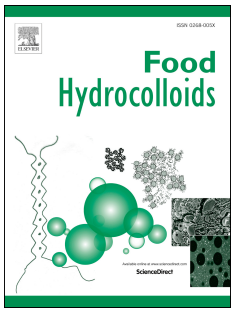

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Fabrication and characterization of highly re-dispersible dry emulsions

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Abstract

Highly re-dispersible dry emulsions were obtained through drying konjac glucomannan (KGM) or monoglyceride (MG) structured O/W emulsions. Emulsion powders showed different morphologies, particle size and surface microstructures, depending on the drying method (spray/freeze-drying), and the emulsion compositions. The introduction of a low level of KGM (0.15wt%) and MG (1wt%) significantly reduced the level of maltodextrin as wall material. All powdered emulsions showed rapid re-hydration in water. Compared with original emulsions before drying, re-constituted emulsions from spray-dried powders showed slightly increased mean droplet size while that from freeze-dried ones showed slightly decreased mean droplet size. KGM significantly decreased the initial viscosity (p<0.05) but increased the creaming stability (p<0.05) of re-constituted emulsions. Measurement of β-carotene content in re-constituted oil droplets fractions indicated that emulsion powders have good re-dispersibility in water (>93% in average). The findings in this study make it possible to obtain emulsion powders and their reconstitutions with desired properties by structuring the original emulsions before drying, and confirmed the possibility of KGM and MG in producing low-cost emulsion powders and the potential of these dry emulsions as novel solid delivery carriers for lipophilic components.

Key words: emulsion, konjac glucomannan, drying, emulsion powder, re-dispersibility

1. Introduction

Emulsions have been widely used for different objectives in the food, nutrition, and pharmacy industries (McClements, 2015). One of their major applications is as encapsulants and delivery carriers for functional ingredients, due to their ease of preparation, maintenance of the physical and chemical stability of encapsulated compounds, potential controlled release and target delivery, and low cost. Emulsion-based carriers can be employed to functionally deliver a variety of lipophilic nutrients, such as carotenoids(Mao et al., 2017; Wei et al., 2018), polyphenols (Lu et al., 2016), vitamins (Parthasarathi, 2016), ω-3 fatty acids (Karthik & Anandharamakrishnan, 2016), and probiotics (Gbassi & Vandamme, 2012). Incorporation of these health-beneficial nutrients into structured emulsions can not only increase their stability and shelf-life, but also can significantly improve their oral bioavailability and thus their health benefits.

However, liquid emulsions are dynamically unstable systems, and their stability decreases with storage time, leading to shortened shelf-life and thus limited application in food industry. In addition, transportation, storage and packaging of liquid emulsions can incur high cost. Hence, strategies must be applied to increase the long-term stability (shelf-life) of liquid emulsions and decrease their transportation/and storage cost at the same time. Several approaches have been developed to improve the long-term stability of liquid emulsions. Among these, microencapsulation technology is always considered to be an ideal way of achieving this (Rosenberg, 1988).

Microencapsulation is a packaging technology by which liquid droplets or solid particles are packed into continuous shells. The shells (or 'walls') are designed to protect the encapsulated material ('core') from factors that may cause its deterioration. In the food industry, the technology has been mainly used for the encapsulation of volatiles and environment-sensitive materials. Spray-drying is one of the mostly used microencapsulation

technique for food preservation, which is also a good way of extending the shelf-life of liquid emulsions through drying them into powders (Vega, 2006; Gharsallaoui et al., 2010).

Spray-drying process can potentially promote the instability of emulsions by altering their interfacial properties (Gharsallaoui et al., 2010). It is therefore important to properly formulate emulsions those are stable to drying, and/or suitable for conversing into powders. In addition, formulation of liquid emulsions can significantly influence their drying process, the properties of obtained emulsion powders (Jafari, 2017), and the properties of re-constituted powdered emulsions in water. In addition, properties of emulsion droplets is closely related to their digestion, release of ingredients from droplets and subsequent absorption of these ingredients in the gastrointestinal tract (GIT) (Lu et al., 2017a, 2017b; Lu et al., 2018; McClements & Li, 2010). Hence, maintaining the uniformity of emulsion droplet structure before and after drying becomes a critical issue in the drying of emulsions. If the powdered emulsions show good re-dispersibility in water and re-constituted emulsions still have intact droplet structure and good stability, such a drying process (including the formulation of liquid emulsions) is always preferred by researchers and manufacturers.

Many strategies have been developed to obtain optimized formulations of liquid emulsions suitable for spray-drying, such as multilayer emulsions (Wei et al., 2018a, 2018b), addition of soluble ingredients as ‘wall’ materials into the water phase of emulsions before drying process, or combined use of both. Commonly used ‘wall’ materials include maltodextrin, gum arabic, dairy proteins, lactose, and cellulose (Aghbashlo et al., 2012; Calvo et al., 2010; Jayasundera et al., 2009). However, a high levels of wall ingredients were always used in drying process, which not only can decrease the content of bioactive ingredients encapsulated in the emulsions but also can significantly increase the cost of the production of powdered emulsions. For example, maltodextrin (MD), mostly used ‘wall’ materials in drying liquid emulsions, was added to the water phase of emulsion in a level of 8%-30% (w/w in liquid

emulsion) with the objective of obtaining stable and highly re-dispersible emulsion powders (Gharsallaoui et al., 2010; Jang et al., 2014). Therefore, new wall materials, which can produce stable emulsion powders at a low level of addition, are required.

Our previous studies showed that konjac glucomannan (KGM) in the water phase of emulsions can form an intermolecular entanglement, which can significantly enhance the stability of whey protein-stabilized emulsion droplets and thus can potentially act as the protective skeleton and 'wall' material in spray-, or freeze-drying of emulsions (Lu et al., 2018). Meanwhile, emulsions containing KGM in the water phase demonstrated sustained release of entrapped nutrients. In addition, emulsion-based carriers with monoglyceride (MG) in the oil phase (Lu et al., 2017b) can significantly improve the bioavailability of encapsulated bioactive nutrients. However, whether these previously-formulated emulsion-based functional delivery systems can be dried into stable powders is still not clear. Meanwhile, little is known about the influence of KGM and MG on the properties of obtained dried emulsions and the properties of their reconstitutions in water.

This study was therefore conducted to prepare dry emulsions with KGM and MG structured liquid emulsions. The effects of KGM and MG on the properties of dry emulsions by both spray-drying and freeze-drying was also studied. β-carotene was incorporated into the oil phase of liquid emulsions as an indicator to provide potential useful information of using structured powdered-emulsion as functional delivery systems for functional lipophilic ingredients.

2. Material and Methods

2.1 Materials

All-*trans*-β-carotene (>93%, UV) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Whey protein isolate (70% β-lactoglobulin and 18% α-lactalbumin) was purchased from Davisco Food International (Le Sueur, MN, USA). Sunflower oil (Solesta, >98% fat)

was purchased from a local supermarket (ALDI, Fermoy, Co. Cork, Ireland). Monoglyceride (glycerol monostearate, Danisco, Denmark) was purchased from Cloverhill Food Ingredients Ltd (Cork, Ireland). Konjac glucomannan (KGM) powder was obtained from Konjac Food (Cupertino, CA, USA). MALTRIN® M180 maltodextrin (DE 16.5-19.5) was obtained from Grain Processing Corporation (Muscatine, IA, USA). All other chemicals and reagents used were of AR-grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Preparation of Emulsions for Freeze Drying

Whey protein isolate (WPI) was dispersed (2%, w/w in final emulsion) in distilled water containing sodium azide as antimicrobial agent (0.01% w/w). The dispersion was stirred for 4 h and kept at 4 °C overnight for complete dissolution of WPI. The oil phase was prepared by dissolving β-carotene (0.05%, w/w in oil phase) or β-carotene (0.05%, w/w in oil phase) and monoglyceride (2%, w/w in final emulsion) in sunflower oil (10%, w/w in final emulsion) at 140 °C, followed by cooling and mixing at room temperature with the water phase (WPI dispersions) at 10,000 rpm for 2 min using an Ultra-Turrax (IKA, Staufen, Germany) followed by homogenization (APV 1000, SPX Flow Technology, Charlotte, North Carolina, USA) at 50 MPa for 3 passes, also at room temperature, to obtain primary emulsions.

The primary emulsions were mixed (1:1, w/w) with 12% or 4% maltodextrin (MD), 0.3% konjac glucomannan (KGM), or maltodextrin-KGM (0.3% KGM with 12% or 4% MD) solutions. The mixtures were then stirred for 1 h at room temperature to obtain final emulsions. Thirty mL of each final emulsion was then dried in a freeze-dryer (FreeZone 6 liters Benchtop Freeze Drying System, Labconco Corporation, Kansas City, MO, USA) at -40°C for 72h.

2.3 Preparation of Emulsions for Spray Drying

The compositions and preparation of emulsions for spray drying was similar to that of emulsion for freeze-drying described above with some modifications. The oil phase containing β-carotene (0.05%, w/w in oil phase) with or without MG was mixed with water phase with emulsifier (WPI, 2%, w/w in final emulsions) at 10,000 rpm for 4 min at room temperature using an Ultra-Turrax (IKA, Staufen, Germany) followed by further two-stage homogenization (TwinPanda 400, GEA Mechanical Equipment Italia, Parma, Italy) for 3 passes at room temperature to obtain primary emulsions. The pressure was 35 MPa for the first stage and 7 MPa for the second stage.

Primary emulsions with different compositions were mixed (1:1, w/w) with maltodextrin (MD) (12% or 4%), or maltodextrin-KGM (0.3% KGM with 12% or 4% MD) solution. The mixtures were then stirred for 1 h at room temperature to obtain final emulsions. About 5 kg of each final emulsion were dried by a pilot-scale ANHYDRO spray dryer with a centrifugal atomizer (Copenhagen, Denmark) at Teagasc Food Research Centre (Moorepark, Fermoy, Co. Cork, Ireland). The inlet temperature was 185 °C and the outlet temperature was 85 °C.

2.4 Preparation of reconstituted emulsions

The obtained freeze-dried emulsion powders were re-constituted with distilled water (25°C) to the same volume (30 mL) as before drying. The obtained spray-dried emulsion powders were reconstituted with distilled water (pre-heated to ~50 °C) to the same total solids content as it was before drying. The re-constituted emulsions were stirred for 30 min before testing their droplet size, surface charge, creaming stability and viscosity.

2.5 Droplet Size and Surface Charge

The droplet size and zeta potential of KGM emulsions were measured by a laser particle analyzer (Nano-ZS, Malvern Instruments, Worcestershire, UK) as described previously (Lu

et al., 2016). Emulsions were diluted to a final oil content (w/w) of 0.01% before testing. The refractive index (RI) of samples was set at 1.47 for sunflower oil.

2.6 Creaming Stability

The creaming stability of emulsions was evaluated using a Lumisizer (LUM GmbH, Berlin, Germany) as described previously (Lu et al., 2016). Emulsions were centrifuged at 2,300 g at 25 °C with a scanning rate of once every 10 s for 1,200 s. Following the test, curves of the integrated level of transmitted light against time were plotted, and the slope of each curve was taken as the light transmission rate or Creaming Index (CI).

2.7 Rheological Analysis

Rheological measurements were performed using an ARG2 rheometer (TA Instruments, Crawley, UK) based on the method of (Lu et al., 2017b). A concentric cylinder geometry was used, and 20 mL of each sample was placed into the inner cylinder and equilibrated for 2 min before measurement. Viscosity testing was performed over a shear rate range of 0-300 s^{-1} at 25 °C.

2.8 Laser Scanning Confocal Microscope Observation

A Leica TCS SP5 confocal laser scanning microscope (CLSM; Leica Microsystems CMS GmbH, Wetzlar, Germany) was used for powder particles visualization. Spray-dried and freeze-dried powder particles were placed onto a glass slide and labeled using a mixture of Fast Green and Nile Red. The dye mixture containing Fast Green (0.1 g/L) and Nile Red (0.1 g/L) were dissolved in polyethylene glycol in a ratio 1:40 of Fast Green to Nile Red, which allowed diffusion of the dye molecules into the particles whilst not influencing the particle morphology and preventing solubilization. Dual excitation at 488 nm/633 nm was used. Confocal images of each systems were taken using 63x oil immersion objective with numerical aperture 1.4 z-Stacks were obtained in order to generate a three-dimensional

structure of the particle and to identify surface lipid staining. Red and Green pseudo-colored pictures (8-bit), 512x512 pixels in size, were acquired using a zoom factor of 1-3.

2.9 Scanning Electronic Microscopy

Spray-dried and freeze-dried emulsion powders were attached to double-sided adhesive carbon tabs mounted on scanning electron microscope stubs, and then coated with chromium (K550X, Emitech, Ashford, UK). Scanning electron microscopy images were collected using a Zeiss Supra 40P field emission SEM (Carl Zeiss SMT Ltd., Cambridge, UK) at 2.00 kV. Representative micrographs were taken at 200×, 500×, 1000×, 5000× and 10000× magnification.

2.10 Quantification of β-carotene

β-carotene was extracted from re-constituted emulsions with ethanol/n-hexane (sample: ethanol: n-hexane=1:2:10, v/v). The n-hexane extracts were combined and dried under a stream of nitrogen gas, and dissolved in ethanol for HPLC analysis.

Reversed-phase high performance liquid chromatography (RP-HPLC) was used to quantify β-carotene as described previously (Lu et al., 2016). Briefly, an Agilent 1200 series system with a DAD UV-Vis detector (Agilent, Santa Clara, CA, USA) and a reversed-phase TSKgel ODS-100v C_{18} column (4.6×250 mm, 5 μm, TOSOH) was employed. Chromatography conditions were as follows: column operation temperature at 30 °C; elution performed with 90% ethanol and 10% acetonitrile from 0-30 min; flow rate of 1 mL/min; detection wavelength of 450 nm, and injection volume of 20 μL.

2.11 Re-dispersibility of powdered emulsions

The dried emulsion powders were re-constituted with distilled water to the same total solids content as it was before drying, and stirred for 2 hours at room temperature before testing. Re-dispersibility of dry emulsions was calculated based on the following equation:

$$\text{Re-dispersibility (\%)} = \frac{\textit{β-carotene content in reconstituted emulsions}}{\textit{β-carotene content in emulsions before drying}} \times 100$$

2.12 Statistical analysis

All experiments were repeated at least three times. One-way analysis of variance (ANOVA) was employed to compare means of data. A t-Test was used to determine the differences between means. Significant differences were determined at the 0.05 level ($p<0.05$).

3. Results and Discussion

3.1 Particle Morphology

Scanning electronic microscopy (SEM) observation

Five liquid emulsions were formulated to investigate the possibility of obtaining spray-dried emulsion powders by using low level of 'wall' materials. As is shown in **Fig.1**, all liquid emulsions were successfully spray-dried into dry powders, which usually showed approximately spherical particles with a concavo-convex surface (**Fig. 1f-j**). The liquid oil droplets with irregular shapes (**Fig. 1h, red arrow**) were embedded within the 'wall' materials (MD or KGM) and located on the surface of the powders. Emulsions containing 2wt% MD and 0.15wt% KGM were spray-dried into dry powders with similar morphology properties to that containing high level of MD (6 wt%), indicating that the addition of KGM can significantly reduce the level of MD (from 6 wt% to 2 wt%), which is frequently used as a protective wall material for emulsion oil droplets during drying (Anwar & Kunz, 2011; Balasubramani et al., 2015; Gharsallaoui et al., 2007). The results indicate the potential of KGM as an ideal protective 'wall' material in spray-drying of liquid emulsions. In addition, KGM has been reported to have many potential health benefits (Chua et al., 2010) and thus the utilization of KGM can also add nutritional value to the obtained dried emulsions.

In addition, an empty-inside structure of these spray-dried powders was observed (**Fig. 2b**). During drying, solutes carrying water migrate to the surface of the atomized liquid droplets,

leading to an empty inside of the droplets (Gaiqing, 2006). In addition, solutes can rapidly deposit at the surface of the atomized droplets and form a shell (**Fig. 2b, red arrows**), which can prevent the mass transfer of the droplets, although the heat transfer is not affected. Thus, the temperature of the liquids inside the droplets keeps increasing. When the internal gas pressure is higher than the mechanical strength of the shell layer, gaseous molecules inside the droplets can break through the shell layer, forming cavities on the shell of particles (**Fig. 2b, blue arrows**) or creating empty-inside particles.

Freeze-dried emulsion powders had significantly different morphologies compared with spray-dried ones. Freeze-dried powders showed flake-like shapes with smooth or folded surfaces depending on different formulas (**Fig. 3**). Freeze-dried powder containing 6wt% MD had a very smooth surface, while that containing 0.15 wt% KGM showed a coarse surface; powders containing both MD and KGM showed a transition from a smooth surface to coarse one with decreasing MD content. In addition, oiling-out was not observed in freeze-dried powders, suggesting that the introduction of KGM (only 0.15 wt%) can significantly reduce the level of MD from 6 wt% to 0 wt%) without affecting the preparation of freeze-dried emulsion powders. The results accordingly confirm the potential of KGM as a functional agent in maintaining the structure of oil droplets in a freeze-drying process, potentially by delaying the growing of ice crystals and/or reinforcing the interfacial layer (Lu et al., 2018).

Particle size

Spray-dried powders showed a wide particle size distribution under SEM, mainly falling in the range of 3~35 μm (**Fig.1**). Similar results were also obtained by laser diffraction (Mastersizer 3000) (**Fig. 2a**). The addition of KGM or MG and reducing MD level all led to increased mean particle size. The powders containing MG showed very large particles (**Fig. 2a, red arrow**), which may be attributed to their larger droplets formed by atomizing due to a high density of droplets induced by crystallization of MG.

The particle size of freeze-dried powders was not tested with Mastersizer 3000 due to their irregular shape. Therefore, the comparison of particle size between freeze-dried and spray-dried powders was mainly based on the SEM images. As was observed in many previous studies, freeze-dried powders showed larger particle size than spray-dried ones (**Fig. 1& Fig. 3**), and it was hard to conclude the difference in particle size of different formulas.

Composition distribution

Confocal laser scanning microscope (CLSM) was employed to further investigate the distribution of oil droplets in powders. As described above, spray-dried powders showed irregular spherical particles with porous surface and hollow structures (**Fig. 4a-e**). The 'wall' materials (MD and/or KGM) formed the shell layer of these particles, and oil droplets were (green color) distributed among the shell layers.

It was clearly observed by CLSM that freeze-dried powders immediately re-dispersed into O/W emulsion with regular spherical emulsified oil droplets after mixing with dye solution containing water (**Fig. 4f-i**), indicating that the freeze-dried powders have an excellent water solubility. Freeze-dried powders are always porous, and can be easily and completely re-hydrated, forming stable dispersions (Ratti, 2009), which explains why the freeze-dried powders immediately re-disperse into liquid emulsions after mixing with aqueous solutions.

Generally, morphology of dried emulsion powders (shape, size, or composition distribution) is influenced by several parameters, such as the drying temperature, solids content in the feed, the drying rate (Walton & Mumford, 1999), compositions, or viscoelastic properties of the adsorbed surface film of the drying drop (Nuzzo et al., 2014). In this study, the spray-drying and freeze-dry parameters were similar for all samples treated with each process. Hence, the differences in the particle morphology (microstructure and droplet size) are mainly attributed to the MD content (6% or 2%), the compositions of liquid emulsions (KGM or MG), and the viscosity of liquid emulsions (KGM). The decrease in MD content and the introduction of

MG both resulted in an increased particle size of spray-dried powders. MD at a low concentration cannot effectively separate neighboring oil droplets, lead to their increased chance of colliding and aggregating in drying process and thus increased mean particle size of final powders. In addition, KGM led to a minor increase in the particle size (**Fig.2a**), but significantly reduced the level of MD and thus the cost of obtaining emulsion powders. This has an enormous market prospect based on the fact that food emulsions are increasingly used as functional delivery carriers in food, nutrition, and biomedical industries. KGM is therefore considered as an ideal protective agent which can be widely used in the drying process of functional food emulsion systems without any toxic and side-effects.

3.2 Properties of Re-constituted Emulsions

One of the most important properties of dried emulsion powders is their ability to re-disperse into liquid emulsions with original properties. Thus, all dry emulsion powders were re-dispersed into water and properties of the re-constituted emulsions were tested, including droplet size, surface charge, viscosity, creaming stability, and re-dispersibility.

Droplet size

Spray-drying process always cause a significantly increase in the droplet size of emulsions (Gharsallaoui et al., 2010; Klinkesorn et al., 2006; Serfert et al., 2013), which accordingly can influence their stability and functionality. Thus, the droplet size of emulsion before and after spray-drying process was first tested. Compared with previous studies (Drapala et al., 2017; Gharsallaoui et al., 2010; Klinkesorn et al., 2006; Serfert et al., 2013), the increase in the droplet size of re-constituted emulsions was significantly improved in this study. The average droplet size of re-constituted spray-dried emulsions varied from 260 nm to 310 nm (**Table 1**), which were slightly larger than their original emulsions before drying. However, such differences in droplet size may be not enough to significantly affect their stability and

relevant functionality, e.g., digestion and bioavailability of bioactive nutrients in emulsion-based delivery carriers (Lu et al., 2017a). In addition, size distribution of reconstituted emulsions showed a shift towards larger particle sizes (data not shown).

In terms of re-constituted emulsions from freeze-dried powders, these showed slightly decreased average droplet size (**Table 2**) and their size distributions were also shifted towards smaller particle sizes (data not shown), as compared to original emulsions. A decrease in the average droplet size after freezing process was also observed in our previous study (Lu et al., 2018), and was mainly attributed to the break-down of some large oil droplets during freezing due to the formation of ice crystals. The ice crystals can break down the interfacial emulsifier layers surrounding the droplets and lead to oiling-off of emulsions (Mao et al., 2015). In addition, significant droplet aggregation of re-constituted emulsion containing MG was observed (data not shown), which accordingly led to its dramatically increased average droplet size (**Table 1**).

Surface Charge

All original emulsions and reconstituted emulsions were negatively charged due to negatively charged whey proteins used as emulsifiers. Reconstitution of spray-dried powder containing 6 wt% MD and 0.15 wt% KGM showed a significantly increased surface charge as compared to the original emulsion ($p<0.05$) (**Table 1**). Similar result was observed for reconstitutions of freeze-dried powders containing MG ($p<0.05$) (**Table 2**). The pH value of the re-constituted emulsions and original emulsions were almost the same (around 6.8), and thus the difference in surface charge is not induced by pH. It is probably due to the absorption of negatively-charged free whey protein molecules onto the surface of oil droplets during drying process. The increased surface charge of reconstitutions actually can be considered as a positive change, because increased surface charge of oil droplets can potentially improve the emulsion stability by increasing electrostatic repulsion between droplets (McClements,

344 2015). Surface charge of other emulsions did not show significant differences before and
345 after drying process.

346 *Viscosity*

347 Viscosity of all emulsions and re-constituted emulsions showed shear-thinning behavior
348 (data not shown), as previously reported (Lu et al., 2017a, 2017b; Lu et al., 2018). Original
349 emulsions containing KGM showed the highest initial viscosities, followed by emulsions
350 containing MG and MD, respectively (**Fig. 5**). The results indicate that KGM and MG can
351 significantly influence the viscosity of emulsions, but MD had little impact on the emulsion
352 viscosity. KGM or MG induced increase in the viscosity of emulsions could be also seen in
353 our previous studies (Lu et al., 2017; Lu et al., 2018; Mao et al., 2014; Mao et al., 2012).

354 Compared with original emulsions before drying, reconstituted emulsions containing both
355 KGM and MD showed significantly decreased viscosity (p<0.05), while no significant
356 differences were observed for others. This is probably attributed to two reasons: (i) de-
357 polymerization and/or ordered arrangement of KGM molecules during the freezing process,
358 which led to a weak intermolecular interaction and/or chain entanglement and thus a low
359 viscosity (Villay et al., 2012); and (ii) absorption of KGM molecules to the surface of
360 droplets in the spray-drying process, which accordingly reduced the content of free KGM in
361 the water phase and thus led to decreased emulsion viscosity.

362 *Creaming Stability*

363 For the creaming stability test, curves of the integrated light transmission against time were
364 plotted, and the slope of each curve was taken as the light transmission rate (%/second) or
365 creaming index (CI). A higher value of this parameter indicates a lower creaming stability of
366 emulsions.

367 Original emulsions containing KGM showed more rapid creaming than those without
368 KGM (**Fig. 6 a,b**), which was also observed in our previous study (Lu et al., 2018). This is

mainly attributed to depletion flocculation of emulsion droplets by non-absorbed KGM, which can generate an attractive osmotic force between droplets (Dickinson, 2019). This osmotic force increases with increasing concentration of KGM until it is large enough to overcome the repulsive forces between droplets and cause their flocculation. In addition, the existence of high concentration of MD can even result in a faster creaming of original emulsion containing KGM (p<0.05). In contrast, the introduction of MG can significantly decrease the creaming velocity, which is mainly attributed to the MG-induced crystallization of the oil phase (Mao et al., 2014).

Compared with original emulsions before drying, reconstituted emulsions containing KGM (from both spray-dried and freeze-dried powders) showed better creaming stability (p<0.05) (**Fig. 6a, b**), while others showed a slightly decreased creaming stability. The viscosity of these re-constituted emulsions all significantly decreased as compared with the original emulsions. Therefore, based on Stokes' law, the increased creaming stability (**Fig. 6a, b**) may be mainly attributed to: (i) increased particle density (ρ) of the droplets by the potential absorption of KGM to the oil droplets surface during spray-drying, and reduced depletion flocculation effect due to a lower content of KGM in the water phase; (ii) smaller droplet size (r^2) of re-constituted emulsions from freeze dried powders (**Table 2**), and a reduced depletion flocculation effect due to the de-polymerization and/or ordered arrangement of KGM molecules during the freezing process.

In addition, creaming velocity of re-constituted emulsion containing MG dramatically increased nearly 20-fold after freeze-drying, and a significant creaming layer in the top was observed. This is mainly caused by the aggregation of droplets after re-dispersing into water, as described above **(Table 2).**

Re-dispersibility

Re-dispersibility is one of the most important factors that will be considered to evaluate the quality of powdered emulsions, and a good re-dispersibility of powdered emulsion is also crucial to their application in food industry. Thus, the re-dispersibility of powdered emulsions was analysed in this study.

All spray-dried powders showed rapid re-dispersing in water at room temperature, and freeze-dried powders showed an even faster re-dispersing than spray-dried ones. Freeze-dried powders were found to re-constitute into O/W emulsions immediately after contacting water, with clearly visible spherical intact oil droplets (**Fig. 4i-f)**. For powders with similar formulas, re-dispersibility is mainly determined by microstructure and particle size of powders (Selomulya., 2013). Compared with a spherical particle shape of spray-dried powders, irregular flake-like shape and porous structure were apparently better at facilitating the wetting and reconstituting of freeze-dried powders in water. In addition, a significant larger particle size of freeze-dried powders (**Fig. 1)** than spray-dried powders can be seen (**Fig. 3**). Generally, food powders with larger particle size can be more easily rehydrated than those with smaller particles (Selomulya., 2013). All these factors can potentially explain why freeze-dried powders showed faster re-dispersing.

The re-dispersibility of powdered emulsion was quantitively analyzed by testing the content of β-carotene in the re-constituted oil droplets fraction. As shown in **Fig. 7a**, spray-dried powders all showed good re-dispersibility (>90%). The introduction of KGM (0.15wt%) or MG (1wt%) can significantly reduce the level of MD (from 6wt% to 2wt%), confirming the possibility of obtaining powdered forms of KGM or MG structured liquid emulsions and the potential of KGM as protective ‘wall’ materials in spray-dry of emulsions as shown in **Fig.1**.

Freeze-dried powders also showed good re-dispersibility (>85%), except for the powder containing MG, which only showed a tested re-dispersibility of 44% (**Fig. 7b**). Huge aggregates which cannot be re-dispersed can be seen on the top of the emulsions. Similarly, the addition of KGM also significantly reduced the level of MD (from 6 wt% to 0 wt%) required in freeze-drying of emulsions. Powder containing 0.15wt% KGM showed the highest re-dispersibility of 96%, but did not significantly differ from other samples without MG. Freezing of emulsions can lead to the formation of ice crystals, and the ice penetration can potentially induce the break-down of the interfacial emulsifier layers surrounding the oil droplets, resulting in oiling-off of emulsions. The incorporation of some food biopolymers, e.g., maltodextrin or KGM, can significantly enhance the freeze-thaw stability and reduce the oiling-off of emulsions during freezing (Lu et al., 2018; Mao et al., 2015). The results further confirm the potential of KGM as an ideal protective agent in the freeze-drying of O/W emulsions.

Obtaining stable and highly-dispersible powdered emulsions is a topic of significant interest in the field of food emulsions. A 'wall' material is always required in the drying process of emulsions, and the main purpose of using 'wall' materials is to coat oil droplets and protect them from aggregating during drying process. However, utilization of high levels of wall materials (>50wt% in powders) can lead to a high cost and decreased content of functional components encapsulated in emulsions. Thus, it is valuable to develop optimized emulsion formulas with significantly reduced levels of 'wall' materials. The main findings in this study demonstrated that utilization of very low level of KGM (<1wt% in the final powders) can obtain stable spray-, and freeze-dried emulsion powders with high re-dispersibility. Reduced levels of wall material accordingly results in lower cost of the powders. Therefore, edible and health-beneficial KGM has clear potential in acting as a wall material of liquid dispersions being subjected to drying process in the food industry.

4. Conclusions

Emulsion powders were obtained through spray-, or freeze-drying of KGM or MG structured O/W emulsions. The introduction of KGM and MG significantly reduced the level of wall material (MD). All emulsion powders showed rapid re-hydration in water. Spray-drying process increased the mean droplet size of KGM, and MD structured emulsions, while the opposite result was observed for freeze-drying process. KGM significantly decreased the initial viscosity (p<0.05) but increased the creaming stability of reconstituted emulsions. (p<0.05). The results of β-carotene content in re-constituted oil droplets fractions indicated that obtained emulsion powders have good re-dispersibility in water.

The findings in this study confirmed the possibility of using low level of KGM or MG-structured emulsions to prepare spray-dried or freeze-dried powders containing bioactive nutrients (β-carotene as an example). The results overall contribute to a better understanding of the relationship between liquid emulsion structure and the properties of their dried powders, making it possible to obtain emulsions powders and well-reconstituted emulsions with desired properties by structuring the liquid emulsions with proper biopolymers. The results also confirmed the great potential of KGM and MG in industrial production of low-cost emulsion powders.

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Notes

The authors declare no conflict of interest.

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Figure captions

Figure 1. Scanning electron microscopy images of spray-dried emulsions containing (a, f) 6% MD; (b, g) 6% MD and 0.15% KGM; (c, h) 2% MD and 0.15% KGM; (d, i) 6% MD and 1% MG; (e, j) 2% MD and 1% MG. MD indicates maltodextrin; KGM indicates konjac glucomannan; MG indicates monoglyceride. a-e:1000×, f-i:5000×

Figure 2. (a) Particle size distribution of spray-dried emulsion powders. MD indicates maltodextrin; KGM indicates konjac glucomannan; MG indicates monoglyceride. Insert (table): mean particle size (D[*3,2*]) of spray-dried powders. (b) SEM images of hollow structures in spray-dried powders

Figure 3. Scanning electron microscopy images of freeze-dried emulsions containing (a, f) 6% MD; (b, g) 6% MD and 0.15% KGM; (c, h) 2% MD and 0.15% KGM; (d, i) 0.15% KGM; (e, j) 6% MD and 1% MG. MD indicates maltodextrin; KGM indicates konjac glucomannan; MG indicates monoglyceride.

Figure 4. Confocal laser scanning microscopy images of spray-dried (a-e) and freeze-dried (f-i) emulsions. **Spray dried emulsions:** (a) 6% MD, (b) 6% MD and 0.15% KGM, (c) 2% MD and 0.15% KGM, (d) 6% MD and 1% MG, (e) 2% MD and 1% MG; **Freeze-dried emulsions:** (f) 6% MD, (g) 6% MD and 0.15% KGM, (h) 2% MD and 0.15% KGM, (i) 0.15% KGM. MD indicates maltodextrin; KGM indicates konjac glucomannan; MG indicates monoglyceride. Mixed dyes of Nile red and fast green were used to dye fat and protein, respectively, i.e., Green color indicates fat and red color indicates proteins.

Figure 5. Initial viscosity of reconstituted (a) spray-dried emulsions and (b) freeze-dried emulsions. KGM indicates konjac glucomannan; MD indicates maltodextrin; MG indicates monoglyceride (* indicates a difference at $p<0.05$).

Figure 6. Creaming stability of reconstituted (a) spray-dried emulsions and (b) freeze-dried emulsions. KGM indicates konjac glucomannan; MD indicates maltodextrin; MG indicates monoglyceride.

Figure 7. Re-dispersibility of (a) spray-dried and (b) freeze-dried emulsions.; KGM indicates konjac glucomannan; MD indicates maltodextrin; MG indicates monoglyceride; KGM indicates konjac glucomannan (* indicates a difference at $p<0.05$).

Table 1 Mean droplet size, zeta potential (ZP), and polydispersity index (PdI) of liquid emulsions and their re-constituted spray-dried emulsions (n=3)

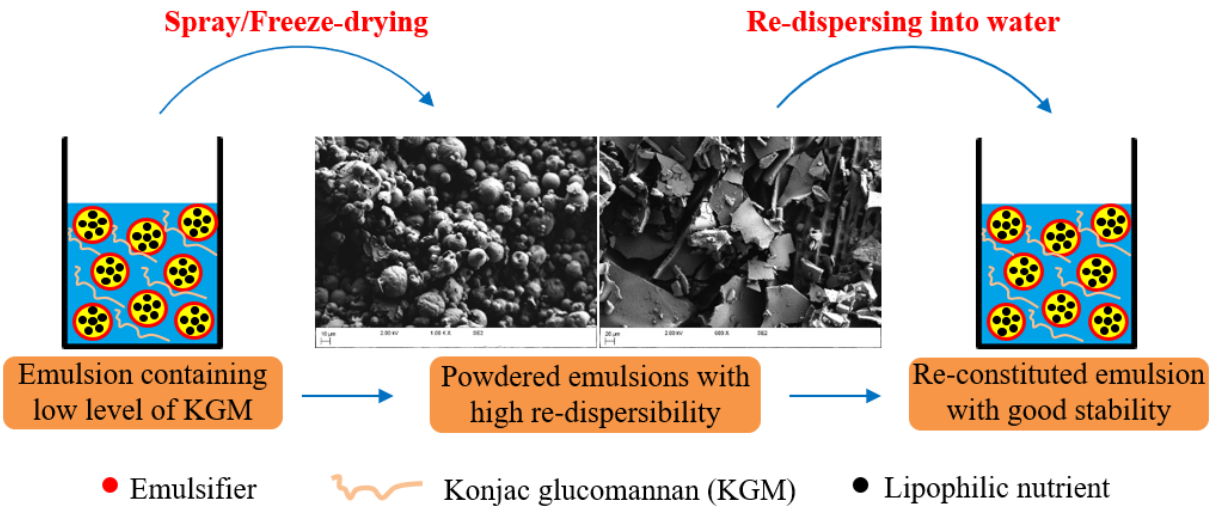
	Before spray-drying			After re-constitution		
Emulsions	Size (d.nm)	ZP (mV)	PdI	Size (d.nm)	ZP (mV)	PdI
6%MD	250±7a	-27.9±0.6^{a}	0.194±0.044^{a}	265±9^{a}	-33.2±0.3^{b}	0.283±0.016^{b}
6%MD +0.15%KGM	278±7a	-27.6±0.6^{a}	0.191±0.044^{a}	311±20^{a}	-34.9±0.7^{b}	0.377±0.007^{b}
2%MD +0.15%KGM	239±9a	-29.1±0.6^{a}	0.220±0.014^{a}	284±1^{b}	-35.5±1.2^{b}	0.290±0.050^{a}
6%MD +1%MG	233±5a	-34.7±0.7^{a}	0.312±0.005^{a}	261±10^{b}	-36.4±1.2^{a}	0.306±0.031^{a}
2%MD +1%MG	184±14a	-31.3±0.8^{a}	0.314±0.011^{a}	265±7^{b}	-35.1±1.4^{b}	0.314±0.011^{a}

* MD indicates maltodextrin; KGM indicates konjac glucomannan; MG indicates monoglyceride. ^{a}Different letters indicate significant difference between values of before spray-drying and after re-constitution (p<0.05)

641 **Table 2** Mean droplet size, zeta potential (ZP), and polydispersity index (PdI) of liquid emulsions and re-constituted freeze-dried emulsions (n=3)

	Before freeze-drying			After re-constitution		
Emulsions	Size (d.nm)	ZP (mV)	PdI	Size (d.nm)	ZP (mV)	PdI
6%MD	220±5[a]	-33.1±0.3[a]	0.223±0.020[a]	212±3[a]	-32.2±0.5[a]	0.260±0.008[b]
0.15%KGM	219±6[a]	-33.5±0.3[a]	0.235±0.016[a]	208±19[a]	-33.5±0.2[a]	0.261±0.023[a]
6%MD+0.15%KGM	217±4[a]	-32.0±0.6[a]	0.238±0.019[a]	210±5[a]	-32.9±0.6[a]	0.258±0.022[a]
2%MD+0.15%KGM	219±3[a]	-33.6±0.7[a]	0.237±0.014[a]	207±6[b]	-35.3±0.7[b]	0.274±0.023[a]
6%MD+1%MG	228±3[a]	-29.8±0.4[a]	0.341±0.006[a]	3229±911[b]	-37.8±2.8[b]	0.874±0.191[b]

642 * MD indicates maltodextrin; KGM indicates konjac glucomannan; MG indicates monoglyceride. [a]Different letters indicate significant difference between
643 values of before freeze-drying and after re-constitution (p<0.05)
644
645
646



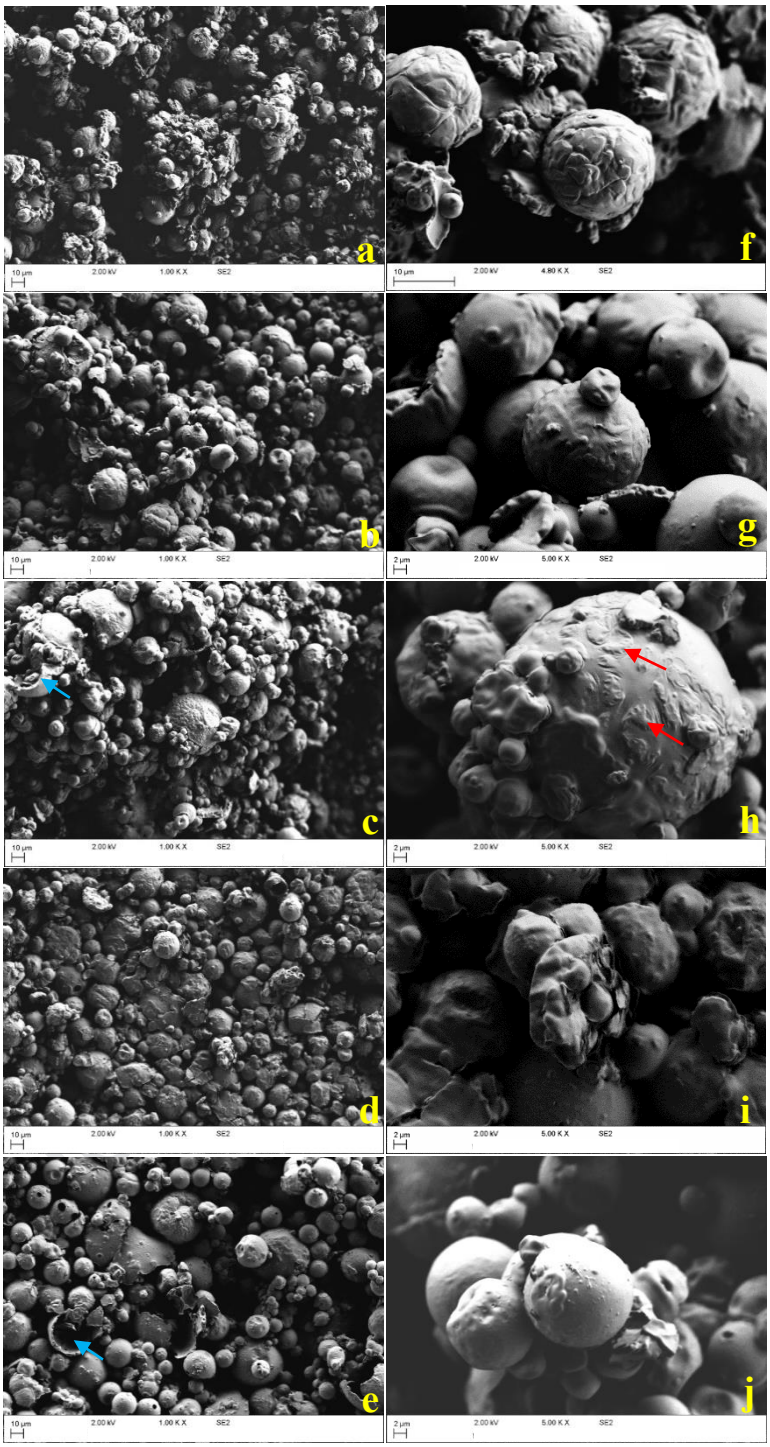
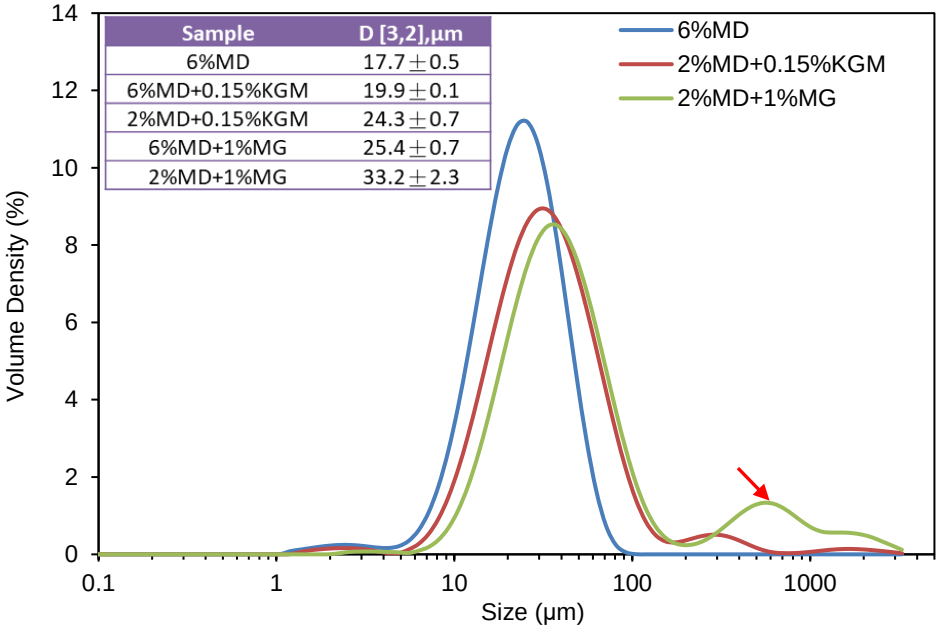
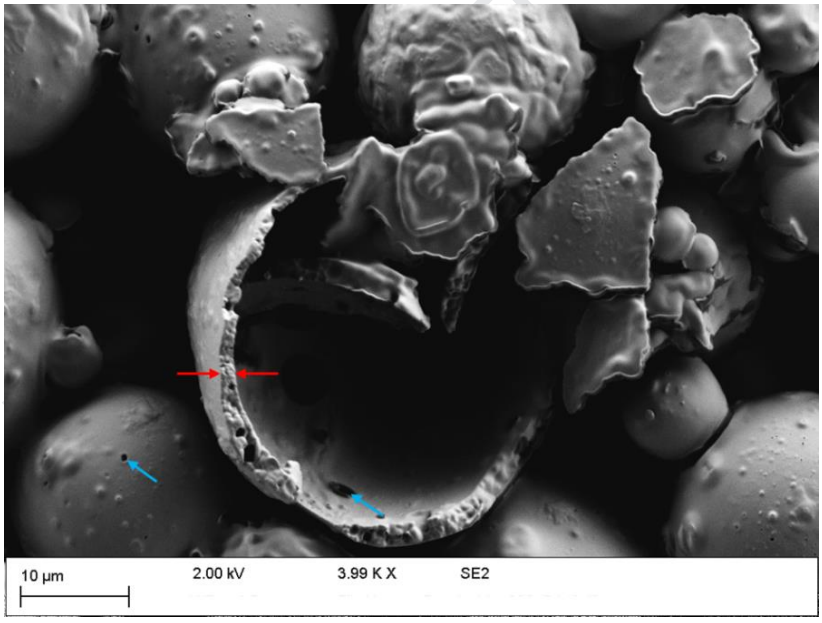


Fig.1


Sample
D [3,2],μm
6%MD
17.7±0.5
6%MD+0.15%KGM
19.9±0.1
2%MD+0.15%KGM
24.3±0.7
6%MD+1%MG
25.4±0.7
2%MD+1%MG
33.2±2.3
6%MD
2%MD+0.15%KGM
2%MD+1%MG
Volume Density (%)
Size (μm)
0
2
4
6
8
10
12
14
0.1
1
10
100
1000

(a)


10 μm
2.00 kV
3.99 K X
SE2

(b)

Fig.2

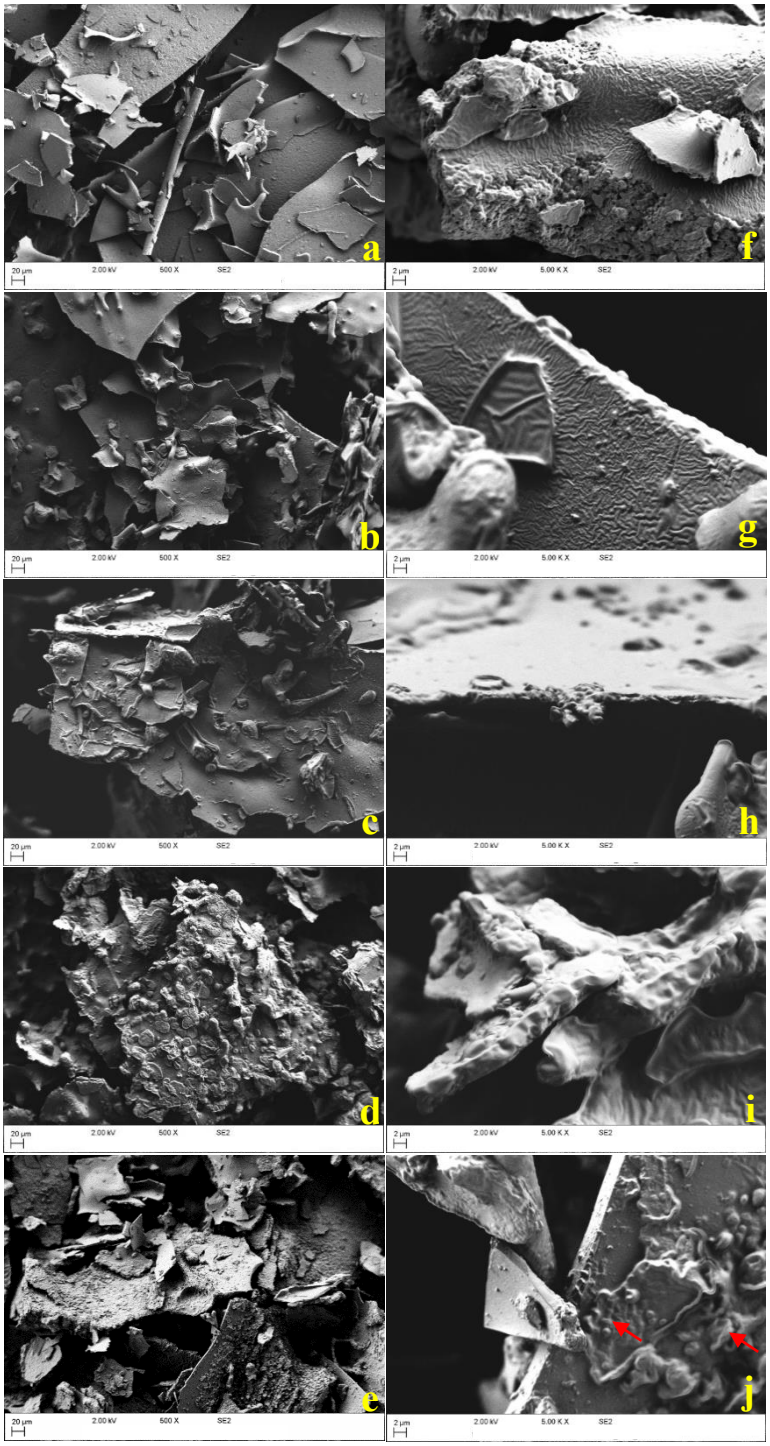


Fig.3

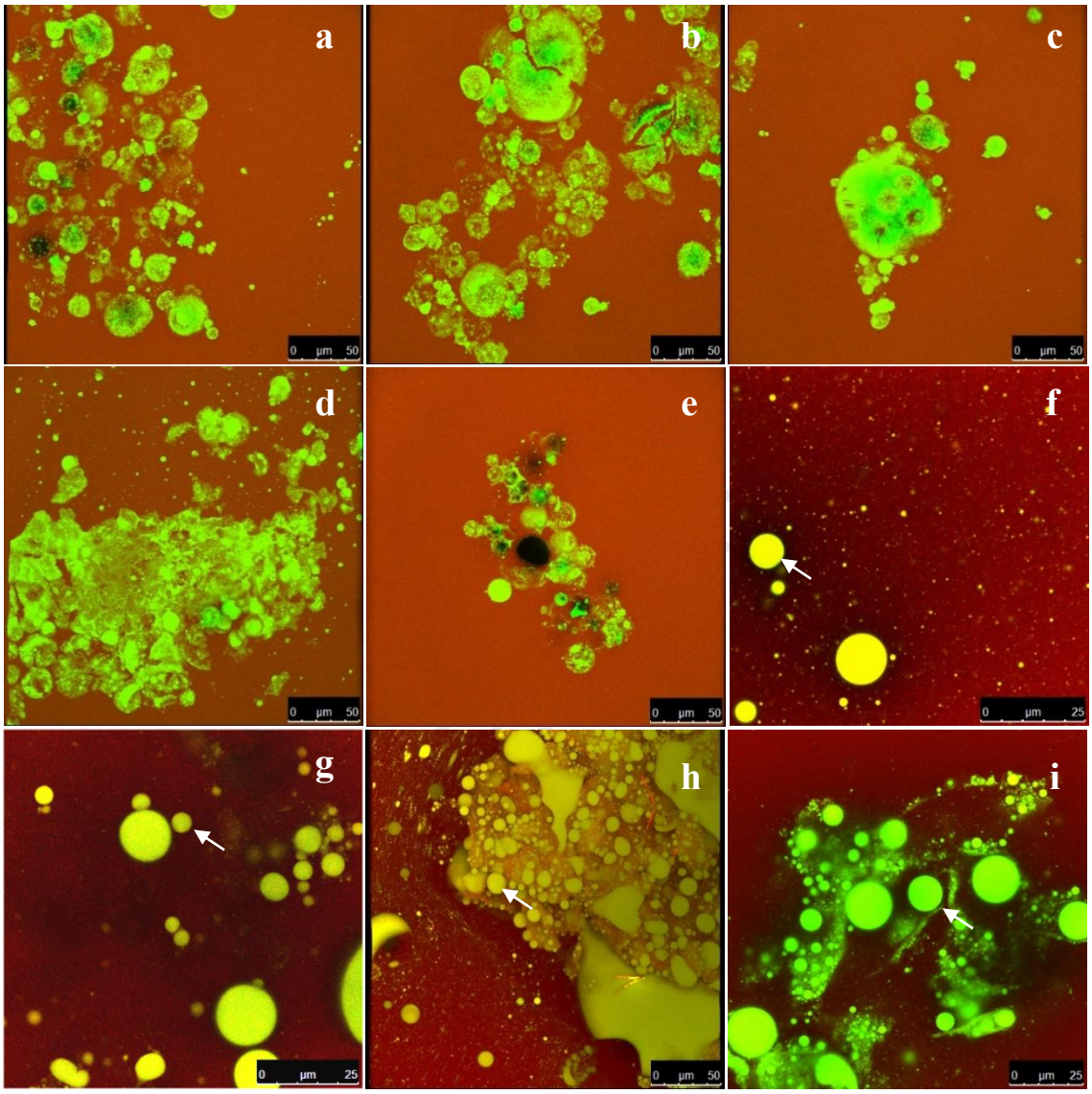


Fig. 4

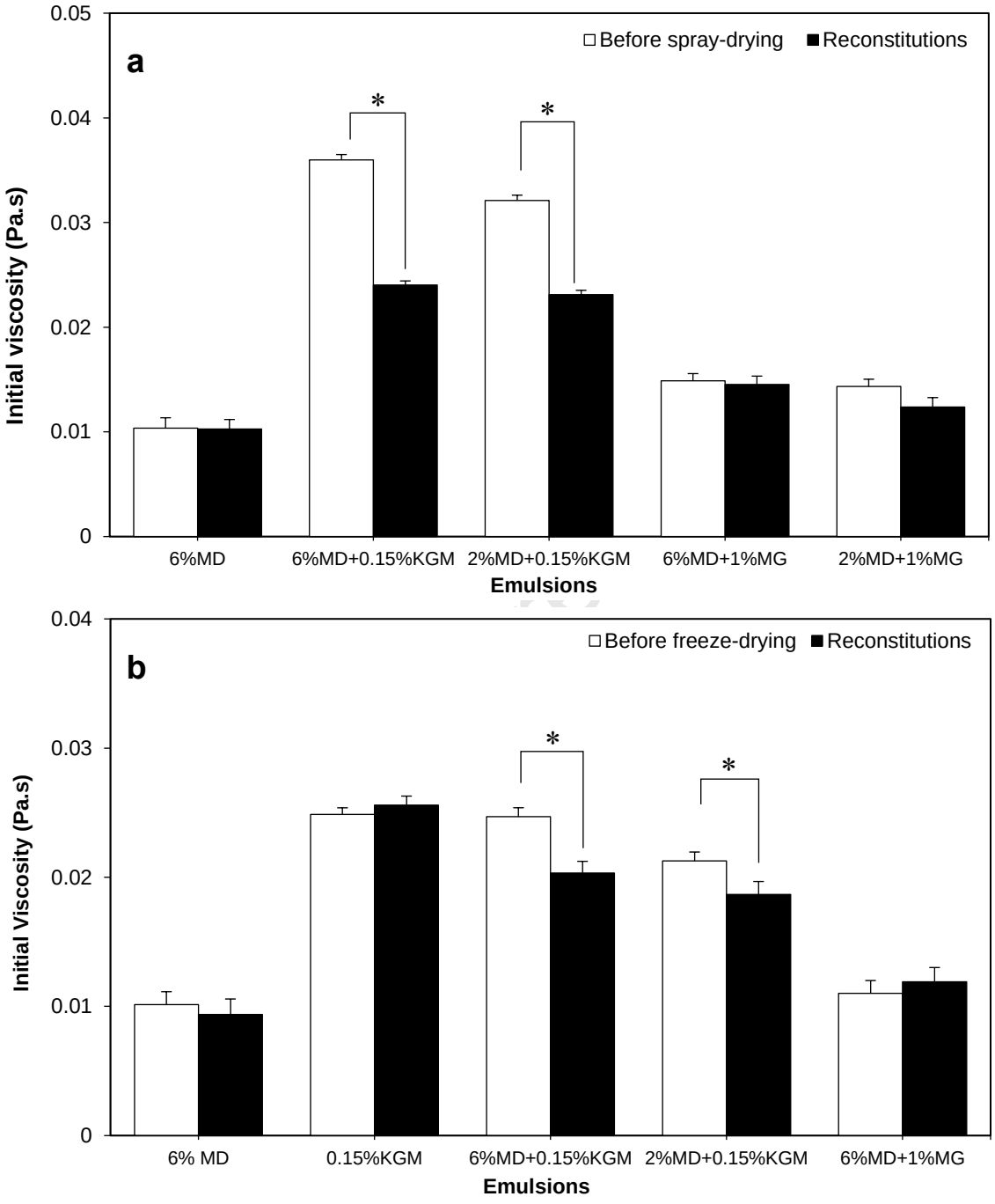

a
□Before spray-drying ■Reconstitutions
Initial viscosity (Pa.s)
0.05
0.04
0.03
0.02
0.01
0
*
*
6%MD
6%MD+0.15%KGM
2%MD+0.15%KGM
6%MD+1%MG
2%MD+1%MG
Emulsions
b
□Before freeze-drying ■Reconstitutions
Initial Viscosity (Pa.s)
0.04
0.03
0.02
0.01
0
*
*
6% MD
0.15%KGM
6%MD+0.15%KGM
2%MD+0.15%KGM
6%MD+1%MG
Emulsions

Fig. 5

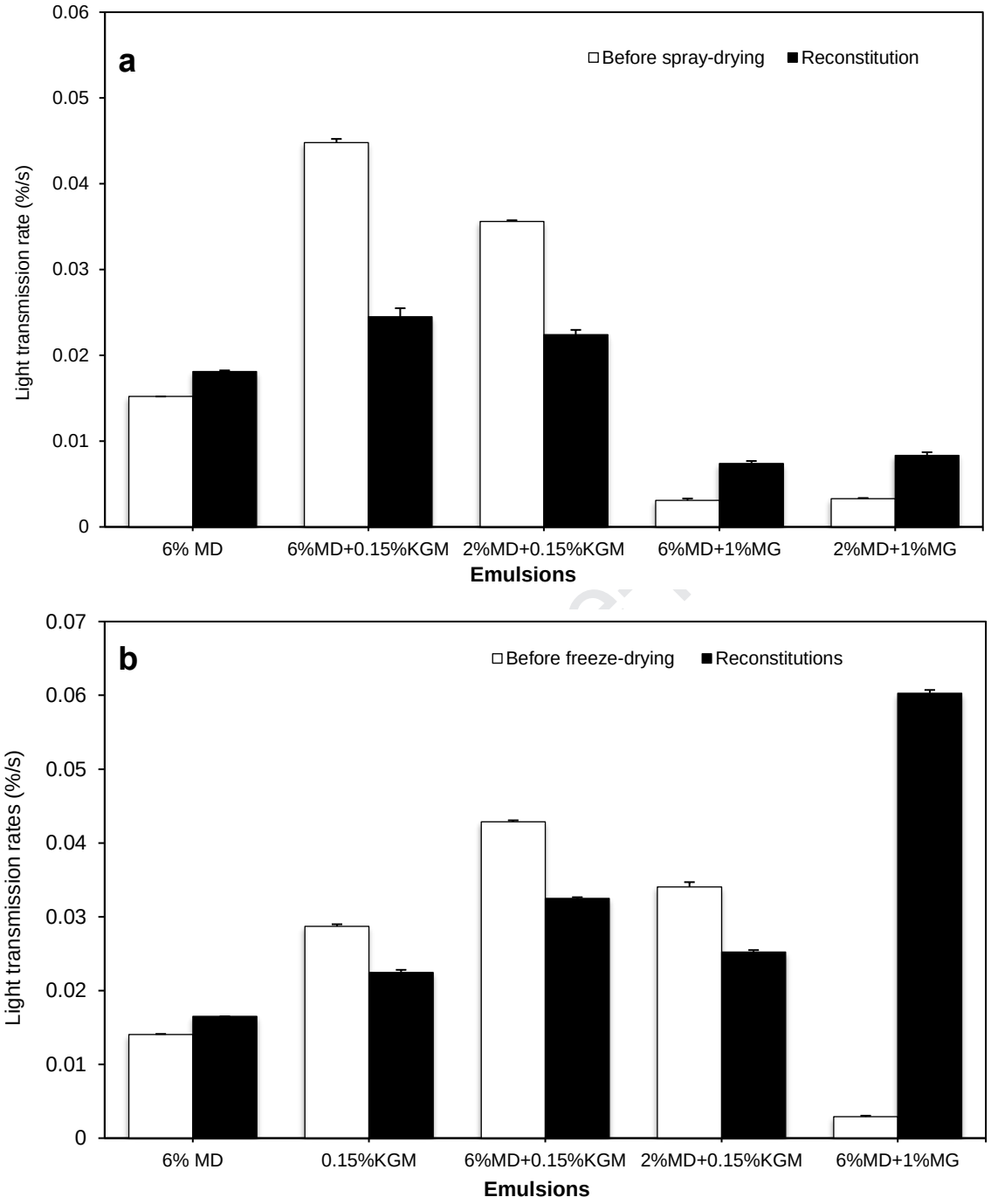

a
□Before spray-drying ■Reconstitution
Light transmission rate (%/s)
0.06
0.05
0.04
0.03
0.02
0.01
0
6% MD
6%MD+0.15%KGM
2%MD+0.15%KGM
6%MD+1%MG
2%MD+1%MG
Emulsions
b
□Before freeze-drying ■Reconstitutions
Light transmission rates (%/s)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
6% MD
0.15%KGM
6%MD+0.15%KGM
2%MD+0.15%KGM
6%MD+1%MG
Emulsions

Fig. 6

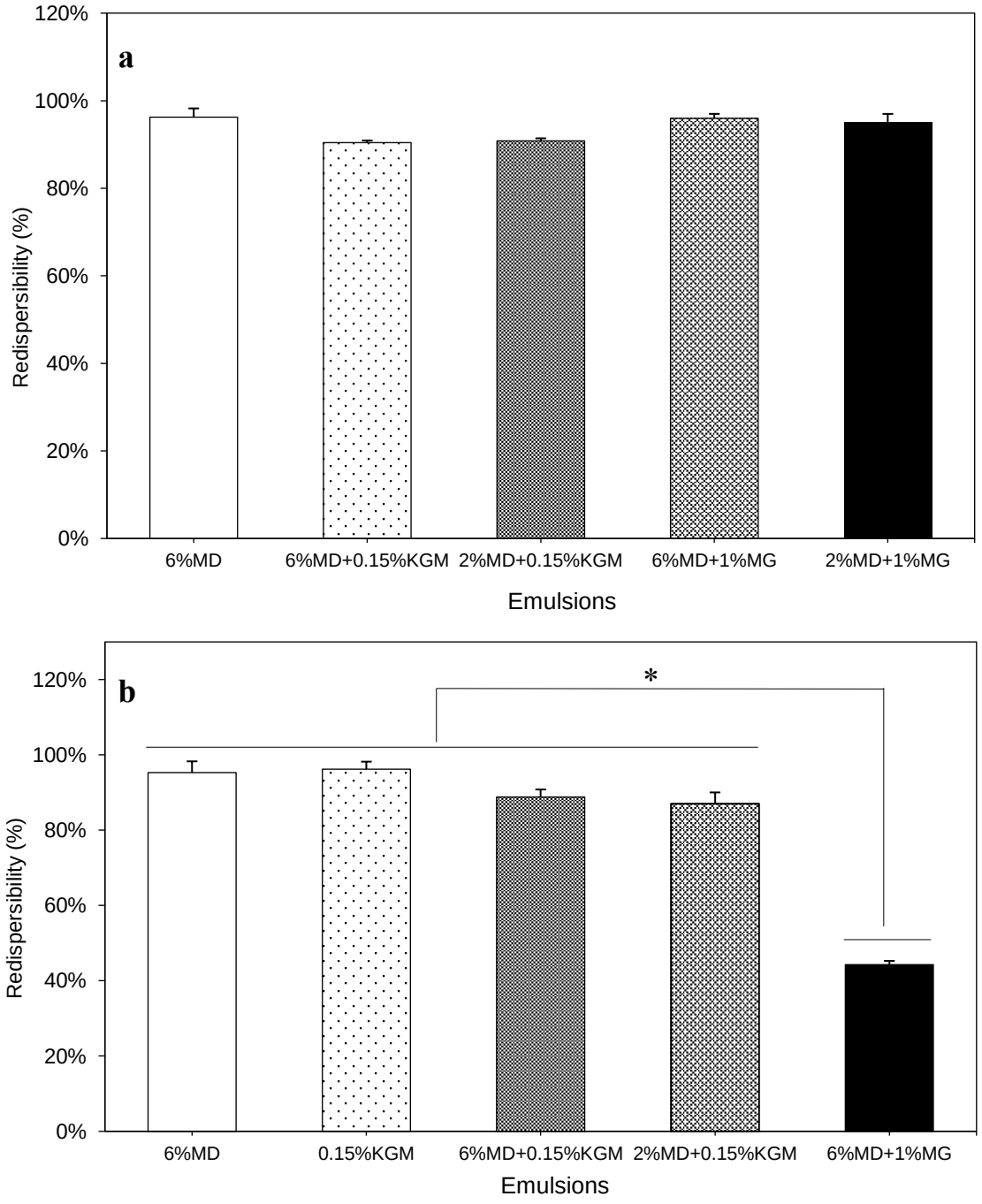

a
120%
100%
80%
60%
40%
20%
0%
Redispersibility (%)
6%MD
6%MD+0.15%KGM
2%MD+0.15%KGM
6%MD+1%MG
2%MD+1%MG
Emulsions
b
*
120%
100%
80%
60%
40%
20%
0%
Redispersibility (%)
6%MD
0.15%KGM
6%MD+0.15%KGM
2%MD+0.15%KGM
6%MD+1%MG
Emulsions

Fig. 7

Highlights of this study:

- Dry emulsions were obtained by drying KGM-, and MG-structured liquid emulsions
- The use of KGM and MG significantly reduced the cost of dry emulsions
- Obtained emulsion powders showed high re-dispersibility of >85%
- KGM can increase the creaming stability of re-constituted emulsions