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High internal phase emulsion gels (HIPE-gels) created through assembly of natural oil bodies

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ABSTRACT

A natural emulsion was used to create a high internal phase emulsion (HIPE) gel with elastic properties, indicated by shear elastic moduli between 10^2 and 10^5 Pa. The elasticity of the gel network was provided from a 2D-gel network of proteins which were naturally adsorbed at the interface of an oil-in-water emulsion formed after aqueous extraction of oil bodies from sunflower seeds. Extensive centrifugation of the obtained emulsion resulted in a stable ultrahighly concentrated emulsion with an oil volume fraction of 0.91 and a protein content of 2.5 wt% only. This high volume fraction of the emulsion cream was achieved due to the large deformability (low rigidity) of the oil body surface. After formation of the HIPE, the rigidity of the interfacial network was increased by addition of small concentrations of Ca^{2+} and heating at 72 °C for 10 min. This led to aggregation of the interfacial proteins, thereby forming a 2D interfacial gel providing a space-spanning network. The behaviour of the self-supporting gel exhibited increased elastic behaviour, determined by the increased elastic modulus of the interfacial network. The balance between the low rigidity upon formation and the increased rigidity after formation offers a tempting strategy to produce structured solid matter that contains edible hydrophobic liquids. Additionally, the followed procedure is cost-effective and friendly to the environment.

1. Introduction

Solid-like hydrophobic matrices have functional usage in different type of materials. They have a large range of applications in food and pharmaceutical formulations, in cosmetics, as lubricants and many others. The solid-like lipophilic phases are generally required to provide "body" to the formulations. They are used to alter the specific rheological behaviour and textural properties, to provide stability against heating or as carriers of lipophilic compounds (Dietsch et al., 2008). Hydrophobic substances with tuneable structural properties are desirable for the formulation of a large range of materials, and often they provide certain elasticity to the product. Controlling the elasticity of such a soft matter is therefore desirable.

An example of such promising new hydrophobic materials with tuneable elastic properties are organogels, which are hydrophobic liquids structured through an assembly of dispersed compounds. In the food and pharmaceutical industry, organogel formation is a strategy to impart solid-fat functionality to liquid oils (Sahoo et al., 2011). Although there are already several paths to create organogels (Co & Marangoni, 2012), a main difficulty faced is tuning their textural properties, such as elasticity.

An alternative approach to form structured hydrophobic liquids is the employment of emulsions in the form of a very concentrated internal oil phase, also known as high internal phase emulsions (HIPE). HIPEs are characterized by a network of the droplet interfaces. The properties of the interfaces on a mesoscopic scale will determine the macroscopic properties of the resulting gel, and therefore commonly depend on a variety of factors, including the type of the surfactant, the thickness of the film between the droplets, the interfacial elasticity and the surface aggregation (Kizling, Kronberg, & Eriksson, 2006). Control over interfacial properties therefore provides opportunities to tune certain properties of the gel. To obtain such high oil content, large deformation of the oil droplets into polyhedral shapes by stretching the droplet surface and formation of plateau borders are necessary. A certain droplet flexibility is therefore required (Israelachvili & Wennerstrom, 1992). These high internal phase

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emulsions (HIPE) can be created by using low molecular weight surfactants, since these interfaces have been shown to be highly deformable (Lissant, Peace, Wu, & Mayhan, 1974). However, they often show low stability as the interfaces are not strong/thick enough to prevent coalescence of the oil droplets (Zhang, Chen, & Perchyonok, 2009). The strength of the interface can be increased by using interfacial proteins to prevent droplet coalescence. Proteins often provide a more rigid 2D-network at the interface, characterized by a viscoelastic behaviour with high elasticity (Lucassen-Reynders, Benjamins, & Fainerman, 2010). However, such a high rigidity may prevent extensive deformation and stretching of the interface, leading to rupture of the interfacial film (van Aken, 2002). Interfacial layers should therefore have a low dilatational modulus, in order to allow surface deformability and provide a sufficient barrier for droplet coalescence. Recently published data have shown that mixtures of phospholipids and oleosins, the main interfacial proteins of oil bodies, form interfacial layers with a low dilatational elasticity (Deleu et al., 2010). This behaviour could be beneficial for the formation of HIPEs.

In the present work, we use a natural emulsion made of sunflower oil bodies to create HIPEs with tuneable rheological behaviour. Oil bodies are intracellular organelles that exist in oleaginous seeds and can be extracted using an environmental friendly aqueous alkaline extraction, which leads to an extremely stable natural oil-in-water emulsion (Nikiforidis, Karkani, & Kiosseoglou, 2011; Nikiforidis, Biliaderis, & Kiosseoglou, 2012). Oil body emulsions consist of oil droplets naturally covered with phospholipids, oleosins and some other minor proteins, called caleosins and steroleosins (Frandsen, Mundy, & Tzen, 2001; Nikiforidis, & Kiosseoglou, 2011). Oleosins are low molecular mass proteins that are distinguished from other proteins for their extended central hydrophobic domain which covers almost half of its entity. This relatively large hydrophobic domain faces the lipid core of the droplet and its size is probably the reason that it is hard to displace oleosins from the surface. Apart from the interfacial proteins, an additional secondary protein layer can provide extra protection of the oil bodies from destabilization (Nikiforidis & Kiosseoglou, 2010; Karkani, Nenadis, Nikiforidis & Kiosseoglou, 2013). Due to economic, environmental and health benefits (Fisk, White, Carvalho, & Gray, 2006; Nikiforidis & Kiosseoglou, 2009), natural highly concentrated oil body emulsions could be a good source for the formation of stable oil-in-protein gels with controlled rheological properties. The rheological properties of sunflower oil body creams with an oil concentration up to 68 wt% has already been studied (White et al., 2008). In this work, we investigate the behaviour and physicochemical properties of high internal phase oil body creams with oil concentration up to 91 wt%, and increased interfacial elasticity.

2. Experimental section

2.1. Materials

Intact sunflower seeds were purchased from the local market. All experiments were performed with seeds from the same batch and from freshly opened containers. All other chemicals were of analytical grade and obtained from Sigma–Aldrich Co. LLC.

2.2. Oil body extraction

The oil bodies were isolated using an aqueous extraction method (Nikiforidis & Kiosseoglou, 2009). The intact sunflower seeds were subjected to comminution, using a domestic grinder fitted with knives (KM 75, Krups, Mexico). The sunflower flour was initially soaked in deionised water (20 wt%) and the pH was adjusted and kept constant at 9.0, using a 0.1 M NaOH solution, while continuously agitating for 24 h with the use of a mechanical stirrer (Kika Labortechnik, Malaysia) at 1200 rpm. The mixture was then subjected to intensive agitation with a domestic blender (8011-ES, Waring, USA) for 40 s and the resulting sunflower seed dispersion was filtered through two layers of cheesecloth. The sunflower seed residue was then again extracted with deionised water at pH 9.0. The oil body dispersions containing both oil bodies and sunflower seed protein debris were combined into one and the pooled dispersion was subjected to centrifugation (Avanti J-26XP, Beckman Coulters, USA) at 4000 *g* for 20 min to remove insoluble solids. 0.01 wt% NaN_3 was added as an antimicrobial agent. The recovered oil body dispersion was stored at 5 °C, and analysed for moisture, fat and protein.

2.3. Centrifugation of the initial emulsion

The recovered oil body dispersion was centrifuged at 5000–30,000 *g* at 4 °C for 30 min and the supernatant sunflower seed oil body cream was recovered and washed once more with water. The specific centrifugal acceleration, g_κ (m/s^2), determines the pressure applied to the oil droplet needed for the deformation of the droplets. As a quantitative measure of emulsion instability, the formation of a thin continuous oil layer on top of the emulsion cream due to coalescence of the droplets is often used (Tcholakova, Denkov, Ivanov, & Campbell, 2002). Droplet coalescence occurs at a critical osmotic pressure Π_{crit}, determined from the difference between the mass densities of the oil and water phase, $\Delta\rho$, the centrifugation acceleration, g_κ, the initial volume fraction in the produced cream, φ_i, and the height of the emulsion, l, as:

$$\Pi_{crit} = \Delta\rho g_\kappa \phi_i l \quad (1)$$

In order to study the effect of applied force on the droplet deformation, equal volume fractions of the initial emulsion, φ_i, were centrifuged at different centrifugal acceleration (until 30,000 *g*). Moisture, fat and protein content of the resulted highly concentrated emulsions were determined according to standard methods of AOAC (AOAC, 1994). Finally, different amounts of calcium (from 0.1 to 0.4 mM) were added in the recovered cream followed by heating at 72 °C for 10 min.

2.4. Confocal Laser Scanning Microscopy imaging (CLSM)

CLSM images were obtained at room temperature on a LEICA TCS SP5 Confocal Laser Scanning Microscope (Leica Microsystems GmbH., Mannheim, Germany) equipped with an inverted microscope (model Leica DMI6000), containing a set of four visible light lasers. The used objectives were HC PL APO 10x/0.40 CS and HC PL APO 20x/0.70 IMM/CORR CS. Digital image files were acquired in 1024 × 1024 pixel resolution. Samples were carefully placed on a microscope slide and stained with Nile Blue.

2.5. Particle size analysis

Particle size distribution of oil body emulsions was determined with the aid of a laser light scattering instrument (Malvern Mastersizer 2000, UK). The refractive index ratio used to calculate the oil body size distribution was taken as 1.09. Measurements are reported as the surface weighted (d_{32}) mean diameter and volume weighted mean diameters (d_{43}). Droplet size distribution measurements were conducted both on fresh and on aged oil body dispersions. Measurements were performed at room temperature following sample dilution with deionized water to an approximate