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Microstructure and long-term stability of spray dried emulsions with ultra-high oil content

Anwesha Sarkar [1, 2]*, Judith Arfsten [2], Pierre-Alain Golay [2], Simone Acquistapace [2] and Emmanuel Heinrich[2]

[1] Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK

[2] Nestlé Research Center, Vers-chez-les-Blanc, P.O. Box 44, CH-1000 Lausanne 26, Switzerland

*Corresponding author:

Dr. Anwesha Sarkar

Food Colloids and Processing Group,

School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT, UK.

E-mail address: A.Sarkar@leeds.ac.uk (A. Sarkar).

Tel.: +44 (0) 113 3432748.

Abstract

The aim of this study was to investigate the microstructure and long-term mechanical as well oxidative stability of a new class of spray dried emulsion containing ultrahigh oil content. Emulsion (20 wt% oil) stabilized by whey protein (1 wt%) was thermally cross-linked at 82°C for 10 minutes and spray dried without any additional wall materials using inlet/ outlet air temperature of 105 ± 2/ 65 ± 2 °C, respectively at a pilot scale. Confocal micrograph showed cohesive cross-linked whey protein film present at the oil-water interface and at the powder surface stabilising the oil powder particles containing 95.3 wt% oil. The mean droplet size of parent emulsion (0.21, 0.38, 0.76, 2.31 μm) significantly influenced the mechanical stability of the resulting oil powder in terms of oil leakage (2.73, 0.93, 4.1, 7.54 wt%) upon compaction. Scanning electron microscopy revealed the level of surface oil and porous "sponge" like internal microstructure of the oil powder with polyhedral, closely packed droplets. Strong correlations existed between the mechanical properties of the oil powder and the oxidative stability over 5 months. The kinetics of oxidation of oil powder was higher than that of corresponding bulk oil with or without added antioxidants as evidenced by evolution of primary oxidation products (hydroperoxides) and secondary oxidation products (hexanal). This might be due to the multi-step processing (e.g. homogenization, thermal cross-linking, spray drying) as well as inability of the cohesive but permeable protein matrix to protect the ultra-high content of oil droplets from diffusion of oxygen and prooxidants.

Keywords

Spray dried emulsion, oil powder, ultra-high oil content, mechanical stability, oil oxidation, cross-linked interface

1 Introduction

The use of solid-like hydrophobic matrices such as high internal phase emulsion gels with tuneable properties have been known for applications in fuels, oil recovery, pharmaceutical and personal care industries (Cameron, 2005). Recently, there has been increasing research interests of creating such high internal phase hydrophobic matrices using food grade ingredients to alter rheological properties as well as to impart solid-fat functionality to liquid oils using environment friendly ingredients and processing conditions (Nikiforidis & Scholten, 2015; Patel, Rodriguez, Lesaffer, & Dewettinck, 2014; Romoscanu & Mezzenga, 2006). An alternative approach to create structured hydrophobic liquids is to create oil powder using emulsion templating as a starting point followed by spray drying where the resulting oil powder will contain ultrahigh content.

Spray drying is one of the oldest and most widely used encapsulation methods in food industries to deliver emulsified food ingredients such as flavours and other lipophilic bioactive ingredients (Ixtaina, Julio, Wagner, Nolasco, & Tomás, 2015; Jafari, Assadpoor, Bhandari, & He, 2008; Taneja, Ye, Jones, Archer, & Singh, 2013). The process of creation of spray dried systems based on oil-in-water emulsion involves creation of emulsions using homogenization, followed by addition of wall material (e.g. starch, lactose, maltodextrin, polysaccharides) before atomization of the mixture into the drying chamber. The addition of carbohydrate based wall materials enables formation of glassy matrices to retard the diffusion of oxygen and enhance oxidative stability (Ubbink & Krüger, 2006). However, the compromise lies in the oil content in resulting powder being 5-30 wt%.

A promising new route to create oil powder with 90 wt% oil content from liquid oil has been developed by Mezzenga & Ulrich (2010). The method has utilized protein-stabilized oil-water interface (β-lactoglobulin), cross-linking the protein absorbed at the interfaces via a heat denaturation process and successively spray drying without the addition of any carbohydrates. Thus, this approach resulted in decreasing the solid dry base and enhancing the content of hydrophobic liquid of the final powder. Thermal cross-linking of the interfacial layer after emulsion provided enough elasticity to the interfaces and sufficient barrier against droplet coalescence. A key open question lies in the oxidative stability of such oil powders with ultra-high oil content. In addition, a critical issue often faced when dealing with encapsulated oil systems is that the oil might leak out during processing food applications in industrial line or bulk compression during shipping over distances due to the insufficient mechanical strength of the matrices to survive shear and normal stresses.

Hence, the objective of this study was to first understand the microstructure and morphology of spray dried emulsions with ultra-high oil levels at a molecular level and then to investigate the long-term mechanical stability and oxidative stability of such new class of microstructure over a period of 5 months. In this study, spray dried whey protein-stabilized emulsion with resulting powder containing ultra-high oil levels (> 95 wt%) was created using appropriate formulation design and a scaled up approach. Post emulsion creation, heat treatment of 82 °C/ 10 minutes was used to enable whey protein denaturation followed by irreversible aggregation reactions at the oil-water interface involving hydrogen bonding, hydrophobic interactions and covalent disulphide linkages resulting in formation of cross-linked film (Euston, Finnigan, & Hirst, 2000; Jiménez-Flores, Ye, & Singh, 2005; Tolkach & Kulozik, 2007; Hammann, & Schmid, 2014) before spray drying. Our hypothesis is that this cross-linked protein film alone without added carbohydrate molecules enables emulsions to dry effectively in a spray dryer without oiling off as well as might protect the oil against any mechanical instability and oxidation during long-term storage.

For studying mechanical stability, we have developed a simple and effective technique to measure quantitatively oil leakage from oil powder under compression (Bahtz, 2010), which might find its use in food industries. The oxidative stability of the oil powders was estimated using characterization of a combination of primary oxidation (hydroperoxides) and secondary oxidation (hexanal) products (Sarkar, Golay, Acquistapace, & Craft, 2015) with or without the addition of relevant antioxidants such as ascorbyl palmitate (AP) and mixed tocopherols (M-TOC) (Velasco, Dobarganes, & Márquez-Ruiz, 2000).

To our knowledge, this is the first study that investigates long-term mechanical and oxidative stability of this new class of spray dried emulsions containing ultra-high oil levels in a pilot scale.

2 Materials and Methods

2.1 Materials

Whey protein isolate (WPI) containing ~ 98% protein (Bipro, Davisco Foods International, US) was used without any further purification. Refined bleached deodorized soy oil (SO) was sourced commercially from the Cargill Inc., Brazil. Upon receipt, oils were stored in their original container in the dark at 4 °C until evaluated for fatty acid (FA) composition and stability. For the oxidative stability study, antioxidants such as ascorbyl palmitate (AP) and mixed tocopherols (M-TOC) purchased from Sigma-Aldrich, US were added to the oils. The M-TOC was a mixture of α-, β-, γ- and δ-tocopherols and contained ≥ 500 mg/g total tocopherol content as stated by the manufacturer. Chloroform, methanol, hexane, acetic acid and isooctane were purchased from Merck, Darmstadt, Germany. Hexanal, purity 98% and hexanal-d_{12}, isotopic enrichment min. 98% atom were purchased from CDN Isotopes, Canada. All the solutions were prepared from analytical grade chemicals unless otherwise specified. Demineralized softened water was used for the preparation of all solutions.

2.2 Preparation of oil powder with ultra-high oil content

Oil powder with ultra-high oil level was prepared at pilot plant level at Nestlé Research Center, Lausanne using an adapted formulation design based on approach described by Mezzenga & Ulrich (2010), which is schematically illustrated in Figure 1 and described below.

2.2.1 Emulsion preparation and thermal treatment

Protein solution (1.0 wt%) was prepared by dispersing 0.48 kg WPI in 47.52 kg dimineralized softened water (160 mg/ L Na, < 0.1 mg/ 100g Fe, < 0.02 mg/ 100g Cu and < 0.1 mg/ 100g Zn) water. Protein solution was stirred for 1 h at 20 °C using a mixer (X50/10, Ystral GmBH, Ballrechten-Dottingen, Germany) to ensure complete dissolution and adjusted to pH 7 using 0.1 M NaOH/ HCl. 20 wt% oil-in-water emulsions were prepared by mixing 12 kg soy oil and 48 kg aqueous protein solution.

Soy oil was used directly for preparation of emulsions used in this study. However, for the oxidative stability study, antioxidants (300 ppm AP, 300 ppm AP + 1000 ppm M-TOC) were added with gentle stirring to the SO before emulsion preparation based on a previous study (Sarkar, et al., 2015). For the pre-emulsification, oil was slowly added into the aqueous solution over a period of 4 min and sheared using conventional rotor–stator type mixer (Polytron PT 120/4 M, Kinematica AG, Lucerne, Switzerland) at a speed of 3000 rpm for 10 minutes, until a target droplet size of 13-15 μm was reached. The pre-emulsions were then homogenized using a two-stage valve homogenizer (Panda Plus 2000, GEA Niro Soavi - Homogeneizador Parma, Italy) in continuous mode operating at first stage/ second stage pressures: 2 passes × 100 / 25, 1 pass × 200 / 100, 1 pass × 400 / 100 and 1 pass × 1200 / 100, to create different droplet sizes as shown in Table 1, respectively. For the long-term stability study and microstructural characterization, emulsions created with 1 × 400/ 100 bar pressures were used to prepare the oil powders.

Plate heat exchanger (Alfa Laval, Lund, Sweden) in line with tubular heat exchanger (Sulzer SMXL DN20, Winterthur, Switzerland) was used for the subsequent thermal cross-linking of the whey proteins at the oil-water droplet interfaces. The temperature was increased to 82°C and held for 10 min using tubular heat exchanger. The cross-linked emulsions were stored at 4°C until its further use. Emulsions were prepared in triplicates for analyses.

2.3 Spray drying

Spray drying of the thermally cross-linked emulsions was performed in a pilot plant scale spray dryer (Niro SD-6.3-N, GEA Process Engineering A/S, Søborg, Denmark). The emulsions were fed into the spray tower through a peristaltic pump at a feed flow rate of 10 L/h and atomized by a spraying rotary disc (25,000 rpm). Inlet and outlet air temperature were 105 ± 2 and 65 ± 2 °C, respectively. The oil powder was collected into aluminium pouches and stored at 4 °C unless otherwise mentioned.

2.4 Chemical composition and physical properties

Moisture (AOAC 925.04) and crude protein content (AOAC 981.10) of the oil powder were determined by standard method (AOAC, 1995). The oil content was measured using adapted Folch extraction method (Dionisi, Golay, Aeschlimann, & Fay, 1998). Briefly, oil powder was treated with methanol/chloroform mixture (2:1, v/v) in dark followed by homogenizing using ultraturrax for 2 min to break the encapsulating protein matrix and then centrifuged at 3000 rpm for 20 min. The liquid phase was filtered and collected. The sample left in the centrifuge tube was homogenized for 2 min with chloroform / methanol (1:1, v/v). After the second centrifugation, the liquid phase was filtered and added to the previous collection. The organic phase was evaporated to dryness under vacuum at 40°C and oil content determined gravimetrically. Fatty acid (FA) profile was determined using preparation of FA methyl esters, followed by gas chromatographic detection using standard method (AOAC, 2012).

Bulk density of the oil powder was measured using JEL jolting density metre (Gemini BV, Apeldoorn, Netherlands). Briefly, volume of a given mass of oil powder after 1250 taps was measured to calculate the tapped bulk density (Fitzpatrick, Iqbal, Delaney, Twomey, & Keogh, 2004).

2.5 Particle size measurement

A static light scattering instrument (Malvern MasterSizer 2000, Malvern Instruments Ltd, Worcestershire, UK) was used to determine the average droplet size and the overall size distribution of the emulsions before and after thermal cross-linking step. The relative refractive index (N) of the emulsion was 1.095, i.e. the ratio of the refractive index of soy oil (1.456) to that of the dispersion medium (1.33). The absorbance value of the emulsion particles was 0.001. The Sauter-mean diameter, d_{32}

($=\sum n_i d_i^3/\sum n_i d_i^2$) where n_i is the number of particles with diameter d_i of the emulsion droplets was measured. The average droplet size and the droplet size distribution of the emulsions were measured by dispersing in aqueous medium using Mie diffraction. The particle size of the oil powder was also analysed using static light scattering. In this case, the powder was dispersed at 3000 rpm for 5 min in medium chain triglycerides (MCT) and then analyzed by dispersing in MCT. All measurements were made at room temperature on at least three freshly prepared samples. Mean droplet diameter of the emulsions and particle diameters of oil powders were calculated as the average of five measurements.

2.6 Determination of surface coverage

The surface coverage was measured using indirect method described by Srinivasan, Singh, & Munro (1996). Briefly, the emulsions before and after heat treatment were centrifuged at 45000 g for 40 min at 20 °C in a temperature-controlled centrifuge (Sorvall RC5C, DuPont Co., Wilmington, DE). The subnatants were carefully removed using a syringe and filtered sequentially through 0.45 and 0.22 μm filters (Millipore Corp., Bedford, MA). The filtrates were analyzed for total protein using the Kjeldahl method (AOAC 981.10). The surface protein concentration (mg/m^2) was calculated from the surface area of the oil droplets, determined by MasterSizer 2000, and the difference in the amount of protein used to prepare the emulsion and that measured in the filtered subnatants.

2.7 Confocal scanning laser microscopy (CSLM)

Oil powder was observed using Zeiss LSM 710 confocal microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany), where the whey protein was stained using 0.1 mL of 1.0% (w/v) Rhodamine 6G dye and imaged using ×63 objective at an excitation of 543 nm.

2.8 Morphology using scanning electron microscopy (SEM)

The particle morphology and internal microstructure of oil powder was characterized immediately after production as well as after 9 months of storage at 25 °C in aluminum pouches. Oil powders were also characterized after removal of hexane extractable surface oil (Carneiro, Tonon, Grosso, & Hubinger, 2013). Briefly, oil powder was washed with hexane (1:10 w/w), shaken for 10 minutes followed by filtration of the powder using Whatman filter paper and the powder collected on the filter was rinsed three times with 20 mL of hexane. Oil powders with or without removal of surface oil were mounted on sample holders with double-side adhesive tape. A part of the sample was cut with a razor blade to reveal the inner structure of the particles. Samples were fixed with 1% OsO_4 vapour during 24 hours. Visualization of samples was carried out in a Quanta 200F FEG microscope (FEI Company, Eindhoven, Netherlands), operated at 10 kV in low vaccum mode and equipped with a Backscatter Detector and no gold was sputtered. The samples were observed with wide range of magnifications of ×1000, ×5000 and ×10,000.

2.9 Oil leakage using mechanical compression

Mechanical stability of oil powders was measured using a simple and reproducible method developed on the basis of underpinning principle of compaction force testing of granulated powder (de Freitas Eduardo & da Silva Lannes, 2007). The amount of oil leaked out from the powder structure upon applying a compaction force was gravimetrically measured using a Texture Analyzer (TA XT PLUS, Stable Micro Systems, Ltd., UK) with a back extrusion A/BE assembly. Compression test was performed for freshly prepared oil powders as well as for the oil powders stored over a period of 15 months at 4 °C/ 70% RH conditions.

Oil powder (0.9 g) was filled into a cylindrical measuring cell and compressed by a compatible piston in a texture analyzer (probe, 20 mm diameter) (Bahtz, 2010). For each measurement, a layer of five filter papers (Whatman; 90 mm in diameter) was placed beneath the cylindrical measuring cell before filling with the oil powder sample. The piston was moved downward with a controlled speed of 0.5 mm/s and force of 30 N. The amount of oil leaked due to the mechanical compression and adsorbed to the filter papers (previously dried in a desiccator) was then determined gravimetrically by weighing the filter papers before and after the compression test. The oil leakage (%) represents the mass percentage of leaked oil related to the total oil present in the powder after compression. Mean value was calculated as average of five measurements for each oil powder formulation.

2.10 Measurement of lipid oxidation

For the oxidative stability tests, the oil powders without or with added antioxidants (300 ppm AP, 300 ppm AP + 1000 ppm M-TOC) were separately sealed in 50g aluminium pouches and amber glass vials (20 mL), and stored at 4 °C and 35 °C for 20 weeks. For comparison, corresponding bulk oils (non-encapsulated) without or with added antioxidants were also stored under the same temperature conditions. Storage of oil powders at 35 °C was carried out as an accelerated storage test to provide insights of oxidative stability for ambient storage. “1 week” at 35 °C is intended to be the equivalent to “1 month” of ambient storage conditions (18 °C) as expressed by Arrhenius equation (Taoukis & Labuza, 1996). The stored oils and oil powders were evaluated for oxidation by two distinct methods selected for primary and secondary oxidation products analyses (i.e. peroxide value and hexanal quantification using isotopic dilution method by headspace solid-phase microextraction (SPME) gas-chromatography mass-spectrometry, respectively).

2.10.1 Peroxide value

Oil was extracted in the dark from the oil powders stored at 4 °C and 35 °C using the modified Folch cold extraction method as described before (Section 2.4). Peroxide value (PV) (meq/kg of oil) of the extracted oil and bulk oils was measured in six replicates by titration with 0.1 N sodium thiosulphate, using starch indicator according to AOCS Method Cd 8b-90 (AOCS, 1997).

2.10.2 Hexanal determination in oil powder

Volatile (hexanal) was quantitatively determined following the method previously described by (Sarkar, et al., 2015). Briefly, oil powders and bulk oils previously weighed (2 ± 0.01g) in 20 mL amber glass vials were spiked with hexanal-d_{12} (120 μg) as internal standard before the analysis. The GC-MS analyses were performed on a Thermo Finningan GC TOP 8000 equipped with a CTC PAL injector coupled to a Thermo Finningan Voyager quadrupole mass spectrometer (Brechbühler Schlieren, Switzerland). A splitless injector with linear velocity of 30 mL/min and Helium as a carrier gas at constant pressure of 150 KPa were used. A DB-5MS (5% Phenyl 95% dimethylpolysiloxane, Brechbühler, Schlieren, Switzerland) capillary column with 60 m length, 0.25 mm internal diameter and 0.25 μm film thickness was employed. The column temperature was held at 50 °C for 10 min and increased to 250° at 10°C/min and held for 10 min at this temperature. The temperatures of the ion source and transfer line were 200 and 220°C, respectively. Electron impact mass spectra were recorded at 500 V in the 35-250 u mass range, at two scans/s. Volatiles were identified by usual MS-libraries. The concentration of volatiles was determined using calibration standard solutions in water containing hexanal and labeled hexanal (hexanal-d_{12}) as internal standards. The quantification was performed by plotting the peak area ratio of hexanal and hexanal-d_{12} of the standard solution against the concentration ratio of hexanal and hexanal-d_{12} determined in the sample in triplicate. The amount of hexanal (expressed in μg/g) was calculated according to the following equation:

$$\text{Content} = \frac{\left(A_A / A_{IS}\right) - I * m}{S * SW}$$

where, AA = area of hexanal in the sample, AIS = area of internal standard in sample, I = intercept of the calibration curve's equation, S = slope of the calibration's curve equation, m = amount of internal standard (μg) and SW = sample weight (g). Mean value is the average of triplicate measurements.

in oil against internal standard (hexanal-d_{12}). Analyses of hexanal in headspace were performed in triplicate.

2.11 Statistical analyses

The results were statistically analyzed by analysis of variance (ANOVA) using Graphpad 5 Prism software and differences were considered significant when $p<0.05$ were obtained.

3 Results and discussion

3.1 Characteristics of oil powder

Figure 1 shows the visual aspect of the resulting dry granular oil powder after the spray drying of the thermally cross-linked oil-in-water emulsions. The oil powder had a light texture and did not have any stickiness or evidence of oil leakage even after several months of storage at a macroscopic level. However, on shearing between fingers or between the oral palate in the mouth, the oil phase was gradually released giving a creamy tactile/ mouth feel. As presented in Table 1, the oil powder had 0.21 wt% moisture content. Ultra-high quantities of oil of 95.3 wt% were encapsulated in the powder. The experimental oil/protein ratio in resulting oil powder (26.1) agreed reasonably well with the theoretical oil/protein ratio in the starting emulsions (25.5). This close agreement of data also confirms the precision of the cold Folch extraction method for complete extraction, where the use of high shear played a significant role to break the wall of encapsulated material and liberate the oil from the cross-linked whey protein matrix. As expected, the fatty acid analysis of the oil powder corresponded to that of soy oil (Sarkar, et al., 2015), suggesting no change in the oil composition during oil powder formation and/or extraction. The bulk density of the oil powder was 0.44 g/ cc, which is within the range of high fat milk powders (Sharma, Jana, & Chavan, 2012).

3.2 Particle size of emulsions and oil powder

Emulsification, cross-linking and spray drying are the three key steps in the preparation of this ultra-high content oil powder (Figure 1). The droplet size distributions of the 20 wt% oil-in-water emulsions prior to, after the heat treatment process and particle size distribution of the resulting oil powder are shown in Figure 2. The emulsions formed by homogenisation at 400/100 bars had a monomodal droplet size distribution with the majority of droplets being in the range of 0.1-4.0 μm, with an average droplet size (d_{32}) of ~0.38 μm (Table 2). As expected at pH 7, which is sufficiently above the pI of whey protein, the whey proteins at the interface had a net negative charge that was sufficient to prevent the emulsions from flocculation through strong electrostatic repulsive forces.

After the thermal cross-linking treatment at 82 °C/ 10 minutes, the emulsions showed a slightly wider size distribution compared with the parent emulsions, ranging from 0.1 to 10 μm, with an increased d_{32} value of 0.67 μm. There was no significant difference between the d_{32} value of the cross-linked emulsion and the emulsion before heat treatment ($p>0.05$). The cross-linked emulsions were homogenous and kinetically stable showing no flocculation during the period of study. The surface protein coverage increased from ~1.1 to ~2.1 mg/m^2 on thermal treatment of the emulsions (data not shown). The significant increase in total surface protein coverage of whey-protein-stabilized emulsions on thermal treatment is in agreement with the results of previous workers (Monahan, McClements, & German, 1996; Sliwinski et al., 2003; Jiménez-Flores, Ye, & Singh, 2005). This suggests that intra-droplet protein-protein interactions were favoured in our study than inter-droplet flocculation, resulting in a sufficiently cohesive and elastic adsorption layer at the interface (Monahan, McClements, & German, 1996; Romoscanu & Mezzenga, 2005). Upon spray drying, the oil powder showed a monomodal distribution and an average droplet size (d_{32}) of ~37 μm and thus was around 50 times larger than the individual cross-linked emulsion oil droplets. The oil powder had partial redispersibility in water after reconstitution, which was also highlighted by Mezzenga & Ulrich (2010). This might be attributed to the ultrahigh content of hydrophobic phase and possible presence of surface oil. Hence, droplet size in the powdered emulsions after reconstitution in water could not be measured with accuracy and hence not reported.

To understand the effect of drying on the emulsion droplet size, CLSM was employed. The CSLM of the oil powder (Figure 3A), which was labelled with protein stain (Rhodamine 6G) showed that the oil powder granules were of a variety of size. Each oil powder granule contained oil droplets separated by a very thin layer of protein film. The oil droplets located within the powder matrix as observed in CSLM images were uniform small droplets evenly distributed within the cross-linked viscoelastic protein matrix. A relatively small proportion of large droplets observed within the powder in the CLSM images could be attributed to the droplet coalescence owing to shear induced rupture of the interfacial layer during the rotary atomisation step (Jafari, et al., 2008; Taneja, et al., 2013). It is worth noting here that the emulsions stabilized by whey protein monolayer alone at this oil/protein ratio without the thermal cross-linking step could not withstand the spray drying conditions and oiled off dramatically. This indicates that the cross-linked protein shell was essential to stabilize the individual droplets against droplet coalescence.

Interestingly, as observed in the higher magnification image (Figure 3B); the protein shell enveloping each powder granule was comparatively thicker than the protein layers surrounding each individual oil droplets within the powder granule. This is expected due to the surface-activity-led diffusion and subsequent adsorption of the excess non-adsorbed protein at the air-water interface of the powder during the final stages of water evaporation during spray drying (Adhikari, Howes, Bhandari, & Langrish, 2009; Mezzenga, et al., 2010). During spray drying and the associated mass transfer, the water in the continuous phase of the emulsions moves along a concentration gradient towards the surface (Jones et al., 2013; Kim, Chen, & Pearce, 2009). As it can expected, water being small molecule diffused faster and carried with it larger whey protein molecules present in the continuous phase, which cannot diffuse as quickly in the opposite direction. The emulsified oil and adsorbed protein were also carried along in the convective flux of water moving toward the surface. This movement continued until the continuous phase became relatively immobile and underwent shrinkage thereafter as the water evaporated. Hence, it appears that whey protein concentration of 3.6 wt% was sufficient to stabilise the emulsion (~ 55% adsorption ratio, data not shown) as well as had non-adsorbed protein contributing to the surface stabilization of drying droplets.

3.3 Influence of emulsion droplet size on mechanical stability of oil powder

During the use of the oil powder in real industrial line for manufacturing of various product applications, the oil powder would essentially be subjected to different levels of mechanical stresses. Depending on the applied stress, one could anticipate that the protective cross-linked protein shell could be weakened, and above a critical level, it might be ruptured, leading to the leakage of the encapsulated emulsified oil droplets. Therefore, the mechanical stability of the oil powder was investigated by quantitatively measuring the oil leakage under normal compression (Bahtz, 2010).

To understand the effect of droplet size of parent emulsion on powder particle size and mechanical stability of the resulting oil powders, the homogenization pressure was varied to create emulsions of a range of size distributions (Table 2). With the same ratio of oil/protein, the increase of homogenization pressure in the range of 100–1200 bars led to a steady reduction of average oil droplet size (d_{32} values between ~2.31 and 0.21 μm, respectively). This decrease in oil droplet size was due to the higher levels of shear forces associated with increased homogenizing pressure applied (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001).

As shown in Table 2, varying emulsion droplet size before drying did not have a significant influence on powder particle diameter ($p > 0.05$), which is in agreement with findings of previous studies (Danviriyakul, McClements, Decker, Nawar, & Chinachoti, 2002; Ye, Anema, & Singh, 2007). However, the level of oil leakage on mechanical compression decreased significantly with decreasing oil droplet size until 0.38 μm ($p < 0.05$). This is in line with previous study, which shows that droplet diameter has a significant effect on burst strength during compression testing, with smaller capsules sustaining higher stresses before bursting out (Keller & Sottos, 2006). However, when the d_{32} of the emulsion droplets further decreased to 0.21 μm at a homogenization pressure of 1200 × 100 bars, the surface area increased significantly and the available whey protein might not have been sufficient to coat the increased surface area of droplets generated. Thus increase of oil leakage to 2.7% was observed.

In the long-term stability experiments, research focusses on oil powder, which was prepared using 0.38 μm sized emulsion droplets (400/ 100 bar pressure) as it had the least oil leakage.

3.4 Long-term mechanical stability of oil powder

To investigate the long-term mechanical stability, oil powder stored at 4 °C for a period of 9 months was tested for oil leakage. As shown in Figure 4, the kinetics of oil leakage followed three-stage behaviour. There was a slow increase in the degree of oil leakage until 5 months. Then, both the kinetics and extent of oil leakage increased significantly until the end of 9 months followed by a plateau thereafter. Even after long storage time of 15 months, the powder did not show more than 10 wt% of oil leakage on compaction. The behaviour upto 5 months might be attributed to the gradual leakage of the surface oil being present. With subsequent storage and inter-particle friction, there might be rupture of the protein matrix and oil might have oozed out of the capillaries (if any) during the 5-9 months period, which might have resulted in such rapid rate of oil leakage.

To understand the microstructure at a deeper length scale, SEM allowed investigation of the internal and external morphologies of the oil powders after production as well as after storage (Figure 5). Analysing the external morphology (Figure 5A), oil powder granules of a variety of sizes showed a spherical shape, smooth surface with no apparent visible cracks or fissures. As compared to previous literatures (Gallardo, et al., 2013; Jafari, et al., 2008), the appearance of characteristic concave surface on oil powder as a result of spray drying was not observed in our study, which might be expected due to substantially higher oil content.

Although there was no visible appearance of free oil or stickiness at the macroscopic level, the surface of the oil powder at the microscopic length scale showed coverage by a layer of oil and also showed “free oil droplets” at the surface. It can be argued that these layers of oil were causing inter-particle bridge formation and (Figure 5A) might have covered any surface cracks or fissures if at all generated. As observed in Figure 5B, the stored sample did not show any visual deterioration or irregularities of structure in terms of emergence of any pores, surface cracks or wrinkling. However, surface oil layer and appearances of “free oil droplets” were further enhanced upon storage (Figure 5B). This suggests that the comparatively higher surface oil available as a function of storage was easier to ooze out of the structure when subjected to mechanical compaction.

The higher magnification images of the internal morphology of the powder with or without storage (Figures 5B and B’) showed less hollowed structure as compared to typical characteristic powder particles obtained in spray drying with higher wall material content. As expected, the structure was supersaturated with oil (> 95%) embedded throughout the oil matrix and the microstructure had some resemblance with cream powder with 75 wt% oil content as observed by Kim, Chen, & Pearce, (2002).

Although there is no clear definition of “surface oil” particularly in this new class of spray dried emulsions with ultra-high oil loads, we can consider it as the hexane extractable oil. This is basically a combination of “free oil” located at the powder surface, oozed out of the cracks or capillaries formed during drying process, insufficiently emulsified oil, coalesced droplets within the powder (Drusch & Berg, 2008; Moisio, et al., 2014) as well as the oil extracted due to the potential hexane-induced breakdown of the hydrophobic protein linkages in the cross-linked whey protein matrix. Hence, to gain deeper microstructure insights, it was interesting to observe the powder after removal of the “hexane extractable” surface oil (Figure 6).

As shown in Figure 6A, the removal of surface oil resulted in dramatic shrinkage of the oil powder granules with clear appearances of wrinkles, dents and open pores. A cross cut of the internal morphology clearly indicated a porous “sponge” like microstructure with deformation of the closely packed oil droplets into polyhedral shapes (Figure 6B). The microstructure showed some larger voids, which might be resulting from hexane-extraction of larger coalesced droplets formed either due to spray drying as seen in CLSM images and also due to the rupture of protein layer due to inter-granular friction on storage (Andersson & Bergström, 2005). Overall, this microstructural evidence together with the quantitative increase of oil leakage as a function of storage suggests that our initial hypothesis of "crosslinked protein film being capable of protecting the encapsulated oil against leakage" cannot be fully validated. Although interfacial cross-linking forms a cohesive film and enables spray drying effectively, the film is not sufficiently dense to completely coat the particle surface, sustain inter-granular friction and protect the encapsulated oil fully from leakage on mechanical compression.

3.5 Long-term oxidative stability of oil powder

Oxidative stability study of oil powder and corresponding bulk oils was carried out at 4 °C and 35 °C, respectively as a function of storage time of 5 months. In an industrial context, many if not most bulk oils contain antioxidants such as AP or M-TOC being added at the suppliers' end to restrict oxidative deterioration. Furthermore, synergistic effects of antioxidants such as AP and M-TOC in microencapsulated system have been well studied (Velasco, et al., 2000). Hence, in this set of experiments, oil powders were produced with added 300 ppm AP or a combination of 300 ppm AP and 1000 ppm M-TOC. As one might anticipate, the bulk oil (unprocessed) and the encapsulated oil fraction (oil extracted from oil powder) displayed different oxidative behaviour. While the free oil oxidized as lipids in continuous phase, oxidation in the encapsulated emulsified oil powder was the result of individual oil droplets oxidizing at different reaction rates.

At 4 °C, PV values of both bulk oil and oil powder with or without any added antioxidants did not show any exceptional increase (≤ 2.0 meq O_2/ kg oil) over the long term storage period of 5 months (Figure 7A). The PV at which soybean oil is unacceptable from a sensory standpoint has been reported to be 2.0 meq O_2/ kg oil (Hawrysh, 1990). Hence, it seems that oil powder without added antioxidant can be stored for 20 weeks without a sharp evolution of hydroperoxides and be still acceptable when stored at 4°C. Furthermore, the PV value was significantly lower than that provided in CODEX STAN 210 for refined vegetable oils, where acceptance limit for PV is 10 meq O_2/ kg oil. However, oil powders had a significantly higher rate and extent of oxidation than the corresponding bulk oil even at 4°C ($p<0.05$). The comparison of oil powder and bulk oil containing antioxidants also elucidated some interesting features (Figure 7A). Addition of AP ($p<0.05$) or AP+M-TOC ($p<0.01$) resulted in significant retardation of generation of hydroperoxides in the bulk oil. However, such beneficial effects of addition of antioxidants were not observed in case of the oil powder.

At 35 °C (Figure 7B), the PV value increased markedly during the storage for both oil powder (54.2 meq O_2/ kg oil) and corresponding bulk oil (38.5 meq O_2/ kg oil). In case of the bulk oils, addition of AP+M-TOC was considerably more effective to prevent the evolution of hydroperoxides above 2.1 meq O_2/ kg oil as compared to AP alone ($p<0.05$) on storage for 5 months. On the other hand, oil powders resulted in faster oxidation kinetics and higher extent of evolution of hydroperoxides (37.2 meq O_2/ kg oil for AP, 48.4 meq O_2/ kg oil for AP+ M-TOC) irrespective of their antioxidant types at 35°C (Figure 7B).

It is worth recognizing that hydroperoxides generated in oil powder was nearly three-fold higher as compared to the corresponding bulk oil at the start of the storage test. This higher extent of hydroperoxides generation in the oil powders at the beginning of the storage study might be expected due to the increased rate of exposure of oil to oxygen, light and higher temperature during multi-step production process of oil powder (i.e. homogenization, thermal treatment, atomization of the feed emulsion followed by spray drying) (Baik et al., 2004, Velasco et al., 2000). In addition, the processing generates oil droplets and thus a huge surface area increasing the degree of oxidation.

One might argue that the cross-linked whey protein film, which is the central concept in this new class of oil powder, was not sufficiently dense enough to provide complete oxygen or light barrier properties to the encapsulated oil. As shown in Figure 8, there existed a strong linear correlation between level of hydroperoxides generated in the oil powder at and oil leakage (%) during the same periods of storage at 4 °C (r^2=0.92). At 35 °C, there existed an exponential relationship between the mechanical and oxidative stability (r^2=0.95). This further highlights that the higher level of oxidative deterioration in case of oil powder was not only driven by the multistep production process but also by the rather porous microstructure of oil powder as well as weaker films incapable of providing sufficient oxygen and light barrier properties.

Although PV provides insights on primary oxidation, the hydroperoxides that might have been converted to secondary oxidation products are generally more informative from off flavour generation and rancidity perspective. To quantify the generation of secondary oxidation products, Figures 9A and B shows the evolution of hexanal concentration (µg/g or ppm) of the oil powders and corresponding bulk oils stored at 4 °C and 35 °C, respectively as function of 5 months' storage time. Hexanal has proved to be a suitable marker for the evaluation of the oxidative status of oil containing linoleic acid, as hexanal is formed from both 9- and 13-hydroperoxides of linoleate and from other unsaturated aldehydes during the oxidation of linoleate (Sarkar, et al., 2015). Since soy oil contained significant amounts of linoleic acid (Table 1), hexanal was identified as a suitable marker for studying the secondary oxidation of the oil powders as a basis for comparison. The higher quantity of hexanal generated can be regarded as a measure of poor sensorial quality. Based on preliminary oxidative stability study (Sarkar, et al., 2015) and sensory tests (data not shown), hexanal generated higher than 1 ppm was considered to be the critical limit to identify any off flavor generation.

At 4 °C (Figure 9A), the bulk oils with and without added antioxidant behaved similarly in terms of evolution of hexanal ($p>0.05$). The evolution of hexanal in bulk oils was much slower and lesser in magnitude than that of corresponding oil powders at 4 °C ($p<0.05$). Interestingly, AP appeared to have slight protective effect in oil powder keeping the hexanal evolution below 1 ppm over the period of study at 4 °C. As it can be expected, at 35 °C, both oil and oil powder showed higher extent of hexanal evolution (5.6 ppm and 5.3 ppm respectively) ($p>0.05$). Although addition of antioxidants had a pronounced protective effect on bulk oils ($p<0.05$), such antioxidant effects were not observed in oil powder. Ascorbyl palmitate (AP) slightly retarded the oxidation kinetics in oil powder as compared to oil powder without any added antioxidants. On the other hand, presence of M-TOC accelerated the oxidation kinetics and extent in case of oil powder. The poor effect of M-TOC might be due to the high levels of tocopherols already present in the soy oil used in the systems and αtocopherol being known to have prooxidative effects in high concentrations (Jacobsen, Let, Nielsen, & Meyer, 2008).

It should be noted that surface area of the oil powder is significantly higher than the surface area of the corresponding bulk oils. One would therefore expect higher lipid oxidation kinetics in this kind of emulsion based encapsulated system as compared to the corresponding bulk oils. In general, the slowing down of such deteriorative lipid oxidation reactions in encapsulated powders is largely dependent on the ability of the encapsulant to keep the oxidizable lipid substrates in the emulsion droplet core (i.e. hydroperoxides generated) well segregated from aqueous phase pro-oxidants (e.g. metals) (Waraho, McClements, & Decker, 2011). It also depends on the ability of the encapsulant to enable lesser oxygen diffusion to the encapsulated oil droplets during storage, thus contributing to longer ingredient shelf life. Furthermore, emulisifier such as whey proteins are known to provide antioxidant effect on the emulisified oil through availability of free sulfhydryl groups (Faraji, McClements, & Decker, 2004; Tong, Sasaki, McClements, & Decker, 2000).

In our study, although thermal treatment might have generated reactive sulfhydryl groups, they were possibly involved in formation of disulphide linkages during creation of cross-linked interfacial film and might not be available to exert the antioxidant effect. It is also noteworthy that although a cohesive film was formed at the droplet interface due to the thermal crosslinking of whey protein, it might be still permeable to proactive species such as iron. This is in line with the results of enzymatically cross-linked sodium caseinate film at oil-water interface, where the inability to restrict pro-oxidants promoted the decomposition of hydroperoxides into free radicals that further oxidized the unsaturated fatty acids in the emulsion droplet core (Kellerby, Gu, McClements, & Decker, 2006). Moreover, the highly porous microstructure (as observed in Figure 6) possibly allowed oxygen, free radicals generated or prooxidants in the aqueous phase to diffuse through the emulsion droplet interface where they reacted with the oil inside the droplets. This resulted in faster oxidation kinetics which is in agreement with literatures showing higher degree of lipid oxidation in encapsulated as compared to non-encapsulated systems (Kellerby, et al., 2006). The increased level of lipid oxidation in oil powder during storage indicates that the matrix to encapsulated oil ratio of 1:25 seems to be not sufficient to prevent diffusion of oxygen during storage of the oil powders. Hence, besides the processing aspects of oil powder, the kind of microstructure generated during oil powder production, significantly contributed to enhance lipid oxidation as compared to the bulk oils (unprocessed).

It is worth noting here that this study is not sufficient to identify the technological conditions, which promote or inhibit the lipid oxidation rate in the oil powder when it is present in the final product applications. For instance, the presence of pro-oxidants in the formulation of the finished product applications, water activity, processing conditions during the manufacturing of the products (e.g. heat treatment, mechanical shear), product packaging and storage conditions are supposed to dramatically impair the stability of encapsulated oil powders. Contrastingly, presence of natural antioxidants in the application recipe might retard lipid oxidation. For these reasons, a systematic storage test (trained sensory panel analysis) of the final product applications containing oil powder with or without added antioxidants needs to be performed to clearly predict a more complete picture of the oxidative stability of the oil powders when used as a fat replacer in the finished product applications. Furthermore, quantitatively describing the mechanism of lipid oxidation in this type of oil powders with ultra-high oil content because of processing and/or microstructural aspect needs to be elucidated in future.

Conclusions

Our study showed a scaled up approach to create spray dried emulsions with ultra-high oil loading of 95.3 wt%. The cross-linking of the interfacial layer enabled spray drying of the emulsion droplets without the requirement of any additional wall materials. The initial emulsion droplets size influenced the mechanical stability of the oil powder as evidenced by oil leakage upon compaction. Our study also established an easier approach to study mechanical stability of oil powder in an industrial context. The oil leakage upon compression during storage was due to not only the surface oil but also coalesced oil droplets formed during spray drying as well as inter-granular friction in the capillaries oozing out of the porous “sponge”-like permeable microstructure of the oil powder as seen in the SEM. The processing aspects of oil powder (e.g. homogenization, thermal cross-linking and spray drying) aggravated the lipid oxidation kinetics as compared to the unprocessed bulk oils. Furthermore, the cohesive cross-linked whey protein film in absence of any added glassy matrix was not sufficiently dense to protect the oil from oxidation and allowed diffusion of oxygen and prooxidants during storage period. The insights generated in designing this “sponge” like porous microstructure and the microscopic detailing of the mechanism of instability is expected to serve as a reference for the designing of new class of microstructures for use in food, pharmaceutical, personal care and home care industries.

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Table 1.

Parameters	**(%)**
Moisture (%)	0.21
Fat (%)	95.31
Crude protein (%)	3.65
Ash (%)	0.10
Bulk density (g/ cc)	0.44
Lipid analysis	**(g FA/ 100g fat)**
Total saturated fatty acids	15.16
C18:1 n-9 & n-7 (Oleic & other cis) acids	22.9
C18:2 n-6 cis (Linoleic acid)	49.9
C18:3 n-3 cis (Linolenic acid)	5.5

Table 2.

2-Stage homogenization pressure (number of passes × bars)	Droplet size (d_{32}) of initial emulsion (µm)	Droplet size (d_{32}) of cross-linked emulsion (µm)	Particle size (d_{32}) of spray dried emulsion (µm)	Oil release (wt%)
2 x 100/ 25	2.31 ± 0.12	2.42 ± 0.36	42.88* ± 0.95	7.54** ± 0.21
1 x 200/ 100	0.76 ± 0.08	1.21 ± 0.21	37.27* ± 0.87	4.1** ± 0.58
1 x 400/ 100	0.38 ± 0.05	0.67 ± 0.04	37.73* ± 1.1	0.93** ± 0.11
1 x 1200/ 100	0.21 ± 0.02	0.33 ± 0.01	39.49* ± 1.26	2.73** ± 0.52

Figure 1.

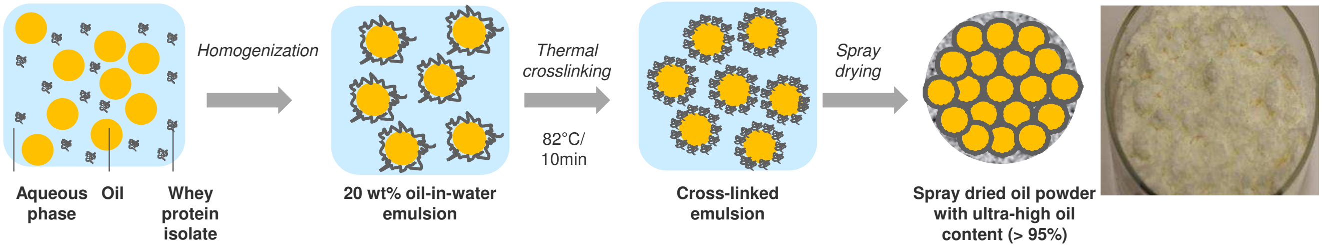

Homogenization
Thermal crosslinking
82°C/ 10min
Spray drying
Aqueous phase
Oil
Whey protein isolate
20 wt% oil-in-water emulsion
Cross-linked emulsion
Spray dried oil powder with ultra-high oil content (> 95%)

Figure 2.

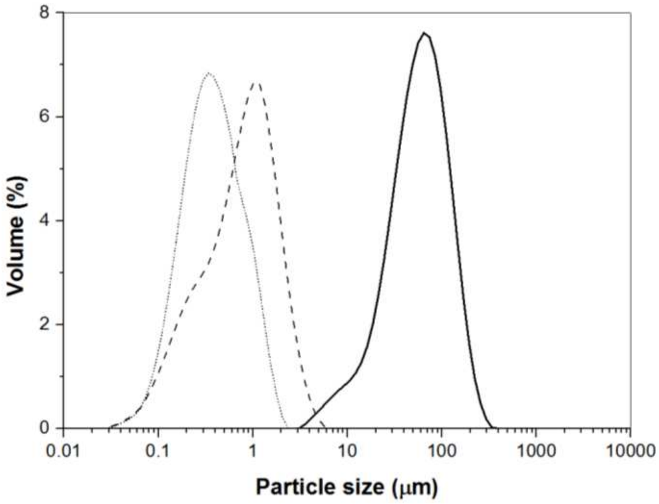


Figure 3.

(A) **(B)**

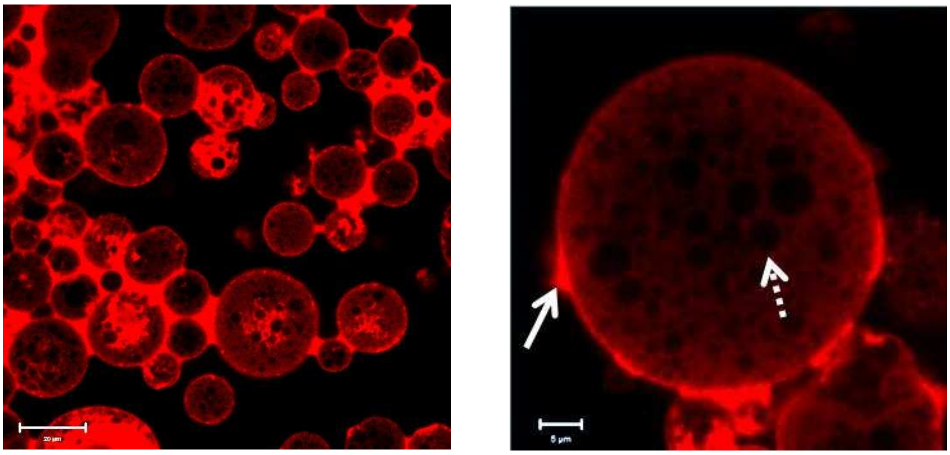


Figure 4.

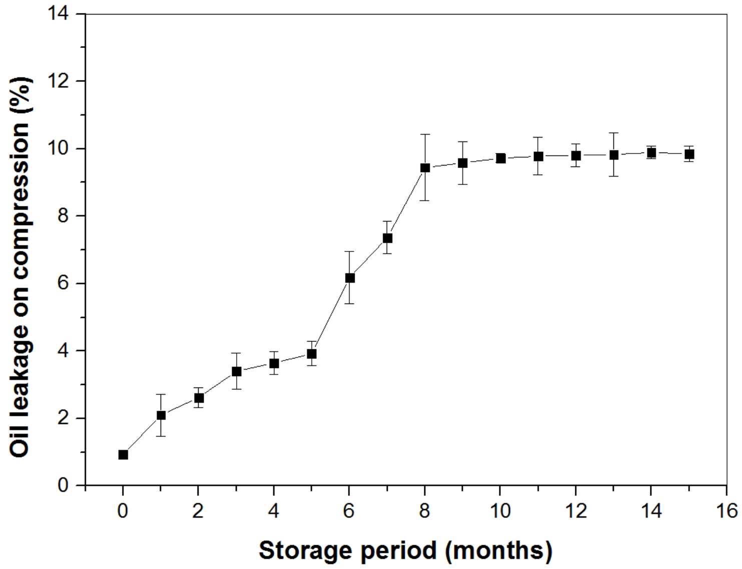
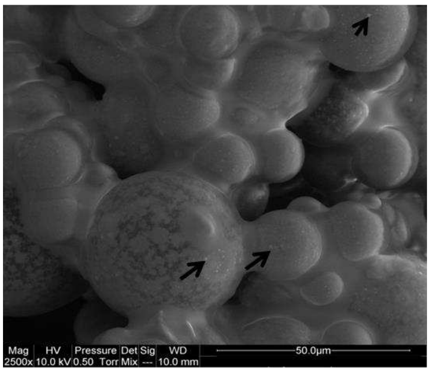
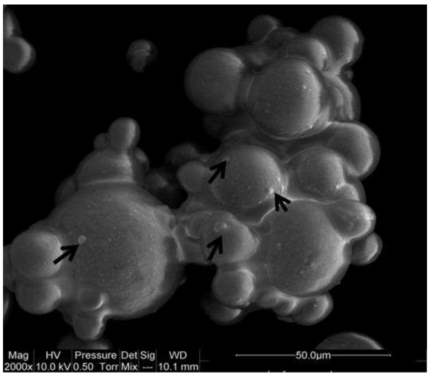

Oil leakage on compression (%)
0
2
4
6
8
10
12
14
0
2
4
6
8
10
12
14
16
Storage period (months)

Figure 5

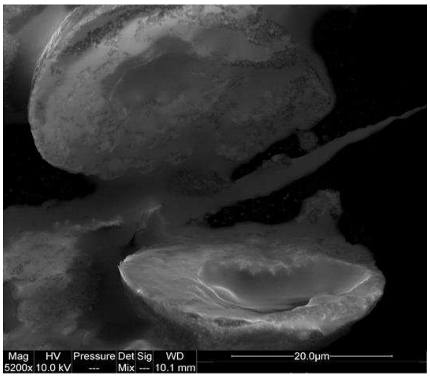
(A)



(B)



(A´)



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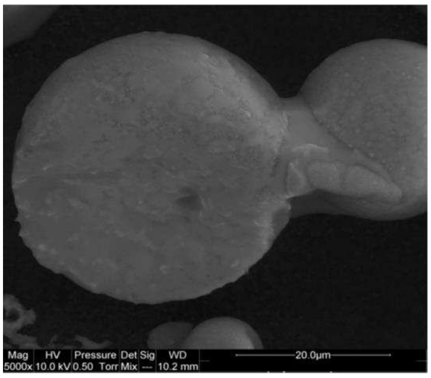
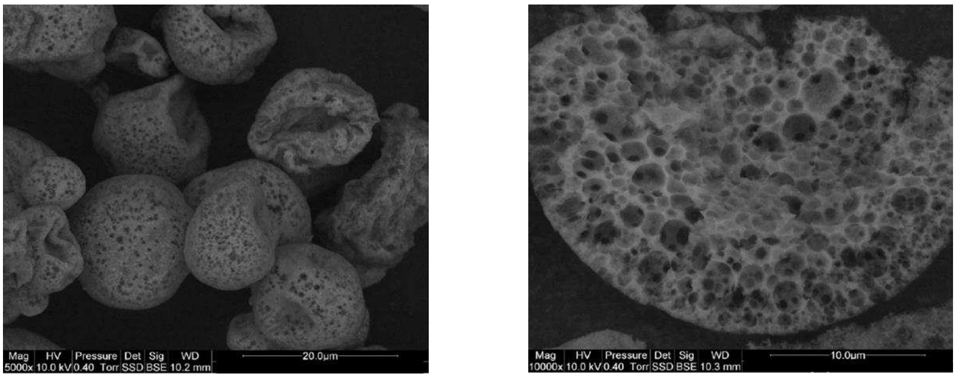
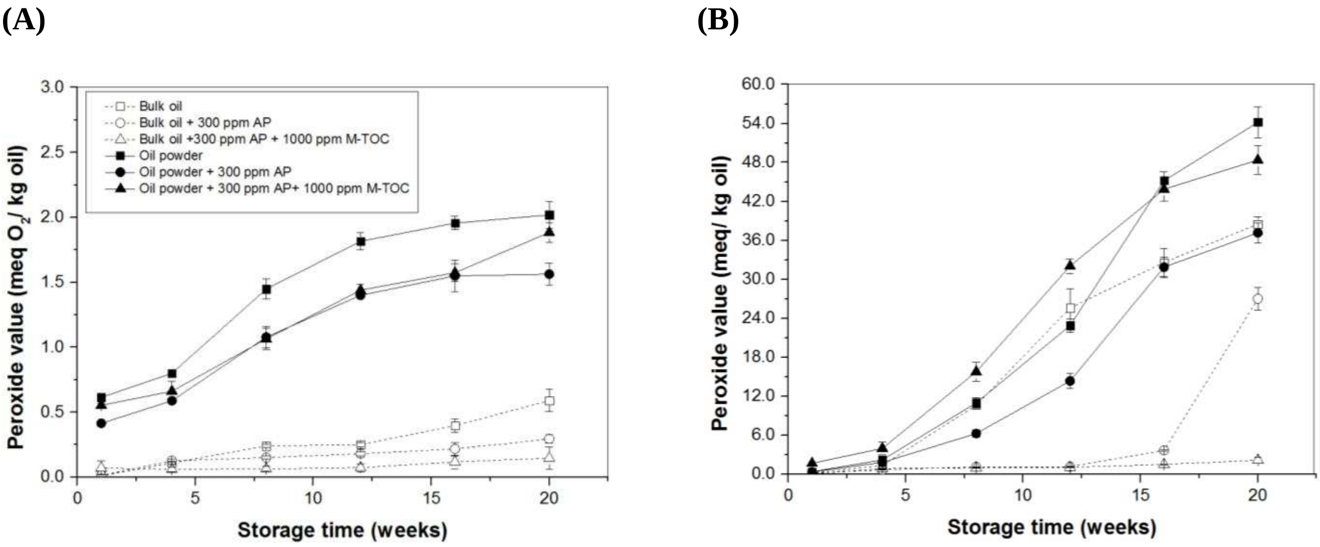


Figure 6

(A) **(B)**


Mag HV Pressure Det Sig WD
5000x 10.0 kV 0.40 Torr SSD BSE 10.2 mm
20.0µm
Mag HV Pressure Det Sig WD
10000x 10.0 kV 0.40 Torr SSD BSE 10.3 mm
10.0um

846 **Figure 7**



847

Figure 8

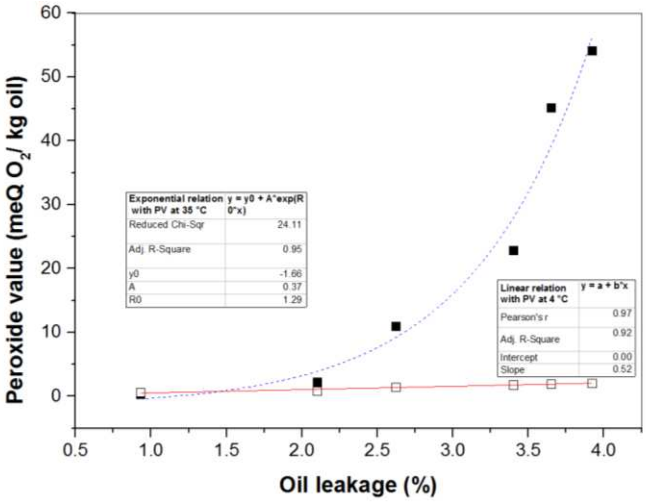
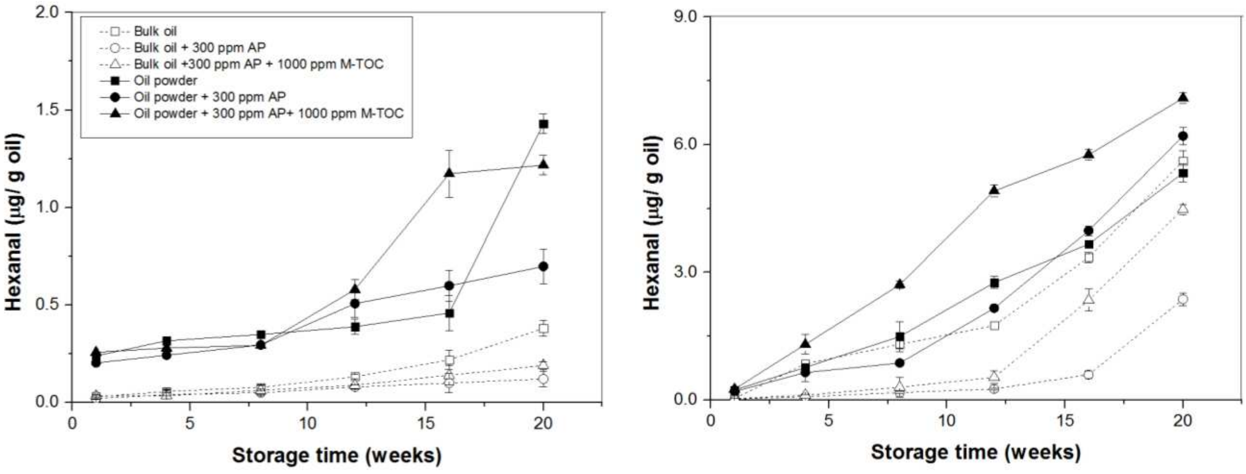

Exponential relation with PV at 35 °C
y = y0 + A*exp(R0*x)
Reduced Chi-Sqr 24.11
Adj. R-Square 0.95
y0 -1.66
A 0.37
R0 1.29
Linear relation with PV at 4 °C
y = a + b*x
Pearson's r 0.97
Adj. R-Square 0.92
Intercept 0.00
Slope 0.52
Peroxide value (meQ O2/ kg oil)
Oil leakage (%)
60
50
40
30
20
10
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Figure 9

(A) **(B)**



Bulk oil
Bulk oil + 300 ppm AP
Bulk oil +300 ppm AP + 1000 ppm M-TOC
Oil powder
Oil powder + 300 ppm AP
Oil powder + 300 ppm AP+ 1000 ppm M-TOC
Hexanal (μg/ g oil)
Storage time (weeks)

Captions for Tables

Table 1. Composition of oil powder.

Table 2. Mean particle size and oil release on compression as a function of homogenization speed of manufacturing of emulsion. Values are means of five measurements with ± standard deviation. *T-test indicates significant difference ($p < 0.05$) when compared to d_{32} values of both emulsions with and without crosslinking; ** indicates significance of linear correlation of oil release (%) to droplet size, $p < 0.05$.

Captions for Figures

Figure 1. Schematic illustrations of steps of production of oil powder with ultra-high oil content showing the visual aspect (macrostructural image).

Figure 2. Droplet size distributions of the 20 wt% oil-in-water emulsion (dotted line), after heat treatment at 82 °C/ 10 minutes (dash line) and particle size distribution of the resulting oil powder (solid line).

Figure 3. Confocal micrograph of oil powder, scale bar represents 20 μm (A) and higher magnification image showing the internal structure of one powder granule containing the cross-linked emulsion droplets, scale bar represents 5 μm (B). Colour in red represents the protein stained by Rhodamine 6G. Dotted arrow represents protein layers protecting individual oil droplets within a powder granule and solid arrow represents the protein shell of the granule.

Figure 4. Long-term mechanical stability of oil powder as a function of storage period. Mean value is the average of five measurements. Error bars represent standard deviations.

Figure 5. Scanning electron micrographs of spray-dried oil powders on (A) 0 day and after storage for (B) 9 months. Corresponding internal images of the microstructures are showed by A´ and B´, respectively. Arrows in the micrographs indicate “free” surface oil.

Figure 6. Scanning electron micrographs of stored spray-dried oil powder after removal of “hexane extractable” surface oil. (A) External and (B) internal images of the microstructures.

Figure 7. Evolution of hydroperoxides in bulk oil and oil powders containing different antioxidants when stored at 4°C (A) and 35 °C over a period of 5 months. Mean value is the average of five measurements. Error bars represent standard deviations.

Figure 8. Evolution of hydroperoxides in oil powders when stored at 4° C (□) and 35 °C (■) over a period of 5 months as a function of oil leakage. Solid and dashed line represents correlation at 4° C and 35 °C, respectively.

Figure 9. Evolution of hexanal in bulk oil and oil powders containing different antioxidants when stored at 4°C (A) and 35 °C over a period of 5 months. Mean value is the average of five measurements. Error bars represent standard deviations.