Resveratrol loaded Pickering emulsions stabilized by OSA modified rice starch granules

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Abstract

Resveratrol is a photosensitive, bioactive molecule which has received increasing research interest during the past decade for its antioxidant properties. However, it has low solubility in water or common triglyceride oils. Resveratrol solubilization in oil can only be achieved in essential oils, such as flavour oil, but the stability of emulsions produced with this type of oils is low as they are prone to creaming phenomena and Oswald ripening.

In this study, resveratrol was first dissolved in orange oil which was mixed into a medium chain triglyceride (Miglyol) at different ratios and used as the internal phase of oil-in-water emulsions (O/W). The emulsions were stabilized by Octenyl Succinic Anhydride (OSA) modified rice starch granules using two different ratios of starch particle:oil to study the influence of interfacial coverage on final emulsion droplet size and emulsion stability.

The study indicated stable Pickering emulsions could be prepared using OSA-modified rice starch granules even at partial coverage conditions. Emulsions prepared at an oil fraction of 0.5 using 30% v/v mixture of orange oil:Miglyol as the dispersed phase seemed to be an appropriate resveratrol carrier system, obtaining encapsulation efficiency values close to 90% which results in emulsions with a resveratrol concentration of 8.45 mg/L. Hence, the emulsions prepared are suitable for food fortification applications.

Keywords: Resveratrol, Pickering emulsions, Rice starch granules, Encapsulation, Orange oil, Emulsion stability

1. Introduction

Resveratrol is a natural polyphenol, produced by plants, that is primarily found in the seeds and the skin of grapes and red wine, but it is also present in nuts and berries. Resveratrol shows geometric isomerism, but only trans-resveratrol (trans-3,4,5-trihydroxystilbene), the most abundant form in nature, appears to have biological effects (Hung, et al.,2000, Howitz et al., 2003,Krist, et al., 2009, Machado, et al., 2019). Resveratrol can be considered as a novel nutraceutical which can be used as an ingredient in functional foods to provide consumers with a

wide range of benefits such as anti-cancer activity, cardio-protection, antioxidant activity, and anti-inflammatory activity among others (Machado et al., 2019, Murtaza et al., 2013, Ruivo et al., 2015, Yang et al., 2014, Saiko et al., 2008). Although resveratrol is present in several foods, due to its low solubility in water and common triglycerides, its absorption is limited (Murtaza et al., 2013, Novelle et al., 2015). In addition,

resveratrol has low chemical stability when exposed to light, high temperature, pH changes, and some enzymes (Machado et al., 2019, Silva et al, 2013)

Microencapsulationis an approach to increase bioavailability and protect unstable bioactive compounds. Microencapsulation is a process where the bioactive compound is retained within an encapsulating matrix or membrane. These systems consist of a semipermeable or non-permeable spherical and strong membrane surrounding a solid or liquid core. Emulsification is one of the common microencapsulation techniques used to encapsulate bioactive compounds (Agustin et al., 2009).

Emulsification involves mixing at least two immiscible phases (usually oil and water), where one is dispersed in another as droplets. Since this mixture is thermodynamically unstable, the dispersed droplets tend to merge (coalesce) which in turn will result in destabilization through phase separation. Therefore, the interface between emulsion droplets needs to be stabilized by some type of amphiphilic or surface-active compounds, such as surfactants, biopolymers, andparticles. The emulsion droplets can be used to encapsulate bioactive compounds for several industrial applications as cosmetics, food, and pharmaceutical products (Shi et al., 2020; Tang 2020, Matos et al., 2018a).

Particle stabilized emulsion, also known as Pickering emulsions, have demonstrated long-term stability against destabilization mechanisms such a coalescence and Ostwald ripening. When particles are at the interface are closely packed, capillary forces are formed between the two adjacent particles at the same droplet interface what leads to the formation of interfacial pressure that impedes mass transfer across the interface which in turn, arrest Ostwald ripening (Matos et al., 2017; Schröder et al., 2017). Moreover, particle-stabilized emulsions have high biocompatibility compared to emulsions ; stabilized by surfactants (Binks, 2001, Matos, et al., 2016, Marefati., et al., 2017, Marefati, et al. 2018, Albert al., 2019, Tang et al., 2019).
Starch particles have been extensively studied for application as Pickering emulsifiers (Timgren, et al., 2011, Yusoff, & Murray, 2011). Particles should have a partial affinity for both phases (oil and water) to have efficient stabilizing properties. Since starch particles are naturally hydrophilic, they need to be modified to optimize their emulsifying efficiency. Starch esterification with dicarboxylic acids to give Octenyl Succinic Anhydride(OSA) is found to beone of the more

effective approaches (Rayner, et al., 2012,Timgren, et al., 2011). A recent study by Marefati and Rayner (2020) has reported that Pickering emulsions stabilized by OSA modified quinoa starch granules remained stable for 8 years with no sign of coalescence. However, quinoa starch is not commercially available which makes other types of starch, such as rice or maize, to have a clear advantage since they are commercially available. Timgren et al., 2011 and Marefati et al, 2018, had studied the emulsification properties of several starches and had demonstrated that after quinoa rice was the type of starch that presented better stabilizing properties.

Previously, resveratrol has been encapsulated in nanovesicles (Pando, et al. 2013). Furthermore, double emulsions have been used for this purpose, in which resveratrol was encapsulated in the inner aqueous droplets (Matos et al., 2015, Matoset al., 2018b, Gan et al., 2019, Santos, et al. 2019, Shao, et al. 2019). These systems present a clear limitation since the aqueous core can have the potential affinity to swell and leak into the external aqueous phase (Khadem, et al., 2019).

The encapsulation of resveratrol in oil-in-water emulsions has not been largely studied, probably due to the low oil solubility of the resveratrol. Recent works have been conducted using orange oil as the oil phase to encapsulate resveratrol obtaining promising results (Davidov-Pardo & McClements, 2015), with solubilities up to 0.13 mg/g, which is higher than the resveratrol solubility in pure water (0.03 mg/g).

However, emulsion stability was shown to be not very high when orange oil was used as dispersed phase (Davidov-Pardo & McClements, 2015, Karthik, et al. 2019, Meroni,&Raikos, 2018). The main reason for this poor emulsion stability is the water solubility of some orange oil components that favours Oswald ripening phenomena which have found to be decreased by the use of other hydrophobic compounds in combination with the orange oil (Lim et al. 2011, Meroni & Raikos, 2018, Davidov-Prado & McClements 2015). In all these studies the concentration of orange oil wasfound to be very small (5-10% of internal phase), therefore, the knowledge regarding the effect of higher concentration of orange oil on the emulsion stability is scarce.Moreover, oil-in-water emulsions used in previous studies as resveratrol carrier presented low oil fraction phase (10-30%of internal phase), which makes thefinal resveratrol concentration low despite the high encapsulation efficiency(Lim et al. 2011, Davidov-Pardo & McClements, 2015, Matos, et al., 2018a, Matoset al., 2018b).

In the present work,the effect of increasing the internal phase concentration ofoil-in.water emulsions formulated using a mixture of orange:Miglyol as oil phase and OSA-rice starch granules as stabilizer was explored up to their colloidal stability limit using several internal oil fractions, particles:oil concentration and ratios of orange:Miglyol. Emulsions were characterized in terms of emulsion droplet size, emulsion stability, and encapsulation efficiency.

2. Material and methods

2.1. Materials

Resveratrol (purity is 99%, CAS 501-36-0, MW=228,24 g/mol) was provided by Sigma Aldrich (USA). The dispersed phase of the emulsions prepared was a mixture of orange oil from Sigma-Aldrich (USA) and Miglyol 812 from Sasol GmbH (Germany).Native starch granules from rice grains were supplied by Lyckeby-Culinar AB, Sweden. Starch particles wereOSA-modified with a degree of substitution of 2.7% using the method described by Rayner et al. (Rayner at al., 2012). As an external continuous phase, for all the emulsions 0.1 M NaCl deionized water solution was used. The presence of 0.1M NaCl in the aqueous phase was because it has been suggested that the presence of electrolytes lowers the attractive force between water droplets, decreasing the dielectric constant of the aqueous phase and therefore reducing collision frequency (Márquez et al., 2010; Frasch-Melnik et al., 2010; Frasch-Melnik, Spyropoulos et al., 2010; Matos et al., 2018, Matos et al., 2015). NaCl, methanol of HPLC-grade and absolute ethanol were purchased from Sigma Aldrich (USA).

2.2. Emulsion formulation and preparation

2.2.1. Formulation with varying concentration of starch

To determine the full coverage conditions, emulsions were prepared at different ratios of particle:oil varying theOSA-modified rice starch concentrations in the range 100-1000 mg/g (mg of starch/g oil). The oil volume factions (ϕ) selected for these preliminary experiments were 0.1 and0.3 . To eliminate the effect of orange oil on the stability of emulsions, pure Miglyol was used in this set of experimentsEmulsions were prepared by rotor-stator high shear homogenizer using a Silent Crusher Mixer (Heildolph, Germany), equipped with 8F type agitator. Firstly, the starch granules were added to the aqueous solution and mixed by vortex to disperse the starch in the aqueous phase. Then the oil phase was added. Samples were mixed at 20000 rpm for 1 min, which was the similar conditions used in previous work when resveratrol loaded quinoa starch Pickering emulsions were prepared (Matos., 2018b).

2.2.2. Formulations with varying oil volume fractions

To see the effect of different oil fractions on the stability of emulsions, Pickering emulsions with several dispersed phase volume fractions (ϕ) (i.e. 0.1, 0.3, 0.4, 0.5 and 0.6) were prepared and characterized using the emulsification procedure described in section 2.2.1.

2.2.3. Formulations with varying orange oil:Miglyol ratios

To determine the influence of the combination of orange oil with Miglyol as the dispersed phase, pure Miglyol and mixtures of orange oil:Miglyol in volumetric ratios 1:9 and 3:7 were compared. Resveratrol was first dissolved in orange oil in a concentration of 0.068 mg/g and mixed for 72

h. Davidov-Pardo & McClements 2015, had demonstrated that resveratrol can be dissolved in orange oil in a concentration of up to 0.13 mg/g. The concentration used in the present work was close to half of that tested value so their total solubilization could be assumed. The solution containing resveratrol and orange oil wasthen mixed with Miglyol as it was reported in previous work (Matos, 2018b), since Miglyol has been used in formulations of starch stabilized Pickering emulsions which demonstrated to have high stability (Matos et al, 2016; Timgren et al., 2011). The final mixture obtained was then used as the dispersed phase using the emulsification procedure described in section 2.2.1.

2.3. Characterization of microstructure of the emulsions

Mastersizer S long bench equipment (Malvern Instruments Ltd., UK) was used to measure the mean droplet diameter, $D_{(0,5)}$, of emulsions prepared and the corresponding droplet size distributions.

Micrographs of the emulsions were also obtained with a light optical microscope (Olympus BX50, Tokyo, Japan) with 10-40×objective magnifications, with a cover glass on the slide.

2.4.Evaluation of emulsion stability

The stability of the emulsions has been measured by Static Multiple Light Scattering (MLS) using a Turbiscan Lab Expert (Formulation Co. France). The stability of the selected emulsions was measured at 30° C for 10 days.

The Turbiscan Stability Index (TSI) was measured for all samples. TSI is the sum of all the variations detected in the samples in terms of size and/or concentration, and is defined by equation 1:

$$TSI = \sum_i \frac{scan_i - scan_{i-1}}{H} \quad (1)$$

where H is the total height of the Turbiscan cell at time intervals i.

2.7. Determination encapsulation efficiency (EE)

Resveratrol content in the external aqueous phase was determined by reverse phase high-performance liquid chromatography (RP-HPLC, HP series 1100 chromatograph, Hewlett Packard, US). The system was equipped with a UV/VIS absorbance detector HP G1315A and a fluorescence detector 1260 Infinity A (Agilent Technologies, USA). The column used for the separation was a reversed-phase column Zorbax Eclipse Plus C_{18} of 5 μm particle size, 4.6 mm × 150 mm (Agilent Technologies, USA). The mobile phase consisted of a mixture of (A) 100% MilliQ-water and (B) 100% methanol with gradient elution at a flow rate of 0.8 mL/min. The step

gradient started at 80% mobile phase (A) running 100% of the mobile phase (B) in min 5 for 10 min. The mobile phase (B) was running for 2 min after each injection to prepare the column for the next run. The separation was carried out at room temperature. A wavelength of 305 nm was used for the UV/VIS detector while the fluorescence detector was used at $\lambda_{excitation}/\lambda_{emission}$ at 310/410 nm. The column was cleaned after each analysis by running the first mobile phase (A) for 20 min and a mobile phase (C) consisting of 50% acetonitrile, 25% MilliQ-water, 25% 2-propanol and 0.01% acid acetic for 40 min at a flow rate of 0.25 mL/min. Finally, the column was rinsed with 50% of the mobile phase (A) and 50% of the mobile phase (B) for another 20 min.

The method followed was the one described in precious works (Matos etl al., 2018b) with some modifications. Emulsions were centrifugated at low speed (1000 rpm for 20 min) in order to separate the aqueous phase from the oil droplets. The collected aqueous phase was filteredwith a 0.22 μm polyvinylidene difluoride (PVDF) syringe filter, to eliminate all the cream oily phase residues and any free starch. These filtered samples were then injected into RP-HPLC.

As a blank, a sample was prepared for each formulation studied by dissolving the same amount of resveratrol ina water:methanol solution (volume ratio 1:1) since resveratrol presents poor solubility in water and the use of methanol will ensure its complete solubilization. Methanol was not necessary to be added to the aqueous phases recovered from the emulsions since in all cases the amount of resveratrol collected was lower than the resveratrol solubility in water.

The encapsulation efficiency (EE%) was calculated using the following equation:

$$EE\% = \left(1 - \frac{A_S^i}{2 \cdot A_c}\right) \cdot 100 \qquad (2)$$

where A_S^i is the area of the peak of the sample detected through RP-HPLC at the day i, and A_c is the area of the blank sample.

2.8. Statistical analysis

Data were expressed as the mean ± SD (standard deviation) of three independent experiments, and statistical analysis of the data was carried out (ANOVA). Fisher's test ($p<0.05$) was used to calculate the least significant difference (LSD) using statistical software (Microsoft Excel 2010).

3. Results

3.1. Formulations with varying amount of starch

In order to determine the full coverage conditions, emulsions were prepared at different ratios of starch particles:oil. As a general trend, droplet size decreases when the ratio of particle:oil

increases until a constant valuethat indicates that full coverage conditions are achieved (Tcholakova et al., 2008, Rayner, Timgrem et al., 2012). Emulsions with oil fractions of 0.1 and 0.3 were prepared with particle concentrations ranging from 100 to 1000 mg/g (Figures 1A and 1B).

Two peaks appeared on all particle size distribution correspondingthe smaller one to the presence of free starchwhile the larger one to the presence of oil dropletsas it was reported in previous studies(Marefati et al. 2017a and Marefati et al. 2017b, Li et al., 2019). To select the appropriate starch:oil ratio the mean size of this second peak was studied.A significant droplet size reduction was observed when a ratio of 400 mg/gwas usedBeing the size constant after the addition of 700 mg/g.Similar behavior was observed for an oil fraction of 0.1 and 0.3 when pure Miglyol was used as the oil phase (Figure 1A, 1B). Previous works, where quinoastarch granules were used (Matos, et al., 2016) have shown that the minimum ratio of starch particles/oil required necessary to ensure the stability of the interface oil/water was independent of the oil fraction used..

The starch:oil ratio obtained of 700 mg/g was similar to another type of Pickering corn starch stabilized emulsions (Li et al., 2013) but higher compared to the other types of starch, as is the case of quinoa starch (Marefati, et al, 2013, Matos,et al., 2018b,Matos, at al., 2016). This is probably due to the larger size of OSA-rice starch granules which presents a particle size distribution that varies from 0.5 to 20 μm with a volume weighted mean diameter (d_{43}) of 5.2 μm (Li et al. 2013, Sato et al. 2018, Timgren, et al., 2013) compared to OSA-quinoa starch which presented particle size distributionsranging from 0.5 to 10 μm with a d_{43} of 2.5 μm (Rayner at al., 2014), resulting in lower total available area to coverthe droplet surfaces at the same mass of starch compared to quinoa starch granules(Timgren, et al.. 2013). So in that sense, two different starch:oil ratios were selected in order to see the influence of partial and full coverage conditions (i.e. 400 and 700 mg/g).

Figure 1 shows a micrograph of the emulsions prepared with pure Miglyol at 400 (C) and 700 (D) mg/g. It is possible to see that partially covered emulsion droplets had part of the droplets uncovered and the initial coalesce can occur while fully coveredemulsion droplets present droplets completely covered by starch particles.Droplet size observed in the micrographs are in good agreement with the ones obtained by particle size distributions analysis using Mastersizer, indicating that the larger peak observed was the one responsible of the emulsion droplets.

Previous works showed that when orange oil was used as the oil phase a larger amount of starch is required in order to obtain the oil droplets in full coverage conditions (Matos, et al., 2018b). In the present work, the ratio of starch:oil was decided to be fixed in the two indicated concentrations (400 and 700 mg/g) for the three different oil phases studied (pure Miglyol, orange:Miglyol 1:9

ratio, orange:Miglyoloil 3:7 ratio). In that sense, the effect of the presence of the orange oil on emulsions properties could be easily identified.

Figure 1

3.2. Formulations with varying oil volume fractions

Droplet size distribution was measured for emulsions prepared with pure Miglyol as the oil phase. Figure 2 shows the droplet size distribution of the emulsions using Miglyol as the internal phase with different internal oil fractions, for the emulsions with partial coverage (400 mg/g) and complete coverage (700mg/g).

Figure 2

As as aformetntioned, for lower ϕ (0.1 and 0.3) the small peak was observed at the lower size, typically due to the presence of free starch also observed in other works where emulsions were stabilized by starch particles (Matos et al., 2016, Matos et al., 2018b, Li et al., 2019).

At higher ϕ the starch particles probably tended to aggregate producing agglomerates of large size. This can be the reason why the pick of a few microns was not observed at high ϕ values as was the case of the work done by other authors (Li et al. 2020) when they study particle size distributions of stach –stabilized emulsions with ϕ of 0.2-0.6.

In both series of samples, it seemed that when ϕ of 0.5 was reached the emulsions started to change their main droplet size. However, only the emulsions with ϕ of 0.6 seem to had a non-homogeneous aspect regardigthe presence of free oil indication that the oil was not completely emulsified. Hence, emulsionsreachedits colloidal stability limit and the main particle size distribution started to be divided into two peaks, since coalescence was taking place. Similar results were obtained by Li et al. (2020) when corn oil was emulsified by quinoa starch, obtaining satisfactory emulsification up to a ϕ of 0.6. Moreover, Binks et al. (2002) have shown that from ϕ of 0.6-0.7 the emulsion inversion usually takes place when particles are used as stabilizers.

It should be pointed out that when ϕ increasesthe starch particle concentration also increases, which leads to interparticle interactions and causes the formation of networks of starch and droplets leading to wider particle size distributions. In addition, the larger oil and starch concentration of higherϕemulsions resulted in a less efficient mixing and hence resulted in wider particle size distributions merging in abimodal particle size distribution.

Most of the instability phenomena (such as flocculation, creaming, coalescence or Oswald ripening) seemed to have their major effect during the first 24 hours (Raikos, 2017, Zhao et al., 2014, Meroni & Raikos, 2018)so the stability of emulsions with ϕ of 0.4 and 0.5 was measured during 24 hours for emulsions with both starch:oil ratios tested.

TSI values were measured being in all cases in the range of 2 to 3.5 units, which indicates high stability when compared to other works where this parameter was measured (Raikos, 2017).No creaming phenomena wereobserved since the clarification layer was not present. This is probably due to the high internal phase concentration that hindrance oil droplets migration to the top of the emulsion vessels.Similar behaviour was observed in previous works where high internal phase emulsions were prepared with surfactants as the stabilizer (Gutierrez et al., 2014). Droplet migration velocity (V) to the surface can be calculated by equation 3 for systems in which the internal fraction is medium of high and hence particle movement is affected by the presence of their neighboring particles (Snabre & Mills., 1994).

$$V = \frac{gd^2(\rho_w - \rho_o)}{18\mu\rho_w} \frac{(1-\varphi)}{\left[1+\frac{4.6\varphi}{(1-\varphi)^3}\right]} \quad (3)$$

where d is the droplet diameter (median size on the volume weighted distribution $D_{(0,5)}$), ρ_w and $\rho_o$the densities of the continuous phase and dispersed phase respectively, μ is the viscosity of the continuous phase, g is the gravitational forceand ϕ is the dispersed phase volume fraction. However, the use of a large amount of starch as stabilizer increasedthe droplets' densities up to values of 1030-1090 kg/m^3 which was similar to continuous phase density, and even larger. Hence, not significant migration velocities were expected in the studied systems.

For the following experiments, emulsions as dispersed phase mixtures of orange oil and Miglyol were used. Emulsions were prepared with ϕ of 0.4 and 0.5.

If the internal phase fraction (ϕ_c) was calculated taking into account, the amount of stabilizer used (Equation 4).

$$\varphi_c = \frac{V_o + V_s}{V_o + V_s + V_w} \quad (4)$$

where V_o is the volume of the oil phase, V_s is the volume of rice starch and V_w volume of the continuous phase.

At 700 mg/g ratio the real internal fraction was larger than at 400mg/g conditions since the amount of stabilizer used is higher for the same amount of oil. The real internal phase of emulsions with ϕ of 0.4 was 0.54 and 0.65 for 400 mg/g and 700 mg/g conditions respectively. While for emulsions ϕ of 0.5 the real internal fraction was 0.58 and 0.72 for 400 mg/g and 700 mg/g conditions respectively. Hence, an increase of starch concentration in the emulsion could increase

oil droplet flocculation within starch networks. Other authors (Li et al, 2019) had studied the effect of the degree of substitution of quinoa starch on the emulsification properties observing that a low degree substitution showed poor emulsification properties obtaining bimodal distributions.

3.3. Formulations with different orange oil:Miglyol ratios

Two differentorange oil:Miglyol ratios were studied, 1:9 and 3:7 v/v using rice starch as a stabilizer for partial and fully covered droplets (400 and 700 mg/g particle:oil ratios).The effect of the presence of orange oil mixed in the oil phase on the particle size distribution is presented in Figure 3 for emulsions with ϕ of 0.4 and 0.5.

Figure 3

For emulsions with particle:oil ratio of 400 mg/g the addition of orange oil at several concentrations seemed to not have an important effect on emulsions size distribution, specially at ϕ of 0.4. However in the case of ϕ 0.5 the presence of orange oil resulted in bimodal size distributions.

For 700 mg/g formulations, the presence of a high concentration of oil and particles and a low amount of water in the formulations resulted in larger sizes at higher oil volume fraction ϕ of 0.5.On the other hand, the presence of orange oil caused droplet coalescence specially at ϕ of 0.5, as it was clearly indicated by the bimodal distribution obtained for emulsions with both orange oil concentration tested compared to those withpure Miglyol as the dispersed phase.

It seems that the presence of orange oil alters the surface properties of the droplets making the particles less efficient for the adsorption at the surface of the droplets.This is in line with the previous work (Matos, 2018b), where larger ratio (particles:oil) was required when orange oil was present in the oily phase, and hence in the present study coalesce can be taking place due to their poor coverage when orange oil was used. The fact that was specially noticeable at a coverage ratio of 700 mg/g, since probably at 400 mg/g coalescence was already taking place even when no orange oil was used.

Theoretical full coverage was also calculated for emulsions, for this purpose. The diameter used was the mean value of the peak observed at the particle size distribution that represents the oil droplets (D_{oil})since the specific surface area of an emulsion is by definition:

$$S = \frac{6\phi}{D_{oil}} \quad (5)$$

For full coverage calculations,it was assumed that rice starch particles are spherical and half volume of each of them is submerged into the oil phase. The same assumptions were made in previous works for theoretical full coverage calculations when starch particles are used (Matos, Laca et al., 2018, Timgren, Rayner, Dejmek, Marku, & Sjöö, 2013)

Equation 6 was followed to calculate the full coverage of the mass of particles per mass of oil ($\Gamma_{p/o}$):

$$\Gamma_{p/o} = \frac{N_{p/d} V_p \rho_p}{V_d \rho_{oil}} \quad (6)$$

where $N_{p/d}$ is the number of particles to cover an oil droplet, V_p and V_d are the volume of a particle and the oil drop respectively and ρ_p is the density of the starch particles (1.5 kg/L) and ρ_{oil} is the densities of the oils used (0.945 kg/L for pure Miglyol and 0.976 kg/L for a mixture of orange oil:Miglyol ratio 1:9 v/v and 0.935 kg/L for a mixture of orange oil:Miglyol ratio 3:7 v/v) and the $N_{p/d}$ is calculated by equation 7:

$$N_{p/d} = \frac{A_d}{A_{tp}} \quad (7)$$

where A_d and A_{tp} are the areas of a droplet and the transversal area of a starch particle.

Results obtained from theoretical full coverage calculations were shown in Table 1. Theoretical full coverage was similar to the ratio of particles:oil used. Only for emulsions prepared with pure Miglyol at ϕ of 0.4, theoretical values were much larger than the experimental values used indicating that the droplets formed were probably not fully covered by the starch particles. However, it is important to point out the wide size distributions obtained, and hence theoretical calculations based on mode values can produce some errors on theretical calculations. Theoretical values for emulsions prepared with the ratios of particles:oil of 700 m/g at ϕ of 0.5 were not calculated since samples presented l extremely wide and bimodal size distributions (Figure 3).

Table 1

Figure 4 shows the stability of the formulated emulsions for one week time. All samples presented high stability since backscattering profiles remained constant with time. Just a small clarification layer was observed at the top part of the cell. Indicating a modest movement of oil droplets to the bottom part of the cell. Similar behaviour was observed in previous works (Matos et al., 2016) since starch stabilized oil droplets presented higher density than the continuous phase.In the mentioned study,the emulsifying ability of starch isolated from quinoa in the granular form, in the dissolved state and a combination of both were compared. For this purpose, emulsions formulated using Miglyol 812 as oily phase were prepared. When starch granules were used as

stabilizer it was observed that at larger ratios of starch particles:oil (200 - 400 mg starch/ml oil) droplets migrated to the bottom of the cell ,instead of to the upper part, promoted by the sedimentation of the unadsorbed granules in the continuous phase or small starch granule stabilized emulsion droplets dense enough to sink due to a large starch to oil ratio, and a density increase since the relative volume of oil to the starch layer covering it was smaller (Rayner et al.,2012). Another effect reported caused byt the use of high starch concentration was that the accumulation of starch granules between droplets what also increased their total effective density (Timgren et al., 2013).

Previous works verified the Oswald ripening behaviour observed when orange oil was used due to their partial water solubility (Zhang et al, 2015, Raikos 2017, Meroni & Raikos, 2018). On the otherhand, some authors have observed that Oswald ripening was hindered when orange oil was encapsulated in other more lipophilic carrier oils. Carrier oils demonstrated an Oswald ripening inhibition effect by the generation of entropy mixing which was found more thermodynamically stable (Meroni & Raikos, 2018, Zhang, 2015). The favourable effect of entropy mixing seemsto be present in this study since all emulsions formulated demonstrated to have high stability which could be the explanation of their high stability. Any significant increase on droplet size was observed over time (Lemarchand et al., 2003).

It is important to point out that the mean oil droplet sizes of the emulsions in the present study were considerably larger than the one measured in previous works, in which surfactant or proteins were used to stabilize orange oil-in-water emulsions (Meroni & Raikos, 2018, Zhang, 2015, Zaho et al. 2014, Davidov-Pardo & Mc Clements 2015),being even larger than other works were other types of starch were used as a stabilizer (Li et al., 2013). This is due to the larger particle sizes of rice starch granules, as explained earlier, and the high particle concentration which could result in the formation of aggregated network of droplets due to interparticle interactions.

In that sense, the oil migration due to the density difference between the oil and the aqueous phases could be more favourable in this study. However, the larger internal phase concentration raised in this work, compared to previous works (Meroni & Raikos, 2018, Zhang, 2015, Zaho et al. 2014, Davidov-Pardo & Mc Clements 2015, Li et al., 2013), could limit droplet movement due to their higher viscosity.

Figure 4

3.4Encapsulation of resveratrol

Emulsions with ϕ of 0.5 were selected for this resveratrol encapsulation since even the wider droplet size distribution achieved for this formulation was not exceeding the 200 μm and had shown high stability. Taking into account that the concentration of resveratrol on orange oil was

388 kept constant (0.068 mg/g). The use of ϕ of 0.5 could produce final emulsions with a resveratrol
389 concentration of 0.0034 and 0.0102 mg/g for orange:Miglyol ratio 1:9 and 3:7, respectively. On
390 the contrary, the use of ϕ of 0.4 could produced final emulsions with a resveratrol concentration
391 of 0.00272 and 0.00816 mg/g for orange:Miglyol ratio 1:9 and 3:7,respectively. Hence the
392 selection of a ϕ of 0.5 offers a potential clear advantage for further emulsion applications.

393 The results of the effect of the two different internal phase ratios of orange:Miglyol oil phase(1:9
394 and 3:7) and two different ratios of starch:oil, 400 and 700 mg/g are presented in Figure 5.

395 As a general trend it can seen that the increase of orange oil concentration and the increase of
396 starch/oil ratio increased encapsulation efficiency. However, significant differences encapsulation
397 efficiency were observed for emulsions with orange:Miglyol ratio of 3:7 and a ratio of starch:oil
398 of 700 mg/g respect and emulsion with orange:Miglyol ratio of 1:9 and a ratio of starch:oil of 400
399 mg/g, with values of 89% and 81 % respectivetly. The fact that emulsion with orange:Miglyol
400 ratio of 3:7 and a ratio of starch:oil of 700 mg/g had bimodal and wider size distribution did not
401 negatively affect the encapsulation capacity of the emulsions.Not significant differences were
402 observed when the orange oil ratio was increased at a constant starch/oil ratio and for and increase
403 of the starch/oil ratio at a constant orange oil concentratrion, indicanting a synergic effect between
404 both variables.

405 All emulsions showed high encapsulation efficiencies(larger than 80%), being the encapsulation
406 efficiency values higher than those previously obtained for the similarformulation when
407 surfactant Tween 20 or whey isolated proteins were used as the stabilizer(values around 60%
408 were registered) (Sha et al., 2019; Bao et al., 2020;Chen et al., 2020). However, similarvalues
409 obtained when quinoa starch particles were used (values around 80-95% were registered) (Matos
410 et al., 2018).However, even the EE values are quite similar to the ones obtained with quinoa starch
411 , the high content of internal oil fraction and orange oil in the oil phase increase resveratrol
412 concentration from 1.72 to 8.45 mg/L.

413 A recent study reported encapsulation efficies up to 97% for systems prepared by membrane
414 emulsification and further spray drying using palm oil as oil phase, the final resveratrol
415 concentration on the obtained powder was 0.8 mg/g (Consolo et al., 2020). Other type of
416 emulsions such as gel filled emulsions reported maximum resveratrol encapsulation efficiencies
417 up to 60%. Encapsulation efficiencies close to 90% were also reported when resveratrol was
418 encapsulated in double emulsions, being resveratrol entrapped in that case in an ethanolic internal
419 aqueous phase (Diaz-Ruiz et al., 2020)

420

421 **Figure 5**

Stability of all emulsions were recorded for ten days to study the effect of different concentration orange oil in the dispersed phase mixture used on the emulsion stability for partial and full coverage formulations with and without encapsulated resveratrol. Backscattering profiles are presented in Figure 6.

Figure 6

In general terms, from the stability backscattering profiles a clarification layer at the top part, higher than the registered in samples without resveratrol could be observed (figure 4). The results obtained,indicated that the presence of resveratrolslightlynegatively affected the stability of the emulsion. Clarification at the top part of the celloccurred in all samples and differences on the backscattered light in the middle part of the cell indicated growth of the droplet size.
The negative effect of the presence of resveratrol on the stability could be due to the migration of resveratrol to the surface of the droplets as was observed by other authors (Davidov-Pardo & McClements, 2015) that may alter the interfacial activity of the droplets.

In the present study, the backscattering variations observed were not high and TSI values lower than 15 were recorded in all cases, indicating high stability.

4. Conclusions

Pickering emulsions with an oil fraction of maximum 0.5 stabilized by OSA- modified rice starch granules showed high stability against creaming phenomena. Homogenous and stable emulsions with higher internal phase were not possible to be obtained with the equipment and operating conditions used in the present study.

Oil droplets with partial coverage of OSA- modified rice starch granules showed high stability indicating that it is not necessary to have full coverage conditions. However, larger mean droplet size was observed for these formulations.

Orange oil can be used up to a 30% v/v in combination with Miglyol as the oil phase to obtain emulsions with high stability. However, the use of orange oil as a portion of the oily phase increasedthe mean droplet sizes of the resulting emulsions.

Pickering emulsions stabilized by OSA- modified rice starchgranules using a mixture of orange oil in combination with Miglyol as the dispersed phase seemed to be an appropriate resveratrol carrier system, obtaining resveratrol encapsulation efficiency values up to 89±2.3% which corresponds to aconcentration of 8.45 mg/L on the final emulsions. The system formulated could be considered suitable for further use in functional food formulations.

Acknowledgements

This study was financed by the Consejería de Economía y Empleo del Principado de Asturias (Plan de Ciencia, Tecnología e Innovación, 2013e2017) through the Grant Ref. IDI/2018/000185. A travel Grant from Lunds Tekniska Högskola (LTH) and Ersasmus + from the University of Oviedo (Ref. STT1819) was also received to perform a research stay at Department of Food Technology, Engineering, and Nutrition (Lund University)

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Highlights

- OSA-rice starch Pickering emulsions containing Resveratrol were prepared
- Stable Pickering emulsions with internal volumetric fraction up to 50% were prepared
- Orange oil was used as Resveratrol carrier in the oily phase
- Stable emulsions with 30% of orange oil on the oily phase were prepared
- OSA-rice starch Pickering emulsions contained 8.45 mg/L of Resveratrol (90% EE)

Figure Captions

Figure 1. Particle size distribution of emulsions prepared at several starch/Miglyol ratio, φ of 0.1 (A) and φ of 0.3 (B). Microscope images of emulsions formulated with pure Miglyol 812 as dispersed phase stabilized with OSA-modified rice starch granules at 400 mg/g (C) and 700 mg/g (D) conditions with φ of 0.3.

Figure 2. Emulsion particle size distributions with different oil fractions (0.1 to 0.6) at partial (400 mg/g) and full coverage (700 mg/g).

Figure 3. Effect of the presence of orange oil on the droplet size distribution of the emulsions. M: Pure Miglyol, O+M (1:9): orange oil:Miglyol ratio (1:9 v/v) and O+M (3:7): orange oil:Miglyol ratio (3:7 v/v)

Figure 4. Backscattering profiles of emulsions with 400 and 700 mg/g of starch:oil and ratios 1:9 and 3:7 or orange oil:Miglyol during 10 days. All samples contain internal oil fraction of 0.5

Figure 5. Encapsulation efficiency of resveratrol in emulsions with different oil ratios and partial and full coverage conditions

Figure 6. Backscattering profiles of resveratrol loaded emulsions with 400 and 700 mg/g of starch:oil and orange oil:Miglyol ratios of 1:9 and 3:7 or during 10 days. All samples contain internal oil fraction of 0.5.

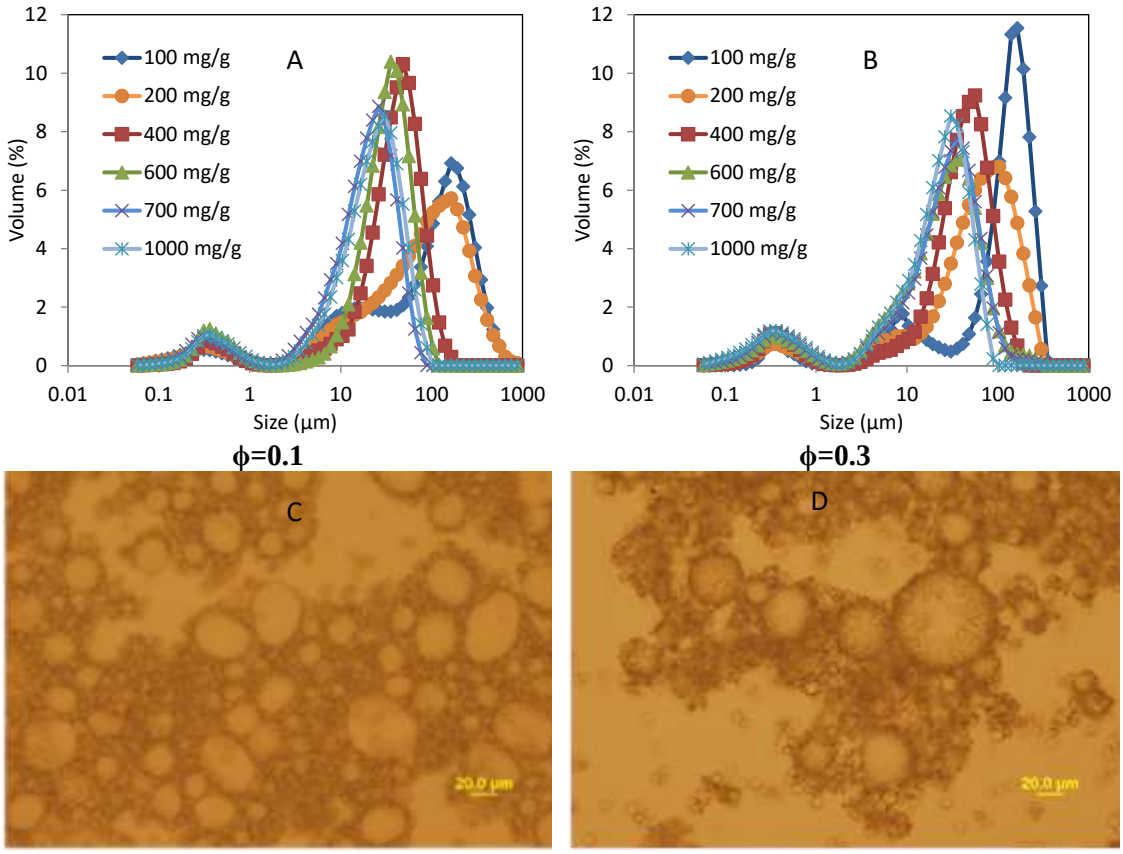

A
B
100 mg/g
200 mg/g
400 mg/g
600 mg/g
700 mg/g
1000 mg/g
Volume (%)
Size (µm)
0.01
0.1
1
10
100
1000
0
2
4
6
8
10
12
ϕ=0.1
ϕ=0.3
C
D
400 mg/g
700 mg/g

Figure 1

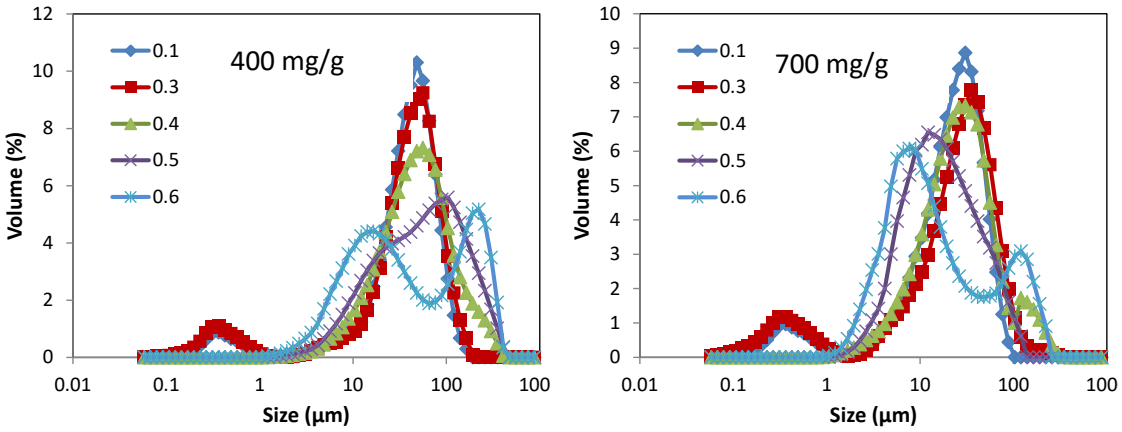
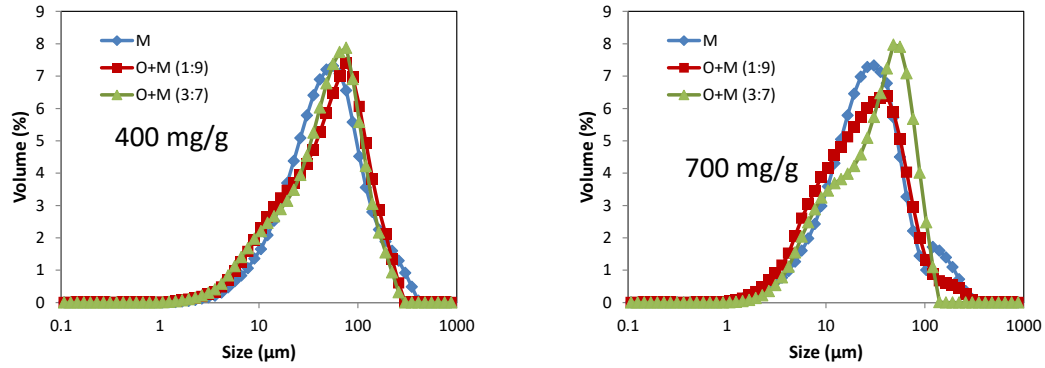
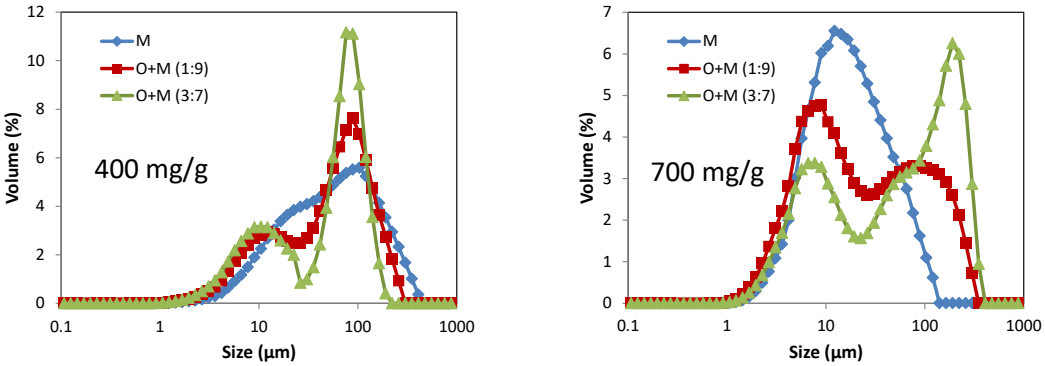

400 mg/g
0.1
0.3
0.4
0.5
0.6
Volume (%)
Size (µm)
700 mg/g
0.1
0.3
0.4
0.5
0.6
Volume (%)
Size (µm)

Figure 2

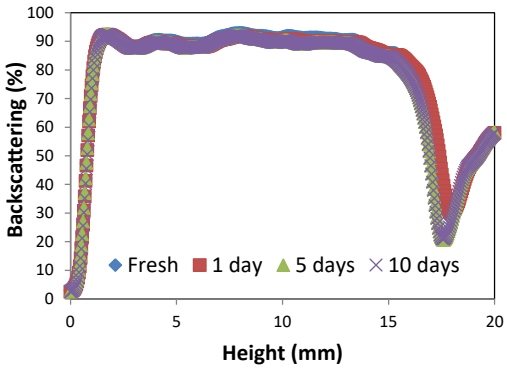


ϕ=0.4

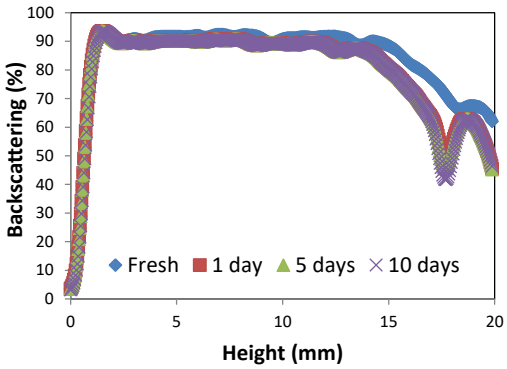


ϕ=0.5

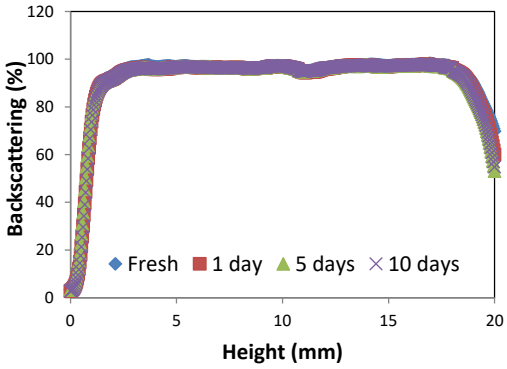
Figure 3



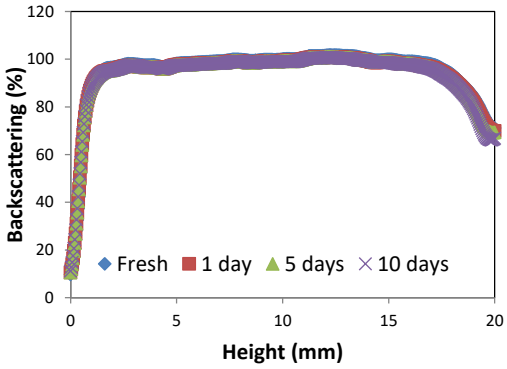
400 mg/g, orange:Miglyol, 1:9



700 mg/g, orange:Miglyol, 1:9



400 mg/g, orange:Miglyol, 3:7



700 mg/g, orange:Miglyol, 3:7

Figure 4

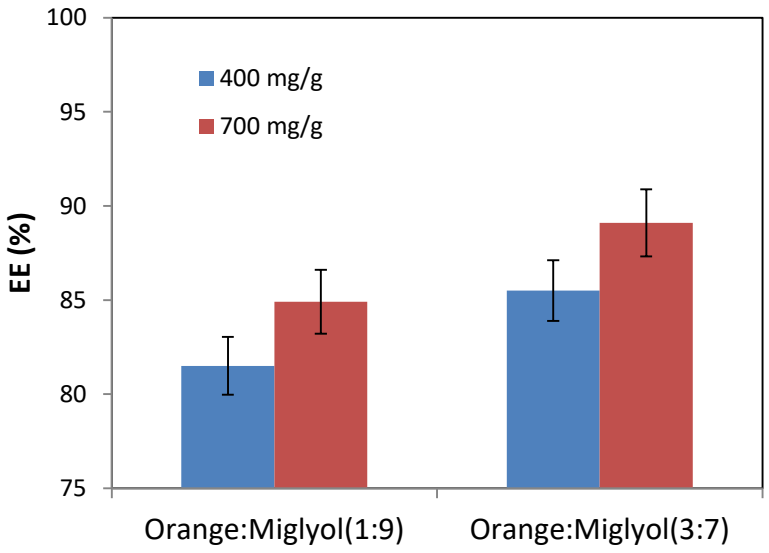
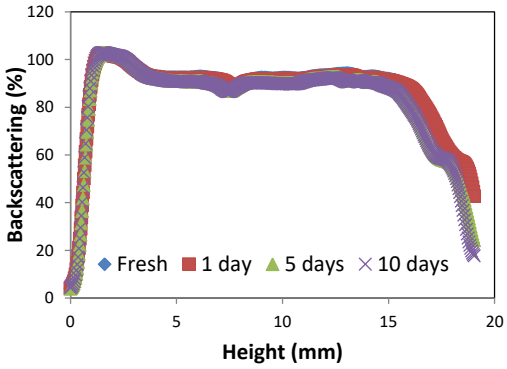
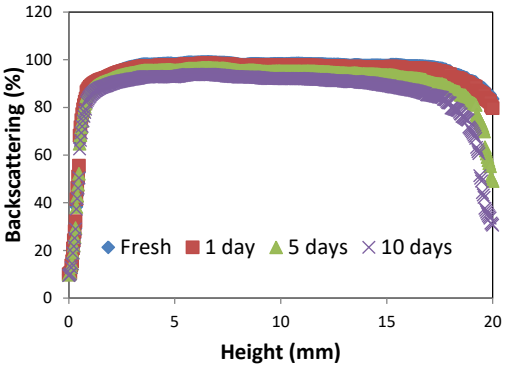

100
95
90
85
80
75
EE (%)
400 mg/g
700 mg/g
Orange:Miglyol(1:9)
Orange:Miglyol(3:7)

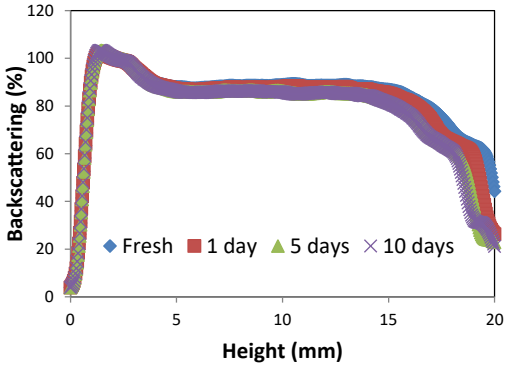
Figure 5



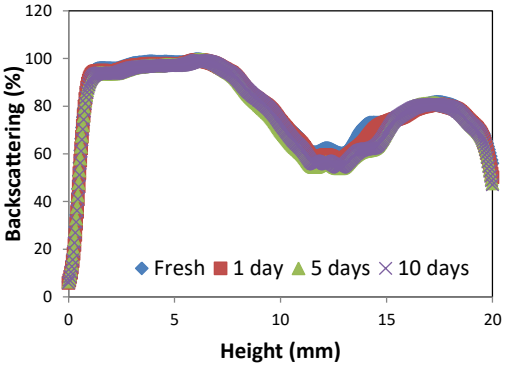
400 mg/g, orange:Miglyol, 1:9



700 mg/g, orange:Miglyol, 1:9



400 mg/g, orange:Miglyol, 3:7



700 mg/g, orange:Miglyol, 3:7

Figure 6

Table 1. Theoretical full coverage and surface particle size for emulsions prepared at internal oil phase fraction of 0.4 and 0.5

	Coverage used 400 mg/g					
	Miglyol		**Orange:Miglyol (1:9)**		**Orange:Miglyol (3:7)**	
	mode (µm)	$\Gamma_{p/d}$ (mg/g)	mode (µm)	$\Gamma_{p/d}$ (mg/g)	mode (µm)	$\Gamma_{p/d}$ (mg/g)
ϕ=0.4	48.3	717	76.3	454	76.3	454
ϕ=0.5	76.3	454	75.8	457	76.7	452
	Coverage used 700 mg/g					
	Miglyol		**Orange:Miglyol (1:9)**		**Orange:Miglyol (3:7)**	
	mode (µm)	$\Gamma_{p/d}$ (mg/g)	mode (µm)	$\Gamma_{p/d}$ (mg/g)	mode (µm)	$\Gamma_{p/d}$ (mg/g)
ϕ=0.4	26.2	1322	35.6	976	48.27	718