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Comparison of simple, double and gelled double emulsions as hydroxytyrosol and n-3 fatty acid delivery systems

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ABSTRACT

The purpose of this study was to compare three different emulsion-based systems, namely simple emulsion, double emulsion and gelled double emulsion, for delivery of n-3 fatty acids (perilla oil at 300 g/kg) and hydroxytyrosol (300 mg/kg). Considering that their structural differences may affect their physical and oxidative stability, this was studied by storing them at 4 °C for 22 days in the dark. The results showed that the oxidative status was maintained in all systems by the addition of hydroxytyrosol. However, there was some loss of hydroxytyrosol, mainly during sample storage and during preparation of the gelled double emulsion. Moreover, the antioxidant loss was more pronounced in more compartmentalized systems, which was attributed to their increased surface area. However, the double emulsion was found to be less stable than the gelled emulsion. Overall, the encapsulation of labile compounds in more complex systems needs to be carefully studied and adapted to specific technological and/or nutritional requirements.

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1. Introduction

In recent years, different systems have been proposed for the delivery of hydrophobic and hydrophilic bioactive compounds in foods (Dickinson, 2012; Garti, 1997; Sagalowicz & Leser, 2010). In this context, oil-in-water (O/W) emulsions, hereafter also called simple emulsions (SEs), have been widely used for the delivery of hydrophobic compounds such as tocopherol and n-3 fatty acids in foods and beverages (Lee et al., 2006; McClements, Decker, & Park, 2009; Sagalowicz & Leser, 2010). Multiple or water-in-oil-in-water (W/O/W) double emulsions (DEs) are more complex liquid dispersions in which oil globules containing small water droplets are dispersed in an aqueous continuous phase. This type of emulsion offers a number of promising opportunities for the food industry, e.g. the delivery of hydrophobic compounds in aqueous systems, it allows for the encapsulation of hydrophilic bioactive compounds and can help improve the fat content of foods (by reducing fat and providing a healthier fatty acid profile) (Jiménez-Colmenero, 2013).

In fact the consumption and development of food products enriched with n-3 fatty acids is of great interest in the context of Western diets in view of a variety of reported beneficial health effects, mainly relating to cardiovascular and inflammatory diseases (Ruxton, Reed, Simpson, & Millington, 2004; Simopoulos, 2006). In this regard, the use of perilla oil is of special interest as it is one of the richest terrestrial sources of linolenic acid (Ciftci, Przybylski, & Rudzinska, 2012). However, systems with high n-3 fatty acid contents are prone to oxidation and hence need to be protected, since that oxidation causes loss of nutritional properties and poses a potential hazard in that various of the compounds thus formed have been associated with neurodegenerative and cardiovascular diseases (Esterbauer, Wag, & Puhl, 1993; Perluigi, Coccia, & Butterfield, 2012).

Addition of antioxidants offers a strategy to tackle this problem (Decker, Elias, & McClements, 2010b), especially if these are natural compounds. In this regard, there are some phenolic compounds that are naturally present in olive oils and have been reported to act as antioxidants (Tripoli et al., 2005). These have two advantages. Firstly, they may provide protection to food sensitive components such as n-3 fatty acids during processing and storage. And secondly, they can exert beneficial effects *in vivo* after food consumption. In this connection, hydroxytyrosol (HTy), a major

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phenolic compound in olive oils (averaging 200–500 mg/kg), has recently been receiving attention for the wide range of its biological activities and its capacity to protect against cardiovascular disease (Bulotta et al., 2014; Tripoli et al., 2005). Therefore, the addition of HTy is not only of nutritional benefit when added in sufficient amounts but also of technological utility as it minimizes the oxidation extent (Cofrades et al., 2011; Pazos, Alonso, Sanchez, & Medina, 2008). Recently, Cofrades et al. (2014) studied the oxidative stability of DEs containing chia oil and HTy and that of a cooked meat system prepared with these DEs. These authors reported that DEs showed good antioxidant capacity in the early days of storage; and similarly, DEs containing HTy protected cooked meat systems against oxidation better than DEs without HTy. However, the addition of HTy appeared to exert less antioxidant effect when incorporated in this way than when added to the meat system directly. In fact it was unclear whether these differences were caused by the encapsulation itself or by a reduction in the amount of HTy, which can occur during preparation or storage of DEs as a consequence of the increased surface area. It is therefore important to know the amount of HTy that is encapsulated within the DE and how much is released and lost during preparation and storage.

Novel proposals for liquid phase oil stabilization and structuring (e.g. multiple emulsions, multilayer emulsions, Pickering stabilization) have recently been reviewed (Jiménez-Colmenero et al., 2015; Mao & Miao, 2015). A number of them can be used to improve the quality of reformulated (healthier) foods. For instance, gelled emulsions can be used in different food applications to improve their nutritional, functional and/or sensory characteristics (Li, Cheng, Tatsumi, Saito, & Yin, 2014; Lobato-Calleros et al., 2008; Weiss, Scherze, & Muschiolik, 2005). Gelled DEs (GDEs) thus offer a range of interesting possibilities for the food industry in that they may serve at once to improve the lipid composition, encapsulate bioactive compounds and provide certain plastic properties. Their physical and structural characteristics (stability, viscoelasticity, encapsulation efficiency, phase separation minimization, etc.) offer technological advantages with important effects on the desired quality attributes of foods when used as intermediate products (food ingredients) to optimize the presence of bioactive compounds. Additionally, this strategy may influence the bioavailability and oxidizability of bioactive compounds, linked as these are to their molecular structure and physicochemical properties.

Given that the physical and oxidative stability of emulsion-based systems depends on their structural differences, the aim of this study was to characterize three emulsion-based systems (SEs, DEs and GDEs) for delivery of n-3 fatty acids (perilla oil) and HTy. The oxidative stability was followed over 22 days of storage at 4 °C in the dark. It is crucial to gain an understanding of the behaviour of these systems during storage, as this needs to be considered when designing a delivery system for a particular food application. In this case it will further help to understand the changes that these potential emulsion-based delivery systems may undergo as part of a food product (e.g. meat matrix) and hence estimate how this may affect the reformulated product's characteristics. As far as the authors are aware there have been no studies on the encapsulation process of HTy in PUFA-enriched gelled DEs.

2. Material and methods

2.1. Materials and Reagents

HTy (purity $\geqslant$ 99%) was purchased from Seprox Biotech (Madrid, Spain) and perilla oil (fatty acid composition: 6% 16:0, 2% 18:0, 12% 18:1, 14% 18:2, 65% 18:3) was purchased from Grupo Nutracéutico Chiasa, SL (Meliana, Spain). Upon arrival this oil was stored at 4 °C until the elaboration of the different emulsions systems (less than 1 month) and its hydroperoxide content was 0.22 ± 0.01 mmol cumene hydroperoxide/kg (Shantha & Decker, 1994). The thiobarbituric acid-reactive substances (TBARS) value was under the limit of detection (Cofrades et al., 2014). Sodium caseinate (Excellion EM 7) was purchased from FrieslandCampina DMV (Veghel, The Netherlands), polyglycerol polyricinoleate (PGPR) was purchased from Bavaro Chemicals S.L. (Sant Cebrià de Vallalta, Spain), gelatine (type B, 200–220 bloom) from Manuel Riesgo, S.A. (Madrid, Spain), and microbial transglutaminase (Activa GS) from Ajinomoto (Tokyo, Japan). According to the supplier the activity of the enzyme was 47–82 units of hydroxamate per g. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ), iron(III) chloride hexahydrate, 2,4-dinitrophenylhydrazine (DNPH) and orthophosphoric acid were acquired from Sigma-Aldrich (Madrid, Spain). MilliQ water was used throughout. All other reagents and solvents used were of a suitable grade for spectrophotometric or chromatographic analyses.

2.2. Preparation of simple emulsions (SE), double emulsions (DE) and gelled double emulsions (GDE)

The aqueous phase (W) of the SE consisted of 0.584 g NaCl, 0.04 g sodium azide, 0.441 g sodium caseinate and 44.1 mg HTy dissolved in 100 mL distilled water. The lipid phase (O) consisted of perilla oil (94 g/100 g) plus PGPR (6 g/100 g). The simple O/W coarse emulsion was prepared by gentle addition of the lipid phase (32 g/100 g) to the outer (68 g/100 g) aqueous phase (W) in a Thermomix food processor (TM-31 Vorwerk, Wuppertal, Germany) set at 37 °C on speed 3. Sample was then passed twice through a two-stage high pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Parma, Italy) at 15 and 3 MPa (first and second stage respectively), and the SE was collected in a beaker immersed in ice. This sample is hereafter designated SE-HTy-PO. Sample aliquots were then transferred to screw-capped tubes and immediately stored at 4 °C in the dark.

A two stage procedure was used to prepare stable DEs, as reported elsewhere (Bou, Cofrades, & Jimenez-Colmenero, 2014b) with minor modifications. The inner (W_1) phase consisted of 0.584 g NaCl plus 375 mg HTy and 0.04 g sodium azide in 100 mL distilled water. The outer (W_2) phase was prepared by dispersing 0.584 g NaCl, 0.04 g sodium azide and 0.5 g sodium caseinate in 100 mL distilled water at room temperature until fully dissolved. The lipid phase (O) used was the same as in the SE. The primary coarse emulsion (W_1/O) was prepared by gentle addition of the inner (W_1) aqueous phase (20 g/100 g) to the lipid phase (80 g/100 g) in the Thermomix food processor set at 37 °C for 15 min on speed 6 (3250 rpm). This primary coarse emulsion was passed twice through a two-stage high pressure homogenizer (Panda Plus 1000) at 55 and 7 MPa respectively and collected in a beaker immersed in ice. The resulting primary fine emulsion (W_1/O) was immediately used for preparation of the DE by gradual addition (40 g/100 g) to the outer (60 g/100 g) aqueous phase (W_2) in the Thermomix food processor set at 37 °C, speed 3 (700 rpm). The resulting coarse W_1/O/W_2 emulsions were passed twice through a two-stage high pressure homogenizer (Panda Plus 1000) at 15 and 3 MPa respectively to obtain the final DE, which was collected in a beaker in ice. This sample is designated DE-HTy-PO. Sample aliquots were then transferred to screw-capped tubes and immediately stored at 4 °C in the dark. The pH of these systems was measured (1/1 v/v, sample/distilled water) using an Orion Research 720A pH meter (Instrumentación Analítica SA, Madrid, Spain). Values were in the range 6.8–7.0.

The GDEs were prepared by mixing the freshly prepared DE with gelatine (4% of the initial weight of the DE). The emulsion

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