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Oil bodies: An insight on their microstructure — maize germ vs sunflower seed

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ABSTRACT

Storage triacylglycerols in oleaginous seeds are surrounded by a layer that consists of phospholipids and proteins, mainly oleosins. These entities are intracellular organelles, known as oil bodies. It is often reported that they have a spherical shape, but imaging using cryo-SEM analysis showed that they are rather elastic and their shape depends on their surrounding environment. In this research we have shown that in maize germ, which has a relatively low moisture content, the oil body geometry depends on the available space they have. On the other hand, oil bodies in sunflower seeds, which contain double amounts of water, appear with an almost spherical shape. Oil bodies can be extracted from oleaginous seeds using an aqueous alkaline extraction, which leads to a stable natural oil-in-water emulsion. As no additional energy is required, this method can be considered as sustainable and may find a lot of potential uses in industry. Extraneous co-extracted proteins most likely form a second layer around the oil body surface, which protects the oil bodies from coalescence, even at high oil concentration. The extraneous proteins of maize germ oil body emulsions could be removed by applying aqueous washing steps, but not in the case of sunflower seed oil bodies.

1. Introduction

During recent years numerous research articles have appeared concerning the extraction, isolation and characterization of oil bodies from different sources (Acevedo et al., 2012; Adams et al., 2012; Nikiforidis & Kiosseoglou, 2009; Rodelas et al., 2008). The aqueous extraction of oil bodies results in a natural oil-in-water emulsion that can potentially have a lot of applications in food or other industrial products (Chiang, Lin, Lu, & Wang, 2011; Nikiforidis, Biliaderis, & Kiosseoglou, 2012). Industry could be highly benefited from this natural emulsion, due to the environment-friendly and cost-effective process since no volatile organic solvents are used to extract the oil and final emulsion preparation does not require a homogenization step. Moreover, the traditional procedure of oil extraction, involving the use of organic solvents, often leads to severe deterioration of seed protein functionality, making them useful for animal feed only (Moure, Sineiro, Dominguez, & Parajo, 2006; Rosenthal, Pyle, & Niranjan, 1996).

Electron micrographs indicated that oil bodies are particles formed in eukaryote cells at the surface of vesicles (Herman, 2009) to store lipids as food reserves, which will be mobilized during the period of active metabolism (Huang, 1992). The particles consist of an electron-opaque core of triacylglycerides surrounded by an electron-dense layer. This indicates the existence of phospholipids at the surface, which are embedded in a protein layer, mainly consisting of the low molecular weight protein called oleosin. The relatively high stability of oil bodies in the seeds, is attributed to the formation of this dense surface layer. According to SEM and TEM analyses (Heneen et al., 2008) the intracellular oil bodies have a spherical or elliptical shape with an apparent diameter higher than 1.0 μm, which is probably affected by environmental factors and differs between seeds of different origins. A peculiar fact is that the size of the isolated oil bodies in the natural emulsion appears to be smaller when determined by laser diffraction. This discrepancy might be due to the method used for size determination or to the sample pre-treatment applied for SEM or TEM analysis.

Recently published results indicate that the recovery method of the natural oil body emulsion has an appreciable influence on the particle size, which can range from nanoscale (Nikiforidis, Karkani, & Kiosseoglou, 2011) to microscale (Huang, 1996). The process of aqueous extraction that initially involves mixing the grinded seeds with water, followed by intensive agitation, leads to the preparation of a dilute oil body extract. To separate the oil bodies from the additional extracted material a centrifugation step is necessary. Depending on the conditions during the oil body recovery process, a certain degree of oil body destabilization may take place (Nikiforidis & Kiosseoglou, 2010). For higher stability, another recovery method has also been suggested. This method involves the combination of the aqueous extraction

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with ultrafiltration (Nikiforidis et al., 2011). This procedure results in an oil body nanoemulsion with extreme physical stability, even after boiling for 1 h or after freeze-thaw cycles using a temperature of −70 °C.

Apart from their physical stability, oil bodies also appear to be remarkably stable against oxidation (Chen, McClements, Gray, & Decker, 2012; Gray, Payne, McClements, Decker, & Lad, 2010). Additionally, recent research shows that the recovery method has a large effect in the oxidative stability of oil body emulsions (Karkani, Nenadis, Nikiforidis, & Kiosseoglou, 2013). In general, hydroperoxide and secondary oxidation product formation is significantly lower in natural emulsions based on oil bodies compared with technically-prepared emulsions formulated with other surfactants. The higher chemical stability of the natural emulsions is probably related to the extraneous proteins found in the continuous phase and their stable interface (Chen et al., 2012; Gray et al., 2010). The oxidative stability of the unsaturated fatty acids in oil bodies, combined with the presence of bioactive compounds like tocopherol (Fisk & Gray, 2011; Fisk, White, Carvalho, & Gray, 2006), may also contribute to the increasing interest for their use in the preparation of food or other products.

Due to the rather high interest on the physicochemical properties of oil bodies, the aim of the present study was to understand the factors that influence their behavior both in the seeds and in the recovered oil body cream (by aqueous extraction). For this reason microscopy techniques were applied for the first time in order to investigate two different biological materials that are rich in oil; maize germ and sunflower seeds. The advantage of the used microscopy technique (cryo-SEM) is that no extensive pre-treatment is required, as it is with the use of Transmitted and Scanning Electron Microscopy techniques. Furthermore, the physical characteristics of their oil body emulsions and creams obtained by applying such an aqueous extraction were also studied.

2. Material and methods

2.1. Materials

Maize germ was purchased from a milling industry in North Greece and intact sunflower seeds from a local market. All other chemicals, which were of analytical grade, were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

2.2. Cryo-Scanning Electron Microscope imaging (cryo-SEM)

Dry and rehydrated maize germ and sunflower seeds were glued on a brass sample holder by carbon glue (Leit-C, Neubauer Chemicalien, Germany) and subsequently frozen with the use of liquid nitrogen. The same procedure was also followed for the maize germ and sunflower seed oil body creams. The sample holder was fitted into the transfer cryogenic Leica holder. All manipulations were carried out under liquid nitrogen.

The Leica sample holder was transferred in a non-dedicated cryo-preparation system (MED 020/VCT 100, Leica, Vienna, Austria) onto a sample stage at −93 °C. In this cryo-preparation chamber the samples were immediately fractured and freeze-dried for 23 min at −93 °C at 1.3×10^{-6} mBar to remove contaminating water vapor. The samples were sputter-coated with a layer of 4 nm Tungsten at the same temperature and transferred cryo-shielded into the field emission scanning microscope (Magellan 400, FEI, Eindhoven, the Netherlands) onto a sample stage equilibrated at −122 °C at 4×10^{-7} mBar. The analysis was performed with SE at 2 kV, 13 pA. All images were recorded digitally.

2.3. Oil body extraction

The oil bodies were isolated using an aqueous extraction method (Nikiforidis & Kiosseoglou, 2010). The intact maize germs or sunflower seeds were subjected to comminution, using a Brown mill fitted with knives. The maize germs or sunflower flour were initially soaked in deionized water (20% w/v) 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 Braun blender (Type: 4249, Germany) for 40 s (speed set at position 2) and the resulting maize germ or sunflower seed dispersion was filtered through two layers of cheesecloth. The maize germ or sunflower seed residue was then again extracted with deionized water at pH 9.0, the oil body dispersion containing both oil bodies and maize germ or sunflower seed debris was combined into one and the pooled dispersion was subjected to centrifugation (Avanti J-26XP, Beckman Coulters, USA) at 40,000 *g* for 20 min, at 4 °C to remove insoluble solids. The recovered oil body dispersion was then centrifuged at 20,000 *g*, at 4 °C. The maize germ or sunflower seed cream at the top was then recovered and washed once more with water (1:5). The pH of both recovered creams was around 7.0. Moisture, fat and protein contents were determined according to standard methods of AOAC (AOAC, 1994) and are described in detail in a previous article (Nikiforidis & Kiosseoglou, 2009). Part of the recovered sunflower oil body cream was also washed once with a 9.0 M urea solution (1:5). All experiments were performed at room temperature.

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 10×/0.40 CS and HC PL APO 20×/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. Protein analysis

The extracted and purified material was analyzed by SDS-PAGE (Laemmli, 1970) using a 12.5% w/v Novex® Tris-Glycine polyacrylamide gel (Invitrogen). The oil body samples were treated with a 0.0625 M Tris buffer containing 2% w/v SDS, 10% w/v glycerol, 0.1% w/v bromophenol blue and 5% β-mercaptoethanol. After boiling the samples for 2 min and the application of two freeze-thaw cycles, the subnatant containing only the protein was recovered by centrifugation and applied onto the electrophoresis gel. The gels were stained with Simply Blue™ SafeStain which contains Safe Coomassie® G-250 Stain (Invitrogen™, Life Technologies, CA, USA) and scanned (Bio-5000 VIS Gel Scanner, Serva electrophoresis, Germany). Determination of the protein molar mass was performed with the aid of the Electrophoresis Programme Gel Pro.

2.6. Particle size analysis

Particle size distribution of oil body emulsions was determined with the aid of a laser light scattering instrument (Malvern Mastersizer 2000, Malvern Instruments Ltd, UK). To determine the size of single oil bodies and to make sure that all the oil body aggregates were completely redispersed into single droplets, the dispersions were treated with a solution of 1 wt.% SDS and 0.5 wt.% 2-mercaptoethanol at 40 °C. The refractive index ratio used to calculate the oil body size distribution was 1.09. Measurements are reported as the surface weighted ($d_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$) mean diameter, where n_i is the number of droplets with a diameter of d_i. Droplet size distribution measurements were conducted both on fresh and on aged (for up to 15 days) oil body dispersions.