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Sunflower-seed oil body emulsions: Rheology and stability assessment of a natural emulsion

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Abstract

The viscoelastic characteristics of a purified oil body cream (67.6 ± 0.7% lipid, 5.4 ± 0.7% protein and 25.2 ± 0.1% moisture) recovered from sunflower seeds (*Helianthus annuus* L.) have been determined. Moreover, the effects of pH (2–7) and $CaCl_2$ concentration (0–150 mM) on rheology and physical stability of oil body emulsions have been studied. Oscillatory measurements showed that the purified oil body cream exhibited weak gel-like behaviour. Diluted oil body emulsions (⩽20 wt% oil) showed extensive creaming (creaming index = 56–59%) at pH 5–6 resulting in significant ($P<0.05$) increases in perceived droplet size ($D(3,2)$ = 11–13 μm compared with 0.3 μm at pH 7) and viscosity (0.025–0.035 Pa s at shear rate 10 s^{-1} compared with 0.008 Pa s at pH 7). Microscopic examination revealed that the emulsion droplets aggregated at pH 5–6 but did not coalesce. The influence of $CaCl_2$ was investigated at pH above (pH 7) and below (pH 3) the isoelectric point (IEP) of the intrinsic oleosin proteins (pH 5⩽IEP⩽pH 6) associated with the surface of the oil bodies. At pH 7, oil bodies were stable up to a $CaCl_2$ concentration of 1.5 mM; at 5–150 mM $CaCl_2$ creaming occurred (66–70%), and significant ($P<0.05$) increases in perceived droplet size ($D(3,2)$ = 10–13 μm) and viscosity (0.015 Pa s at shear rate 10 s^{-1}) were observed. At pH 3 there was no significant ($P>0.05$) influence of $CaCl_2$ on emulsion stability or rheology. These findings demonstrate that rheology and stability of oil body emulsions are, like processed emulsions, affected by pH and by ionic concentration when the pH is above the IEP of the surface components of the oil body. The novelty in this work therefore lies in the source of the emulsion, a natural, pre-formed oil-in-water emulsion derived from seed tissue.

Keywords: Rheology; Oil bodies; Sunflower; pH; $CaCl_2$

1. Introduction

Plant seeds store cellular triacylglycerols (TAGs) in distinct spherical droplets called oil bodies that act as energy stores for post-germinative growth. These oil bodies, typically 0.5–2.5 μm in diameter, consist of a TAG core surrounded by a monolayer of phospholipids embedded with small alkaline proteins (15–26 kDa molecular mass) specific to oil bodies called oleosins, some minor proteins termed caleosin and steroleosin (Chen, Tsai, & Tzen, 1999; Lin, Tai, Peng, & Tzen, 2002; Naested et al., 2000; Tzen, Cao, Laurent, Ratnayake, & Huang, 1993; Tzen & Huang, 1992) and some additional lipophilic compounds, such as tocopherol (Fisk, White, & Gray, 2006). Oleosin proteins have three structural regions; an amphipathic N-terminal region, a central hydrophobic antiparallel β-strand domain and an amphipathic C-terminal domain of variable length (Tzen, Lie, & Huang, 1992). The topology of oleosin in the oil body membrane exposes negatively charged residues giving oil bodies an overall electronegative charge; given that the pH of mature seeds is near to neutral, it is therefore likely that oil bodies in vivo are stabilised, at least in part, through electrostatic repulsion. Several authors also claim that the steric hindrance, conferred by oleosin proteins at the surface of oil bodies, prevents coalescence in vivo and when oil bodies are dispersed in an aqueous phase (Tzen & Huang, 1992; Tzen et al., 1992). The use of the term 'steric hindrance' in this context may however be misleading, as we discuss later.

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Oil bodies dispersed in an aqueous media represent a natural emulsion system. Indeed, the use of oil bodies isolated from plant seeds may offer a strategy to deliver stable, pre-emulsified oil into appropriate food systems. The stability and rheology of oil-in-water emulsions is dependent on the interactions between droplets which are in turn dependent on the interfacial composition. In most emulsions stabilised with proteins, among the many colloid interactions present between droplets, the electrostatic interactions are often the most significant in influencing droplet aggregation (ultimately leading to coalescence). Subsequently, the stability of protein-stabilised emulsions is susceptible to changes in ionic/electrolyte concentration and pH (McClements, 1999). When the pH of this kind of emulsion is close to the isoelectric point (IEP) of the stabilising protein, droplet aggregation occurs (electrostatic repulsion between droplets negated). When the pH is far away from the protein's IEP, the charge on the emulsion droplet increases, raising stability against droplet aggregation. Electrolyte ions influence protein-stabilised emulsions essentially in four different ways: by electrostatic screening thereby reducing droplet repulsion; binding to emulsion droplets lowering electrostatic repulsion; increasing hydration repulsion between droplets and by altering structural organisation of water molecules, changing hydrophobic interactions between non-polar groups (Israelachvili, 1992; McClements, 1999). Indeed, the effects of variable pH and ionic strength on emulsions stabilised with proteins have been well documented (Demetriades, Coupland, & McClements, 1997a, b; Demetriades & McClements, 1998; Hunt and Dalgleish, 1994, 1995; Kulmyrzaev, Silvestre, & McClements, 2000a, 2000b). Apart from interfacial properties, the volume fraction of the dispersed phase, the average size of the droplets and the droplet size distribution can also affect the stability of emulsions (McClements, 1999).

To date, only one study has reported the rheology (viscoelastic properties) of an oil body emulsion. In this study, Mason and Friis (2001) used a rapeseed oil body preparation recovered in an acidic medium and minimally washed; no indication of the purity of the preparation was included. The authors also acknowledge that the medium droplet size was 8 μm which is outside the range of native oil bodies; this is probably due to aggregation and limited purification of the oil body emulsion. A fundamental understanding of a purified oil body emulsion's physical stability, under variable conditions such as pH and ionic strength, would allow us to incorporate oil body emulsions effectively into selected food systems. In this study, the rheology and stability of oil body emulsions recovered and purified from sunflower seeds and exposed to variable conditions of pH and $CaCl_2$ concentration have been assessed.

2. Materials and methods

2.1. Materials

Sunflower seeds were purchased from Lembas Ltd. (Sheffield, UK). Sodium azide and $CaCl_2$ were purchased from Sigma Chemical Company (Dorset, UK). Deionised, polished water (14–18 MΩ, total organic content <30 ppb, pH 6.8) was used to recover oil bodies and diluted where appropriate.

2.2. Recovery and washing of oil bodies

Sunflower seeds were homogenised (Kenwood BL315 blender, Havant, UK) in deionised water (1:5 w/v, 0.02% w/v sodium azide) for 2 min. The slurry was then filtered through three layers of cheesecloth and the filtrate centrifuged at 10,000*g* (Beckman J2-21 centrifuge, fixed rotor JA-10) for 30 min at 10 °C. The crude oil bodies, which collected as a creamy pad at the top of the mixture, were then carefully picked from the media and resuspended in 9 M urea (1:5 w/v), vigorously shaken and then left on a rotary roller mixer (SRT 2 roller, Stuart Scientific, Staffordshire, UK) for 10 min. The mixture was then centrifuged as above and the creamy pad isolated. This pad was then washed with deionised water for another three times, to remove urea from the preparation. Finally, the preparation was washed with hexane to eliminate the influence of free lipid on the behaviour of dispersed oil bodies (Tzen, Peng, Cheng, Chen, & Chiu, 1997). The final washed oil body preparation contained 67.6 ± 0.7% lipid, 5.4 ± 0.2% protein and 25.2 ± 0.1% moisture using methods described below.

2.3. Proximate composition

The proximate composition of the oil body cream was measured sequentially beginning with the moisture content followed by the lipid content and finally the protein content. The moisture content of the oil body cream was determined gravimetrically following vacuum drying (Gallenhamp −900 mBar, 50 °C, 24 h). The lipid content of the dried oil body preparation (ca. 0.5–1 g) was quantified gravimetrically using repeated extraction with diethyl ether (Murphy & Cummins, 1989). The protein content of the defatted dried oil bodies was determined using the BCA assay (Smith et al., 1985) following solubilisation of proteins in 2% sodium dodecyl sulphate (SDS) solution at 90 °C. Bovine serum albumin was used as a protein standard. All measurements were conducted in triplicate.

2.4. Protein separation by SDS-PAGE

Samples following protein quantification using the BCA assay were diluted to 10 μg protein/mL and 10 uL was loaded for SDS-PAGE separation. Proteins were resolved by SDS-PAGE using 15% and 4.0% polyacrylamide gels in the separating and stacking gel, respectively; gels were positioned within a SE 600 BioRad separation unit and suspended in tank buffer (0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3). Proteins were then resolved with a fixed current of 40 mA. After electrophoresis, the gel was washed (15 min) three times with distilled water then