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Rheological characteristics and physicochemical stability of dressing-type emulsions made of oil bodies–egg yolk blends

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ABSTRACT

Two oil body creams, differing in oil volume fraction and surface protein composition, were obtained by applying alkaline aqueous extraction to comminuted maize germ and then recovering the oil bodies from the extract by centrifugation, either in the presence of 0.5 M sucrose (OB-W) or following isoelectric precipitation at pH 5.0 (OB-A). Oil bodies in the former cream are stabilised by natural oil body surface proteins while in the OB-A cream, exogenous proteins, in addition to natural oil body proteins, are also present. The creams were blended with appropriate amounts of water, NaCl and liquid yolk, and the pH was adjusted to 3.8 to obtain 20% or 45% (w/w) in oil OB-W and 20% (w/w) in oil OB-A model dressing-type emulsions. The physical stability of the emulsions, against creaming and coalescence, was monitored upon storage, while the development of emulsion structure during ageing was probed by applying steady shear and small deformation oscillatory rheometry. The adsorbed to oil bodies' surface proteins were analysed by applying SDS–PAGE. Since no yolk protein constituents were detected at the oil body surface layer of the emulsions, it is hypothesised that the presence of unadsorbed yolk particles in the emulsion continuous phase results in the intensification of interdroplet interaction effects, due to depletion events, and may therefore have an indirect but nevertheless strong influence on emulsion structure and physical stability.

1. Introduction

Oil bodies are small-sized organelles where lipids are found in many oil-rich plant raw materials, including maize germ (Tzen & Huang, 1992). One common feature of all oil bodies is the presence, at their surface, of a mixed phospholipids-protein membrane that confers physical and chemical protection to the oil core against environmental stresses, such as moisture and temperature fluctuations (Shimada & Hara-Nishimura, 2010; Shimada, Shimada, Takahashi, Fukao, & Hara-Nishimura, 2008). The oil core is mainly composed of TAG and a small amount (<0.1%) of free fatty acids (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993). The fatty acid profile of maize germ oil bodies depends on the variety, with linoleic acid (49.7–62.7%) being the predominant fatty acid component, followed by oleic (23.5–34.9%) and palmitic (9.5–11.5%) acids (Saoussem, Sadok, Habib, & Mayer, 2009). The dominant proteins of the oil body surface are called oleosins (Chen, Lin, Huang, & Tzen, 1997; Jolivet et al., 2004; Simkin et al., 2006; Tzen & Huang, 1992). These are very hydrophobic proteins of a relatively low molecular mass (15–20 kDa). Other minor groups of proteins present at the oil body surface are the caleosins (27 kDa) and steroleosins (40 kDa) which also possess a hydrophobic central domain, but with fewer residues (Lin, Liao, Yang, & Tzen, 2005; Purkrtova, Jolivet, Miquel, & Chardot, 2008).

Traditionally, maize germ oil is used for food preparation after extraction with an organic solvent and refinement. This process is characterised by high extraction efficiency and low cost. However, the use of volatile organic solvents very often results in environmental pollution, while safety problems, because of organic solvent inflammability, also constitute a matter of concern. Moreover, the organic solvent extraction processes may have a detrimental impact on protein functionality (Moure, Sineiro, Dominguez, & Parajo, 2006; Rosenthal, Pyle, & Niranjan, 1998). It could, therefore, be more advantageous to apply aqueous extraction to oil seeds in order to recover the oil, as an aqueous dispersion of oil bodies, as suggested by a number of researchers (Iwanaga et al., 2007; Kapchie, Wei, Hauck, & Murphy, 2008; Nikiforidis & Kiosseoglou, 2009; Tzen et al., 1993; White et al., 2008). Application of aqueous extraction to oil seeds results in a natural emulsion of oil bodies dispersed in water and, as such, it may be exploited in this form as a pre-emulsified oil food ingredient. For example, these oil bodies could be used in the preparation of liquid or semi-liquid food products, appearing in the form of oil-in-water emulsions, such as mayonnaise, salad dressings, creams, imitation milk and cream liqueurs. The use of the very small-sized (<1.0 μm)

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oil bodies in the preparation of such products, in place of vegetable oil, would help to avoid the application of the high energy-consuming step of homogenisation required for oil droplet size reduction and prepare the food dispersion by simply mixing the oil bodies with the rest of the ingredients. Additionally, oil bodies appear to be stable against oxidation, and hydroperoxide formation is significantly reduced in emulsions based on oil bodies compared to emulsions formulated with other surfactants (Fisk, White, Lad, & Gray, 2008).

According to the results of a number of studies, the physicochemical stability of oil body dispersions, against aggregation and coalescence, depends on the method applied for oil body recovery, the presence of exogenous proteins, the pH value and NaCl concentration (Iwanaga et al., 2007; Nikiforidis, Karkani, & Kiosseoglou, 2011; Nikiforidis & Kiosseoglou, 2010). For instance, the mean diameter of the maize germ oil bodies in the matrix extract is around 0.3 μm (Nikiforidis & Kiosseoglou, 2010), which changes, depending on the method applied to recover the oil bodies. When applying isoelectric precipitation, the droplet diameter is not higher than 0.45 μm, while, if the oil bodies are recovered after the addition of sucrose, the d_{32} value is around 0.7 μm. In addition, a number of recently published research papers have been dealing with the improvement of oil body stability to environmental stresses (Chen, McClements, Gray, & Decker, 2010; Iwanaga, Gray, Decker, Weiss, & McClements, 2008; Nikiforidis & Kiosseoglou, 2010; Nikiforidis & Kiosseoglou, 2011). Successful exploitation of oil bodies in the preparation of food products, however, should depend on an adequate understanding of their interaction with other product ingredients. Proteins, in particular, which tend to adsorb to oil–water interfaces, may or may not interact with oil body surfaces and influence (in a different way) the product rheological and structural characteristics and hence consumer acceptability.

As stated above, oil bodies could find use in the preparation of salad dressings and the aim of this study was to provide the scientific basic information for the exploitation of oil bodies extracted from maize germ in the preparation of such emulsions. Taking into account that these products are characterised by a low pH, and the presence of NaCl and egg yolk, the objective of this paper is to elucidate how the yolk constituents and the oil bodies behave when mixed together under the conditions prevailing in the system and what is the effect of oil bodies–yolk protein interaction on a number of key emulsion physicochemical properties, such as rheological characteristics and physical stability upon storage.

2. Materials and methods

2.1. Materials

A corn milling industry by-product, rich in maize germ, was used for the aqueous extraction of oil bodies. The germs were separated by hand and subjected to comminution, using a Brown mill fitted with knives. Fresh hen eggs were purchased from the local market. The eggs were broken manually and the yolks were separated from the albumen. The vitelline membrane was then pierced and the liquid yolks of a number of eggs were collected and pooled.

2.2. Oil body extraction and emulsion preparation

Two oil body creams, one rich in exogenous germ proteins (OB-A) and a second practically free from their presence (OB-W), were prepared (Nikiforidis & Kiosseoglou, 2010). The overall process was divided into two steps. The first step was common for both types of creams and involved extensive alkaline extraction that resulted in an aqueous oil body dispersion. The extract was then divided into two parts and the oil body cream samples were recovered either by acid coagulation or by extensively washing with a sucrose solution to remove extraneous proteins. More precisely, the comminuted germ material (250 g) was initially soaked in deionised water (20% w/v) and the pH was adjusted and kept constant at 9.0, using a 0.1 M NaOH solution, while constantly 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 (speed set at position 2) for 40 s by employing a Braun Blender (Type: 4249, Germany) and the resulting dispersion was filtered through three layers of cheesecloth. The germ residue was then again extracted with deionised water at pH 9.0; the two extracted oil body dispersions were combined into one, and the resulting pooled dispersion was subjected to centrifugation (Firlabo SV11, France) at 4000*g* for 15 min to remove insoluble solids. The supernatant containing the oil bodies was divided into two parts. In the first, the recovery of oil bodies in the form of a cream was effected by adjusting the pH to 5.0 with 0.1 M HCl and centrifuging at 4000*g* for 30 min. The creamed layer at the top of the centrifuge tube was carefully removed, dispersed in deionised water (1:5) and the pH was adjusted to 9.0 with 0.1 M NaCl. Following stirring for 1 h to break the aggregates, the pH value was again lowered to 5.0, the dispersion was centrifuged at 4000*g*, and the resulting cream (OB-A) was collected. The second part of the suspension was mixed with an equal volume of a 0.5 M sucrose solution at pH 9.0 and centrifuged at 4000*g*. The cream at the top was then recovered and treated one more time with the alkaline sucrose solution; the latter washed cream (OB-W) was collected. Both creams, obtained by applying the two different isolation schemes, were analysed for moisture, fat and protein according to standard methods of AOAC (1994). To prepare dressing-type emulsions, cream samples were mixed with suitable amounts of deionised water, NaCl and liquid egg yolk and the pH of the blends was adjusted to 3.8. Depending on the type of oil body cream, 20% (w/w) oil-in-water emulsions using the OB-A and 20% or 45% (w/w) oil-in-water emulsions using the OB-W, were prepared. The contents of liquid egg yolk and NaCl in all emulsions were 2.5% and 1.5% (w/w) in the aqueous phase, respectively. In addition, control emulsions were also prepared by omitting the yolk from the formula. The samples were stored at 4 °C and studied over a period of 75 days.

2.3. Protein characterisation by SDS–PAGE

Emulsion samples were centrifuged at 10,000*g* for 30 min and their cream layers were recovered and dispersed in distilled water (1:5). Following centrifugation the creams were dispersed in a buffer containing 50 mM Tris-HCl, 5 M urea, 1% SDS and 4% 2-mercaptoethanol at pH 8.0, at a concentration of 1.5 mg protein/ml of buffer. After incubation for 1 h at room temperature, an equal volume of an electrophoresis sample buffer of 125 mM Tris-HCl, 5 M urea, 1% SDS, 20% glycerol and 4% 2-mercaptoethanol, was added. Following boiling for 2 min and the application of two freeze-thaw cycles, the supernatant was removed and analyzed by SDS–PAGE (Laemmli, 1970). The electrophoresis gels were prepared using 4.5 and 12.5% acrylamide solutions for the stacking and separating gels, respectively. Protein fractions were fixed by immersing into a 12.5% trichloroacetic acid solution. The gels were stained with Coomassie brilliant blue G-250 and photographed with the aid of a digital camera (Sony, DSC-W90). Determination of protein molar mass was performed with the aid of the Gel-Pro Analyzer (Media Cybernetics, Maryland, USA).