol polyricinoleate (HLB 0.6), decaglycerine, and polyoxyethylene sorbitan monolaurate (HLB 16.7), were provided by Ilshin Wells Co. Ltd. (Seoul, Korea). Pepsin (catalog no. P7000; EC 3.4.23.1, 250 unit/mg solid, from porcine gastric mucosa) and pancreatin (catalog no. P7545; EC 232–468-9, 8 × USP specification, from porcine pancreas) were purchased from Sigma-Aldrich. Ultra-high temperature and whole market milk (pasteurized at 130°C for 2 s, no composition adjustment) was purchased from Seoul Dairy Cooperative (Seoul, Korea). The market milk contained, per 200 mL, 9 g of carbohydrates, 8 g of fat, 6 g of protein, 0.1 g of sodium, and 0.2 g of calcium.

**Microencapsulation of Lactase: Emulsion Preparation**

Preparation of the secondary enteric coating material followed the method of Ahn et al. (2018b). In brief, the secondary enteric coating materials being tested, HPMC and shellac, were each dissolved in 80 mL of distilled water, where the pH was adjusted to 8.0 with 0.1 N sodium hydroxide. After that, the solutions were neutralized with 0.1 N hydrochloric acid, and the final volume was adjusted to 100 mL using distilled water. These solutions were desalinized by an ion exchange resin (Sigma-Aldrich) to eliminate salts.

Enteric-coated lactase microcapsules were produced using the method of Quispe-Condori et al. (2011), with slight modification. First, the primary emulsion was produced by dropwise addition of lactase (aqueous core material) into the oil-based primary coating material MCT, which included 0.75% polyglycerol polyricinolate as a hydrophobic emulsifier. The primary emulsification was performed with a high-speed homogenizer (HMZ-20DN, Poonglim Co., Seoul, Korea) at 9,000 rpm for 1 min. Second, the primary emulsion and the secondary enteric coating material solution, which contained 0.25% PSML, were homogenized at 2,500 rpm.
for 4 min. This final emulsion was frozen at −80°C (MDF-U53V, Sanyo Denki Co. Ltd., Tokyo, Japan) and freeze-dried (FDA 8212, Ishin Labs Co. Ltd., Dongduchun, Korea).

**Particle Size Analysis**

The sizes of the microcapsules with the two different coating materials were analyzed on a particle size analyzer (Mastersizer 2000, Malvern Panalytical Ltd., Malvern, UK). The particle size analysis was conducted in triplicate by dispersing samples in 10 mL of distilled water at 25°C at a 1:1,000 (wt/vol) ratio. The particle size was indicated as the volume-weighted mean particle size ($D_{[4,3]}$), and the distribution span was calculated using Eq. [1]:

$$\text{Span} = \frac{d(0.9) - d(0.1)}{d(0.5)},$$  \[1\]

where $d(0.1)$, $d(0.5)$, and $d(0.9)$ represent the diameters at which the accumulative sample volumes were under 10, 50, and 90%, respectively.

**Zeta Potential**

The zeta potentials of the lactase microcapsules were measured using a particle size analyzer (ELSZ2-PLUS, Otsuka Electronics Co. Ltd., Osaka, Japan). The zeta-potential analysis followed the method of Ahn et al. (2018a). In brief, 10 mg of each microcapsule sample was dispersed in 10 mL of deionized water and sonicated for 5 min. After 15 min standing, 1 mL of supernatant was collected and analyzed using the particle size analyzer (ELSZ2-PLUS, Otsuka Electronics Co. Ltd.). All the samples were analyzed in triplicate at 25°C.

**In Vitro Analysis**

**Simulated Gastric Digestion.** To survey the release behavior of the microcapsules in gastric conditions, simulated gastric fluid (SGF) was manufactured according to the method adopted from Luan et al. (2006) and Papagianni and Anastasiadou (2009), with minor modification. One gram of pepsin was added to 25 mL of 0.1 M hydrochloric acid to prepare the SGF. Next, for each type of microcapsule, 1 g of the microcapsule sample was dissolved in a 5% (wt/vol) lactose solution, and its pH was adjusted to 2.0, 3.0, or 4.0 with 6 M hydrochloric acid. After addition of 1 mL of SGF, the samples were incubated in a 37°C shaking water bath, with 100 rpm shaking, for 2 h. Samples were collected every 30 min for 2 h and incubated in ice for approximately 10 min to discontinue the pepsin-catalyzed digestion. The samples were centrifuged at 3,000 × $g$ for 3 min to collect the supernatant.

**Simulated Intestinal Digestion.** The release behavior of each type of microcapsule in a small intestinal environment was determined using the method from Miquel et al. (2006) and Papagianni and Anastasiadou (2009), with slight modification. To prepare the simulated intestinal fluid (SIF), 0.04 g of pancreatin and 0.25 g of deoxycholate were dissolved in 10 mL of 0.1 M sodium bicarbonate. Next, 1 g of each type of microcapsules was added to 100 mL of 5% (wt/vol) lactose solution. After that, the pH was adjusted to 6.0, 7.0, or 8.0 with 5 N sodium hydroxide by dropwise addition, and 1 mL of SIF was added. The samples were incubated in a 37°C shaking water bath for 2 h, with 100 rpm shaking, and collected at 30-min intervals for 2 h. The samples were placed in ice for 10 min to stop further digestion by SIF and were centrifuged at 3,000 × $g$ for 3 min to collect the supernatant.

**Physicochemical and Sensory Properties of Milk Containing Lactase Microcapsules**

**Powders of Microencapsulated Lactase Supplements.** In a preliminary test, it was confirmed that 0.15 g of lactase hydrolyzed the lactose of 200 mL of market milk at 37°C within 60 min. Both types of microcapsules contained approximately 7.4% lactase. Adding these capsules to 200 mL of market milk at 1% (wt/vol) was equivalent to the addition of 0.148 g of lactase (data not shown). Each sample was stored at 4°C for 12 d to evaluate pH, color, release of lactase from the microcapsules, and sensory characteristics.

**pH.** The pH levels of each milk sample was assessed at 4°C using a pH meter (Orion 900A, Thermo Fisher Scientific, Waltham, MA). Sample analyses were performed in triplicate.

**Color Measurement.** The color values of each milk sample were measured by means of a colorimeter (CT-310, Minolta, Tokyo, Japan) after calibration with a standard white plate ($X = 97.83$, $Y = 81.58$, $Z = 91.51$). The measured lightness, red-green color, and yellow-blue color were represented by $L^*$, $a^*$, and $b^*$, respectively, under artificial light. The chroma ($C^*$), which is a measurement of vividness, and hue angle ($h$), which is related to the color tone perceived by the human eye, were calculated using Eq. [2] and Eq. [3], respectively (Sah et al., 2016):

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2}$$  \[2\]  

and
Different values of hue angle represent various colors: 0° = red-purple, 90° = yellow, 180° = bluish green, and 270° = blue. Samples were analyzed in triplicate.

Quantitation of Residual Lactose. The concentrations of residual lactose in the milk supplemented with the powdered lactase microcapsules were assayed using a slightly modified method from Kwak and Jeon (1988). A mixture of 5 mL of sample and 25 mL of de-ionized water was transferred into a 50-mL volumetric flask, and the flask was filled with acetonitrile up to the volume mark. After mixing, the supernatant was collected from the suspension by centrifugation at 5,000 × g for 10 min. The supernatant was percolated with a 0.45-μm polyvinylidene difluoride syringe filter. The filtrate was analyzed by means of an HPLC system (Dionex Co., Sunnyvale, CA) using a refractive index detector (RI-101, Showa Denko K. K. Co., Tokyo, Japan). Lactose was analyzed on an NH2 column (4.6 × 250 mm, Hector-M, RS Tech Co., Daejeon, Korea). As a mobile phase, 75% (vol/vol) acetonitrile was used with a flow rate of 2.0 mL/min. The sample injection volume was 20 μL. The sample analyses were performed in triplicate.

Sensory Evaluation. Ten panelists (3 men and 7 women) who were nonsmokers, in good health, and with a mean age of 26 yr (range: 23–39 yr) were selected from the graduate student population in the Department of Food Science Technology at Sejong University, Seoul, Korea. Three 1-h training sessions were held for the panelists with hydrolyzed lactose milk, for sweetness and for off-taste, using the method of Jimenez et al. (2008), Sensory Evaluation Technology at Sejong University, Seoul, Korea. Three 1-h training sessions were held for the panelists with hydrolyzed lactose milk, for sweetness and for off-taste, using the method of Jimenez et al. (2008). The commercial milk containing 1% (wt/vol) of lactase microcapsules was stored at 4°C for 12 d. The samples were poured into 50-mL transparent plastic cups with lids. Four-digit random codes were attached to the cups, and the samples were served in triplicate.

RESULTS AND DISCUSSION

Physicochemical Properties of Enteric-Coated Lactase Microcapsules

Particle Size. The size of the microcapsules coated with HPMCP or shellac is shown in Table 1. The microcapsules coated with HPMCP were smaller those coated with shellac. The sizes of both HPMCP- and shellac-coated microcapsules were less than 10,000 nm. Homogenized milk fat globules are usually 2,000 to 5,000 nm in size and dispersed stably in milk. The span values of the microcapsules coated with HPMCP and shellac were 1.005 and 3.257, respectively. Decrease of span value implies narrow particle size distribution, which indicates that particles are distributed with similar size fraction (Bitra et al., 2009). According to this finding, both types of microcapsules can be expected to be evenly dispersed in milk.

Size profile can also be used to predict the release properties of microcapsules. According to Bezemer et al. (2000), the release profile of a microsphere is affected by its size; release rate was observed to decrease with increasing particle size. Berkland et al. (2002) also reported that drug release increases as particle size decreases, and the release profile of microspheres can be controlled by their size. Therefore, due to their larger size than the microcapsules coated with HPMCP, those coated with shellac could be expected to be more degradable than those coated with HPMCP.

Zeta Potential. The zeta potentials of the lactase microcapsules coated with HPMCP or shellac are presented in Table 1. The absolute values of the zeta potential of the microcapsules coated with HPMCP sig-
nificantly increased as the pH increased from 6 to 8 ($P < 0.05$). In contrast, that of the microcapsules coated with shellac somewhat decreased as the pH increased, and there was no significant difference in its zeta potential ($P > 0.05$). According to Salopek et al. (1992) and Freitas and Müllerä (1998), the zeta potential can be indexed to explain the stability of a colloidal system. If a colloidal system has a zeta potential over ±30 mV, then the system is considered to have good stability. Systems with values over ±60 mV are considered to have very good stability. In this study, the zeta potentials of the microcapsules coated with HPMCP or shellac were approximately −37.70 and −88.50 mV at pH 6.0, −43.81 and −87.10 mV at pH 7.0, and −49.95 and −87.00 mV at pH 8.0, respectively. Generally, the pH of fresh milk is approximately 6.6 to 6.8. Therefore, we expected that these microcapsules can be stably scattered in milk. The shellac-coated microcapsules had a higher zeta potential than the HPMCP-coated microcapsules; this finding is probably due to the negative charge of the carboxylic ion (−COO⁻). Soradech et al. (2013) reported that when a 50% (wt/wt) gelatin solution, which has a positive charge because of the −NH₃⁺ groups, was added to shellac, it caused a reduction of the zeta potential during composite film formation. According to the results of the particle size and zeta potential, our microcapsules could be finely dispersed in milk to ensure adequate sensory properties during milk consumption.

**Simulated Gastric Digestion.** The release rate of lactase from the microcapsules in SGF and SIF is indirectly represented using the hydrolysis rate of lactose. Figure 1 illustrates the rate of hydrolyzed lactose following the lactase release from the microcapsules coated with HPMCP or shellac in simulated gastric environment; this release was feeble. As the pH increased from 2.0 to 4.0, the amount of hydrolyzed lactose was increased. The microcapsules coated with HPMCP hydrolyzed 0.1, 0.2, and 0.3% of lactose at pH 2.0, 3.0, and 4.0, respectively. Those coated with shellac hydrolyzed 0.2, 0.3, and 0.4% of lactose at pH 2.0, 3.0, and 4.0, respectively. However, there were almost no significant differences between before (0% at initial) and after hydrolysis ($P > 0.05$). This means that both microcapsules were stable in the simulated gastric environment, and the release rate of the microcapsules coated with HPMCP was less than that of those coated with shellac. In addition, we found no significant difference between HPMCP and shellac when the release rates of the capsules were compared at the same pH and hydrolysis time ($P > 0.05$). This result showed a similar tendency to the report by Kim et al. (2003), who demonstrated that diclofenac nanocapsules coated with HPMCP released ~0.2% of their core material under simulated gastric conditions after 120 min. Furthermore, Qussi and Suess (2005) reported that a drug pellet coated with various materials, such as aqueous shellac, polyvinyl alcohol, hydroxypropyl methylcellulose, or carbomer 940, hardly released the drug in SGF after 120 min. According to the report by Toorisaka et al. (2005), less than 4% of insulin was released after 120 min in SGF from an insulin-loaded enteric-coated dry emulsion. According to Kwak et al. (2002), single-layered lactase microcapsules coated with MCT or PGMS released lactase at ~13% and 15% in SGF within 60 min. It appears that our enteric-coated microcapsules effectively protected lactase as a core material under gastric conditions and were superior in this regard to single-layered microcapsules coated with MCT or PGMS.

**Simulated Intestinal Digestion.** The in vitro release behavior of the lactase capsules coated with HPMCP or shellac under a simulated intestinal environment is presented in Figure 2. As the pH increased from 7.0 to 8.0, more microcapsules released lactase under the simulated intestinal conditions. The capsules coated with HPMCP or shellac showed a rapid release behavior: 55 to 75% and 61 to 87% of lactase released, respectively, at pH 7.0; and 73 to 93% and 83 to 97% of lactase released, respectively, at pH 8.0. Meanwhile, the release rates were 1.5 to 2% and 2.5 to 5%, respectively, at pH 6. All samples in SIF showed significant differences before (0% at initial) and after hydrolysis ($P < 0.05$ or $P < 0.01$). Also, as a result of comparing release rates of the capsules at the same pH and hydrolysis time, significant differences between HPMCP and shellac were found at 90 and 120 min at pH 7, and at 60 and 90 min, at pH 8 ($P < 0.05$). This result is different from hydrolysis in SGF. Current evidence suggests that HPMCP and shellac are enteric coating materials and can easily release the core material from microcapsules coated with them in SIF condition. This result is not inconsistent with the data reported by Limmatvapirat et al. (2007), that the degradation rate of shellac is relatively slow in the intestinal environment because the degradation pH of the shellac is 7.0 to 7.3. Nonetheless, the results of our experiment are in agreement with reports by Kim et al. (2003), Patel et al. (2013), and Farag and Leopold (2011). Kim et al. (2003) demonstrated that diclofenac nanocapsules coated with HPMCP released 70 to 90% of their core material within 30 min. Furthermore, Patel et al. (2013) found that 90% of epigallocatechin gallate was released during a 1-h span from microcapsules coated
with a shellac-gelatin mixture. Additionally, according to the study by Farag and Leopold (2011), as the pH of the simulated conditions increased from 6.8 to 7.4, the release rate of the core material increased from 60 to 90% within 30 min from theophylline capsules coated with shellac by means of a bed coater. The results of this study showed a release rate of the core material from lactase microcapsules slightly lower than the results from other studies, and we believe this to be due to the double layer of our microcapsules. Nonetheless, we anticipate no likelihood of serious problems with digestion of lactose in the human intestine after drinking these experimental milks, since the small intestinal transit time is known to be approximately 255 to 275 min (Worsøe et al., 2011).

Makino et al. (2000) determined the drug release rate of fabricated microspheres with different molecular weights (MW). In their study, the release rate of poly(lactide-co-glycolide) microspheres tended to increase as the MW decreased. Tuncay et al. (2000) and Ravivarapu et al. (2000) reported similar results on the MW of blends of polyglycolic acid and poly(lactic-co-glycolic) acid, respectively, finding that the microparticles containing the lower-MW polymer released their core materials more rapidly. Generally, the MW of shellac and HPMCP are 1006 and 37,860 Da, respectively (Fukasawa and Obara 2003; USDA, 2014). We suspect that the release rate of microcapsules coated with shellac could be higher than that of microcapsules coated with HPMCP because the MW of shellac is lower than that of HPMCP.

According to the report by Harshna and Solanki (2012), enteric coating materials are generally designed to withstand gastric conditions and dissolve rapidly in the intestine. All enteric coating materials possess an ionizable free carboxylic acid group derived from a phthalyl moiety. In general, the pH of the medium and pKₐ of the polymer determine the equilibrium between an unionized insoluble and ionized soluble polymer. The equilibrium between ionized and unionized polymers can determine the release properties of enteric coating materials; therefore, release properties can be predicted by the Henderson–Hasselbalch equation. Although the capsules coated with shellac have better release properties than those coated with HPMCP in SIF, as shown in our study, it is likely that the capsules coated with HPMCP are more suitable for supplementation of milk than those containing shellac, owing to the stability of HPMCP in milk during storage and its white color.

Properties of Milk Supplemented with Lactase Microcapsules

Changes in pH. The changes in the pH of milk supplemented with a powder of microencapsulated lactase coated with HPMCP or shellac and maintained at

![Figure 1](image-url). In vitro lactase release from lactase microcapsules coated with hydroxypropyl methylcellulose phthalate (HPMCP) or shellac, in a simulated gastric environment, at pH 2.0, 3.0, and 4.0, over 120 min, measured as % hydrolyzed lactose. Values are expressed as mean ± SD (n = 3). Means marked with * and ** differ significantly from “before hydrolysis” (0 at initial) values at P < 0.05 and 0.01, respectively.
4°C for 12 d are illustrated in Figure 3. The pH values of all the milk samples were nearly constant during the 12 d of storage. The pH ranges of the control and of milk supplemented with lactase microcapsules coated with HPMCP or shellac were 6.79 to 6.80, 6.65 to 6.53, and 6.81 to 6.80, respectively. The pH values of milk supplemented with shellac-coated microcapsules were similar to those of the control. Shellac is a material that consists of a weak acid such as aleuritic acid, shelloic acid, or aliphatic dicarboxylic acid, and it has a pH-dependent dissolution profile (Challinor, 2007; Farag and Leopold, 2009). The acid value of shellac decreased due to polymerization by esterification during storage. Nonetheless, the dissolution properties of shellac were not affected by this (Farag and Leopold, 2009). We consider this the reason that the pH of milk supplemented with shellac-coated microcapsules was similar to that of the control.

The pH values of HPMCP-coated microcapsules were the lowest and slightly decreased during storage. This change may have been caused by the slight liberation of the phthalyl group, which confers enteric properties (such as pH sensitivity) to HPMCP; however, this characteristic cannot influence the quality of milk. The MW of HPMCP is approximately 37,000 to 60,000 Da (Fukasawa and Obara, 2003), whereas low-MW phthalate esters such as diethyl phthalate, diisodecyl phthalate, and diisononyl phthalate used for plasticizers, cosmetics, and food packaging, are approximately 194 to 530 Da (Schettler, 2006). Nevertheless, low-MW phthalate esters have been linked with breast cancer (Lopez-Carillo et al., 2010), endocrine disruption (Swan et al., 2005), abdominal obesity (Desvergne et al., 2009), allergies (Bornehag et al., 2004; Bertelsen et al., 2013), low birth weight (Zhang et al., 2009), and attention deficit hyperactivity disorder (ADHD; Kim et al., 2009).

In contrast, Kitagawa et al. (1970) could not find any toxicity, mortality, clinical signs, or histopathologies due to HPMCP in a subacute-toxicity and chronic study on rats. Furthermore, Ito and Toida (1972) reported that oral medication of HPMCP up to 2,400 mg/kg per day during pregnancy caused no maternal toxicity, embryo-fetotoxicity, or teratogenic effects, and no adverse effects arose in mice fetuses following oral administration of up to 4,000 mg/kg per day on d 7 to 12 of pregnancy. Furthermore, Kitagawa et al. (1971,
1974) conducted 2 studies on absorption, distribution, metabolism, and excretion in rats using $^{14}$C-labeled methoxyl and phthalyl groups of HPMCP at doses of 3,000 and 1,300 mg/kg, respectively. Approximately 96% of each dose was excreted in feces within 72 to 96 h, and 0.7 to 1.2% of doses were excreted in urine in these studies. These data indicate poor oral absorption of this high-MW polymer.

Changes of Color. Table 2 depicts the changes in the color of the samples of milk supplemented with a powder of lactase microcapsules during storage at 4°C for 12 d. The lightness ($L^*$) significantly decreased with the addition of lactase microcapsules coated with shellac for 4 d; however, there were no significant changes thereafter ($P > 0.05$). Milk supplemented with capsules coated with HPMCP did not show any significant differences from the control ($P > 0.05$). Milk supplemented with shellac-coated microcapsules showed the highest $L^*$ values throughout the storage period compared with the others, with significance $P < 0.05$. We believe this to be caused by the color of shellac. In general, shellac has a yellow or light brown color. Thus, we speculate that the addition of microcapsules coated with shellac changed the $C^*$ value of the milk. On the other hand, the addition of microcapsules coated with HPMCP or shellac did not affect the hue value ($h^*$), and the values were not significantly different than those of the control ($P > 0.05$). According to the results of this study, it is likely that the color parameters of milk were not influenced by supplementation with HPMCP-coated lactase microcapsules.

Stability of Powdered Lactase Microcapsules in Milk. The residual lactose content of the milk samples during storage was measured to examine the stability (lactase holding capacity) of the outer layer of the powdered lactase microcapsules coated with HPMCP or shellac, as depicted in Figure 4. The concentrations of residual lactose in the milk samples supplemented with HPMCP- or shellac-coated microparticles decreased to

![Figure 3. Changes in the pH of milk supplemented with a powder of lactase microcapsules coated with hydroxypropyl methylcellulose phthalate (HPMCP) or shellac compared with unsupplemented market milk (control), stored at 4°C for 12 d. Each point indicates mean ± SD (n = 3).](image-url)
81.81 and 64.91%, respectively, during the 12-d storage period. This means that approximately 18.82 and 35.09% of lactose in the milk samples, respectively, were hydrolyzed. These results may have been due to the release of lactase from the microcapsules. The concentration of residual lactose in milk supplemented with the shellac-coated microcapsules decreased dramatically from 4 d, and decreased more than the milk containing microcapsules coated with HPMCP. This result suggests that HPMCP as a coating material is more stable than shellac in milk. The lactase encapsulated with HPMCP was gradually released during storage, which resulted in a gradual increase of sweetness in the sensory evaluation (Table 3). However, we did not find a significant difference in sweetness between the control and the milk supplemented with lactase microcapsules coated with HPMCP. During the storage period, release rate of the shellac-coated microcapsules was higher than that of the HPMCP-coated microcapsules, causing a significant increase of sweetness in the milk supplemented with shellac-coated microcapsules from d 8 of storage compared with the control milk (Table 3; $P > 0.05$). According to Kwak et al. (2001), microcapsules coated with PGMS and MCT, which were washed once, hydrolyzed 28.81% of the lactose in milk in 12 d. Therefore, the stability of lactase microcapsules coated with HPMCP in milk was better than that reported in the study by Kwak et al. (2001).

**Sensory Evaluation.** The sensory properties of the milk supplemented with the microcapsules during storage at 4°C for 12 d are given in Table 3. The sweetness of the milk supplemented with the HPMCP-coated microcapsules slightly increased during the 12-d storage; however, significant differences were not observed ($P > 0.05$). The sweetness of the milk supplemented with the shellac-coated microcapsules also increased during the 12-d storage, but no significant changes in this type of milk were observed after 8 d ($P > 0.05$). As shown in Figure 4, more shellac-coated lactase capsules degraded than did HPMCP-coated lactase capsules; therefore, more lactose was hydrolyzed during storage in the milk containing shellac-coated microcapsules compared with the microcapsules coated with HPMCP, owing to the stability of HPMCP, thereby influencing the sweetness.

The off-taste of milk supplemented with both types of microcapsules slightly increased, and a significant difference was observed after 8 d ($P < 0.05$). Nonetheless, the off-taste of milk supplemented with the HPMCP-coated microcapsules did not show a significant change throughout storage at 4°C for 12 d, whereas milk supplemented with the shellac-coated microcapsules showed a significant change from d 0 to d 10 and thereafter ($P < 0.05$). We suspect that the off-taste was caused by the properties of shellac. Shellac is known as a tasteless enteric coating material. However, it can have some odor, which is a result of the complex fragrance system (Buchbauer et al., 1993). Therefore, although it is a tasteless enteric coating material, the sensory properties of materials containing shellac are thought to be influenced by its odor. On the basis of the results of this study, we conclude that the powder of lactase microcapsules coated with HPMCP is more stable and suitable for the supplementation of milk than that coated with shellac, and milk supplemented with the former was found to have properties similar to those of unsupplemented market milk during the sensory evaluation for approximately 1 wk. The shelf life of market milk was reported to be approximately 10 d during storage at 4°C (Allen and Joseph, 1985; Zygoura et al., 2004; Grandy et al., 2008). According to Van Boxstael et al. (2014), among dairy products, the consumption of market milk is most influenced by

### Table 2. Color changes in milk supplemented with a powder of lactase microcapsules coated with hydroxypropyl methylcellulose phthalate (HPMCP) or shellac during storage at 4°C for 12 d

<table>
<thead>
<tr>
<th>Color value</th>
<th>Treatment</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lightness ($L^*$)</td>
<td>Control$^a$</td>
<td>90.45$^{-AB}$</td>
<td>90.05$^{-AB}$</td>
<td>89.96$^{-B}$</td>
<td>90.09$^{-AB}$</td>
<td>91.01$^{-A}$</td>
<td>90.11$^{-AB}$</td>
<td>90.17$^{-AB}$</td>
</tr>
<tr>
<td>HPMCP</td>
<td>90.44$^{-A}$</td>
<td>89.99$^{-A}$</td>
<td>89.83$^{-A}$</td>
<td>89.89$^{-A}$</td>
<td>89.46$^{-A}$</td>
<td>90.05$^{-A}$</td>
<td>90.14$^{-A}$</td>
<td></td>
</tr>
<tr>
<td>Shellac</td>
<td>88.90$^{-AB}$</td>
<td>89.30$^{-A}$</td>
<td>89.30$^{-A}$</td>
<td>89.44$^{-A}$</td>
<td>89.31$^{-A}$</td>
<td>89.58$^{-A}$</td>
<td>89.85$^{-A}$</td>
<td></td>
</tr>
<tr>
<td>Chroma ($C^*$)</td>
<td>Control</td>
<td>6.91$^{-B}$</td>
<td>6.80$^{-AB}$</td>
<td>6.96$^{-AB}$</td>
<td>6.88$^{-AB}$</td>
<td>7.06$^{-A}$</td>
<td>6.90$^{-AB}$</td>
<td>6.87$^{-B}$</td>
</tr>
<tr>
<td>HPMCP</td>
<td>6.91$^{-B}$</td>
<td>6.75$^{-C}$</td>
<td>6.84$^{-BC}$</td>
<td>6.86$^{-BC}$</td>
<td>7.18$^{-A}$</td>
<td>6.76$^{-BC}$</td>
<td>6.82$^{-BC}$</td>
<td></td>
</tr>
<tr>
<td>Shellac</td>
<td>7.53$^{-A}$</td>
<td>7.33$^{-AB}$</td>
<td>7.18$^{-ABC}$</td>
<td>6.95$^{-BC}$</td>
<td>7.57$^{-A}$</td>
<td>6.89$^{-C}$</td>
<td>7.28$^{-ABC}$</td>
<td></td>
</tr>
<tr>
<td>Hue angle ($h^*$)</td>
<td>Control</td>
<td>118.79$^{-C}$</td>
<td>120.86$^{-A}$</td>
<td>119.44$^{-BC}$</td>
<td>120.11$^{-B}$</td>
<td>118.89$^{-C}$</td>
<td>120.39$^{-A}$</td>
<td>119.10$^{-C}$</td>
</tr>
<tr>
<td>HPMCP</td>
<td>119.28$^{-BC}$</td>
<td>121.05$^{-A}$</td>
<td>120.02$^{-BC}$</td>
<td>120.31$^{-AB}$</td>
<td>118.99$^{-B}$</td>
<td>120.89$^{-A}$</td>
<td>119.91$^{-A}$</td>
<td></td>
</tr>
<tr>
<td>Shellac</td>
<td>119.36$^{-BC}$</td>
<td>119.44$^{-BC}$</td>
<td>119.00$^{-BC}$</td>
<td>120.33$^{-B}$</td>
<td>119.73$^{-A}$</td>
<td>122.34$^{-A}$</td>
<td>118.48$^{-C}$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Values with different superscript letters within a color value in columns are significantly different at $P < 0.05$ according to Duncan’s multiple range test.

$^A$Values with different superscript letters in rows are significantly different at $P < 0.05$ according to Duncan’s multiple range test.

$^1$Control: market milk.
the shelf life. Although recently, the shelf life of market milk has been extended by the introduction of the extended shelf life process (Goff and Griffiths, 2006), and consumers perceive that the only way to stop the spoilage process of market milk is to consume it immediately (Tsiros and Heilman, 2005). Thus, the stability of the microcapsules coated with HPMCP in milk for 1 wk should be sufficient to provide consumers with a unique taste of milk similar to regular market milk. Furthermore, Adhikari et al. (2010) stated that the acceptability of lactose-hydrolyzed milk is lower than that of regular market milk because of its sweetness. Judging by the results of this study, we determined that milk containing lactase microcapsules coated with HPMCP was highly acceptable and could be consumed similarly to regular market milk.

**Table 3.** Sensory evaluation of milk supplemented with a powder of lactase microcapsules coated with hydroxypropyl methylcellulose phthalate (HPMCP) or shellac during storage at 4°C for 12 d

<table>
<thead>
<tr>
<th>Sensory description</th>
<th>Storage period (d)</th>
<th>Treatment</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.00a,A</td>
<td>1.00a,A</td>
<td>1.00a,A</td>
<td>1.00a,A</td>
<td>1.00b,A</td>
<td>1.00b,A</td>
<td>1.00b,A</td>
</tr>
<tr>
<td>Sweetness</td>
<td></td>
<td>HPMCP</td>
<td>1.00a,A</td>
<td>1.10a,A</td>
<td>1.20a,A</td>
<td>1.20a,A</td>
<td>1.20ab,A</td>
<td>1.30ab,A</td>
<td>1.40ab,A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shellac</td>
<td>1.00c,C</td>
<td>1.10b,BC</td>
<td>1.30b,BC</td>
<td>1.30b,ABC</td>
<td>1.40b,ABC</td>
<td>1.50b,AB</td>
<td>1.70a,A</td>
</tr>
<tr>
<td>Off-taste</td>
<td></td>
<td>Control</td>
<td>1.00a,A</td>
<td>1.00a,A</td>
<td>1.00a,A</td>
<td>1.00a,A</td>
<td>1.00b,A</td>
<td>1.00b,A</td>
<td>1.00b,A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPMCP</td>
<td>1.00c,C</td>
<td>1.10b,BC</td>
<td>1.30b,BC</td>
<td>1.40b,BC</td>
<td>1.60b,ABC</td>
<td>1.70b,AB</td>
<td>1.90a,A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shellac</td>
<td>1.00d,B</td>
<td>1.30c,CD</td>
<td>1.40c,CD</td>
<td>1.70b,BC</td>
<td>1.90b,AB</td>
<td>2.30b,A</td>
<td>2.60a,A</td>
</tr>
</tbody>
</table>

*a–c Values with different superscript letters within a sensory description category in columns are significantly different at *P* < 0.05 according to Duncan’s multiple range test.

A–D Values with different superscript letters in rows are significantly different at *P* < 0.05 according to Duncan’s multiple range test.

Sweetness and off-taste were evaluated on a 5-point scale: 1 = none, 3 = moderate, 5 = very strong. Values shown are means across 10 trained panelists.

Control: market milk.
CONCLUSIONS

The present study indicates that lactase microcapsules coated with HPMCP or shellac have good size distribution, zeta potential, and in vitro release characteristics for the supplementation of milk. In addition, the pH of milk containing these microcapsules is almost identical to that of market milk. Furthermore, the HPMCP-coated microcapsules added to milk do not influence its color. The stability of the outer layer of the microcapsules coated with HPMCP is superior to that of the microcapsules coated with shellac according to the sensory evaluation of milk. Overall, we find that powdered lactase microcapsules coated with HPMCP can be added into milk to help reduce the effects of lactose intolerance and may be expected to contribute to increased milk consumption by adults. Furthermore, because encapsulation techniques and new types of enteric coating materials are continuously being developed, future study will likely find still more suitable and effective techniques and new enteric coating materials that have high stability, good safety ratings, and proper capsule formation.

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REFERENCES


