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The emulsification properties of octenyl- and dodecenyl- succinylated inulins.

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Key words:
Inulin, alkenyl succinylated inulin, zeta potential, oil-in-water emulsions, emulsification
Abstract

The emulsification properties of a series of inulin samples modified using varying amounts of octeny1- and dodeceny1- succinic anhydride have been studied by determining the droplet size as a function of time, temperature and in the presence of electrolyte using laser diffraction. It was found that ~2% modified inulin was required to stabilise 15% w/v medium chain triglyceride emulsions and that the minimum droplet size was achieved when the % hydrophobe content was > 8 mole %. The dodecenyl derivatives produced emulsions with a smaller droplet size than the octenyl derivatives. Apart from OSA-inulin with a low degree of modification, stability was maintained when stored for a period of up to 21 days at room temperature and at 50°C. The zeta potential of the emulsion droplets was determined by Laser Doppler Velocimetry and was found to increase from ~5mv to ~60mv as the pH increased from 2 to 7 due to the presence of carboxylate ions present in the linkage between the inulin and alkenyl chains. The droplet size was found to increase on the addition of electrolyte indicating that the adsorbed polymer layer was insufficient to provide a steric repulsive barrier and that stabilisation was mainly due to electrostatic repulsive forces.
INTRODUCTION

Inulin is a storage polysaccharide and is found in a range of fruits, vegetables and cereals including chicory, leek, garlic, Jerusalem artichoke, banana, rye and barley (Franck, 2006; van Loo et al., 1995). It is produced commercially from chicory, which contains about 15-20% inulin (Franck, 1995; Meyer.D, 2009; van Loo et al., 1995). Inulin is a polyfructan and consists of linear chains of β-(2,1) fructose units which terminate with a glucose unit at the reducing end (Franck, 2006; Meyer.D, 2009; Verraest et al., 1995). It is a polydisperse material and the degree of polymerisation (DP) varies with the source. Inulin from chicory has a DP between 2 to 60 (Franck, 2006; Meyer.D, 2009). It is classified as dietary fibre since it is not digested in the human digestive tract and is fermented by colonic microflora to give short chain fatty acids which have a beneficial effect on overall health (Franck 2006; Meyer.D, 2009; Gibson and Roberfroid, 1995). The solubility of inulin is dependent on the DP and it will form very low viscosity solutions even at concentrations of ~20% (Franck 2006; Meyer.D, 2009). On standing inulin will form gels at concentrations above 15% as a result of precipitation and crystallisation of the inulin molecules (Meyer, 2009; Alvarez-Sabatet et al., 2015). This behaviour has led to the use of inulin in a variety of food systems including dairy and low fat food products (Meyer et al 2011; Teeuwen, Thone and Vandorpe, 1992; Franck, 2002; Solowiej et al., 2015).

There has been considerable interest recently in the chemical and enzymatic modification of inulin to produce fatty acid ester derivatives which have potential application as surfactants to stabilise foams and emulsions (Kokubun et al., 2013; van Kempen et al., 2014). It has been shown that modification can be readily
achieved by the interaction of inulin with alkenyl succinic anhydride (ASA) in aqueous solution at room temperature and that the products are surface active and form micellar aggregates in solution (Kokubun et al., 2013). Alkenylated starch and gum Arabic are used in the Food Industry as emulsifiers and a particular application is in the stabilisation of concentrated flavour oil emulsions used in beverages etc. (Bhandari and Singhal, 2002; Ward, 2002; Wurzburg, 2006). Most of the studies reported have involved the octenyl succinylated derivative and the degree of substitution (DS) has been very low to comply with food regulations. Very few studies have been reported at higher DS and using longer alkenyl chains (Wang, Williams and Senan, 2014). The aim of the present work, therefore, is to investigate the emulsification properties of inulin modified using octenyl- and dodecenyloctenyl-succinic anhydrides.

MATERIALS AND METHODS

Materials

The alkenyl succinylated inulin samples used in this study were produced by chemical modification of Fibruline® DS2 and Inutec N10 using 2-octen-1-yl-succinic anhydride (OSA) and 2-dodecen-1-yl-succinic anhydride (DDSA) as reported previously (Kokubun et al., 2013). Fibruline® DS2 and Inutec N10 were obtained from Cosucra and Beneo Biobased Chemicals respectively and previous work using Gel permeation Chromatography coupled to multiangle light scattering has indicated that they are polydisperse. The Inutec N10 was found to have a weight average and
number average molar mass of ~3,120 and ~2,160 g/mol respectively (Evans et al., 2014; Evans et al, 2015) while the Fibruline DS2 was found to have values of 3,760 and 3,000 g/mol respectively (Kokubun et al., 2013). Details of the various samples used in this study are listed in Table 1. Medium chain triglyceride (MCT) gold oil was obtained from Trec Nutrition UK and was used as supplied.

Methods

Preparation of emulsions

Oil-in-water (O/W) emulsions were prepared by mixing 1.5 g MCT oil with 8.5 g of ASA-inulin solution at varying concentrations for 3 minutes at 24 000 rpm, using an IKA T25 digital Ultra-Turrax mixer. This time was chosen since it was found to give the minimum droplet size for the emulsions.

Emulsion droplet size

The droplet size was determined immediately after emulsion preparation and further measurements were made periodically for up to 21 days using a Malvern Mastersizer 2000 at room temperature (25°C). Before measurement, background readings were made and subtracted from the total scattering received from the samples. Two or three drops of the sample were introduced into the dispersion unit containing distilled water. The dispersion unit pump speed was 2000 rpm. The obscuration was between 10% and 30%. The refractive index of the dispersing medium and the dispersed particles were 1.33 and 1.45 respectively. Measurements were performed in duplicate and the average value reported. Accelerated aging
and DDSA- modified inulins and it was concluded that stabilisation was achieved mainly through electrostatic repulsive forces created by the presence of a carboxylate ion present in the surfactant head group. It is evident that alkenylated inulins have considerable potential as emulsifiers in the Food Industry.

Acknowledgements

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References


experiments were performed by storing samples in an oven at 50°C and droplet size
measurements were performed as described above. The influence of adding
electrolyte (0.5M NaCl) after emulsification on the droplet size was also investigated.

Droplet zeta potential

The zeta potential of inulin-coated emulsion droplets was determined by Laser
Doppler Velocimetry using a Zetasizer Nano ZS (Malvern Instrument Lab, Malvern,
UK) equipped with a 5 mw He-Ne laser ($\lambda_0$ 633 nm) and a digital correlator.
Measurements were carried out using a folded capillary cell DTS1060 (Malvern
Instrument Lab, Malvern, UK). The cell was washed with ethanol and deionised
water several times and dried before measurements. Two drops of the emulsion
prepared as above were added into 10mL 0.01M NaCl in deionised water and mixed
for 30 seconds and the pH was adjusted to between 2 and 10 using 0.1M NaOH or
0.1M HCl. Ten runs were performed for each sample. Zetasizer Software 6.20 ©
2002-2010 Malvern Instruments Ltd was used for data analysis and the zeta
potential was determined from the electrophoretic mobility using the Smoluchowski
equation.

RESULTS AND DISCUSSION

Effect of ASA-inulin concentration

The droplet sizes for emulsions prepared using OSA- and DDSA- inulin at varying
concentration are presented in Figures 1 and 2 respectively. Although the surface
weighted mean diameter ($d_{3,2}$) was found to remain relatively constant as the
concentration increased, the volume weighted mean ($d_{4,3}$) diameter, which is more
sensitive to the presence of larger droplets, was found to decrease significantly with increasing concentration for both OSA- and DDSA-inulin. The minimum droplet size was found to occur at a concentration of 2.0-2.5% indicating that this concentration was necessary to fully coat all of the droplets produced during the homogenisation process. The droplet size was generally smaller for the DDSA-inulins compared to the OSA-inulins over the concentration range studied indicating their increased affinity for the droplet surface. We have previously shown that DDSA-inulins are more effective at reducing the surface tension compared to OSA-inulins. For example, a 1% solution of DDSA-inulin with DS 12 mole % gave a surface tension of 30mN/m, while a 1% solution of OSA-inulin with DS 12mole% gave a surface tension of 35mN/m (Kokubun et al., 2013). It is assumed that the molecules adsorb onto the surface of the oil droplets through the alkenyl chains and that the carbohydrate moieties extend away from the surface into the aqueous phase. The extent to which the molecules extend away from the surface will depend on the DS and the position of the hydrophobic groups along the inulin chain. van Kempen et al. (2014) determined the area per molecule for oligofructose fatty acid esters adsorbed at the air-water interface from surface tension measurements and found that they had a greater cross sectional area than surfactants with one or two sugar units in the head group. They concluded, therefore, that the oligofructose residues do not extend vertically away from the surface but adopt a more tilted orientation.

The succinylation reaction used in the synthesis of the ASA-inulins gives rise to the formation of a half ester and hence there will be a carboxylate group present in the linkage between the alkenyl chain and the inulin molecule. The zeta potential for emulsion droplets with the DS values 7.9% OSA and 11.4% DDSA were determined as a function of pH and the results are shown in Figure 3. It was noted that the zeta
potential increased as the pH increased and had a value of ~ -5mv and ~60mv at pH 2 and pH 7 respectively. The increase is due to the ionisation of the carboxylate group as the pH increased (Wurzburg, O.B., 2006).

Effect of DS

The droplet size for emulsions prepared using 2.5% OSA- and DDSA- inulin are plotted as a function of the DS in Figure 4. The surface weighted mean (d_{3,2}) and the volume weighted mean (d_{4,3}) diameters of both OSA- and DDSA-inulin were found to decrease as the DS increased up to a value of ~ 8 mole % and then remained constant. It is estimated that this level of substitution corresponds approximately to 1.5 OSA- / DDSA- residues per inulin chain. Wang, Williams and Senan (2014) determined the droplet size of MCT emulsions using DDSA-modified gum Arabic and found the droplet size to be smaller for emulsions prepared with gum containing 10% hydrophobe compared to 5% hydrophobe. The droplet size was found to be smaller for emulsions prepared with DDSA-inulin compared to OSA-inulin at equivalent DS values indicating that the increased alkenyl chain length for the former facilitates more effective adsorption onto the oil droplets during the homogenization process as discussed above.

Emulsion stability on aging

The droplet sizes for emulsions prepared using 2.5% OSA- and DDSA- inulin were monitored over time at room temperature (25°C) to assess their effectiveness on aging and the results are presented in Figures 5 and 6. With the exception of OSA-inulins with DS values of 5.2 and 5.6 mol %, the surface weighted mean (d_{3,2}) and
the volume weighted mean \(d_{4,3}\) diameters for both the OSA- and DDSA-inulins remained relatively constant over the 21 day period both at room temperature and 50°C indicating that flocculation and/or coalescence did not occur.

*Emulsion stability in the presence of electrolyte*

The droplet sizes for emulsions prepared using 2.5% OSA- and DDSA- inulin after the addition of electrolyte are given in Figures 7a and 7b. The surface weighted mean \(d_{3,2}\) and the volume weighted mean \(d_{4,3}\) droplet diameters for emulsions prepared using OSA-inulin increased significantly at room temperature (25°C) and oil separation was noted after 14 days. For the emulsions prepared using the DDSA-inulin, the surface weighted mean \(d_{3,2}\) diameter remained constant but the volume weighted mean \(d_{4,3}\) diameter increased indicating that some droplet aggregation or coalescence had occurred. This finding indicates that electrostatic repulsive forces, which arise due to the presence of the carboxylate group in the linkage between the alkenyl chain and carbohydrate head group, play a key role in the ability of the modified inulins to stabilise the emulsions. It also suggests that the adsorbed layer thickness is insufficient to provide stability through steric repulsions indicating that the molecules must adopt a fairly flat conformation at the interface.

**Conclusions**

It has been shown that OSA- and DDSA- modified inulins are able to stabilise 15% w/v O/W emulsions at concentrations above ~2%. DDSA-inulins produced emulsions with a smaller droplet size than OSA-inulins. The minimum droplet size was achieved when the % hydrophobe incorporation was above ~8 mole% for both OSA-


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Table 1.

DS of modified inulin samples.

<table>
<thead>
<tr>
<th>Inulin</th>
<th>Reagents</th>
<th>DS* / moles %</th>
</tr>
</thead>
<tbody>
<tr>
<td>N10</td>
<td>OSA</td>
<td>5.2</td>
</tr>
<tr>
<td>DS2</td>
<td>OSA</td>
<td>5.6</td>
</tr>
<tr>
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<td>OSA</td>
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<td>7.9</td>
</tr>
<tr>
<td>DS2</td>
<td>DDSA</td>
<td>11.4</td>
</tr>
</tbody>
</table>

* Determined by NMR spectroscopy (Kokubun et al., 2013)
Figure 1. Droplet size for emulsions prepared using various OSA-inulins in the presence of different OSA-inulin concentrations.
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7(a)

Droplet size [µm]

Day 0 5 10 15 20

- 7.9% d 4,3 water
- 7.9% d 3,2 water
- 7.9% d 4,3 electrolyte
- 7.9% d 3,2 electrolyte

OSA
Figure 7 Droplet size for emulsions prepared using (a) 2.5% OSA-inulin and (b) 2.5% DDSA-inulin after addition of 0.5 NaCl.