Food Hydrocolloids 25 (2011) 1063–1068

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Competitive displacement of oil body surface proteins by Tween 80 – Effect on physical stability

Constantinos V. Nikiforidis, Vassilios Kiosseoglou*

Laboratory of Food Chemistry and Technology, Department of Chemistry, Aristotle University of Thessaloniki, GR-54124, Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 15 June 2010
Accepted 1 October 2010

Keywords:
Oil body
Emulsion
Maize
Tween
Stability

ABSTRACT

A concentrated oil body cream, prepared from maize germ by aqueous extraction, was dispersed in water to obtain a natural 5% o/w emulsion. To improve the emulsion physical stability, the hydrophilic surfactant Tween 80 (polyoxyethylene sorbitan monooleate) was incorporated at levels ranging from 0.25 to 2%, and the increase of the oil body mean diameter and the volume of serum separated from the emulsion system, was followed with storage time. In addition, the amount and composition of oil body surface proteins competitively displaced by the surfactant was studied. The improvement in oil body stability against coalescence and creaming, resulting from Tween addition, is discussed in terms of the development at the oil body surface of an adsorbed film of a mixed nature, made up of surfactant- and phospholipid-rich domains, with the non-displaced surfactant protein molecules, mainly oleosins, remaining embedded in the latter.

1. Introduction

In maize germ, as well as in many oil-rich seeds, the lipids are encountered in the form of small-sized organelles called oil bodies (Tzen, Lie, & Huang, 1992). One common feature of all oil bodies, irrespective of their origin, is the presence at their surface of a mixed phospholipids–protein membrane, responsible for maintaining oil body integrity. In electron micrographs the oil bodies appear to be surrounded by an electron-dense layer, attributed to a single layer of phospholipids, with the acyl moieties directed towards the oil body core (Frandsen, Mundy, & Tzen, 2001). Depending on plant origin, the phospholipids content may range between about 0.6 and 2%. In maize germ oil bodies, the phospholipids content is about 0.9%, with phosphatidyl choline and phosphatidyl serine constituting, respectively, 64.1 and 20.2% of the phospholipids fraction (Chen, Chyan, Lee, Huang, & Tzen, 2004; Tzen, Cao, Laurent, Ratnayake, & Huang, 1993).

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 proteins of relatively low-molecular mass (15–20 kDa) with a triblock structure, comprising two amphiphilic N- and C-terminal regions and a central hydrophobic region of about 70 residues. According to the predicted model of Tzen and his co workers (Huang, 1992), the central hydrophobic oleosin domain, formed by two antiparallel strands, connected by a "proline knot", is inserted into the triglycerides core and forms a hairpin-like structure while the C- and N-terminal moieties reside on the oil body surface where they interact with the phospholipids. Caleosins are another group of oil body surface proteins. Compared to oleosins, caleosins are minor proteins with a molecular mass close to 27 kDa and a molecular structure comprising a fairly hydrophilic N-terminal moiety, a C-terminal hydrophilic region and a central hydrophobic region inserted, as in the case of oleosins, in the triglyceride core (Lin, Liao, Yang, & Tzen, 2005; Purkrtova, Jolivet, Miquel, & Chardot, 2008; Purkrtova et al., 2008). Steroleosins are the third group of oil body surface proteins. They are also minor proteins with a molecular mass close to 40 kDa that anchor to the oil body surface through their hydrophobic N-terminal domain (Lin, Tai, Peng, & Tzen, 2002; Lin & Tzen, 2004).

Oil recovery from oil-rich plant materials is performed industrially using organic solvents, such as hexane or petroleum ether. The extracted crude oil is then refined and consumed as bulk vegetable oil or used in the preparation of products appearing in the form of oil-in-water emulsions. A novel idea, recently put forward by a number of investigators, involves extraction of oil in the form of oil bodies by employing aqueous media in place of organic solvents (Chen et al., 1997; Kapchie, Wei, Hauck, & Murphy, 2008; Nikiforidis & Kiosseoglou, 2009, 2010; Towa, Kapchie, Hauck, & Murphy, 2010). This "green technology" approach may lead to the development of a concentrated oil-in-water emulsion made up of small-sized oil droplets protected by a natural mixed protein–phospholipids layer which could be used in the preparation of food

* Corresponding author. Tel.: +30 231 0 997834; fax: +30 231 0 997779.
E-mail address: kiosse@chem.auth.gr (V. Kiosseoglou).

0268-005X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2010.10.002

products appearing in the form of emulsions such as salad dressings imitation milk and cream by simply mixing the oil droplets with the other product constituents.

Although it is generally believed that oil body-based dispersions are relatively stable upon storage (White et al., 2008), recently published data demonstrated that maize germ oil body dispersions tend to exhibit a rather limited stability against coalescence (Nikiforidis & Kiosseoglou, 2010). This instability constitutes a serious disadvantage when considering oil body exploitation for the preparation of physically stable, over long time storage periods, food emulsion products.

Addition of low-molecular weight hydrophilic emulsifiers such as Tween (polyoxyethylene sorbitan fatty acid esters) to protein-stabilized emulsions may modify their behaviour with regard to oil droplet aggregation and coalescence, especially when adverse processing treatments, such as heating, are applied (Demetriades & McClements, 1998; Nikiforidis & Kiosseoglou, 2007). Nikiforidis & Kiosseoglou (2007) reported that heat destabilization of yolk-stabilized emulsions is halted in the presence of Tween 40. Since the structure of the stabilizing membrane at the oil droplet surface of yolk-stabilized emulsions presents many similarities to that of oil bodies in being made up of a phospholipids layer with embedded apolipoprotein molecules, it is expected that Tween addition to maize germ oil body dispersions could also enhance their physicochemical stability and this was the main target of the present study. A second, equally important target was to investigate how the highly hydrophobic proteins of the oil body surface behave in the presence of hydrophilic surfactants, often incorporated both in food as well as in non-food systems, and whether these proteins are competitively displaced from the surface by the surfactant molecules.

2. Materials and methods

2.1. Materials

A milling industry by-product, rich in maize germ, was used for the aqueous extraction of oil bodies. Tween 80 was obtained from TCI (Shanghai) Development Co., Ltd. All other chemicals were obtained from Sigma–Aldrich Chemicals Co.

2.2. Oil body extraction and dispersion preparation

The oil bodies were isolated using an aqueous extraction method (Nikiforidis et al., 2010). The intact germs were first collected by hand and then subjected to comminution, using a Brown mill fitted with knives, to pass through a 0.8 mm mesh sieve. The germ flour was initially soaked in deionised water (20% w/v) and the pH was adjusted and kept constant at 9, using 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 (speed set at position 2) for 40 s, by employing a Braun Blender (Type: 4249, Germany), and the resulting dispersion was filtered through a filter made up of three layers of cheesecloth. The germ residue was then again extracted with deionised water at pH 9, the two oil body dispersions containing both oil bodies and debris, were combined into one and the pooled dispersion was subjected to centrifugation (Firlabo SV11, France) at 3800 g for 20 min to remove insoluble solids. The recovered oil body dispersion was then mixed with an equal volume of a sucrose solution (pH 6.5) to obtain a final sucrose concentration of 0.5 M and the diluted dispersion was centrifuged at 3800 g. The cream at the top was then recovered and washed once more with the sucrose solution. Following moisture, fat and protein determination, according to standard methods of AOAC (AOAC, 1994), the cream was suitably mixed with deionised water or a 4% Tween 80 solution in order to obtain a series of dispersion samples of 5% (v/v) in oil and from 0.0 to 2.0% (w/v) in Tween 80. To prevent microbial growth, NaN_3 at a concentration of 0.01% (w/v), was added. The samples were stored at 4 °C and studied over a period of 15 days.

2.3. Protein characterization by SDS-PAGE

To determine oil body surface protein composition, the oil body dispersion was centrifuged at 16000 g (4 °C, 30 min), and the cream layer was dispersed (1:5) in a buffer containing 50 mM Tris–HCl, 5 M urea, 1% SDS and 4% 2-mercaptoethanol at pH 8.0. 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 subnatant containing the solubilized protein was removed with the aid of a syringe 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 fixated by immersing into a 12.5% trichloroacetic acid solution. The gels were stained with Coomasie 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).

In order to determine the amount of desorbed protein from the oil body surface by the surfactant, the serum obtained from oil body dispersions, by centrifugation at 16000 g (4 °C, 30 min), was analyzed colorimetrically, using the Modified Lowry protein assay (Markwell, Haas, Bieber, & Tolbert, 1978). In addition, the serum sample was lyophilized and the dry matter was washed with acetone, chloroform and diethylether, then treated with a 62.5 mM Tris buffer containing 2% SDS, 10% glycerol, 0.1% bromophenol blue and 5% 2-mercaptoethanol, boiled for 2 min and analyzed by SDS-PAGE.

2.4. Particle size analysis

Particle size distribution of oil body dispersions was determined with the aid of a laser light scattering instrument (Malvern Mastersizer 2000, UK). To determine the size of single oil bodies, the dispersions were initially treated with a solution of 1% SDS and 0.5% 2-mercaptoethanol at 40 °C, in order to make sure that all the oil body aggregates were completely dispersed into single droplets. Measurements were performed at room temperature following sample dilution with deionised water to an approximate oil content of 0.005%. 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.

2.5. ζ-potential measurement

Aged for 30 min or 1 day oil body dispersions were diluted with deionised water to an approximate oil concentration of 0.01%. The pH of the diluted sample was adjusted using either 0.1 M HCl or NaOH. The diluted oil body suspension was subjected to electrophoresis with a Lazer Zee Meter 501 (PenKem, Bedford Hills, NY, USA) type 501 Lazer Zee Meter (USA) at 100 V in the instrument's cell while the droplets were visualized on a monitor. This instrument determines the ζ-potential of the particles in an emulsion by