Food Research International 83 (2016) 14–24

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

The interplay between diverse oil body extracts and exogenous biopolymers or surfactants

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ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 8 February 2016
Accepted 11 February 2016
Available online 13 February 2016

Keywords:
Oil bodies
Natural emulsion
Physical stability
Hazelnut
Sesame
Soybean
Xanthan
Tween
Caseinate

ABSTRACT

Hazelnuts, sesame seeds and soybeans were selected as three diverse sources of oil bodies. Application of aqueous extraction and centrifugation steps resulted in concentrated oil body creams that were studied for their physical stability after dilution to a series of 5.0 wt.% oil-in-water emulsions incorporating sodium caseinate (1.0 wt.%), Tween 80 (1.0 wt.%) or xanthan gum (0.1 wt.%). In terms of aggregation/coalescence and creaming, the stability of the oil body based emulsions was ruled to a large extent by the initial natural oil droplet size and the presence of co-extracted exogenous proteins and secondarily by the added biopolymers and the surfactant. More specifically, soybean oil bodies exhibited the highest physical stability, even though incorporation of Tween 80 into all three oil body emulsions improved the stability against aggregation/coalescence, while xanthan gum was an effective stabilizer against creaming.

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1. Introduction

Oil bodies are dynamic intracellular organelles that are actively involved in cellular lipid homeostasis and metabolism (Murphy, 2012; Walther & Farese, 2012). Therefore, in contrast to what is broadly believed, oil bodies are not only serving as an energy source during the germination period (Murphy & Vance, 1999), but they are sophisticated structures with important physiological or pathological functionality (Khor, Shen, & Kraemer, 2013). Oil bodies consist of triglycerides and other lipophilic nutrients that are surrounded by a phospholipid/protein membrane (Tzen, Cao, Laurent, Ratnayake, & Huang, 1993). These structures are analogues to the fat droplets of mammal's milk (Murphy & Vance, 1999) and this automatically implicates that if we can extract them in an intact form, we can use them to fabricate all type of dairy-like products, but with enhanced nutritional value. Under specific conditions due to the nature of their interface (protein–phospholipid complexation), they may exhibit remarkable physical and chemical stability in comparison to "artificial' oil-in-water emulsions that are conventionally prepared (Fisk, White, Lad, & Gray, 2008; Gray, Payne, McClements, Decker, & Lad, 2010; Kapchie, Yao, Hauck, Wang, & Murphy, 2013; Karkani, Nenadis, Nikiforidis, & Kiosseoglou, 2013). The membrane proteins (oleosins, caleosins and steroleosins) are embedded in an interfacial phospholipid layer and due to their unique extended hydrophobic domain, they are strongly bonded on the interface (Frandsen, Mundy, & Tzen, 2001; Furse et al., 2013; Lin & Tzen, 2004; Shimada & Hara-Nishimura, 2010). Apart from contributing to the organelle stability, a growing body of evidence indicates that the interfacial proteins have advanced physiological functionalities as well (Chapman, Dyer, & Mullen, 2012; Hanano et al., 2006; Lin, Tai, Peng, & Tzen, 2002). It is still not clear enough, but depending on the plant and its needs, the size and composition of oil bodies might differ. Aqueously extracted oil bodies might have sizes from nanoscale level (<0.1 μm) up to a few microns (>1 μm) (Borem, Marques, & Alves, 2008; Frandsen et al., 2001; Nikiforidis, Karkani, & Kiosseoglou, 2011). In legumes and grains, where the protein content is high, they appear with smaller sizes (Chen, McClements, Gray, & Decker, 2012; Nikiforidis & Kiosseoglou, 2009; White, Fisk, & Gray, 2006) while in seeds with low protein content like avocados and olives they have a very different structure (Ting et al., 1996). As expected, these differences in size and composition play a dominant role in the physicochemical stability of the extracted oil bodies (Nikiforidis, Kiosseoglou, & Scholten, 2013).

Exploitation of oil bodies in foods and pharmaceuticals is a rising scientific area of interest due to the fact that they are environmentally friendly pre-emulsified droplets, since no organic solvents are required

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http://dx.doi.org/10.1016/j.foodres.2016.02.007
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for the oil extraction and, furthermore, no additional emulsification step is needed. The understanding of their behavior and properties could lead to novel structures with enhanced functionality (Acevedo et al., 2014; Chiang, Lin, Lu, & Wang, 2011; Fisk, Linforth, Trophardy, & Gray, 2013; Nikiforidis, Matsakidou, & Kiosseoglou, 2014; Nikiforidis & Scholten, 2015). In addition to the environmentally friendly process and, subsequently, the economic profit originating from the preparation of a sustainable natural oil-in-water emulsion, the nutritional benefit is also high (Adams et al., 2012; Chen, Cao, Zhao, Kong, & Hua, 2014). The extracted oil bodies are still in their natural state containing all the lipophilic bioactive nutrients. Moreover, they are free from any traces of organic solvents in contrast to vegetable oils obtained by applying the conventional oil extraction process (Chen et al., 2014).

Efficient exploitation of oil bodies requires the understanding of their behavior when present in real complex systems, where extraneous biopolymers, surfactants or low molecular weight composites will also be present. Previously published articles investigated the interactions and enhancement of soybean and maize germ oil body stability by ionic polysaccharides, such as pectin, carrageenan, xanthan gum or Tween 80 (Iwanaga, Gray, Decker, Weiss, & McClements, 2008; Nikiforidis & Kiosseoglou, 2010, 2011; Wu et al., 2012). In this study we focused on two different oleaginous raw materials, hazelnuts and sesame seeds that are known for their important nutrients and healthy fatty acid profile (Altun et al., 2013; Tey et al., 2011). Previously published studies have reported on their behavior after processing (Ahmadian-Kouchaksaraei, Varidi, Varidi, & Pourazarang, 2014; Bernat, Cháfer, Rodríguez-García, Chiralt, & González-Martínez, 2015). In the present work their oil body behavior and stability during the extraction process, upon heating and when embedded in a complex matrix are studied. The soybean oil bodies were chosen as a reference material, since they are well-studied and tend to exhibit high physical and chemical stability (Iwanaga et al., 2007). Furthermore, the effect of heating on the physical stability of the oil bodies was studied due to the high importance of heat processing to enhance their stability against microbial degradation and/or enzyme attack (Bernat et al., 2015; Chen et al., 2012).

2. Materials and methods

2.1. Materials

Intact sesame seeds, hazelnuts and soybeans were purchased from a local market. Sodium caseinate from bovine milk (Grade III), xanthan gum from *Xanthomonas campestris*, Tween® 80 and all other chemicals, which were of analytical grade, were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

2.2. Extraction of oil bodies and preparation of natural emulsions

An aqueous extraction method based on the modification of the method previously described (Nikiforidis & Kiosseoglou, 2010) was applied to extract the oil bodies from the three oleaginous materials. Intact sesame seeds, hazelnuts or milled soybeans (<1.0 mm) were initially soaked in deionized water (1:4) for 24 h, while the pH of the suspension was adjusted and kept constant at 9.0, using a 0.1 M NaOH solution. The mixture was then filtered through two layers of cheesecloth. The solid residue was re-dispersed in deionized water (1:4) at pH 9.0 while vigorously agitating for 2 h and filtered again through a two layered cheesecloth. Finally, the two oil body extracts (filtrates) were combined into one and subjected to centrifugation (Firlabo SV11, France) at 3000 *g* for 15 min to remove debris. The clear from insoluble material stock oil body emulsion was divided into two parts. The first part was mixed with a sucrose solution (pH 6.5) to a final sucrose concentration of 0.5 M and the diluted dispersion was centrifuged at room temperature at 4000 *g* for 30 min to remove extraneous proteins. The resulting cream was recovered and dispersed once more in the sucrose solution (1:5) and the final oil body cream free of extraneous proteins was recovered by centrifugation at 15,000 *g* for 30 min at 4 °C. The second part of the extract was initially subjected to heat treatment at 98 °C for 30 min before recovering the cream with the aid of the sucrose solution as described above. Moisture, fat and protein content of both creams, the control and the heat-treated one, was determined according to standard methods of AOAC (AOAC, 1994). After determining the oil content, the two creams were mixed with deionized water or with the appropriate volume of Tween 80, xanthan gum or sodium caseinate solutions to obtain a series of emulsions (5.0 wt.% in oil); the control, one emulsion containing 1.0 wt.% sodium caseinate, one containing Tween 80 and, finally, one containing 0.1 wt.% xanthan gum. The pH of each emulsion was checked and, adjusted if needed to 6.5, using 0.1 M HCl or NaOH. To prevent microbial growth, NaN_3 (0.01 wt.%) was added. The emulsions were stored at 4 °C and their physical stability was studied over a storage period of 15 days.

2.3. Protein characterization by SDS-PAGE

To determine the protein composition of the oil body surface membrane, samples of 2.0 g from each cream were dispersed (1:5) in a buffer containing 50 mM Tris–HCl, 5.0 M urea, 1.0 wt.% SDS and 4.0 wt.% 2-mercaptoethanol at pH 8.0. Following incubation for 1 h, an equal volume of an electrophoresis sample buffer of 125 mM Tris–HCl, 5 M urea, 1.0 wt.% SDS, 20.0 wt.% glycerol and 4.0 wt.% 2-mercaptoethanol, was added. The mixture was boiled for 2 min and then subjected to freeze–thaw cycles. The subnatant containing the soluble proteins was removed and analyzed by SDS-PAGE (Laemmli, 1970). The stacking and separating gels were prepared using 4.5 and 12.5 wt.% acrylamide solutions; the gels were stained with Coomassie brilliant blue G-250 and photographed with the aid of a digital camera.

2.4. Particle size and ζ-potential measurements

Particle size distribution of the oil body emulsions was determined using a laser light scattering instrument (Malvern Mastersizer 2000, UK). The emulsion samples were initially treated with a solution of 1.0 wt.% SDS and 0.5 wt.% 2-mercaptoethanol at 40 °C for 15 min to effect the complete dispersion of the oil body aggregates into single droplets, as inter-droplet hydrophobic attractive forces and disulfide bonding are disrupted. Measurements were performed following sample dilution with deionized water to an approximate oil content of 0.005 wt.%. 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.

To determine the ζ-potential of the oil bodies aged for 1 day, oil body dispersions were diluted with deionized water to an approximate oil concentration of 0.01 wt.%. The pH of the diluted sample was adjusted using either 0.1 M HCl or NaOH. Measurements were performed using a BIC Zeta Potential Analyzer instrument, employing the dedicated “Particle Solutions” Software, v.2.5 (Brookhaven Instruments Corporation, Brookhaven, Holtsville, NY), at 10 mg/mL sample concentration in ultrapure water.

2.5. Physical stability over storage

The physical stability of emulsions against aggregation/coalescence was determined by following the droplet size increase with aging at 4 °C. To assess the emulsion stability against creaming a quantity of 10 mL of each emulsion was placed in sealed cylindrical vessels and stored at room temperature. The appearance of serum at the bottom of the vessel and/or a cream layer at the top at the end of a 15 day storage period was used to discriminate the stable from the unstable emulsions.

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