# Development of Encapsulation Methods to Control Garlic Flavor Release by Oil-in-Water Emulsion and Alginate Microgels

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# Development of Encapsulation Methods to Control Garlic Flavor Release by Oil-in-Water Emulsion and Alginate Microgels

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**Table of Contents**

**Table of Figures**

**Fig. 2.11.** Influence of additives on particle size distributions and microstructures of emulsions (10% corn oil, 1% Sodium Caseinate) (A). Left: Regular image (Scale bar: 10 μm); Right: Confocal image (Red is oil). Appearance (B) B: before heating; A; after heating. Mean particle diameter (C). The relationship between Mean particle diameters (d43) of after heating and Retention half-time (D).

**Fig. 2.12.** Influence of additives on the apparent shear viscosity on the applied shear rate (1-100 $s^{-1}$) of 0.5% aqueous solutions (A). The apparent shear viscosity of each additive at the shear rate 10 $s^{-1}$ (B). The relationship between shear viscosity and retention half-time (C).

**Fig. 2.13.** Influence of maltodextrin concentration on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B).

**Fig. 2.14.** Influence of maltodextrin concentration on particle size distributions and microstructures of emulsions (10% corn oil, 1% Sodium Caseinate) (A). Left: Regular image (Scale bar: 10 μm); Right: Confocal image (Red is oil). Appearance (B) B: before heating; A; after heating.

**Fig. 2.15.** Influence of maltodextrin concentration on the apparent shear viscosity on the applied shear rate (1-100 $s^{-1}$) of 0.5% aqueous solutions (A). The apparent shear viscosity of each maltodextrin concentration at the shear rate 10 $s^{-1}$ (B). The relationship between apparent shear viscosity and retention half-time (C).

**Fig. 2.16.** Influence of xanthan gum concentration on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B).

**Fig. 2.17.** Influence of xanthan gum concentration on particle size distributions and microstructures of emulsions (10% corn oil, 1% Sodium Caseinate) (A). Left: Regular image (Scale bar: 10 μm); Right: Confocal image (Red is oil). Appearance (B) B: before heating; A; after heating.

**Fig. 2.18.** Influence of xanthan gum concentration on the apparent shear viscosity on the applied shear rate (1-100 $s^{-1}$) of 0.5% aqueous solutions (A). The apparent shear viscosity of each gum xanthan concentration at the shear rate 10 $s^{-1}$ (B). The relationship between apparent shear viscosity

and retention half-time (C).

**Fig. 3.1.** Appearances and particle distributions of different delivery systems prepared in buffer solution (5 mM phosphate buffer, pH 7). From left to right: emulsion (10% oil), unfilled alginate microgels, and emulsion-filled alginate microgels (10% oil) (A); Particle size distributions and microstructures of different delivery systems at pH 7 (B).

**Fig. 3.2.** Appearance of AMDS-loaded emulsions and filled alginate microgels in phosphate buffer (pH 7) before and after heating (A); The volume-based mean particle diameters ($d_{43}$) of emulsions and microgels before and after heating (B).

**Fig. 3.3.** Particle size distribution and microstructures of emulsion (10% oil) prepared in buffer solution (5 mM PBS, pH 7) (A); Filled microgels (10% oil) prepared in buffer solution (5 mM PBS, pH 7) (B) as a function of boil time (ambient temperature, 0min, 10min, 20min, 30min boil).

**Fig. 3.4.** Temperature profile and AMDS retention in oil-in-water emulsions (10% oil) and alginate microgels (10% oil) during heating in phosphate buffer (pH 7) (A); Time for a 50% decrease in the AMDS level ($t_{50}$) in the emulsions (10% oil) and microgels (10% oil) during heating in phosphate buffer (pH 7) (B).

**Fig. 3.5.** Impact of lipid droplet level on: Initial appearance (A); Initial mean particle diameters ($d_{43}$) (B); Initial particle size distributions (C); Appearance before and after heating (D); Microstructure below and after heating (E).

**Fig. 3.6.** Impact of lipid level on flavor retention versus time profiles for microgels during simulated cooking (A); Impact of lipid level on retention half-time (B).

**Fig. 3.7.** Microstructures of alginate calcium microgels formed with 0.5%, 1% and 2% sodium alginate. *Left*: Confocal image; *Right*: Regular image (Scale bar: 100 μm). Red is oil, green is protein.

**Fig. 3.8.** Appearances of alginate calcium microgels formed with 0.5%, 1% and 2% sodium alginate (A); volume-based mean particle diameters ($d_{43}$) of alginate calcium microgels formed with 0.5%, 1% and 2% sodium alginate (B).

**Fig. 3.9.** Impact of alginate level on flavor retention versus time profiles for microgels during

simulated cooking (A); Impact of alginate level on retention half-time (B).

**Fig. 3.10.** Microstructures of alginate calcium microgels formed with 1%, 2% and 3% sodium caseinate.

**Fig. 3.11.** Appearances of alginate calcium microgels formed with 1%, 2% and 3% sodium caseinate (A); volume-based mean particle diameters ($d_{43}$) of alginate calcium microgels formed with 1%, 2% and 3% sodium caseinate (B).

**Fig. 3.12.** Impact of sodium caseinate level on flavor retention versus time profiles for microgels during simulated cooking (A); Impact of sodium caseinate level on retention half-time (B).

**Fig. 3.13.** Appearances (A), particle size distributions (B), mean particle diameters (C), and microstructures (D) of calcium alginate microgels.

**Fig. 3.14.** Particle size distribution and microstructures of microgels prepared in distilled water (0mM sodium chloride) (A); Microgels prepared in 500mM sodium chloride (B) as a function of boil time (ambient temperature, 0min, 10min, 20min, 30min boil). *Left*: Confocal image; *Right*: Regular image (Scale bar: 100 μm). Red is oil, green is protein. Appearance of microgels prepared in distilled water (0mM sodium chloride) (C); Microgels prepared in 500mM sodium chloride (D) as a function of boil time (ambient temperature, boil point 0min, 10min, 20min, 30min boil).

**Fig. 3.15.** Appearance (A) and volume-based mean particle diameters ($d_{43}$) (B) of flavor-loaded alginate microgels (10% corn oil, 1% sodium caseinate, 0.05% allyl methyl disulfide) incubated in NaCl solutions (0-500 mM) before (25 °C) and after thermal treatment (held at boiling temperature for 30 min after boiling was observed).

**Fig. 3.16.** Temperature and flavor retention profiles (A) and retention half-time (B) of alginate microgels (10% corn oil, 1% sodium caseinate, 0.05% allyl methyl disulfide) prepared in different NaCl solutions (0- 500 mM) before (25 °C) and during heating (25 - 100°C, 0-30 min after boiling).

**Fig. 3.17.** Appearance and microstructures of microgels prepared in double distilled water, 500mM calcium cloride, 500mM sodium cloride solutions stored at ambient temperature for 0 days (A), 2 days (B), 4 days (C), 7 days (D).

**Fig. 3.18.** Mean particle diameters ($d_{43}$) of microgel beads (10% corn oil, 1% sodium caseinate, 1% allyl methyl disulfide) incubated in double distilled water, 500 mM calcium chloride, and 500 mM sodium chloride after storage for 0, 2, 4 and 7 days at room temperature.

**Fig. 3.19.** Flavor retained in alginate microgels (10% corn oil, 1% sodium caseinate, 1% allyl methyl disulfide) incubated in double distilled water (one group was stored in GC vials sealed with cap, another group with the same formulation was stored in open GC vials), 500 mM calcium chloride and 500 mM sodium chloride after storage in sealed GC vials for 0, 2, 4 and 7 days at room temperature.

# 1. Introduction

## 1.1 Background

Volatile aroma molecules play an important role in determining the desirable flavor attributes of many foods. It is, however, often difficult to control the flavor profile of foods because many of the aroma constituents are lost during their manufacture, storage, or preparation [1)]. These losses may be a result of evaporation due to their high volatility or transformation due to their chemical instability. It would, therefore, be useful to develop effective strategies to mitigate the loss of volatile flavors during thermal processing. Moreover, for certain applications, it is advantageous to control the flavor release profile during cooking and eating so as to create a more desirable sensory experience for the consumer [2-6)]. Consequently, there is also a need to develop strategies to control the flavor release profile of foods during cooking and eating.

Many of the food products containing volatile flavor compounds are oil-in-water (O/W) emulsions, which consist of small lipid droplets dispersed in an aqueous medium [7)]. The functional performance of emulsion-based encapsulation systems can often be controlled by altering their composition or preparation conditions to create systems with different oil phase compositions, droplet concentrations, particle size distributions and/or interfacial properties [8-13)]. Indeed, a number of recent studies have shown the possibility of controlling flavor release profiles by modifying emulsion structures and properties [14, 15)]. Nevertheless, the design of emulsion-based encapsulation systems with tunable flavor release profiles is still a challenge since so many factors can contribute to the release profile. Moreover, these emulsion-based foods may also contain other ingredients, such as proteins, carbohydrates, fats, or surfactants, which can alter the thermodynamic and kinetic aspects of flavor release e.g., partitioning and mass transport of the aroma compounds [7, 16)]. In principle, the flavor release profile of foods can therefore be manipulated by carefully controlling the type and level of ingredients they contain. To achieve this goal, however, a good understanding of the impact of specific food ingredients on the flavor release profile of emulsions is required. The elucidation of the major factors impacting the release profile of volatile flavors from food emulsions has been the focus of many studies [17-21)].

Meanwhile, encapsulation technologies have been developed to protect flavors from evaporation and degradation in the food industry [22 - 25]. Many of these technologies involve trapping the flavor molecules within colloidal particles specifically designed to inhibit their volatilization and degradation during storage and cooking [26]. A range of food-grade materials can be used to construct these colloidal particles, including polysaccharides (e.g., starches and gums), proteins (e.g., milk, egg, meat, and plant proteins), and lipids (e.g., triacylglycerol, essential, and mineral oils). In addition, a range of fabrication technologies are available to assemble these materials into colloidal particles, including molecular complexation [27], homogenization, milling, injection, phase separation [28, 29], spray drying, spray chilling, extrusion [25, 30], and freeze drying [31] methods. The art and science of developing an effective delivery system for a particular application is to identify the most appropriate wall materials and fabrication technology to create particles with the desired encapsulation, protection, retention, and release properties.

In this study, we focus on the utilization of biopolymer microgels prepared from a natural polysaccharide (alginate) to encapsulate a model volatile lipophilic garlic component: allyl methyl disulfide (AMDS) [32]. Alginate is a food-grade biopolymer commonly used in the pharmaceutical industry to fabricate microgels designed to encapsulate and deliver drugs [33-35]. The biopolymer network inside alginate microgels consists of linear anionic alginate chains held together by cationic calcium ions in an "egg-box" structure [34, 36-38]. The relative amounts of α-L-glucuronic acid (G) and β-D-mannuronic acid (M) groups in the alginate molecules (i.e., the G/M ratio) determines the permeability and physicochemical properties of the microgels formed [39, 40]. The functional properties of alginate microgels can also be varied by altering their particle size, shape, alginate concentration, calcium concentration, and additive contents [37]. Consequently, there is considerable scope for tailoring their properties to particular food applications [41].

## 1.2 Purpose

The overall objectives of this study were to elucidate the main factors influencing AMDS retention in oil-in-water emulsions and alginate microgels containing flavor during simulated cooking. (e.g., oil droplet concentration, oil type, droplet size, and emulsifier type, biopolymer additives) In the current study, we investigated the possibility of controlling the release of AMDS, from oil-in-water emulsions by varying their droplet characteristics. AMDS was used as a model aroma compound, which has an allylic structure and is one of the key contributors to the desirable flavor profile of garlic-based products [42-45]. These characteristics were manipulated by altering the components and homogenization conditions used to prepare the emulsions [46, 47]. In addition, we examined the influence of various food-grade biopolymers (proteins and polysaccharides) on flavor retention and release from oil-in-water emulsions during cooking. These results have important implications for the design of food matrices that can control flavor retention and release during food preparation.

Similarly, we investigated the possibility of controlling the release of AMDS, from alginate microgels by varying their biopolymer network characteristics. Consequently, lipophilic AMDS molecules were initially mixed with an oil phase and then converted into an oil-in-water emulsion. The AMDS-loaded lipid droplets were then incorporated into biopolymer microgels fabricated from alginate using a simple injection method. It was hypothesized that encapsulation of the flavor molecules within biopolymer microgels would reduce the extent of flavor loss during cooking. The impact of lipid droplet loading level on the physicochemical and retention properties of the microgels was determined during simulated cooking. The information obtained in this study may be useful for the development of novel encapsulation technologies to control the flavor profile of foods.

# 2. Flavor release control from oil-in-water emulsions during simulated cooking

## 2.1 Introduction

The design of emulsion-based encapsulation systems with tunable flavor release profiles is still a challenge since so many factors can contribute to the release profile. The overall objectives of this study were to elucidate the main factors influencing AMDS retention in oil-in-water emulsions during simulated cooking, including oil droplet concentration, oil type, droplet size, and emulsifier type.

Initially, it was hypothesized that the physical stability and rheology of the emulsions during simulated cooking would affect their flavor retention profile. In particular, we postulated that aggregation of the oil droplets during cooking would cause them to move to the top of the emulsions, thereby increasing the rate of flavor release because the ADMS molecules would be closer to the emulsion surface. In the current study, the possibility of controlling the release of AMDS, from oil-in-water emulsions by varying their droplet characteristics and rheology was investigated. AMDS was used as a model aroma because the type of flavor compound is easily lost from foods during thermal processing and so there is a need to identify effective strategies to improve its retention and modulate its release profile. The insights gained from this study may be useful for the design of emulsion-based foods with improved flavor retention or tunable flavor release profiles during cooking.

## 2.2 Materials and Methods

### Materials

Corn oil and palm oil were obtained from a regional supermarket and used as received. Mineral oil (Crystal Plus Food Grade Mineral Oil P350FG - 4 oz) was purchased from STE Oil Company, Inc. (San Marcos, TX). The following chemicals were purchased from the Sigma Chemical Company (St. Louis, MO): Nile Red (N3013-100MG); Polysorbate 80 (Tween®80), n-hexane, corn starch, sodium alginate (medium viscosity type), xanthan gum, β-cyclodextrin, and methyl cellulose. AMDS was purchased from TCI America (Portland, OR). Sodium phosphate monobasic and sodium phosphate dibasic anhydrous were purchased from Fisher Science (Fair Lawn, NJ). Whey protein isolate (BiPro, WPI), was obtained from AGUROPUR (Le Sueur, MN). Casein sodium salt was purchased from MP Biomedicals (Solon, OH). Quillaja saponin extract (Q-Naturale® 100) was obtained from Ingredion Incorporated (Westchester, IL). Maltodextrin (MALTORIN® M180) was obtained from the Grain Processing Corporation (Muscatine, IA). All chemicals used were of analytical grade. Double distilled and deionized water was used to prepare all solutions.

### Methods

#### Emulsion preparation Materials

Unless stated otherwise, the following general procedure was used for preparing the oil-in-water emulsions used in this research. Oil phases were prepared by dissolving AMDS (500 ppm) into bulk oil. Aqueous phases were prepared by dissolving emulsifier (2% w/w) into phosphate buffer solution (5 mM, pH 7.0). Coarse emulsions were created by blending 10% w/w oil phase and 90% w/w aqueous phase using a high-shear mixer for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). Fine emulsions were prepared by passing the coarse emulsions 3-times through a high-pressure homogenizer (Microfluidizer, M110Y, Microfluidics, Newton, MA) with a 75-µm interaction chamber (F20Y) at an operational pressure of 12,000 psi (82.7 MPa).

The impact of oil type, droplet concentration, emulsifier type, and droplet size were examined

by modifying this basic procedure. Oil phase type was examined by using corn oil, palm oil, or mineral oil to prepare the emulsions, using Tween 80 as the emulsifier. Emulsifier type was examined by using sodium caseinate, whey protein isolate, quillaja saponin, or Tween 80 to prepare the emulsions, using corn oil as the oil phase. Droplet concentration was examined by preparing emulsions containing 5, 10, or 20 wt% corn oil, using Tween 80 at a fixed (1:5) emulsifier-to-oil ratio. Emulsions with different sizes were prepared by comparing coarse and fine emulsions prepared from corn oil and Tween 80. The resulting emulsions were stored in a refrigerator at 4℃ prior to utilization.

The impact of biopolymer addition on emulsion properties was examined by modifying the basic procedure described above. Maltodextrin, sodium alginate, sodium caseinate, corn starch, WPI, methyl cellulose, β-cyclodextrin, and xanthan gum were used as additives. In the first set of experiments, the effect of additive type on emulsion properties was examined by adding 0.5 wt% of each biopolymer to the original emulsions. In another set of experiments, the effect of additive concentration was examined by adding 5, 10, 20, 40 wt% maltodextrin or 0.01, 0.05, and 0.1 wt% xanthan gum to the original emulsions.

**Simulated cooking conditions**

A fixed volume (200 mL) of emulsion sample was poured into a glass beaker (volume, 400 mL; height, 110 mm; outer diameter, 77 mm) and then heated on a hotplate (Isotemp Digital Stirring Hotplate, Fisher Scientific, Waltham, MA). Each emulsion was heated from ambient temperature to boiling temperature and then held at this temperature for 30 min. All samples were continuously stirred (300 rpm) throughout this process. The temperature-time profile was determined using a digital thermometer. Emulsion samples were collected at particular time points during the cooking process and then analyzed to determine any changes in flavor retention and physicochemical properties.

**Gas chromatography**

The AMDS content of the emulsions was determined by gas chromatography (GC2010, Shimadzu, Columbia, MD) equipped with an auto sampler (AOC-20i, Shimadzu, Columbia, MD). To this end, 2 mL of hexane were added to 2 mL of samples for liquid–liquid extraction. The mixture was shaken for 2 h at 200 rpm using a shaking device (Gyrotory® shaker model G2, New Brunswick Co. Inc, New Brunswick, NJ). The organic phase was then separated and placed in a vial. 1 μL of the organic phase was injected directly by an auto sampler set at 250 °C in the injector at a split ratio of 1:10, and then separated on a SH-Rxi-5ms capillary column (15 m × 0.25 mm inner diameter × 0.25 μm). The GC column temperature program was as follows: initial temperature of 80 °C for 1 min, then a temperature ramp of 5 °C/min until a temperature of 100 °C was reached, followed by holding for 2 min. Helium was used as the carrier gas at a flow rate of 0.91 mL/min. Concentrations were determined from peak areas using a standard curve prepared using an AMDS standard. The retention of the flavors in the delivery systems was determined by the ratio of the remaining to the initial quantity of flavors in the liquid phase during heat treatment as a function of time.

**Particle size analysis**

The particle size distribution of the samples was measured using static light scattering (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). Samples were diluted with phosphate buffer (5 mM, pH 7.0) prior to analysis to avoid multiple scattering effects. The samples were diluted until they reached an appropriate obscuration level for the light scattering measurements. The refractive indices of the particles and dispersing medium used in the calculations were 1.507 and 1.33, respectively.

**Microstructure analysis**

Sample microstructure was assessed using conventional light microscopy and/or confocal scanning laser fluorescence microscopy with a 60× objective lens and 10×eyepiece (Nikon D-Eclipse

C1 80i, Nikon, Melville, NY, U.S.). A drop of sample was positioned onto a glass microscope slide and then covered with a cover slip. For confocal fluorescence microscopy, the oil phase was dyed with an oil-soluble stain by adding about 0.1 mL of Nile red solution (1 mg/mL ethanol) to 2 mL of sample prior to analysis. The excitation and emission wavelengths used for Nile red were 543 nm and 605 nm. The acquired microstructural images were analyzed using the image analysis software associated with the microscope (NIS-Elements, Nikon, Melville, NY).

**Shear viscosity measurements**

The apparent shear viscosity of 0.5 wt% aqueous solutions of each additive was measured at the cooking temperature using a dynamic shear rheometer with a temperature-controlled concentric cylinder measurement cup (Kinexus, Malvern, Worcestershire, UK). Initially, each sample was transferred into the measurement cup and allowed to reach the set temperature (95 °C) before starting the measurements. The apparent shear viscosity of the samples was then measured over a shear rate of 1 to 100 s-1 over a 400 sec period. Similarly, maltodextrin (5, 10, 20, 40 wt%) and xanthan gum (0.01, 0.05, 0.1 wt%) solutions containing different levels of additive were measured.

**Statistical analysis**

All measurements were carried out on two or three separately prepared samples and reported as means and standard deviations. Statistical differences of the experimental results were determined by analysis of variance (ANOVA) using a statistical software package (SAS Inst. Inc., Cary, NC). The mean values were compared using the Duncan's multiple-range test to determine significant differences ($p < 0.05$).

## 2.3 Results and Discussion

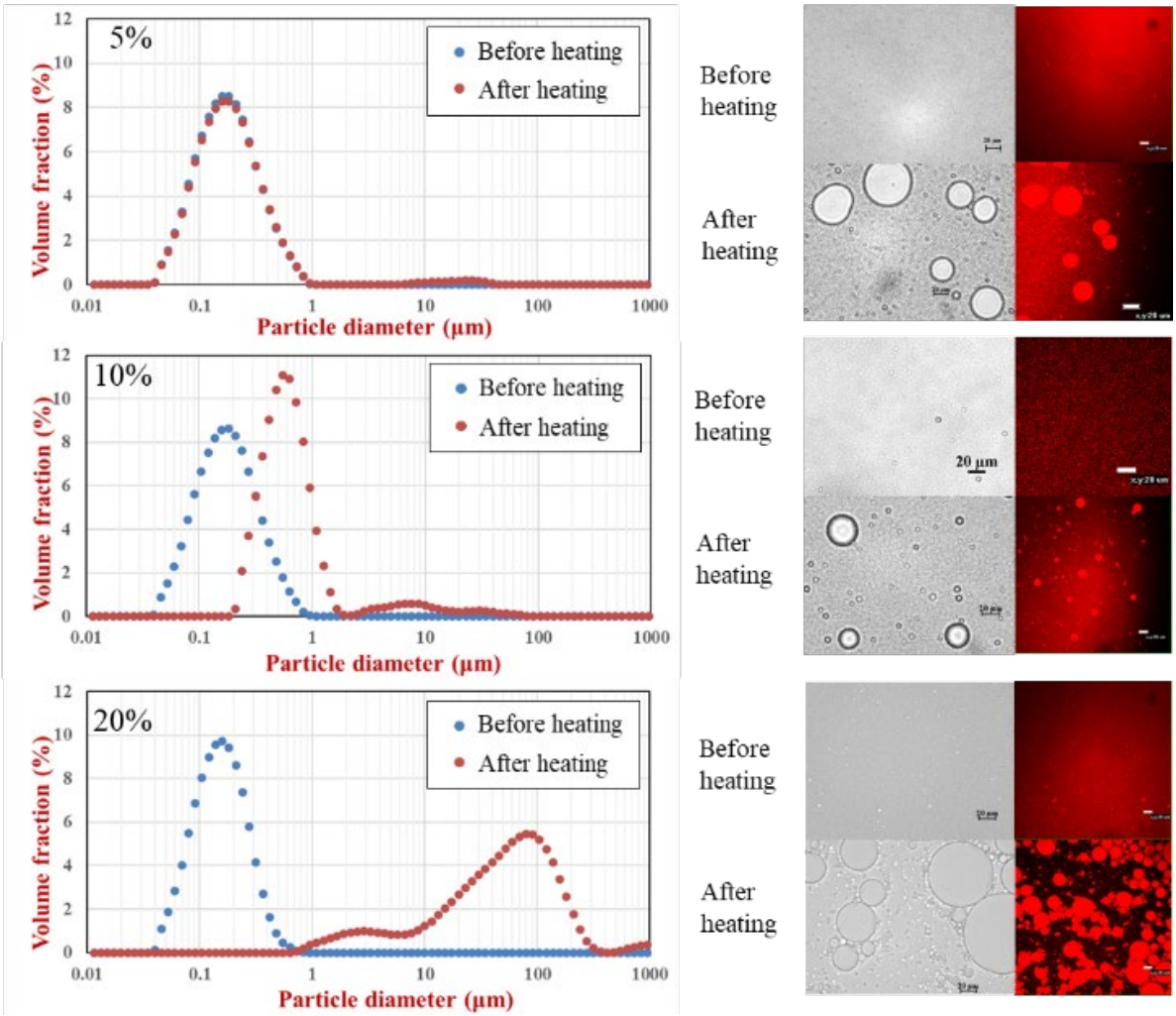
### 2.3.1 Impact of oil droplet concentration on flavor release

(1) AMDS used in the research is a hydrophobic molecule (Log P = 2.87), and so it was hypothesized

that its retention within the emulsions during cooking would depend on the oil droplet concentration. For this reason, 5, 10, and 20 wt% corn oil-in-water emulsions were fabricated, and then the effect of droplet concentration on their particle size, stability, and flavor retention were determined during simulated cooking (**Figs. 2.1.1 and 2.1.2**).

**Impact on physical properties and structure**

A



B

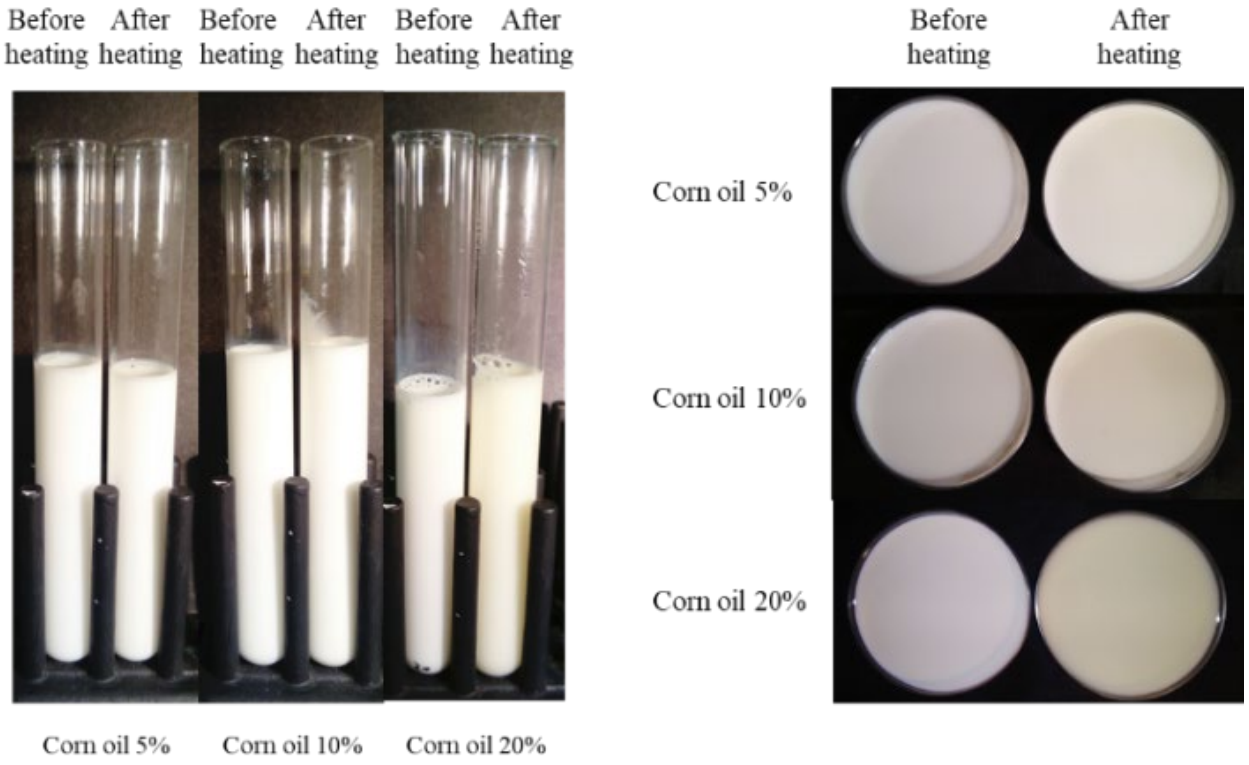


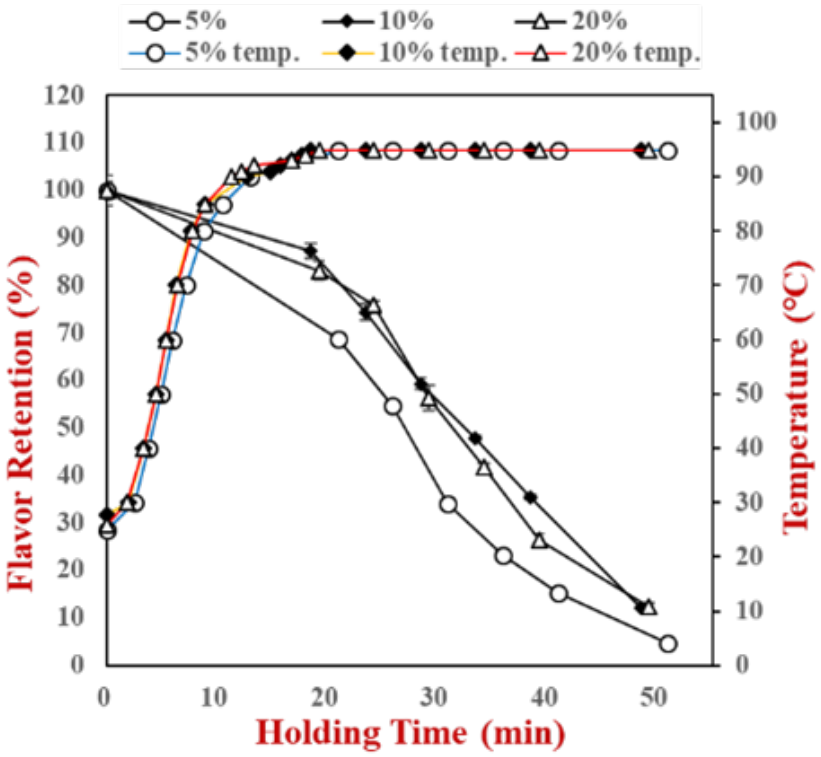

**Fig. 2.1.** Impact of initial droplet concentration on particle size distributions and microstructures of emulsions (1:5 w/w Tween 80-to-corn oil) (A). Left: Regular image (Scale bar: 20 μm); Right: Confocal image (Red is oil). Appearance (B)

The particle size distributions (PSDs) and microstructures of all the emulsions were fairly similar before heat treatment, indicating that the initial systems contained relatively small droplets that were stable to aggregation. However, there was clear evidence of extensive droplet coalescence after heat treatment in all the emulsions (**Fig. 2.1.A**). The PSDs obtained by light scattering suggested that the fraction of droplets that had undergone coalescence increased as the initial droplet concentration increased. However, the microscopy measurements indicated that some droplet coalescence occurred in all of the emulsions. Indeed, after heating, many of the oil droplets in the concentrated emulsions were relatively large (10-100 μm). Despite this, visual observation of the emulsions indicated that they still had a fairly uniform whitish appearance after cooking, but that they were

slightly more yellow than the original samples (**Fig. 2.1.B**). Overall, these results indicated that the oil droplets used were not particularly stable to thermal processing, which may have been because Tween80 is non-ionic surfactant, which this type of non-ionic surfactant loses its functionality at elevated temperatures. In particular, the hydrophilic head groups of the surfactant become increasingly dehydrated as the temperature is raised, thereby altering their interfacial packing and interactions, making the droplets more prone to coalescence [48]. The reason the emulsions appeared more yellow after cooking may have been because the increase in droplet size during cooking reduced the degree of light scattering, thereby allowing the light waves to penetrate further into them [49]. Moreover, the thermal decomposition of AMDS may have generated some colored reaction products. As a result, more of the light was absorbed by yellow pigments in the oil phase. Moreover, the greater level of droplet coalescence observed in the more concentrated emulsions was probably because the frequency of droplet-droplet encounters increased, giving more chance for the droplets to merge together.

**Impact on flavor retention**

**A**



**B**

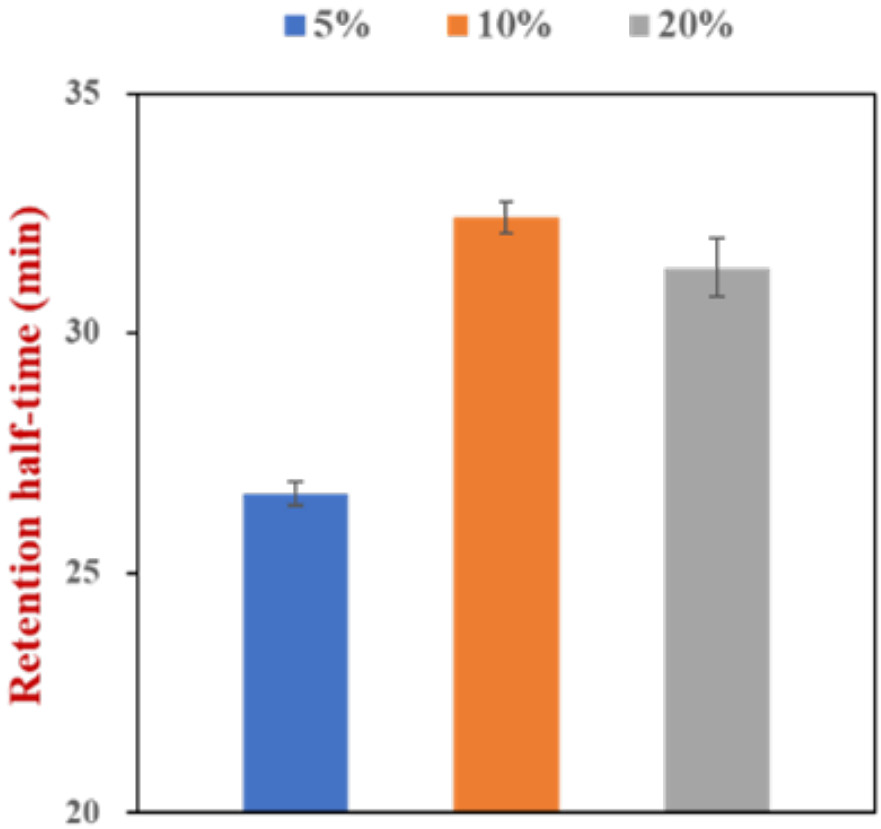



**Fig. 2.2.** Impact of droplet concentration on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B).

The impact of droplet concentration on the kinetics of flavor retention during cooking was also measured (**Fig. 2.2.A**). In general, there was a relatively slow reduction in flavor retention during the period when the samples were heating up to the final cooking temperature (0 to 20 min), but then the flavor retention decreased more rapidly afterwards. This kind of behavior would be expected because the volatility of the flavor molecules increases with increasing temperature. The *retention half-time* was estimated from the flavor retention-time profiles by extrapolation to the time where half of the flavor had been lost (**Fig. 2.2.B**). The flavor retention profiles of the 10% and 20% emulsions were fairly similar, with a slower rate of flavor loss than for the 5% systems. For instance, the retention half-time increased from around 27 min for 5% oil to around 32 min for 10 and 20% oil

(**Fig. 2.2.B**). The results agree with previous studies that also showed that the loss of hydrophobic flavors from O/W emulsions decreased as the oil droplet concentration increased [50].

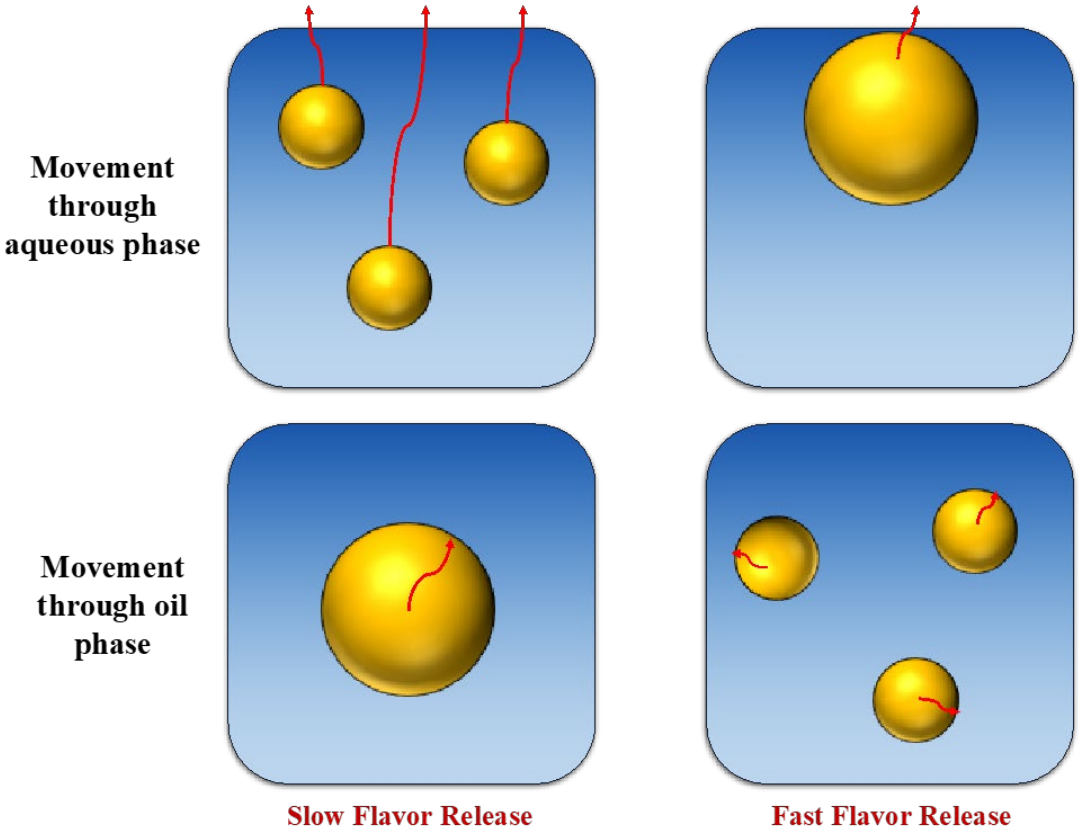



**Fig. 2.3.** Flavor release depends on the transport of flavor molecules through the oil droplets and the aqueous phase to reach the gas phase.

The origin of the observed effect can be attributed to the physicochemical processes occurring in the emulsions during cooking (**Fig. 2.3.**). First, the flavor molecules must travel through the oil phase to the droplet surfaces and then be released into the surrounding aqueous phase. Second, the flavor molecules must travel through the aqueous phase to the water-air interface where they are released into the gas phase. The rate of these processes will depend on the rheology of the oil and aqueous phases, the droplet dimensions, and the location of the oil droplets in the emulsion.

Typically, the rate of flavor release from an oil droplet decreases as its dimensions increase. To a first approximation, the time for half of the flavor molecules to diffuse out of an oil droplet is given by the following equation [7]:

$$t_{1/2} = \frac{0.0146 d^2 K_{ow}}{D} \qquad \textbf{(Equation 1)}$$

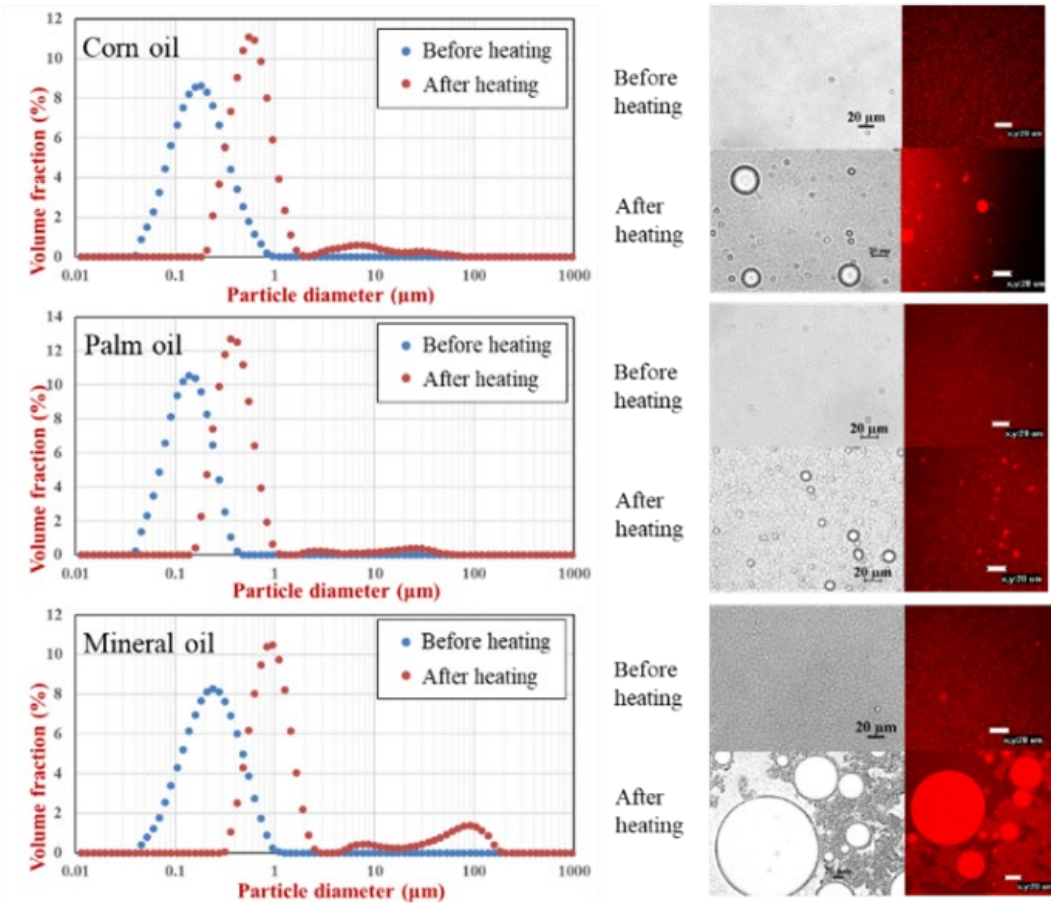
Here, $d$ is the droplet diameter, $K_{ow}$ is the oil-water partition coefficient (≈ 740 for ADMS), and $D$ is the translational diffusion coefficient (≈ $4 \times 10^{-10}$ $m^2$ $s^{-1}$) of the flavor molecules through the oil phase. For droplets with diameters of 1, 10 and 100 μm, the calculated half-times are around 0.11 seconds, 11 seconds, and 18 min, respectively. Consequently, there may have been a slower release in emulsions containing relatively large oil droplets. As noted earlier, droplet coalescence occurred in many of the emulsions after heating, particularly those with higher droplet concentrations, which may explain why there was a slower release of flavor from these systems. On the other hand, the location of the oil droplets within the emulsions during boiling may also have been important. The closer the droplets are to the upper surface of the emulsions, the faster one would expect the flavor molecules to be released into the headspace because they have a shorter distance to travel before reaching the air. In this case, one would expect that flavor molecules would be released more quickly from emulsions containing larger oil droplets because they would have a greater tendency to be near the top of the emulsions due to gravitational separation effects. This phenomenon may account for the fact that the flavor retention did not change much when the droplet concentration was increased from 10 to 20%.

### 2.3.2 Impact of oil type on flavor release

Food emulsions may be formulated using different kinds of oils and so we examined the impact of oil phase type on emulsion stability and flavor release during cooking. All the emulsions used in these experiments were prepared using 10 wt% oil phase and 90 wt% aqueous phase (containing 2 wt% Tween 80). Flavor release from emulsions depends on the partitioning and mass transfer of flavor molecules amongst the oil, interfacial, water, and air phases [51]. We therefore postulated that oil phase properties, such as melting point and viscosity, would influence the flavor release rate. For this reason, corn oil (melting point : -15 to -10 °C, viscosity: 31cP at 40 °C), palm oil (melting point : 33 to 39 °C, viscosity: 35 cP at 40 °C), and mineral oil (melting point : -60 to -9 °C, viscosity: 59.5cP at 40 °C) were used to prepare emulsions, and then the effect of oil type on their size, stability, and flavor retention during cooking were determined (**Figs. 2.4. and 2.5.**).

**Impact on physical properties and structure**

**A**



B

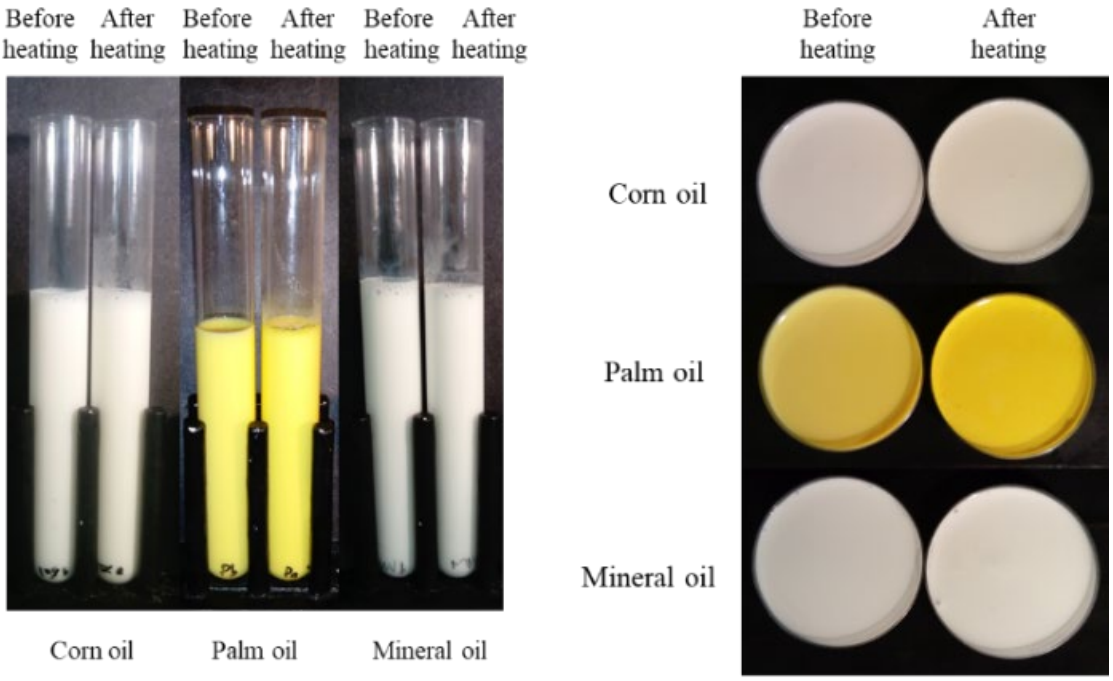
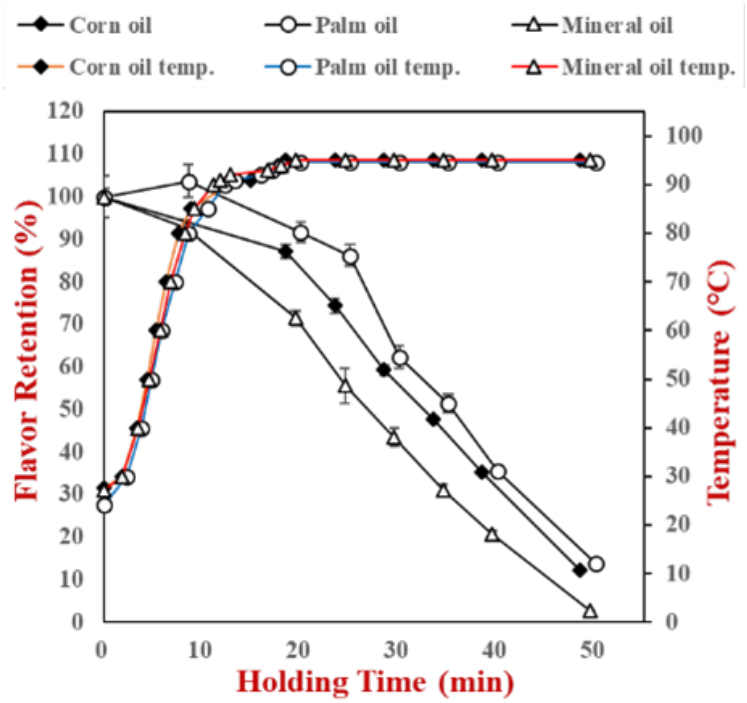



**Fig. 2.4.** Impact of oil type on particle size distributions and microstructures of emulsions (10% corn oil, 2% Tween 80) (A). Left: Regular image (Scale bar: 20 μm); Right: Confocal image (Red is oil). Appearance (B)

The PSDs of the emulsions prepared using the three kinds of oil were fairly similar before heat treatment (**Fig. 2.4.**). After cooking, there was a large increase in particle size in all of the emulsions, indicative of droplet coalescence (**Fig. 2.4.A**). However, there were some differences in the size of the droplets present in the emulsions after cooking. The microscopy images suggested that the size of the oil droplets in the emulsions after cooking followed the order: mineral oil > corn oil > palm oil. These results suggest that the nature of the oil used to formulate the emulsions had an impact on their thermal stability. However, the origin of this effect is currently unknown – all of the oils should have been liquid over the range of temperatures used. Interestingly, the appearance of the emulsions was fairly similar before and after heat treatment (**Fig. 2.4.B**). However, the emulsion prepared from palm oil had a distinct yellowish color, which was attributed to the presence of natural yellow pigments in the palm oil itself.

**Impact on flavor retention**

A




B

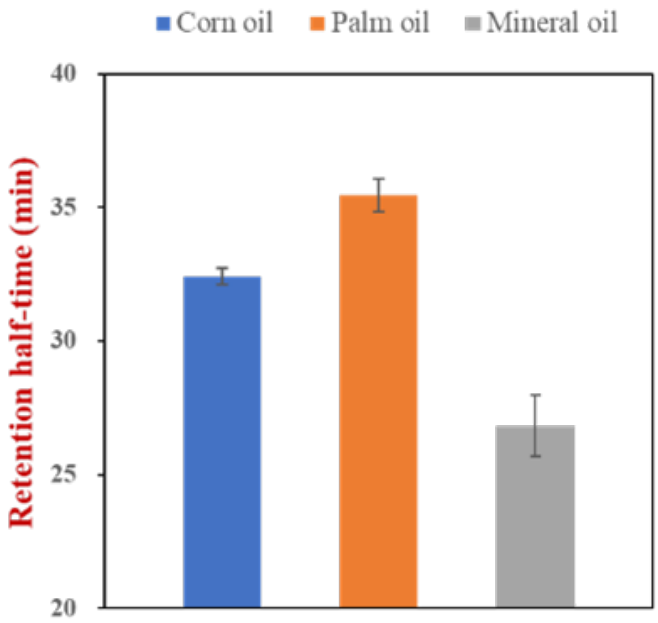



**Fig. 2.5.** Impact of oil type on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B).

The impact of oil type on the flavor retention profile and retention half-time of emulsions subjected to simulated cooking was also determined (**Fig. 2.5.**). There were clearly some differences in the ability of the three emulsions to retain flavor during cooking. The emulsions prepared with palm oil had the longest flavor retention half-time (36 min), while those prepared with mineral oil had the shortest (27 min). Initially, it was hypothesized that the rate of flavor loss would decrease as the oil phase viscosity increased because then the flavor molecules would travel more slowly through the oil droplets. However, this was not the case, since the viscosity of the oil phase increased in the following order: corn oil (31 cP) < palm oil (35 cP) < mineral oil (60 cP). Hence, the emulsion with the highest oil phase viscosity (mineral oil) actually led to the fastest flavor release. This result suggests that some other factors impacted the rate of flavor release from the emulsions.

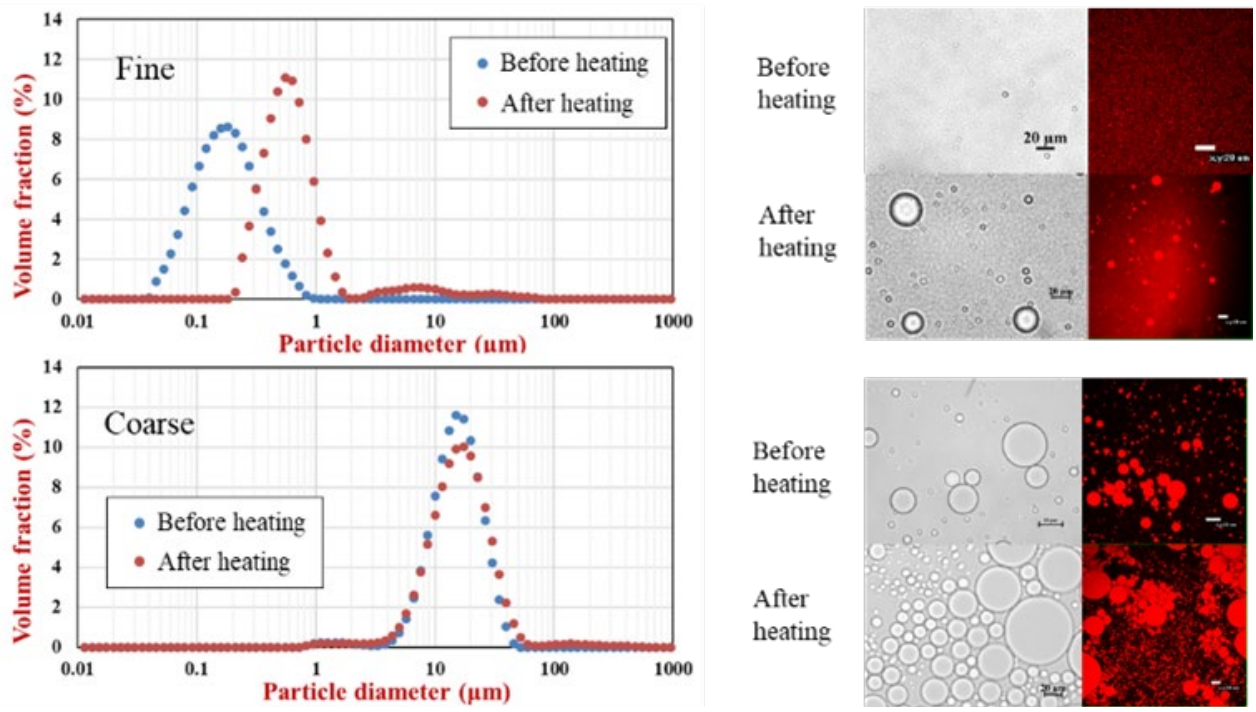
The delayed release of the flavor from the palm oil emulsions may have been because the oil droplets were either fully or partly crystalline at the start of the experiment, thereby inhibiting the movement of the flavor molecules inside the droplets. Conversely, the rapid release of flavor from the mineral oil emulsions, may have been because they were the most prone to droplet coalescence during cooking. Normally, an increase in droplet size would be expected to delay flavor release from the oil droplets, but it may also have reduced the distance the flavor molecules had to travel from the oil droplets to the headspace (**Fig. 2.3.**). Very large droplets would be expected to be closer to the upper surfaces of the emulsions because of creaming effects.

### 2.3.3 Impact of oil droplet size on flavor release

The initial size of the droplets in the emulsions would be expected to impact the stability of the emulsions during cooking, as well as their flavor release profiles (**Equation 1**). For this reason, two emulsions with different initial droplet sizes were prepared by varying the homogenization conditions. These two emulsions were prepared using 10 wt% oil phase and 90 wt% aqueous phase (containing 2 wt% Tween 80). The effects of their initial droplet dimensions on their physical stability and flavor retention during cooking were then determined (**Figs. 2.6. and 2.7.**).

**Impact on physical properties and structure**

**A**



**B**

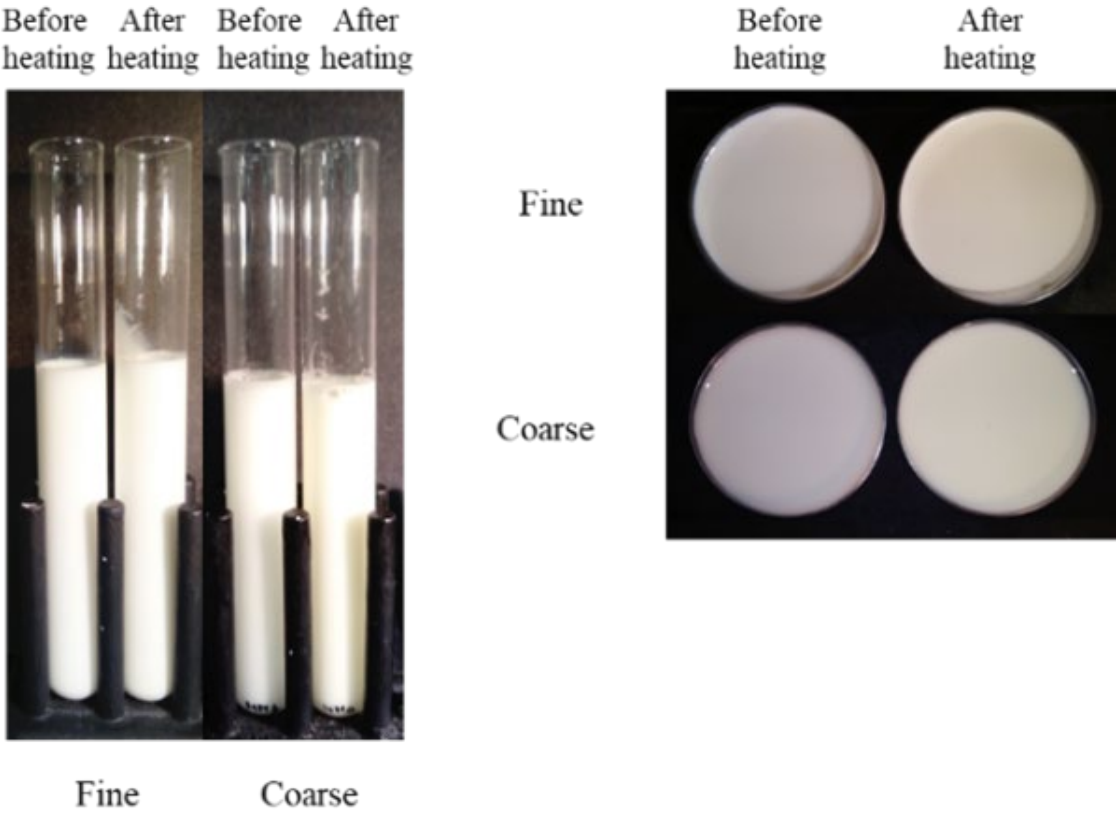
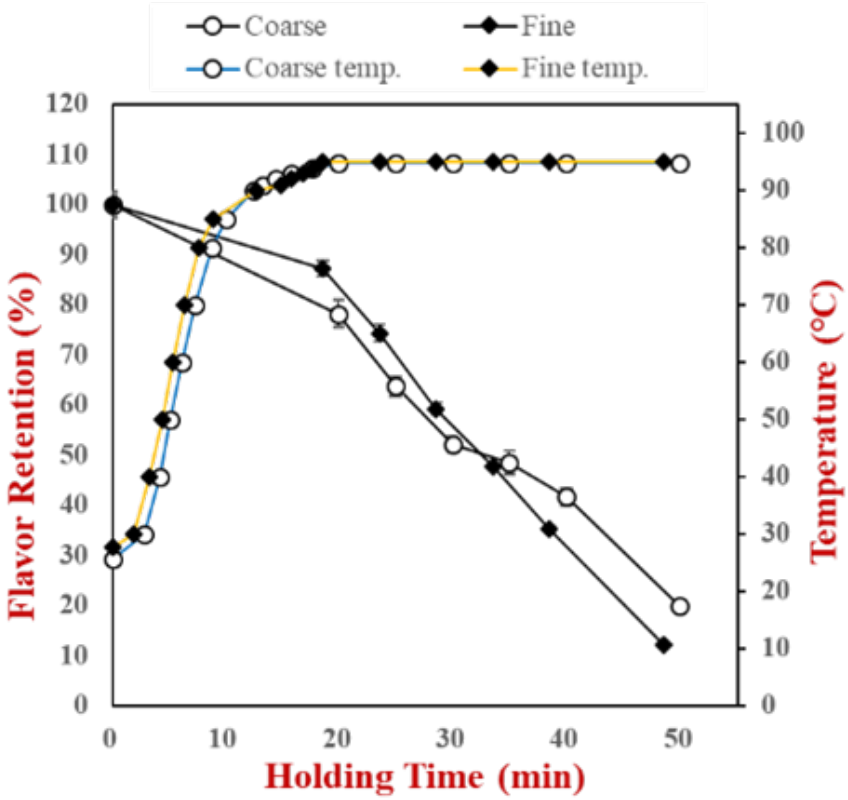



**Fig. 2.6.** Impact of initial droplet size on particle size distributions and microstructures of emulsions (10% corn oil, 2% Tween 80) (A). Left: Regular image (Scale bar: 20 μm); Right: Confocal image (Red is oil). Appearance (B)

The mean particle diameters ($D_{32}$) of the fine and coarse emulsions before heat treatment were 0.13 μm and 11.3 μm, respectively. After cooking, there was evidence of extensive droplet coalescence in the fine emulsions, as seen by a large increase in the dimensions of the individual oil droplets present (**Fig. 2.6.A**). Conversely, the oil droplets in the coarse emulsions were large both before and after cooking. Interestingly, the overall appearances of the emulsions were fairly similar before and after cooking (**Fig. 2.6.B**).

**Impact on heat retention**

A




B

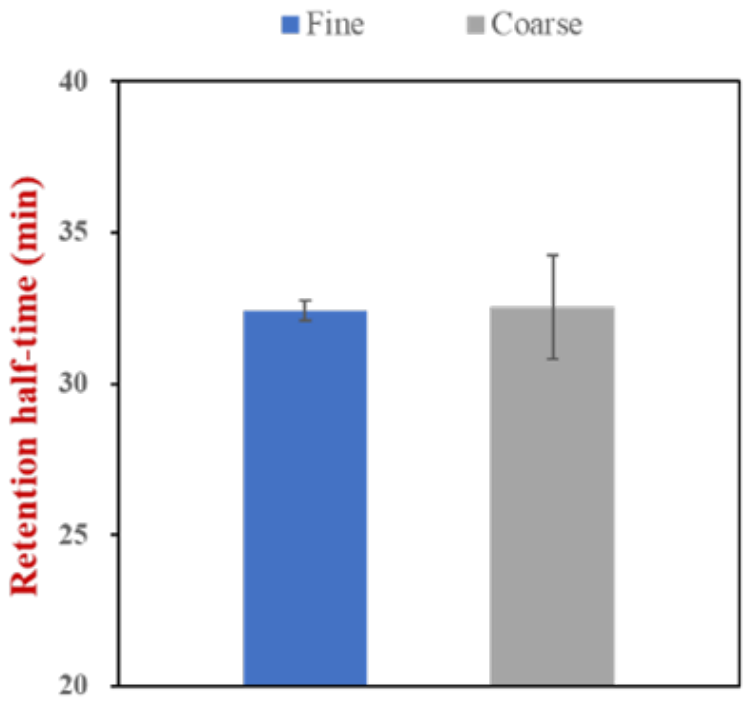



**Fig. 2.7.** Impact of droplet size on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B).

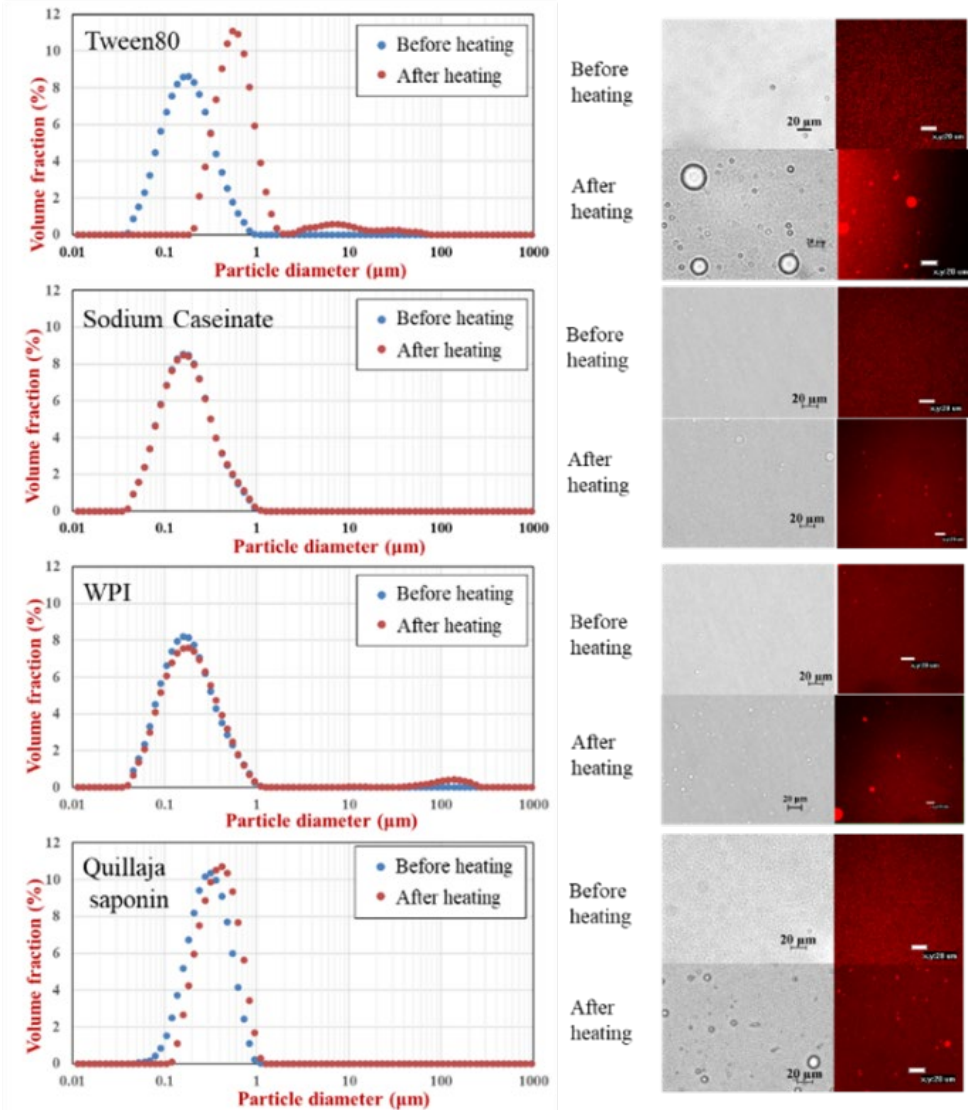
The impact of oil droplet size on the flavor retention profile and retention half-life of emulsions exposed to simulated cooking was also measured (**Fig. 2.7.**). Interestingly, the retention profiles and half-times of both emulsions were very similar, which suggests that the initial oil droplet size did not have a major impact on flavor loss during cooking. A previous study also showed that oil droplet size had little impact on the release of volatile ester compounds from oil-in-water emulsions to the head space of closed glass vials at 25 °C [52]. In the case, the flavor retention profiles may have been fairly similar because the droplets in the fine emulsions rapidly coalesced during cooking, so that the droplet size of the two systems was actually fairly similar.

### 2.3.4 Impact of emulsifier type on flavor release

Food emulsions can be stabilized by a variety of different kinds food-grade emulsifier, including surfactants, phospholipids, and biopolymers. We therefore investigated the impact of emulsifier type on the flavor release profile of the emulsions. Emulsions were prepared using four kinds of food-grade emulsifier: a non-ionic surfactant (Tween 80), a flexible protein (sodium caseinate), a globular protein (whey protein isolate), and a biosurfactant (quillaja saponin). All the emulsions used in these experiments were prepared using 10 wt% oil phase and 90 wt% aqueous phase (containing 2 wt% emulsifier). The effect of emulsifier type on their PSDs, stability, and flavor retention during cooking were then determined (**Figs. 2.8 and 2.9**). Quillaja saponin was included in this study because it is a natural surfactant reported to have good emulsion formation and stabilization characteristics [53, 54].

**Impact on physical properties and structure**

**A**




**B**

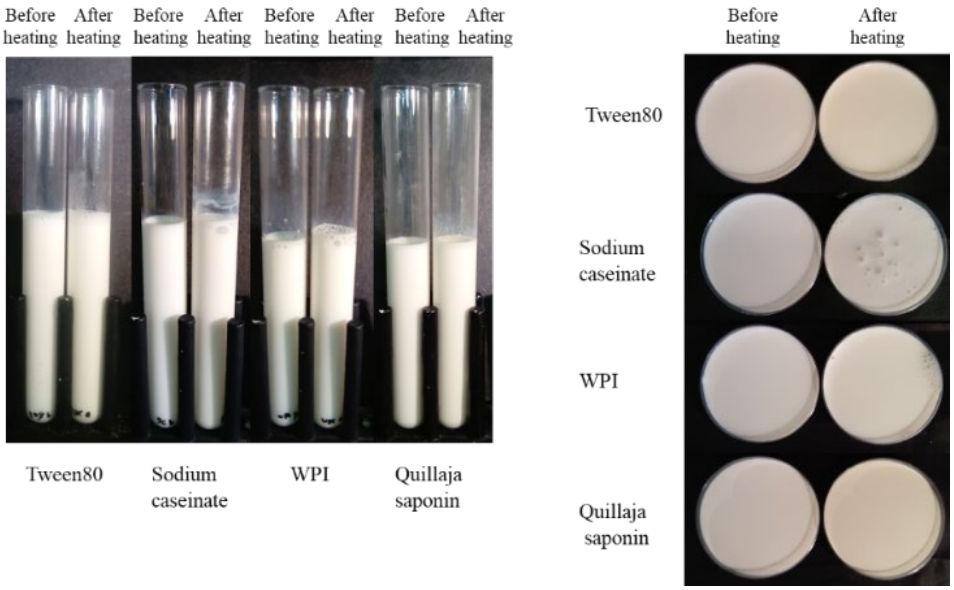


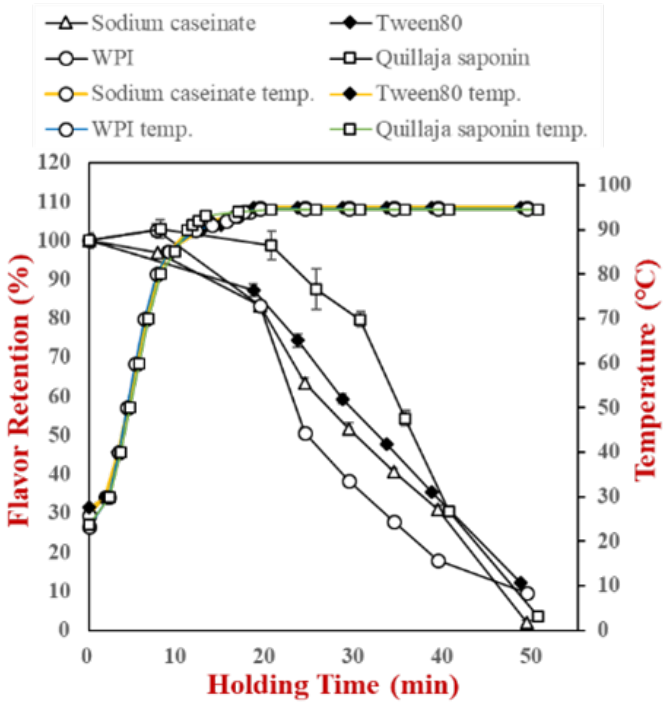
**Fig. 2.8.** Impact of emulsifier type on particle size distributions and microstructures of emulsions (10% corn oil, 2% emulsifier). (A). Left: Regular image (Scale bar: 20 μm); Right: Confocal image (Red is oil). Appearance (B)

All of the emulsions had monomodal PSDs and contained relatively small droplets before cooking indicating that all of the emulsifiers used were effective (**Fig. 2.8.A**). However, there were appreciable differences in the particle sizes of the emulsions after cooking. In particular, the emulsions formulated using Tween 80 had the worst thermal stability, exhibiting a large increase in droplet dimensions after cooking, indicative of extensive droplet coalescence. As discussed earlier, this effect can be attributed to dehydration of the non-ionic surfactant head-groups at elevated temperatures [48]. When the temperature is increased, some of the water molecules that were bound to the head-groups are liberated, which reduces the range of the steric repulsion, as well as changing the optimum curvature of the surfactant monolayer [55]. A small amount of droplet aggregation was also observed in the emulsions formulated with WPI after cooking, which can be attributed to denaturation and aggregation of the globular whey proteins at elevated temperatures [56, 57]. Previous studies have shown that heating sodium caseinate at 120 °C for prolonged times reduced its molar mass, viscosity and turbidity by an amount that depended on the heating time and protein concentration [58]. However, the study showed that the emulsions formulated with sodium caseinate maintained their stability when heated at 100 °C for 30 min. Finally, there appeared to be a small amount of droplet coalescence in the emulsions formulated with quillaja saponin after heating, but the oil droplets were still much smaller than those observed in the systems stabilized by Tween 80 after cooking. Previous studies have also reported some aggregation of saponin-coated oil droplets at elevated temperatures, which may again be due to partial dehydration of the hydrophilic regions on the surfactants [59, 60]. Again, the overall appearances of all the emulsions were fairly similar before and after heating (**Fig. 2.8.B**). These results indicate that the type of emulsifier used to stabilize the droplets impacts their stability during thermal processing, which

may have an impact on their functional performance in commercial products. Moreover, the level of emulsifier present in the emulsions may also have impacted the stability of the droplets to coalescence because this will have impacted the nature of the interfacial levels formed and their susceptibility to rupture.

**Impact on heat retention**

**A**




**B**

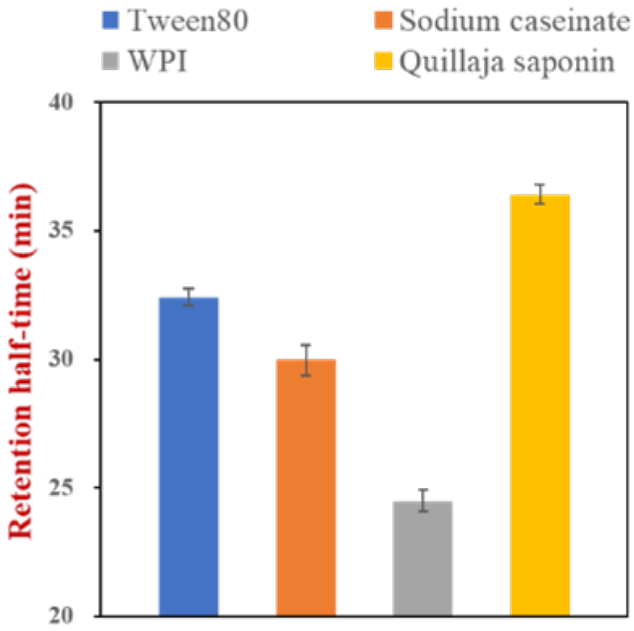


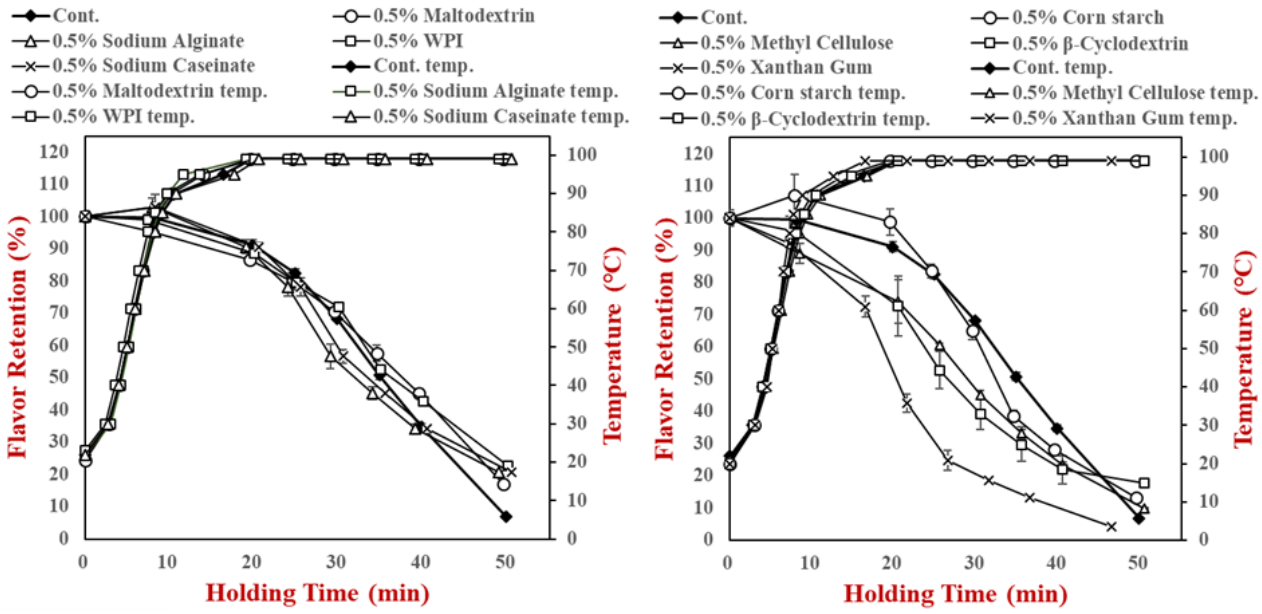
**Fig. 2.9** Impact of emulsifier type on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B).

The impact of emulsifier type on the flavor retention profile and retention half-time was also measured (**Fig. 2.9.**). The extent of flavor loss from the emulsions during cooking clearly depended on emulsifier type: quillaja saponin < Tween 80 < sodium caseinate < WPI. Thus, the emulsions stabilized by quillaja saponin appeared to give the best flavor retention, whereas those stabilized by the whey protein gave the worst. The physicochemical origin of this dependence on flavor retention on emulsifier type is not obvious. The emulsions stabilized by the small molecule surfactants (quillaja saponin and Tween 80) had the slowest flavor loss but were the least stable to oil droplet growth during cooking. Conversely, the emulsions stabilized by the proteins (sodium caseinate and WPI) had the fastest flavor loss but were the most stable to droplet aggregation during cooking. This effect could be due to differences in the size of the oil droplets in the emulsions during cooking. The emulsions that were more susceptible to coalescence contained larger droplets, which led to a reduction in the rate at which flavor molecules were released from the oil phase (**Equation 1**). On the other hand, larger oil droplets are more likely to be near the surface of the emulsions during cooking, which would lead to a shorter distance for them to travel into the gas phase (**Fig. 2.3.**). This could account for the better stability of the quillaja saponin system compared to the Tween 80 system: the droplets in the former may have been more uniformly dispersed through the system than those in the latter. In addition, there may have been differences in the ability of the different surfactants to solubilize or bind to the flavor molecules. Clearly, further research is required to establish the precise origin of the differences between the different kinds of emulsifier.

### 2.3.5 Impact of food additives on flavor release

A wide range of biopolymer additives that are frequently used as functional ingredients in food products was selected for testing. Maltodextrin and corn starch were selected because they are digestible oligosaccharides and polysaccharides often used to modify food texture [61, 62]. Methyl cellulose, xanthan gum, and sodium alginate were selected because they are indigestible polysaccharides often used as thickening agents, gelling agents, or stabilizers [61, 62]. Sodium caseinate and whey protein isolate were selected because they are widely used protein-based emulsifiers and protein sources in foods [63, 64]. In addition, we selected β-cyclodextrin because it has the potential to bind non-polar flavor molecules within its hydrophobic cavity [65], which might be expected to retard flavor release.

**A**



**B**

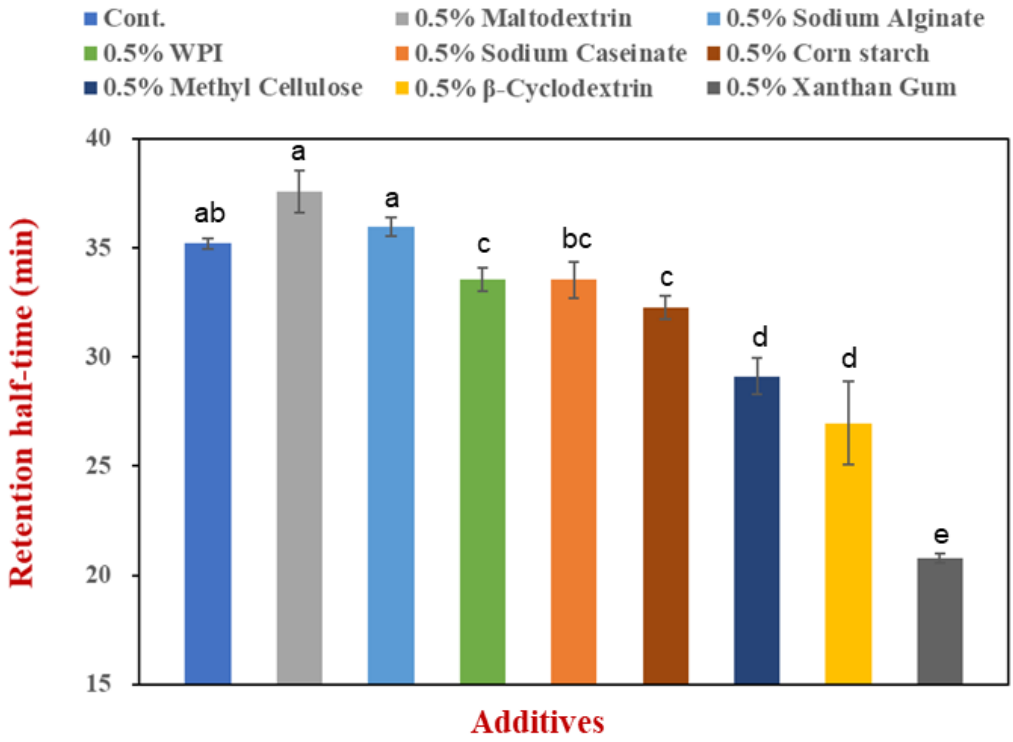



**Fig. 2.10.** Influence of additives on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B). Values (mean ± SD, n = 3) with the same lowercase letters indicate no significant difference ($P < .05$, ANOVA)

The oil-in-water emulsions used in this series of experiments all contained 10 wt% corn oil (flavor-loaded oil droplets), 1 wt% sodium caseinate (emulsifier), and 0.5 wt% of additive. The impact of the additives on the flavor retention profiles of the emulsions during simulated cooking were measured (**Fig. 2.10.**). All of the emulsions had similar temperature-time profiles: there was a rapid increase in temperature from 0 to 10 min, followed by a slow increase from 10 to 20 min, followed by a relatively constant value at longer times. The flavor retention-time profiles depended on the nature of the additive used. For the sake of clarity, we divided the samples into two categories; one group with similar retention profiles as the control emulsion (no additive) and another

group with different profiles (**Fig. 2.10.A**).

For the control, the amount of flavor retained by the emulsion remained relatively constant from 0 to 10 min but then decreased progressively with increasing cooking time. This effect can be attributed to the fact that appreciable volatilization of the garlic flavor only occurred when the temperature of the emulsion was close to boiling. The emulsions containing 0.5% sodium alginate, sodium caseinate, maltodextrin, or whey protein all exhibited fairly similar behavior as the control emulsions, suggesting that these additives did not have a major impact on the flavor release profile. Conversely, the emulsions containing 0.5% methyl cellulose, xanthan, corn starch, or β-cyclodextrin exhibited a distinctly different flavor retention-time profile to the control.

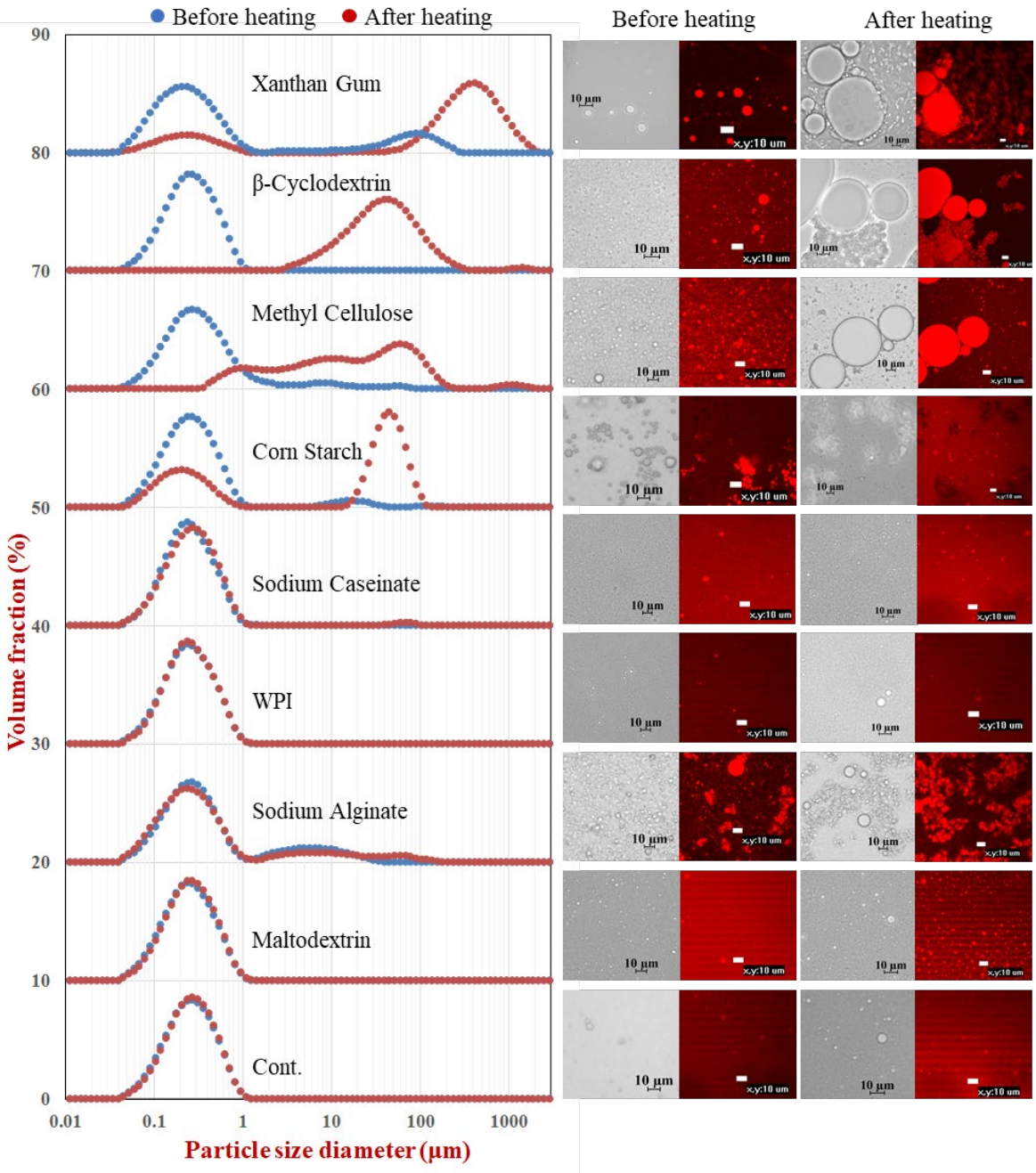
The retention half-time, that is the time required for half of the flavor molecules to be lost, was calculated from the flavor retention profiles for each sample (**Fig. 2.10.B**). These calculations show that the majority of the additives actually increased the rate at which the garlic flavor was lost from the emulsions during cooking, *i.e.,* they reduced the retention half-time. In particular, the addition of xanthan gum led to the quickest loss of flavor from the emulsions. These results are discussed in terms of the impact of the various additives on emulsion stability and rheology in the following sections.

**Impact of emulsion stability on flavor release**

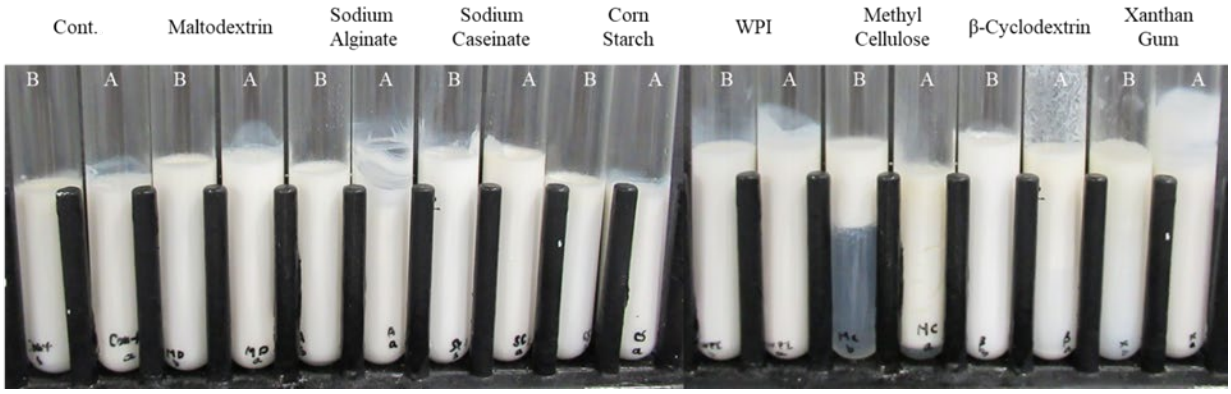
The particle size distributions (PSDs) and microstructures of the emulsions before and after simulated cooking were recorded **(Fig. 2.11.A**). After heating, there was a large increase in particle size in the emulsions containing corn starch, methyl cellulose, β-cyclodextrin, and xanthan gum. The size increase observed in the emulsions with methyl cellulose, β-cyclodextrin, and xanthan gum appeared to be due to extensive coalescence since large individual oil droplets were observed in the confocal fluorescence microscopy images. However, the large increase in the size of the particles in the emulsions containing corn starch was attributed to gelatinization of the starch granules because the particles did not appear to consist of oil (no red dye). Previous studies have also shown that

starch gelatinization can occur in oil-in-water emulsions during cooking without breaking the emulsions [66]. Moreover, other researchers have shown that starch from quinoa, rice, corn, and potato can also stabilize emulsions [67-69]. Based on the results and those reported by other previous researchers, it therefore seems that the addition of corn starch did not promote droplet coalescence during heating, although a small amount of droplet flocculation may have occurred.

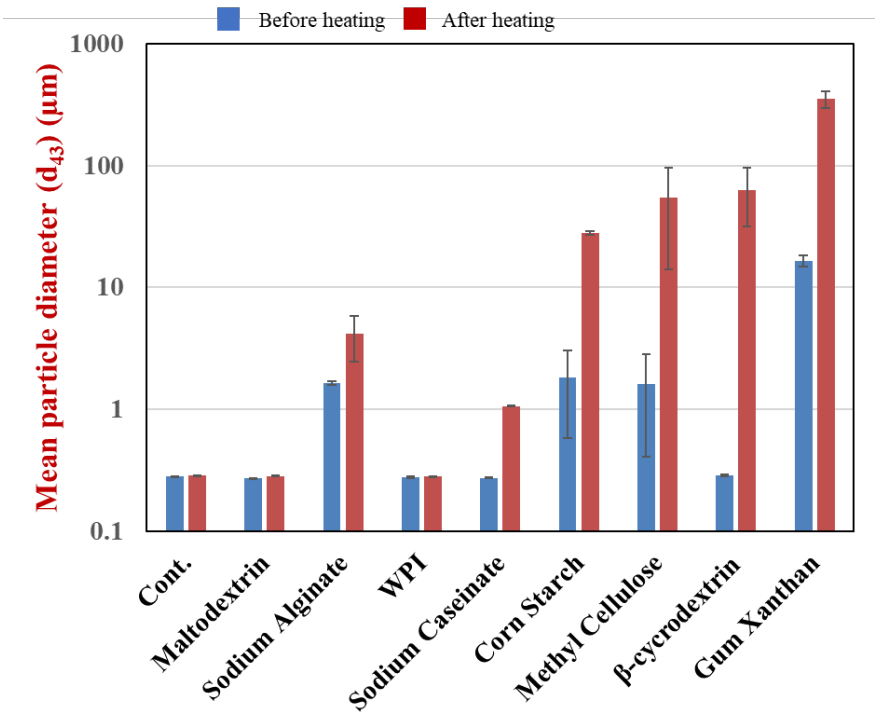
**A**



**B**


Cont.
Maltodextrin
Sodium Alginate
Sodium Caseinate
Corn Starch
WPI
Methyl Cellulose
β-Cyclodextrin
Xanthan Gum
B
A

**C**


Before heating
After heating
Mean particle diameter (d43) (μm)
1000
100
10
1
0.1
Cont.
Maltodextrin
Sodium Alginate
WPI
Sodium Caseinate
Corn Starch
Methyl Cellulose
β-cycrodextrin
Gum Xanthan

**D**

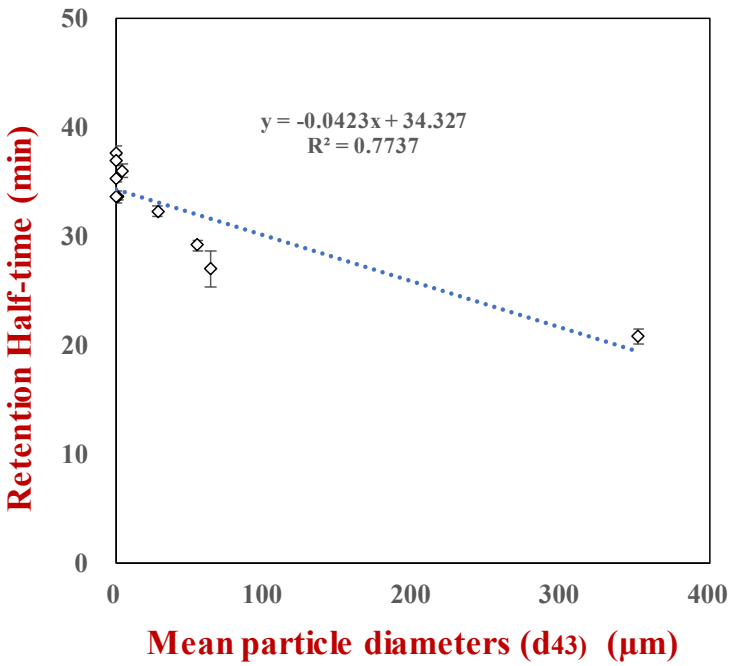

y = -0.0423x + 34.327
R² = 0.7737
Retention Half-time (min)
50
40
30
20
10
0
0
100
200
300
400
Mean particle diameters (d43) (μm)

**Fig. 2.11.** Influence of additives on particle size distributions and microstructures of emulsions (10% corn oil, 1% Sodium Caseinate) (A). Left: Regular image (Scale bar: 10 μm); Right: Confocal image (Red is oil). Appearance (B) B: before heating; A; after heating. Mean particle diameter (C). The relationship between Mean particle diameters ($d_{43}$) of after heating and Retention half-time (D).

As mentioned earlier, methyl cellulose, β-cyclodextrin, and xanthan gum all caused extensive droplet coalescence within the emulsions during heating. Interestingly, the flavor retention half-time values for these emulsions were considerably shorter than that of the control (**Fig. 2.10.B**). In fact, the positive correlation was found between Mean particle diameters ($d_{43}$) of after heating and Retention half-time (**Fig. 2.11.D**) This result suggests that droplet coalescence accelerated the rate of flavor release from the emulsions during cooking. We postulate that the large oil droplets rapidly moved to the top of the samples due to gravitational forces, where they formed an oil layer on the surfaces of the emulsions. As a result, the flavor molecules could easily diffuse from the oil phase into the gas phase, *i.e.,* they only had a short diffusion pathway compared to if they were present within the interior of the emulsions.

The ability of methyl cellulose, xanthan gum, and β-cyclodextrin to promote droplet coalescence during cooking may have been a result of various physicochemical phenomena. First, biopolymers with hydrophobic moieties, such as methyl cellulose, may have bound some of the emulsifier molecules to their surfaces, thereby reducing their ability to stabilize the oil droplets from coalescence. Other studies have also shown that the addition of methyl cellulose can cause creaming and oil separation in emulsions [70]. Second, sufficiently high levels of non-adsorbed biopolymers, such as methyl cellulose and xanthan gum, can generate a strong osmotic attraction between the oil droplets due to a depletion mechanism [7]. This osmotic pressure forces the droplets into close contact, which can disrupt the interfacial layers and promote coalescence. Other studies have also shown that addition of xanthan gum can lead to accelerated coalescence in emulsions due to this phenomenon [71, 72]. Third, certain types of additives, such as β-cyclodextrin, may form insoluble inclusion complexes

with the oil phase, thereby making the oil droplets more susceptible to coalescence. Previous studies have shown that β-cyclodextrin can form inclusion complexes with oils, thereby leading to the formation of physically unstable systems [73]. Originally, we had thought that the β-cyclodextrin would bind the flavor molecules and increase their retention during cooking. In practice, however, their addition actually led to faster flavor release (**Fig. 2.10.B**). It therefore seems that the propensity of the β-cyclodextrin to destabilize the emulsions is more important than any flavor binding properties.

Interestingly, the addition of sodium alginate to the emulsions caused extensive droplet flocculation in the emulsions, both before and after heating (**Fig. 2.11.A**), but did not have any effect on flavor retention (**Fig. 2.11.B**). This may have been because the osmotic pressure generated by the sodium alginate was less than that generated by the xanthan gum, and so the propensity for coalescence to occur was reduced. The presence of additives that did not promote droplet coalescence or flocculation in the emulsions, such as maltodextrin, sodium caseinate, and whey protein also did not appear to impact the flavor release rate during cooking. In summary, these results may have important consequences for the formulation of foods with controlled flavor release profiles, since different ingredients appear to have distinctly different effects.

**Impact of rheology on flavor release**

The rheological properties of an emulsion may impact the mass transport of volatile flavor molecules into the gas phase by altering molecular diffusion or mixing phenomena [7, 16]. It is, however, important to distinguish between micro-viscosity and macro-viscosity effects. Some biopolymers can greatly increase the macro-viscosity of aqueous solutions by altering the fluid flow profile but still have little effect on the micro-viscosity experienced by small molecules in solution. For instance, small flavor molecules can diffuse through the large pores that separate the biopolymer molecules in solution. As a result, at the molecular level they experience a viscosity similar to that of pure water, rather than that of the overall system. On the other hand, large increases in the macro-

viscosity of an emulsion can alter flavor release by changing the mixing behavior and movement of the flavor-loaded oil droplets.

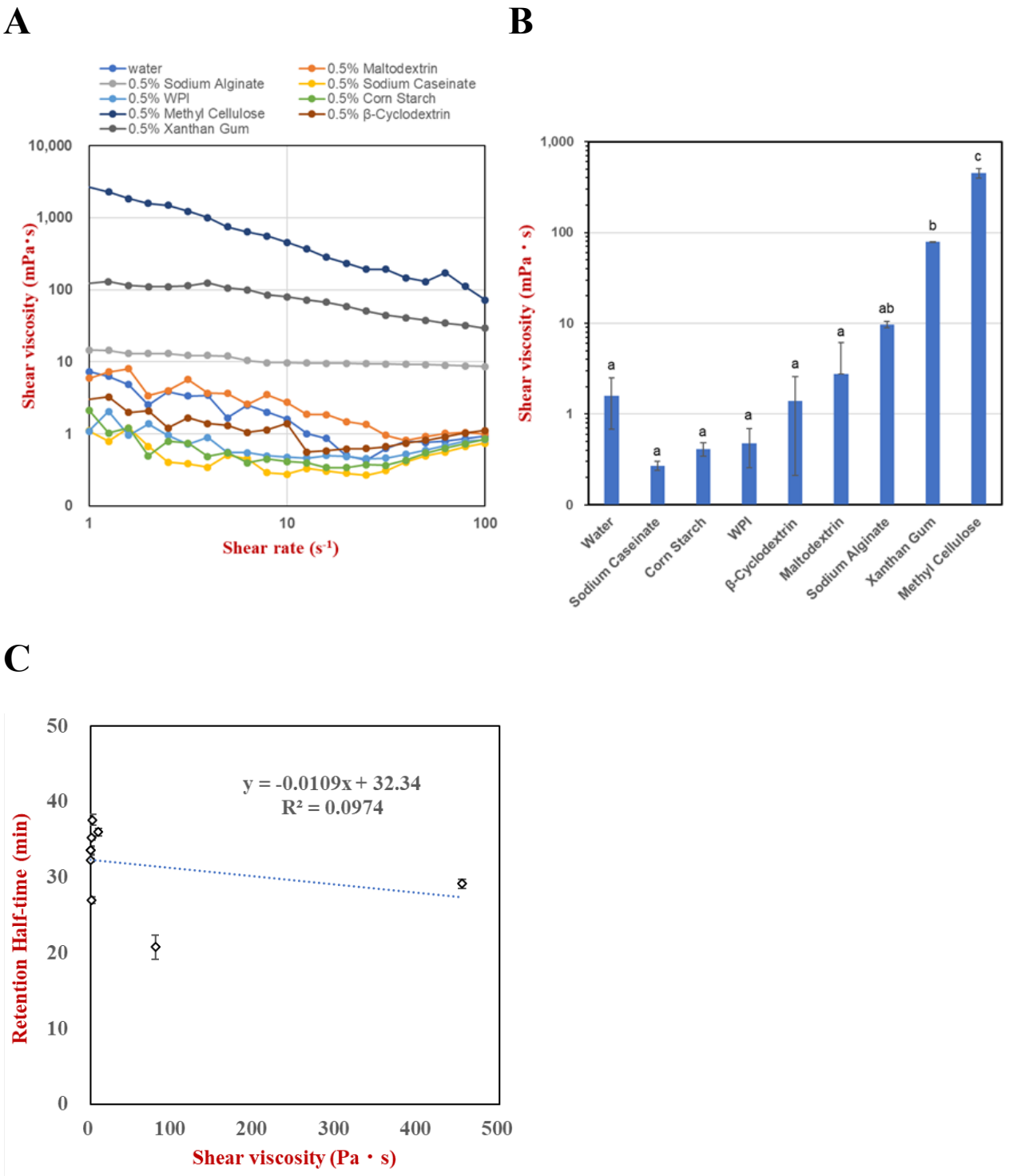



**Fig. 2.12.** Influence of additives on the apparent shear viscosity on the applied shear rate (1-100 $s^{-1}$) of 0.5% aqueous solutions (A). The apparent shear viscosity of each additive at the shear rate 10 $s^{-1}$ (B). The relationship between shear viscosity and retention half-time (C).

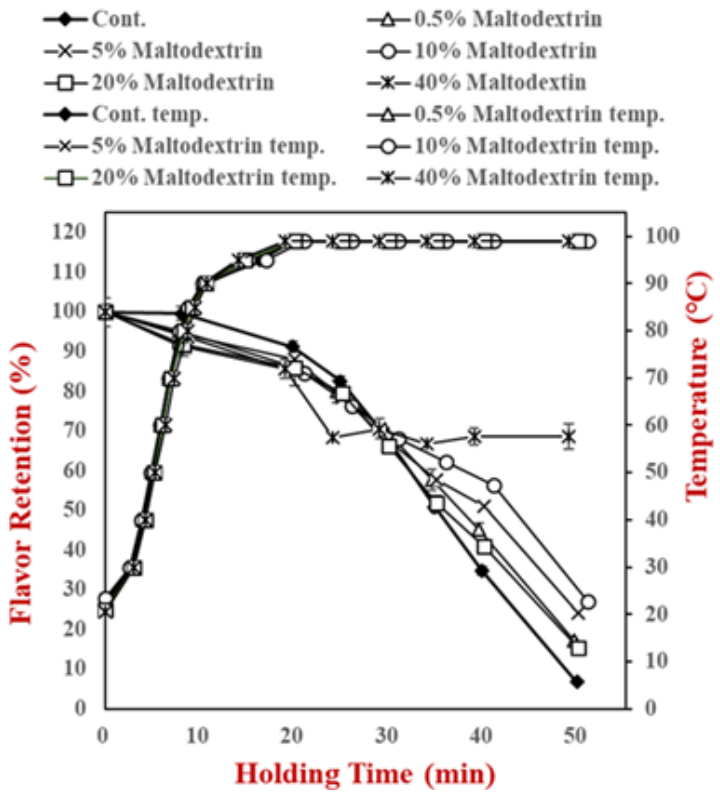
In this series of experiments, the impact of the different additives on the rheological properties of the aqueous solutions used to formulate the emulsions was measured to determine if the macro-viscosity influenced flavor release properties (**Fig. 2.12.**). The results showed that the apparent shear viscosity of the 0.5% aqueous solutions at 95 °C decreased in the following order: methyl cellulose > xanthan gum > sodium alginate > others. There appeared to be no correlation between the viscosity of the aqueous phase and the flavor release profile during cooking (**Fig. 2.12.C**). For instance, the viscosity of the β-cyclodextrin solution was fairly similar to that of pure water, whereas the viscosities of the methyl cellulose and xanthan gum solutions were much greater than that of pure water, despite the fact that they both led to rapid flavor release.

Overall, these results suggest that emulsion stability is the dominant factor impacting flavor release rate during cooking rather than the aqueous phase viscosity. Further insights were obtained by examining the impact of additive concentration on flavor release for two selected additives, one where there was little effect (maltodextrin) and another where there was a big effect (xanthan gum).

### 2.3.6 Impact of maltodextrin concentration on flavor release

As described earlier, the addition of relatively low levels of maltodextrin (0.5%) to the emulsions did not have much impact on their stability, viscosity, or flavor release profile during cooking. For this reason, the impact of much higher levels of maltodextrin on emulsion properties was examined, since the additive would be expected to have a bigger impact at a higher concentration.

**A**




**B**

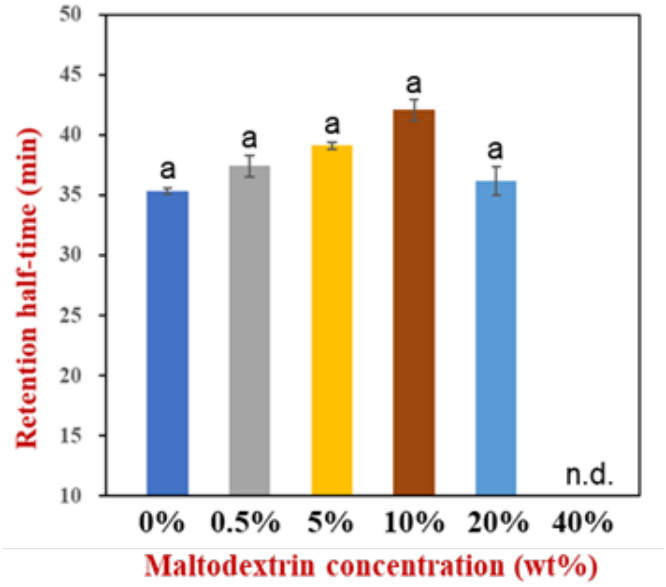


**Fig. 2.13.** Influence of maltodextrin concentration on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B). Values (mean ± SD, n = 3) with the same lowercase letters indicate no significant difference ($P < .05$, ANOVA)

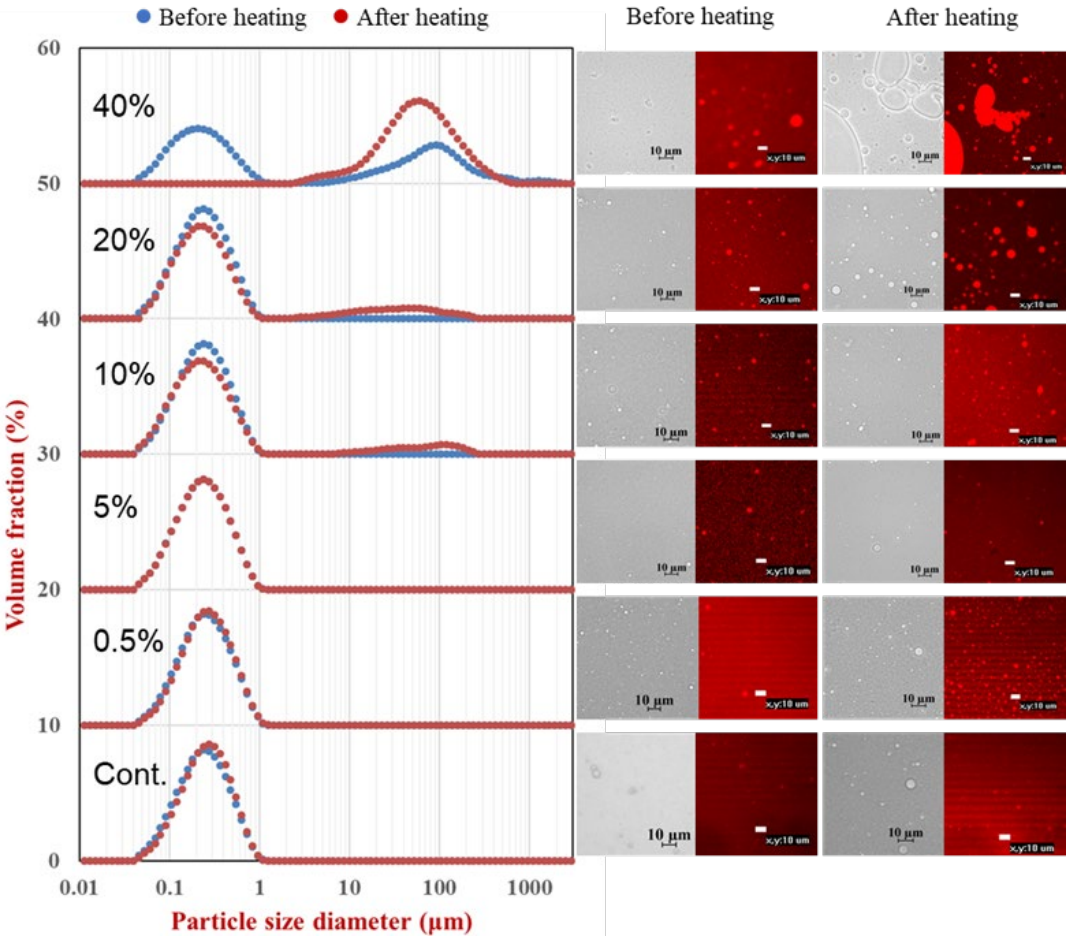
The flavor retention profile and retention half-time of the emulsions during simulated cooking were measured (**Fig. 2.13.**). From, 0 to 20 wt% maltodextrin, there was no significant differences between the retention half-times of the emulsions. At 40 wt% maltodextrin, however, we observed some unusual behavior – a thick crust formed at the surface of the emulsions after some of the water evaporated, which prevented any more flavor molecules from being released. Consequently, the flavor retention remained relatively constant upon further heating.

**Impact of emulsion stability on flavor release**

The PSDs and microstructures of emulsions containing different maltodextrin levels were also measured before and after heat treatment (**Fig. 2.14.**). From 0.5 to 5 wt% maltodextrin, the PSDs of the emulsions appeared fairly similar before and after heat treatment, with little evidence of droplet aggregation. At maltodextrin levels of 10 wt% and higher, however, there was evidence that some of the droplets in the emulsions had coalesced, especially after heating. Based on previous studies, we postulate that this effect was due to the presence of non-adsorbed maltodextrin molecules in the aqueous phase surrounding the oil droplets [74]. Once the maltodextrin exceeds a critical level, the attractive interactions (van der Waals and depletion) between the oil droplets exceed the repulsive interactions (steric and electrostatic), which promotes droplet flocculation. As a result, the droplets remain in contact for extended periods and are forced together due to an osmotic pressure, which promotes coalescence. Other studies have also reported that maltodextrin addition influences the stability of sodium caseinate-stabilized emulsions, which was again attributed to a depletion effect [75]. However, the level of emulsion destabilization observed in the study did not appear to have a major

impact on flavor release, except at the highest maltodextrin level used. For example, the retention half-times were fairly similar from 0.5 to 20 wt% maltodextrin even though some droplet coalescence was observed at 10 and 20 wt% maltodextrin. This phenomenon may have occurred because the majority of oil droplets in the emulsions remained relatively small, as seen in the microscopy images and the photographs of the samples. At 40 wt% maltodextrin, the emulsions had clearly undergone extensive flocculation, coalescence, and creaming, but they still had a relatively slow flavor release rate. As mentioned earlier, it was hypothesized that an impermeable crust was formed at the top of these emulsions during heating, which inhibited flavor release.

**A**



**B**

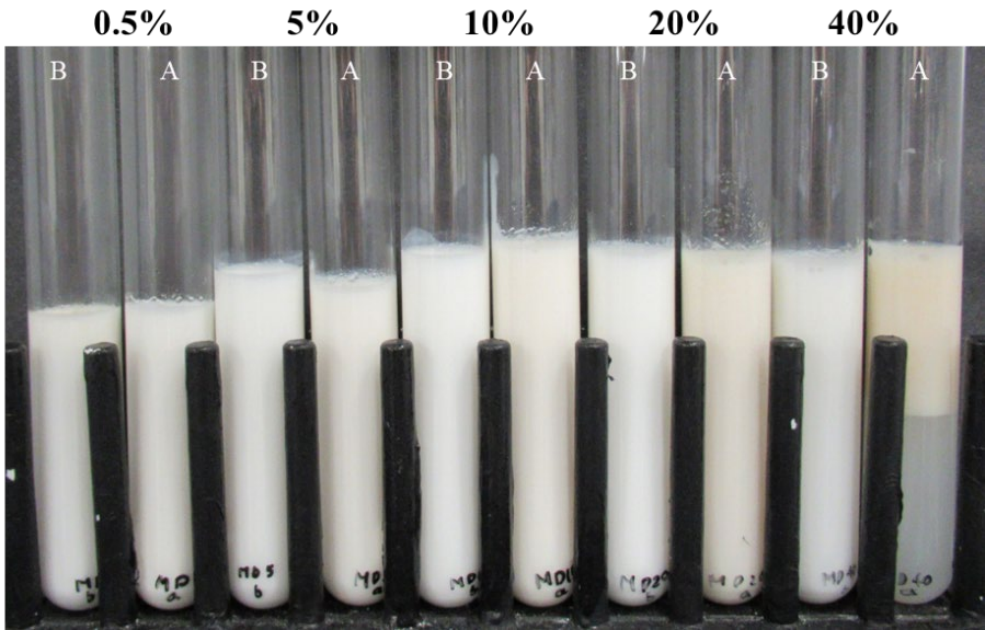



**Fig. 2.14.** Influence of maltodextrin concentration on particle size distributions and microstructures of emulsions (10% corn oil, 1% Sodium Caseinate) (A). Left: Regular image (Scale bar: 10 μm); Right: Confocal image (Red is oil). Appearance (B) B: before heating; A; after heating.

**Impact of rheology on flavor release**

The rheological properties of the aqueous maltodextrin solutions were also measured as a function of concentration (**Fig. 2.15.A**). As expected, the apparent shear viscosity increased with increasing maltodextrin concentration (**Fig. 2.15.B**). However, we did not observe a change in the flavor retention half-time as the shear viscosity increased (**Fig. 2.15.C**), which suggests that the macro-viscosity of the continuous phase did not play a major role in favor release for this system.

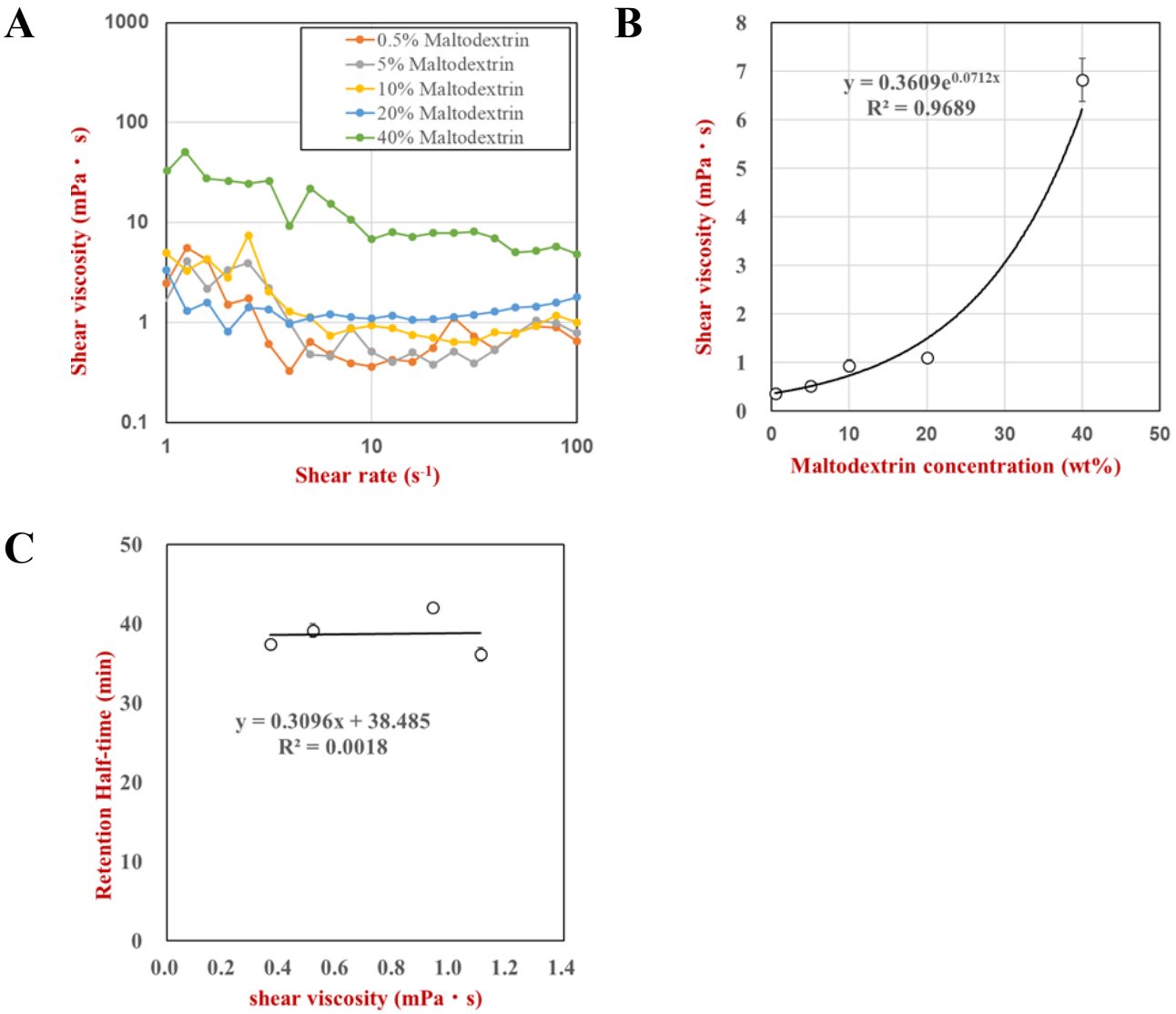
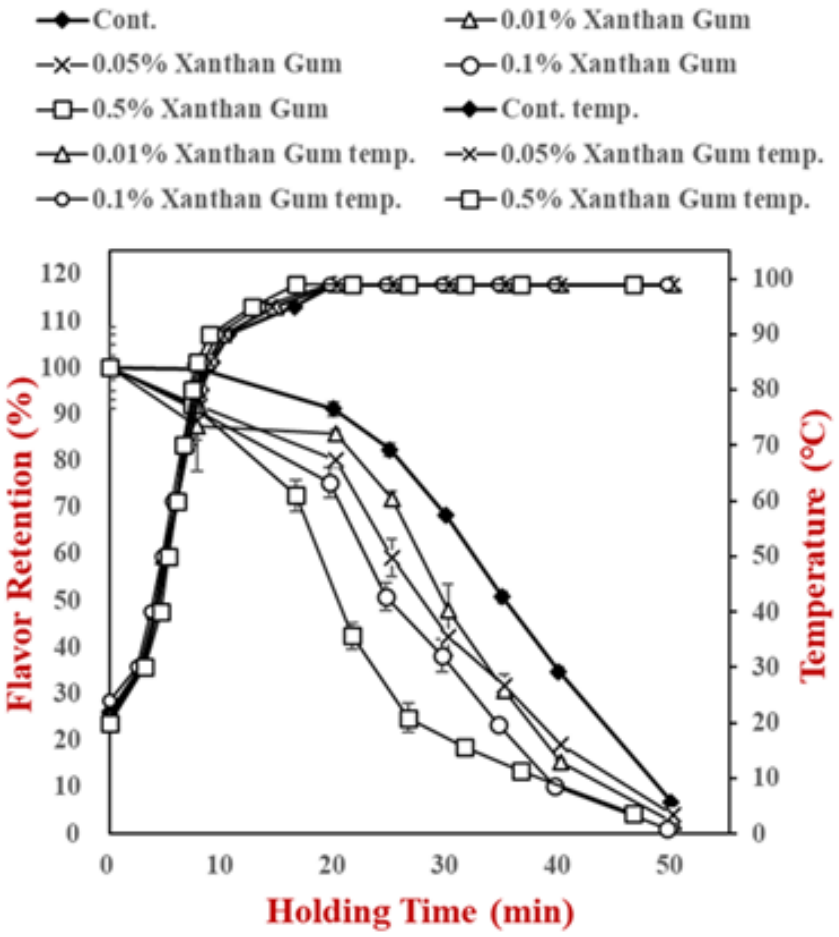



**Fig. 2.15.** Influence of maltodextrin concentration on the apparent shear viscosity on the applied shear rate (1-100 $s^{-1}$) of 0.5% aqueous solutions (A). The apparent shear viscosity of each maltodextrin concentration at the shear rate 10 $s^{-1}$ (B). The relationship between apparent shear viscosity and retention half-time (C).

### 2.3.7 Impact of xanthan gum concentration on flavor release

The impact of xanthan gum concentration on emulsion properties was examined because this biopolymer was shown to have the largest influence on flavor release in the earlier studies (**chapter 2.3.5.**). Only relatively low levels (0.01 - 0.5 wt%) of xanthan gum could be evaluated because higher concentrations led to extremely viscous solutions that were difficult to prepare and work with. The impact of xanthan gum concentration on the flavor retention profile and retention half-time of emulsions subjected to simulated cooking was measured (**Fig. 2.16.**). In general, the rate of flavor release increased (retention time decreased) with increasing xanthan gum concentration.

**A**



**B**

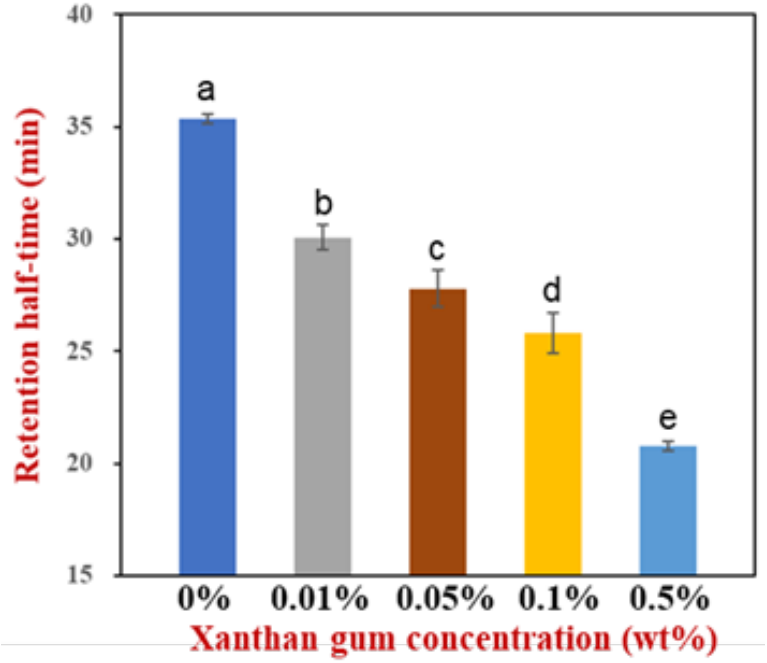
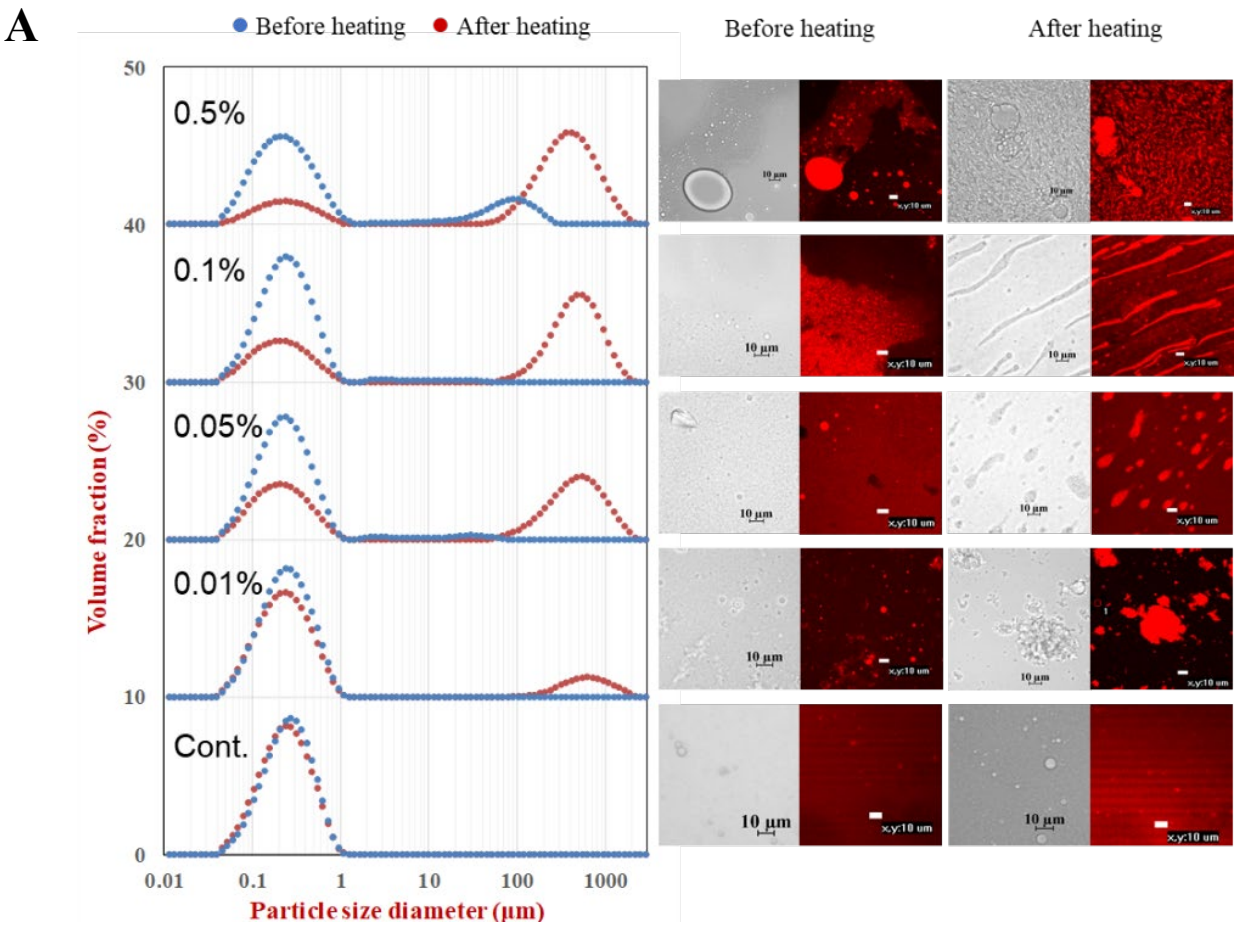



**Fig. 2.16.** Influence of xanthan gum concentration on the temperature and flavor retention profiles of emulsions during simulated cooking (A); Retention half-time for a 50% decrease in AMDS level (B). Values (mean ± SD, n = 3) with the same lowercase letters indicate no significant difference ($P < .05$, ANOVA)

**Impact of emulsion stability on flavor release**

The PSDs, microstructures, and appearances of emulsions with different xanthan gum levels were measured before and after heat treatment (**Fig. 2.17.**). These results showed that addition of xanthan gum promoted depletion flocculation, coalescence, and gravitational separation at higher levels. Previous studies have also reported that the presence of non-adsorbed biopolymers can promote instability in protein-stabilized emulsions during heating due to a strong depletion effect [76,77]. Creaming was observed in the emulsions containing 0.01 to 0.05 wt% xanthan gum after heat treatment and 0.1 wt% xanthan gum before heat treatment. At higher levels, creaming was not observed, which can be attributed to the fact that the viscosity of the aqueous phase was so high that

the droplets could not move, even if they were aggregated [78]. Nevertheless, droplet flocculation and coalescence can still occur at high xanthan levels because of the strong osmotic attraction generated by the non-adsorbed biopolymers. There are a number of factors that might account for the differences in the properties of the heated and non-heated emulsions. The xanthan gum concentration in the emulsions may have been higher after heating due to some water evaporation. There may also have been changes in the structure and interactions of the biopolymers in the system at elevated temperatures, which effected their functionality.



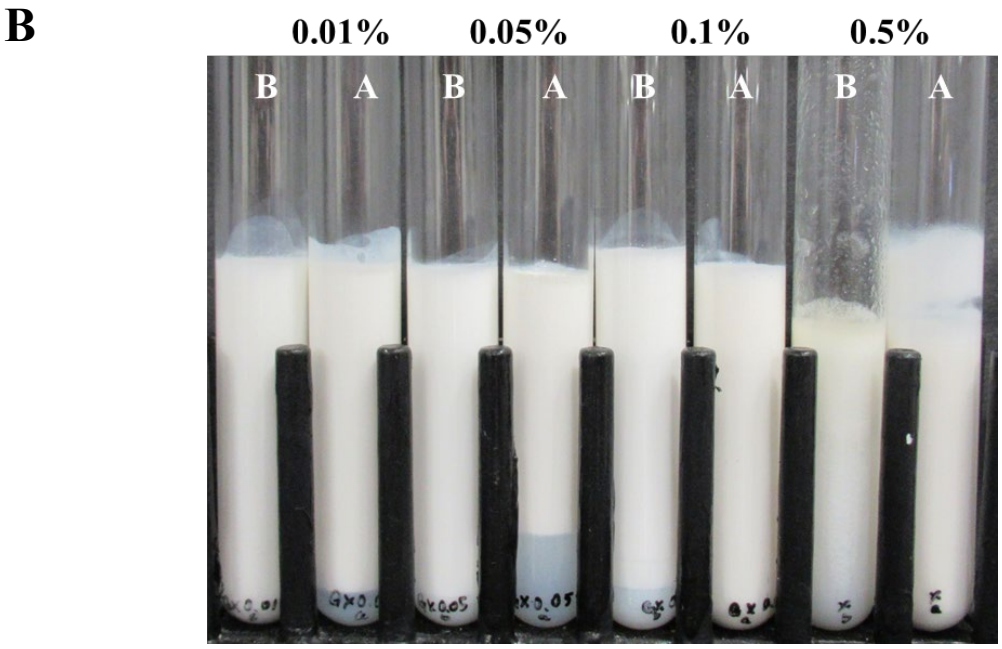



**Fig. 2.17.** Influence of xanthan gum concentration on particle size distributions and microstructures of emulsions (10% corn oil, 1% Sodium Caseinate) (A). Left: Regular image (Scale bar: 10 μm); Right: Confocal image (Red is oil). Appearance (B) B: before heating; A; after heating.

Xanthan gum is typically used as a stabilizer in food emulsions [79, 80]. Indeed, previous studies have shown that it can delay creaming in emulsions during storage at room temperature and thereby enhance emulsion stability [81]. In contrast, other studies have shown that xanthan gum can promote droplet aggregation and creaming when emulsions are exposed to elevated temperatures [77], which is in agreement with the study.

**Impact of rheology on flavor release**

The rheological properties of the heated xanthan gum aqueous solutions were measured (**Fig.2.18.A**). As expected, the apparent shear viscosity increased as the xanthan gum concentration was increased (**Fig.2.18.B**). The flavor retention half-time, however, decreased when the viscosity of the xanthan gum solution increased (**Fig.2.18.C**), which means that faster flavor release occurred at higher xanthan concentrations. Before starting the experiments, it was hypothesized that a higher aqueous phase viscosity would decrease flavor release during cooking by slowing down mass

transport processes. In reality, we found the opposite result – the flavor release became faster as the xanthan gum concentration was raised. This result again suggests that the thermal stability of the emulsions plays a more important role in determining flavor release kinetics than the viscosity of the aqueous phase. An increase in xanthan gum concentration promoted flocculation, coalescence, and creaming, which may have caused more of the flavor-loaded oil droplets to be at the surfaces of the emulsions leading to faster flavor release.

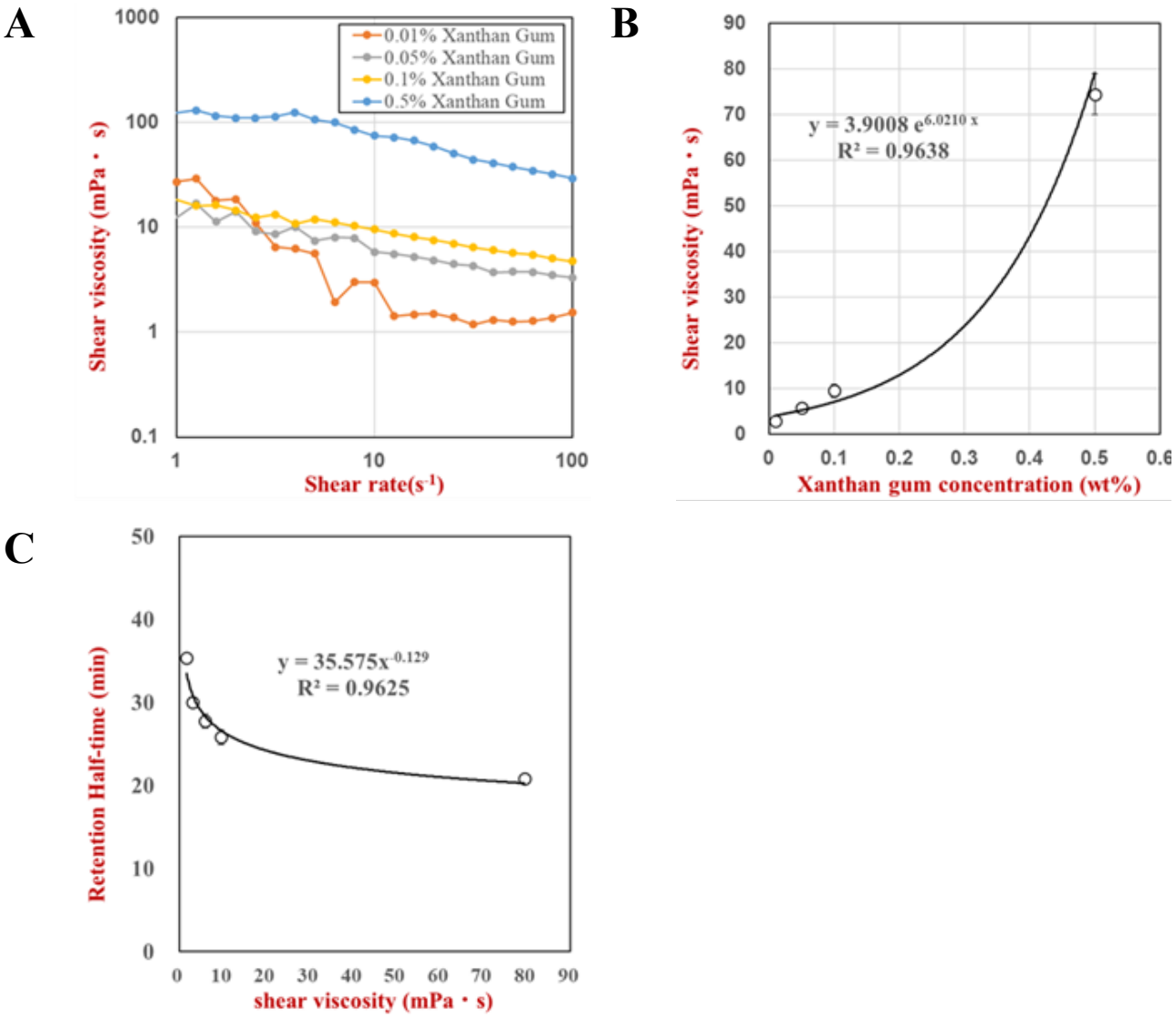



**Fig. 2.18.** Influence of xanthan gum concentration on the apparent shear viscosity on the applied shear rate (1-100 $s^{-1}$) of 0.5% aqueous solutions (A). The apparent shear viscosity of each gum xanthan concentration at the shear rate 10 $s^{-1}$ (B). The relationship between apparent shear viscosity and retention half-time (C).

## 2.4 Conclusions

This study showed that a number of droplet characteristics influenced the rate of flavor retention in oil-in-water emulsions during cooking. Emulsions containing higher oil droplet levels were more effective at retaining the hydrophobic flavors, but less physically stable. The nature of the oil phase used to formulate the emulsions also had an impact on flavor release, with the rate of flavor loss increasing in the following order: mineral oil > corn oil > palm oil. These differences were attributed to differences in the diffusion of the flavor molecules through the oil phases, as well as to differences in the coalescence stability of the oil droplets during cooking. Emulsifier type also impacted emulsion stability and flavor retention. Emulsions stabilized by small molecule surfactants (quillaja saponin and Tween 80) were most prone to droplet growth during thermal processing but gave the slowest flavor release. Conversely, emulsions stabilized by proteins (whey protein or caseinate) were the most resistant to droplet aggregation but gave the fastest flavor release. Additionally, this study investigated the impact of various biopolymer additives, namely proteins and polysaccharides, on the stability, rheology, and flavor release characteristics of oil-in-water emulsions during simulated cooking. The flavor retention profiles of emulsions containing maltodextrin, sodium alginate, whey protein, sodium caseinate, and corn starch were similar to the control, which was attributed to the fact that the individual oil droplets remained relatively stable to coalescence during cooking. On the other hand, a faster rate of flavor release was observed in emulsions containing methyl cellulose, β-cyclodextrin, and xanthan gum, which was attributed to their tendency to promote extensive droplet coalescence. It was postulated that the oil droplets moved to the top of the emulsions where they could release the flavor molecules into the surrounding air more easily. In contrast, the viscosity of the aqueous phase did not appear to play a major role in determining the flavor release profiles. Experiments on the impact of additive concentration on flavor retention in the emulsions during heating also supported the hypothesis: emulsions that were more unstable to coalescence gave a faster rate of flavor release.

Overall, these results suggest that a variety of factors contribute to flavor loss during cooking, including initial droplet concentration, composition, size, and interfacial properties. The composition and structure of the initial emulsions impacts the size and location of the oil droplets during the cooking process, which may influence the movement of flavor molecules from the model food to the headspace. This study also provides some valuable insights into the impact of additives on the thermal stability, rheology, and flavor release characteristics of emulsions. In summary, these results suggest that emulsions can be designed to control flavor retention and release during food preparation. This may lead to food products with enhanced sensory attributes, e.g., flavors that last longer during the cooking process.

# 3. Flavor release control from Alginate Microgel during simulated cooking

## 3.1 Introduction

Encapsulation technologies have been developed to protect flavors from evaporation and degradation in the food industry [22 - 25]. Many of these technologies involve trapping the flavor molecules within colloidal particles specifically designed to inhibit their volatilization and degradation during storage and cooking [26].

Alginate acids are natural ingredients extracted from brown algae that consist of linear anionic polysaccharide chains [82, 83]. The alginate acid molecule consists of varying amounts of α-L-guluronic acid (G) and 1,4'-linked β-D-mannuronic acid (M) blocks [84]. The -COOH groups of both residues (but mainly guluronic acid) can be crosslinked by divalent cations such as calcium ions ($Ca^{2+}$) to form an egg-shell structure [85]. The resulting hydrogel formed by the cross-linking of the alginate chains tends to be relatively strong and optically clear. The physical properties of calcium alginate hydrogels depend on their composition. Typically, the stability and gel strength of the hydrogels increases as the level of α-L-guluronic acid in the alginate molecules increases. The gel strength also increases as the alginate and calcium level increases because these factors lead to an increase in the cross-linking density [86]. The dimensions of calcium alginate microgels depends on the method used to fabricate them. The most common method of producing calcium alginate microgels is the injection-gelation method [87]. Basically, an aqueous solution containing a mixture of alginate and active agent is extruded dropwise into a calcium solution, which cross-links the alginate molecules and encapsulates the active agent inside the microgels formed. The diameter of the injection device used to extrude the alginate solution determines the size of the microgels.

One objective of the current study was to evaluate whether alginate microgels could be used to control flavor retention and release during simulated cooking. It was hypothesized that alginate microgels can keep the structure and oil droplets containing flavor molecules during simulated cooking because alginate microgels formed with Ca ion by electrostatic interactions are heat resistant materials. It is considered that the flavor release profile could be controlled by altering alginate microgels factors like oil concentration, alginate concentration, and emulsifier concentration. For this

reason, we examined the flavor release profile of an encapsulated model garlic flavor under simulated cooking and the impact of oil, alginate, and casein concentration. The information obtained in this study may be useful for the development of novel encapsulation technologies to control the flavor profile of foods.

Another objective of the current study was to determine how ionic strength impacted the integrity and release profile of the microgels during storage and simulated cooking. Salts are commonly found at relatively high levels in some foodstuffs where it may be desirable to control flavor release during cooking (such as soups and cooking sauces). It was hypothesized that the presence of the salt would weaken the electrostatic interactions holding the calcium alginate (Alg-Ca) microgels together, which would impact their thermostability and flavor release profiles. Previous studies have shown that calcium alginate hydrogels are stable under low ionic strength conditions (distilled water) because of the strong electrostatic attraction in the system [88]. However, they become less stable when they are placed in aqueous environments containing cationic ions because they can replace the calcium ions normally holding the alginate molecules together in the hydrogels [88]. Indeed, it has been shown that calcium alginate microgels become unstable when the $Na^+$: $Ca^{2+}$ ratio exceeds 25:1 for high G-block and 3:1 for low G-block alginates [97]. For food applications, it is therefore important to understand how the stability of calcium alginate microgels behaves in different ionic environments. For this reason, we examined the impact of salt concentration on microgel stability and the flavor release profile of an encapsulated model garlic flavor under simulated cooking and storage conditions.

## 3.2 Materials and Methods

### Materials

Corn oil was obtained from a regional supermarket and used as received. The following chemicals were purchased from the Sigma Chemical Company (St. Louis, MO): alginic acid (sodium salt) (Lot# SLBT1081, viscosity of 1% alginic acid in water is 4-12 cP); Nile Red (N3013-100MG); and, Fluorescein isothiocyanate isomer I. AMDS was purchased from TCI America (Portland, OR). Calcium chloride dehydrate was purchased from Fisher Science Education. Casein sodium salt was purchased from MP Biomedicals (Solon, OH). All chemicals used were of analytical grade. Double distilled and deionized water was used to prepare all solutions.

### Methods

#### Preparation of AMDS-loaded emulsions

An oil-in-water emulsion was prepared using a method described previously [90]. Briefly, an aqueous phase was prepared by mixing 2% (w/w) sodium caseinate with 5 mM phosphate buffer (pH 7.0) and stirring for at least 2 h to ensure dissolution. An oil phase was prepared by dissolving AMDS (500 ppm) in corn oil. A coarse emulsion was then prepared by homogenizing 20% (w/w) oil phase with 80% (w/w) aqueous phase using a high-shear mixer for 2 min (M133/1281-0, Biospec Products, Inc., ESGC, Switzerland). The droplet size in the coarse emulsion was then reduced by passing it through a high-pressure homogenizer (Microfluidizer, M110Y, Microfluidics, Newton, MA) with a 75-µm interaction chamber (F20Y) at an operational pressure of 12,000 psi (82.7 MPa) for 3 passes. The resulting emulsions were stored in a refrigerator at 4°C prior to utilization.

#### Fabrication of alginate microgels

Aqueous alginate (1% w/w) solutions were prepared by dissolving powdered sodium alginate in distilled water and then stirring continuously at 60 °C for an hour. The solution was then mixed with the AMDS-loaded emulsion (1:1 mass ratio) for 2 h with continuous stirring to form a dispersion that

contained 10% oil (w/w) and 0.5% alginate (w/w). AMDS-loaded alginate microgels were then prepared using a semi-automatic encapsulation unit (Encapsulator B-390, Buchi, Switzerland) with a nozzle size of 120 μm operated at a vibrating frequency of 800 Hz, an electrode potential of 800 V, and a pressure of 250–300 mbar. The AMDS-loaded emulsion/alginate mixtures were sprayed into 10 mL of 10% (w/w) calcium chloride solution with continuous stirring. The microgels formed were incubated in the calcium chloride solution for two hours at ambient temperature to promote cross-linking of the alginate molecules. The microgels were then collected by filtration and washed with distilled water and phosphate buffer to remove any excess calcium ions from their surfaces.

Unfilled alginate microgels were fabricated using the same approach but without the addition of the emulsion. Briefly, 0.5% alginate solution was extruded into 10% $CaCl_2$ solution under continuous agitation. The working parameters and washing steps were the same as those for the preparation of the filled alginate microgels. Filled alginate microgels with oil levels of 2.5%, 5%, 15%, 20%, 30% were fabricated by incorporating different levels of emulsions (10:1 oil-to-emulsifier) in the alginate solutions prior to injection. Similarly, filled alginate microgels with 0.5%, 1% and 2% sodium alginate solutions were fabricated in the alginate solutions prior to injection. And filled alginate microgels with 1%, 2% and 3% sodium caseinate solutions were fabricated by incorporating different levels of emulsions in the alginate solutions prior to injection. All the other steps were the same as already described.

**Simulated cooking conditions**

Test samples (microgels or emulsions) were mixed with double distilled water or 100, 200, 300, 400 or 500 mM sodium chloride solutions with continuous stirring at 300 rpm (1:10 mass ratio) in a 400 mL beaker (height 110 mm, outer dimension 77 mm). The resulting mixture was then heated using a hotplate (Isotemp digital stirring hotplates, Fisher Scientific, Waltham, MA) from room temperature to 100°C with continuous stirring at 300 rpm until the end of the treatment. The change in temperature over time was measured using a thermometer and recorded. Samples were collected

throughout the cooking process for analysis of flavor retention and particle properties.

**Gas chromatography**

The headspace concentration of AMDS above the samples was determined using gas chromatography (GC2010, Shimadzu, Columbia, MD) equipped with a headspace sampler (AOC-6000, Shimadzu, Columbia, MD). Samples (0.8 mL) were incubated at 50 °C for 10 min before being exposed to a divinylbenzene/carboxen/polydimethylsiloxane (DVB/carboxen/PDMS) solid-phase microextraction (SPME) fiber (50/30 μm, Supelco, Bellefonte, PA) for 1 min to adsorb volatile components. The volatile compounds collected were desorbed for 2 min at 250 °C in the injector at a split ratio of 1:10, and then separated on a fused-silica capillary column (30 m × 0.32 mm inner diameter × 1 μm) coated with 100% polydimethylsiloxane (Equity$^{-1}$, Sigma–Aldrich, Natick, MA). The GC column temperature program was as follows: initial temperature of 80 °C for 1 min, then a temperature ramp of 30 °C/min until a temperature of 100 °C was reached, followed by holding for 3 min. Helium was used as carrier gas at flow rate of 26.6 mL/min. Concentrations were determined from peak areas using a standard curve prepared using an AMDS standard. The retention of the flavors in the delivery systems was determined by the ratio of the remaining to the initial quantity of flavors in the liquid phase during heat treatment as a function of time.

**Particle size analysis**

The particle size distribution of the samples was measured using a static light scattering device that measures the angular dependence of the intensity of scattered light (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). Samples were diluted with phosphate buffer (5 mM, pH 7.0) prior to analysis to avoid multiple scattering effects. The refractive index of the particles used in the calculations was 1.507. The average particle sizes are reported as the volume-weighted mean diameter ($d_{43}$).

**Microstructure analysis**

The microstructure of all systems was examined using optical and/or confocal scanning laser microscopy with a 60× objective lens and 10×eyepiece (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, U.S.). A small aliquot of sample was placed on a microscope slide and covered with a cover slip prior to analysis. For confocal microscopy, the oil phase in the microgels was dyed by adding about 0.1 mL of Nile red solution (1 mg/mL ethanol) to 2 mL of sample prior to analysis. Similarly, the protein phase in the microgels was dyed by adding about 0.1 mL of fluorescein thiocyanate isomer I (FITC) solution (1 mg/mL dimethyl sulfoxide) to 2 mL of sample before analysis. The excitation and emission wavelengths used for Nile red were 543 nm and 605 nm, respectively while they were 488 nm and 515 nm for FITC, respectively. The acquired microstructural images were analyzed using the image analysis software associated with the microscope (NIS-Elements, Nikon, Melville, NY).

**Statistical analysis**

All experiments were carried out at least in triplicate using freshly prepared samples and the results are expressed as the mean ± standard deviation. Statistical differences of the experimental results were determined by analysis of variance (ANOVA) using the SAS statistical software package (SAS Inst. Inc., Cary, NC). The Duncan's multiple-range test was used to determine differences between means, and $p < 0.05$ was considered to be statistically significant.

**Storage test**

Microgels were mixed with double distilled water, 500 mM calcium chloride solution or 500 mM sodium chloride solution at a mass ratio of 1:10 and then stored at ambient temperature (25 °C) for 7 days. Samples were collected at day 0, 2, 4, and 7, and their appearance and physical characteristics were determined.

**Physical properties of oil-in-water emulsion and microgels**

The optical microscopy images showed that both the filled and unfilled microgels had spherical shapes with diameters ranging from about 100 to 1000 μm (**Fig. 3.1.1.**). The filled microgels were slightly bigger than the unfilled ones, which suggests that the presence of the lipid droplets may have interfered with the formation of the biopolymer network inside the microgels.

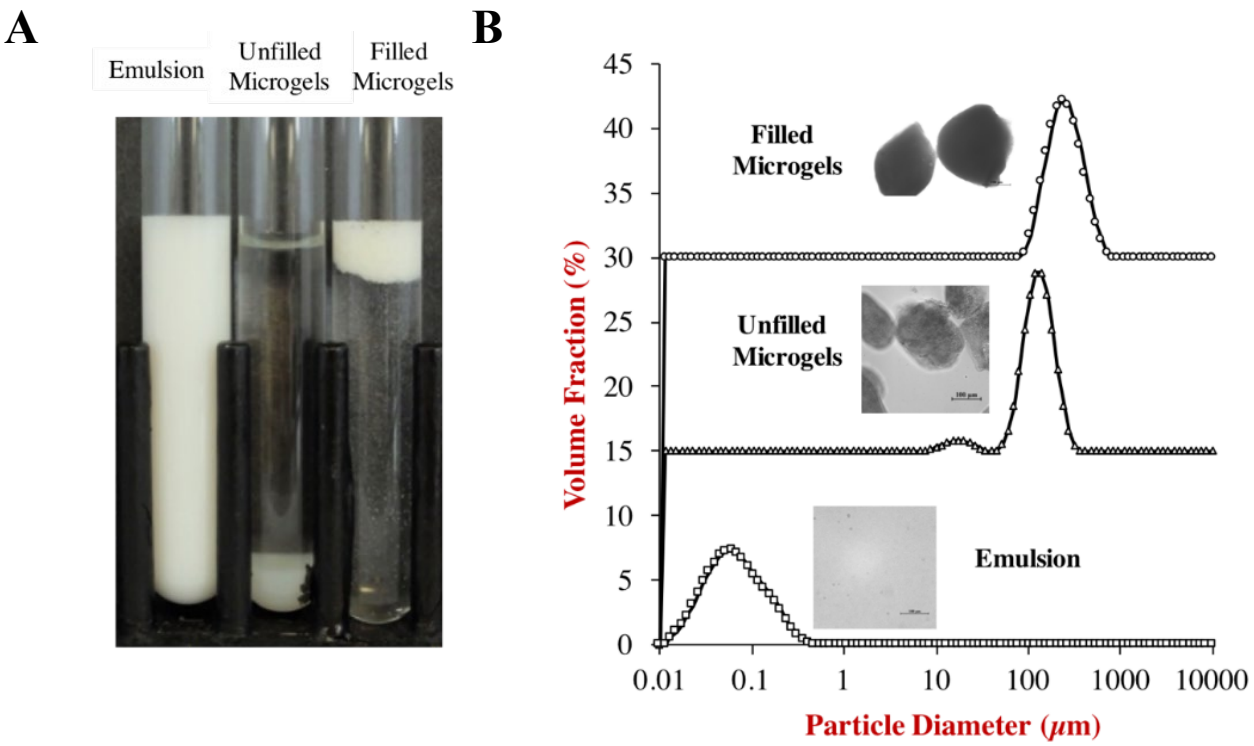



**Fig. 3.1.** Appearances and particle distributions of different delivery systems prepared in buffer solution (5 mM phosphate buffer, pH 7). From left to right: emulsion (10% oil), unfilled alginate microgels, and emulsion-filled alginate microgels (10% oil) (A); Particle size distributions and microstructures of different delivery systems at pH 7 (B).

After storage, the unfilled microgels sedimented to the bottom of the test tubes, whereas the filled microgels and lipid droplets creamed to the top. This behavior is due to the difference in densities of the colloidal particles in the various delivery systems [91]. Corn oil ($\rho$ = 920 kg/m$^3$) has a lower density than water ($\rho$= 1000 kg/m$^3$), whereas alginate ($\rho$ = 1500 kg/m$^3$) has a higher density [91]. Consequently, the lipid droplets in the emulsions move upwards due to gravity, whereas the unfilled alginate microgels move downwards. Presumably, the filled microgels moved upwards because the

contribution of the lipid droplets to the overall density of the particles was greater than the contribution of the alginate molecules [92]. There was also an appreciable difference in the optical properties of the microgels depending on whether they contained lipid droplets or not. The unfilled microgels appeared visibly transparent whereas the filled microgels appeared opaque (white), which can be attributed to the influence of the lipid droplets on the light scattering properties. The unfilled microgels have a refractive index fairly similar to that of water, and therefore do not scatter light strongly and appear relatively transparent. In contrast, the lipid droplets within the microgels have a relatively large refractive index contrast with the surrounding aqueous phase and have dimensions similar to the wavelength of light, and therefore scatter light strongly leading to an opaque whitish appearance.

**Physical properties change during heating process**

After fabrication, the emulsions and microgels were subjected to simulated cooking conditions, which involved heating them from room temperature to boiling, and then holding them for 30 min. The appearance (**Fig. 3.2.A**), mean particle diameters ($d_{43}$) (**Fig. 3.2.B**) of the emulsions and microgels were then measured after the samples had been exposed to the simulated cooking conditions.

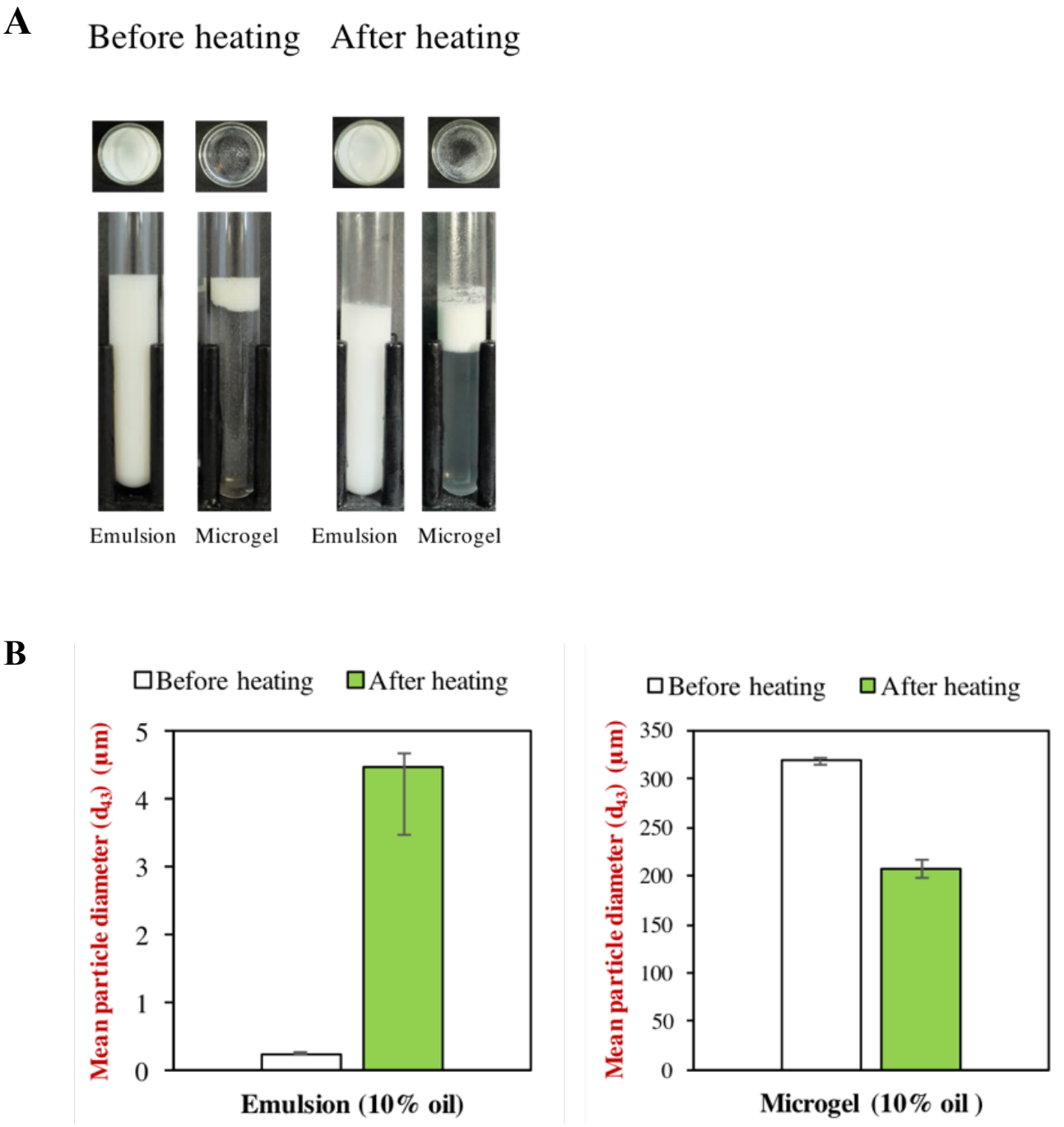



**Fig. 3.2.** Appearance of AMDS-loaded emulsions and filled alginate microgels in phosphate buffer (pH 7) before and after heating (A); The volume-based mean particle diameters ($d_{43}$) of emulsions and microgels before and after heating (B).

*Emulsions:* After simulated cooking, large oil droplets and oiling-off were visible at the top of the emulsion samples, suggesting that some droplet coalescence and phase separation occurred during heating. Previous studies have also reported droplet coalescence and phase separation in caseinate-

stabilized oil-in-water emulsions after thermal treatment [58]. The particle size distribution was monomodal before cooking but became bimodal after cooking. Moreover, an increasing number of relatively large oil droplets ($d$ = 10-100 μm) was observed within the microscopy images as the boiling time increased, again indicating that droplet coalescence occurred during heating. These results indicate that caseinate-coated lipid droplets are not stable to aggregation during long-term boiling.

*Microgels:* After simulated cooking, the aqueous phase surrounding the microgels became slightly cloudy, which suggested that some of the lipid droplets were released from them. Nevertheless, the microgels themselves remained largely intact after exposure to boiling for 30 min, which suggested that they were relatively heat-stable. Moreover, the particle size distribution of the microgel suspensions remained monomodal throughout the cooking process (**Fig. 3.3.**). However, we did observe a slight decrease in the mean diameter of the microgels after heating, which may have occurred because of shrinkage or surface erosion during heating. A similar phenomenon has also been reported for glyceryl palmitostearate-loaded calcium alginate microgels during heating [93]. Overall, these results suggest that the microgels have better heat-resistance during simulated cooking than the emulsions.

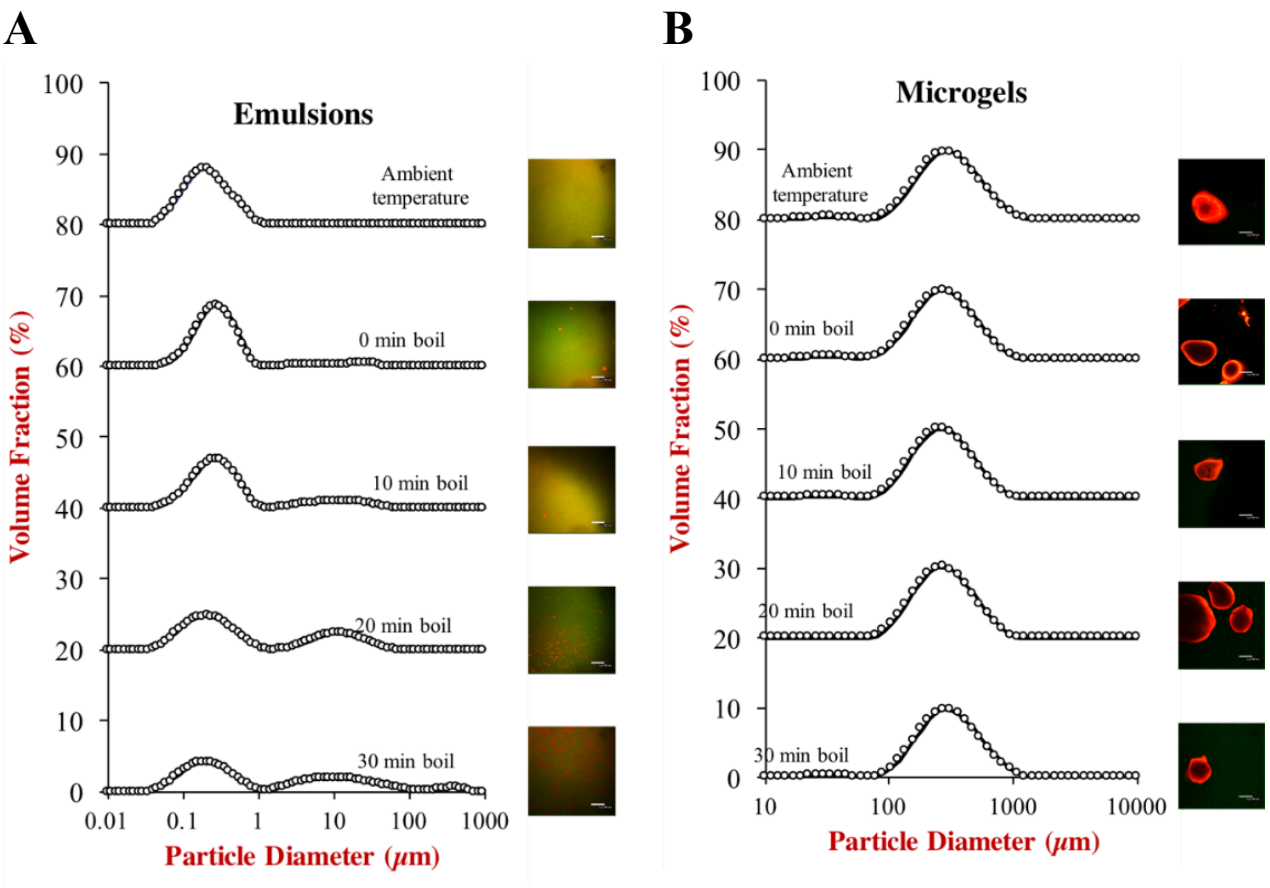



**Fig. 3.3.** Particle size distribution and microstructures of emulsion (10% oil) prepared in buffer solution (5 mM PBS, pH 7) (A); Filled microgels (10% oil) prepared in buffer solution (5 mM PBS, pH 7) (B) as a function of boil time (ambient temperature, 0min, 10min, 20min, 30min boil).

**Flavor release characteristics of alginate microgels**

The retention of the flavor throughout the simulated cooking process was then measured using headspace analysis for emulsions and microgels containing 10% oil phase (**Fig. 3.4**). The samples took about 10 min to increase from around 30 to 93 $^{o}$C, and then remained at this temperature during the 30 min of cooking. The flavor retention profiles were distinctly different for the emulsion and microgel samples. For the emulsions, there was only a slight reduction in flavor retention during the first 10 min as the sample reached boiling temperature, but then there was a steep decline, with almost no flavor remaining after 30 min. For the microgels, there was again only a slight reduction in flavor retention during the first 10 min, but then the decline in flavor retention during boiling was much less steep than for the emulsions. The time for 50% of the AMDS to be released from the particles ($t_{50}$) in both colloidal delivery systems were calculated to compare their flavor retention properties during cooking. The $t_{50}$ value for the microgels (12.6 min) was more than 3-fold longer than that for the emulsions (3.2 min), indicating that the alginate microgels were able to inhibit flavor loss during boiling.

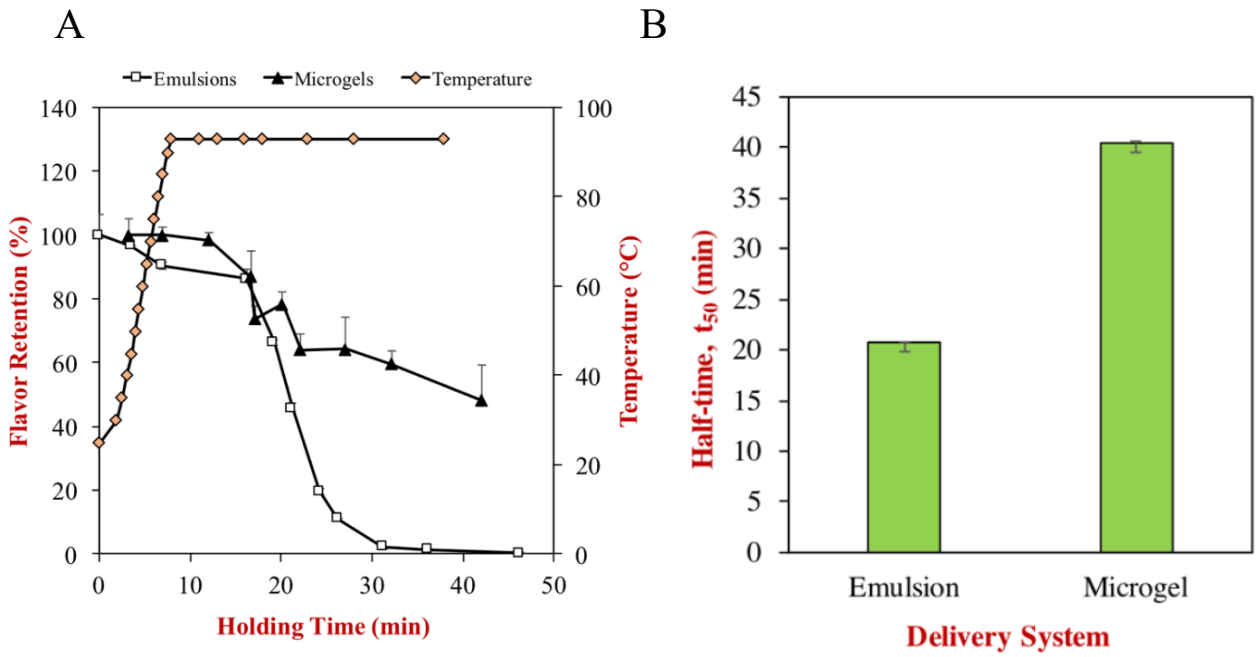



**Fig. 3.4.** Temperature profile and AMDS retention in oil-in-water emulsions (10% oil) and alginate microgels (10% oil) during heating in phosphate buffer (pH 7) (A); Time for a 50% decrease in the

AMDS level ($t_{50}$) in the emulsions (10% oil) and microgels (10% oil) during heating in phosphate buffer (pH 7) (B).

There are a number of possible reasons for the delayed loss of the flavor molecules from the microgels compared to the emulsions [91]. First, the release rate of molecules from spherical particles decreases as the particle size increases due to the longer diffusion path length and lower specific surface area [94]. The diameter of the microgels ($d \approx 270$ μm) was initially over 1000-fold larger than the diameter of the lipid droplets ($d \approx 0.26$ μm) in the emulsions, which should therefore lead to a much slower release rate. Second, the flavor molecules trapped in the lipid droplets only have to travel through the oil phase before being released, but in the filled microgels they must travel through the oil phase and then through a biopolymer network, which may hinder their diffusion [95]. Third, the flavor molecules may bind to the biopolymer network due to attractive physical interactions, which again slows down their release [96]. It should be noted that the lipid droplets coalesced, and phase separated during heating, which would increase their effective size, and therefore may impact the release profile at longer boiling times. Similarly, the microgel particles shrank during the simulated cooking process, which may also alter their release profiles.

The impact of microgel properties on the amount of AMDS retained during heating from room temperature to boiling for 30 min was determined to simulate cooking conditions. Encapsulation of AMDS-loaded lipid droplets in microgels delayed flavor release appreciably (3-fold longer), and the microgels were found to remain intact throughout the boiling process. These results suggest that biopolymer microgels is useful for controlling flavor release during cooking.

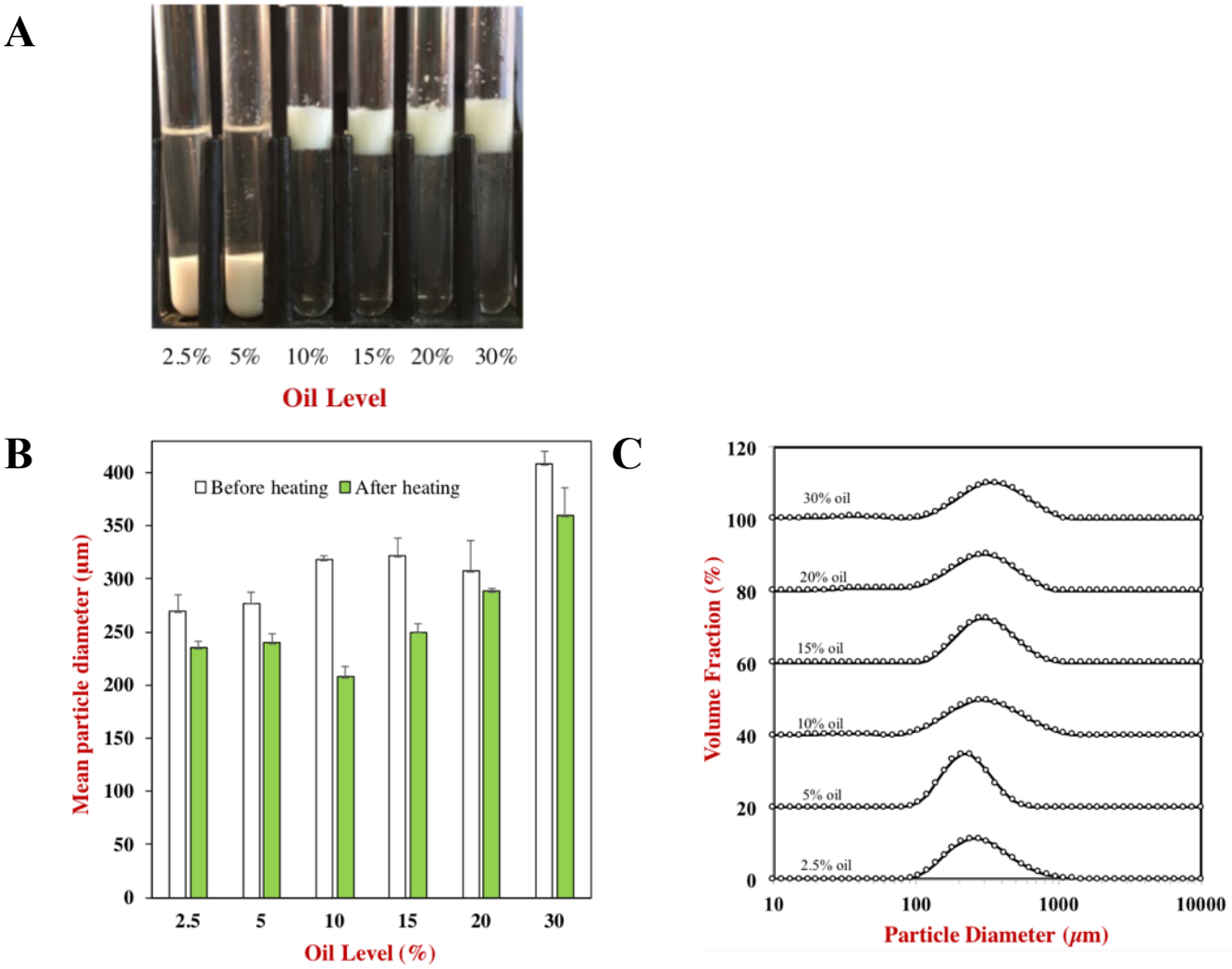
## 3.3 Results and Discussion

### 3.3.1 Impact of oil concentration on flavor release

Based on previous investigation, it has been identified calcium alginate microgels as the major delivery system for AMDS retention. It has been further investigated the influences of oil concentration, alginate concentration as well as casein concentration of microgels on flavor retention.

**Impact on physical properties and structure**

AMDS used in this study is a relatively hydrophobic molecule (Log P = 2.87), and therefore we postulated that its retention within the microgels would be increased at higher lipid loading levels. For this reason, alginate microgels containing 2.5%, 5%, 10%, 15%, 20% and 30% lipid droplets were fabricated as described earlier, and then the impact of lipid loading level on their size, stability, and flavor retention during simulated cooking were assessed (**Fig. 3.5.**).



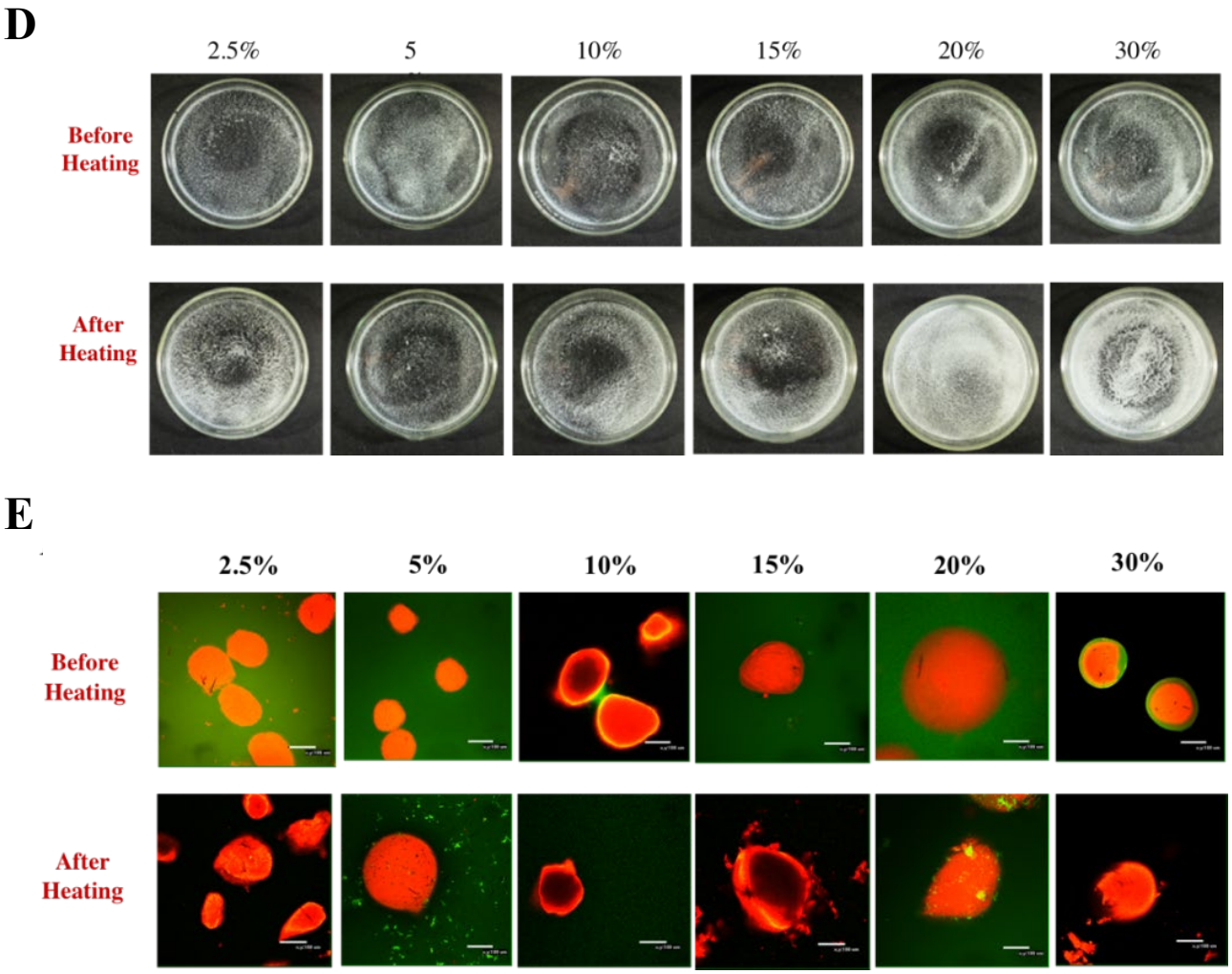



**Fig. 3.5.** Impact of lipid droplet level on: Initial appearance (A); Initial mean particle diameters ($d_{43}$) (B); Initial particle size distributions (C); Appearance before and after heating (D); Microstructure below and after heating (E).

Visual observation of the initial samples indicated that microgels containing 2.5% and 5% oil sedimented to the bottom of the test tubes, whereas microgels containing 10% oil and higher floated to the top (**Fig. 3.5.A**). These results indicate that oil concentration played an important role in determining the gravitational separation of this type of delivery system, which can be attributed to the fact that oil is lighter than water whereas alginate is heavier. Interestingly, one would expect that there is a microgel composition (between 5% and 10% oil) where the particles have a similar density to that of the surrounding aqueous phase, which should inhibit gravitational separation. In future

studies, we intend to determine if there are particle compositions where density matching can be achieved, as stability to gravitational separation is important in many food applications.

All of the initial microgel samples had monomodal particle size distributions (**Fig. 3.5.C**) and mean particle diameters between about 270 and 410 μm (**Fig. 3.5.B**). There was a general trend of an increase in microgel size with increasing lipid droplet level, which suggested that the lipid droplets may have interfered with biopolymer network formation during the injection process. This may have happened because the lipid droplets acted as physical barriers that did not allow the alginate chains to get as close together as they would normally. These results are in agreement with those reported previously for the influence of lipid droplet levels on the properties of calcium alginate microgels formed by injection methods [97]. There was no difference in the visible appearance of microgels with different lipid levels before and after heat treatment – they all appeared as small white spheres (**Fig. 3.5.D**). At all lipid levels, there was an appreciable decrease in the mean particle diameter of the microgels (from 6 to 35%) after simulated cooking (**Fig. 3.5.B**), which may have been due to some shrinkage or surface erosion. Confocal microscopy indicated that the microgels remained largely intact after simulated cooking, and that some of the lipid droplets remained trapped inside of them. However, there did appear to be some irregularities on the surfaces of the microgels after the heat treatment, especially at the higher lipid droplet levels used. This may have occurred because the presence of the lipid droplets led to the formation of a weaker biopolymer network inside the microgels, which was then more easily disrupted during heating.

**Impact on heat retention**

The impact of the lipid droplet level inside the microgels on the flavor retention during the simulated cooking process was measured (**Fig. 3.6.**).

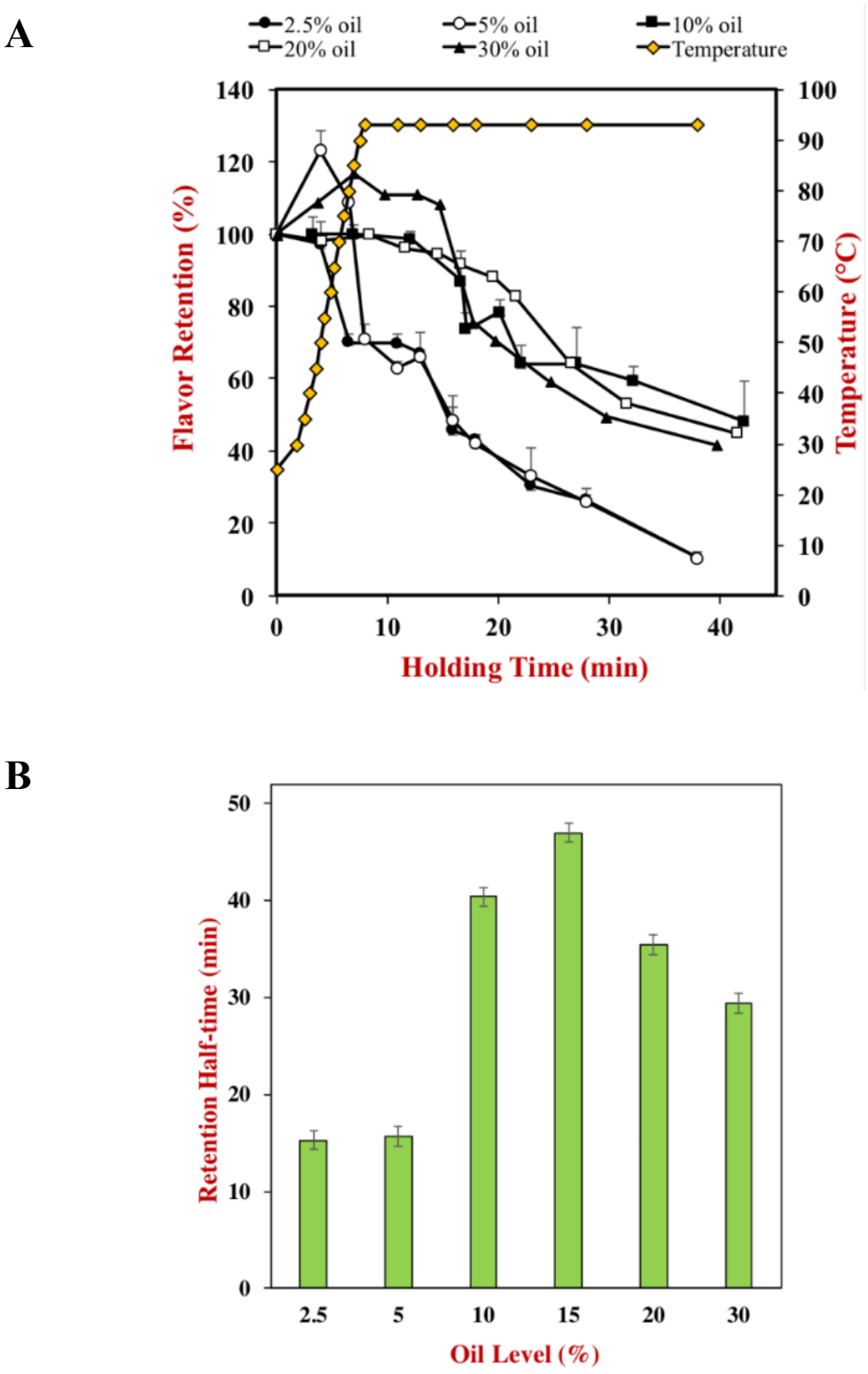



**Fig. 3.6.** Impact of lipid level on flavor retention versus time profiles for microgels during simulated cooking (A); Impact of lipid level on retention half-time (B).

In general, there was relatively little flavor loss during the initial cooking stage when the

temperature of the samples was increased from ambient temperature to boiling, but then the amount of flavor retained by the microgels decreased appreciably, which can be attributed to increased volatilization of AMDS at elevated temperatures. Nevertheless, there were differences in the retention-time profiles depending on the lipid level inside the microgels. At relatively low lipid levels (2.5% and 5%), the rate of flavor loss was much faster than at higher lipid levels (10% to 30%). The time for half of the flavor to be lost from the microgels was estimated from the experimental data of retention ($R$) *versus* time ($t$) by extrapolation. The impact of lipid level on the retention half-time is shown in **Fig. 3.6.B**. The retention half-time increased from around 15 min at the lowest lipid level (2.5%) to around 47 min at an intermediate lipid level (15%), but then decreased upon a further increase in lipid level. The initial increase in retention with lipid content may have been because there were more lipid droplets inside the microgels to solubilize the flavor molecules, *i.e.,* greater partitioning. Conversely, the decrease in retention observed at higher lipid contents may have been because the lipid droplets interfered with the structural integrity of the alginate microgels, thereby allowing faster diffusion of the flavor molecules out of the microgels. These results suggest that there is an optimum lipid content that should be utilized to obtain a sustained release profile. Interestingly, more rapid flavor release was observed for those microgels that underwent sedimentation (2.5 and 5% oil), than those that underwent creaming (≥ 10% oil). This may have been because microgels at the bottom of the containers were subjected to higher temperatures and more vigorous agitation than those at the top of the containers during cooking, which led to faster flavor release.

### 3.3.2 Impact of alginate concentration on flavor release

**Impact on physical properties**

We postulated that alginate network in microgel works to prevent from oil droplet release. Therefore, higher alginate concentration can form higher density microgel and its retention within the microgels would be increased at higher alginate concentration. For this reason, alginate microgels with 0.5%, 1% and 2% sodium alginate solutions were fabricated as described earlier, and then the impact of alginate concentration on their size, stability, and flavor retention during simulated cooking were assessed (**Fig. 3.7.** and **Fig. 3.8.**).

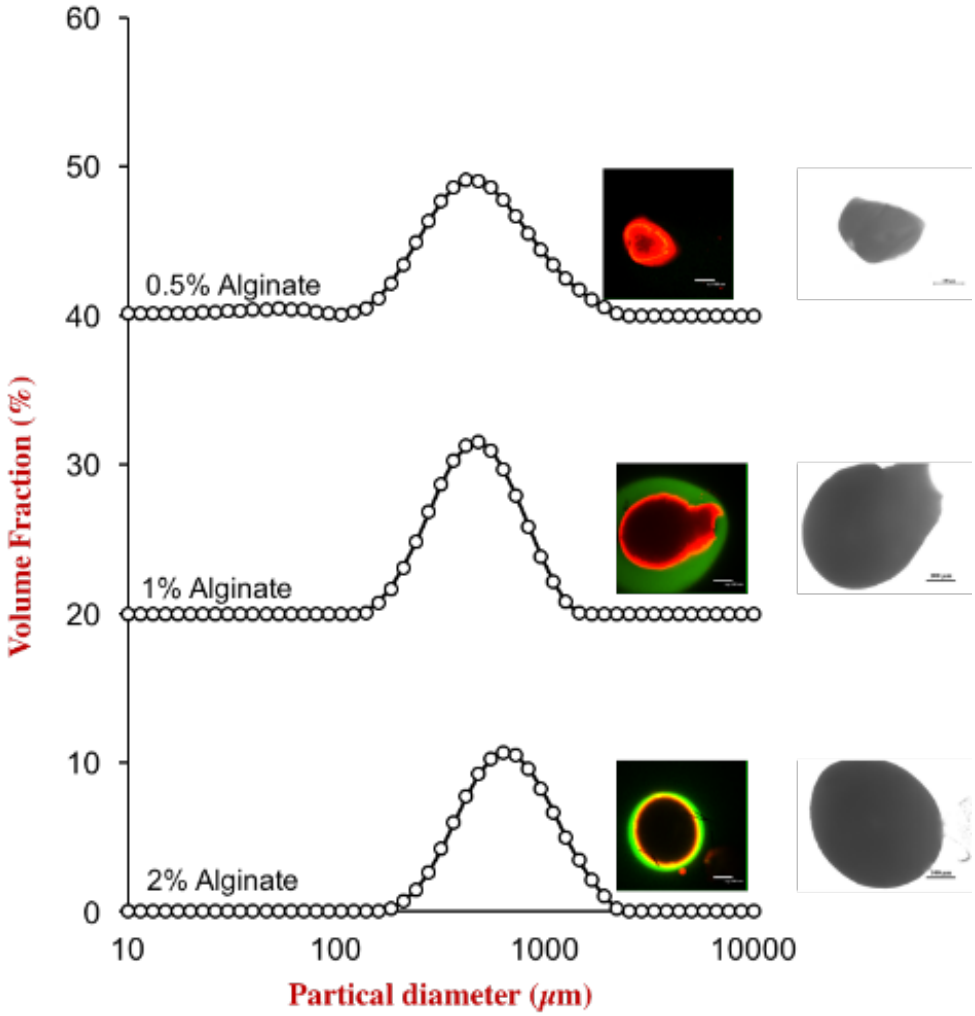



**Fig. 3.7.** Microstructures of alginate calcium microgels formed with 0.5%, 1% and 2% sodium alginate. *Left*: Confocal image; *Right*: Regular image (Scale bar: 100 μm). Red is oil, green is protein.

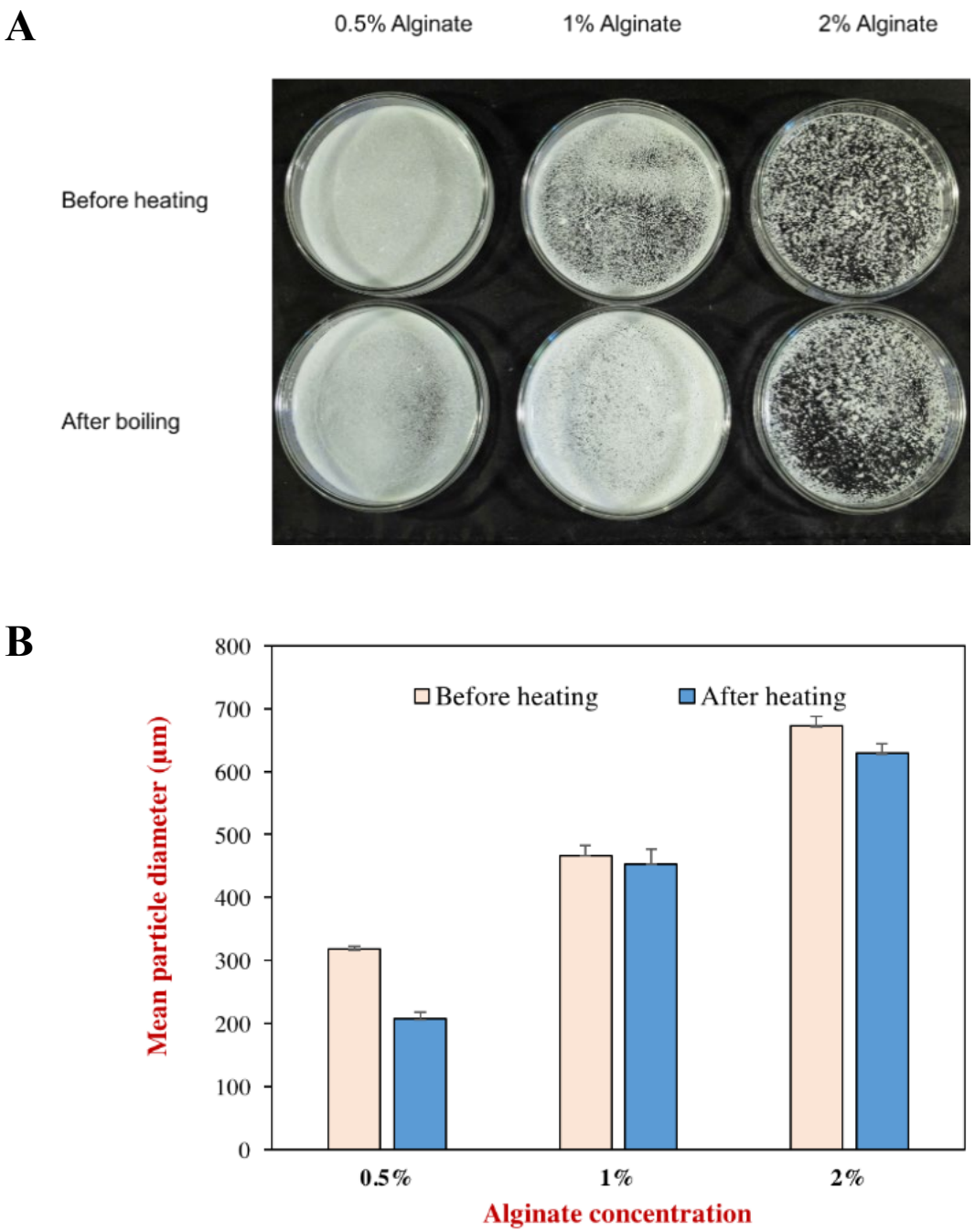



**Fig. 3.8.** Appearances of alginate calcium microgels formed with 0.5%, 1% and 2% sodium alginate (A); volume-based mean particle diameters ($d_{43}$) of alginate calcium microgels formed with 0.5%, 1% and 2% sodium alginate (B).

The particle sizes of alginate calcium microgels were larger as alginate concentration became higher. However, the sizes became smaller as boiling processes went on. The structures stayed intact

during heating. There was a general trend of an increase in microgel size with increasing alginate concentration, which suggested that higher alginate concentration increased the viscosity of solution for injection and droplet size also increased. There was no difference in the visible appearance of microgels with different alginate concentration before and after heat treatment – they all appeared as small white spheres (**Fig. 3.8**).

At all alginate concentrations, there was a little decrease in the mean particle diameter of the microgels after simulated cooking (**Fig. 3.8**), which may have been due to some shrinkage or surface erosion. At higher alginate concentration, the decrease in the mean particle diameter of the microgels after simulated cooking was smaller. It is considered that higher alginate concentration formed a stronger biopolymer network which could prevent from shrinkage during simulated cooking.

**Impact on flavor retention**

The impact of the alginate concentration in alginate microgels on the flavor retention during the simulated cooking process was measured (**Fig. 3.9.A**). The time for half of the flavor to be lost from the microgels was estimated from the experimental data of retention ($R$) *versus* time ($t$) by extrapolation. The impact of alginate concentration on the retention half-time is shown in **Fig. 3.9.B**.

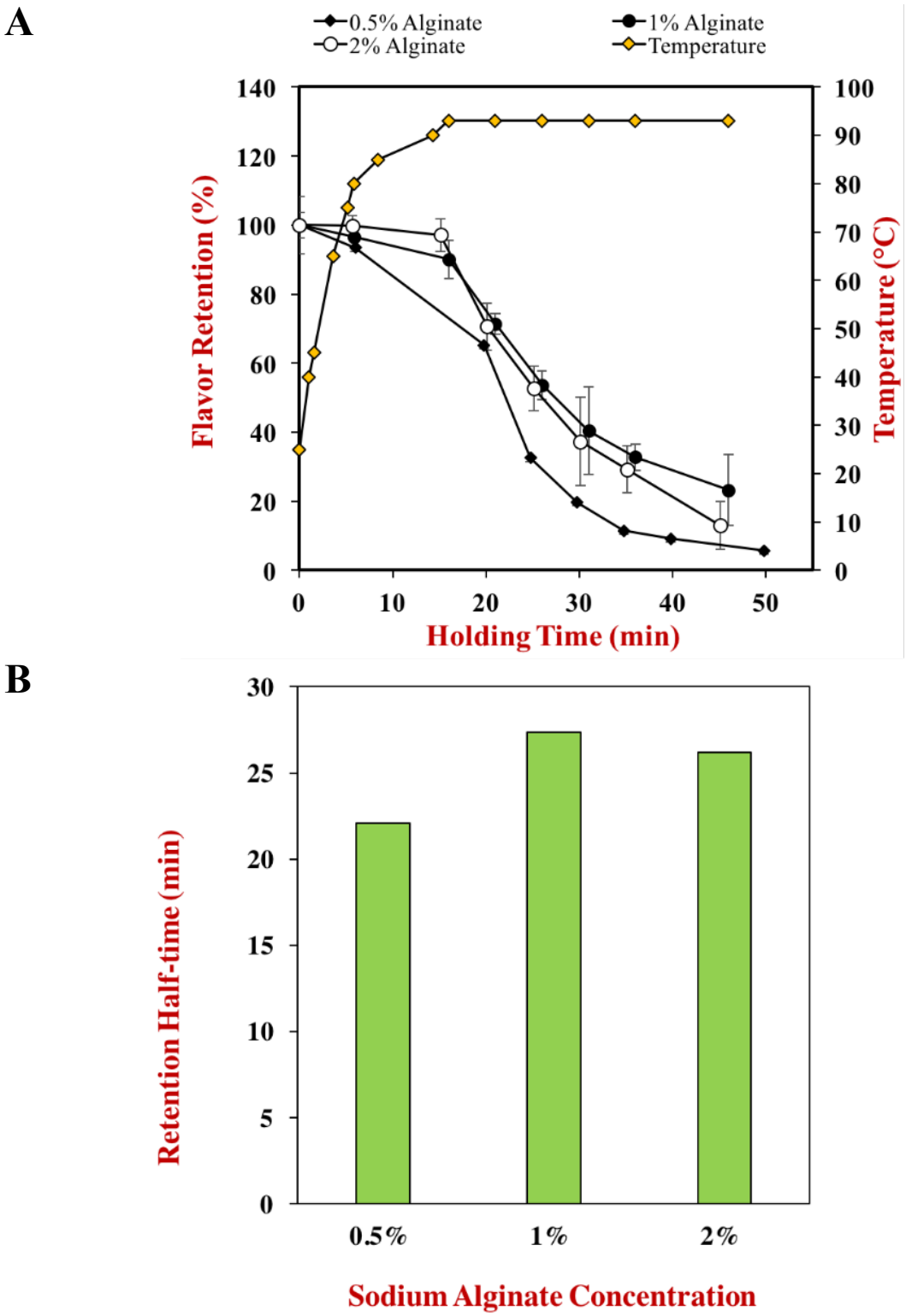



**Fig. 3.9.** Impact of alginate level on flavor retention versus time profiles for microgels during simulated cooking (A); Impact of alginate level on retention half-time (B).

According to the results, there were differences in the retention-time profiles depending on the

alginate concentrations. Higher alginate concentration (>1%) in microgels improved the flavor heat retention during boiling process. However, elevation of alginate concentration above 1% in formulation cannot further improve the heat retention properties. At lower alginate concentration in microgels, the decrease of retention half-time may have been because the amount of alginate molecule was not enough to form strong biopolymer network with calcium ions. Conversely, the increase in retention observed at higher alginate concentrations (>1%) may have been because enough amount of alginate molecule reacted with calcium ions and formed stronger biopolymer network which led to slower flavor release.

### 3.3.3 Impact of sodium caseinate concentration on flavor release

**Impact on physical properties**

We postulated that emulsifier concentration affects to stabilize oil droplets in microgel and flavor release. Therefore, emulsion would be stabilized at optimum caseinate concentration and its flavor retention within the microgels would be increased. For this reason, alginate microgels with 1%, 2% and 3% sodium caseinate solutions were fabricated as described earlier, and then the impact of caseinate concentration on their size, stability, and flavor retention during simulated cooking were assessed (**Fig. 3.10.** and **Fig. 3.11.**).

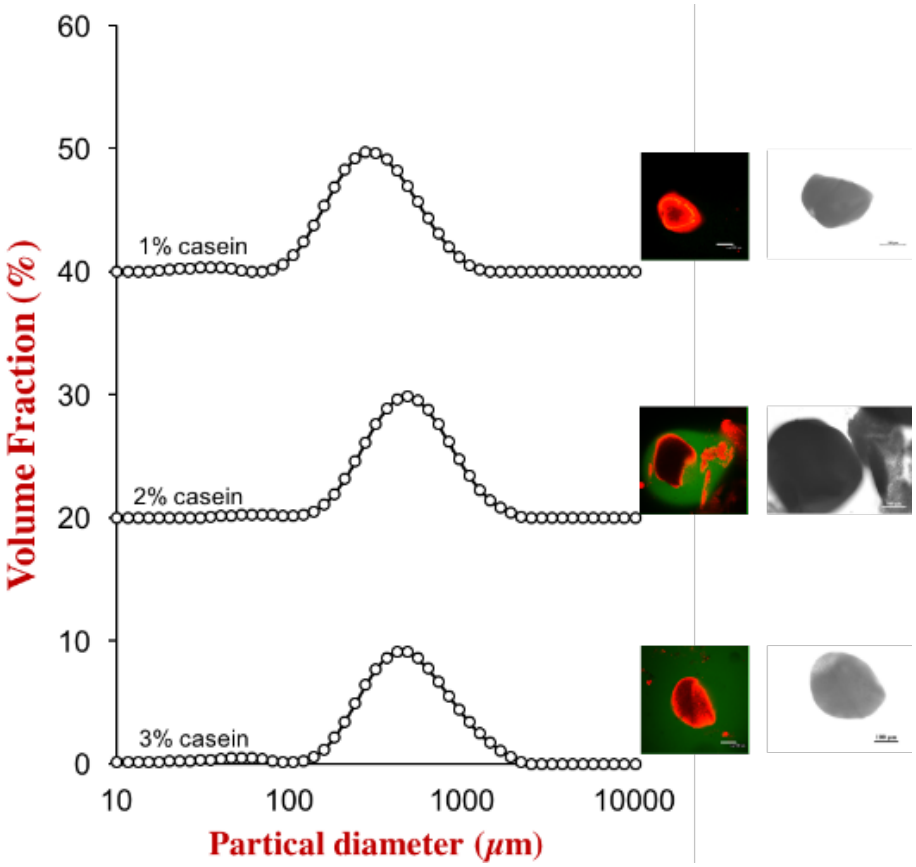



**Fig. 3.10.** Microstructures of alginate calcium microgels formed with 1%, 2% and 3% sodium caseinate. *Left*: Confocal image; *Right*: Regular image (Scale bar: 100 μm). Red is oil, green is protein.

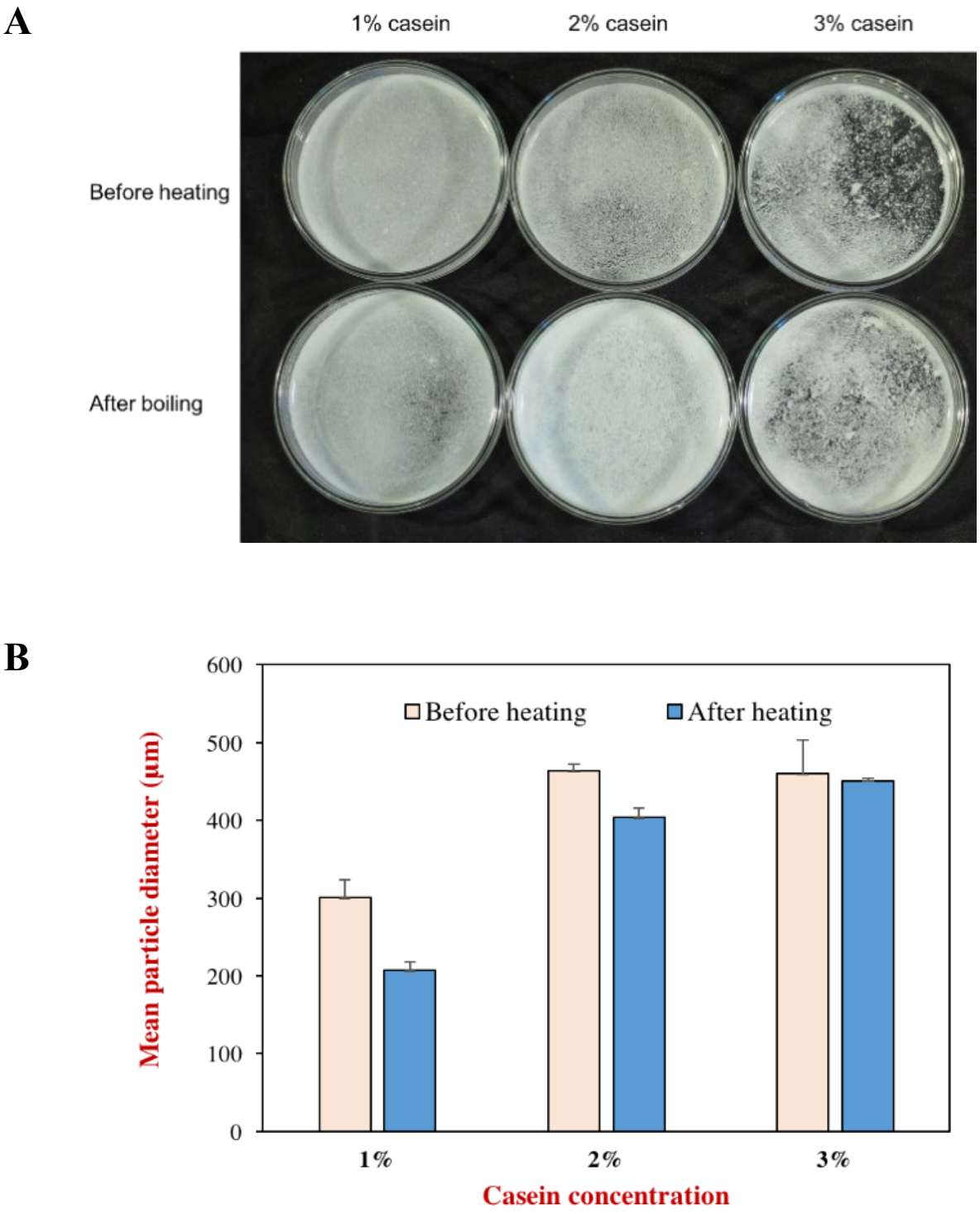



**Fig. 3.11.** Appearances of alginate calcium microgels formed with 1%, 2% and 3% sodium caseinate (A); volume-based mean particle diameters ($d_{43}$) of alginate calcium microgels formed with 1%, 2% and 3% sodium caseinate (B).

The particle sizes were larger as caseinate concentration increased (from 1% to 3%). However,

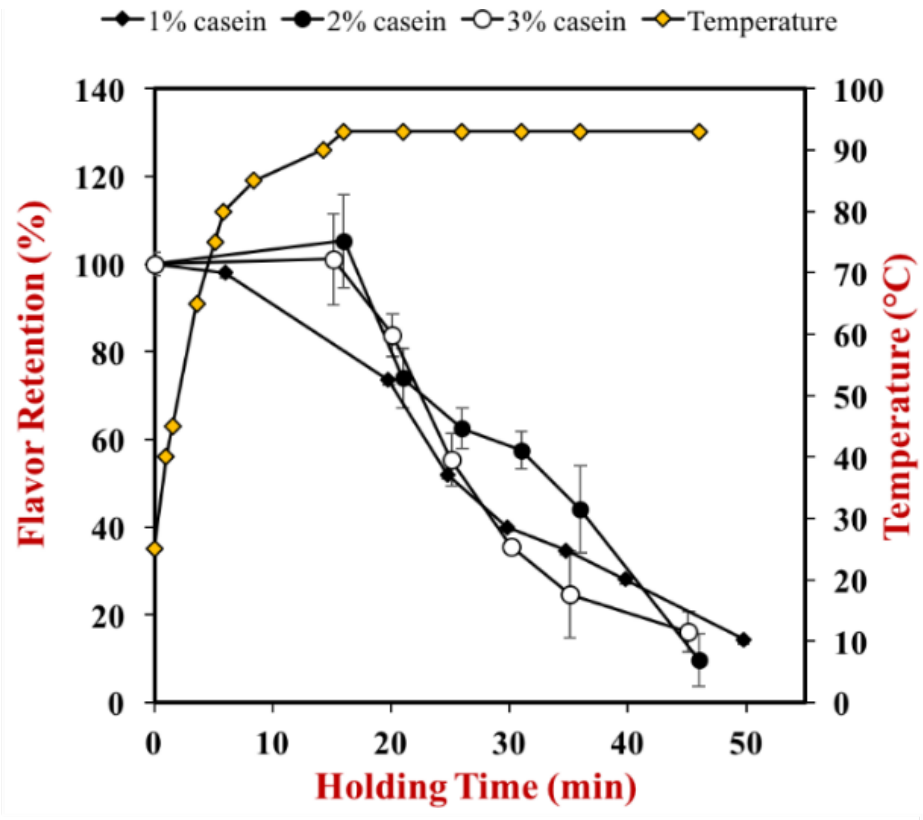
the sizes became smaller as boiling processes went on. The structures stayed intact during simulated cooking process. There was no difference in the visible appearance of microgels with different caseinate concentration before and after heat treatment during simulated cooking process.

At lower caseinate concentration, the decrease in the mean particle diameter of the microgels after simulated cooking was bigger. It is considered that lower caseinate concentration formed unstable emulsion which could coalesce easily and release out from microgel during simulated cooking. Therefore, the coalescence of emulsion may have caused shrinkage of microgel.

**Impact on flavor retention**

The impact of the caseinate concentration in alginate microgels on the flavor retention during the simulated cooking process was measured (**Fig. 3.12.A**). The time for half of the flavor to be lost from the microgels was estimated from the experimental data of retention ($R$) *versus* time ($t$) by extrapolation. The impact of alginate concentration on the retention half-time is shown in **Fig. 3.12.B**.

**A**



**B**

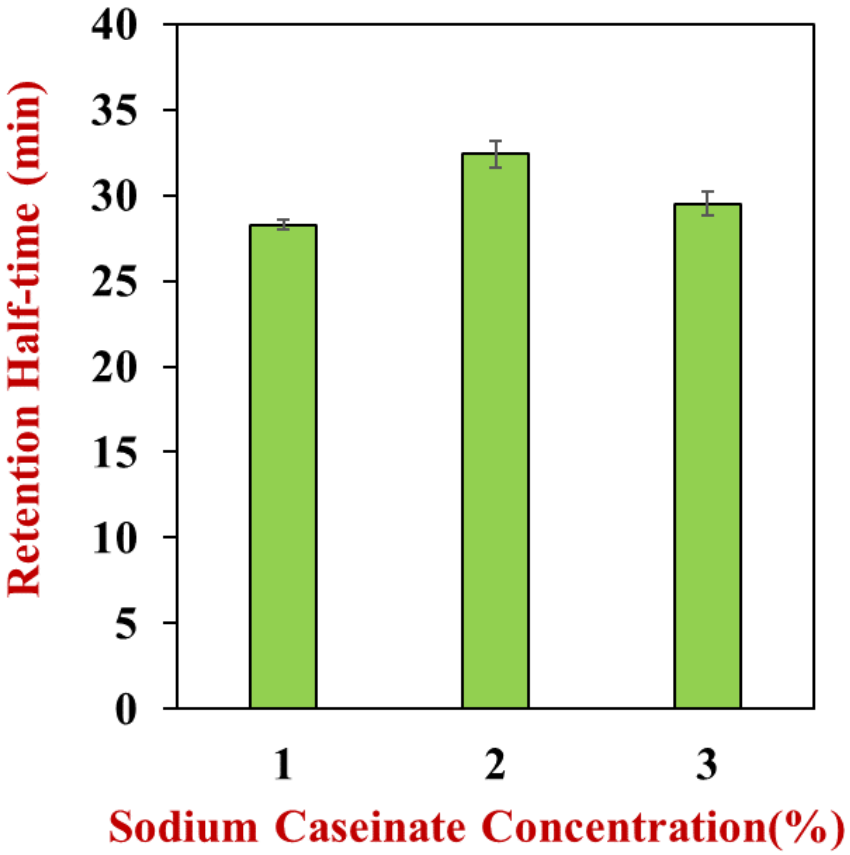



**Fig. 3.12.** Impact of sodium caseinate level on flavor retention versus time profiles for microgels during simulated cooking (A); Impact of sodium caseinate level on retention half-time (B).

According to the results of simulated cooking test (**Fig. 3.12.**), higher caseinate concentration (>1%) in microgels can slightly improve the flavor heat retention during boiling process. However, elevation of caseinate concentration above 2% in formulation cannot further improve the heat retention properties. Instead, 3% caseinate showed reduced half-time retention during heat test.

At lower 1% caseinate in microgels, the decrease of retention half-time may have been because the amount of caseinate was not enough to form stable emulsion with oil. Unstable oil droplets in emulsion may have caused coalescence during heating, and coalesced oil released from microgel. It is considered the release rate of flavor molecule from an oil layer on the surfaces of solution is higher than that from oil droplets in microgel network.

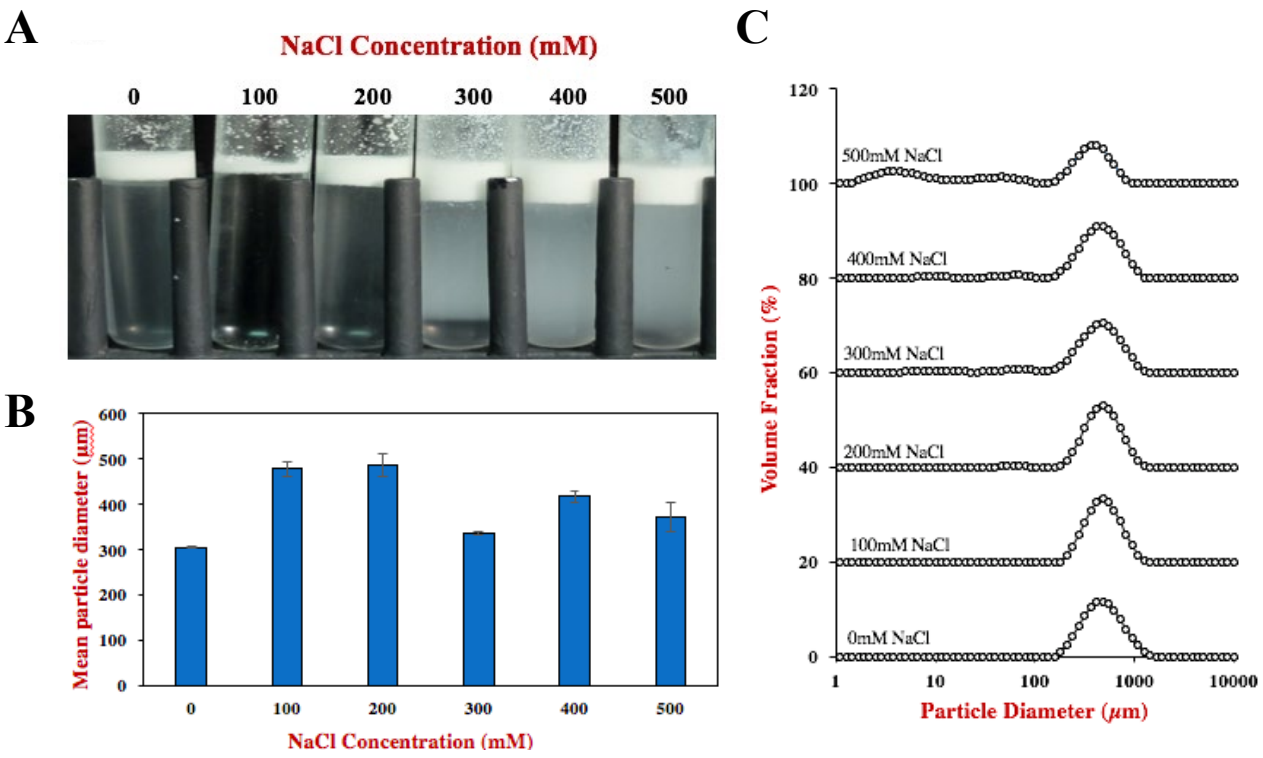
At the 3% caseinate in microgels, the decrease of retention half-time may have been because the amount of caseinate was excess to form stable emulsion. Once the caseinate exceeds a critical level, the attractive interactions (van der Waals and depletion) between the oil droplets exceed the repulsive interactions (steric and electrostatic), which promotes droplet flocculation. Then destabilized oil droplets in emulsion caused coalescence during heating and moved to the surface in the solution. As a result, the release rate became fast. These results suggested that the optimum concentration of emulsifier exists to keep emulsion stable.

### 3.3.4 Impact of salt concentration on microgel properties

In the previous study, we found that encapsulation of flavor-loaded lipid droplets within alginate microgels improved their flavor retention during simulated cooking [98]. The rate at which the flavor molecules was released decreased as the dimensions of the microgels increased because they had a longer pathway to travel. The purpose of the current study was to determine how ionic strength impacted the integrity and release profile of the microgels during storage and simulated cooking. AMDS was used as a model hydrophobic volatile flavor. Salts are commonly found at relatively high levels in some foodstuffs where it may be desirable to control flavor release during cooking (such as soups and cooking sauces). It was hypothesized that the presence of the salt in the aqueous solution surrounding the calcium alginate microgels impacted their thermostability and flavor release profiles by weakening the electrostatic interactions holding the microgels together.

**Characteristics of microgels in different NaCl concentrations**

The physical properties, appearance, and microstructures of microgels incubated in different sodium chloride concentrations were compared (**Fig. 3.13.**).



**D**

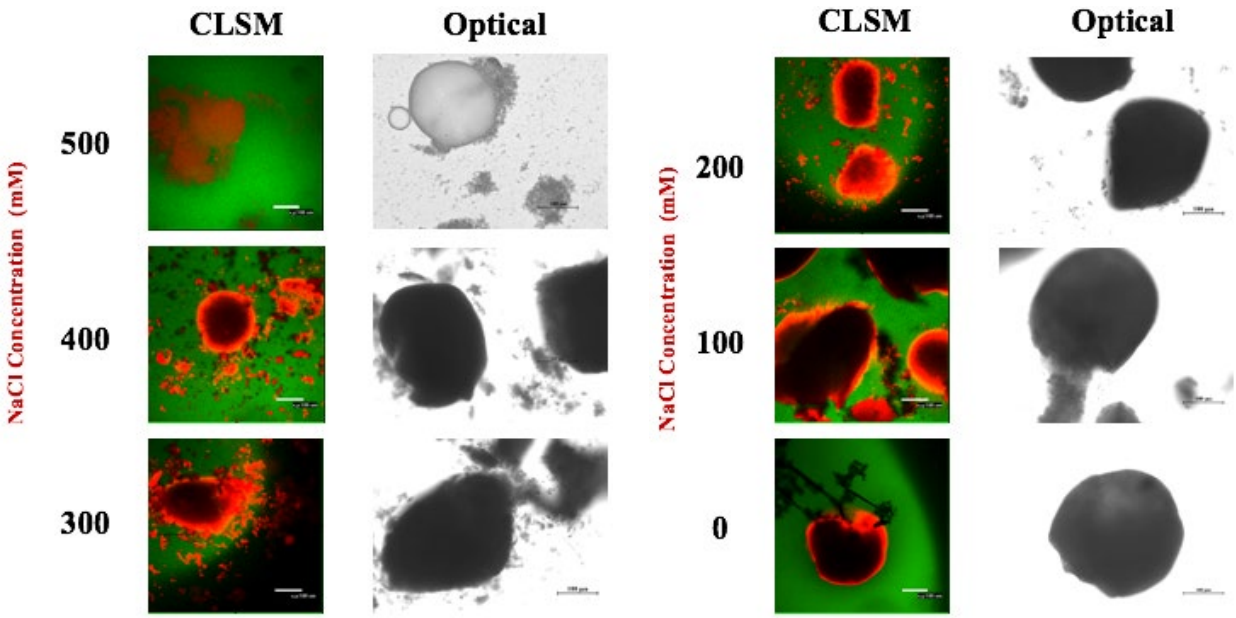


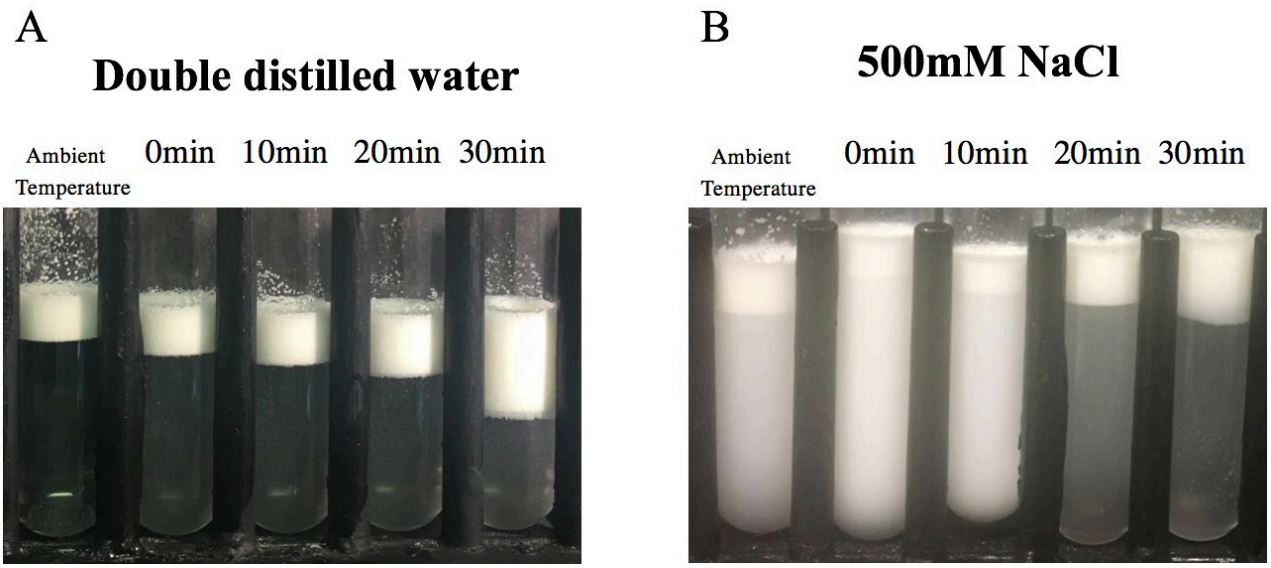

**Fig. 3.13.** Appearances (A), particle size distributions (B), mean particle diameters (C), and microstructures (D) of calcium alginate microgels. The microgels were prepared using 10% corn oil, 1% sodium caseinate, 0.5% sodium alginate, and 0.05% allyl methyl disulfide injected into calcium solutions. The microgels were mixed with various NaCl solutions (0-500 mM) at a 1:10 mass ratio and stored at room temperature. *Left*: confocal microscopy image. *Right*: optical microscopy image (Scale bar: 100 μm for both confocal and optical microscopy). Red is oil dyed with Nile red, green is protein dyed with fluorescein isothiocyanate isomer I.

These samples were prepared using only mild agitation when mixing the beads with the salt solutions and the time between sample preparation and particle characterization was less than 30 min. The aqueous phase of the microgels incubated in the 0, 100, and 200 mM NaCl solutions was optically transparent indicating that the lipid droplets were retained inside the microgels (**Fig. 3.13.A**). However, the aqueous phase became cloudy at higher NaCl levels, which suggests that there was some alteration in the properties of the microgels that led to release of the encapsulated lipid droplets. The lipid droplets have dimensions similar to the wavelength of light and therefore scatter light strongly leading to a turbid solution when they are released.

The particle size distribution of the microgels did not change much when the NaCl concentration was increased from 0 to 400 mM (**Fig. 3.13.B**), which suggested that the light scattering signal was mainly dominated by the presence of the microgels and that they largely stayed intact. However, at 500 mM NaCl, two peaks were observed in the particle size distribution profile: one around 500 µm which represents the microgels and another around 5µm which represents the released lipid droplets. These results suggest that in the short term, the microgels largely remain intact at all salt levels but that some of the lipid droplets are released at high NaCl levels. This effect may have been a result of a decrease of the electrostatic attraction between the protein-coated lipid droplets and the alginate network inside the microgels and/or due to an increase in the pore size of the alginate microgels at high salt levels.

**Impact of salt on microgel characteristics during simulated cooking**

The influence of salt concentration on the stability of the microgels during simulated cooking was then investigated (**Fig. 3.14.**).



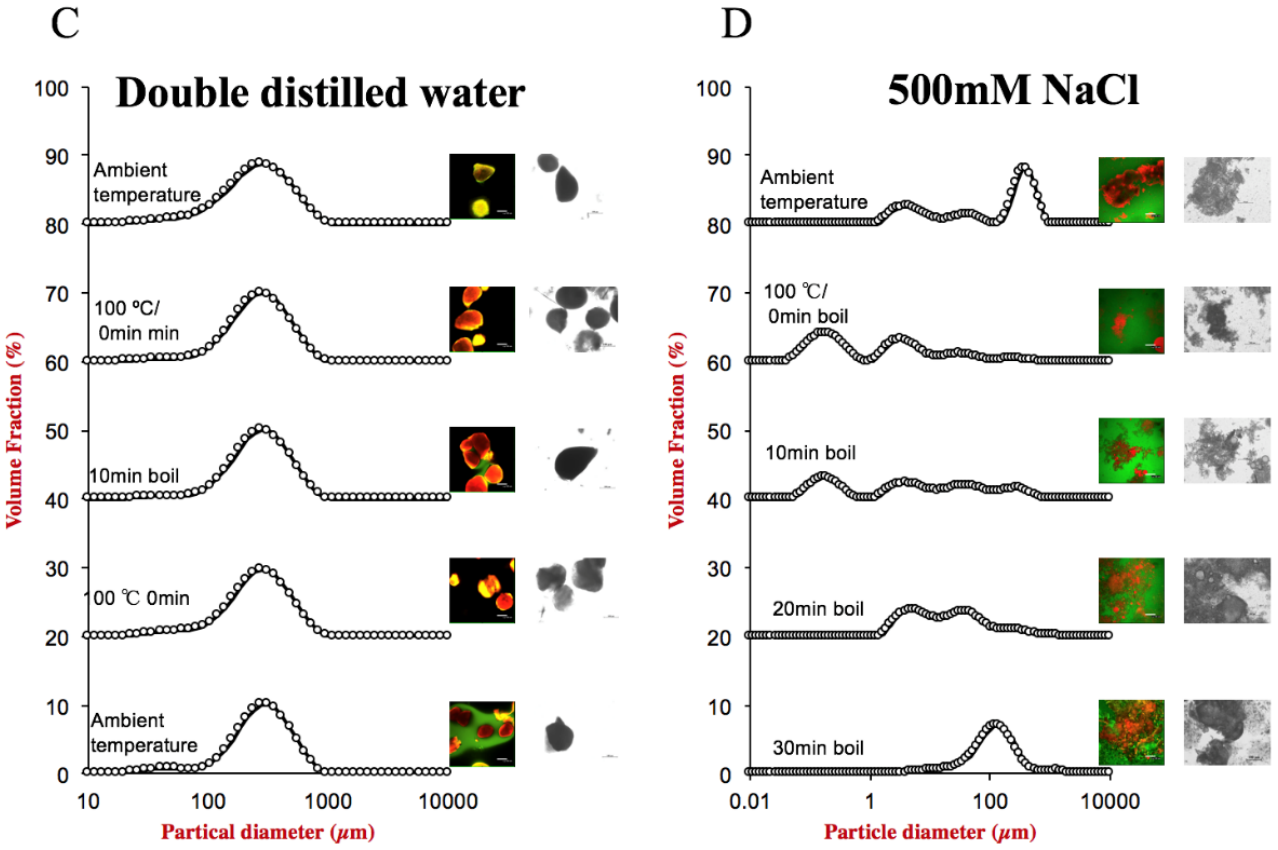



**Fig. 3.14.** Particle size distribution and microstructures of microgels prepared in distilled water (0mM sodium chloride) (A); Microgels prepared in 500mM sodium chloride (B) as a function of boil time (ambient temperature, 0min, 10min, 20min, 30min boil). *Left*: Confocal image; *Right*: Regular image (Scale bar: 100 μm). Red is oil, green is protein. Appearance of microgels prepared in distilled water (0mM sodium chloride) (C); Microgels prepared in 500mM sodium chloride (D) as a function of boil time (ambient temperature, boil point 0min, 10min, 20min, 30min boil).

In these experiments, the microgels were dispersed in aqueous solutions with either low ionic strength (distilled water) or high ionic strength (500 mM NaCl). The particle size distribution and morphology of the microgels mixed with distilled water remained almost the same throughout the cooking process, indicating that they retained their integrity during prolonged heating. In addition, visual observations showed that there was no increase in the turbidity of the aqueous solution surrounding the microgels, while confocal microscopy indicated that the fats were located inside the

microgels. Taken together, these results suggest that the fat droplets remained trapped inside the microgels during the cooking process. Visual observation of the microgels indicated that they tended to move to the top of the test tubes after a few minutes' storage (**Fig. 3.14.A and B**), which can be attributed to their relatively large size and the fact that they contain oil droplets that are less dense than water. Interestingly, the thickness of the white layer formed at the top of the test tubes increased throughout the cooking process, which suggests that the microgels either swelled in size or that they packed less efficiently after heating. The particle size measurements determined by laser diffraction suggested that the size of the microgels did not increase after cooking. For instance, the mean particle diameter ($d_{43}$) changed from 306 μm before cooking to 261 μm after 30 min cooking.

The microgels heated in the presence of 500 mM NaCl exhibited quite different behavior (**Fig. 3.14.C and D**). There was a large change in the particle size distribution and microstructure of the microgels throughout the simulated cooking process, suggesting that there was some disintegration of the microgel structure during heating. In addition, there was evidence of release of the fat droplets from the microgels and some droplet coalescence (large individual fat droplets) in this system. The change in the general appearance of these samples also reflected these changes in microgel characteristics. At relatively short cooking times there was an increase in the turbidity of the aqueous solution outside the microgels, suggesting that more lipid droplets had been released. However, at longer cooking times this aqueous solution became clearer, which may have been because the lipid droplets rapidly moved to the top of the test tubes due to the increase in their size caused by coalescence.

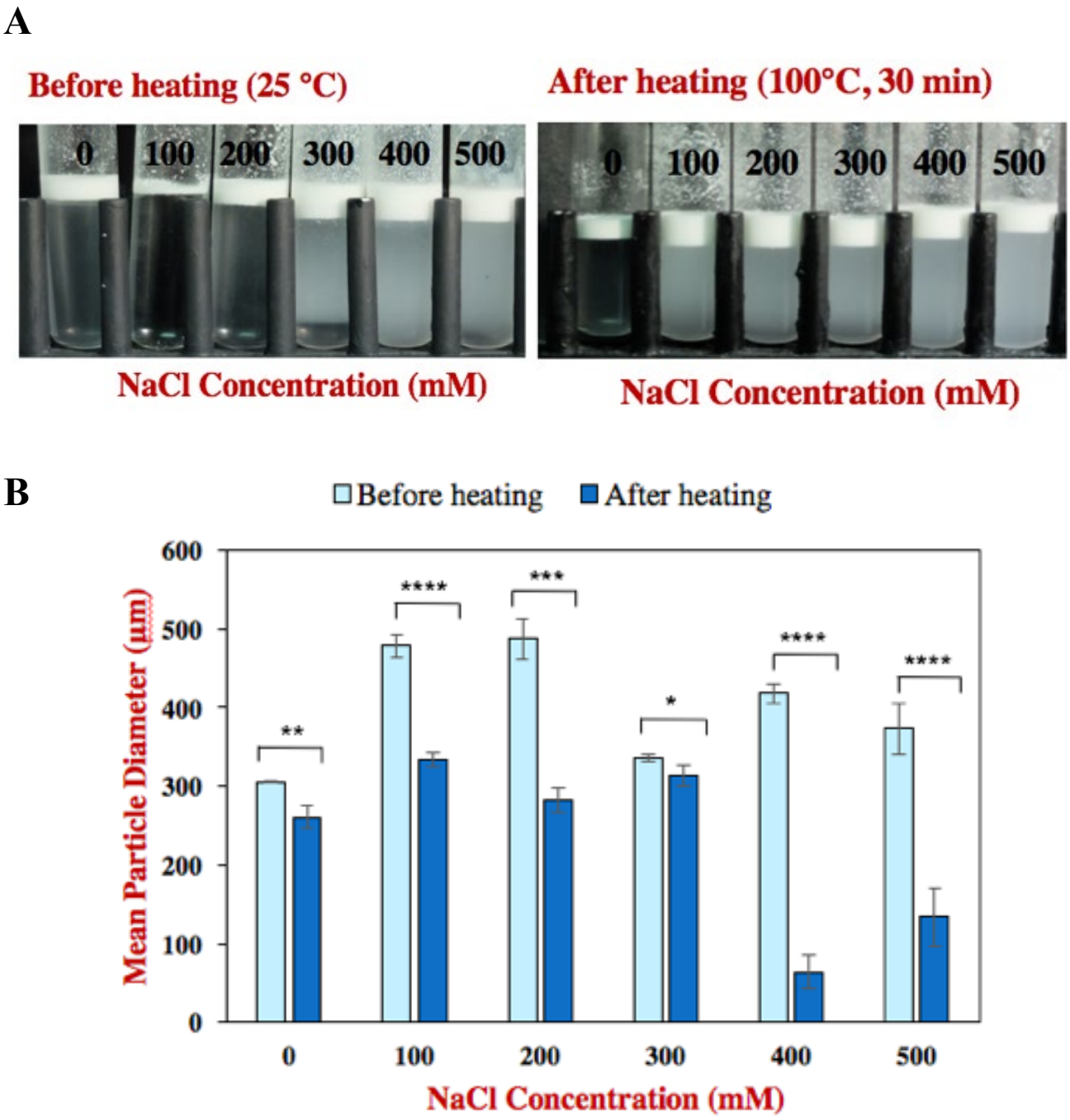
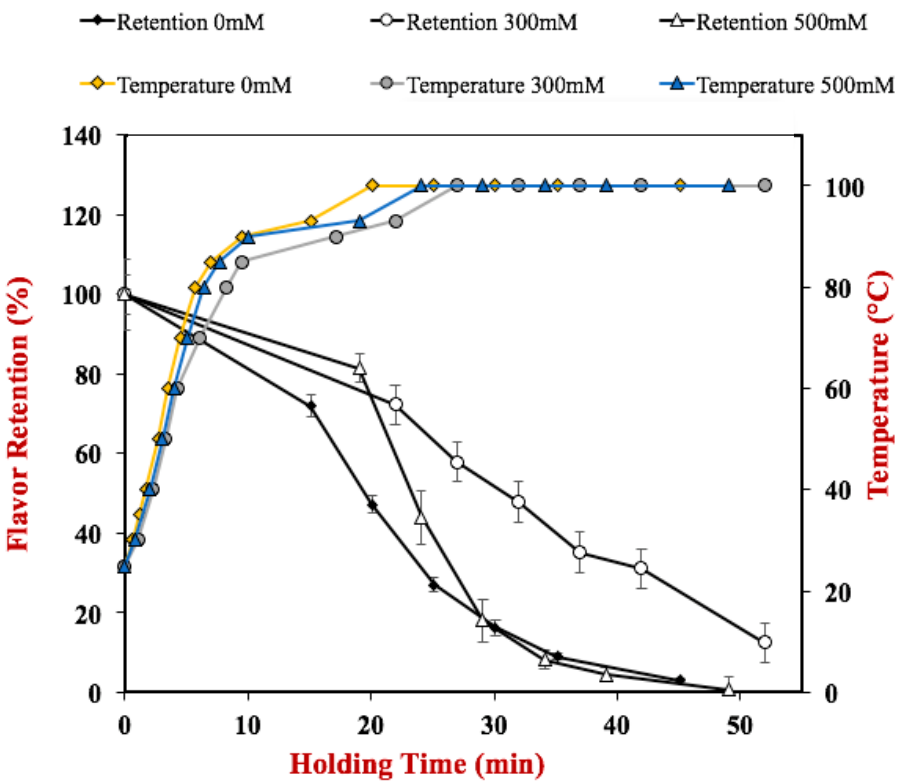



**Fig. 3.15.** Appearance (A) and volume-based mean particle diameters ($d_{43}$) (B) of flavor-loaded alginate microgels (10% corn oil, 1% sodium caseinate, 0.05% allyl methyl disulfide) incubated in NaCl solutions (0-500 mM) before (25 °C) and after thermal treatment (held at boiling temperature for 30 min after boiling was observed). Student's t-test was used to compare each sample before and after heating (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ****$P \leq 0.0001$).

The impact of salt concentration on the appearance and mean particle diameter ($d_{43}$) of the microgels before and after simulated cooking were compared (**Fig. 3.15.**). As discussed earlier, the microgels remained intact at all salt concentrations before heating, but some lipid droplets were released at the higher salt levels. After heating, there appeared to be shrinkage of the microgels at all salt concentrations but particularly at the highest salt levels (400 and 500 mM NaCl). Moreover, lipid droplets were observed in the aqueous phase in all the samples containing salt after heating. These results show that the calcium alginate microgels are highly unstable to heating in the presence of salt. Presumably, the combined effects of weakening the electrostatic interactions, high temperatures, and mechanical agitation during boiling were sufficient to disrupt the hydrogel network, leading to microgel disruption and lipid droplet release.

### 3.3.5 Impact of salt concentration on flavor release

**A**




**B**

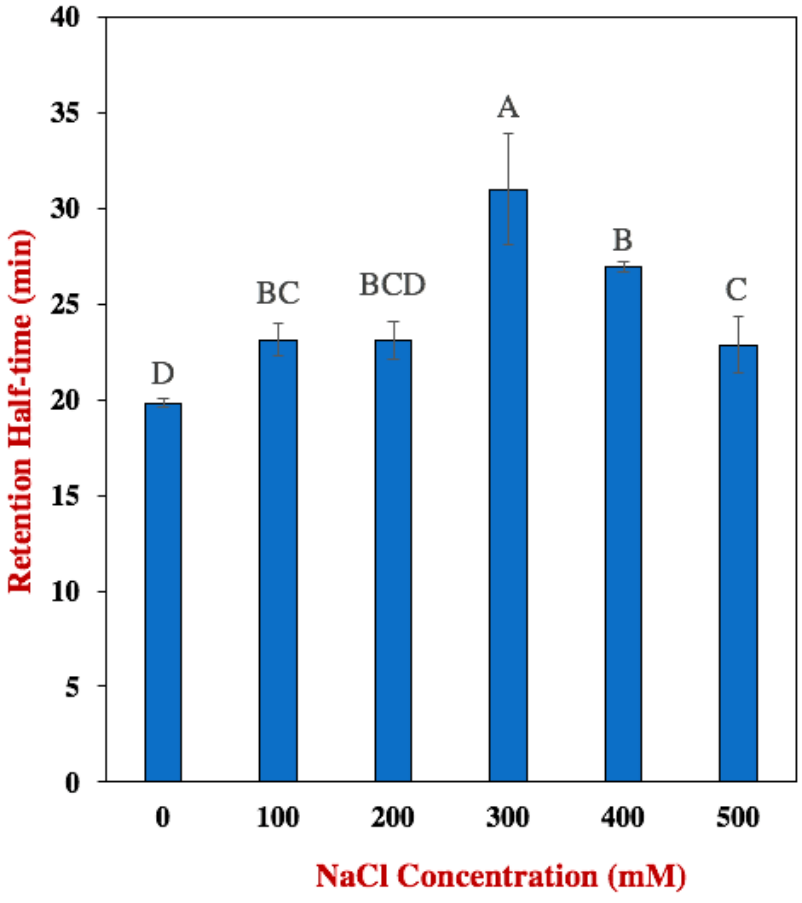


**Fig. 3.16.** Temperature and flavor retention profiles (A) and retention half-time (B) of alginate microgels (10% corn oil, 1% sodium caseinate, 0.05% allyl methyl disulfide) prepared in different NaCl solutions (0- 500 mM) before (25 °C) and during heating (25 - 100°C, 0-30 min after boiling). Only the 0, 300, 500 mM data are shown for the profiles. Error bars indicate SD. Values followed by the same letter do not differ statistically according to the one-way ANOVA, post-hoc Tuckey test at $P<0.05$.

In many food applications, it is important to control the release of flavors during the cooking process to obtain a desirable flavor profile. The impact of salt on flavor retention during cooking was therefore determined. The flavor retention of the microgels was characterized by the *retention half-times i.e.,* the time required for 50% of flavor to be lost.

For the flavor retention profiles, three salt levels were selected to produce delivery systems with different characteristics during cooking: *0 mM NaCl* – the hydrogels remained intact, and the lipid droplets remained encapsulated; *300 mM NaCl* – the hydrogels remained intact, but some of the lipid droplets were released; *500 mM NaCl* – the hydrogels disintegrated and some of the lipid droplets were released. The temperature-time profiles of all the delivery systems was fairly similar, which suggested that they experienced similar cooking conditions. Interestingly, the flavors appeared to be lost most readily from the system containing no added salt and most slowly from the system containing 300 mM salt (**Fig. 3.16.**). The salt may have changed the flavor retention profiles through a number of mechanisms: (i) altering the size of the microgels thereby altering the diffusion path length; (ii) altering the pore size of the microgels thereby altering the degree of restricted diffusion; (iii) altering the molecular interactions between the lipid droplets and the hydrogel matrix inside the microgels; (iv) altering the location of the lipid droplets (free or encapsulated); (v) altering the size of the individual lipid droplets (small or large). All of these factors could contribute to the observed effects and at present it is not possible to determine their relative importance. Having said this, the effects of salt were fairly modest, with the half-time being within the range of about 20 and 31 min.

### 3.3.6 Impact of salt concentration on microgel stability in storage

In this series of experiments, the impact of salt concentration on the stability of the microgels during storage at ambient temperature was determined (**Fig. 3.2.5. and 3.2.6.**).

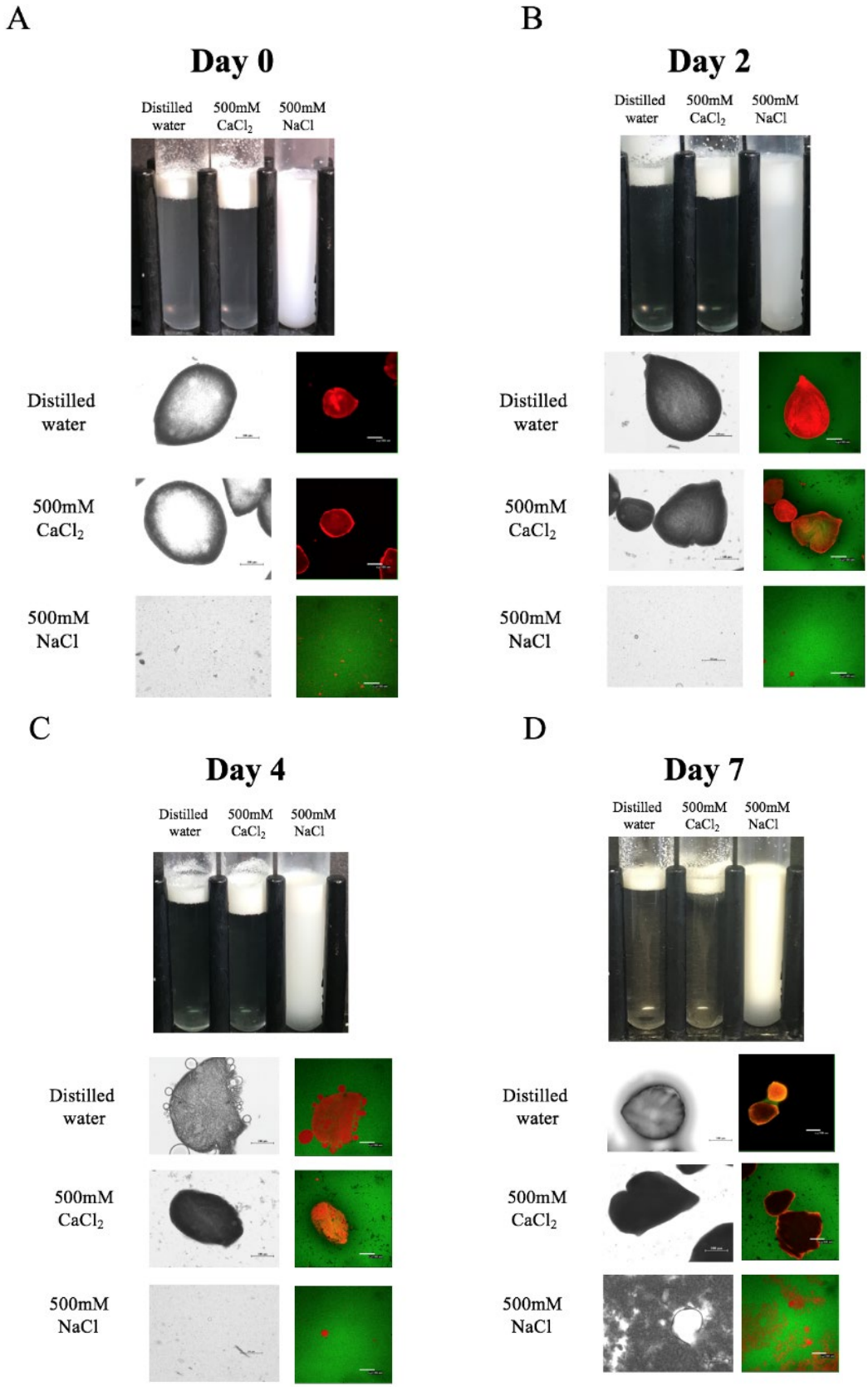


**Fig. 3.17.** Appearance and microstructures of microgels prepared in double distilled water, 500mM calcium cloride, 500mM sodium cloride solutions stored at ambient temperature for 0 days (A), 2 days (B), 4 days (C), 7 days (D). *Left*: Regular image; *Right*: Confocal image (Scale bar: 100 μm). Red is oil, green is protein.

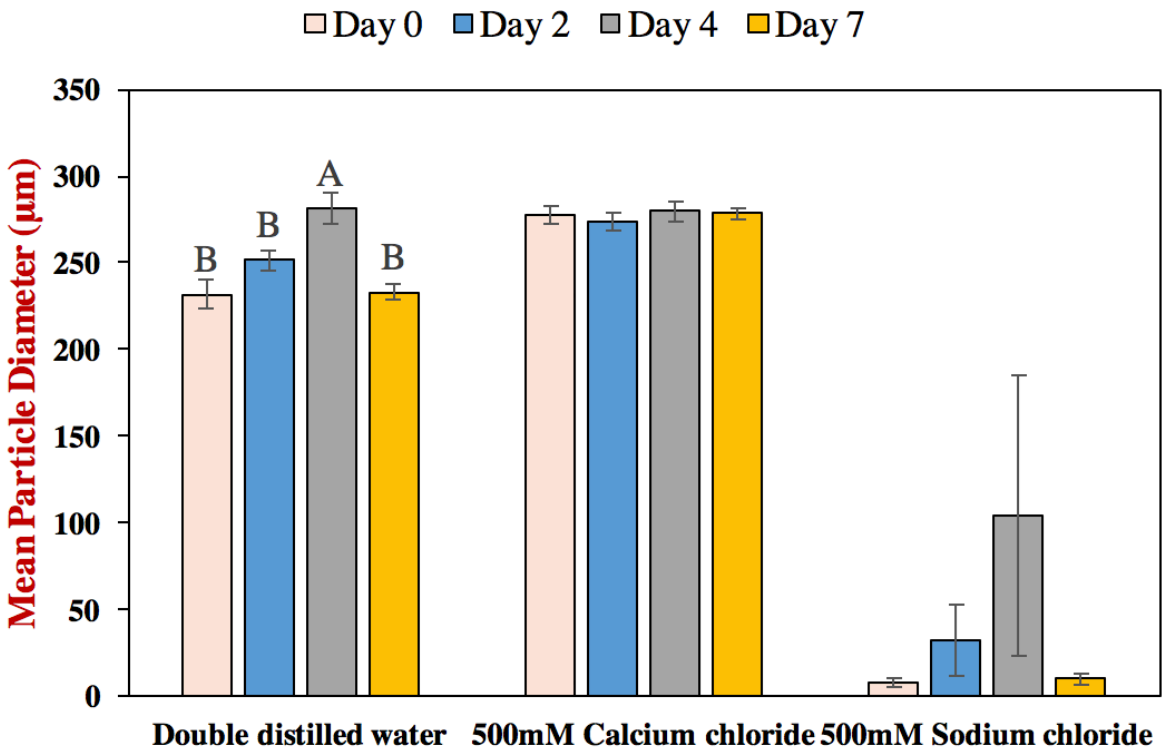



**Fig. 3.18.** Mean particle diameters ($d_{43}$) of microgel beads (10% corn oil, 1% sodium caseinate, 1% allyl methyl disulfide) incubated in double distilled water, 500 mM calcium chloride, and 500 mM sodium chloride after storage for 0, 2, 4 and 7 days at room temperature. Values followed by the same letter do not differ statistically according to the one-way ANOVA, post-hoc Tuckey test at $P<0.05$.

Microgels were dispersed in solutions containing distilled water, 500 mM calcium chloride, or 500 mM sodium chloride to determine the impact of ion level and type on their stability. The microgels stored in calcium chloride remained physically intact throughout storage with little change in their particle size or microstructure over 7 days. In addition, there was no evidence of the oil droplets leaking out of the microgels during storage in this system. Presumably, the high level of calcium ions in this system led to a strongly cross-linked alginate network that maintained the

integrity of the microgels.

The microgels stored in distilled water also maintained their physical integrity throughout 7 days storage, but there was some evidence of lipid droplets leaking out of them after 4 days storage as seen in the confocal microscopy images. There also appeared to be some evidence of droplet coalescence, since large lipid droplets were seen on the surfaces of the microgels after 4 days. After 7 days, it seems that the lipid droplets had all coalesced into a single mass that linked the alginate beads together.

The microgels stored in sodium chloride rapidly disintegrated, with their structures being completely lost soon (day 0) after preparation so that only small particles remained. In addition, the lipid droplets were rapidly released into the surrounding aqueous phase as seen in the confocal microscopy images and photographs of the samples. Indeed, visual observations showed that the aqueous phase of this sample became cloudy only a few minutes after the microgels were mixed with the salt solution. In this case, the sodium ions may have competed with the calcium ions and weakened the alginate network structure leading to disintegration of the alginate network thereby promoting lipid droplet release.

**Flavor retention during cooking after microgel storage**

In this series of experiments, the flavor retention of the microgel suspensions after cooking was measured after they had been stored for different times at ambient temperature. It was hypothesized that samples that had been stored longer may have contained less flavor molecules due to evaporation effects. We therefore carried out some experiments where the samples were stored in either open containers or closed containers. (**Fig. 3.19.**)

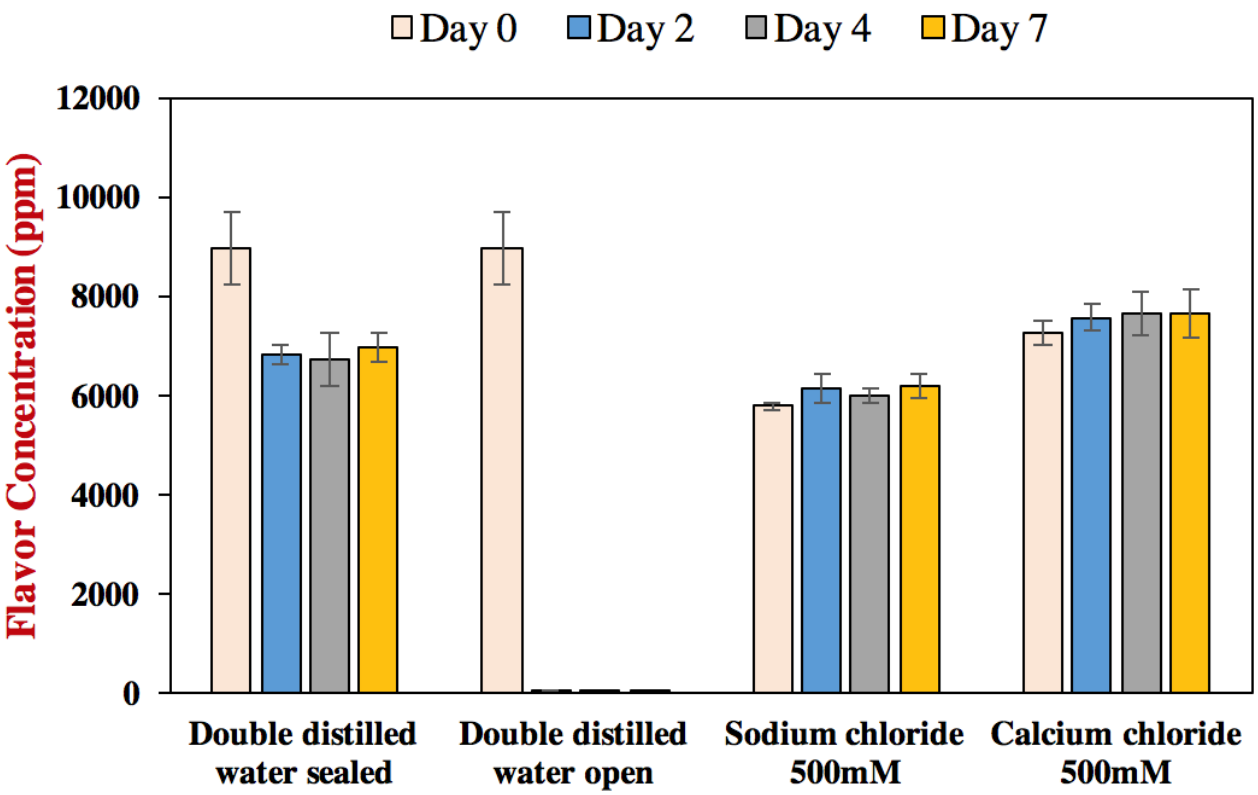



**Fig. 3.19.** Flavor retained in alginate microgels (10% corn oil, 1% sodium caseinate, 1% allyl methyl disulfide) incubated in double distilled water (one group was stored in GC vials sealed with cap, another group with the same formulation was stored in open GC vials), 500 mM calcium chloride and 500 mM sodium chloride after storage in sealed GC vials for 0, 2, 4 and 7 days at room temperature.

When the samples were stored in closed containers, there was little influence of storage time on their flavor retention after cooking, regardless of the nature of the solution they were incubated in, *i.e.,* distilled water, 500 mM NaCl, or 500 mM $CaCl_2$. This is presumably because the samples were stored in sealed containers and so the volatile flavor molecules could not escape. In contrast, when microgels were stored in an open container, very little flavor was detected after 2 days' storage, indicating that much of the flavor had been lost due to evaporation. The fact that the flavor levels in all the sealed vials remained relatively constant throughout storage indicated that no chemical degradation occurred under these conditions.

## 3.4 Conclusions

***Impact of oil concentration on flavor release***

In conclusion, this study has shown that there is an optimum lipid content in alginate microgels that should be utilized to obtain a sustained release profile. More rapid flavor release was observed for those microgels that underwent sedimentation (2.5 and 5% oil), than those that underwent creaming (≥ 10% oil)

***Impact of alginate concentration on flavor release***

At higher alginate concentration, the decrease in the mean particle diameter of the microgels after simulated cooking was smaller. It is considered that higher alginate concentration formed a stronger biopolymer network which could prevent from shrinkage during simulated cooking. Higher alginate concentration (>1%) in microgels improved the flavor heat retention during boiling process. However, elevation of alginate concentration above 1% in formulation cannot further improve the heat retention properties.

***Impact of oil casein concentration on flavor release***

At lower caseinate concentration, the decrease in the mean particle diameter of the microgels after simulated cooking was bigger. It is considered that lower caseinate concentration formed unstable emulsion which could coalesce easily and release out from microgel during simulated cooking. Therefore, the coalescence of emulsion may have caused shrinkage of microgel. Higher concentration of caseinate in oil-loaded alginate calcium microgels did not show strong impact on particle structure. It was suggested that the optimum concentration of emulsifier exists to keep emulsion stable.

***Impact of salt concentration on flavor release***

The results showed that salt addition promoted disintegration of the alginate microgel structures during simulated cooking and storage, with the effect becoming more pronounced at higher NaCl levels. It is considered that the cationic sodium ions ($Na^{+}$) displace some of the cationic calcium ions ($Ca^{2+}$) that normally hold the alginate network together, thereby leading to lipid droplet release and

microgel disintegration. The extent of disintegration and droplet release increased as the NaCl content of the system increased. Microgel disintegration was further promoted by the cooking process, which may have been due to the elevated temperatures and mechanical agitation occurring during boiling.

In summary, this study has shown that the retention of a model garlic flavor can be increased by encapsulating the flavor-loaded lipid droplets within filled alginate microgels. Overall, these results suggest that a variety of factors contribute to flavor loss from alginate microgels during cooking, including initial oil concentration, alginate concentration, casein concentration, and salt concentration. This may lead to food products with enhanced sensory attributes, e.g., flavors that last longer during the cooking process.

However, further work is still required to determine the impact of the microgels on the sensory attributes (texture, appearance, stability, and mouthfeel) of foods, and to determine whether the microgels can be produced economically on large scale.

# 4. Conclusions

The objective of this study was to elucidate main factors influencing a model volatile lipophilic flavor (Allyl methyl disulfide) retention in emulsion-based encapsulation systems during simulated cooking for the design of the encapsulation systems with tunable flavor release profiles.

In the chapter 2, we investigated the impact of factors on model flavor retention in oil-in-water emulsions during simulated cooking, including oil droplet concentration, oil type, droplet size, and emulsifier type, and biopolymer additives. These results suggest that each factor has great influence on emulsion stability and flavor release characteristics. These study makes it clear that the influence of each factor on flavor release. These results are useful to design emulsion-based encapsulation system for desirable flavor release profile. However, further research to elucidate the influence of combination of each factor is necessary to design the flavor release system with more certainly.

In the chapter 3, we fabricated alginate microgels containing AMDS-loaded oil droplets and evaluated its flavor release profile during simulated cooking. As a result, it was able to delay flavor release appreciably (3-fold longer), and the microgels were found to remain intact throughout the boiling process. These results suggest that biopolymer microgels may be useful for controlling flavor release during cooking. Moreover, the impact of oil, alginate, casein concentration on flavor release from alginate microgel was evaluated. These results suggest that a variety of factors contribute to flavor release from alginate microgels during cooking, including initial oil concentration, alginate concentration, casein concentration, and salt concentration. The studies have shown that increasing oil concentration may improve flavor heat retention. Sodium caseinate concentration and alginate concentration showed little correlation to the flavor retention time. Additionally, the impact of salt concentration on flavor release profile on alginate microgel was evaluated. As expected, salt addition promoted disintegration of the alginate microgel structures during simulated cooking and storage, with the effect becoming more pronounced at higher NaCl levels. It seems reasonable to conclude that the cationic sodium ions ($Na^+$) displace some of the cationic calcium ions ($Ca^{2+}$) in alginate network hereby leading to lipid droplet release and microgel disintegration.

In summary, the study makes it clear that each factor of both O/W emulsion and alginate microgels has optimum value to realize desirable flavor release profile for the application to commercial food products. These findings may lead to food products with enhanced sensory attributes, e.g., flavors that last longer during the cooking process.

# 5. Future work

To further improve the flavor retention during cooking and producing colloidal delivery systems that have commercial viability and efficacy, it has been identified a number of approaches to optimize emulsion/microgel.

***Microgel formulation optimization***

Other ingredients that can increase particle density could be added into emulsion/ microgels for flavor heat retention. Possible system: microparticulated protein such as particle WPI (high density) or particle beta-lactoglobulin.

***Enzymatic approach for flavor formation and controlled release***

As an alternative of encapsulating AMDS, the precursor of garlic flavors can be encapsulated, and release can be triggered by enzyme at the end of cooking period.

***Simulated digestion in mouth***

After encapsulation of garlic flavors, release can be controlled by triggering in mouth for enhanced flavor intensity. In this case, starch could be incorporated, and the release of flavors could begin when amylase reacts with the starch.

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