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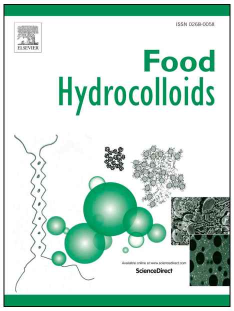

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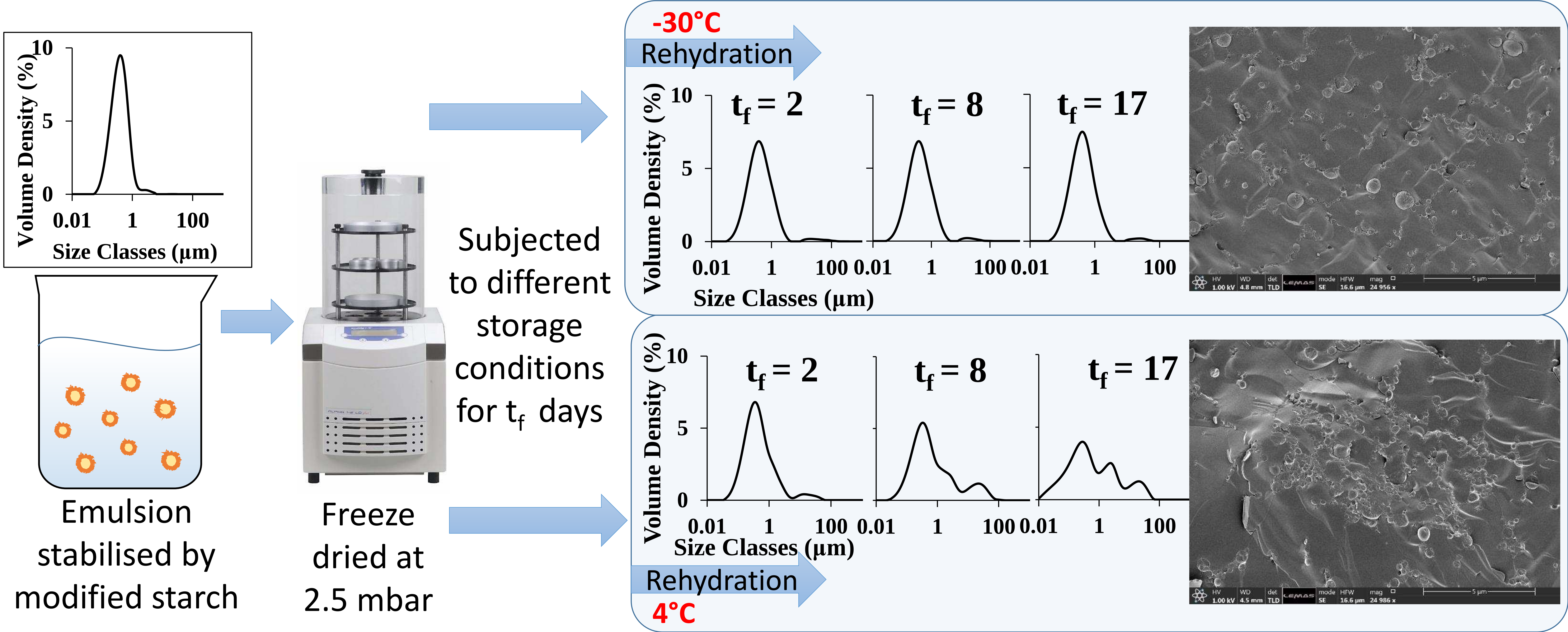

Emulsion stabilised by modified starch

Freeze dried at 2.5 mbar

Subjected to different storage conditions for t_f days

Effect of storage temperature and relative humidity on long-term colloidal stability of reconstitutable emulsions stabilised by hydrophobically modified starch

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Abstract

Dried emulsions leading to formulations exhibiting a high level of colloidal stability post rehydration would have many potential industrial applications and are of significant interest to food scientists in that the dry formulations can be easily stored and more cheaply transported. The influence of powder storage time and conditions on the long-term colloidal stability of reconstituted oil-in-water emulsion has been examined here. Emulsion systems of 20% oil were prepared with 2.5% hydrophobically modified starch acting as the emulsifier. These were subjected to freeze drying followed by up to 3 weeks of powder storage under different conditions varying in relative humidity and temperature. Rehydration was performed at specific time intervals during storage for each set of powders. The change in droplet size and morphology of the reconstituted emulsion showed that powder storage temperature has a significant effect on the long-term colloidal stability of reconstituted emulsions. Powders stored under the lowest temperature condition produced the smallest droplet size and were the most colloidally stable emulsions once rehydrated, whereas those stored at higher temperatures showed inferior performance in this respect. Freeze-dried emulsion powder, stored at -30±1 °C for 3 weeks, once rehydrated gave liquid emulsions that were stable for at least 2 weeks. In contrast, flocculation was observed upon reconstitution of dry powders that were stored at relatively high storage temperatures (4 ˚C and 20 ˚C), but neither creaming nor extensive coalescence were present, post rehydration. It is often assumed that little change to the colloidal state of the system occurs during storage, once the system has been fully dried. Our results indicate otherwise. Even in the dried form, emulsion droplets still undergo substantial changes in their surface properties, impacting the subsequent colloidal interactions and thus their colloidal stability during storage and particularly post reconstitution.

1. Introduction

Despite their high technological desirability in many different industries, formulating fully reconstitutable dry oil-in-water (O/W) emulsions continues to be a demanding and complex problem for colloid scientists. We specifically define a fully reconstitutable emulsion as one where, following the drying and after an extended period of storage in dried form, droplets of the same size as the original emulsion are retrieved by simple and gentle rehydration. Furthermore, the reconstituted emulsion thus formed without the need for further homogenisation, should exhibit a comparable level of long-term colloidal stability as it had prior to its drying. The difficulties of realising such formulations are probably nowhere more challenging than in food related systems. Food colloid scientists are rather limited in the variety of the stabilisers that they can include in food related dispersions. With an increasing demand on reducing, and the eventual phasing out of synthetic ingredients in foods, this choice is likely to become even narrower in the future. Central production of dry reconstitutable emulsions in one large facility may offer economies of scale efficiencies, as well as cost savings in such respect as the storage and transportation of raw materials to a single location. Of course, the extent of such savings will depend somewhat on the size and geographic distribution of local production sites.

The preparation of emulsions, followed by their drying, is also a common practice for making encapsulated (or microencapsulated) products. However, we wish to emphasise that the main aims of microencapsulation are quite different to those pursued here and often are focused on production of a dry powder that entraps an otherwise volatile food ingredient or flavour (Adachi, Imaoka, Hasegawa, & Matsuno, 2003; Madene, Jacquot, Scher, & Desobry, 2006; Tang & Li, 2013), or alternatively to protect a component from oxidative and other kind of damage during storage (Aberkane, Roudaut, & Saurel, 2014; Ghouchi-Eskandar, Simovic, & Prestidge, 2012; Klinkesorn, Sophanodora, Chinachoti, McClements, & Decker, 2005; Naik, Meda, & Lele, 2014; Zhang et al., 2014). Other possible reasons for encapsulation are the provision of vehicles for obtaining a desirable controlled release profile (Ahmed & Aboul-Einien, 2007; Giardiello, McDonald, Martin, Owen, & Rannard, 2012) and more recently, to achieve novel porous structures (Akartuna, Studart, Tervoort, & Gauckler, 2008; Qian & Zhang, 2011) which for example can enhance the water dissolution behaviour of the resulting powder (Klinkesorn, Sophanodora, Chinachoti, Decker, & McClements, 2006). In all of these applications, it is seldom the case that the rehydration of the dried systems is required to result, or indeed does lead to, the formation of colloidally stable emulsion droplets of the same size as those prior to drying (Christensen, Pedersen, & Kristensen, 2001; Domian, Cenkier, Górska, & Brynda-Kopytowska, 2018; Hogan, McNamee, O'Riordan, & O'Sullivan, 2001; Holgado, Marquez-Ruiz, Dobarganes, & Velasco, 2013; Jena & Das, 2012; Li, Woo, & Selomulya, 2016; Millqvist-Fureby, Elofsson, & Bergenstahl, 2001; Serfert et al., 2013; Tang & Li, 2013).

An emulsion system undergoes major environmental changes during the drying operation (Garti & McClements, 2012), whether this is achieved through spray drying, freeze drying, heat drying or any other kind of drying process. Freeze drying has been considered the most suitable technique for drying food emulsions, where it is necessary to preserve most of the structures and properties of the matrix (Desai & Park, 2005; Ray, Raychaudhuri, & Chakraborty, 2016). The porous structure of freeze-dried emulsion accounts for the relatively easy and quick rehydration process (Anwar & Kunz, 2011; Domian et al., 2018). However, the formation of ice crystals during the freeze part of the cycle can cause an increased concentration of droplets in the remaining unfrozen regions (Mun, Cho, Decker, & McClements, 2008). The same also occurs for many salts and other ingredients originally dissolved in water as these are also excluded from the frozen ice (Thanasukarn, Pongsawatmanit, & McClements, 2004). More tightly packed emulsions, in the presence of an increasing concentration of electrolyte are more prone to destabilisation, particularly if a part of the contribution to emulsion stability is through electrostatic means (Dickinson, 1992; Hunter, 2000). Similarly, the provision of steric repulsion by colloidal stabilisers relies heavily on the suitability of the dispersion medium being a satisfactory solvent, for at least some sections of the macromolecules (Dickinson, 1992; Russel, Saville, & Schowalter, 1992) that are adsorbed at the surface of the droplets. It is known that the solubility of amino acids, including the hydrophilic residues, whether charged or polar, tends to decrease significantly as the temperature of water is lowered towards the freezing point (Amend & Helgeson, 1997; Dunn, Ross, & Read, 1933). Much of the same is also true for sugar moieties that make up the polysaccharide molecules. Thus, this decrease in repulsive colloidal forces, induced by the lowering of temperature, enhances the tendency of protein stabilised emulsion droplets to aggregate. When combined with the possible formation of solid fat crystals in the dispersed phase, this makes the aggregated emulsion droplets, only separated from each other by thin protein layers, quite susceptible to the well-known phenomenon of partial coalescence (Walstra, Wouters, & Geurts, 2006). Subsequently, full coalescence and breakup of the emulsion dispersion follows when the fat crystals begin to melt during the thawing part of the process. While each different drying process possesses its own particular difficulties, the above example typifies some of the challenges that are faced in formulating a reconstitutable dry emulsion.

Several relatively novel food-grade dispersants have been tried in preparation of emulsions for the purpose of microencapsulation in recent years. However, the suitability of these in formation of reconstitutable fine emulsions, possessing long-term colloidal stability has rarely been explored. One such technique is the layer by layer deposition method first introduced to food systems by McClements and co-workers (2005). This involves the adsorption of a layer of polysaccharide onto a primary emulsion, already stabilised by protein

(Guzey & McClements, 2006; McClements, 2006). An alternative involves the covalent bonding of polysaccharide chains to proteins via Maillard reactions to produce amphiphilic conjugates. Yet one further method is to make the polysaccharides the actual emulsifying agents. This can be achieved by incorporation of an adequate number of hydrophobic side groups into the structure of the otherwise hydrophilic polysaccharide, thus turning it into an amphiphilic molecule (Nilsson & Bergenstahl, 2006, 2007; Yusoff & Murray, 2011). Each technique exhibits its own distinct advantages and disadvantages, as discussed in our recent review (Ettelaie, Zengin, & Lishchuk, 2017).

Several authors have studied the stability of food emulsions stabilised by hydrophobically modified starch (HMS). Only octenyl succinic anhydride (OSA) is a permitted food-grade reagent for the modification of starch (Liu, Z. et al., 2008). Interestingly, in most of these studies the HMS remains in the form of granule particles. That is to say that the resulting emulsions are particle-stabilised, i.e. the so called “Pickering” emulsions. Yusoff and Murray (2011) produced starch particles by reacting non-swelling starch granules with OSA followed by freeze-milling process. They found that just like many Pickering type emulsions, the oil droplets stabilised by these starch particles showed excellent stability to coalescence, even after several months, but that the mean droplet size was relatively large, as big as 20 µm in some cases. Insensitivity to pH variations, increases in background electrolyte and changes in temperature are other features associated with Pickering emulsions that have also been found to hold true for droplets stabilised by HMS granules (Marefati, Rayner, Timgren, Dejmek, & Sjoo, 2013; Murray, Durga, Yusoff, & Stoyanov, 2011). Marefati et al. (2013) also considered the effects of freeze-thawing and freeze drying on the stability of such O/W dispersions. For cases where no thermal treatment had been applied to the emulsion before freeze drying, the mean droplet size in the freeze-dried-rehydrated system was seen to be similar to the original emulsion, although in both cases the droplets were quite large ~ 50 µm.

Most food-grade particles suitable for making Pickering emulsions, even in the case of nano-sized primary particles, have been found to produce relatively coarse emulsions and bubbles, which are therefore more susceptible to creaming. In practice, it is relatively difficult to obtain an ideal dispersion of such individual particles due to their tendency to aggregate in the aqueous phase. This hinders the rapid diffusivity of the particles onto the interface (Ettelaie & Murray, 2014, 2015) during high-pressure homogenization and high-intensity ultrasound (Dickinson, 2012; Murray et al., 2011). In contrast, the adsorption and formation of macromolecular HMS layers can produce significantly finer emulsions, ~ 1 μm, (Chanamai & McClements, 2001; Tesch, Gerhards, & Schubert, 2002). Recent theoretical work involving molecularly adsorbed HMS provide further evidence for the ability of such layers to provide strong long ranged steric repulsion between droplets (Ettelaie, Holmes, Chen, & Farshchi, 2016).

Freezing and drying of emulsions is nowadays quite a common practice, with the use of the technique for microencapsulating and protecting valuable active ingredient against oxidation well-studied in the literature. In contrast very few of such reported researches have reported on the long-term colloidal stability of reconstituted emulsions. Furthermore, in the few examples where such investigation has indeed been carried out, the reconstituted emulsions produced for long-term stability were obtained from dry powders immediately after their drying (Cheuk et al., 2015; Gallarate, Mittone, Carlotti, Trotta, & Piccerelle, 2009; Matsuura et al., 2015; O'Dwyer, O'Beirne, Eidhin, & O'Kennedy, 2013), involving no powder storage period between the end of drying and the start of rehydration processes. The assumption has been that once dried, any surviving droplets in the solid matrix undergo insignificant changes during the powder storage period, due to their immobilisation and the arrest of their Brownian motion. In this study, amongst other things, we wish to re-examine this assumption. Given the already reported potential of HMS for producing reconstitutable emulsions (Cheuk et al., 2015; Domian et al., 2018), we have chosen this as the emulsifying agents for our investigation here. An additional reason for our choice was that, unlike protein + polysaccharide conjugates, for HMS, one only needs to consider the behaviour of a single type of biopolymer under the various encountered storage conditions. This makes an initial understanding of results somewhat easier to accomplish in such a preliminary study of the behaviour of colloids in a rehydrated system obtained post drying.

2. Materials and Methods

2.1. Materials

A commercial octenyl succinic anhydride (OSA) modified waxy maize starch was a gift from Ingredion UK Ltd. Typical molecular weight of OSA-modified starch is 10 – 30 MDa, with 3% modification (Nilsson, Leeman, Wahlund, & Bergenstahl, 2006). The average molecular weight of this particular commercial product was determined to be ~10 MDa (PDI = 11.9) by asymmetrical flow field-flow fractionation (AF4), using the method described by Modig, Nilsson, Bergenstahl, and Wahlund (2006) with modification (see Supplementary Material S1). Such molecular weight indicates some likely degree of hydrolysis as one of the processing steps during the commercial production of this HMS. Tesco® Pure Sunflower Oil was purchased from a local supermarket. Sodium phosphate monobasic and sodium phosphate anhydrate were purchased from Acros Organics (USA). All other chemicals used were obtained from Sigma Chemical Co. (USA). Milli-Q water (Millipore Corp., USA) was used in all experiments.

2.2 Preparation of HMS solution

Phosphate buffer of 0.2 M at pH 5.5 was heated on a hotplate to 50 °C while stirred magnetically. HMS of different concentrations by weight was added slowly to the meniscus of the solution created by stirring. The solution was then left on the hotplate for 60 mins to ensure complete dissolution.

2.3 Preparation of O/W emulsion

Oil-in-water emulsions contained 20 wt% sunflower oil and 80 wt% HMS solution with various emulsifier concentrations, expressed as wt% of the total emulsion. Emulsions were prepared using a University of Leeds in-house made Jet homogenizer operating at a constant pressure of 250 bar (Burgaud, Dickinson, & Nelson, 1990). After emulsion preparation, 20 ml of each emulsion was transferred into a screw-cap glass sample tube.

2.4 Freeze drying, storage and reconstitution of emulsion

Emulsions used for freeze drying were made with 20 wt% sunflower oil and 2.5 wt% HMS at pH 5.5, using the method described. Exact 20 ± 0.1 g of each emulsion was weighed into six Petri dishes (internal diameter 92 mm), then stored in a freezer overnight at -30 ± 1˚C, which has an estimated freezing rate of 0.42 ˚C/min. The frozen samples were dried with a Christ Alpha 1-4 LD plus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) freeze dryer at a constant vacuum pressure of 2.5 mbar, which corresponds to a temperature of -10˚C. The dried powder was stored at six different powder storage conditions varying in temperature and relative humidity:

1. 20 ± 1 °C, 72% RH (achieved by a desiccator with oversaturated NaCl solution), coded as HRH (high relative humidity)
2. 20 ± 1 °C, 2% RH (achieved by a desiccator with silica beads), coded as LRH (low relative humidity)
3. 20 ± 1 °C, sealed, coded as R (room temperature)
4. 4 ± 1 °C, sealed, coded as F (fridge temperature)

5. -18 ± 1 °C, sealed, coded as L (low temperature)
6. -30 ± 1 °C, sealed, coded as VL (very low temperature)

The amount of water lost during drying was determined by calculating the weight loss of sample. After certain periods (0, 1, 2, 5, 8, 11, 14, 17, 21 days) of powder storage in the above conditions, reconstitution was performed by adding back the weight loss with MilliQ water containing 0.02 wt% sodium azide. The tube containing reconstituted emulsion was then capped and placed on a Vortex Mixer for 15 mins. Droplet size measurements were taken 60 mins after the mixing. All reconstituted emulsions were stored under refrigeration temperature.

To clearly identify different samples and the conditions of their dry powder storage, the following naming system is adopted:

Non-freeze-dried emulsions are called fresh. All freeze-dried samples are coded by their storage conditions with the convention $Tt_f.t_s$ where T is the powder storage condition as coded above, t_f is defined as the powder storage time (in days) prior to reconstitution, and t_s is defined as the post reconstitution storage time. For example, R2.7 refers to a sample that was freeze-dried, sealed and stored as powder under room temperature condition, i.e. 20 ± 1 °C and sealed (R) for 2 days. Once rehydrated, the resulting reconstituted emulsion was then kept for further 7 days before it was subjected to various measurements. Samples reconstituted immediately after freeze-drying are designated as $D0.t_s$, (with t_s once again signifying the post reconstitution storage period).

2.5 Size measurement for starch in solution and oil droplets in emulsion

Hydrated size of starch in solution was determined using a dynamic light scattering instrument Zetasizer nano ZS (Malvern Panalytical, UK). Before the measurement, the samples were diluted to the appropriate concentration with 0.2 M phosphate buffer of pH 5.5

and then transferred to a disposable sizing cuvette. The refractive indices of water and starch were set at 1.330 and 1.520, respectively.

The droplet size of emulsions was measured using a laser light scattering instrument Mastersizer 3000 (Malvern Panalytical, UK). Before droplet size measurement, emulsions were shaken by hand to ensure homogeneity. Sample was added to the dispersion unit connected to the laser light scattering instrument until an obscuration between 1% and 4% was obtained. The mean droplet size was reported as the volume-weighted mean diameter, $\mathrm{d}_{43} = (\sum \mathrm{n_i}\,\mathrm{d_i^4} / \sum \mathrm{n_i}\,\mathrm{d_i^3})$, where $\mathrm{n_i}$ denotes the number of droplets with a diameter $\mathrm{d_i}$.

2.6 Rheological measurements

The apparent viscosity of both HMS solutions and emulsions was measured within 3h after their preparation using a Kinexus Ultra rheometer (Malvern Panalytical, UK) and a double gap concentric cylinder DG25 geometry (cup diameter 26.25 mm, bob internal diameter 24 mm, bob external diameter, 25 mm). The samples were gently mixed, poured into the temperature-controlled measurement cell, and allowed to equilibrate at 25 ˚C for 10 min prior to the measurement. Apparent viscosity of emulsions was measured at shear-rates in the range 0.2-200 s^{-1} using continuous shear, at 25 ˚C.

2.7 Water activity (a_w) and moisture determination

The value of a_w in the freeze-dried powders were determined using HygroLab C1 with HC2-AW accessary (Rotronic Instruments, UK). Care was exercised to ensure sufficient equilibration time before readings were taken. The moisture content of the powders (1 g) was determined gravimetrically by vacuum oven drying at 105 °C and 29 in Hg for 24 h.

2.8 Scanning Electron Microscopy (SEM), and Cryo-SEM

Scanning Electron Microscopy on dried samples was performed by using Carl Zeiss EVO MA15 SEM (Carl Zeiss Microscopy, Jena). Cryo SEM images were obtained using an FEI Helios G4 CX (Fei, USA), and a Quorum PP3010 cryo-FIB/SEM preparation system (Quorum Technologies, UK) as the cryo system.

2.9 Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (Perkin Elmer DSC 8000, Perkin-Elmer, Norwalk, US) was used to record the DSC thermogram of the dried powders. Approximately 10 mg powder was placed in an aluminium pan and the pan was sealed hermetically. An empty pan was used as reference. The thermal analysis was performed using a three-cycle scan model, with temperature range from 25 °C to -45 °C, with heating and cooling rates of 10 °C/min under a stream of nitrogen with a flow rate of 20 mL/min.

2.10 Cold-Stage X-Ray Diffraction

Dried emulsion powder and bulk sunflower oil was observed for X-ray pattern using a Phillips P’Analytical XPert pro MPD X-ray diffractometer (Malvern Panalytical Ltd., UK) with CuKα radiation (K-Alpha1 wavelength = 1.54,0598 Å) generated from a copper source operating at a voltage of 40 kV and a current of 40 mA. The test samples were packed into an AP TTK-450 sample holder. The samples were scanned over the range of 4 – 40° 2θ (scan step size = 0.0334, scanning time per step = 135 s). Scans were performed at -70 °C, -18 °C, and 25 °C.

2.11 Statistical analysis

All measurements, unless stated otherwise, were repeated in triplicates. The mean value of the three readings was calculated and reported in each case. Pearson correlation and linear regressions with associated coefficient of determination R^2 were performed where applicable.

All calculations were completed using Microsoft Excel 2013 and statistical significance was assigned at the level $p < 0.05$.

3. Results and discussions

3.1 Hydrophobically modified starch solution

Rheological behaviour of emulsions subjected to shear can often provide valuable information regarding the colloidal state of the droplets. In order to be able to interpret such data correctly, it is necessary that any possible complications arising from the flow properties of the continuous phase itself are appropriately taken into account. In systems considered here, the continuous phase contains HMS. Depending on the level of the hydrophobic modification, as well as the value of pH and method for preparing the solution, HMS may remain in granular aggregated form. Alternatively, it can also be present as dissolved individual macromolecules in the solution and may or may not associate to form weak networks (Sweedman, Tizzotti, Schaefer, & Gilbert, 2013). As mentioned in the introduction, in both of these forms, HMS can stabilize emulsion droplets. In the latter case (Chanamai & McClements, 2002), the general stabilising mechanism will be similar to that for emulsions stabilised by other amphiphilic type macromolecules, whilst in the former the emulsions will be of particle stabilised “Pickering” type (Marefati et al., 2013; Yusoff & Murray, 2011). Measurements of low shear viscosity for dilute solutions can be used to determine the typical size of entities that are dispersed within the solution (Chanamai & McClements, 2001), and thus potentially allow us to distinguish between these alternative possible scenarios. In this section, we present and discuss the results of such rheological measurements for HMS solution in the absence of oil droplets.

At sufficiently low volume fractions of the dispersed phase/dissolved macromolecules, the viscosity of the solution, η (Pa·s), varies in a linear fashion with the concentration c (mol/L) of the molecules as shown in equation (1)

$$\eta = \eta_0 \left(1 + [\eta]c\right) \tag{1}$$

where η_0 is the viscosity of the pure solvent phase and $[\eta]$ the intrinsic viscosity of the macromolecules (or colloidal particles) added to the solution (Barnes, 2000). Indeed, Chanamai and McClements (2002) have already shown that the viscosity variation of well dissolved HMS solutions, at low concentrations, can reasonably be approximated by Eq. (1). Following their work, we also measured the viscosity variation of our HMS solution, as a function of biopolymer content in the low concentration limit. This was conducted for both the solution just prior to homogenisation and once it had passed through the homogeniser (without addition of any oil phase). In Fig. 1 the data for both cases have been plotted as $[(\eta/\eta_0) - 1]$ vs. c. For both the homogenised and the non-homogenised solutions, a very reasonable fit to equation (1) was obtained. However, the value of intrinsic viscosity, $[\eta]$ was found to be somewhat higher at ~0.52 prior to homogenisation, as compared to 0.37 dl/g for that following homogenisation. We suspect that this is due to small residual starch clusters that are not fully broken up and dissolved until the solution is subjected to the higher shears encountered in the homogeniser. More likely, it is also the result of some degradation of the HMS, known to take place in the homogenisation process (Modig et al., 2006).

The value of intrinsic viscosity obtained from the slope of the graphs in Fig. 1 can be used to obtain an estimate of the size of macromolecules (or their aggregates) present in the solution. In the low dilution limit, HMS molecules in the solution will not overlap with each other due to the strong excluded volume interactions between them. Therefore, the total effective volume fraction occupied by such molecules will be $\phi \sim 4\pi R_H^3 n/3$, where R_H is the hydrodynamic radius of the chains (approximately the same as their radius of gyration, R_g) and n the number density of the HMS molecules as given by $10000cN_A/Mw$, if c is expressed in g/dl. Here, Mw denotes the average molecular weight of the HMS molecules and $N_A = 6.022 \times 10^{23}$ is the Avogadro's constant. For spherical dispersed entities, occupying a volume fraction ϕ, Eq. (1) can also be expressed in term of ϕ, as given by Einstein equation:

$$\eta = \eta_0 (1 + 2.5\phi) \qquad . \qquad (2)$$

From a comparison of Eqs. (1) and (2) it follows that

$$R_H^3 = \frac{3M_w[\eta]}{10^5 \pi N_A} \qquad . \qquad (3)$$

Molecular weight for modified starch varies largely from one to several hundred MDa. Typical values following degradation due to shear are measured to be around 30 MDa (Nilsson et al., 2006). Taking this value together with our measured $[\eta]$=0.37 dl/g, we obtain R_H = 56 nm. The absolute size of the measured entities by itself is not an indication of their particulate nature or otherwise. However, the measured radius here agrees well with the value of the radius of gyration for a single HMS molecule, as reported by Nilsson et al. (2006) measured using dynamic light scattering (DLS). The value is also in accord with our own data using AF4 (R ~ 50 nm), as well as our DLS results with d =112 nm (PDI < 0.1). This strongly indicates that our HMS was not in the form of granules, but much more likely that it had dissolved to form a starch solution. Subsequently, oil droplets that are emulsified in this solution cannot be classified as Pickering type, but are instead stabilised by adsorbed macromolecular layers of modified starch. There have been studies reporting on modified starch stabilised Pickering emulsions with droplet size of 391.5 nm (Liu, W., Li, Chen, Xu, & Zhong, 2018). However, this probably requires starch granules no larger than ~ 40 nm, which is as small as, if not smaller than the size of a single hydrated starch chain. Thus, even if such granules truly exist, they cannot contain more than very few HMS molecules.

3.2 Fresh liquid emulsion

In order to find the optimum HMS concentration for a 20% O/W system, emulsions with different HMS content were prepared. A very interesting relationship was observed when the normalised viscosity of emulsions was plotted against HMS concentration. The presence of a biopolymer such as starch in a solution can by itself cause a significant change in the solution viscosity. Therefore, here the normalised viscosity (η/η_0) was plotted to compensate for the effect of increasing starch concentration, where η is the viscosity of emulsion, and η_0 is the viscosity of HMS solution at the same corresponding bulk concentration, but in the absence of oil droplets. It can be seen that the relative viscosity of the emulsion dropped to a minimum as the concentration of HMS increased to 2 wt%, and then started to rise again beyond a concentration of 4 wt% (Fig. 2A). Increase in viscosity is often associated with emulsion instability, especially flocculation, which often is considered as the very first step in the possible destabilization of emulsion (Dickinson, 2009). Bridging flocculation tends to occur at low emulsifier concentration, while depletion flocculation tends to occur at high emulsifier concentration (Dickinson, 1989) when significant excess biopolymer remains non-adsorbed in the bulk solution. Related to the results in Fig. 2B, we also find a mild shear-thinning behaviour at HMS concentration of 1 wt%. This behaviour disappears and becomes completely Newtonian for emulsions at higher HMS concentrations from 2 wt% to 4 wt%. Yet, at still higher concentrations of HMS, the shear-thinning behaviour once again manifests itself (Fig. 2B). These graphs in our opinion result from the classic bridging flocculation—stable emulsion—depletion flocculation trend as the concentration of HMS increases. It is particularly interesting to note that this situation is very similar to what occurs for other types of biomacromolecular emulsions, such as sodium caseinate emulsions (Berli, Quemada, & Parker, 2002). While the depletion flocculation part of this trend has been experimentally observed and presented for HMS in the research work of Chanamai and McClements (2001), the complete curve, showing both types of bridging and depletion flocculation occurring for the same HMS stabilised system over the varying range of HMS concentrations, has not been reported previously to the best of our knowledge. Such a common feature between the colloidally-induced behaviour in protein stabilised and HMS stabilised emulsions, is interesting and worthy of further investigation in future.

From the above results, 2.5 wt% HMS was determined to give excellent emulsion stability and therefore emulsions with this HMS concentration were used for subsequent freeze drying studies. Emulsions stabilised with 2.5% HMS gave Sauter mean diameter d_{32}, ($\mathrm{d}_{32} = (\sum \mathrm{n_i}\,\mathrm{d_i^3}/\sum \mathrm{n_i}\,\mathrm{d_i^2})$, of approximately 300 nm, and d_{43} of 480 nm at pH 5.5. Fresh emulsion's stability was monitored over the course of four weeks. By appearance, there was no creaming visible to the naked eye after two months of storage at 4 °C. The values of both d_{32} and d_{43} remained stable over the whole storage period (Fig. 3B) indicating very little or no flocculation in the emulsion system. Samples tested at different storage times all gave Newtonian type behaviour, as seen in Fig. 3A, thus once again supporting the view that no aggregation of droplets occurred prior to drying. These results are largely in line with previously reported studies regarding the stability of properly prepared HMS stabilized emulsions.

3.3 Effect of freeze drying

Through the freeze-drying procedure, dried powders containing 80 wt% oil and 10 wt% HMS were prepared. The other 10% of the powder consisted of retained buffer salt and 3.02% ± 0.74% moisture, with water activity of 0.087 ± 0.035. The non-sticky, white-in-colour dried emulsion had a flaky texture, and became powdery once broken gently. The droplet size distributions of both the fresh emulsion, as well as emulsions reconstituted straight after drying are shown in Fig. 4. For the latter sample, the particle size was determined both immediately after reconstitution (D0.0) and 14 days post rehydration (D0.14). Comparing the fresh and D0.0 samples, the value of d_{43} is seen to have changed four fold from 0.50 μm to almost 2 μm. The distribution has also widened and a second peak appeared at approximately 10 μm as a result of the drying process. Upon either ultrasonication or addition of SDS, the second peak was reduced and the first peak increased in its height (data not shown). This indicates that the appearance of the second peak was mainly due to aggregation of droplets either during drying or at the point of rehydration. However, once reconstituted, the average droplet size and its distribution did not change substantially over the next 14 days (Fig. 4, D0.0 and D0.14). It is worth noting that even though sub-micron droplet size was lost after freeze-drying, the reconstituted 2 μm droplets are still reasonably fine and considerably smaller than HMS granular-based Pickering emulsions (Yusoff & Murray, 2011). Coupled with their excellent stability after rehydration, this should make them of useful practical interest in many potential applications.

During the freezing step, formation of ice crystals may have started to destabilise the emulsion droplets by limiting their spatial arrangements, by increasing the local electrolyte concentration in the none frozen regions, and possibly also by penetrating the adsorbed layer of emulsifier, as discussed extensively in previous studies (Marefati et al., 2013; Mun et al., 2008; Zhu, Zhang, Lin, & Tang, 2017). The freezing point of sunflower oil is -17 ˚C, but homogenized oil droplets in 300 nm size range are expected to have a very high degree of supercooling, (Cramp, Docking, Ghosh, & Coupland, 2004; Elwell, Roberts, & Coupland, 2004). Despite this, the freezing temperature of -30 ˚C was sufficiently low for fat crystallization to take place, as the crystallization temperature of freeze-dried emulsion powder was determined here to be -24 °C by DSC (See Supplementary Material S2). The lipid crystals penetrating the adsorbed interfacial layers would cause some desorption of the HMS. This in turn could cause further disruption to the provision of steric repulsion forces (Cramp et al., 2004; Marefati et al., 2013). When the drying phase of the freeze-dry cycle is initiated at a temperature of -10 ˚C, oil crystals would start to melt. As drying proceeds, there is an increasing reduction in the volume of the free aqueous phase. Despite this, the bulky hydrophilic parts of HMS, responsible for provision of strong steric forces, could still physically provide some protection against total coalescence (Donsì, Wang, & Huang, 2011), though not necessarily against aggregation of droplets anymore. With a compromised adsorbed layer of HMS, and the combination of competing factors discussed above, some degree of aggregation and even partial coalescence may well be expected (Cramp et al., 2004). After all, the adsorption of HMS occurred at an oil-water interface during preparation of the original emulsion. In contrast, after drying, the interface is essentially one between the starch in the matrix and the oil phase. The adsorption behaviour of HMS molecules is not necessarily expected to be the same at these two rather different interfaces. The degree of aggregation (and possibly partial coalescence) proceeded further once rehydration happened and the droplets became more mobile again. We believe this is the main cause of the increase in droplet size and the wider size distribution seen for dried and then immediately rehydrated emulsions, when they are compared to fresh ones (Fig. 4).

Fig. 5 shows the SEM images of the above freeze-dried emulsion, at several levels of magnification. The irregular flaky structure can be observed in Fig. 5A, with internal porous structures evident on the breaking sites of the flakes. This is typical of freeze-dried materials (Laine, Kylli, Heinonen, & Jouppila, 2008; Sousdaleff et al., 2013). Reconstituted droplet size reduction upon ultrasonication or addition of SDS suggests aggregation, and this is supported by the fact that only a very small number of larger droplets can be observed in

SEM micrographs. However, coalescence cannot be ruled out completely, as the arrows in Fig. 5B indicate, there is possible evidence for partial coalescence of oil droplets. Despite these, as seen in Fig. 5C, many oil droplets have survived the drying process, and retained their submicron droplet size. Considering that the dry powder consisted of 80 wt% oil and only 10 wt% HMS, the spherical entities observed are very likely oil droplets, as there would not be enough HMS to form so many particulate entities, even in the extreme case where all HMS was to desorb from the surface of droplets.

3.4 Storage condition and its effect on reconstituted emulsions

By freeze-drying samples under the same conditions and carefully characterising them both before and after the process, it is ensured that the influence of freeze-drying on HMS stabilized emulsions would be the same for all samples. Thus, the only variables remaining are the powder storage conditions and the duration of the dry storage before rehydration. SEM images were taken of powders, after varying periods of storage (ranging from 0 to 21 days) and for all of the different powder storage conditions considered in this work. No obvious differences were observed between the powders, with all of them being visually similar to D0.0 shown in Fig. 5 (See Supplementary Material S3). In particular the sizes of droplets in various powders seem approximately the same in all of the SEM micrographs. Thus, any differences in the behavior of reconstituted emulsions are not simply due to the variation of droplet size caused during the powder storage period. However, this does not mean that the adsorption behavior of HMS remains the same. For example, HMS may get desorbed from the surface to varying degrees, depending on the length and conditions of powder storage. However, these differences cannot fully manifest themselves as changes in the size of droplets, due to lack of aggregation resulting from very low mobility of droplets in all powders. As noted, when powders are rehydrated and droplets become mobile once again, these differences result in quite dissimilar reconstituted droplet sizes.

In Fig. 6, the normalised droplet size, defined as the ratio of droplet sizes between emulsions reconstituted after a powder storage time of t_f and those reconstituted straight after freeze-drying (sample D0.0), is plotted as a function of powder storage period. Fig. 6A compares samples R, HRH and LRH to examine the impact of relative humidity during powder storage, and Fig. 6B compares samples R, F, L, VL to examine that of powder storage temperature. The results demonstrate that all dry powders, with the exception of VL, gave reconstituted emulsion that became more coarse with longer powder storage times, t_f. In contrast, for VL samples, the reconstituted droplet size seemed to remain relatively stable irrespective of t_f. The Pearson correlation analysis (Table 1) confirms that in all samples but VL, the reconstituted droplet size was significantly ($p < 0.05$) correlated to the period of powder storage, t_f. These differences imply that any possible destabilization occurring during reconstitution was sensitive to the period of powder storage, even though visually no major differences between powders may be seen prior to rehydration.

It is interesting to note that in all reconstituted emulsions no creaming was visible. Similar to D0.0, all other samples showed no additional instability during a further 14 days of observation after rehydration (t_s) (Fig. 7), albeit having produced quite different emulsion sizes when initially reconstituted. In particular, for VL samples freeze-dried under the aforementioned processing condition, with the dry powder stored at -30 ± 1 °C and sealed for up to 3 weeks, the reconstituted droplets maintained their stability with an average droplet size of less than 2 μm, for at least 2 further weeks. The ability to keep dried emulsion powders for 3 weeks and then produce such stable fine emulsions simply by rehydration, is a particularly important first step in designing truly reconstitutable submicron emulsion systems, capable of transportation and storage as dry powders, for long periods of time.

3.4.1 Effect of relative humidity during storage of powder

The impact of relative humidity was examined by comparing HRH, LRH and R, as shown in Fig. 6A. In these three conditions, all powders were stored at the same temperature of 20 ˚C. The powder R was sealed at room humidity to prevent any further moisture exchange subsequently. In LRH, the environment of 2% RH suppressed moisture uptake of the powder. On the other hand, in HRH case, where moisture exchange with an environment having 72% RH was allowed, the powders rehydrated slowly during storage by absorbing moisture from the surrounding air. These were evidenced by a small variation in weight (See Supplementary Material S4) and more prominently a noticeable change in the texture of the powders. Both R and LRH powders stayed relatively dry until reconstitution, where they were rehydrated quickly.

Even though several authors reported higher degree of agglomeration with higher RH during powder storage in encapsulation studies of anthocyanins (Alvarez Gaona, Bater, Zamora, & Chirife, 2018; Garcia-Tejeda, Salinas-Moreno, Barrera-Figueroa, & Martinez-Bustos, 2018), as seen from Fig. 6A, the slopes of the linear regression lines for HRH and LRH (0.2066 and 0.2049 respectively) are comparable. This shows that the rates of change in reconstituted droplet size with respect to powder storage time were equivalent for HRH and LRH samples, despite the difference in relative humidity of their powder storage conditions. Therefore, relative humidity during powder storage did not have a strong impact on reconstituted droplet size. The relatively large standard deviation between triplicate samples prepared under same condition, for both HRH and LRH cases, indicates high level of emulsion breakdown and instability as the powders were rehydrated. The rather mild impact of humidity may be the result of hydrophobic modification of starch, which makes the affinity of matrices consisting of HMS for the uptake of water lower than those of unmodified starch.

When Fig. 6A is contrasted to Fig. 6B, it is clear that humidity at best played only a minor role, at the studied temperature range. The effect of humidity, being a less significant and more subtle parameter, should be looked at in more detail for a wider range of storage temperatures, but this was beyond the scope of the current preliminary study. The more prominent factor, temperature, will be discussed in the next section.

3.4.2 Effect of temperature during storage of powder

After storage under various temperatures, moisture content of powders increased slightly, varying from 3.39% to 5.13% (See Supplementary Material 5), but otherwise remained low. During freeze drying, the adsorbed layer of HMS covering the surface of oil droplets will tend to be disrupted to some extent by the crystalline structure of oil (Cramp et al., 2004; Marefati et al., 2013). This is likely due to the much altered nature of the interface between the dispersion medium and dispersed phase, if one or both solidify. Once the dried powders are removed from the freeze-dryer, the temperature of the subsequent storage determines the morphology and kinetic mobility of the constituents of the system. Compared to room temperature storage conditions (sample R), it is obvious that the sensitivity of change in droplet size to powder storage time, t_f, is reduced with lowering temperature (Fig. 6B). Emulsions formed by rehydration of the powder, stored at -30 ˚C, showed no variation with the duration of powder storage. The beneficial effect of lower temperature might be due to a lower kinetic and reduced mobility, and therefore a slowing down of aggregation (Su, Guo, Mao, Gao, & Yuan, 2018). Nevertheless, the important point here is that this reduction in mobility does not only refer to the Brownian motion of droplets, as for example occurs when the continuous phase becomes a gel, as also seen in other type of applications. For all of the sufficiently dried and sealed powders, at any storage temperature, the mobility of droplets is expected to be very small. Thus, the reduction in mobility with temperature that we refer to here, is that which also includes the movement of actual oil molecules themselves, as well as desorption kinetics of HMS from the surface. This suggests that processes such as Ostwald ripening may have a bearing on destabilisation of droplets when in the dry powder form. On one hand, the larger significance of Ostwald ripening in the dry system seems reasonable, given that in such samples the oil volume fraction is approaching 80%. This is a very concentrated emulsion system, indeed. But on the other hand, the solid (glassy) nature of the dispersion medium should resist and slow down any shrinkage of droplets, unless if HMS can only form rather weak and mechanically fragile matrices prone to rearrangement between droplets. In any case, the absence of obvious visual signs of change in the size of droplets, between powders stored at different temperatures (See Supplementary Material S3) prior to rehydration, seems to rule out Ostwald ripening being the main contributor for the formation of more coarse droplets observed upon rehydration. Thus, the exact mechanism through which the mobility of oil molecules in the dry powder contributes to this observed coarsening of droplets, is more likely to be desorption of HMS from the surface of droplets and/or some degree of arrested partial coalescence, as was alluded to above.

To better understand how components of the dry powders were affected by low temperatures, oil crystallization and glass transition were also considered and characterised. Samples of powders stored for 20 days at -30 ˚C and 4 ˚C, as well as bulk sunflower oil, were scanned for X-ray diffraction pattern while held at scanning temperatures of 25 ˚C, -18 ˚C and -70 ˚C (Fig. 8). A temperature of 25 ˚C is well above the crystallization temperature of bulk sunflower oil, typically between -20 to -17 (-20 ˚C here as determined by DSC, see Supplementary Material S2). Therefore, the oil component in the dry powders remained fluid with no characteristic peak associated with oil crystallisation detectable (Fig. 8A). Dry emulsion powder stored under -30 ˚C and bulk oil showed no crystallinity, while that stored at 4 ˚C was overall molten with some small peaks. However, these peaks are most certainly not associated with the oil phase, but seem most likely to be contributed by the buffer salt

Na_3PO_4 in tetragonal structure (standard pattern 04-015-4964). As seen in the patterns scanned at -18 ˚C (Fig. 8B), the supercooling effect is quite obvious as bulk sunflower oil showed distinctive peaks for crystallinity while the emulsion powders still remained molten, with no such peaks visible. As the scanning temperature was further lowered to -70 ˚C (Fig. 8C), pronounced peaks from oil crystallization could be observed in all three samples. The appearance of additional peaks compared to bulk oil scanned at -18 ˚C indicates a possible transition from α-form crystalline to β' structure (Calligaris, Arrighetti, Barba, & Nicoli, 2008). Phase transition involving crystallization was also captured by the thermogram generated by DSC, involving a temperature scan in the range -45 ˚C to 25 ˚C. For the three stored emulsion powders (20 days at 4 ˚C, -18 ˚C, and -30 ˚C respectively) examined by DSC, the crystallization temperature (during cooling) was approximately -24.6 ˚C (Fig. 9A), the melting point (during heating) was -27.5 ˚C (Fig. 9B), and no glass transition was identified in the tested temperature range (Floros, Leao, & Narine, 2014). The observation that all dried emulsion powders, irrespective of their original storage temperature, exhibited the same degree of supercooling is a reflection of a similar size of the droplets in all these powders (see Supplementary Material S3). As mentioned previously, oil droplet size in dry powder did not change during powder storage, and the effect of powder storage temperature on droplet size only manifested itself once rehydration was performed. As evidenced here, destabilization upon reconstitution does not seem to be the result of alterations in the oil phase, but most likely related to desorption and re-adsorption behaviour of HMS in dried oil/starch matrix, as well as on the reformed O/W interface upon rehydration. Nonetheless, the fact that the oil droplets in VL powder (powder storage temperature of -30 ˚C) crystallised, rather than remaining in molten or amorphous states during their storage, can in itself have some bearing on the desorption of HMS from the surface of droplets. This difference may be a possible reason for the superior resistance of VL sample against aggregation/coalescence upon reconstitution, when compared to other powders stored at higher temperatures, i.e. where droplets remained molten (Bhandari & Howes, 1999). A study of the processes involving desorption of HMS, as occurring throughout the storage period within the dry state, is not trivial. This remains an area for future investigation.

Normal starch in dry state has an estimated glass transition temperature (T_g) no lower than 227 ˚C (Bhandari & Howes, 1999; Bizot et al., 1997; Orford, Parker, Ring, & Smith, 1989). Even though the presence of water is known to significantly reduce T_g, it has been established that a moisture content as high as 22% is required to lower T_g of high molecular weight (> 10 MDa) starch down to room temperature (Bizot et al., 1997). With the amount of moisture in our powders determined at 3-5%, the T_g of our HMS is estimated to be above 50 ˚C, very unlikely to be as low as room temperature let alone below zero degrees (Lim & Roos, 2018; Liu, P., Yu, Liu, Chen, & Li, 2009; Liu, P. et al., 2010; Mizuno, Mitsuiki, & Motoki, 1998). Therefore, the HMS matrix in all the emulsion powders studied here are thought to have remained in glassy state throughout the powder storage period (i.e. the only differentiating factor between various samples). Hence, the rubber-glass transition is not considered here as a process playing a significant role in altering the colloidal stability behaviour of different samples, seen post rehydration. Had for example the matrix consisted of a low molecular weight hydrocarbon (e.g. maltodextrin) instead, then clearly this could have been a very different proposition.

Interestingly, in Fig. 6B where the droplet sizes of reconstituted emulsions are plotted against powder storage time t_f, three different regimes can be identified for R and F samples. There is a short period of initial plateau, where droplet size did not seem to change much with t_f. When powder storage period exceeded 2 days, the average rehydrated droplet size became larger and showed higher sensitivity to t_f. A second plateau was reached when the powders were stored for 8 days or more before reconstitution, where the droplet size showed no further changes with increasing t_f once again. It is possible that dry powders stored at -18 ˚C (i.e. sample L) actually followed the same pattern of rehydrated size variation with t_f as the R and F samples. However, in this case the second regime, in which large variation of reconstituted droplet size was observed, was considerably delayed due to the prolonged first plateau regime (no droplet size change with t_f). In other words, had our observation time been far longer, samples for all three storage temperatures that were higher than crystallization temperature of the oil phase, would have resulted in the same pattern of droplet size change with the duration of the powder storage period prior to rehydration. This delaying effect with lower storage temperature is quite clear from Fig. 10, which shows the change in reconstituted droplet size distribution at different t_f. Similar effect of different storage temperatures on the deterioration of freeze-dried entities was found in the microcapsules produced by Malacrida, Ferreira, Zuanon, and Nicoletti Telis (2015), but their focus was on retention percentage of encapsulated material rather than reconstituted droplet size, unlike that here.

3.4.3 Long-term stability of reconstituted emulsion from dry stored powders

It was observed that the powders stored at room temperature (samples R) developed a weak gel-like texture subsequent to post reconstitution storage. These seem similar in appearance to those often encountered in the presence of weak attractive depletion interactions between droplets. The structure was found to break down easily with gentle hand shaking. The samples were subjected to rheological measurements, but even at the very lowest shear rates, the gel was already too fragile and had sufficiently broken down to show any pronounced rheological characteristics. Presumably, as was discussed before, some HMS molecules desorbed from the interface during freeze-drying (and possibly powder storage period). Upon reconstitution the free HMS did not re-adsorb quickly enough, if at all, back onto the surface of the droplets. Presence of a small amount of free starch in solution could lead to depletion flocculation of oil droplets. The effect was disrupted by much higher dilution and the gentle shear that droplets suffer in the Mastersizer 3000. The weak flocs took a short period to separate. This manifests itself as an initial evolution of droplet size distribution in the Mastersizer over a period of 10 minutes or so, reaching a steady final value after that period. Interestingly a similar result was reported by Farshchi et al. (2013) with regards to a delay in complete break up of depletion flocculated aggregates occurring during similar measurements, though in their case the droplets were stabilised by soy bean protein.

Figure 11 shows Cryo-SEM images on reconstituted emulsions F11.87 and VL11.153, in which flocculation was suspected despite the lack of creaming. These samples were rehydrated and then flash frozen before imaging, as described in the method section. Like all other samples, in these two cases the droplet size remained fairly constant once samples were reconstituted (6.7 μm for F11.87 vs 3.91 μm for F11.0, and 1.8 μm for VL11.153 vs 1.18 μm for VL11.0). Occasionally, single droplets of a size over 10 μm were detected, but the vast majority of droplets were only visible under high magnification, as shown in Figure 11.

In Fig. 11A, sample VL11.87 (with powder storage temperature of -30 ˚C) showed a uniform spatial distribution of droplet positions, having typical sizes of 1 μm. Some of the droplets aligned along the ridges, which was the result of getting pushed together by flash freezing of water. On the other hand, in Fig. 11B for powder storage of 4 ˚C samples (samples F), it can be seen that a large cluster (~6 μm) was formed by aggregation of small droplets, with sizes less than 1 μm. Similar clusters were also seen in all the other F samples. Again, this seems to indicate some degree of flocculation in such cases. It is interesting that the droplets in these large flocs remained intact as individual droplets, rather than coalescing into bigger ones. This was the case even for our samples after a long period of time, i.e. 153 days post rehydration. The molecularly adsorbed layer of HMS must still be mostly intact, as indicated by the clear edges around each droplet within the flocs. Presumably the thick HMS layer still could act as a physical barrier preventing extensive coalescence, even after freeze-drying, long period of powder storage, and many days post reconstitution. In Fig. 11C, we show one interesting rare droplet with an approximate size of 8 μm. On the surface of this large droplet, there is a small region that does not seem to be fully covered, and in this loosely packed area, particle-like material can be observed. One could speculate that the surface of this droplet was covered by aggregated HMS particles, judging by the non-spherical shape of the small particulates. Presence of these droplets, though quite rare, could indicate that a very small amount of HMS may not have been completely dissolved, remaining in residual particulate form. These in principle can adsorb onto the surface of droplets and lead to the formation of Pickering droplets. Alternatively, some of the molecularly adsorbed HMS on the surface of droplets may be reverting back to form particle aggregates during freeze-drying or in the subsequent storage period. In any case, the observation of these types of large droplets was too rare to allow us to perform a more systematic detailed examination, or for it to have any significant practical implications.

4. Conclusions

Extensive attention has been paid to encapsulation in studies involving drying of emulsions. In contrast, long-term stability of rehydrated emulsions has rarely been considered, either as part of such microencapsulation studies or other research involving drying of emulsions. Similarly, the effect of powder storage conditions on the degree of entrapment of numerous active ingredients, their retention during dry storage and their protection against possible oxidation have been widely researched. Once again, far less research has involved the impact of powder storage on colloidal stability of the reconstituted emulsions. In the present study, we have shown that powder storage temperature plays a major role on the size and emulsion stability of droplets that are obtained after rehydration of the dried powder. This most likely is resulted from the limited diffusion of oil molecules, since the oil phase was found to be in crystalline form at temperatures below ~ -24 ^{0}C, showing some degree of supercooling as may be expected. The relative humidity on the other hand plays a rather minor role. This is perhaps due to hydrophobic modification of starch, somewhat reducing its affinity for uptake of moisture. Flocculation was observed in rehydrated emulsions, for samples reconstituted from powders stored at higher storage temperatures. However, extensive coalescence and emulsion breakdown were absent once the powder was rehydrated back to an emulsion. It is shown that during the powder storage period, oil droplets in the emulsion powder were not altered in morphology or size. Future studies should focus on characterising the HMS adsorbed layers on the surface of droplets within the dried powders. In particular, desorption/re-adsorption behaviour of HMS, as affected by powder storage temperature, should be investigated. We note that a study of such processes, occurring on the surface of droplets while in the dry form, remains quite challenging. Nonetheless, we have demonstrated in the current work that dry reconstitutable emulsions can be made and stored for considerable periods of time (more than 3 weeks), where upon simple rehydration (i.e. not needing any additional re-homogenisation), colloidally stable droplets of size < 2 μm were achieved (stable for more than 100 days after reconstitution). The result can have major commercial potential such as the possibility of the mass production of the dried emulsion powders in one central site, shipment of the powder to other locations, and storage and rehydration of these as and when required. However, with further studies on optimising the relevant drying and storage conditions mentioned above, we believe that the realisation of even smaller, eventually stable sub-micron reconstitutable emulsions, formed by gentle rehydration of already dried emulsions, is a viable proposition.

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Figure Captions

Fig. 1. **$[(\eta/\eta_0) - 1]$ plotted as a function of the HMS concentration for starch solutions before and after homogenization, where η and η_0 are the apparent viscosity of the solution and of the pure solvent, respectively. The slopes of the best-fit lines in each case provide the corresponding intrinsic viscosities of the HMS solutions.**

Fig. 2A. **Normalised viscosity (at a shear rate of 2 s^{-1}) of 20 wt% O/W HMS stabilised emulsions with different HMS concentrations (wt%).**

Fig. 2B. **Apparent viscosity of emulsions plotted as a function of shear rate. Curves for three different HMS concentrations (wt%), 1% (dotted), 2% (dashed) and 8% (solid line) are displayed.**

Fig. 3. **Apparent viscosity vs shear rate for non-freeze-dried emulsions, on the day they were made (dashed line) and after 22 days (dotted line). These emulsions contained 20 wt% oil and 2.5 wt% HMS. The inset shows the variation of the mean droplet size for non-freeze-dried emulsions over a period of 28 days.**

Fig. 4. **Droplet size distributions for non-freeze-dried emulsions (—), emulsions reconstituted immediately after freeze drying D0.0 (– –), and the latter sample 14 days post rehydration D0.14 (….).**

Fig. 5. SEM images of freeze-dried emulsion powders that underwent no powder storage (i.e. straight after drying). Arrows indicate some possible evidence for partial coalescence. Micrographs are taken at different magnifications, A) 500 X, B) 2.50K X, C) 30.00K X.

Fig. 6. Normalised average reconstituted droplet size plotted versus the powder storage period (t_f). All measurements were performed immediately after rehydration (t_s=0). Normalised droplet sizes are obtained as the ratio of droplet sizes between emulsions reconstituted after a powder storage time of t_f and those reconstituted straight away after freeze-drying (sample D0.0). Error bars represent calculated standard deviation. Linear regression lines are shown with equations and R^2 values. (A) Effect of relative humidity with samples that underwent powder storage with high (HRH), low (LRH), and typical room (R), humidity conditions. (B) Effect of temperature with samples that underwent powder storage at room temperature (R), 4 °C (F), –18 °C (L), and –30 °C (VL).

Fig. 7. Assessing the long-term stability of reconstituted emulsions by monitoring d_{43} (μm) for samples VL11.t_s and R11.t_s at different t_s (days) post rehydration.

Fig. 8 XRD patterns for 20-day stored emulsion powders and bulk sunflower oil, scanned while holding the samples at A) 25 °C, B) -18 °C, C) -70 °C.

Fig. 9 **DSC thermograms for freeze-dried powders after 20 days of powder storage, A) cooling from 25 °C to -45 °C, B) heating from -45 °C to 25 °C.**

Fig. 10 **Droplet size distributions of reconstituted emulsions from powders that had been stored for 2, 8 and 17 days in conditions VL (–30 °C, sealed), L (–18 °C, sealed) and R (20 °C, sealed). All the distributions were obtained shortly after rehydration.**

Fig. 11 **Cryo-SEM micrographs of reconstituted emulsions; A and C were for samples VL11.153 (i.e. 11 days of powder storage, following by 153 days post rehydration) while B was for sample F11.87.**

Table 1 **The correlation coefficients and associated p values for Pearson correlation analysis, on reconstituted droplet sizes vs powder storage time, showing data size (n) and correlation coefficient (r).**

Fig. 1

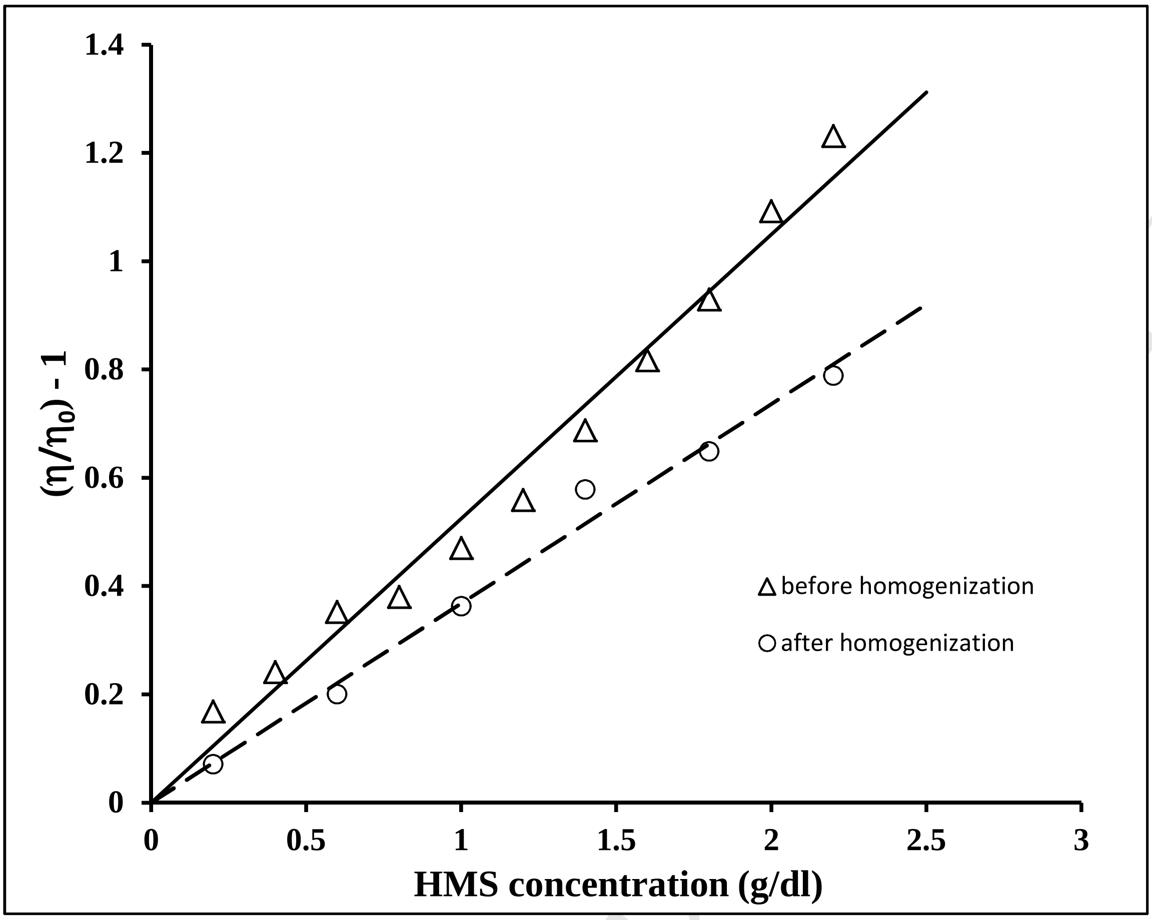
1.4
1.2
1
0.8
0.6
0.4
0.2
0
(η/η₀) - 1
0
0.5
1
1.5
2
2.5
3
HMS concentration (g/dl)
△ before homogenization
○ after homogenization

Fig. 2

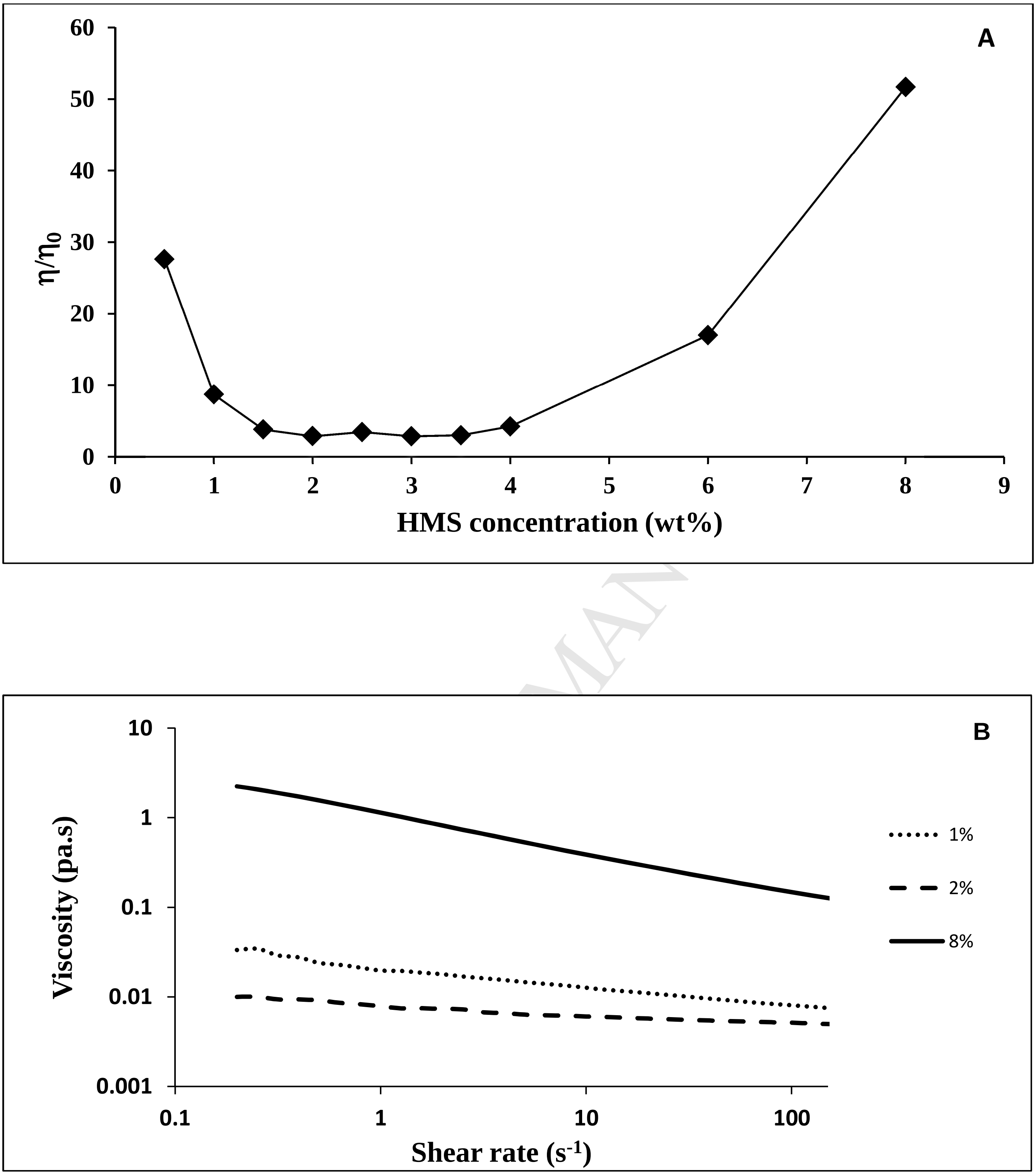
A
η/η_0
0
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8
9
HMS concentration (wt%)
B
Viscosity (pa.s)
10
1
0.1
0.01
0.001
0.1
1
10
100
Shear rate (s^{-1})
1%
2%
8%

Fig. 3

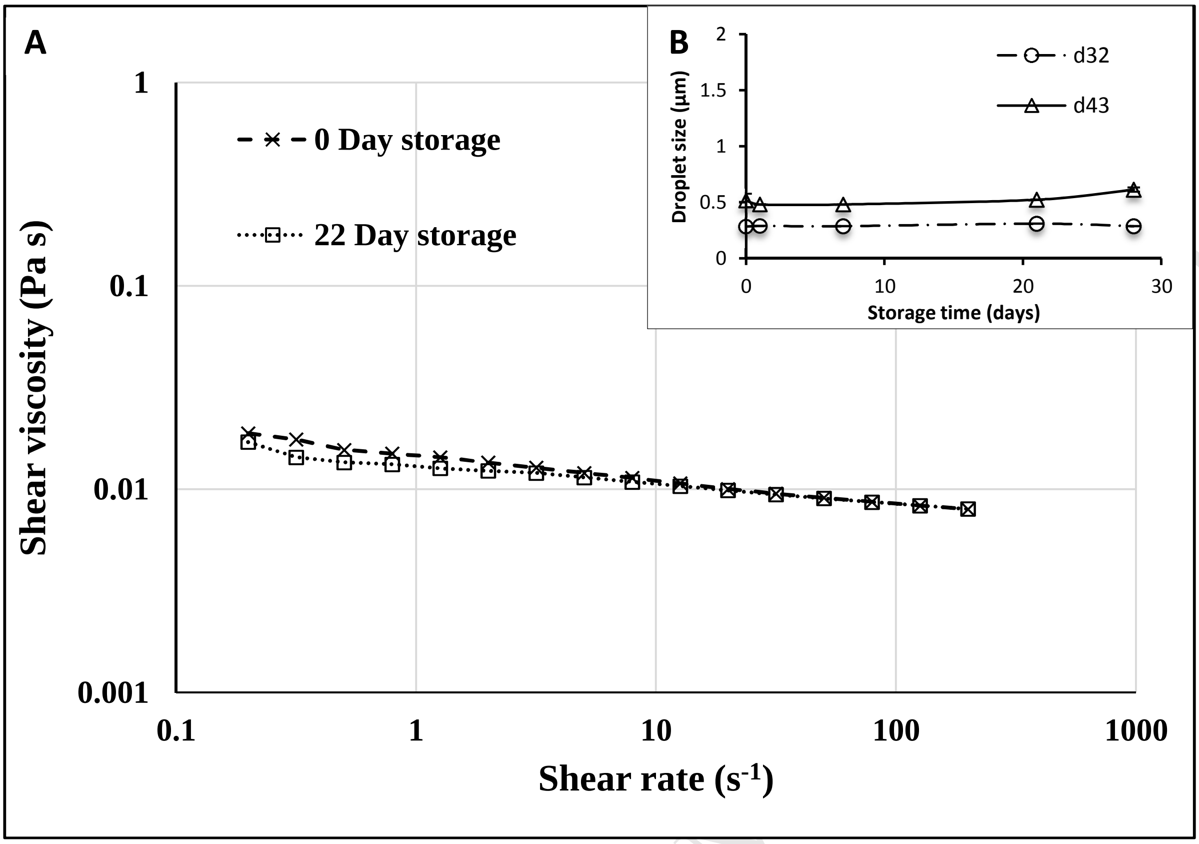
A
Shear viscosity (Pa s)
1
0.1
0.01
0.001
0.1
1
10
100
1000
Shear rate (s-1)
0 Day storage
22 Day storage
B
Droplet size (µm)
2
1.5
1
0.5
0
0
10
20
30
Storage time (days)
d32
d43

Fig. 4

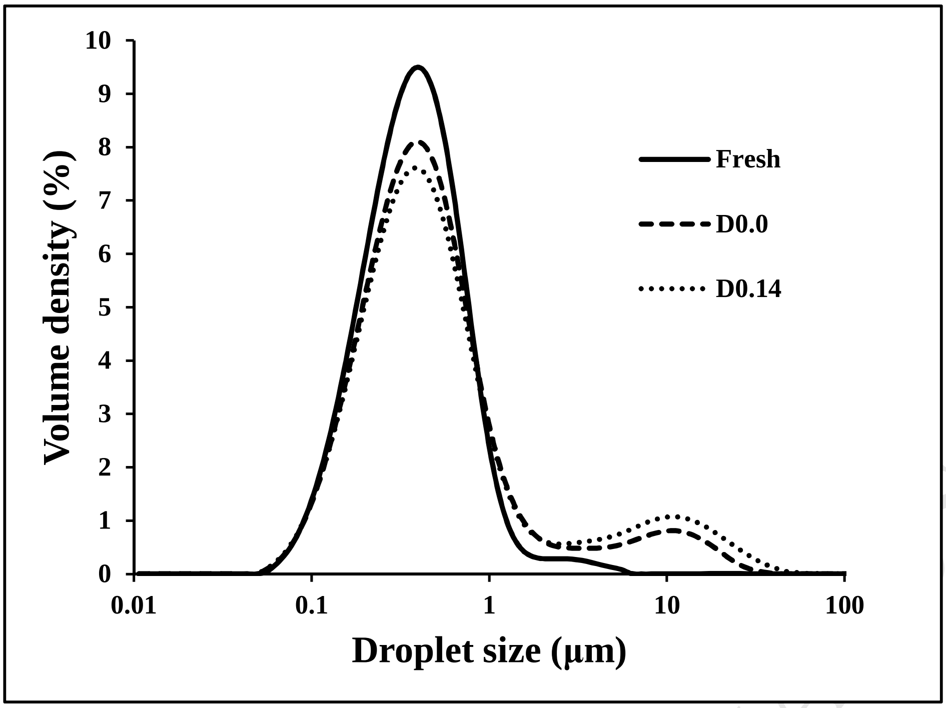
Volume density (%)
10
9
8
7
6
5
4
3
2
1
0
0.01
0.1
1
10
100
Droplet size (µm)
Fresh
D0.0
D0.14

Fig. 5A

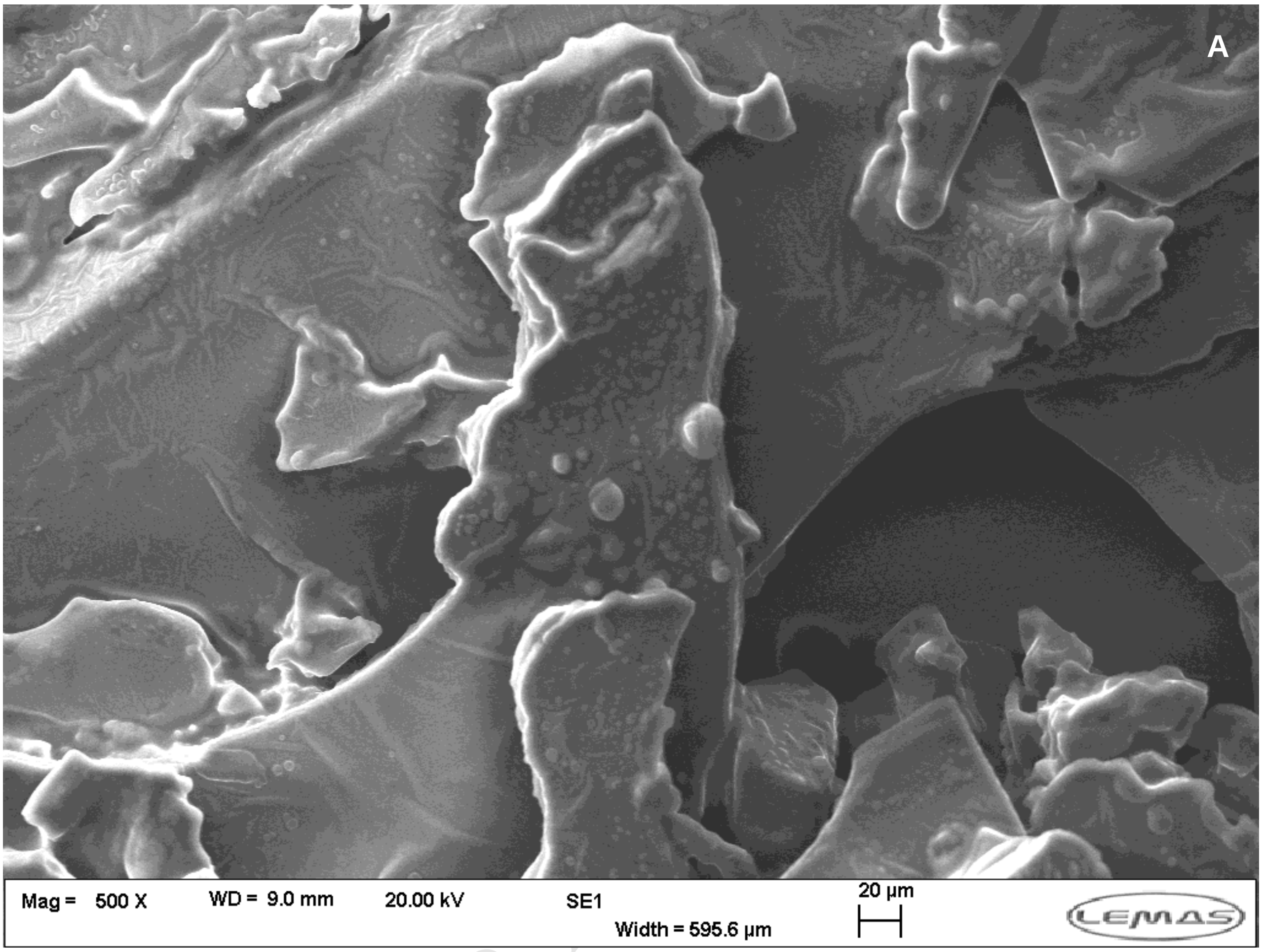

Fig. 5B

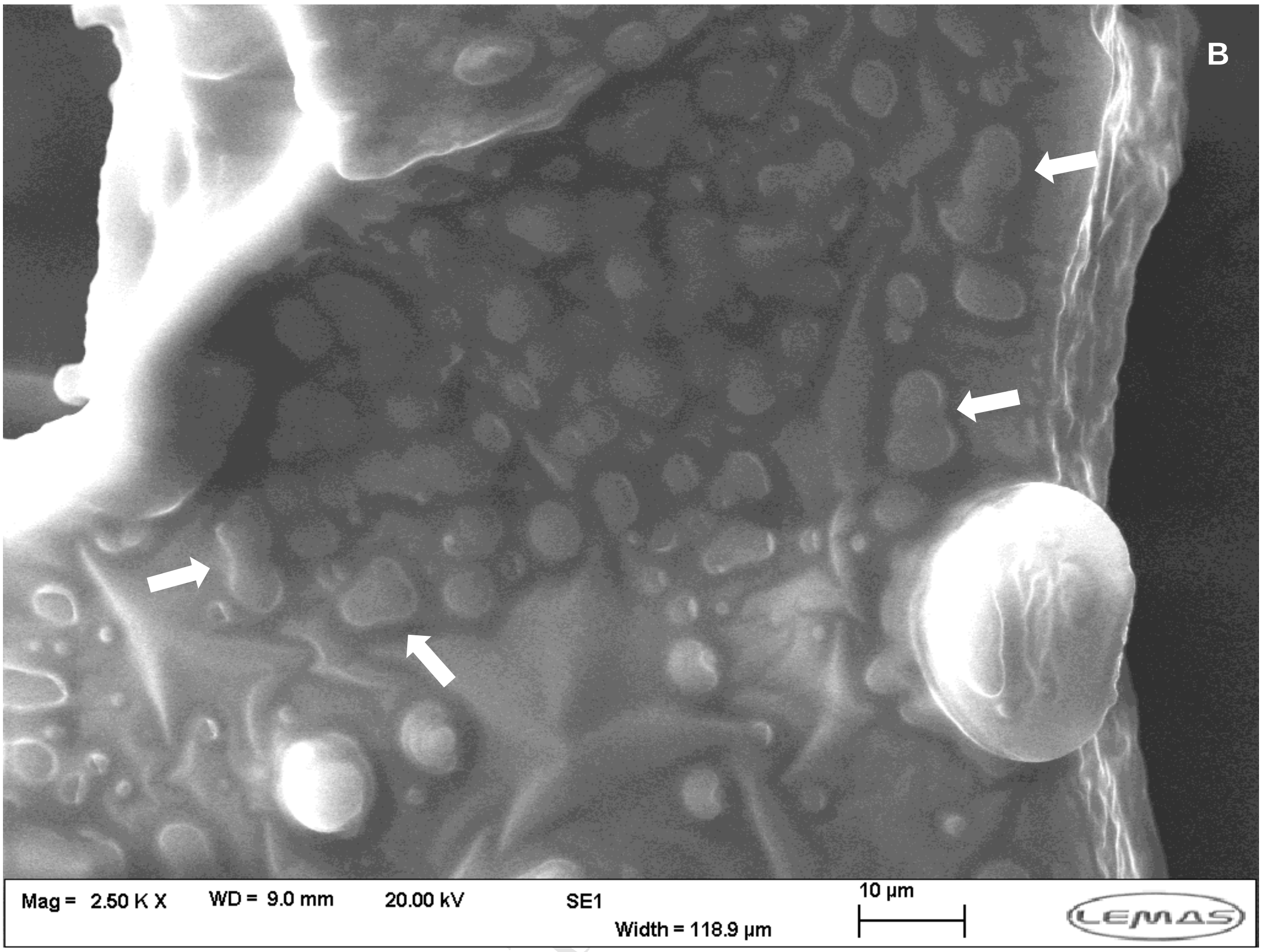

Fig. 5C

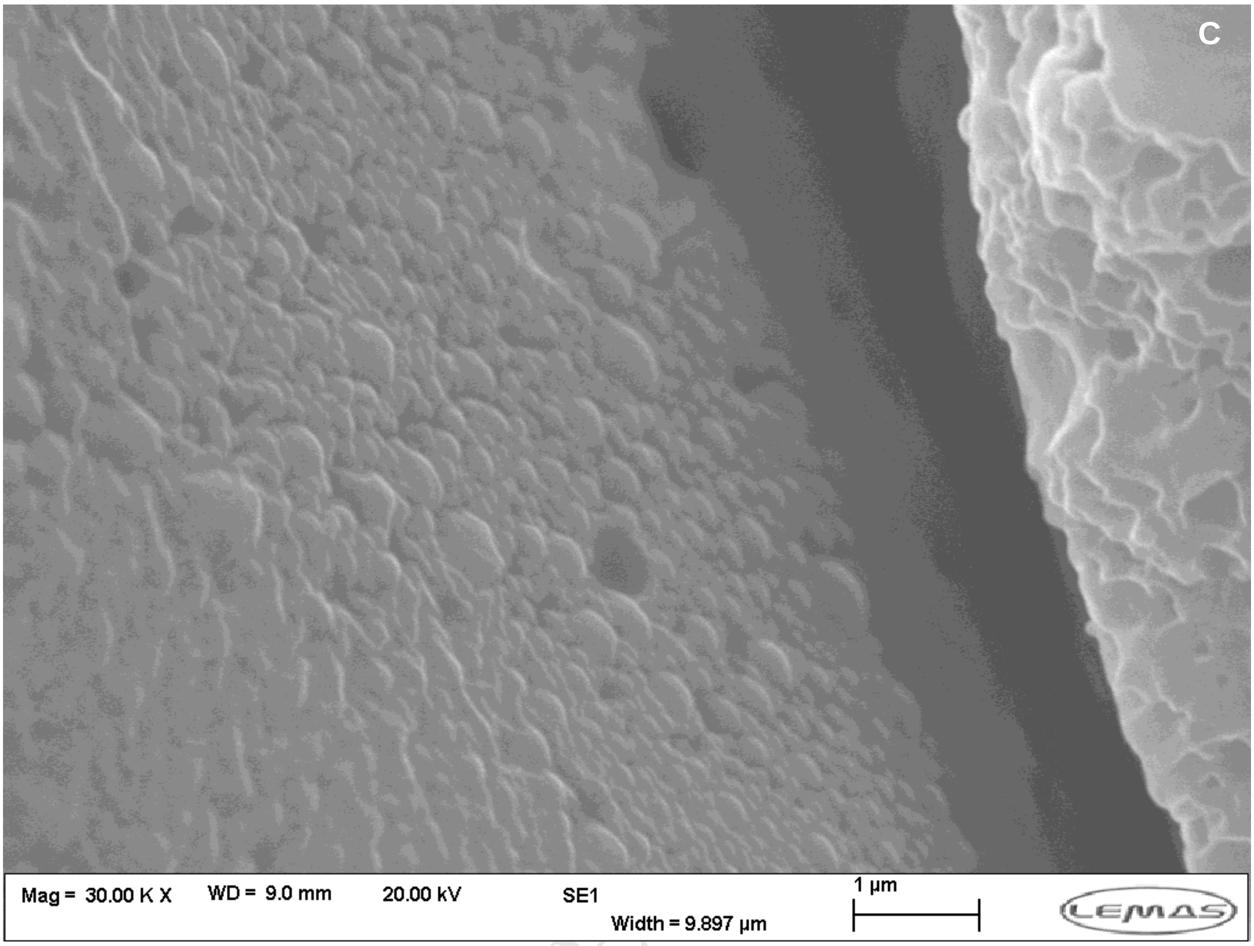

Fig. 6A

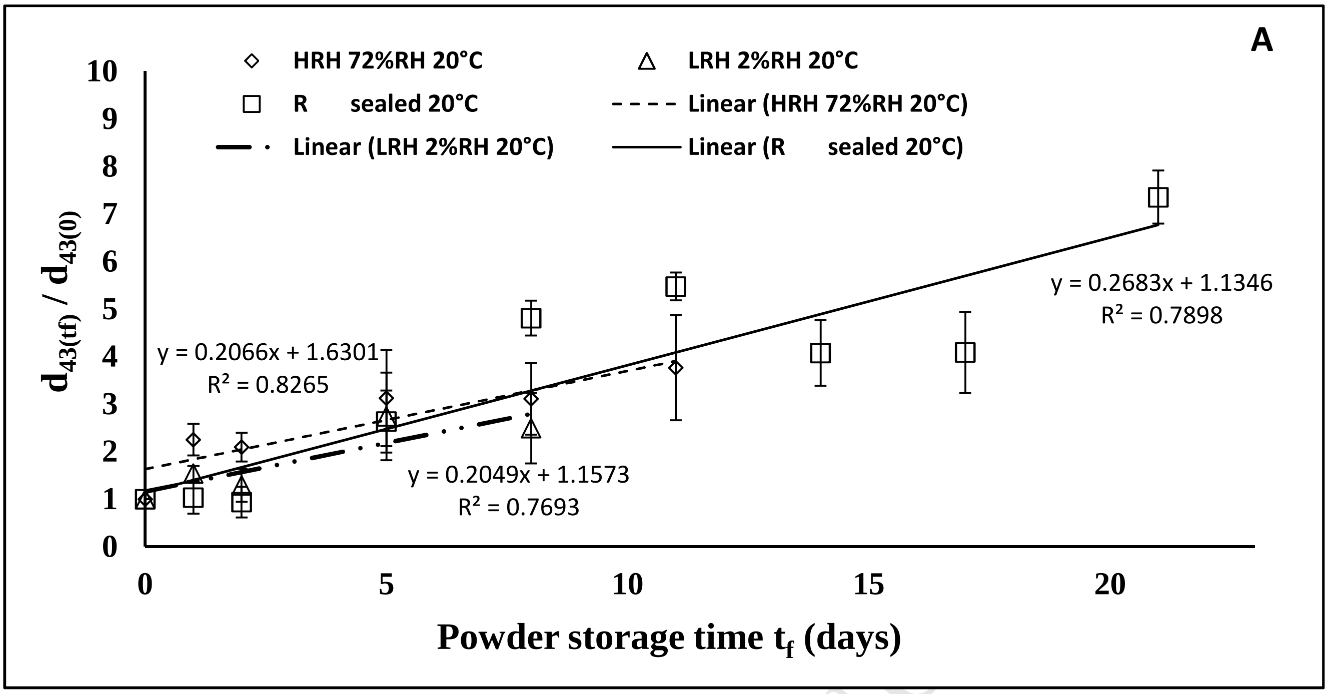
A
HRH 72%RH 20°C
LRH 2%RH 20°C
R sealed 20°C
Linear (HRH 72%RH 20°C)
Linear (LRH 2%RH 20°C)
Linear (R sealed 20°C)
y = 0.2066x + 1.6301
R² = 0.8265
y = 0.2049x + 1.1573
R² = 0.7693
y = 0.2683x + 1.1346
R² = 0.7898
d43(tf) / d43(0)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
Powder storage time tf (days)

Fig. 6B

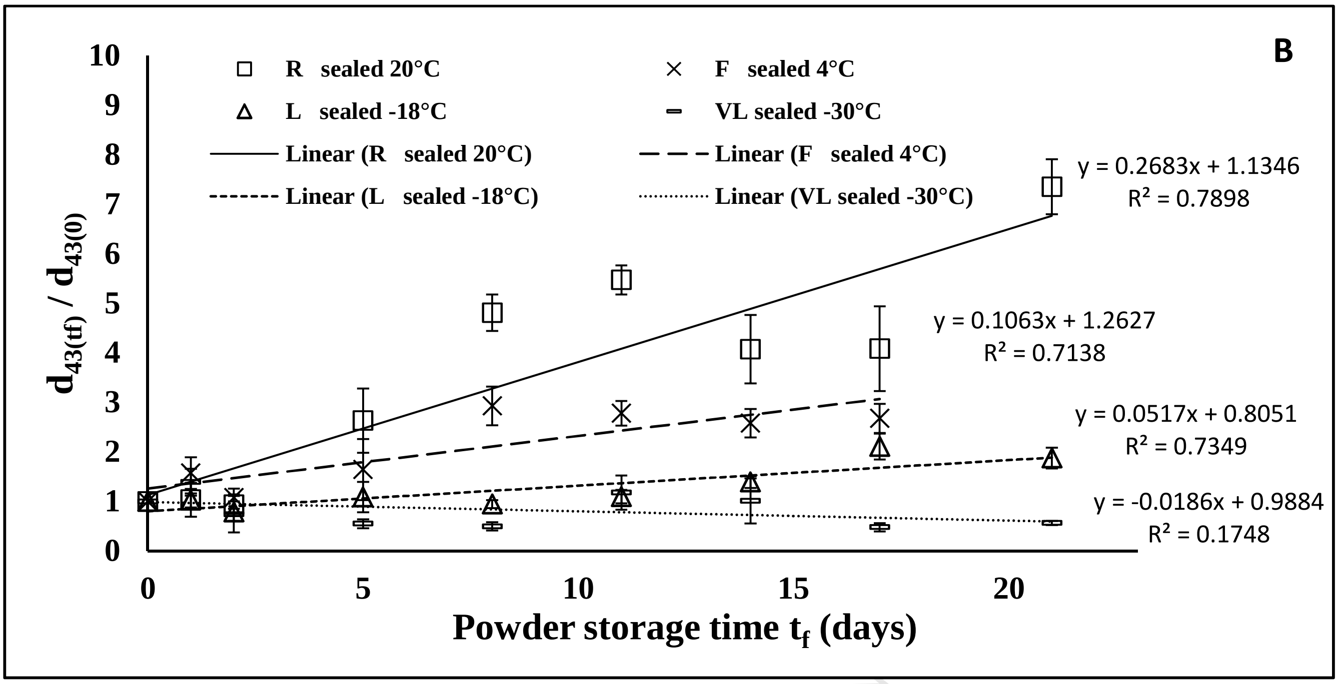
B
R sealed 20°C
F sealed 4°C
L sealed -18°C
VL sealed -30°C
Linear (R sealed 20°C)
Linear (F sealed 4°C)
Linear (L sealed -18°C)
Linear (VL sealed -30°C)
y = 0.2683x + 1.1346
R² = 0.7898
y = 0.1063x + 1.2627
R² = 0.7138
y = 0.0517x + 0.8051
R² = 0.7349
y = -0.0186x + 0.9884
R² = 0.1748
d43(tf) / d43(0)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
Powder storage time tf (days)

Fig. 7

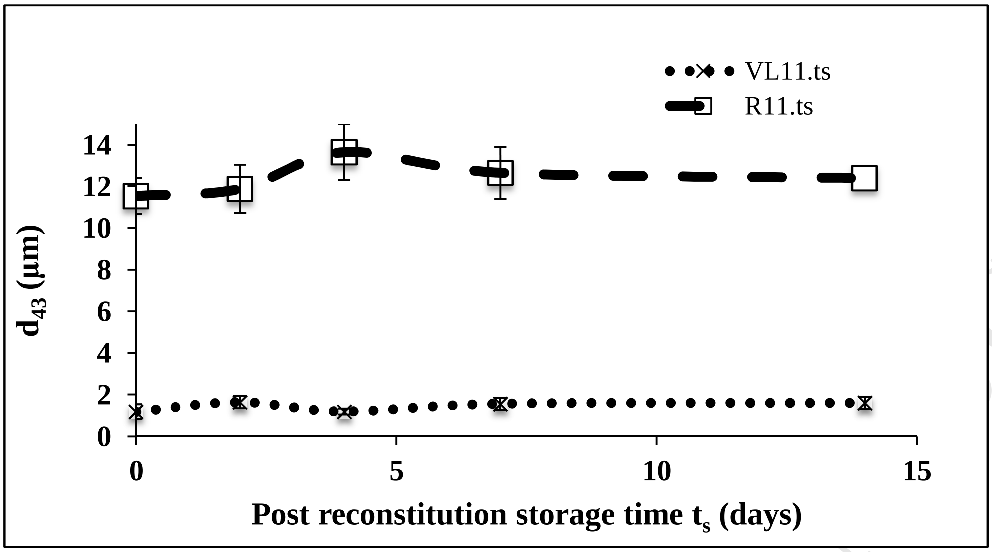
VL11.ts
R11.ts
d43 (µm)
14
12
10
8
6
4
2
0
0
5
10
15
Post reconstitution storage time ts (days)

Fig. 8A

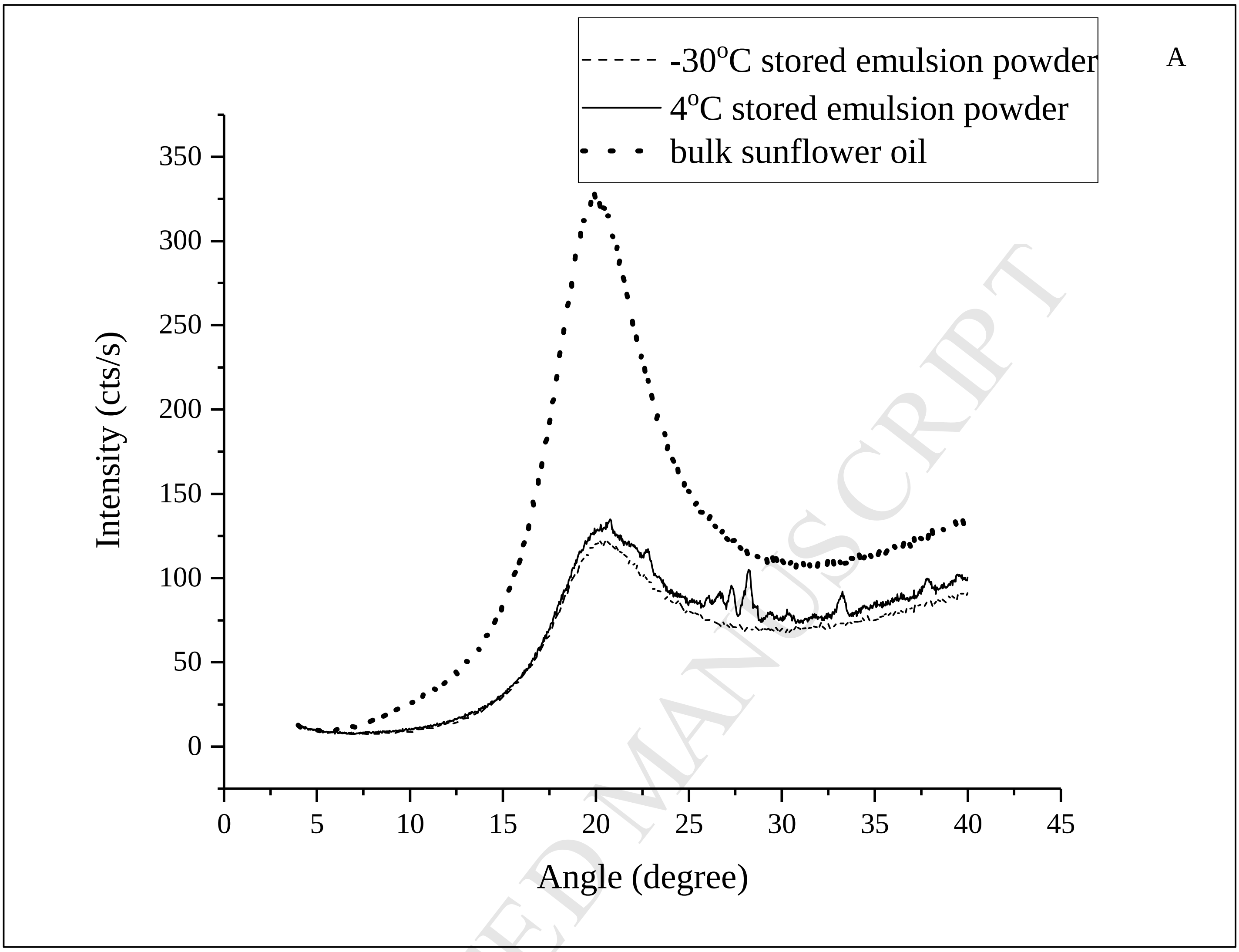
-30°C stored emulsion powder
4°C stored emulsion powder
bulk sunflower oil
A
Intensity (cts/s)
0
50
100
150
200
250
300
350
0
5
10
15
20
25
30
35
40
45
Angle (degree)

Fig. 8B

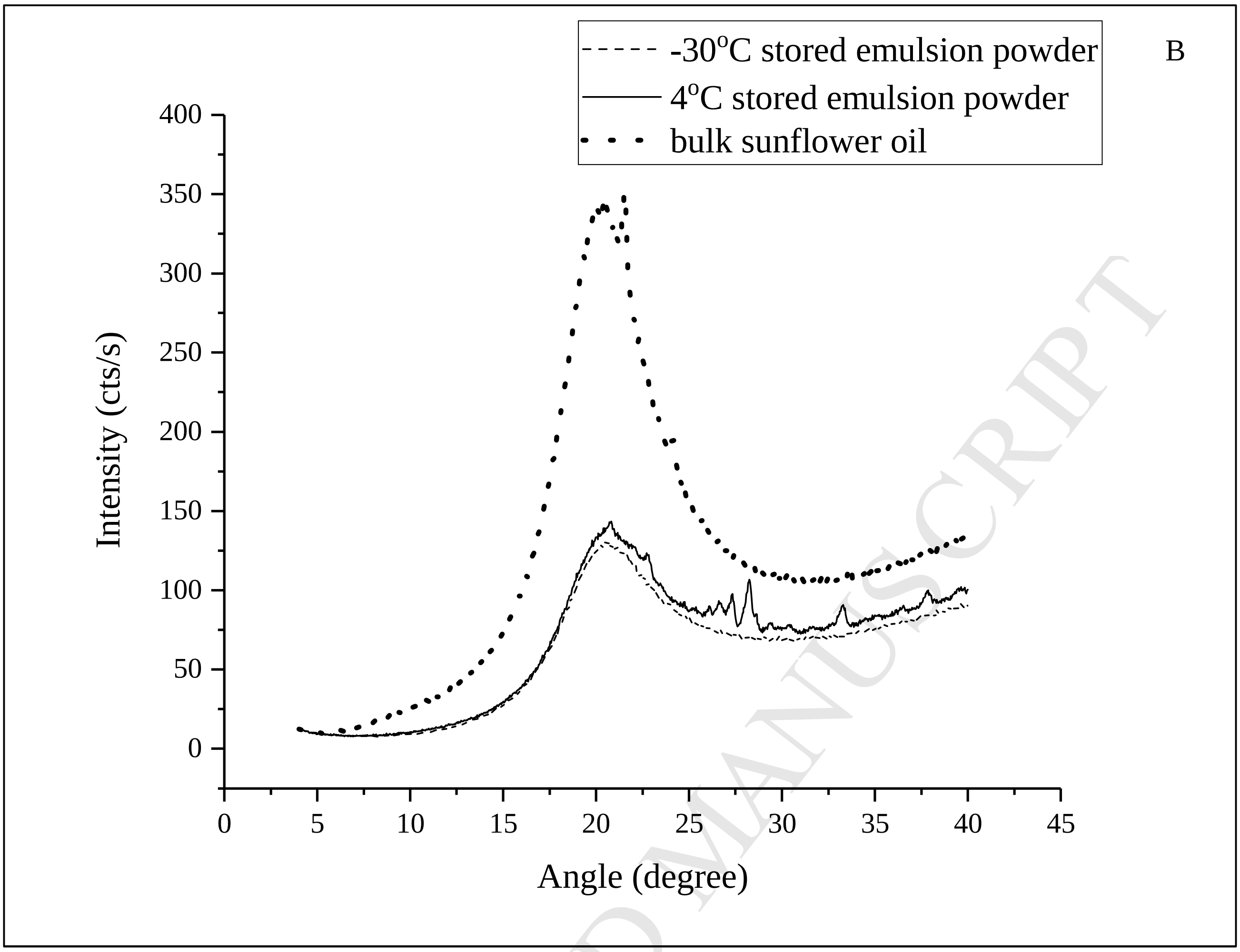
-30°C stored emulsion powder
4°C stored emulsion powder
bulk sunflower oil
B
Intensity (cts/s)
400
350
300
250
200
150
100
50
0
0
5
10
15
20
25
30
35
40
45
Angle (degree)

Fig. 8C

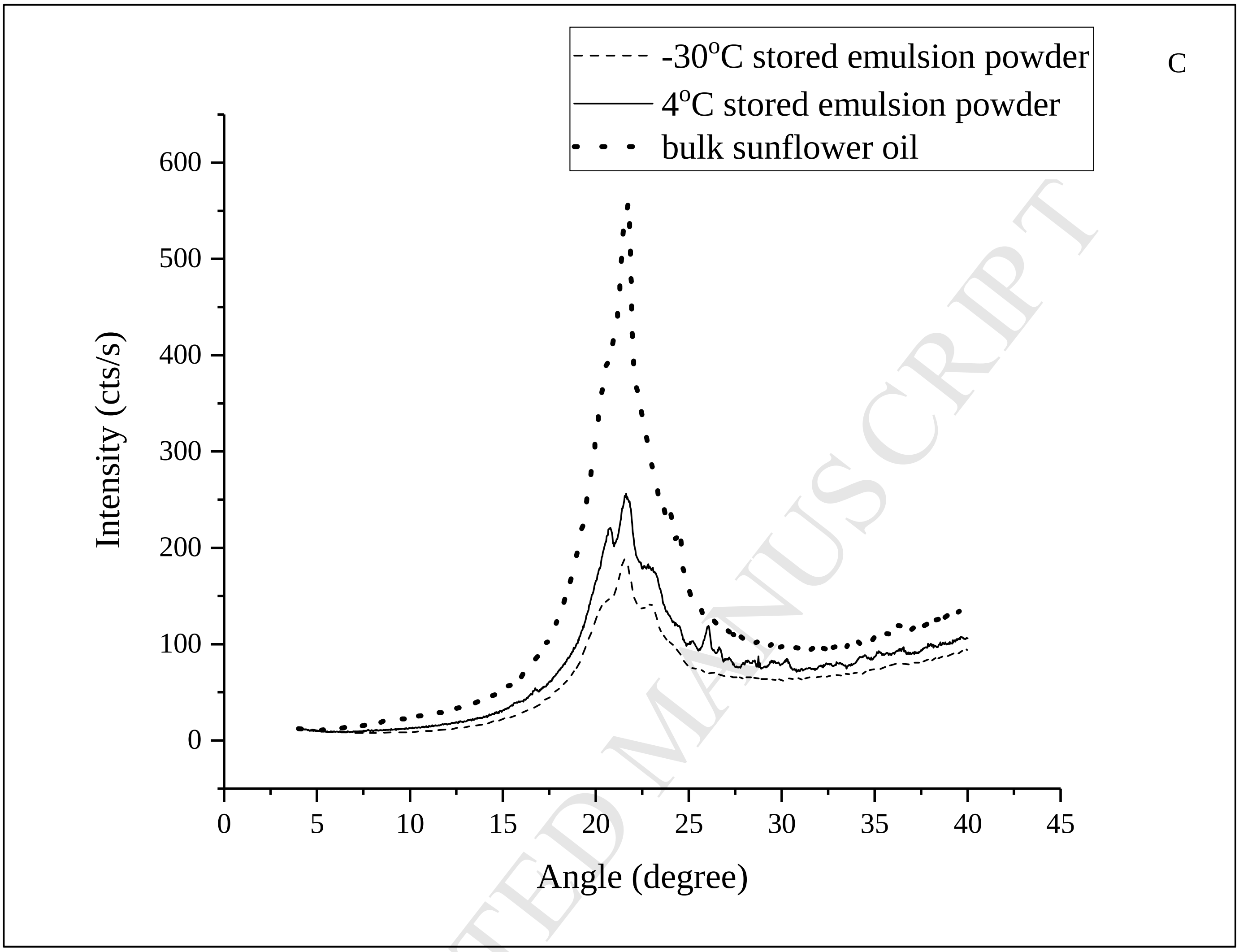
-30°C stored emulsion powder
4°C stored emulsion powder
bulk sunflower oil
C
Intensity (cts/s)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
Angle (degree)

Fig. 9A

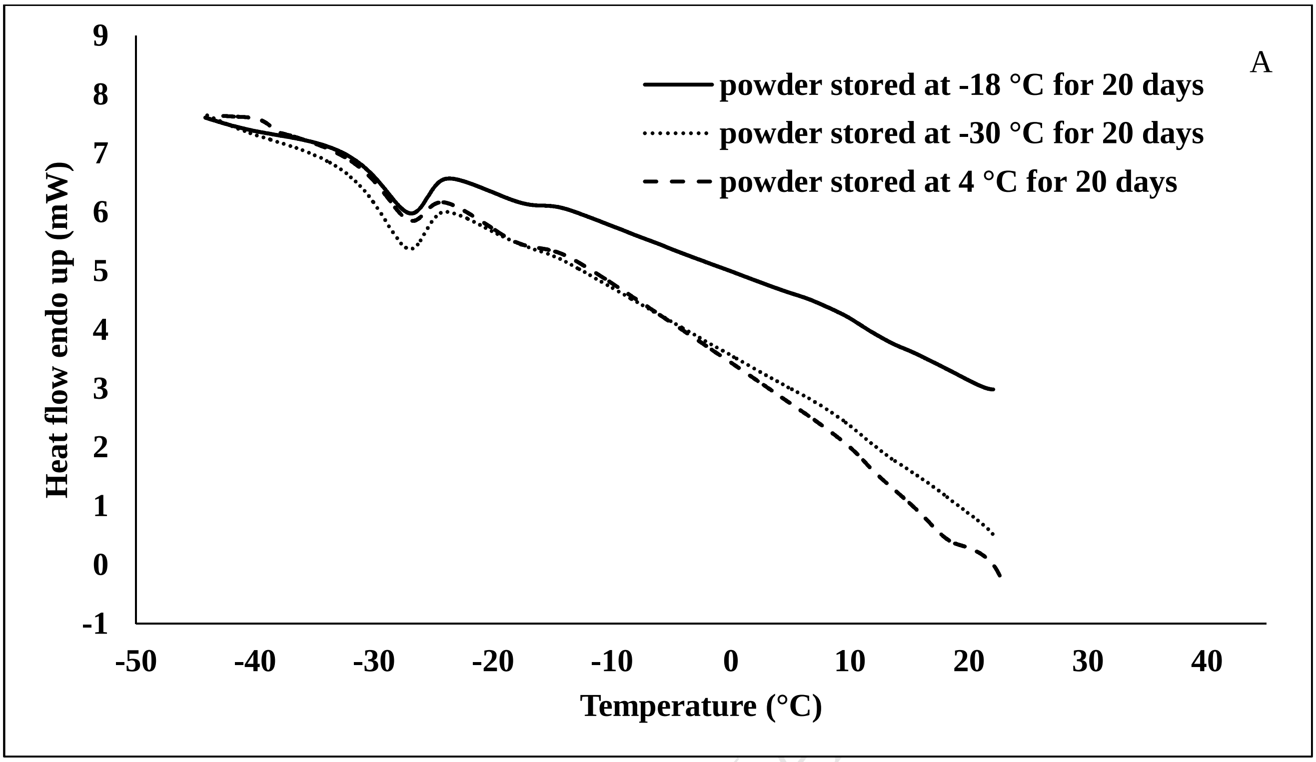
A
powder stored at -18 °C for 20 days
powder stored at -30 °C for 20 days
powder stored at 4 °C for 20 days
Heat flow endo up (mW)
9
8
7
6
5
4
3
2
1
0
-1
-50
-40
-30
-20
-10
0
10
20
30
40
Temperature (°C)

Fig. 9B

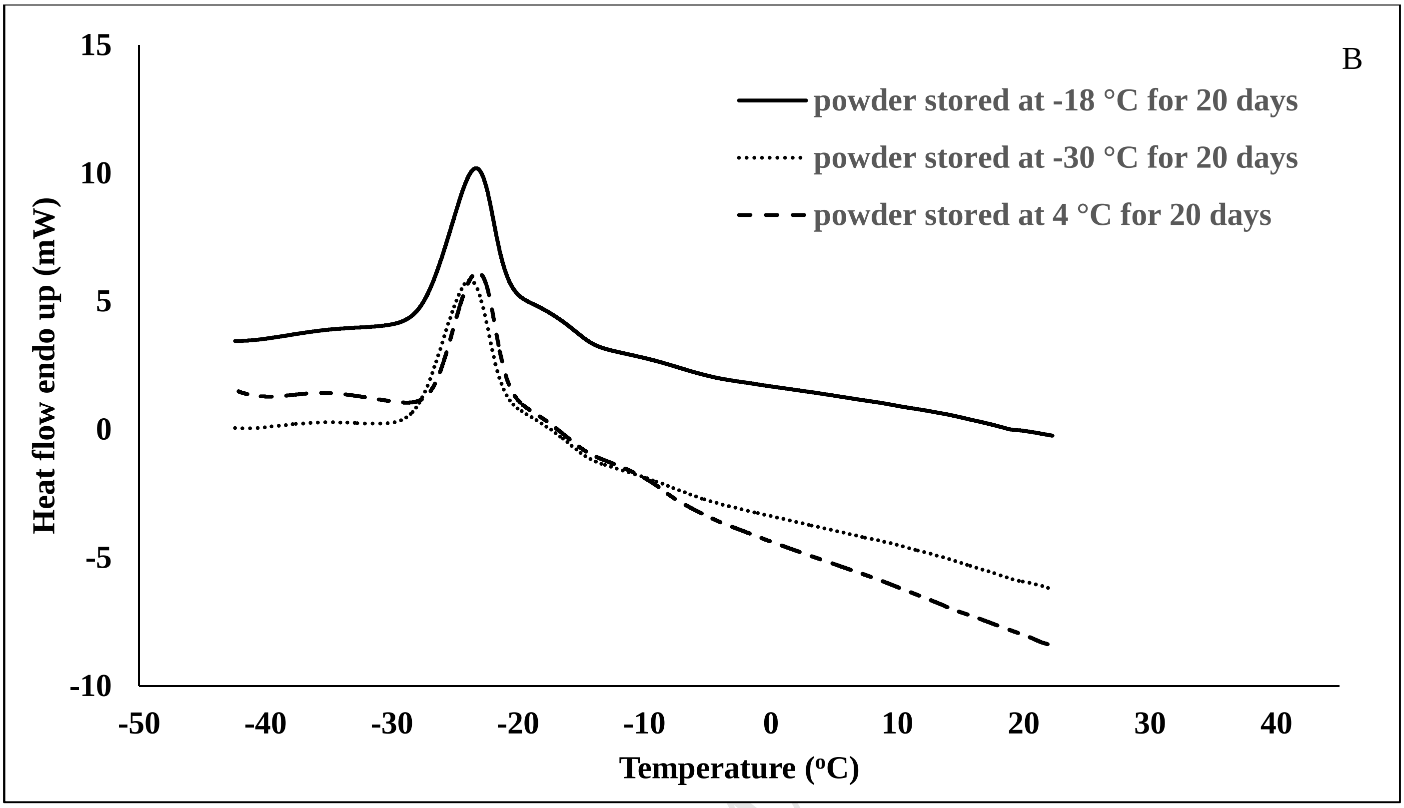
B
powder stored at -18 °C for 20 days
powder stored at -30 °C for 20 days
powder stored at 4 °C for 20 days
Heat flow endo up (mW)
15
10
5
0
-5
-10
-50
-40
-30
-20
-10
0
10
20
30
40
Temperature (°C)

Fig. 10

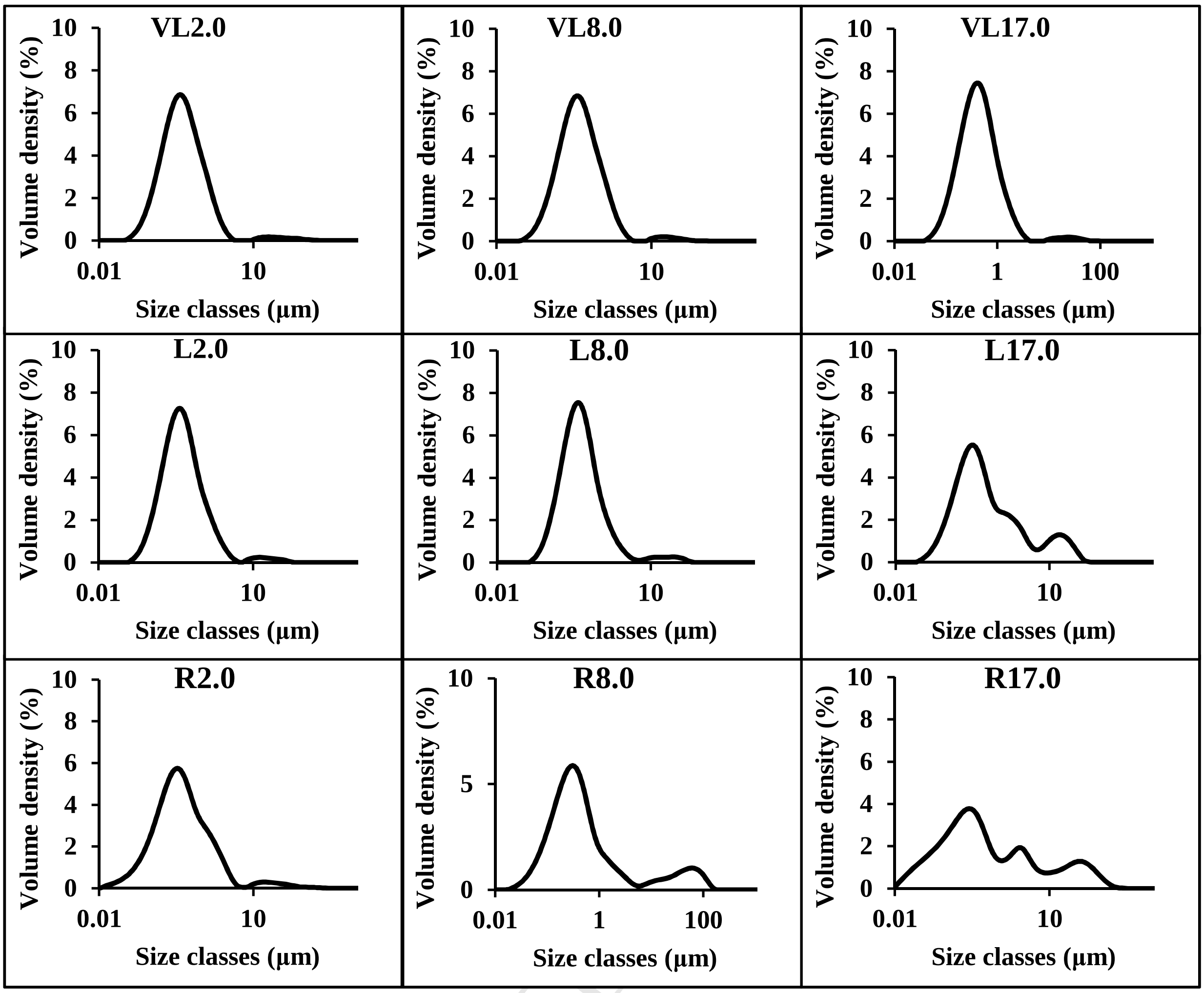
VL2.0
VL8.0
VL17.0
L2.0
L8.0
L17.0
R2.0
R8.0
R17.0
Volume density (%)
Size classes (µm)
0
2
4
5
6
8
10
0.01
1
10
100

Fig. 11A

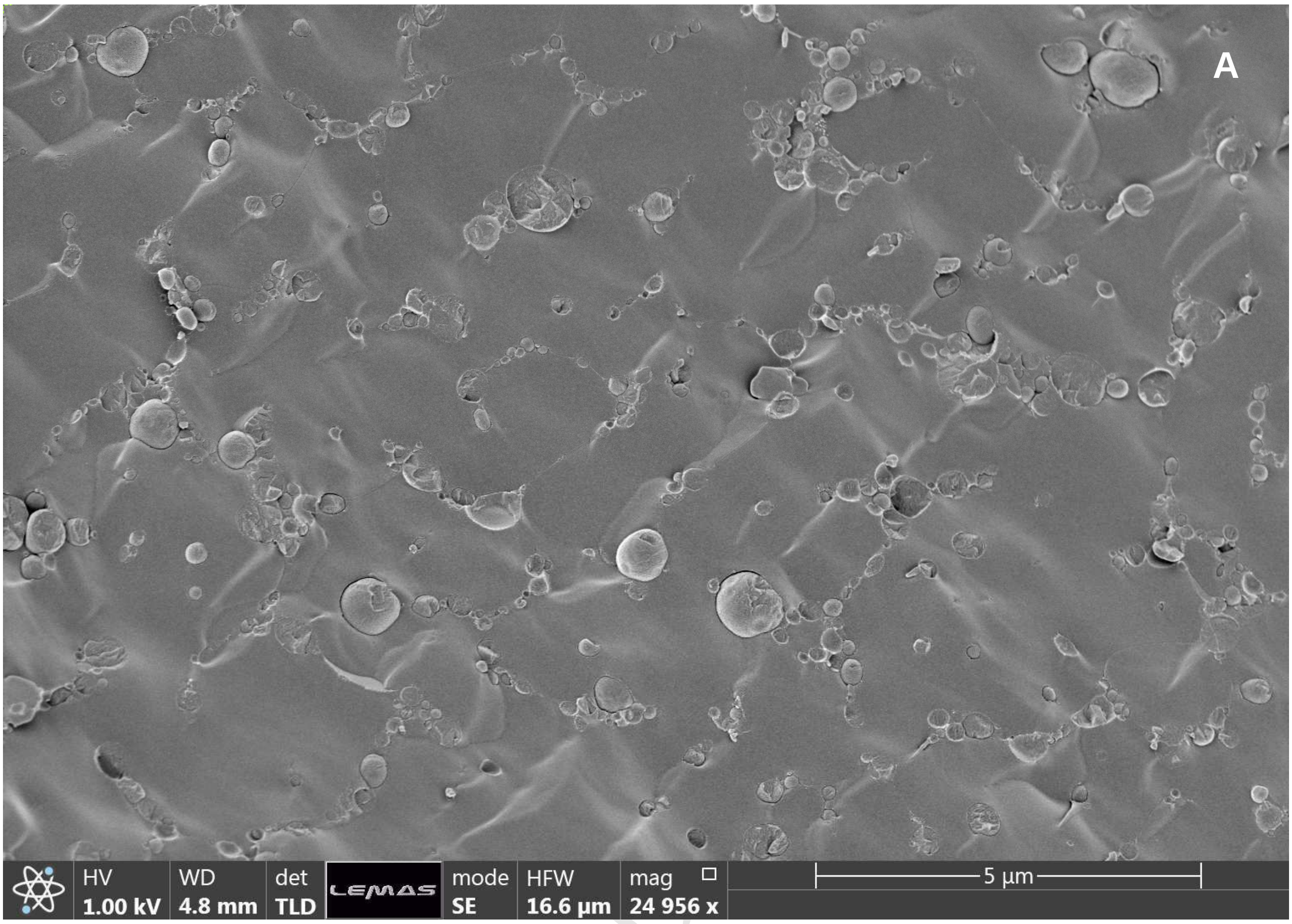
A
HV 1.00 kV
WD 4.8 mm
det TLD
LEMAS
mode SE
HFW 16.6 µm
mag 24 956 x
5 µm

Fig. 11B

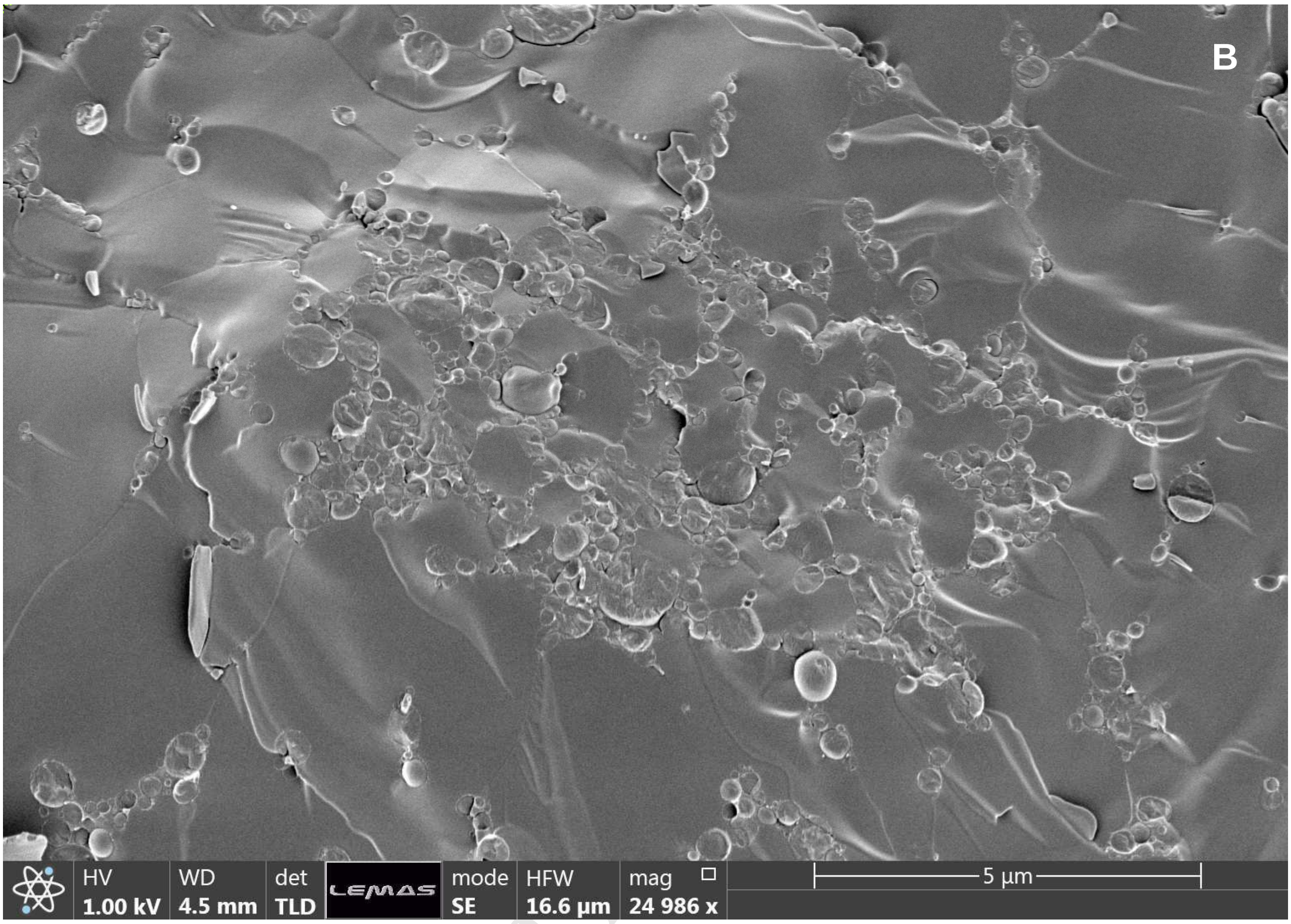
B
HV
1.00 kV
WD
4.5 mm
det
TLD
LEMAS
mode
SE
HFW
16.6 µm
mag
24 986 x
5 µm

Fig. 11C

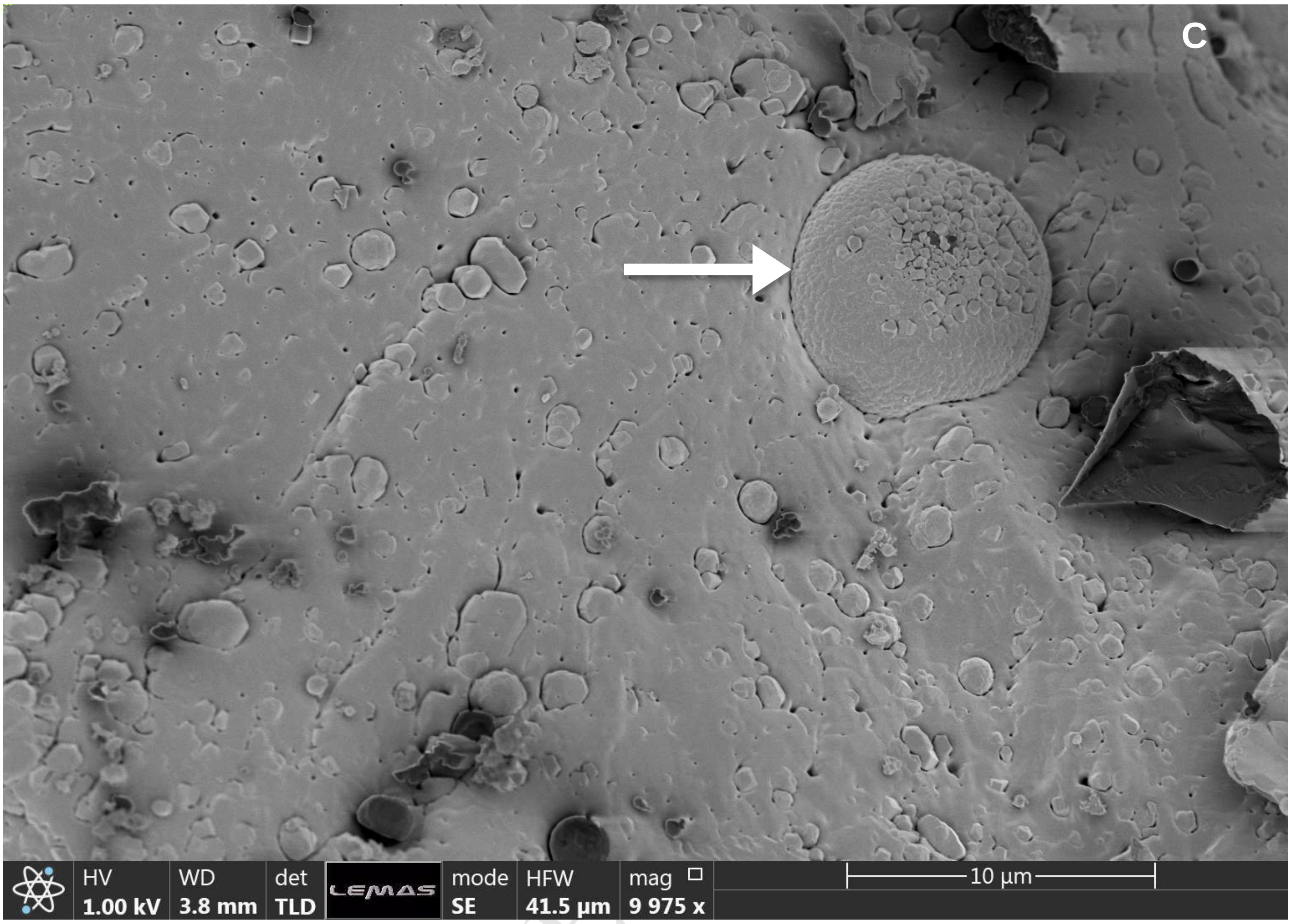

Table 1.

Sample	n	r	p	Sample	n	r	p
R	9	0.89	0.0007	R	9	0.89	0.0007
HRH	6	0.91	0.0060	F	8	0.84	0.0041
LRH	5	0.88	0.0254	L	9	0.86	0.0016
				VL	9	0.42	0.1314

Highlights

1) The impact of dry storage conditions on properties of reconstitutable emulsions has been investigated.

2) Dried OSA starch stabilised emulsions, kept for 3 weeks, produced 2 μm droplets by gentle rehydration, stable for 100 days.

4) Dry storage temperature plays a major role in determining the size and stability of reconstituted emulsions.

5) Humidity, in the temperature range studied, was at best only a minor factor in determining the quality of reconstituted emulsions.