Master's Thesis of Science in Agricultural Biotechnology

A study on the spray-dried Pickering emulsions coated by WPI/CHS as a carrier system for lipophilic bioactive compounds

지용성 생리활성물질의 캐리어 시스템으로 분리유 청단백과 키토산으로 코팅한 분무건조 피커링 에멀션에 관한 연구

February, 2023

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A study on the spray-dried Pickering emulsions coated by WPI/CHS as a carrier system for lipophilic bioactive compounds

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Submitting a Master's Thesis of Science in Agricultural Biotechnology

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Confirming the master's thesis written by Dayeong Kim

February, 2023

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ABSTRACT

Pickering emulsion is a stabilized emulsion using solid particles and is a system that is in the spotlight because of its excellent stability compared to conventional emulsions using surfactants. However, Pickering emulsions are thermodynamically unstable, susceptible to microbial spoilage, and have limited application to various types of food, so there are still difficulties in applying them to the food industry. In this study, Pickering emulsions stabilized with whey protein isolate and surface-coated with chitosan were spray-dried for industrialization of Pickering emulsion. The surface of the Pickering emulsion was coated with chitosan, and the physicochemical properties and encapsulation efficiency of the emulsion powder were controlled using maltodextrin (MD) and gum Arabic (GA) as wall materials. Furthermore, the physicochemical properties and morphological changes of the spray-dried Pickering emulsion during the digestion process and the mucoadhesiveness were investigated. When preparing the Pickering emulsions, the interface between oil (10 wt%) and water was stabilized with whey protein isolate (1 wt%), and 0.4 wt% chitosan was coated to the surface of the Pickering emulsions by electrostatic adsorption. The encapsulation efficiency of the emulsion powder spray-dried by mixing 20 wt% of maltodextrin and gum Arabic increased as the content of the wall materials

increased. When the Pickering emulsions were coated with chitosan, the encapsulation efficiencies were improved by about 20% to be more than 95%, and the moisture content and water activity were reduced. When the Pickering emulsions were coated with chitosan, the particle size rapidly increased after spray drying, and the use of gum Arabic during spray drying improved the dispersibility. The chitosan coating was able to prevent the coalescence of oil during the digestion of the spray-dried Pickering emulsion, and the Pickering emulsion powders exhibited strong mucoadhesiveness due to the positive charge of chitosan regardless of the type of wall material. In summary, these results suggest that the spray-dried Pickering emulsion developed in this study can be used in the food industry as a carrier for lipophilic compounds and nutraceuticals. This study could provide a basis for further research that aims to develop various types of a carrier for food.

Keywords: spray drying, Pickering emulsion, chitosan, maltodextrin, gum Arabic

Student Number: 2021-24069

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I. INTRODUCTION

Recently, as consumers' interest in health and the demand for functional foods are increasing, research on encapsulation technology for the delivery of lipophilic bioactive compounds is attracting attention in the field of food science. Lipophilic bioactive compounds such as carotenoids, flavonoids, polyunsaturated fatty acids, and vitamins have diverse health benefits such as antioxidant, anti-cancer, anti-obesity, and treating allergic, metabolic, and cardiovascular diseases (Batiha et al., 2020).

However, they can easily lose their functional features under physicochemical and biochemical conditions such as changes of temperature, pH, light and hydrolysis by digestive enzymes (Shin et al., 2015). Also, they have very low bioavailability because of their high hydrophobicity and insufficient gut-retention time (Wang et al., 2016; Yang et al., 2014). Therefore, it is necessary to protect them during storage or digestion and to deliver them into the body using a carrier system.

Pickering emulsion-based systems are particularly effective for encapsulating and delivering lipophilic compounds. Pickering emulsions are stabilized by rigid or soft solid particles that can be located at the water-oil interface (Santos et al., 2021). The Pickering stabilizers act as a physical

barrier, so they are less likely to coalescence and have better stability compared to conventional emulsions (Pawar et al., 2011). However, in order to increase dispersion stability, nano-sized Pickering stabilizers are usually used, which require a lot of time and cost. Also, Pickering emulsions have limitations in that they are susceptible to microbial spoilage at room temperature and can be easily destabilized because they are thermodynamically unstable liquid emulsions.

Spray drying of emulsions is one of the most commonly used drying methods in the food industry to overcome the limitations of liquid emulsions because of its low cost and time effective advantages (Gharsallaoui et al., 2007). By spray drying the Pickering emulsions to produce powders, it becomes convenient to transport, can be applied to various types of food including dry food, and can overcome the limitations of liquid emulsions with a short shelf life due to microbial contamination and destabilization (Teo et al., 2021). During spray drying, the droplet structure of emulsions can be damaged and oil leakage can occur by thermal stress and dehydration (Mohammed et al., 2020). For these reasons, wall materials are used in spray drying to protect the core emulsions against unfavorable ambient conditions. Among the numerous wall materials, gum Arabic (GA) and maltodextrin (MD) are most commonly used. Gum Arabic produces low-viscosity solutions at high concentrations, forms very stable emulsions, and has high solubility and film-

forming ability (Karrar et al., 2021). Maltodextrin has high molecular weight, glass transition temperature (Tg), low viscosity, and ability to avoid stickiness and wall deposition (Do & Nguyen, 2018).

In this study, I fabricated Pickering emulsions stabilized by whey protein isolate (WPI), which has a good dispersibility and emulsifying ability. And the emulsions were coated with chitosan (CHS) by layer-by-layer (LBL) method (Jo et al., 2019). Chitosan is a cationic polysaccharides used in medical and pharmaceutical applications due to its biocompatibility, biodegradability, mucoadhesiveness and low toxicity (Sakloetsakun et al., 2016). Then, the Pickering emulsions were spray-dried with gum Arabic (GA) and maltodextrin (MD) as wall materials. The physicochemical properties and encapsulation efficiency of the emulsion powder were analyzed to evaluate whether it could be used as a microcapsule suitable for lipophilic compounds. In addition, an in vitro digestion test based on INFOGEST 2.0, a standardized digestion test method, and a mucoadhesiveness test using porcine small intestine were performed to investigate the potential to be used as a delivery system for lipophilic bioactive compounds.

II. MATERIALS AND METHODS

2.1. Materials

Gum Arabic was provided by Nexira (Rouen, France). Maltodextrin (DE 13.3) was provided by Ingredion Korea Inc. (Icheon, South Korea). Whey protein isolate was provided by Carbery (Cork, Ireland). Canola oil with the brand name Beksul was purchased from CJ Cheiljedang Co. (Seoul, South Korea). Chitosan (C2396; 20-100 mPa·S) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Quercetin, Pepsin from porcine gastric mucosa, α-amylase from porcine pancreas, pepsin from porcine pancreas, lipase from porcine pancreas, pancreatin from porcine pancreas, trypsin from porcine pancreas was purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Rabbit gastric extract (RGE-15) was purchased from Lipolytech (Marseille, France). Porcine intestinal mucosa was purchased from Woojoo Pig (Seoul, South Korea).

2.2. Preparation of multilayer emulsions

Pickering emulsions were prepared by stabilizing by whey protein isolate and coating with chitosan (Figure 1).

Whey protein isolate was dissolved in 0.02 wt% sodium azide

solution and stirred overnight at room temperature, which was used as an aqueous phase. The concentration of WPI was determined to be 1.0 % at a final concentrate of WPI-stabilized emulsions by preliminary experiments, and data are not presented. The oil phase containing 1.0 mg/g of quercetin was prepared by adding quercetin solution (20 mg/mL in 99.5% hot EtOH) into canola oil with stirring at 75°C for 30 min to remove EtOH. 90 wt% aqueous phase and 10 wt% oil phase were homogenized with a high-speed blender (Ultra-Turrax T25D, Ika Werke GmbH & Co., Staufen, Germany) at 8,000 rpm for 1 min and then 11,000 rpm for 1 min to prepare coarse emulsions. The emulsions were reduced in droplet size using a probe-type sonicator (VCX 750, Sonics & Materials Inc., Newtown, USA) for 10 min (pulse-on/-off mode of 1 s each) at 60% amplitude (450 W; 20 kHz) in a jacketed beaker at 20°C to prepare primary emulsions.

Chitosan was dissolved in 6% acetic acid at a concentration of 0-0.4 wt%. The chitosan solution and primary emulsion were adjusted to pH 6.0, and the primary emulsion was added dropwise to chitosan stirring at 450 rpm using a separatory funnel and mixed for 1.5 hours to prepare chitosan-coated emulsions.

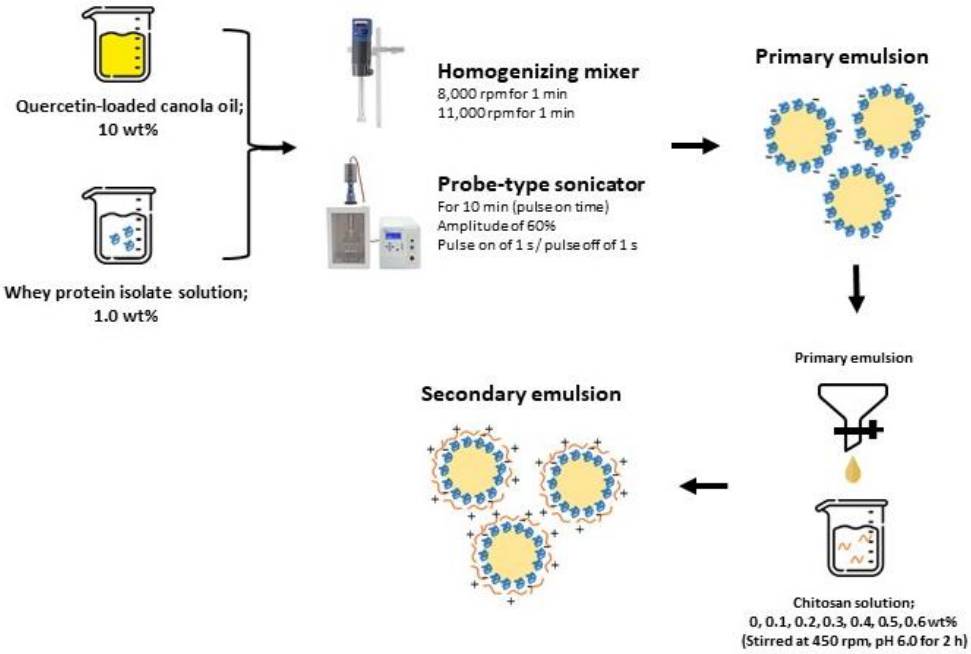

Figure 1. Schematic diagram of Pickering emulsions stabilized by WPI and coated with CHS

2.3. Spray drying of the emulsions

Solutions of wall materials were prepared by dissolving maltodextrin or gum Arabic in double-distilled water (DDW) at different concentrations (20, 30, and 40 wt%) and the pH was adjusted to 6.0. The chitosan-coated emulsions were mixed with the wall material solutions in a 1:1 ratio and homogenized using a high-speed blender (Ultra-Turrax T25D, Ika Werke GmbH & Co., Staufen, Germany) at 14,000 rpm for 3 min.

Spray drying of the emulsions was carried out using a laboratory-scale spray dryer (EYELA SD-1010, Tokyo Rikakikai Co., LTD., Tokyo, Japan) equipped with a two-fluid nozzle atomizer and a peristaltic pump. The type of flow of the feed and hot air was a co-current. The feed solutions were agitated throughout the spray drying and atomized with the air pressure of 140 kPa and the inlet and outlet temperatures of 170°C and 80°C, respectively. The powders were collected from the cyclone and transferred into tubes sealed with caps and stored with silica gel in the dark.

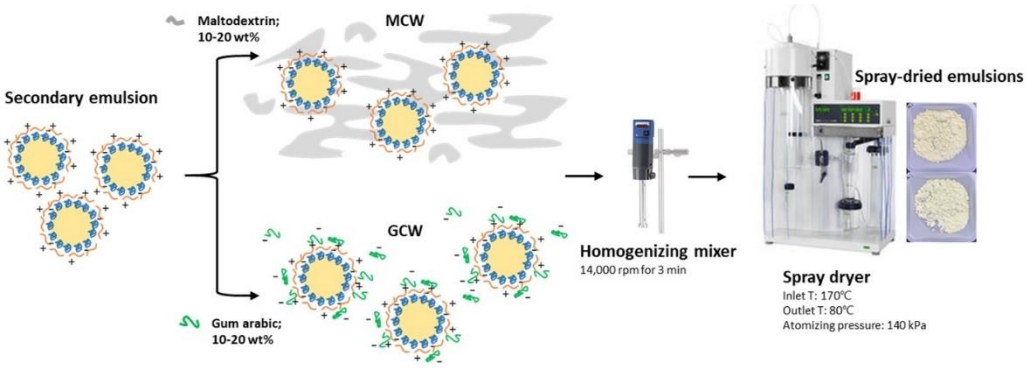

Figure 2. Schematic diagram of spray drying of the emulsions.

2.4. Physicochemical properties of the emulsions

2.4.1. Droplet size and ζ-potential

The droplet size of the emulsions was measured by light diffraction using a Mastersizer 3000 (Malvern Instruments Ltd., Worcestershire, U.K.) equipped with a Hydro EV accessory. The emulsions were added to about 500 mL of DDW, and stirred at 2,500 rpm during the measurement. The refractive index and absorption index were set to 1.52 and 0.100, respectively. The $D_{3,2}$ was calculated by the following Equation (1):

$$D_{3,2} = \sum_{i=1}^{k}(Ni \cdot Di^3) \,/\, \sum_{i=1}^{k}(Ni \cdot Di^2) \qquad (1)$$

The zeta potential of the emulsion was measured using a Nano ZS90 Zetasizer (Malvern Instruments Ltd., Worcestershire, U.K.). In order to avoid multiple scattering, the emulsions were diluted 100× in DDW prior to the measurements.

2.4.2. Optical microscopy

The morphology of the emulsions was investigated by means of optical microscopy using a light microscope (Leica DM3000, Leica, Wetzlar, ermany).

2.4.3. Visual appearance

The emulsions were stored at room temperature and destabilization phenomena such as creaming or oiling-off were observed with the naked eye. Pictures were taken on day 1, 3, 5, 10 and 20 respectively, after the emulsions were prepared.

2.5. Physicochemical properties of spray-dried emulsions

2.5.1. Moisture content and water activity

The moisture content of the spray-dried emulsions was carried out using moisture analyzer (Mettler-Toledo HB43-S, Mettler Toledo LLC, Columbus, OH, USA) with 1.7 g of the samples.

2.5.2. Water activity

The water activity of the spray-dried emulsions was measured using water activity meter (AquaLab 4TE, Meter Group, Inc., USA) at 25℃.

2.5.3. Particle size

The particle size of the spray-dried emulsions was as mentioned in Section 2.4.1.

2.5.4. Encapsulation efficiency

The efficiency of the spray-dried emulsions was determined using the method of (Zhang et al., 2022). with slight modification. The amount of oil encapsulated in the emulsion powders was calculated based on the surface oil (SO) and total oil (TO) content of the emulsion powders by Equation (2). For measurement of surface oil content, 15 mL of petroleum ether was added to 1.5 g of spray-dried emulsion powders and shaken using a vortex mixer for 1 min. The mixture was then filtered through a Whatman filter paper no. 4 and the emulsion powders on the filter were washed twice with 15 mL petroleum ether. The filtered solvent was evaporated on a hot plate over 40℃ in a fume hood to remove petroleum ether, then the flask was dried at 105℃ to constant weight. The SO was calculated as the difference in mass between the initial flask and the flask containing the extracted oil. The TO was determined to be equal to the initial oil content.

$$\text{EE (\%)} = \frac{(TO-SO)}{TO} \times 100 \qquad (2)$$

2.5.5. Scanning electron microscopy (SEM)

The morphology of the spray-dried emulsion was investigated using a field-emission scanning electron microscope (FE-SEM, SIGMA, Carl Zeiss, U.K.). The emulsion powders were placed on carbon tape and then coated with

platinum sputter using a low vacuum coater (EM ACE200, Leica, Wetzlar, Germany). The accelerating voltage was 5 kV, and the magnification was 5,000.

2.6. *In vitro* digestion of the spray-dried emulsions

The *in vitro* digestion test of the spray-dried emulsions was performed using a standardized digestion protocol INFOGEST 2.0 (Brodkorb et al., 2019).

Oral phase (pH 7, 2 min): After dispersing 1.5 g of powder in 3 mL DDW, it was mixed with simulated salivary fluid (SSF, containing a final mixture concentration of 75 U/mL α-amylase) at a 1:1 ratio.

Gastric phase (pH 3, 2 h): Simulated gastric fluid (SGF, containing a final mixture concentration of 2000 U/mL pepsin and 60 U/mL lipase) was added to the mixture obtained in the oral phase at a 1:1 ratio. For enzymatic digestion of samples, RGE and pepsin were used.

Small intestinal phase (pH 7, 2 h): Simulated small intestine fluid was mixed 1:1 (w/w) with the mixture obtained in the above step. Final mixture concentrations of trypsin activity (100 U/mL), lipase activity (2000 U/mL), and α-amylase activity (200 U/mL) were achieved using pancreatin, lipase from porcine pancreas, α-amylase from porcine pancreas, and trypsin from

porcine pancreas.

Throughout the digestion, the samples were shaken at 160 rpm, 37 °C using a shaking water bath (BS-31, JEIO Tech., Seoul, South Korea). Changes of $D_{3,2}$ and ζ-potential during digestion were measured after each step. For measuring ζ-potential, the samples were diluted 10× in DDW prior to the measurements.

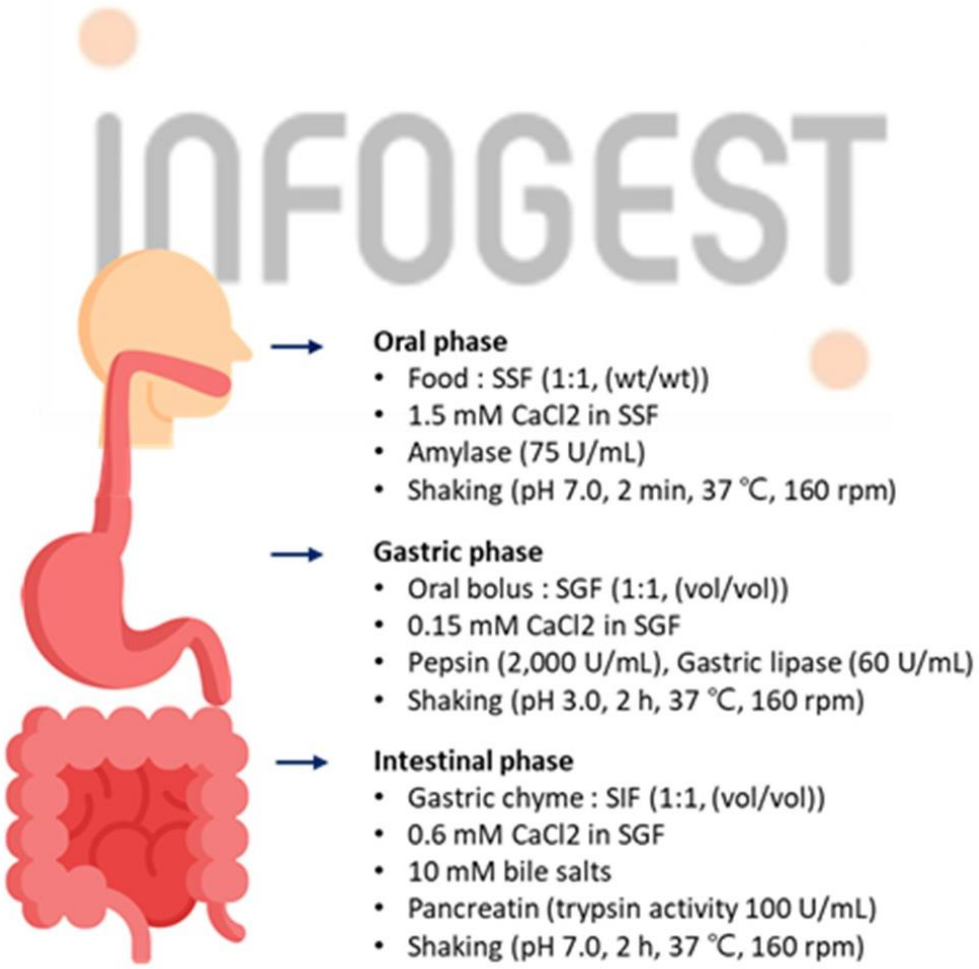

Figure 3. Schematic diagram of in vitro digestion test

2.7. Evaluation of mucoadhesiveness of spray-dried emulsions

The mucoadhesive properties of emulsion powders were evaluated using the method of (Chanburee & Tiyaboonchai, 2017) with slight modifications as follows. A piece of porcine intestinal mucosa (2 cm × 30 cm) was mounted in a long open tube held on an inclined holder. After that, 3 mL of the sample from the small intestine step of digestion was evenly spread to the mucosa. PBS (pH 6.8) flowed over the mucosa using a feeding bag at a flow rate of 1 mL/min for 2 h. Samples for 0 h were prepared without running PBS.

To observe the remaining quercetin on the mucosa, a portion of the mucosa through which PBS had flowed was placed on glass slides and covered with coverslips. At 0 h and 2 h, the mucosa tissues were imaged by a confocal laser scanning microscope (CLSM) (Leica TCS SP8 X Confocal Microscope; Leica Microsystems, Mannheim, Germany) with confocal scan objectives (HC PLAPO CS2 20 × /0.75 IMM). A 405 nm laser was used to excite quercetin, and the emitted fluorescence was detected at 450-550 nm.

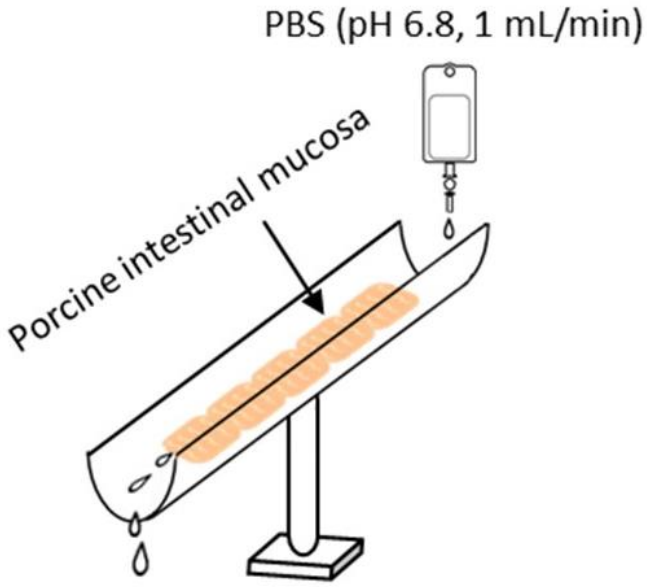

Figure 4. Schematic diagram of Evaluation of the mucoadhesiveness

2.8. Statistical analysis

The data represent an average of at least three independent experiments or measurements, and the results are expressed as mean ± standard deviation (SD).

III. RESULTS AND DISCUSSION

3.1. Preparation and characterization of the chitosan-coated Pickering emulsions

The use of chitosan, a polysaccharide with a positive charge, can improve the stability of emulsions by modifying the surface of oil droplets stabilized by proteins with a negative charge. To determine the ratio of whey protein isolate and chitosan, whey protein isolate and canola oil were fixed at 1.0 wt% and 10 wt% of the primary emulsion, respectively, through preliminary experiments. Then, physicochemical characteristics analysis of the multilayer emulsions stabilized by WPI and coated with chitosan was examined according to the chitosan concentration.

3.1.1. Physicochemical properties of the chitosan-coated Pickering emulsions

The emulsions stabilized by WPI were coated with chitosan at pH 6.0, where chitosan could adhere to the surface of the droplets. Chitosan loses its positive charge as the pH increases and has a pKa of about 6.5 (Hong & McClements, 2007). Also, the zeta potential of the emulsions was measured

to be sufficiently high negative at the pH condition (data are not presented).

The zeta potential for the emulsion uncoated with chitosan was -38.92 mV at pH 6, which was highly negative, and became positive in the presence of chitosan (Figure 5). In the emulsion uncoated with chitosan, the droplet surface was negatively charged because whey protein isolate was negatively charged at a pH above the p*I* (pH ~5). When cationic chitosan was adsorbed on the negatively charged surface of the droplet by electrostatic attraction, the emulsion coated with chitosan had positive surface charge. The absolute value of the positive surface charge on the droplet increased as the concentration of chitosan increased.

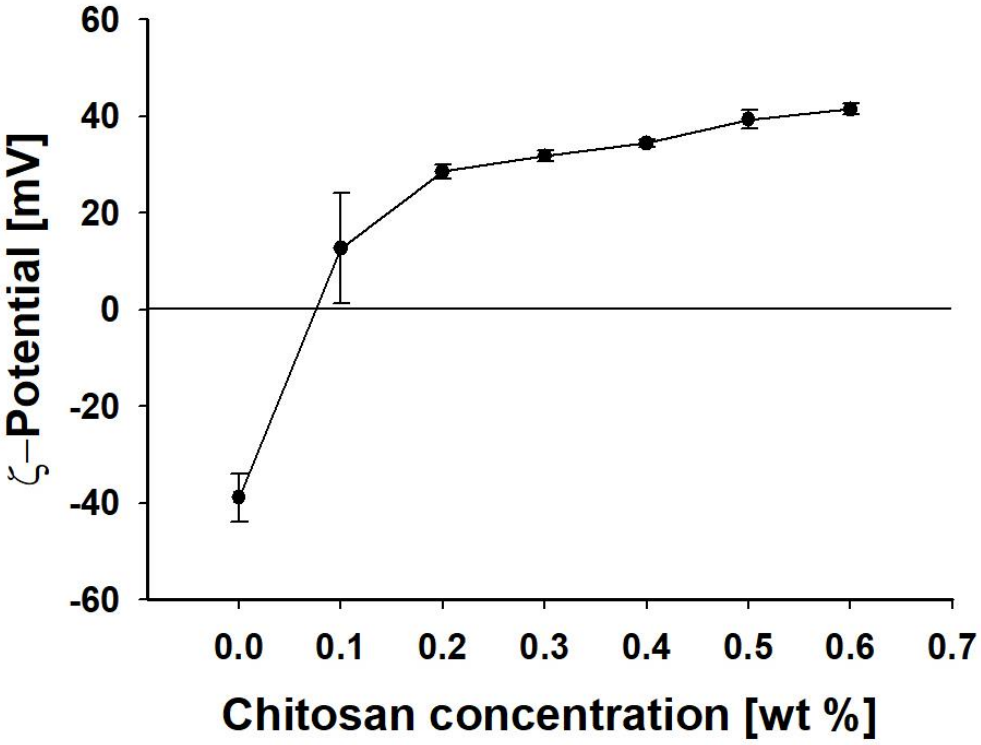

Figure 5. ζ-potential of the whey protein isolate-stabilized Pickering emulsions coated with different concentration of chitosan (0-0.6 wt%).

The mean droplet size and size distribution of quercetin-loaded emulsions were shown in Fig. 6. For 0.1-0.3 wt% of chitosan, D3,2 increased steeply compared to the emulsion without chitosan coating. This is because of the bridging flocculation of the negatively charged emulsion droplets stabilized by WPI, which was caused by a small amount of cationic chitosan molecules by the electrostatic attraction. On the other hand, when 0.5-0.6 wt% chitosan was used, depletion flocculation occurred due to the presence of excess chitosan. As a result of size distribution, 0.4% chitosan did not cause flocculation compared to the distribution of uncoated emulsion, and the distribution was slightly shifted to a larger size by the increase of droplet size due to chitosan coating. On the other hand, 0.1–0.3% chitosan coating formed new peaks in the large size due to bridging flocculation. The emulsions coated with 0.5-0.6% chitosan also showed a large peak and it may have been due to depletion flocculation (Klinkesorn, 2013; Laplante et al., 2005).

These phenomena can also be observed in optical micrographs (Fig. 7) and visual appearance during storage (Fig. 8). The emulsions coated with 0.1-0.3% chitosan had a large number of flocculation (Fig. 7), so syneresis and creaming occurred 5 days after preparation (Fig. 8). The emulsions with 0.4% chitosan coating showed relatively stable dispersion stability during the storage period, while the emulsions with 0.5-0.6% chitosan coating developed

a creaming layer after about 20 days and became destabilized.

Based on these results, emulsions stabilized by WPI were coated with 0.4 wt% chitosan to fabricate stable multilayer emulsions with less flocs.

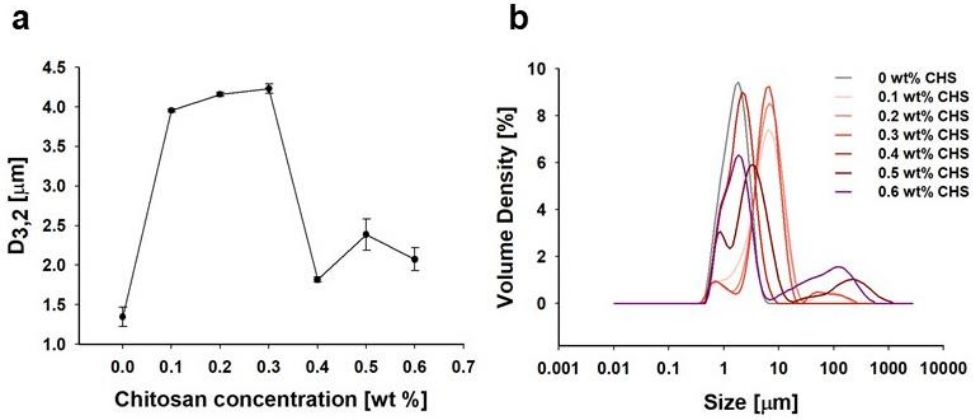

Figure 6. (a) Mean droplet size (D3,2) and droplet size distribution of the whey protein isolate-stabilized Pickering emulsions coated with different concentration of chitosan (0-0.6 wt%).

3.1.2. Morphology of the chitosan-coated emulsions

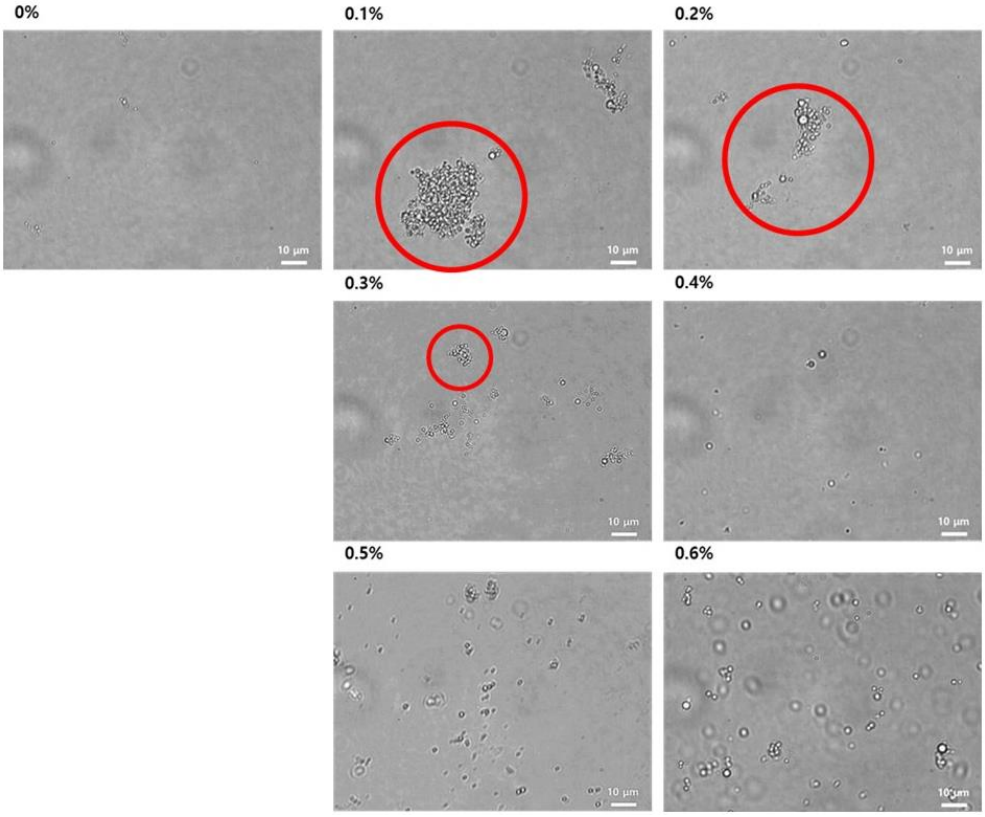

Figure 7. Optical microscope images of the whey protein isolate-stabilized Pickering emulsions coated with different concentration of chitosan (0-0.6 wt%).

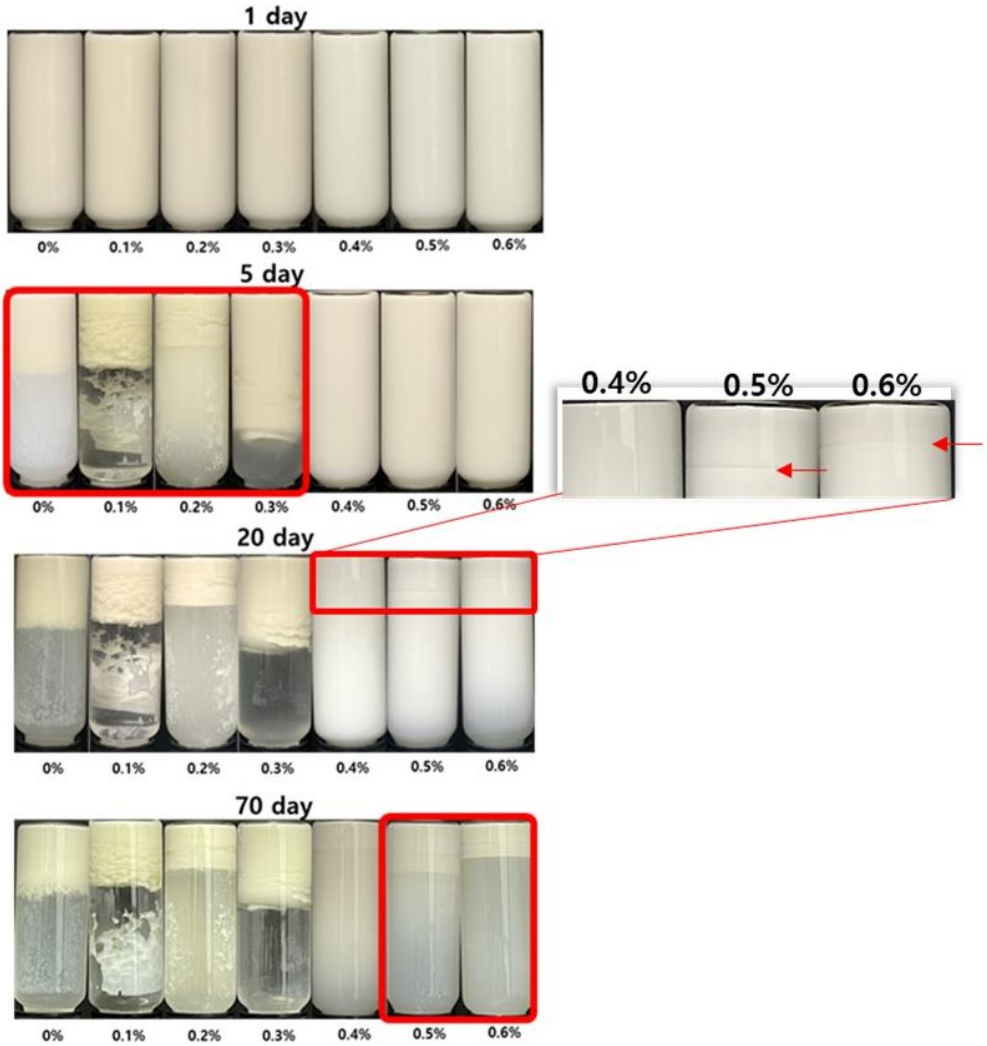

Figure 8. Visual appearance of the whey protein isolate-stabilized Pickering emulsions coated with different concentration of chitosan (0-0.6 wt%) during storage.

3.2. Physicochemical property of spray-dried Pickering emulsions

3.2.1. Physicochemical properties of the spray-dried Pickering emulsions with different content of wall materials

The spray-dried Pickering emulsions were fabricated using GA and MD as wall materials. To determine the content of the wall material, analysis of the physicochemical properties, encapsulation efficiency, and morphological microstructure of the spray-dried emulsions was conducted.

Regardless of the type of wall material, the water content and water activity increased as the content increased (Table. 1). MCW (MD-CHS-WPI emulsion powder) had higher moisture content than GCW (GA-CHS-WPI emulsion powder) at the same content. The size of the emulsions before and after spray drying were compared. After spray drying, the particle size of all the samples increased compared to before drying. This is a common feature of spray-dried emulsions. After drying, the increase in particle size was especially large in MCW. This seems to be attributed to the characteristic of CHS, which has very poor solubility in solvents other than acids. MD is nonionic, so it does not interact electrostatically with CHS coated on the

droplet surface. On the other hand, GA interacts with CHS on the droplet surface by electrostatic attraction. Therefore, the negatively charged GA coated the surface of droplet, and the dispersibility was improved by the electrostatic repulsive force of the droplets. In conclusion, the emulsions spray-dried using GA as a wall material had lower moisture content and water activity, and better reconstitution ability.

Table 1. Moisture content, water activity, and $D_{3,2}$ before and after spray drying of the spray-dried Pickering emulsions with different content of wall materials.

Samples	Moisture content [%]	Water activity	$D_{3,2}$ [μm] of the feed solution	$D_{3,2}$ [μm] of the powders
GCW10	2.10 ± 0.23^{f}	0.15 ± 0.0015^{c}	1.84 ± 0.02^{b}	5.15 ± 0.97^{c}
GCW15	3.12 ± 0.33^{e}	0.14 ± 0.0064^{c}	1.8 ± 0.28^{b}	5.46 ± 0.53^{c}
GCW20	3.85 ± 0.13^{d}	0.18 ± 0.0061^{b}	1.32 ± 0.10^{c}	2.18 ± 0.33^{d}
MCW10	4.77 ± 0.08^{c}	0.04 ± 0.0029^{d}	2.62 ± 0.13^{a}	7.26 ± 0.66^{b}
MCW15	5.96 ± 0.03^{a}	0.14 ± 0.0044^{c}	2.02 ± 0.01^{b}	9.33 ± 0.14^{a}
MCW20	5.45 ± 0.26^{b}	0.27 ± 0.0024^{a}	2.09 ± 0.07^{b}	$5.94 \pm 0.01^{b,c}$

a Data are mean values of triplicate measurements. Values are shown as means ± standard deviation.

As a result of observing the morphological microstructure of the emulsion powders, MCW powders had a relatively smooth powder surface than GCW powders. The surface of the powder may shrink due to moisture evaporation and mechanical stress during spray drying. Similar result was reported by (Akram et al., 2021). This result could be attributed to preventing shrinkage during spray drying because of the higher elasticity of MD than GA. Also, there were no noticeable cracks in any of the powders. As the wall material content increased, an excessive amount of dried wall material was observed. Therefore, the content of the wall material was determined to be 20%. Several studies have reported that samples should contain 10-30% of total solids to achieve an efficient spray drying process for emulsions (O/W) (Frascareli et al., 2012).

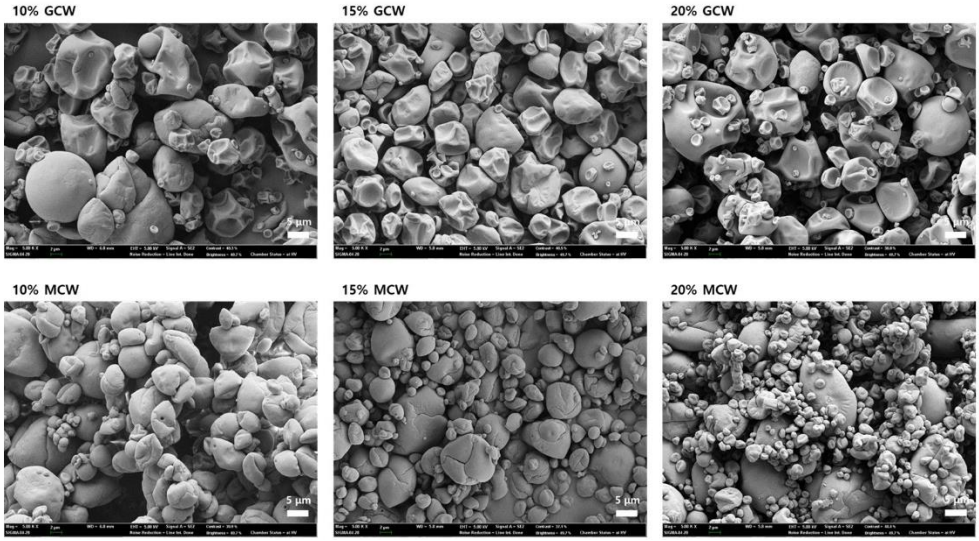

Figure 9. SEM images of the spray-dried Pickering emulsions with different content of wall material.

The encapsulation efficiency was evaluated to determine the ability of the fabricated emulsion powders to encapsulate lipophilic bioactive compounds. The encapsulation efficiency of the spray-dried emulsions increased as the content of both GA and MD increased (Fig. 10). At 20% content, the spray-dried emulsions had very high encapsulation efficiency of 98.6% and 95%, respectively.

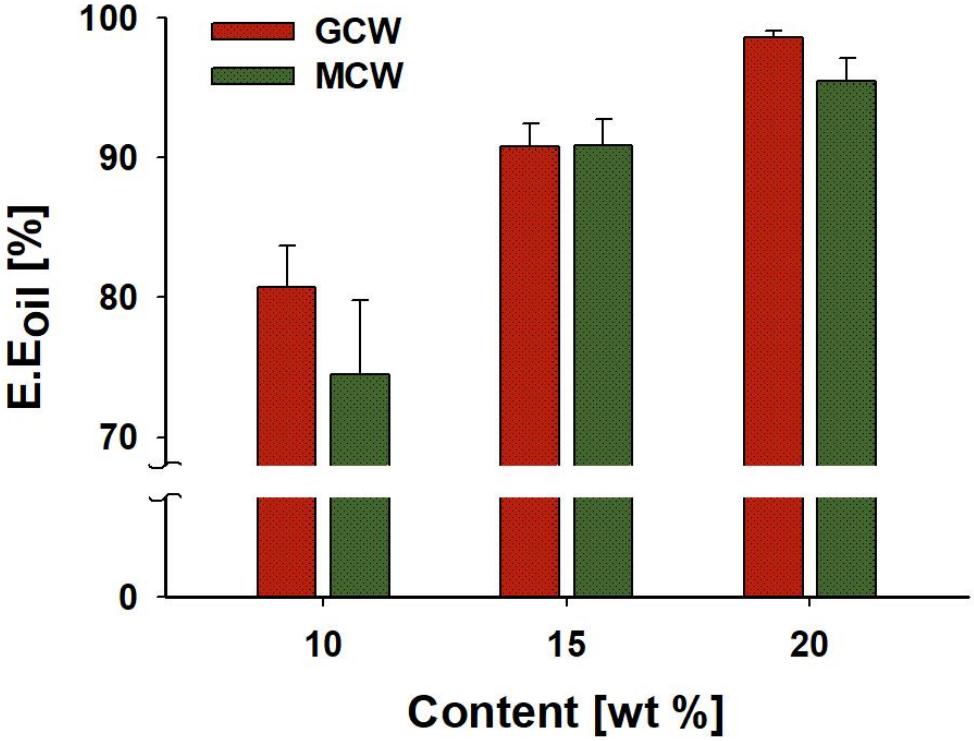

Figure 10. Encapsulation efficiency of the spray-dried Pickering emulsions with different content of wall material.

3.2.2. Effect of chitosan coating on the physicochemical properties of the spray-dried Pickering emulsions

During the study, the effect of chitosan coating on the physicochemical properties and encapsulation efficiency of the emulsion powders was found, so they were also investigated. The moisture content and water activity of GCW and MCW decreased as chitosan coating. Moisture content and water activity play an important role in powder stability, e.g. caking, stickiness, oxidation of bioactive compounds and microbial growth. In this respect, the chitosan coating can give the spray-dried emulsions enhanced physicochemical properties. The results of moisture content of spray-dried emulsions with a different type of wall material showed different aspects in the Pickering emulsion powder with or without chitosan coating. Spray-dried emulsions uncoated with chitosan showed a higher water content in GW (GA-WPI emulsion powder) than in MW (MD-WPI emulsion powder). (Akram et al., 2021) observed similar result that GA can have a higher water content than MD because it can hold more water inside the microcapsule. The particle size of the spray-dried emulsion before and after drying was similar between GW and MW without chitosan coating, whereas the difference was large in the spray-dried emulsion coated with chitosan. As mentioned above, this is due to the low

solubility of chitosan in solvents other than acids. MD did not interact electrostatically with CHS, whereas GA interacted electrostatically with CHS, resulting in a negative charge on the emulsion droplet surface (Fig. 11).

Table 2. Moisture content, water activity, and $D_{3,2}$ before and after spray drying of the spray-dried Pickering emulsions coated with chitosan or not.

Samples	Moisture content [%]	Water activity	$D_{3,2}$ [μm] of the feed solution	$D_{3,2}$ [μm] of the powders
GCW	3.29 ± 0.05^{d}	0.21 ± 0.01^{c}	1.32 ± 0.10^{c}	2.18 ± 0.33^{b}
GW	8.73 ± 0.12^{a}	0.35 ± 0.00^{a}	2.06 ± 0.03^{b}	2.01 ± 0.08^{b}
MCW	5.56 ± 0.03^{c}	0.28 ± 0.01^{b}	2.09 ± 0.07^{b}	5.94 ± 0.01^{a}
MW	6.53 ± 0.08^{b}	0.35 ± 0.02^{a}	2.79 ± 0.22^{a}	2.59 ± 0.15^{b}

a Data are mean values of triplicate measurements. Values are shown as means ± standard deviation.

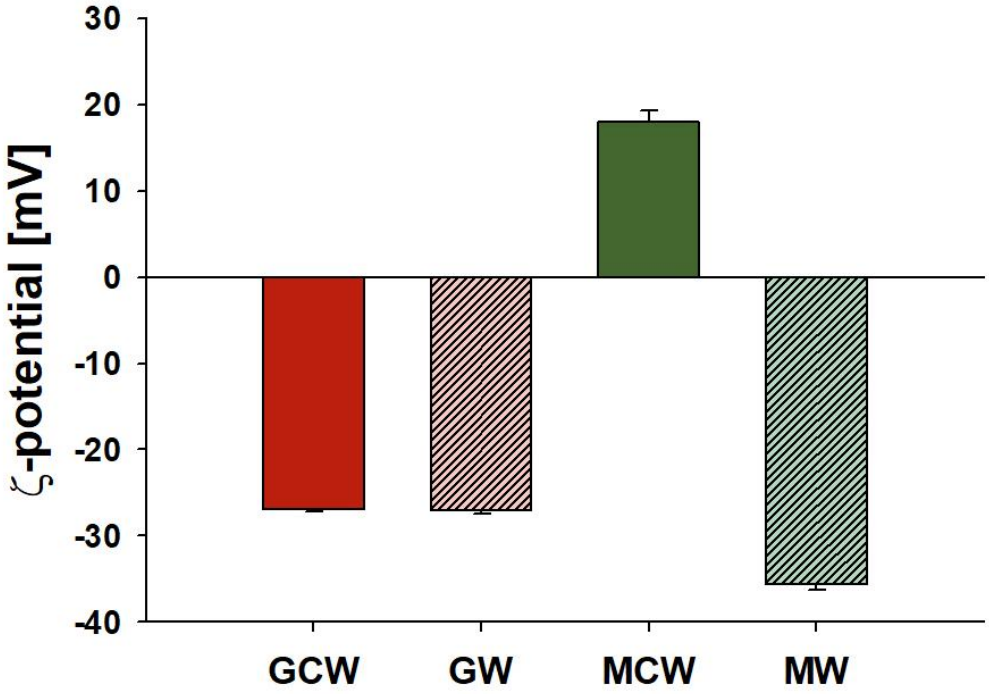

Figure 11. ζ-potential of spray-dried emulsions.

The morphological microstructures of the spray-dried emulsion coated with chitosan and without chitosan coating were not significantly different (Fig. 11). In addition, no cracks were observed, and encapsulation efficiencies of all the spray-dried emulsions were over 75% (Fig. 12). Chitosan coating improved the encapsulation efficiency of the spray-dried emulsion by about 20% (from GW, 75.6%; MW, 78.3% to GCW, 98.6%; MCW, 95.5%).

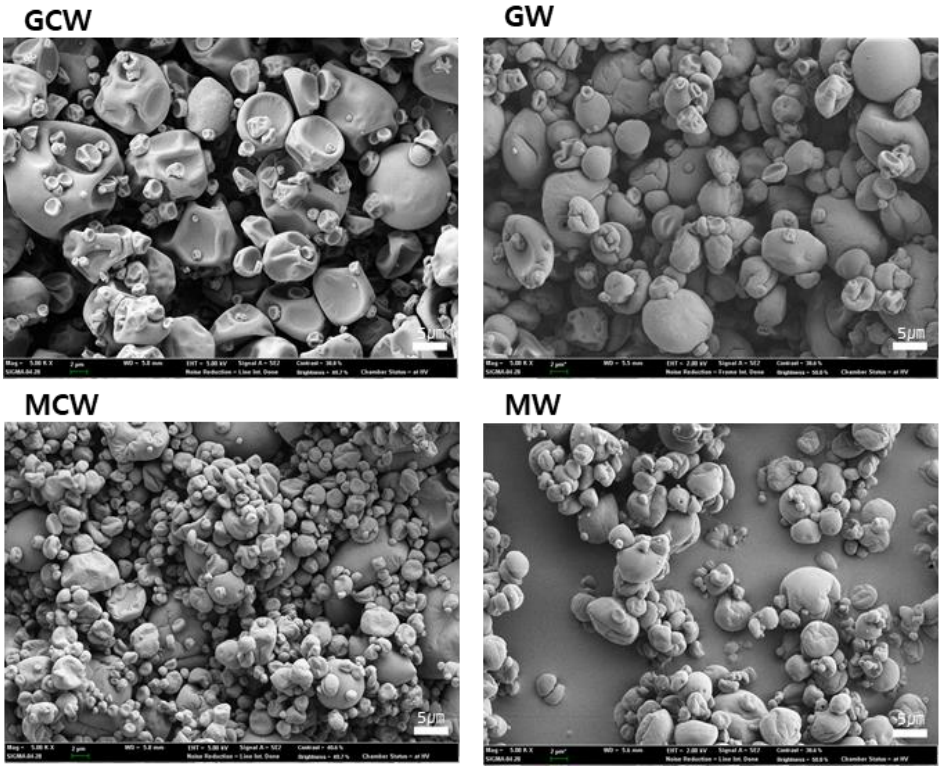

Figure 12. SEM images of the spray-dried Pickering emulsions coated with chitosan or not.

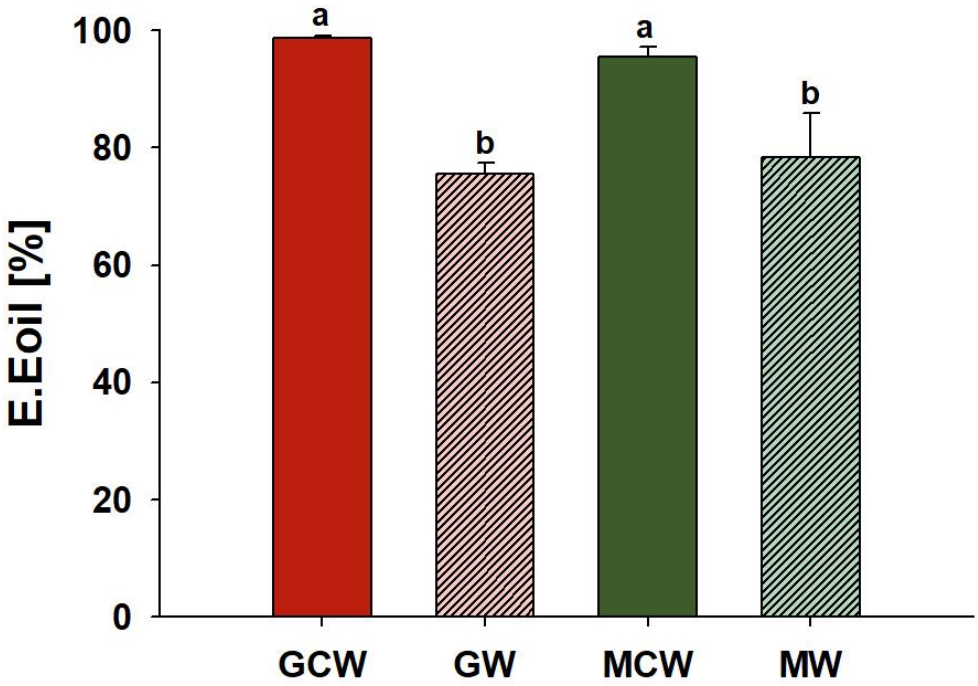

Figure 13. Encapsulation efficiency of the spray-dried Pickering emulsions coated with chitosan or not.

3.3. In vitro digestion property of the spray-dried emulsions

To investigate the potential of using the spray-dried emulsions as a delivery system for lipophilic bioactive compounds, droplet size, surface potential, and morphological microstructure of the samples subjected to in vitro digestion were observed. Since the protein-stabilized emulsion can be degraded by pepsin in the gastric stage of the digestion process and can coalesce while oil is hydrolyzed by gastric lipase, I focused on the digestive aspects of the gastric stage.

In the oral phase, the droplet size of the chitosan-coated spray-dried emulsions (GCW, MCW) was noticeably higher than that of the uncoated samples. This is because the pKa of chitosan is about 6.5, so chitosan loses charge and aggregates under oral digestion conditions (pH 7.0) (Fig. 14). These features also appear in CLSM images of spray-dried emulsions during the digestion process. The chitosan-coated samples (GCW and MCW) showed droplet flocculation by chitosan aggregation in the oral phase (pH 7.0). In the case of the GCW sample, the droplet was already flocculated in the pre-ingestion step due to the electrostatic interaction between GA and CHS.

In the gastric phase, first comparing MCW and MW, MCW maintained almost constant droplet size during gastric digestion, but MW

showed an increase in droplet size (Fig. 14, 15). MCW droplets have a stronger positive charge than MW, indicating that the chitosan coating on the droplet surface was maintained during gastric digestion (Fig. 16). In addition, the droplet size of MW significantly increased during gastric digestion, while the droplets of MCW, which were aggregated in the oral phase, were dispersed into droplets smaller than that of MW due to pH change during gastric digestion (Fig. 17). Therefore, in MW without chitosan coating, the interfacial structure was decomposed by a change of pH and digestive enzymes, and oil coalesced. However, because of the chitosan coating in MCW, decomposition of the structure occurred less in the above step.

The gastric digestion of the spray-dried emulsions with GA shows a different aspect from MD. GA and chitosan form a three-layered structure at the oil-water interface due to the interaction of electrostatic attraction. Unlike MW, the droplet size of GW did not increase and was maintained during digestion in the mouth and stomach (Fig. 14, FIg. 15). This is because the positively charged WPI and GA form a two-layer structure by the electrostatic attraction in the acidic environment at the gastric stage, so the structure was protected. The gastric digestion of the samples with GA was an environment in which GA can interact electrostatically in both GCW and GW. However, chitosan coating had a stronger interaction with gum Arabic in the gastric

phase, resulting in a large amount of droplet aggregation.

In the intestinal phase, the droplet size of all the samples increased significantly (Fig. 14, 15). In the GW and MW samples uncoated with chitosan, the interfacial structure of the droplet was decomposed by digestive enzymes during the digestion process, and oil coalescence was observed (Fig. 17). In addition, all samples exhibited a negative charge, which seems to be due to interfacial adsorption of bile (Fig. 16)

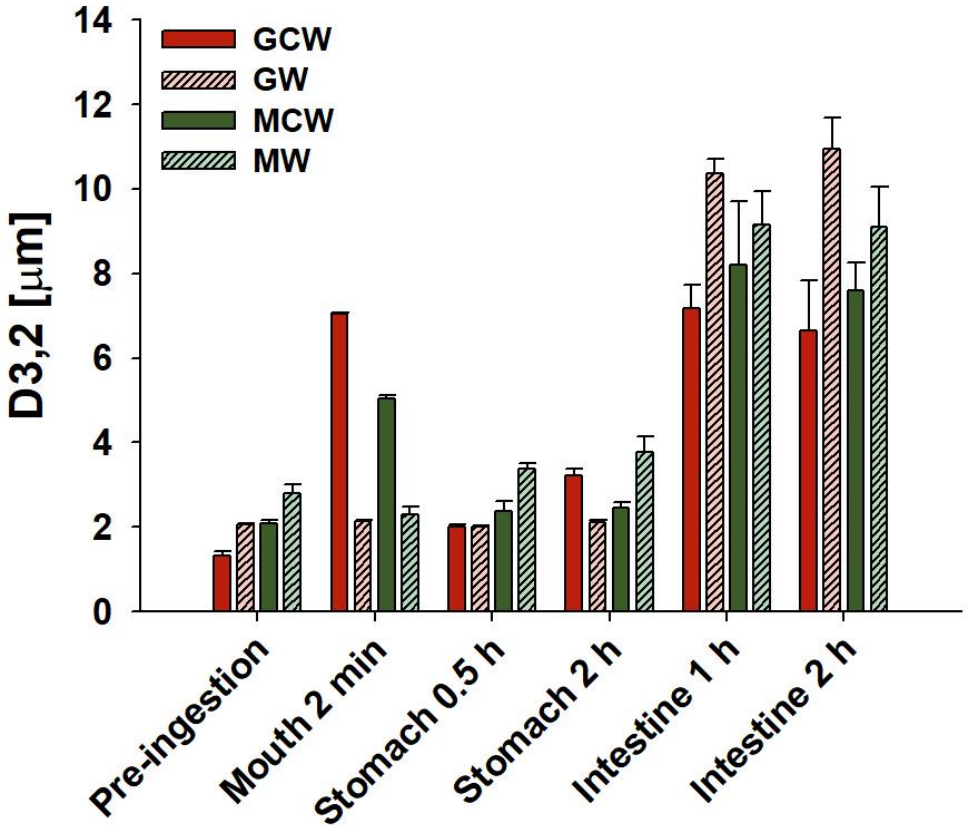

Figure 14. Mean particle size ($D_{3,2}$) of the spray-dried emulsions during

the gastrointestinal digestion procedure.

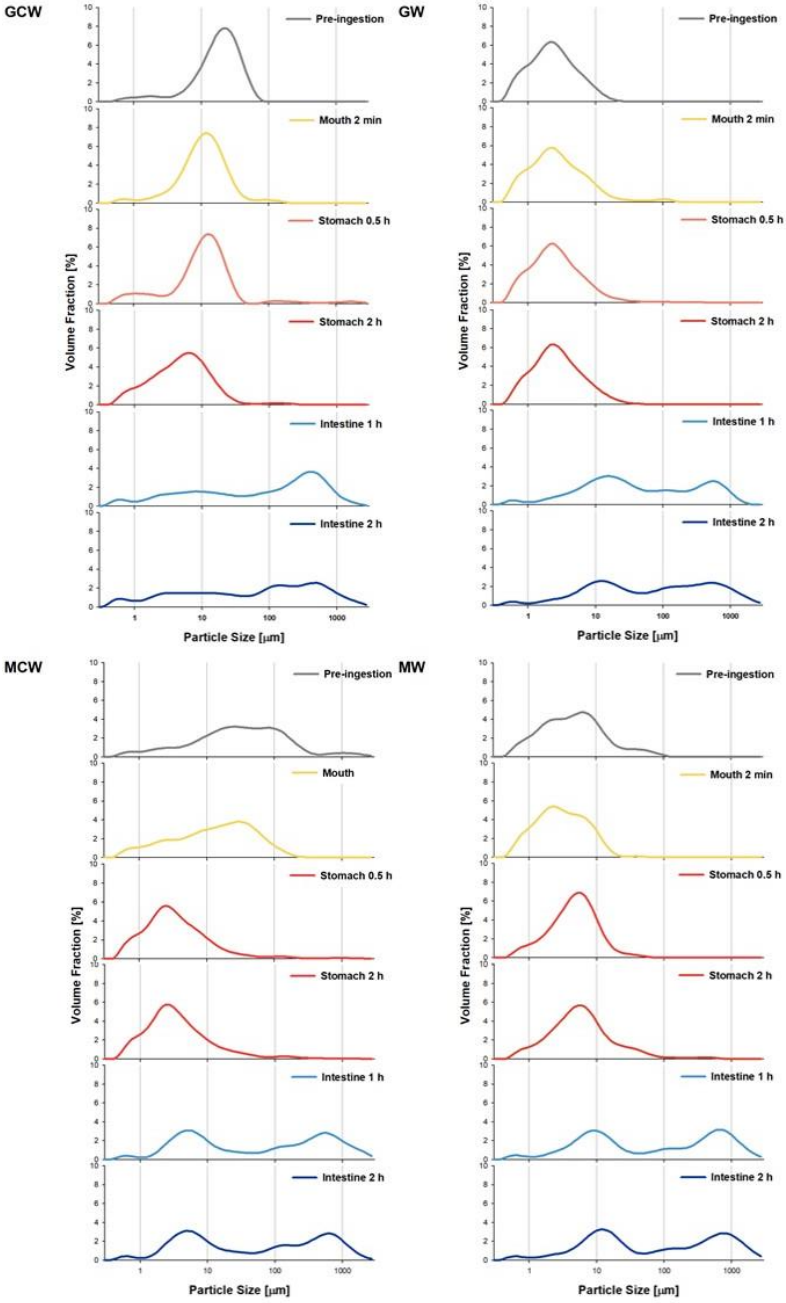

Figure 15. Particle size distribution of the spray-dried emulsions during the gastrointestinal digestion procedure.

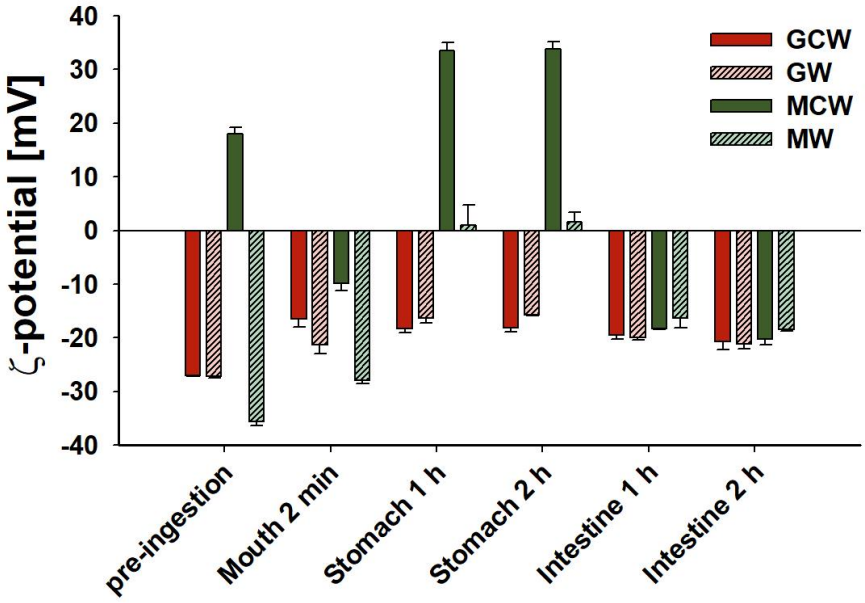

Figure 16. Zeta potential of the spray-dried emulsions during the gastrointestinal digestion procedure.

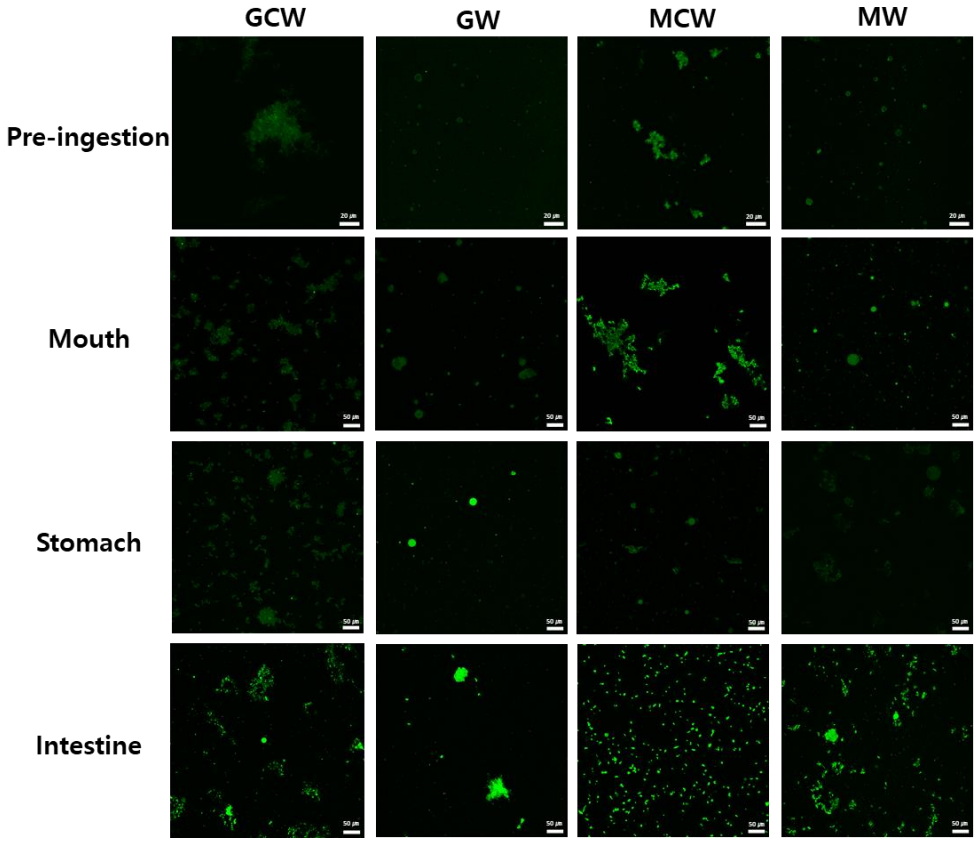

Figure 17. CLSM image of the spray-dried emulsions during the gastrointestinal digestion procedure.

3.4. Evalution of the mucoadhesive characteristics of the spray-dried emulsions

To evaluate the mucoadhesiveness of spray-dried emulsions, a simulated small intestine device was set to simulate the mucus layer flow environment on the surface of the small intestine (Fig. 4). And quercetin was loaded in oil to observe the samples remaining on the small intestinal mucosa (Fig. 18). After flowing PBS on the mucosa for 2 hours, most of the quercetin-loaded emulsions without chitosan were washed away. However, a considerable amount of the emulsions coated with chitosan remained on the surface of the small intestine. The mucoadhesive characteristic of chitosan caused emulsion droplets to adhere to the small intestinal mucosa as the chitosan coating was preserved during the digestion process. These results were also reported in the studies with emulsion stabilized with starch nanoparticles and coated with chitosan (Jo et al., 2021), spray-dried emulsion stabilized by chitosan particles (Singh et al., 2021) and nanostructured lipid carriers coated with polyethylene glycol, polyvinyl alcohol and chitosan (Chanburee & Tiyaboonchai, 2017). Chitosan is known to be have the property of adhering to the surface of the negatively charged mucous layer of the small intestine due to electrostatic interaction and hydrogen bonding (Cazorla-Luna et al., 2021; de Lima et al.,

2022). In addition, in the case of GCW samples, the mucoadhesiveness of the negatively charged GA and positively charged chitosan-coated emulsion droplets did not conflict, and a large amount of quercetin remained on the small intestine mucosa. The interaction between GA's anion and mucoadhesion has not been reported, but (Li et al., 2019) reported that GA can adhere to the tongue mucosa and reduce sodium. These results indicate that the chitosan coating can improve the retention time of the emulsion powder in the intestine.

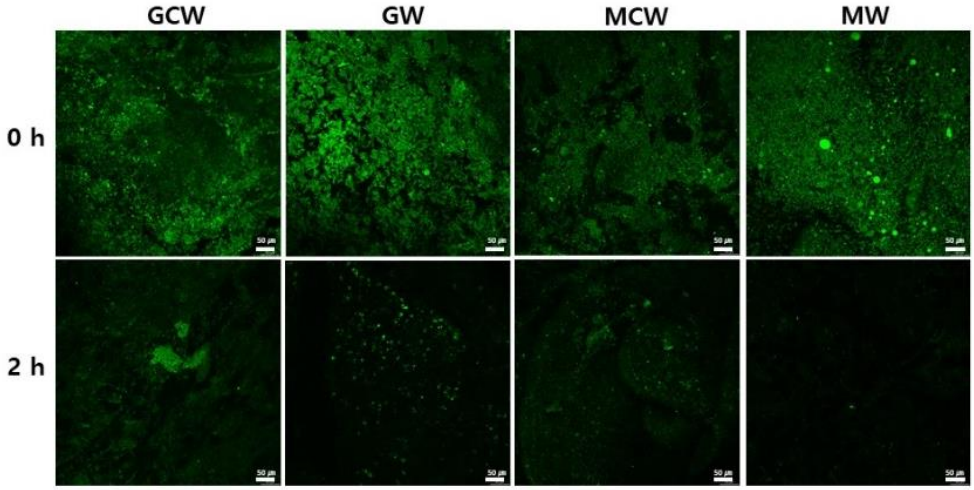

Figure 18. CLSM of porcine intestinal mucosa

CONCLUSION

Lipophilic compounds, which can be easily destabilized by environmental stress and have low bioaccessibility, should be protected. In this study, WPI-stabilized and CHS-coated spray-dried Pickering emulsions with high encapsulation efficiency were fabricated. During spray drying, the CHS-coated Pickering emulsions using GA as a wall material had high encapsulation efficiency, and improved dispersibility due to the electrostatic attraction between GA and CHS. Also, the encapsulation efficiency of the spray-dried emulsions was improved by the chitosan coating of the Pickering emulsion, and the water content and water activity were reduced, giving advantages to the use of powder materials. The fabricated spray-dried Pickering emulsions maintained its structure stable during the digestion process and showed mucoadhesiveness. The spray-dried Pickering emulsions have the potential to be used as a delivery system for lipophilic compounds.

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국문초록

피커링 에멀션은 고체 입자를 이용하여 에멀션을 안정화한 것으로, 계면활성제를 이용한 기존의 에멀션에 비해 안정성이 우수하여 각광받고 있는 시스템이다. 하지만, 피커링 에멀션은 열역학적으로 불안정하며 미생물에 의한 부패에 의해 취약하고, 다양한 유형의 식품으로의 활용이 제한적이기 때문에 여전히 식품산업에 적용하는 데 어려움이 있다. 본 연구에서는 분리유청단백으로 안정화하고 키토산으로 표면을 성형한 피커링 에멀션을 분무건조하였다. 피커링 에멀션의 표면을 키토산으로 성형하고 부형제로 말토덱스트린과 아라비아검을 사용하여 에멀션 분말의 이화학 특성과 포집 효율을 조절하였다. 나아가 소화 과정 중 에멀션의 이화학 특성 및 형상 변화와 장 표면 부착성을 확인하였다. 피커링 에멀션 제조 시 분리유청단백(1wt%)으로 기름(10 wt%)과 물의 계면을 안정화 하였고, 피커링 에멀션의 표면에 0.4 wt%의 키토산을 전기적으로 흡착시켰다. 말토덱스트린과 아라비아검을 20 wt%를 혼합하여 분무건조한 에멀션 분말의 포집 효율은 부형제의 함량이 높을수록 증가하였다. 피커링 에멀션에 키토산을 코팅한 경우 포집 효율은 약 20% 향상되어 95% 이상의 포집 효율을 지녔으며, 수분함량과 수

분활성도가 감소하였다. 한편, 피커링 에멀션을 키토산으로 코팅하면 분무건조 후 재분산 입자 크기가 급격히 증가하였는데, 분무건조 시 아라비아검을 사용하면 재분산성을 향상시킬 수 있었다. 키토산 코팅은 분무건조한 피커링 에멀션의 소화 중 기름의 융합을 방지할 수 있었으며, 피커링 에멀션 분말은 부형제의 유형에 상관없이 키토산의 양전하에 의해 강한 점막 부착성을 나타냈다. 요컨대, 이 결과들은 본 연구에서 개발된 분무건조 피커링 에멀션이 지용성 물질 및 건강기능성물질의 운반체로서 식품산업에 활용될 수 있다는 것을 시사하며, 본 연구는 지용성 물질을 이용하여 다양한 유형의 식품용 운반체를 개발하려는 연구에 기초 자료를 제공할 수 있을 것이다.

주요어: 분무건조, 피커링 에멀션, 키토산, 말토덱스트린, 아라비아검

학번: 2021-24069