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Influence of environmental stresses on the stability of W/O/W emulsions containing enzymatically modified starch

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ABSTRACT

The present study was performed to investigate the stability of W/O/W emulsions containing 4-α-glucanotransferase (4αGTase)-treated starch against environmental stresses such as heating, shearing, and repeated freeze-thawing. W/O/W emulsions were subjected to thermal processing at different temperatures ranging from 30 to 90 °C for 30 min, constant shear for 0–7 min, and freeze–thaw cycling between −20 °C and 30 °C, respectively, and followed by encapsulation efficiency (EE) measurement. As for the case of thermal stress, it was clearly shown that addition of 4αGTase-treated starch in the internal aqueous phase of emulsions helped to maintain higher EE during thermal processing. However, at lower PGPR level (2%), the addition of 4αGTase-treated starch dramatically reduced EE at temperatures higher than 70 °C, which was probably related to the melting of 4αGTase-treated starch gel. The incorporation of 4αGTase-treated starch improved the stability of emulsions during shearing process, but could not prevent W/O/W emulsions from creaming and destabilizing during freeze–thaw cycling.

1. Introduction

Water-in-oil-in-water (W/O/W) emulsions consist of small water droplets contained within larger oil droplets dispersed in a watery continuous phase. Increasing research attention has focused on this double (or multiple)-compartment structure, which gives W/O/W emulsions a high capacity to entrap hydrophilic compounds. These emulsion systems have been successfully formulated for use in pharmaceuticals and cosmetics (Gallarate, Carlotti, Trotta, & Bovo, 1999; Vlaia, Vlaia, Miclea, Olariu, & Coneac, 2009). They also have many potential uses in the food industry, such as in producing supplements containing sensitive micronutrients, masking the unpleasant flavors of bioactive compounds, achieving controlled release of various substances, and producing low-fat foods by incorporating dispersed water into fat droplets (Benichou, Aserin, & Garti, 2004, 2007; Choi, Decker, & McClements, 2009; Garti, 1997; Garti & Aserin, 1996; Leal-Calderon, Homer, Goh, & Lundin, 2012; Sapei, Ali Naqvi, & Rousseau, 2012; Su, Flanagan, Hemar, & Singh, 2006; Weiss, Scherze, & Muschiolik, 2005).

Nevertheless, the application of multiple emulsions is limited by their thermodynamic instability, especially their strong tendency for droplet coalescence. Another problem associated with multiple emulsions is the possible diffusion of water-soluble compounds from one aqueous phase to the other through the oil layer (Benichou et al., 2004; Pays, Giermanska-Kahn, Pouligny, Bibette, & Leal-Calderon, 2001; Su, Flanagan, & Singh, 2008).

Much effort has been devoted to improving the stability of W/O/W emulsions (Benichou et al., 2007; Garti, 1998; Garti, Aserin, Tiunova, & Binyamin, 1999; Hajda & Dickinson, 1996; Hino, Shimabayashi, Tanaka, Nakano, & Okochi, 2001; Su et al., 2006; Weiss et al., 2005). Of these, the incorporation of a thickening or gelling polymer such as xanthan, alginate, gelatin, agarose, bovine serum albumin, sodium caseinate or modified starch within the internal aqueous phase has been attempted to improve their long-term stability (Cho, Shim, & Park, 2003; Garti & Aserin, 1996; Mun, Choi, Shim, Park, & Kim, 2011; Sapei et al., 2012; Weiss et al., 2005). This strategy has been shown to produce relatively stable water-in-oil (W/O) emulsions, which is a necessary part for the preparation of stable W/O/W emulsions. In our previous research, we have prepared 4-α-glucanotransferase (4αGTase)-treated starch and incorporated it into the internal aqueous phase of W/O/W emulsion as a gelling agent. The enzymatically modified starch had unique rheological characteristics, that is, it could remain in a liquid state during emulsion preparation, but with time, the solution became a gel. This interesting behavior of the 4αGTase-treated starch allowed easy preparation of W/O/W emulsions, high encapsulation efficiency of hydrophilic compound, as well as enhanced long-term stability by gelling of

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the internal water phase (Lee, Kim, Park, & Lee, 2006; Mun et al., 2011).

For practical application of W/O/W emulsions to food industries, maintaining stability of the emulsions against various environmental stresses is highly essential. Therefore, in this study, we examined the influences of thermal processing, shearing, and freeze–thaw cycling, which are frequent practices especially in food processing, on the encapsulation efficiency and stability of W/O/W emulsions containing 4αGTase-treated starch in the inner aqueous phase.

2. Materials and methods

2.1. Materials

The PGPR (CRS-75, Sakamoto Yakuhin Kogyo, Co., Ltd.), prepared by esterification of condensed castor oil fatty acids with polyglycerol, was used as a hydrophobic emulsifier in this study. The starch used herein was isolated from native rice (Ilmi byeo, Korea) by using a traditional alkaline method (Lumdubwong & Seib, 2000). To produce the 4αGTase enzyme, we used a recombinant strain of *Escherichia coli* containing thermostable *4αGTase* gene isolated from *Thermus aquaticus* (Food Enzymology Laboratory, Seoul National University, Korea). Polyoxyethylene-sorbitan monolaurate (Tween 20), hydrochloric acid (HCl), sodium hydroxide (NaOH), and sodium azide (NaN_3) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium phosphate (dibasic, anhydrous) and sodium phosphate (monobasic, anhydrous) were purchased from Showa Chemical Co. (Tokyo, Japan). Soybean oil was purchased from a local supermarket and used without further purification. We purchased 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (PTSA) from Fisher Scientific International L.L.C. (Hampton, NH).

2.2. Enzymatic modification of starch

Isolated rice starch was dispersed into water (5%, w/w) and heated in boiling water for 30 min with mechanical stirring. After cooling to 75 °C, starch paste samples were incubated with 4αGTase (5 U/g dry starch) for 96 h. After terminating the reaction by boiling for 30 min, three volumes of ethanol were added to the mixture. The resulting precipitate was separated and dried at room temperature.

2.3. Solution preparation

A hydrophobic emulsifier solution was prepared by dispersing 2 and 4 wt% PGPR into soybean oil and heating to 50 °C. The 4αGTase-treated starch solutions were prepared by dispersing the desired amount (5, 10, 15, and 20 wt%) of 4αGTase-treated starch into 5 mM phosphate buffer solution (pH 7), and then heating for 30 min to ensure complete dissolution. A hydrophilic surfactant solution was prepared by dispersing surfactant (0.5 wt%, Tween 20) in 5 mM phosphate buffer (pH 7) containing 0.02 wt% NaN_3 as an antibacterial agent.

2.4. Preparation of water-in-oil emulsions

Water-in-oil (W/O) emulsions were prepared at an aqueous fraction of 20 wt%. The emulsions were prepared at around 50 °C rather than at room temperature, because previous studies have shown that the viscosity of PGPR–oil mixtures reduces upon heating, and the emulsions produced by homogenization have smaller droplet sizes (Surh, Vladisavljević, Mun, & McClements, 2007). The aqueous phase (0–20 wt% 4αGTase-treated starch, pH 7) was gradually dispersed into the oil phase under agitation with a magnetic stirrer, and then sonicated for 4 min at a frequency of 20 kHz, an amplitude of 40%, and a duty cycle of 0.5 s (VCX 750; Sonics & Materials, Inc., Newtown, CT).

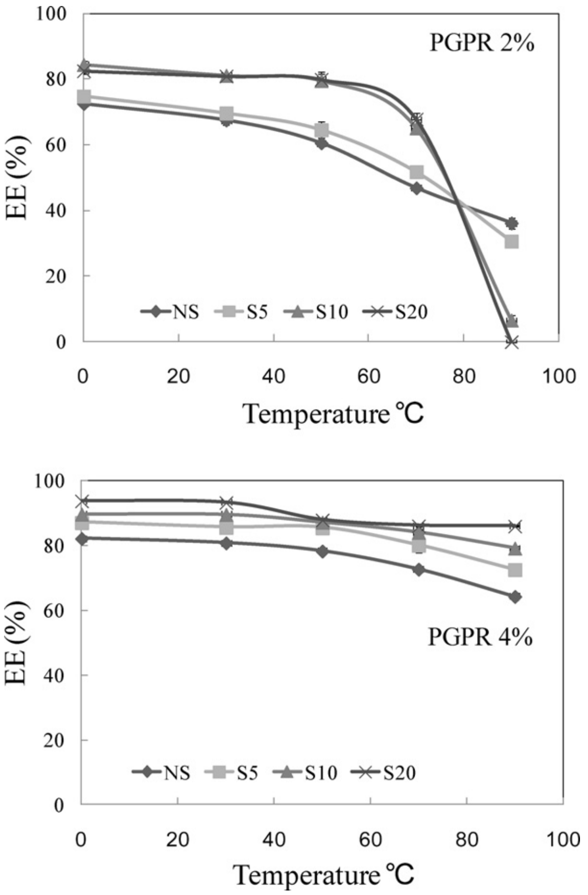

Fig. 1. Influence of heating on EE of W/O/W emulsion prepared with 2 and 4 wt% PGPR and 4αGTase-treated starch. NS: W/O/W emulsion prepared without 4αGTase-treated starch. S5, S10, and S20: W/O/W emulsion containing 5, 10, and 20 wt% 4αGTase-treated starch in the internal aqueous phase.

2.5. Preparation of water-oil-water emulsions

Water-oil-water (W/O/W) emulsions were prepared using a two-stage emulsification method (Surh et al., 2007). First, 20 wt% W/O emulsion was prepared as described above. Second, the 20 wt% W/O emulsion was sonicated with 80 wt% aqueous surfactant solution (0.5% Tween 20, 5 mM phosphate buffer, 0.02 wt% NaN_3, pH 7) for 3 min at a frequency of 20 kHz, an amplitude of 40%, and a duty cycle of 0.5 s (VCX 750, Sonics & Materials, Inc.).

2.6. Confocal laser scanning microscopy

Confocal fluorescence images (excitation: diode 405-nm laser, emission wavelength: 420–480 nm) were captured by confocal laser scanning microscopy (LSM 510 Meta; Carl Zeiss, Göttingen, Germany).

2.7. Determination of encapsulation efficiency (EE)

In this study, the highly water-soluble fluorescent dye PTSA was used as a model ingredient for encapsulation of active food supplements. The EE of the dye in a W/O/W emulsion was defined as

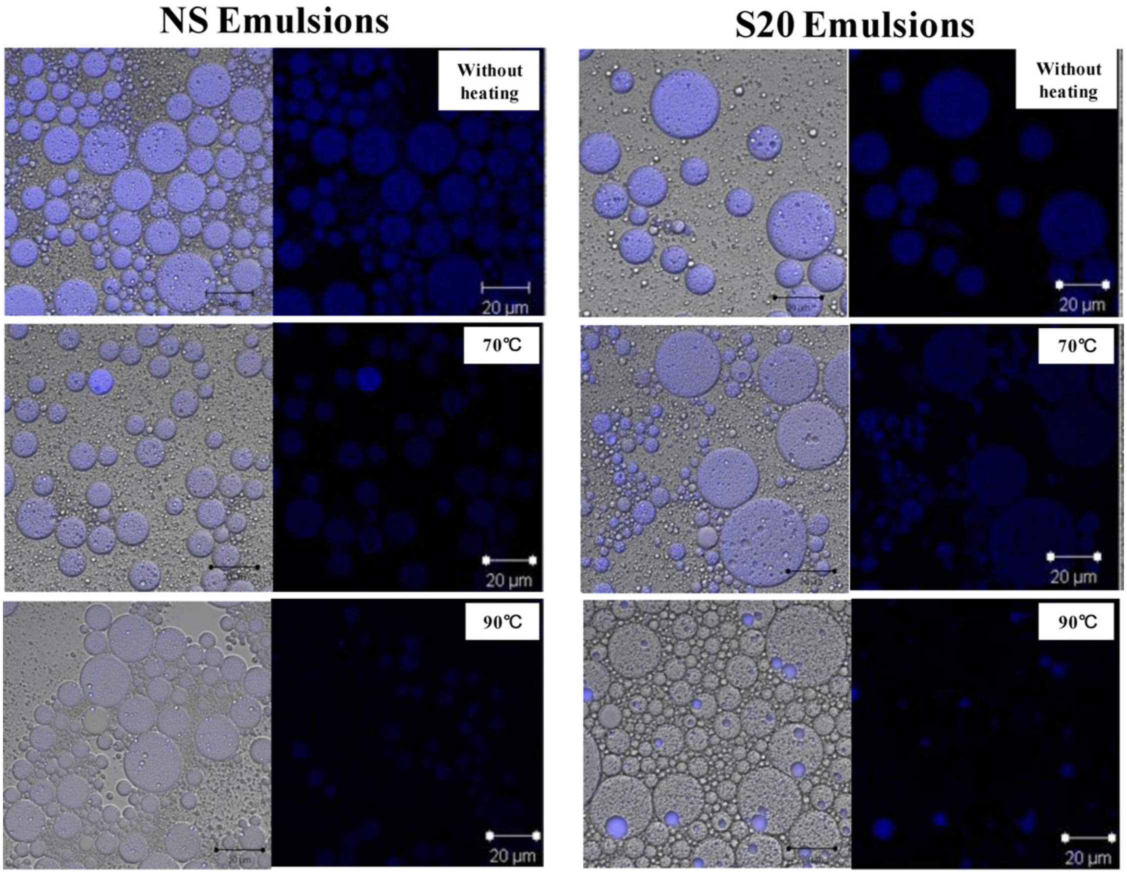

Fig. 2. Confocal micrographs of heated W/O/W emulsions prepared with 2 wt% PGPR in the oil phase and 0.5 wt% Tween 20 in the outer aqueous phase. NS: W/O/W emulsion prepared without 4αGTase-treated starch. S20: W/O/W emulsion containing 20 wt% 4αGTase-treated starch in the internal aqueous phase. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

the percentage of the water-soluble dye retained within the inner aqueous phase droplets following sonication of the W/O emulsion with the aqueous phase. Initially, we prepared a standard curve by plotting fluorescence intensity against PTSA concentration (Adachi, Imaoka, Hasegawa, & Matsuno, 2003; Tokgoz, Grossiord, Fructus, Seiller, & Prognon, 1996). A stock dye solution was prepared by dissolving 0.01% (w/v) PTSA in buffer solution (5 mM phosphate buffer, pH 7). A standard curve was then prepared ($r^2 = 0.996$) by measuring the fluorescence intensity of PTSA (excitation wavelength, 374 nm; emission wavelength, 404 nm) by using a microplate reader (Safire2; Tecan, Männedorf, Switzerland). The concentration of dye in the external aqueous phases collected from the W/O/W emulsions was then determined using this standard curve.

The PTSA (0.2%) was dispersed in the aqueous phase used to prepare the W/O emulsions as described above. The W/O/W emulsions were then prepared by sonication of 20 wt% W/O emulsions and 80 wt% aqueous surfactant solution (0.5% Tween 20, 5 mM phosphate buffer). Aliquots of the W/O/W emulsions were then centrifuged for 20 min at 30,000 rpm by using an ultracentrifuge (Himac CP100β; Hitachi, Tokyo, Japan) to separate them into 2 layers: creamed layer and serum layer. An aliquot of the serum layer from each centrifuged sample was clarified using a syringe-driven filter unit (Millipore Corporation, Bedford, MA), and the fluorescence intensity of the dye was recorded with a microplate reader. This procedure was repeated for similar emulsions that had been prepared without dye in order to obtain blank values, and these were subsequently subtracted from the values obtained for their counterparts with dye. The concentration of dye present in the serum layer was determined from the standard curve.

The EE was expressed as the percentage of PTSA that remained encapsulated within the water droplets after homogenization, as follows:

$$E(\%) = \frac{M_i - M_e}{M_i} \times 100$$

where M_i is the mass of PTSA initially present in the internal water droplets in the W/O emulsion and M_e is the mass of PTSA present in the external water phase in the W/O/W emulsion after sonication. EE can be calculated if one assumes that the amount of PTSA released from the internal water droplets is proportional to the amount of water released, and that PTSA is released because of expulsion of the internal water droplets during the formation of W/O/W emulsion (Surh et al., 2007).

2.8. *Emulsion stability with respect to environmental stress*

The stability of W/O/W emulsions containing 4αGTase-treated starches in the internal water phase was compared to that of W/O/W emulsions prepared without 4αGTase-treated starches by measuring the EE, microstructures, and creaming stability after they were subjected to certain environmental stresses. To investigate the effects of shearing and heating on the stability and EE of W/O/W emulsions, the samples were subjected to different temperatures ranging from 30 to 90 °C in a water bath for 30 min,

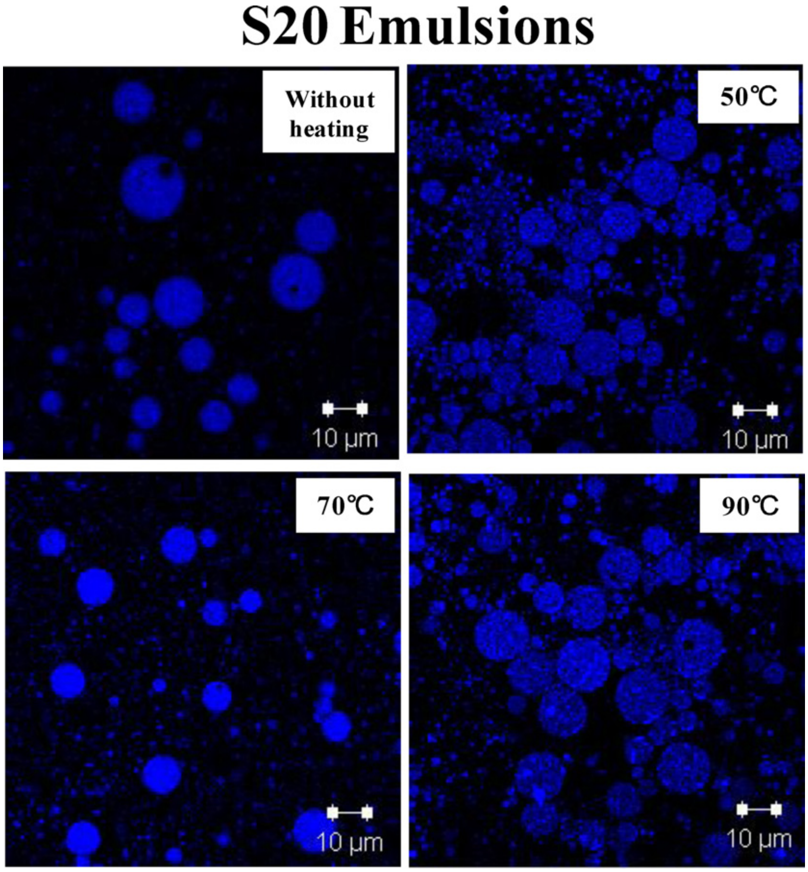

Fig. 3. Confocal micrographs of heated W/O/W emulsions prepared with 4 wt% PGPR in the oil phase and 0.5 wt% Tween 20 in the outer aqueous phase. S20: W/O/W emulsion containing 20 wt% 4αGTase-treated starches in the internal aqueous phase.

and constant shear for 0–7 min at room temperature by using a high-performance disperser (T25 digital Ultra-Turrax, Ika). This was followed by EE measurement. For freeze–thaw processing studies, samples were stored in a freezer (−20 °C) for 22 h. After freezing, the samples were thawed in a water bath at 30 °C for 2 h.

3. Results and discussion

3.1. Influence of thermal processing

3.1.1. W/O/W emulsions prepared with 2 wt% PGPR

In the previous study, incorporation of 4αGTase-treated starch in the internal water phase of W/O/W emulsions not only improved the dye EE but also reduced the concentration of synthetic emulsifiers, such as PGPR, required to prepare stable W/O emulsions. The results suggested that addition of 20 wt% 4αGTase-treated starch to the internal aqueous phase could reduce the concentration of PGPR from 8 to 2 or 4%, and PGPR could be replaced with 4αGTase-treated starch without changing the EE or stability of the emulsion. Therefore, in this study, the influence of thermal processing on the EE of W/O/W emulsions prepared with reduced PGPR concentrations (2 and 4 wt%) and 5–20 wt% 4αGTase-treated starch was evaluated. The W/O/W emulsions prepared in this condition had mean diameters of approximately 5 μm, irrespective of the PGPR and 4αGTase-treated starch concentration. However, full droplet size distributions differed between samples prepared with 2 and 4 wt% PGPR. The majority of droplets in emulsions prepared with 4 wt% PGPR were below 20 μm, whereas large oil droplets (>20 μm) were seen in emulsions prepared with 2 wt% PGPR (data not shown).

The EEs (%) of W/O/W emulsions prepared with and without 4αGTase-treated starches heated at different temperatures (30, 50, 70, and 90 °C) for 30 min were determined as the percentage of dye retained in the internal water droplets (Fig. 1). The PGPR concentration used to prepare W/O emulsions was strongly related to the EE of W/O/W emulsions and their stability during heat processing. In the case of emulsions prepared with 2 wt% PGPR, the EE decreased as temperature increased. Furthermore, the result depended on the concentration of 4αGTase-treated starch incorporated in the internal aqueous phase. The EEs of emulsions NS (no starch) and S5 (5% 4αGTase-treated starch) gradually decreased as temperature increased. In contrast, the EEs of S10 (10% 4αGTase-treated starch) and S20 (20% 4αGTase-treated starch) emulsions hardly changed at ≤50 °C and then rapidly decreased at higher temperature, eventually reaching to near 0% EE at 90 °C. Thus, at 2% PGPR level, the EE of W/O/W emulsions prepared with modified-starch incorporated in the internal aqueous phase greatly depended on the temperature and modified-starch concentration. At ≤70 °C, the incorporation of 10 and 20 wt% 4αGTase-treated starches significantly improved the EE values of emulsions even at 2% PGPR. Little difference was observed between EE values of 10 and 20 wt% 4αGTase-treated starch samples. These results indicated that incorporating an appropriate concentration of 4αGTase-treated starch solution improved the resistance of aqueous droplets to droplet leakage or coalescence during thermal processing. As reported previously, improving the EEs of emulsions by incorporation of 4αGTase-treated starch solutions into the internal aqueous phase might be attributable to the unique rheological properties of 4αGTase-treated starch solutions. At relatively high starch concentrations (20 wt%), 4αGTase-treated starch solutions could be

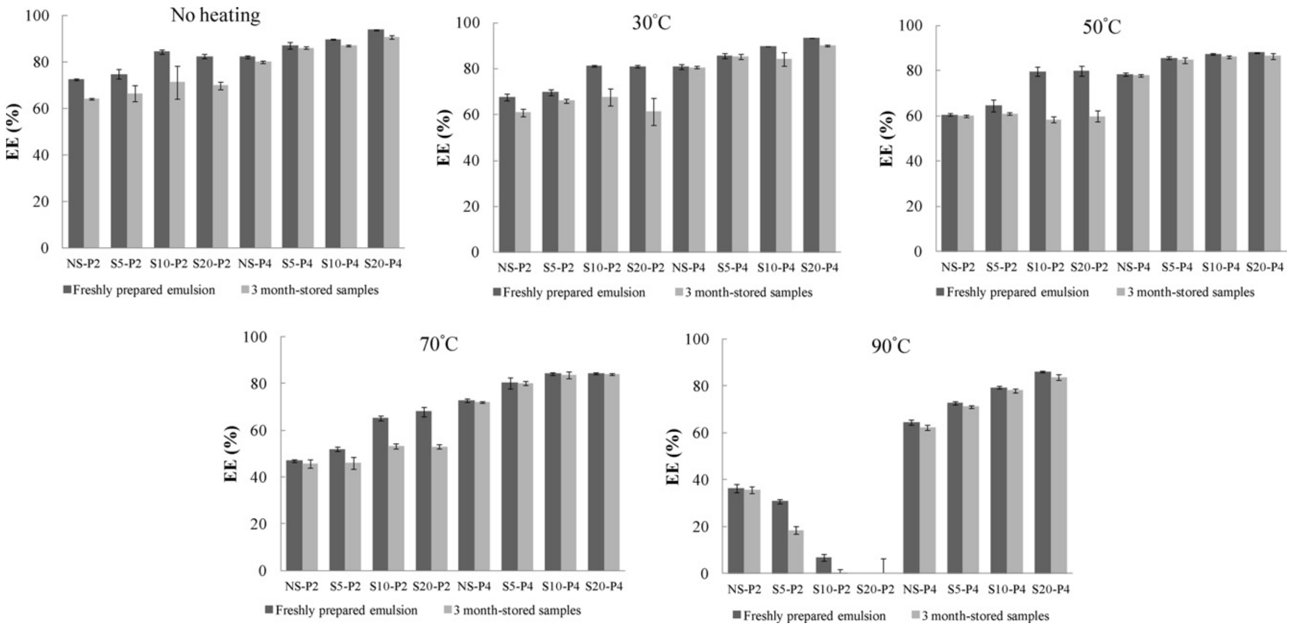

Fig. 4. EEs of W/O/W emulsions stored for three months after heating at different temperatures. NS: W/O/W emulsion prepared without 4αGTase-treated starch. S5, S10, and S20: W/O/W emulsion containing 5, 10, and 20 wt% 4αGTase-treated starch in the internal aqueous phase. P2: W/O/W emulsions prepared with 2 wt% PGPR. P4: W/O/W emulsions prepared with 4 wt% PGPR.

remained as a liquid state with relatively low viscosity during preparation and then become a solid gel during storage (Mun et al., 2011).

However, at high temperature (>70 °C), incorporation of 10 and 20 wt% 4αGTase-treated starches adversely affected the EE. Almost all of the dye was released from W/O/W emulsions containing high concentrations of 4αGTase-treated starch (10 and 20 wt%) at 90 °C. We were unable to elucidate the mechanism underlying this phenomenon. However, we deduced one possibility for the cause of dye release from the microstructure obtained by confocal fluorescence microscopy.

As shown in Fig. 2 (top), without heating, NS and S20 emulsions contained blue-colored W/O/W droplets indicating that the dye remained within the internal aqueous phase. Although the fluorescence intensity seemed to be decrease at 70 °C (middle pictures in Fig. 2), a significant amount of dye remained after heating. The microstructure of emulsion S20 heated at 90 °C appeared to be different from that of other emulsions. W/O/W droplets in S20 emulsions heated at 90 °C included a large nucleus of coalesced aqueous droplets (right bottom picture in Fig. 2). Considering that the 4αGTase-treated starch solution showed sol-gel thermoreversibility between 4 and 70 °C (Lee et al., 2006), the solid starch gel in the internal aqueous phase should change to a liquid state above 70 °C, while the aqueous droplets were not completely coated with 2 wt% PGPR. Consequently, coalescence between water droplets may occur easily. Internal droplet coalescence produced large nuclei which are preferentially adsorbed on the globular inner surface. As a result, massive discharge of aqueouse components with dye in internal aqueous phase into external aqueous phase would occur. Two possible mechanisms responsible for this phenomenon have been reported: (1) coalescence of the thin liquid films separating the internal droplets and the globular inner surfaces, and (2) diffusion and/or permeation of the chemical substances across the oil phase (Ficheux, Bonakdar, Leal-Calderon, & Bibette, 1998; Pays et al., 2001).

Interestingly, massive discharge of aqueous components with dye in internal aqueous phase into external aqueous phase occurred only in case of low PGPR (2 wt%) and high 4αGTase-treated starch (10 and 20 wt%) levels at temperature higher than 70 °C. This phenomena was not detected in emulsions prepared with none or low level (5 wt%) of modified starch (top in Fig. 1 and left in Fig. 2) and/or 4 wt% PGPR (bottom in Figs. 1 and 3). One postulation is that the arrangement of PGPR surrounding the internal aqueous droplets formed during the emulsion preparation might be disturbed by hard gel formation at high concentration of 4αGTase-treated starch. With insufficient amount of PGPR (2 wt%), this might accelerate droplet coalescence and leakage when the gel melts at high temperature (>70 °C). The 5 wt% 4αGTase-treated starch was not hard gel-forming. Further research is required to elucidate the relationship between thermoreversible gel formation and emulsion stability.

In summary, heating temperature and concentrations of 4αGTase-treated starch and PGPR could be factors affecting the dye release from W/O/W emulsions containing 4αGTase-treated starch and these results could be useful in the applications that require temperature-triggered release.

3.1.2. W/O/W emulsions prepared with 4 wt% PGPR

W/O/W emulsions prepared with 4 wt% PGPR showed not only higher EE values than those of emulsions with 2 wt% PGPR, but also greater resistance to changes in EE values induced by heating (Fig. 1, bottom). The EE values of W/O/W emulsions prepared with 4 wt% PGPR did not change significantly throughout the temperature range, even though EE values decreased slightly at above 70 °C regardless of the presence of 4αGTase-treated starch.

These data suggest that 4 wt% PGPR was superior for the preparation of stable W/O emulsions compared to 2 wt% PGPR. Even though temperature altered the viscosity of liquid phases and the solubility and partitioning of molecules between the oil and aqueous phases (McClements, 2004a; Surh, 2009), this result suggested that emulsions prepared with a sufficient quantity of surfactant could conserve a significant amount of dye.

The influence of heat processing on the microstructure of W/O/W emulsions prepared with 20 wt% 4αGTase-treated starch and 4 wt% PGPR was observed using confocal fluorescence microscopy (Fig. 3). W/O/W droplets were well-prepared with small aqueous droplets visible within larger oil droplets. Even at high temperature, W/O/W emulsion system was well-maintained,

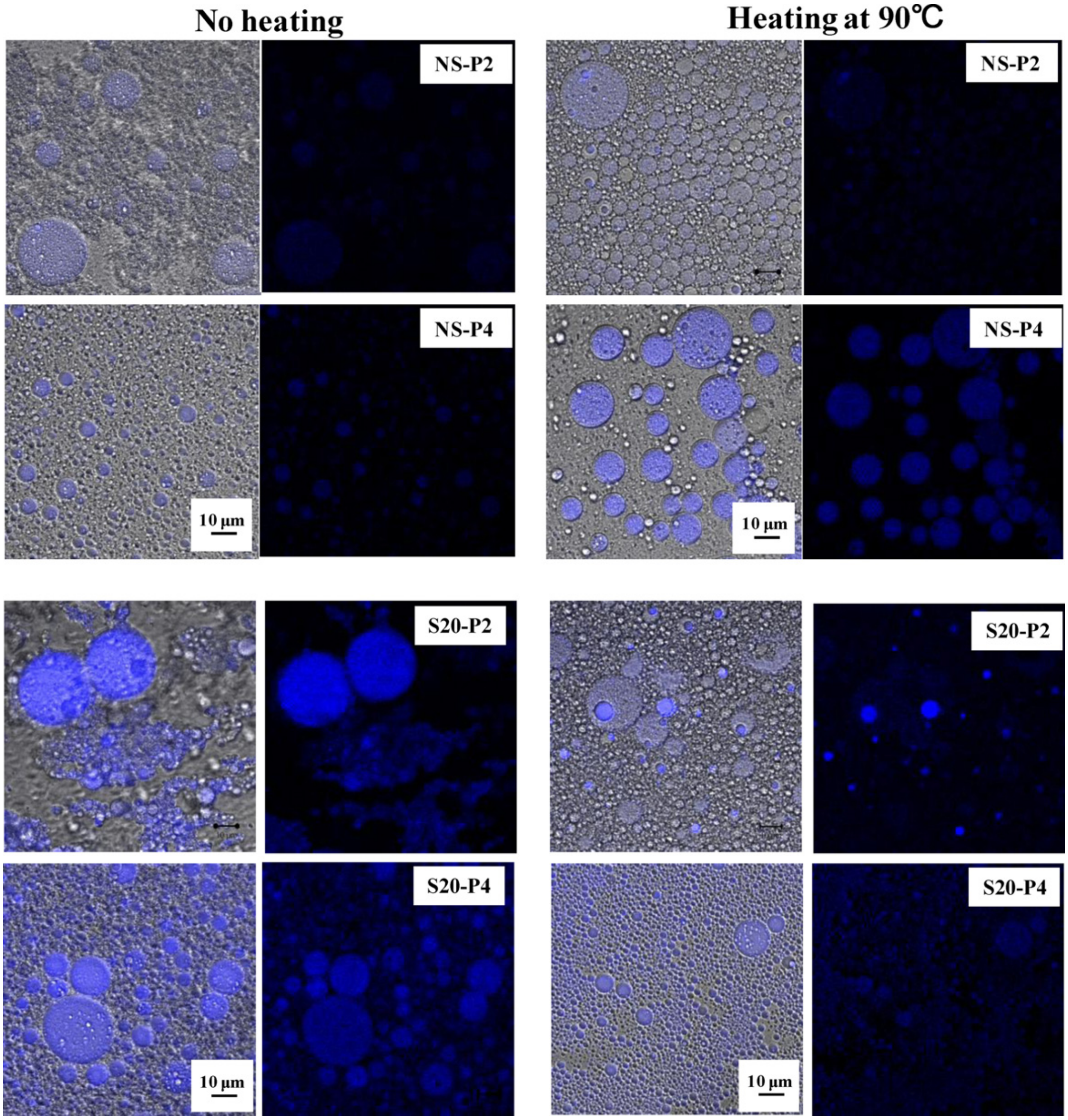

Fig. 5. Confocal micrographs of W/O/W emulsions stored for three months. No heating means stored W/O/W emulsions which were not heated before storing. 'Heating' means W/O/W emulsions heated at 90 °C before being stored. NS: W/O/W emulsion prepared without 4αGTase-treated starch. S20: W/O/W emulsion containing 20 wt% 4αGTase-treated starch in the internal aqueous phase. P2: 2 wt% PGPR used to prepare W/O emulsion. P4: 4 wt% PGPR used to prepare the W/O emulsion.

even though fluorescence intensity slightly decreased as temperature increased. The presence of 4αGTase-treated starch in the internal water phase of W/O/W emulsions prepared with 4 wt% PGPR improved the EE, compared to those of NS emulsions throughout the temperature range tested. As the concentration of starch incorporated into the internal aqueous phase increased, EE values also increased (Fig. 1, bottom). Large nuclei adsorbed on the globule surface as seen in the microstructure of S20 emulsion with 2 wt% PGPR heated at 90 °C (right bottom picture in Fig. 2) were not observed in this case. Several methods have been suggested to improve the stability and EE of W/O/W emulsions, such as stabilizing them by increasing the viscosity of the internal water phase, modifying the nature of the oil phase, stabilizing the internal and/or external phases of emulsion using polymeric emulsifiers, and adding colloidal solid particles to form a stronger and more rigid film at the interface (Benichou et al., 2007; Garti, 1998; Garti & Aserin, 1996; Garti et al., 1999; Hajda & Dickinson, 1996; Hino et al., 2001; Su et al., 2006; Weiss et al., 2005). The strategy used in this study was the incorporation of a gelling polymer in the internal water phase. We hypothesized that if a gel network formed in the internal water phase, the dye could be entrapped more securely to minimize diffusion. The thermoreversible gel forming characteristics of 4αGTase-treated starch made this material suitable for this purpose on the top of another advantage that it showed relatively low liquid viscosity at the stage of emulsion preparation. With sufficient amount of PGPR, gelation of 4αGTase-treated starch solution could be one of the main reasons for the improvement of EE and thermal stability of W/O/W emulsions.

3.1.3. *Stability of W/O/W emulsions during storage*

W/O/W emulsions heated at different temperatures (30, 50, 70, and 90 °C) were stored for three months at room temperature and then EE values were measured. As shown in Fig. 4, W/O/W emulsions prepared with 4 wt% PGPR in the primary W/O emulsions showed increasing EE values with the concentration of 4αGTase-treated starch and did not show a noticeable difference in EE during storage at each heating temperature. Even W/O/W emulsions heated at 90 °C conserved most of the encapsulated dye after 3 months of storage period. As a result, with 20 wt% of 4αGTase-treated starch, the EE could be remained as similar as that of a freshly-made emulsion (no heating, no storage) after heating at 90 °C followed by 3 months of storage. This result indicated that 4 wt% PGPR was sufficient to produce highly stable W/O emulsions (containing 20 wt% aqueous phase) and the improved EE due to the addition of 4αGTase-treated starch could be maintained in this condition. In contrast, a W/O/W emulsion prepared with 2 wt% PGPR showed significantly reduced EE values after storage compared to those before storage. In particular, the extent of EE reduction was greater in W/O/W emulsions containing 10 and 20 wt% 4αGTase-treated starch in the internal aqueous phase. At heating temperatures below 70 °C, even though the EE values of emulsions containing 4αGTase-treated starch were reduced after three months of storage, they retained similar or slightly larger EE values compared to those of emulsions without 4αGTase-treated starch. However, EE values of 4αGTase-treated starch containing emulsions heated at 90 °C were dramatically reduced after storage compared to that without 4αGTase-treated starch. At 2 wt% PGPR level, both thermal stability and storage stability were highly dependent on heating temperature and 4αGTase-treated starch concentration.

Fig. 5 confirmed the above conclusion. In the case of emulsions prepared with 4 wt% PGPR, most of W/O/W droplets showed fluorescent dye retained within the internal aqueous phases after three months of storage regardless of heating and starch addition, although the fluorescence intensity differed depending on samples. At 2 wt% PGPR, fluorescence after 3 months of storage differed dramatically between samples with and without heating at 90 °C, especially for the case of 20 wt% 4αGTase-treated starch addition.

3.2. *Influence of shearing stress*

There are a number of situations where emulsions are subjected to intense mechanical stresses, such as shearing, centrifugation, or homogenization. Under these mechanical stresses, droplet coalescence may be promoted, which may cause some problems for practical applications of these emulsions (McClements, 2004b; Surh, 2009).

The effect of 4αGTase-treated starch incorporated into the internal aqueous phase on the EE of W/O/W emulsions subjected to constant mechanical agitation in a high speed blender was investigated (Fig. 6). There was a gradual decrease in the EE with shearing time in all emulsions regardless of PGPR and 4αGTase-treated starch concentrations. For all the shearing times tested, emulsion samples with higher PGPR and 4αGTase-treated starch levels remained higher EE values. As a result, after 7 min of shearing, the incorporation of 20 wt% 4αGTase-treated starch in the aqueous phase of W/O/W emulsions could maintained the EE value as similar as that of un-sheared sample at 2 wt% PGPR and much higher at 4 wt% PGPR, respectively.

After shearing, all emulsion samples showed creaming more or less, even though they retained >70% of dye. According to McClements (2004b), if a sufficiently large stress is applied parallel to an interface that is covered with emulsifier, then some of the emulsifier molecules may be dragged along the interface, leaving some regions with an excess of emulsifier and other regions depleted of emulsifier. Thus coalescence could then occur if two emulsifier-depleted regions on different droplets come into close proximity during a droplet–droplet encounter. The structure of W/O/W emulsions is more complicated than that of O/W or W/O emulsions, so does the mechanism behind the creaming detected in W/O/W emulsions after shearing. However, this result indicated that emulsion creaming might not be directly connected to its capacity to conserve encapsulant in the internal aqueous phase.

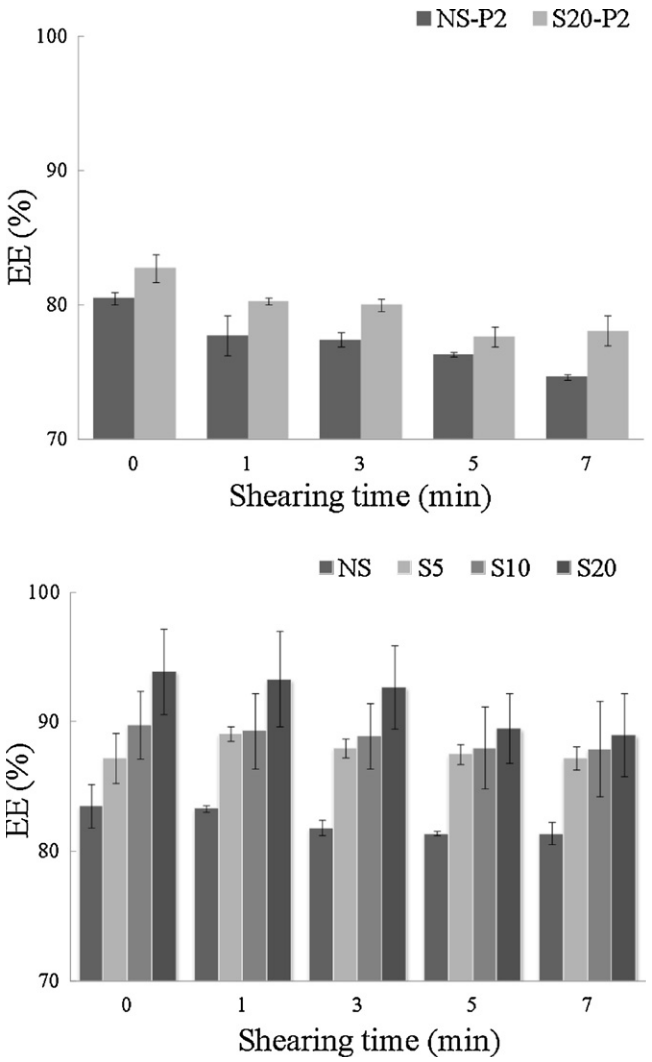

Fig. 6. Influence of shearing on EE W/O/W emulsion prepared with 2 wt% (A) and 4 wt% (B) PGPR and 4αGTase-treated starch.

3.3. *Influence of freeze–thaw cycling*

Many food emulsions are frozen, either to improve their shelf life or as a necessary part of the production process. When frozen emulsions are thawed, they are significantly destabilized and sometimes completely broken down into oily and aqueous phases. Therefore, we also examined the stability of W/O/W emulsions containing 4αGTase-treated starch against the freeze–thaw cycling. Unfortunately, the incorporation 4αGTase-treated starch into the internal aqueous phase could not prevent W/O/W emulsions from creaming and destabilizing. All emulsion samples showed creaming appearance after the first cycle, indicating extensive droplet aggregation (Fig. 7).

A variety of different physicochemical processes may occur during frozen storage that may promote emulsion instability, including fat crystallization, ice formation, freeze-concentration, and interfacial phase transitions (Aronson & Petko, 1993; Ghosh & Coupland, 2008; Ghosh & Rousseau, 2011; Lin et al., 2007). In W/O/W emulsions, two morphological types [oil-in-water (O/W) or water-in-oil (W/O)] occur together within the same system; therefore, various mechanisms governing the destabilization of

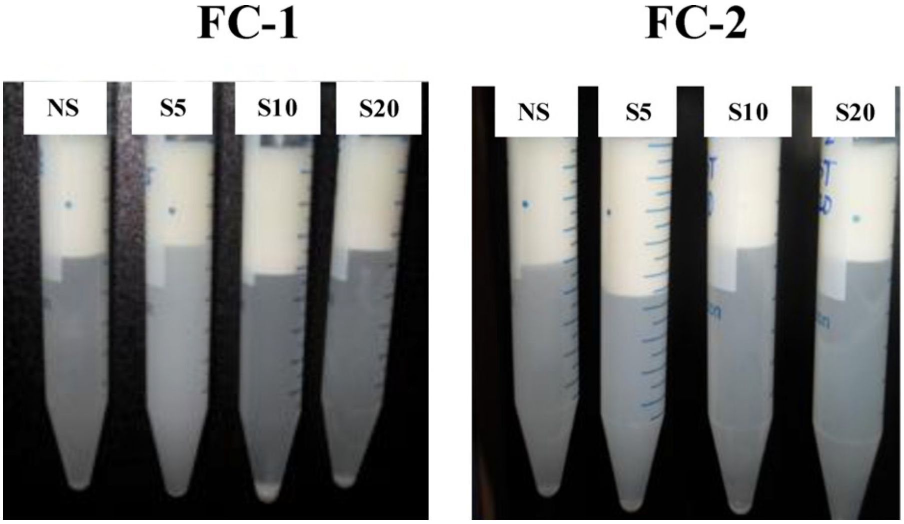

Fig. 7. Creaming appearance of freeze–thaw cycled W/O/W emulsion prepared with 4 wt% PGPR and 4αGTase-treated starch. FC-1: freeze–thaw 1 cycled emulsions. FC-2: freeze–thaw 2-cycled emulsions.

O/W or W/O emulsions by freeze–thaw cycling might also be involved to the destabilization of W/O/W emulsions. The stability and microstructural changes associated with freeze–thaw cycled W/O emulsions remain poorly understood compared to O/W emulsions.

Aronson and Petko (1993) proposed that the inter-droplet oil film separating dispersed water droplets ruptures when ice crystals in adjacent droplets come into contact, leading to coalescence upon thawing. According to previous studies, the choice of surfactant and composition of the continuous oil phase strongly impact the stability of W/O emulsions to freeze-thawing; the sequence of crystallization events (i.e., dispersed after continuous phase or vice versa) is also important for the W/O emulsion stability (Lin et al., 2007). For these reasons, the incorporation 4αGTase-treated starch into the internal aqueous phase may not help prevent destabilization of W/O/W emulsions upon free–thaw cycling.

4. Conclusions

This study investigated the influence of thermal processing, shearing, and freeze-thawing on the EE and stability of a W/O/W emulsion containing 4αGTase-treated starch in the inner aqueous phase. The stability and EE of the W/O/W emulsion after heat processing were closely associated with the PGPR concentration used. With sufficient amount of PGPR (4 wt%), thermoreversible gel forming characteristics of 4αGTase-treated starch made W/O/W emulsion stable to heating processing. However, in the presence of an insufficient PGPR concentration (2 wt%), the result depended on the concentration of 4αGTase-treated starch incorporated in the internal aqueous phase. The addition of large amount of 4αGTase-treated starch (10 and 20 wt%) accelerated dye discharge at high temperature. This phenomenon might be able to be useful in the applications that require temperature-triggered release. The incorporation of 4αGTase-treated starch also improved the resistance of W/O/W emulsions to shearing stress, but did not significantly affect the freeze–thaw stability.

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