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Encapsulation of betalain into w/o/w double emulsion and release during *in vitro* intestinal lipid digestion

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ABSTRACT

A water-in-oil-in-water (w/o/w) double emulsion was prepared with water extract of red beet as the inner water phase, rapeseed oil as the oil phase and polysaccharides solution as the outer water phase. Polyglycerol polyricinoleate and polar lipid fraction from oat were used as emulsifiers for primary water-in-oil (w/o) emulsion and secondary w/o/w emulsion, respectively. Their mean droplet sizes were approximately 0.34 μm and 5.5 μm, respectively. The double emulsion showed a high encapsulation efficiency of 89.1% and had a pink coloration due to encapsulated betalain. The double emulsion was subjected to *in vitro* intestinal lipid digestion and the evolution of structures and release of betalain were monitored. During the first 2 h of digestion, coalescence of the inner water phase droplets was observed, and the sizes of the double emulsion droplets increased quickly because of aggregation. This period also corresponded to release of betalain, reaching about 35%. After 3 h of digestion, no more release was measured, corresponding to no further increase in droplet sizes. In contrast, the encapsulation efficiency and droplet sizes were not affected after 3 h in the same digestion conditions but without the bile salts and lipase, showing they were responsible for the release.

1. Introduction

In recent years, there have been reports of artificial colorants and preservatives having relation to hyperactivity in children (McCann et al., 2007; Nigg, Lewis, Edinger, & Falk, 2012). As consumers are becoming more and more aware of health issues, these findings have made natural pigments, such as carotenoids, anthocyanins and betalains, more favorable to be used as food colorants. However, their use as food colorants is hindered by their instability and solubility properties, which narrow the possible applications. Betalains are water-soluble yellow, red or violet natural pigments, which have antioxidative properties, but are sensitive to high temperature, basic or very acidic pH, light, air (oxygen), and high water activity (Cai, Sun, & Corke, 2003; Cai, Sun, & Corke, 1998; Herbach, Stintzing, & Carle, 2006). The stability of betalains, and also other natural pigments, could be improved with encapsulation technologies, for example spray-drying or emulsification (Gandia-Herrero, Jimenez-Atienzar, Cabanes, Garcia-Carmona, & Escribano, 2010; Rodriguez-Huezo, Pedroza-Islas, Prado-Barragan, Beristain, & Vernon-Carter, 2004).

Double (or multiple) emulsions can be described as being emulsions within emulsions. The first phase is dispersed into the second as small droplets, and this emulsion is again dispersed as droplets into a third phase. There are two major types of double emulsions: water-in-oil-in-water (w/o/w) emulsions, which have water droplets dispersed into oil droplets dispersed into a continuous water phase, and oil-in-water-in-oil (o/w/o) emulsions, which have oil droplets dispersed into water droplets dispersed into a continuous oil phase. Since most foods are constituted of an aqueous continuous phase, the w/o/w double emulsions have more potential for food applications. They offer the possibility to incorporate both lipophilic and hydrophilic compounds which are isolated from the surrounding aqueous environment. Besides food industry, the possibilities of double emulsions as encapsulation systems have been extensively studied for the drug and cosmetic

Abbreviations: CMC, critical micelle concentration; D3,2, surface mean diameter (Sauter mean diameter); D4,3, volume mean diameter (De Brouckere mean diameter); DLS, dynamic light scattering; LD, laser diffraction; NaGDC, sodium glycodeoxycholate; o/w/o, oil-in-water-in-oil (emulsion); o/w, oil-in-water (emulsion); PGPR, polyglycerol polyricinoleate; PTFE, polytetrafluoroethylene; SD, standard deviation; w/o, water-in-oil (emulsion); w/o/w, water-in-oil-in-water (emulsion).

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industries (Jiao & Burgess, 2008; Leal-Calderon, Schmitt, & Bibette, 2007).

In our previous research, we used a polar lipid fraction from oat (*Avena sativa*) to produce o/w emulsions, which were colored yellow with lutein (Kaimainen et al., 2012). In that study we showed a rapid creaming of these emulsions, but we have thereafter managed to significantly delay the creaming by adding small amounts of long chain polysaccharides to the emulsions (data not published). In the present research, the first step was to formulate a w/o/w double emulsion encapsulating hydrophilic betalain colorant and using an oat polar lipid emulsifier to produce natural and stable pink-colored emulsions, with the longer term goal to study the color stability during shelf life. As betalain is also a bioactive displaying antioxidative properties *in vivo*, the next step was to follow the evolution of both the betalain encapsulated and the structure of the double emulsion during *in vitro* intestinal digestion, in order to understand its release.

2. Materials and methods

2.1. Materials

The oat polar lipid fraction used as o/w emulsifier was extracted from oat flakes (*Avena sativa*) using a supercritical fluid process described by Aro et al. (Aro, Järvenpää, Könkö, Huopalahti, & Hietaniemi, 2007). It consists mainly of different glycolipids (monogalactosyldiacylglycerol, digalactosyldiacylglycerol and steryl glucoside) and phospholipids (phosphatidyl choline). The polyglycerol polyricinoleate (PGPR) used as w/o emulsifier was a sample of PGPR 4175 received from Palsgaard (Juelsminde, Denmark). The betalain pigment was extracted with hot water (70 °C, 30 min) from red beets (*Beta vulgaris*) bought at a local grocery store (ratio of water:beet was 2:1). After extraction, the solid material was filtered out through a filter paper under vacuum. The extract was further concentrated to 60% of original volume with a rotary evaporator at 50 °C, 7–8 kPa. The rapeseed oil used for the oil phase of double emulsion was bought at a local grocery store and used as such without any purification. For making the buffer solution, citric acid monohydrate was purchased from Carlo Erba Reagenti (Milano, Italy) and disodium hydrogen phosphate heptahydrate from Riedel-de Haën (Seelze, Germany), and both salts were of analytical grade. Guar gum (Meypro Rein guarin) was purchased from Meyhall Chemical AB (Kreuzlingen, Switzerland) and xanthan gum (Rhodigel® xanthane) from Rhodia (Lyon, France). For *in vitro* digestion, sodium glycodeoxycholate, NaGDC (G9910) and pancreatic lipase type II (L3126, activity 100–400 units/mg protein, using olive oil) were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Water used was purified reverse osmosis water (Milli-Q Plus ultra-pure water system, Millipore, Molsheim, France).

2.2. Preparation of double emulsions

First the oil phase of the primary w_1/o emulsion was prepared by adding PGPR at 20 mg/g into rapeseed oil. Beet extract was added slowly with mixing at 10 000 rpm with a Silent Crusher M high-speed mixer (Heidolph, Schwabach, Germany) so that the amount of the inner water phase w_1 was 0.3 ml/g of the total w_1/o emulsion. After the whole amount was added, the emulsion was homogenized at 20 000 rpm for 5 min. This primary w_1/o emulsion was slowly added to the outer water phase w_2 with mixing at 13 000 rpm so that the amount of w_1/o emulsion was 0.03 ml/g of the total double emulsion, and after the whole amount was added, the double emulsion was homogenized at 18 000 rpm for 5 min. The outer water phase w_2 was prepared as described by Kaimainen et al. for simple o/w emulsions (Kaimainen et al., 2012) with slight modifications. A pH of 5.8 instead of 2.6 was chosen because the polysaccharides used for stabilizing the emulsion are degraded at low pH values. Citrate-phosphate buffer with pH 5.8 was prepared by mixing 0.1 mol/L citric acid solution, 0.2 mol/L disodium hydrogen phosphate solution and water in proportion 197:303:500, respectively. This buffer was used to prepare three solutions containing either 10 mg/g oat polar lipid emulsifier (dissolved at 50 °C with magnetic stirring for 1 h), 10 mg/g guar gum (dissolved at 80 °C with magnetic stirring for 2 h), or 10 mg/g xanthan gum (dissolved at 80 °C with magnetic stirring for 2 h). Different compositions for the outer water phase w_2 were formulated by mixing these four solutions in different proportions as preliminary tests. Particularly, a composition of w_2 phase consisting of 5 mg/g oat polar lipid emulsifier, 2 mg/g guar gum, 2 mg/g xanthan gum and 39 mg/g glucose (for adjusting the osmolarity of the solution) in the pH 5.8 citrate phosphate buffer was used in this study. The osmolarities of inner and outer water phases were measured with a Micro-Osmometer type 13 Autocal (Roebling, Berlin, Germany) and glucose was added to the outer water phase to balance the osmolarity of the inner w_1 and outer w_2 water phases.

2.3. Encapsulation efficiency

Encapsulation efficiency was measured by centrifuging a double emulsion sample at 3000 × g for 10 min and filtering the outer water phase through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter. The absorbance at 530 nm of the filtered sample was measured with a Multiskan GO spectrophotometer (Thermo Scientific, Vantaa, Finland) against a blank sample made of filtered (0.45 μm PTFE syringe filter) outer water phase and the value was compared with a standard curve prepared by adding calculated amounts of beet extract to the filtered (0.45 μm PTFE syringe filter) outer water phase corresponding to 5, 10, 20, 40, 60 or 100% release of the inner water phase, i.e. 95, 90, 80, 60, 40 or 0% encapsulation efficiency. The double emulsion sample and all the standards were prepared as duplicate samples, and each sample was measured twice for a total of four measurements per sample or standard. Duplicate measurements of the two duplicate standard sets were very close to each other; relative standard deviations were all less than 1%, and for most solutions less than 0.1%. The relative standard deviations calculated from all 4 measurements of each standard point were slightly higher, between 0.14% and 7.41%, so the major source of error came from the preparation of solutions and not from the actual measurement. Standard deviation for 90% encapsulation efficiency standard point was 24%, but this was due to an error in the preparation of one of the standard solutions. For this reason, that standard solution was excluded and the point of 90% encapsulation efficiency was calculated only from one standard solution instead of two (and two measurements instead of four). The resulting standard curve had a squared correlation coefficient of 0.9986.

2.4. Droplet size

Droplet sizes were measured by laser diffraction (LD) using a Mastersizer S equipped with a 2 mW He–Ne laser of 633 nm and a 300RF lens (Malvern Instruments Ltd., Worcestershire, UK). The detection limits were 0.05 and 900 μm. Calculations to determine the droplet size distribution were based on an o/w emulsion model with a refractive index n_0 of the aqueous phase of 1.33, and that of rapeseed oil of 1.457. The absorption was set to 0.001. Emulsions were diluted with distilled water in the dispersion unit to reach a droplet volume concentration near 0.03% for the circulation in the measurement cell. For each sample, triplicate measurements were done. For undiluted samples, droplet sizes and overall double