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Calcium-alginate microparticles for sustained release of catechin prepared via an emulsion gelation technique

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Abstract Catechin-loaded Ca-alginate beads and microparticles were prepared by an emulsion gelation method using sunflower oil for efficient sustained release of catechin. The emulsion was prepared by sequential mixing of alginate, oil, and oleic acid ester as an emulsifier. Encapsulation efficiency (EE) and inhibition of catechin release of the beads were significantly increased approximately to 453.83 and 148.71% by the emulsion gelation technique, respectively ($p<0.05$). For the microparticles, the highest inhibition of catechin release after 1 h of incubation (78.82%) was observed at the microparticles prepared by 5% (w/w) oil, 3% (w/w) alginate, 4% (w/v) $CaCl_2$, and 200 mg catechin with the most hydrophilic emulsifier, decaglycerol mono-ester. Moreover, the catechin release was sustained at acidic conditions and increased with increase in pH of release medium. These results suggest that catechin encapsulation within Ca-alginate particles by emulsion gelation method can be an effective delivery system for catechin.

Keywords: catechin, calcium alginate microparticle, ionic gelation, release property, emulsion gelation technique

Introduction

Catechin, a major polyphenolic compound of green tea, has received a great deal of attention due to its pharmacological effects including antioxidant, antibacterial, and antitumor activities (1). However, low stability during digestion and short duration in plasma are regarded as problems of oral administration (2,3). Therefore, methods for protecting the degradation of catechin from the external environment and improving the low bioavailability have been required (4).

Biopolymeric delivery systems using encapsulation techniques have been studied for improving the stability and bioavailability of bioactive ingredients (5,6). Moreover, encapsulation by ionic gelation of natural polysaccharides, avoiding organic solvent and thermal stress have been proposed as attractive oral delivery systems for sensitive ingredients (7). Alginate, a representative natural polysaccharide, which forms strong gel complexes in presence of Ca^{2+} ions has been reported to be suitable for food applications due to its non-toxicity and biocompatibility (8). Particularly, Ca-alginate particles are stable in acidic and degradable in neutral or alkaline conditions, and result in sustained release of core materials in gastric environments (9). Thus, bioactive materials such as probiotics, and vitamins within Ca-alginate particles were gradually released during digestion and delivered effectively to absorption site of intestine, led to increase of acidic stability and bioavailability (10,11). However, encapsulation of hydrophilic or low molecular materials such as ascorbic acid and catechin showed problems of easily diffusion and fast release through the ionic gel network regardless of pH (12).

The emulsion gelation technique using oil based on ionic gelation has been reported to enhance the barrier function of gel network against hydrophilic compounds (13). In previous investigations, metformin hydrochloride, a hydrophilic anti-diabetic drug, was effectively encapsulated in alginate gel beads containing groundnut, castor, and mentha oil (14). Furthermore, olive oil, carnauba, and spermaceti wax incorporated Ca-pectin gel beads with polyethylene glycol and glutaraldehyde as hardening agents were improved drug release behavior (15,16). Therefore, the emulsion gelation techniques are considered as a promising approach for the encapsulation of catechin (17).

Selecting the particle types depending on particle size for appropriate application is important because particle characteristics determine the functionality of the particles in food matrices (18). However, emulsion gelation techniques were mainly investigated in macro beads. Furthermore, macro and microparticles have been investigated in separate independent studies with different preparation conditions and core materials. Therefore, assessing the macro and microparticles with coherent conditions in single study can reveal the characteristics of particles more clearly.

The objective of this study was to investigate the release properties

of catechin-loaded Ca-alginate particles using the emulsion gelation technique. The catechin-loaded particles were prepared in macro-size beads and microparticles and assessed the effect of particle size on catechin encapsulation. Moreover, the effects of different preparation conditions on the characteristics of the beads and microparticles, including encapsulation efficiency (EE), morphology, swelling, and release properties were also investigated.

Materials and Methods

Materials Sodium alginate (350 cps in a 1%(w/v) solution at 20°C) and calcium chloride were purchased from Kanto Chemical Co. (Kyoto, Japan) and Yakuri Chemical Co. (Kyoto, Japan), respectively. Catechin was supplied by Unigen Co. (Cheonan, Korea) and the polyglycerol esters of oleic acid produced by Sakamoto Yakuhin Kogyo Co. (Osaka, Japan) were kindly supplied by Metro B & F Co. (Seoul, Korea). Sunflower oil was purchased from Sempio Co. (Seoul, Korea). Other chemicals were reagent grade and used without further purification.

Preparation of catechin-loaded beads and microparticles Catechin-loaded beads and microparticles were prepared by the emulsion gelation method using a size extrusion process (14). For beads, catechin, sunflower oil, and the 1% (w/w) emulsifier were mixed into 3% (w/w) sodium alginate solution using overhead stirrer at 3,000 rpm (HS-120A; Daihan Co., Seoul, Korea), as outlined in Table 1. The resulting emulsions were extruded into 4% (w/v) $CaCl_2$ solution using a peristaltic pump at 1 mL/min (Masterflex 7520-57; Cole Parmer Inc., Vernon Hills, IL, USA) through a 2 mm diameter plastic tube. The resulting beads were kept with constant agitation in the $CaCl_2$ solution for 5 min at room temperature, filtered, and air-dried at 40°C for 12 h (J-300M; Jisico Co., Seoul, Korea).

For microparticles, the resulting emulsions of alginate, catechin, oil, and emulsifier, prepared as outlined in Table 2, were sprayed into the $CaCl_2$ solution at fixed air pressure of 0.4 MPa using an air atomizing system (151BL-6L; Spraying System Co., Incheon, Korea) (19). The microparticles were collected using a sieve (200 mesh) after hardening in the $CaCl_2$ solution for 5 min at room temperature and lyophilized (FD8508; Ilshin Co., Seoul, Korea).

Encapsulation efficiency Catechin was extracted from the beads and microparticles using a 50% aqueous methanol solution containing 0.1%(v/v) H_3PO_4 with 100 rpm of stirring for 12 h (MS-MP8; Daihan Co.) followed by sonication for 20 min (JAC 1505; Jinwoo Co., Seoul, Korea). The extracted catechin solution was mixed at 1:10 (v/v) ratio with 0.1% (w/v) 4-dimethylamino-cinnamaldehyde (DMACA) in methanol/HCl (9:1, v/v). The mixture was vortexed (G-560; Scientific Industries, Bohemia, NY, USA) and centrifuged at 2,000× *g* for 1 min (Micro 12; Hanil Co., Seoul, Korea). After allowing color formation for 6 min, the absorbance of the resulting supernatant was measured at UV absorbance of 622 nm (Biomate 3S; Thermo Scientific, Madison, WI, USA) (20). The catechin encapsulation efficiencies (EE) of the beads and microparticles were determined as follows (21):

$$\text{Encapsulation efficiency (\%)} = W_1/W_2 \times 100 \quad (1)$$

where W_1 was the measured amount of catechin in the particles and W_2 was the theoretical amount of catechin in the particles.

***In vitro* release properties** Dissolution media for *in vitro* release studies were prepared using 0.05 M sodium chloride adjusted to pH 3.0 with 0.1 M HCl and 0.05 M sodium dihydrogen phosphate buffer adjusted to pH 5.0 and pH 7.0 with 0.1 M NaOH, respectively (22). Catechin-loaded particles were immersed in each different pH solution at 10, 20, and 30°C, respectively, with 100 rpm of shaking (J-USR; Jisico Co.). Each sample was collected at 30, 60, 120, and 240 min and centrifuged at 2,000× *g* for 5 min. The released catechin in the supernatant was determined as described above and calculated as follows (21):

$$\text{Fractional release (\%)} = W_3/W_4 \times 100 \quad (2)$$

where W_3 was the amount of catechin released from the particles and W_4 was the amount of catechin initially entrapped in the particles.

Swelling properties The swelling properties of the microparticles were measured with the weight of the particles after exposure to the different pH solutions and temperature as the same conditions as in the *in vitro* release studies. Accurately weighed amounts of the microparticles were suspended in Eppendorf tubes with each different pH solutions at 10, 20, and 30°C, and shaken at 100 rpm. The tubes were collected at 60, 120, and 240 min, centrifuged at 2,000× *g* for 5 min, and the swollen microparticles were weighed after remove the supernatant. The swelling degree of particles was determined as follows (23):

$$\text{Swelling degree} = (W_5 - W_6)/W_6 \quad (3)$$

where W_5 was the weight of the swollen catechin-loaded micro-particles and W_6 was the initial weight of the dried catechin-loaded microparticles.

Physical characterizations Particle size and dispersity of the microparticles were analyzed in suspensions using laser diffraction particle analyzer (Mastersizer S; Malvern, Worcestershire, UK). The observation of surface morphology was carried out using a field emission scanning electron microscope (FE-SEM; JSM-6330F, JEOL, Tokyo, Japan). The freeze-dried microparticles were mounted on a metal grid using carbon tape, followed by coating with approximately 30-nm thickness of gold. Electron micrographs were taken at 1,500× magnification.

Statistical analysis All experiments were repeated triplicate and

expressed as mean±standard deviation. Significant differences among the corresponding mean values were determined at $p<0.05$ using one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test (SPSS 12.0.1; SPSS Inc., Chicago, IL, USA).

Results and Discussion

Encapsulation efficiency of beads Three types of emulsions were prepared with the following ingredients added in the order listed: AOC type; alginate, oil, and catechin, OCA type; oil, catechin, and alginate, and ACO type; alginate, catechin, and oil. The beads by ACO type emulsion showed significantly higher EE which was approximately 32.38% compared with the other types of beads (Table 1). It can be assumed that, the catechin could not disperse uniformly in the oil contained solution during the mixing of the OCA and AOC type emulsion due to its low lipid solubility. However, mixing the catechin powder with the aqueous alginate solution first led to satisfactory catechin dispersion in the ACO type emulsion (24). Moreover, catechin was entrapped stably within the ACO type emulsion and inhibited diffusion into the $CaCl_2$ solution during the gelation process, resulting in significantly the highest EE. Therefore, the ACO type was revealed to be suitable procedure for further preparations of catechin-loaded particles in this study. EE of the beads was drastically increased by using oil approximately from 9.14 to 22.54%, and significantly increased with an increase in oil concentration from 2.5 to 10%. It can be explained that increase in oil ratio in the beads inhibited catechin diffusion into the aqueous $CaCl_2$ solution during the gelation and hardening processes due to the low lipid solubility of catechin (15). Three types of polyglycerol esters of oleic acids with different hydrophile-lipophile balances (HLB) depending on bond numbers of glycerol were used as emulsifier. The used oleic acid esters were MO-3S, MO-5S, and MO-7S which showed 8.8, 11.6, and 12.9 of HLB values at 4, 6, and 10 of glycerol bonds, respectively (25). EE was increased by using the emulsifiers and significantly increased with an increase of HLB values of the oleic acid esters which indicated degree of hydrophilicity. It can be explained that higher hydrophilic emulsifier resulted smaller droplets of the emulsion and better catechin dispersion which led to higher EE, owing to better efficiency for stabilizing oil-water interfacial surface area (26).

***In vitro* release of beads** ACO beads showed significantly higher inhibition of the catechin release after 2 h of exposure in pH 7 at 20°C compared with the other types (Table 1). The results can be explained that the uniform emulsion within ACO beads seemed effective for suppressing the catechin release from the beads, with good agreement of the results of EE. As the oil concentration was varied from 2.5 to 10%, the beads prepared using 5% oil showed significantly the highest inhibition of catechin release and the release rate was increased below and above the 5% oil. This result could be explained that the oil in Ca-alginate beads acted as the barrier to catechin release (16). On the other hand, the further increase of oil content caused a relative decrease in the ratio of alginate in the beads, which maintains the gel structure. As the decrease of alginate ratio, less cohesive gel structures were generated with low durability in solution, resulting in the accelerated catechin release (27). As mentioned above, more hydrophilic emulsifier worked more effectively in the O/W emulsion, and the beads prepared with MO-7 showed significantly greater inhibition of catechin release. As results, catechin encapsulation by ACO type using 5% oil and MO-7 emulsifier was determined to result in the optimum EE and release properties in our study. These predetermined conditions were

Table 1. Effect of preparation conditions of catechin-loaded Ca-alginate beads on their encapsulation efficiency and release property

Emulsion process[1)]	Oil (%, w/w)	Type of emulsifier[2)]	Encapsulation efficiency (%)	Release rate after 2 h (%)
Effects of different emulsification procedure				
OCA	10	None	29.46±1.35^{b}	85.25±3.13^{a}
AOC	10	None	27.54±2.64^{c}	83.15±2.89^{ab}
ACO	10	None	32.38±2.58^{a}	80.01±3.95^{b}
Effects of oil concentration				
ACO	0	None	9.14±0.07^{d}	97.11±0.28^{a}
ACO	2.5	None	22.54±1.11^{c}	85.89±5.03^{b}
ACO	5	None	29.76±1.97^{b}	73.55±2.53^{d}
ACO	7.5	None	29.29±0.52^{b}	79.08±2.13^{c}
ACO	10	None	32.38±2.58^{a}	80.01±3.95^{c}
Effects of emulsifier				
ACO	5	None	29.76±1.97^{c}	73.55±2.53^{a}
ACO	5	MO-3S	33.99±3.23^{bc}	74.17±3.11^{a}
ACO	5	MO-5S	36.57±1.12^{b}	70.97±2.77^{a}
ACO	5	MO-7S	41.48±0.72^{a}	65.30±3.21^{b}

$^{a-d}$Different letters in the same column indicate significant differences ($p<0.05$).

[1)]OCA, sequential mixing of oil, catechin, and alginate; AOC, sequential mixing of alginate, oil, and catechin; ACO, sequential mixing of alginate, catechin, and oil.

[2)]MO-3S, tetraglycerol mono-ester; MO-5S, hexaglycerol mono-ester; MO-7S, decaglycerol mono-ester.

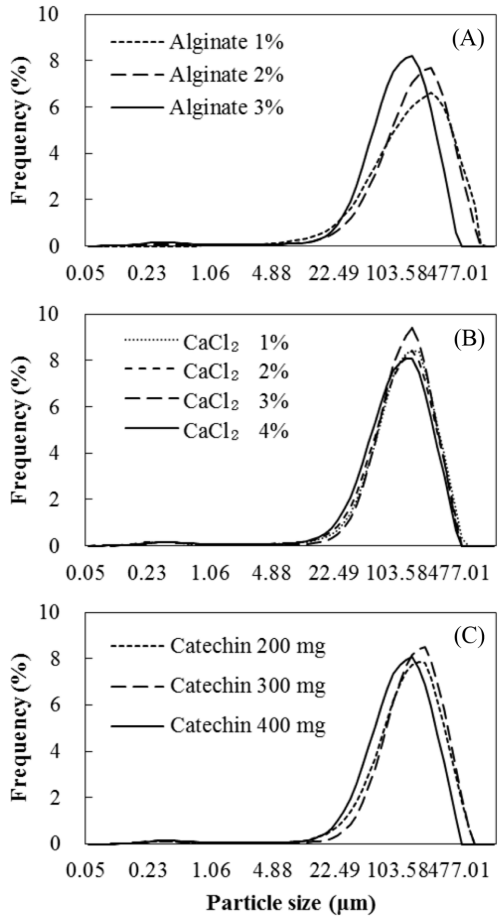

Fig. 1. Size distribution of catechin-loaded Ca-alginate beads prepared by varying concentration of (A) alginate, (B) $CaCl_2$, and (C) catechin.

applied to all of the following preparation of catechin-loaded microparticles.

Physical characteristics of microparticles Particle size of microparticles were decreased with an increase of alginate and calcium concentration while catechin showed no significant effects on tendency of size change (Fig. 1 and Table 2). Ionic gelation between charged materials were closely affected by the proportions of the materials and increased cross-linking properties led to generate smaller and compact gel particles (28). Moreover, microparticles prepared by 3% alginate, 4% $CaCl_2$, and 200 mg catechin showed the smallest particle size of approximately 131.32 μm. The surfaces of microparticles prepared in different $CaCl_2$ concentrations are shown in Fig. 2. In all preparation conditions, tiny grains can be found on the surface of particles, which can be explained by the oil micelles surrounding alginate gel (15).

Encapsulation efficiency of microparticles EE was significantly decreased with an increase of alginate concentration (Table 2). With strong ionic interaction, contraction of the gel structure occurred by syneresis, the process of molecular reconstruction in a hydrogel (29). Therefore, it can be assumed that catechin was squeezed out from the microparticles prepared by higher alginate because the structural density of gel particles was increased and the internal space for core materials was decreased (30). Similar to the case with alginate, more compact gel structure was formed at higher $CaCl_2$ concentration which affected the cross-linking strength and EE was decreased within the microparticles. As an increase in catechin quantity, EE were significantly increased and this seemed to be a typical result given that the actual used catechin during the preparation of microparticle was increased. Generally, EE of microparticles was lower than that of the macro beads. As mentioned in the results of the beads, encapsulation of catechin within alginate particles tended to depend on physical properties of particles such as structure.

Table 2. Effect of preparation conditions of catechin-loaded Ca-alginate microparticles on their encapsulation efficiency, swelling, and release properties[1)]

Alginate (%, w/w)	$CaCl_2$ (%, w/v)	Catechin (mg)	Particle size (μm)	Encapsulation efficiency (%)	Swelling degree after 1 h[2)]	Release rate after 1 h (%)[3)]
Effects of alginate concentration						
1	4	200	212.37±7.82^a	11.53±0.15^a	3.68±0.38^b	96.29±6.60^a
2	4	200	202.78±6.57^a	10.50±0.38^b	4.11±0.27^b	92.80±3.17^b
3	4	200	131.32±6.17^b	5.61±0.40^c	5.72±0.22^a	78.82±5.23^c
Effects of $CaCl_2$ concentration						
3	1	200	149.21±5.93^a	7.14±0.50^a	33.94±0.59^a	92.10±2.14^a
3	2	200	141.09±6.06^a	6.57±0.30^a	13.40±0.47^b	90.32±11.24^a
3	3	200	146.12±5.41^a	5.58±0.25^b	9.45±1.19^c	89.66±5.87^a
3	4	200	131.32±6.17^b	5.61±0.40^b	5.72±0.22^d	78.82±5.23^b
Effects of catechin concentration						
3	4	200	131.32±6.17^b	5.61±0.40^b	5.72±0.22^a	78.82±5.23^b
3	4	300	158.78±5.89^a	5.92±0.32^b	4.69±0.91^{ab}	83.35±9.40^b
3	4	400	138.40±6.22^b	6.90±0.56^a	4.10±0.37^b	91.46±8.72^a

$^{a-d}$Different letters in the same column indicate significant differences (p<0.05).
[1)]The concentration of sunflower oil and MO-7S as the emulsifier were fixed at 5%(w/w) and 1%(w/w), respectively.
[2)]Swelling degree of microparticles was performed at 20°C under pH 7.
[3)]Release rate of catechin was performed at 20°C under pH 7.

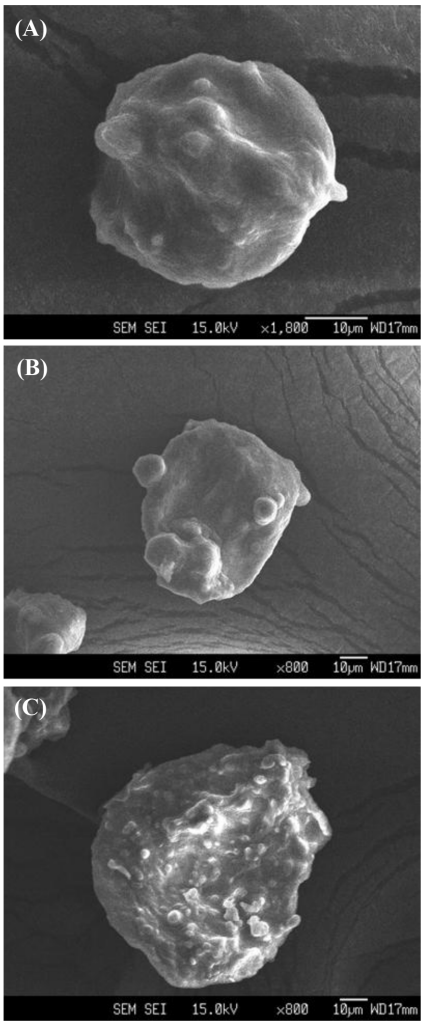

Fig. 2. SEM morphology of catechin-loaded Ca-alginate microparticles with (A) 1%, (B) 2%, and (C) 3% $CaCl_2$ concentration.

Likewise, the increased contact surface area induced by size reduction to microparticles probably increased chances to diffuse of catechin from the particles to $CaCl_2$ solution during the hardening process (31). Low EE of low molecular compounds has been a major problem for encapsulation within the alginate particles due the structural properties of a high water content matrix. Thus, alginate microparticles were reinforced by chitosan coating to enhance the hydrogel structures for effective encapsulation of low molecular drug in the other study (32). By comparison with the results, though the EE were not quantitatively satisfied at microparticles, the emulsion gelation technique using oils still can be used as possible alternative for encapsulation of catechin within alginate particles, regarding the increasing EE of beads and microparticles. However, further research is still required to improve the catechin EE of microparticles.

Swelling properties of microparticles Swelling degree of the microparticles under pH 7 at 20°C was significantly increased from approximately 3.68 to 5.72 with an increase of alginate and significantly decreased from approximately 33.94 to 5.72 with an increase of $CaCl_2$ concentrations (Table 2). Swelling of gel particles were occurred by water absorption and loosen the networks of gel structure, resulting in the accelerated release of catechin from the particles (33). Moreover, when particles exposed to continuous water absorption, gel particles eroded through the excessive swelling. For Ca-alginate microparticles, alginate had mainly influence on the density of the gel particle structure, while Ca^{2+} had influence on the cross-linking strength, specifically (29). Therefore, it can be explained that higher alginate could generate the denser structures which allowed for maintenance of the highly swollen form without erosion. Moreover, higher $CaCl_2$ concentration could form stronger gel structures due to increased gel structure linkage, which were not easily swollen. With an increase of catechin concentration, the swelling degree was decreased significantly. It can be assumed that relative decrease in the alginate and $CaCl_2$ ratio due to the increase of catechin concentration influenced the density of the gel network, and the total water absorption capacity of the particles decreased.

***In vitro* release properties of microparticles** Catechin release from the microparticles under pH 7 at 20°C was significantly inhibited at higher alginate and $CaCl_2$ concentrations (Table 2). With an increase in alginate concentration, the release of catechin showed opposite trend compared with swelling properties of the microparticles. As mentioned above, the microparticles with higher alginate generated higher density gel structure that was not eroded in excessive swelling due to its higher water absorption capacity. Therefore, though the swelling rate increased with an increase of alginate, gel structures of the microparticles were maintained and release of catechin from the microparticles into the dissolution media were prolonged. Contrary to the results with alginate, the release patterns were directly proportional to the swelling properties as an increase of $CaCl_2$. It can be assumed that the microparticles of high durability could be generated using higher $CaCl_2$ and expansion of the particles which led to loosen the gel structure was restrained. Due to the structural firmness of the gel structure, catechin could be retained longer within the microparticles and release into dissolution media was reduced (34). Moreover, catechin release was significantly increased at 400 mg catechin. This result could be explained by the gradient of catechin concentration. The increase of the catechin amount increased the concentration gradient between the inside and the outside of the microparticles in dissolution media. And the catechin release from the microparticles was promoted by increased catechin concentration gradient across the surface of microparticles (35). Therefore, the microparticles prepared with 3% alginate, 4% $CaCl_2$, and 200 mg catechin showed significantly the highest inhibition of catechin release. While catechin release from the beads showed significant results depending on preparation and storage conditions, degree of catechin release at the microparticles was not exposed clearly after 2 h exposure to dissolution media. It can be explained that general release of catechin from the microparticles were

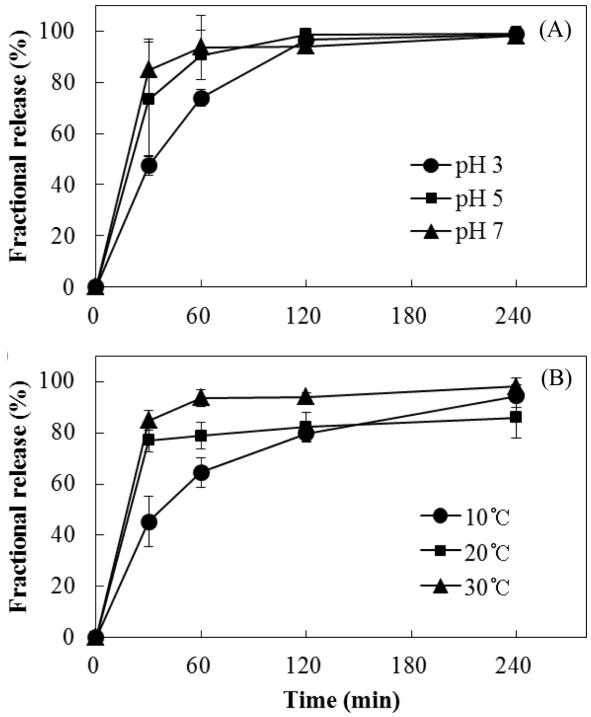

Fig. 3. Fractional release of catechin from the Ca-alginate microparticles with 3% alginate, 4% $CaCl_2$, and 200 mg catechin at varying (A) pH under 30°C and (B) incubation temperature under pH 7.

accelerated compared with macro beads due to the high surface area-to-volume ratio caused by size reduction (36).

In order to investigate the effect of pH and temperature of dissolution media on the catechin release from microparticles, the *in vitro* catechin release patterns of the microparticles by 3% alginate, 4% $CaCl_2$, and 200 mg catechin were analyzed (Fig. 3). The release of catechin was accelerated in higher pH dissolution media and this result can be explained with the swelling properties of microparticles. In acidic conditions, the carboxylate groups of alginate were protonated and the gel structures of the microparticles were constricted (37). However, the charge of carboxylic acid in the polymer backbone was converted into negative in neutral or alkaline conditions and an electrostatic repulsion between the polymer chains resulted the swelling and hydration of polymer network which induce catechin release (38,39). Catechin release increased with an increase of incubation temperature under identical condition of pH 7 (Fig. 3B). The higher temperatures accelerated the physicochemical reaction rate, and therefore degradation of the gel structure that the release of entrapped catechin was promoted.

In conclusion, encapsulation by emulsion gelation techniques using oils showed significantly higher EE and inhibition of catechin release compared with conventional methods without using oil which showed low EE and burst release within a short time period. EE of the catechin-loaded beads were significantly increased from 9.14±0.07% up to 41.48±0.72% and catechin release from the beads was significantly decreased from 97.11±0.28% up to 65.30±3.21% by the emulsion gelation technique. Physicochemical properties of catechin-loaded microparticles including EE, swelling, and release properties were significantly influenced by the preparation conditions such as alginate, $CaCl_2$, and catechin concentrations. Moreover, the microparticles prepared by 5% (w/w) oil, 3% (w/w) alginate, 4% (w/v) $CaCl_2$, and 200 mg catechin with MO-7S as the emulsifier showed significantly the highest inhibition rate of catechin release in the dissolution media. Furthermore, catechin release was increased as increase of pH and temperature of incubation conditions. Therefore, it can be suggested that the Ca-alginate microparticles by the emulsion gelation method is effective delivery system for oral administration of catechin. However, though emulsion gelation method showed a possible approach for the controlled release of catechin, low EE must be improved prior to successful application to food.

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Disclosure The authors declare no conflict of interest.

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