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Technological characteristics of cold-set gelled double emulsion enriched with n-3 fatty acids: Effect of hydroxytyrosol addition and chilling storage

María Freire[a,*], Ricard Bou[b], Susana Cofrades[a], Francisco Jiménez-Colmenero[a]

[a] Institute of Food Science, Technology, and Nutrition (ICTAN-CSIC), 28040 Madrid, Spain
[b] Institut de Recerca i Tecnologia Agroalimentàries (IRTA), XaRTA, Finca Camps i Armet, Monells 17121, Girona, Spain

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ABSTRACT

A study was carried out to analyse the technological characteristics and microbiological content of gelled double emulsions (GDE) formulated with perilla oil (as lipid phase and source of n-3 fatty acids) combined or not with hydroxytyrosol (Hyt) (in the inner aqueous phase) over 30 days storage at 4 °C. Both the control sample without Hyt (GDE-C) and the sample containing Hyt (GDE-Hyt) had an appropriate whitish solid-like structure with rheological (elastic and viscous moduli, and phase angle) and textural (hardness and chewiness) properties of strong gels. In comparison with GDE-C, the presence of Hyt promoted the formation of weaker gels, as evidenced by lower hardness and chewiness values and elastic modulus. Overall, GDEs presented excellent water and fat binding properties irrespective of the formulation and storage time. Changes in hydroperoxides and TBARs contents over storage indicated that GDEs were little prone to oxidation after 30 days. Nevertheless, Hyt increased GDE antioxidant capacity by up to 12 times after preparation, although this declined with storage. Most of the antimicrobial activity of Hyt was observed during the first two weeks of storage.

1. Introduction

Over the last several years novel applications of unsaturated liquid oil structuring methods have been considered as a strategy to improve the fat content of foods. Various different approaches have been proposed to stabilize and structure edible oils to promote solid-lipid functionality for use as an alternative to (semi-solid) animal fat in the development (reformulation) of healthy lipid foods, including meat products (Jiménez-Colmenero et al., 2015; Mao & Miao, 2015). In this regard gelled emulsions, acomplex colloidal material where emulsion and gel structures co-exist, offer interesting possibilities for use as food ingredients with novel functional properties and many industrial applications (Dickinson, 2011; Mao & Miao, 2015; McClements, 2012).

Although different simple emulsion gels have been described in the literature, there have been relatively few studies dealing with the development of gelled double emulsions (Benna-Zayani, Kbir-Ariguib, Trabelsi-Ayadi, & Grossiord, 2008; Delample, Da Silva, & Leal-Calderon, 2014; Patel et al., 2015), and still less involving their use as potential animal fat replacers. Gelled water-in-oil-in-water (W/O/W) double emulsions (GDEs) are a particular type of structured emulsions which offer a number of promising opportunities for food applications. These novel lipid materials can be used to improve lipid composition (reducing fat and providing a healthier fatty acid profile) of foods and encapsulate bioactive (hydrophilic and lipophilic) compounds while providing certain plastic properties. The inclusion of different emulsifiers and the oil and water phase composition are important factors in the final double emulsion (DE) characteristics, but when these systems are also intended to stabilize and structure a soft solid, the embedding of the emulsion droplets within a continuous hydrogel matrix seems the most promising strategy (Jiménez-Colmenero et al., 2015). Such structured hydrogelled double emulsions are typically formed in a two-step procedure: first, a double emulsion (liquid-like) is produced which, in a second step, is transformed into a solid-like emulsion gel by gelling the continuous phase and/or aggregating the emulsion droplets by thermal, enzymatic or chemical means (Dickinson, 2011; Jiménez-Colmenero et al., 2015; Mao & Miao, 2015). In this regard, we are seeing continuous advances based on traditional emulsifiers (proteins, polysacharrides) and novel complex emulsifiers (Liu et al., 2017).

One novel structuring proposal in the context of technological strategies to improve the fatty acid profile of foods is the development of GDEs enriched with n-3 fatty acid. While a number of different oils can be used for this purpose, perilla oil combined with a natural antioxidant such as hydroxytyrosol (Hyt) is especially promising (Flaiz et al., 2016). This is because perilla oil is one of the richest existing

Abbreviatons: MTG, microbial transglutaminase; Hyt, hydroxytyrosol; GDE, gelled double emulsions; DE, double emulsion

* Corresponding author.
E-mail address: maria.freire@ictan.csic.es (M. Freire).

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sources of α-linolenic acid (containing over 18 g/100 g of GDE), with demonstrated preventive effects on atherosclerosis and chemically-induced cancer, and also beneficial effects on immune and mental functions (Jo, Kim, Lee, Kim, & Song, 2013). For its part, Hyt is a phenolic compound (occurring naturally in olive oil) which has recently been receiving attention because of its wide range of biological and technological activities, including antioxidant and antimicrobial effects in foods and protection against cardiovascular diseases (Cofrades, López-López, & Jiménez-Colmenero, 2011; Medina-Martínez, Truchado, Castro-Ibanez, & Allende, 2016; Pazos, Alonso, Sánchez, & Medina, 2008). While different ingredients (pectin, alginate, gellan gum, etc.) have been added to the continuous phase (outer aqueous phase of DEs) to assist gelling and form the emulsions gels (Dickinson, 2011; Jiménez-Colmenero et al., 2015; Mao & Miao, 2015), with a combination of gelatin and microbial transglutaminase, a thermally stable structured cold-set double emulsion gel (hydrogelled) can be achieved with solid-like properties at room temperature (Flaiz et al., 2016).

Although several aspects of the preparation of GDE delivery systems containing Hyt and n-3 fatty acids have been studied (Cofrades et al., 2017; Flaiz et al., 2016), the presence of Hyt in GDE and its interaction with other components of the system needs to be addressed to understand its effect on physicochemical and microbiological characteristics, as well as its behaviour over storage of GDEs. The addition of Hyt is expected to have a considerable influence on the quality and stability of newly-formulated products. A deeper understanding of GDE technological characteristics will facilitate their use as food ingredients and thus help to further elucidate their role when incorporated as fat analogues in the meat matrix structure to obtain healthy food systems.

As a contribution to the successful development of GDEs as food-grade delivery systems with desirable (composition and solid-lipid) characteristics, this study aimed to characterize the technological (rheological, textural, colour, etc.) and microbiological properties of GDEs formulated with perilla oil (as lipid phase and source of n-3 fatty acids) as affected by the presence of Hyt (incorporated in the inner aqueous phase). These characteristics can also be affected during chilled storage given the nature of these new systems and that of the products to which they are added, and therefore these processing conditions were also studied.

2. Material and methods

2.1. Formulation and preparation of gelled double ($W_1/O/W_2$) emulsions (GDEs)

GDEs were prepared as reported in Flaiz et al. (2016). Briefly, a double emulsion (DE) was prepared in a 2-step emulsification procedure. The inner aqueous phase (W_1) consisted of 0.584 g NaCl (Panreac Quimica S.A., Barcelona, Spain) 125 mg Hyt (Seprox Biotech S.L., Madrid, Spain) and 0.04 g sodium azide (Panreac Quimica S.A., Barcelona, Spain) dissolved in 100 mL distilled water. The external aqueous phase (W_2) consisted of 0.5 g sodium caseinate (Excellion EM 7, FrieslandCampina DMV, Veghel, the Neteherlands), 0.584 g NaCl and 0.04 g sodium azide dissolved in 100 mL distilled water. The lipid phase (O) consisted of 6 g polyglycerol ester of polyricinoleic acid (PGPR; Lasenor Emul S.L., Olesa de Montserrat, Spain) in 100 g perilla oil (NutraZell Perilla, Grupo Nutraceutico ChiaSa, S.L., Meliana, Spain). In the first step, a primary emulsion (W_1/O; 20/80) was prepared using a two-stage high pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Parma, Italy) and the emulsion was homogenized twice at 7/55 MPa (first-stage pressure/second-stage pressure). The DE was formed by mixing 40 g/100 g W_1/O with 60 g/100 g W_2 and homogenized twice in a two-stage high pressure homogenizer at 3.5/15 MPa. Similarly, a DE without Hyt in the W_1 was prepared as control. Gelation was achieved by mixing the freshly prepared DEs with 4% bovine gelatine (200–220 bloom. Manuel Riesgo, S.A. Madrid, Spain) dissolved at 40 °C and 2% microbial transglutaminase (MTG; Activa GS, Ajinomoto (Tokyo, Japan). The two systems were rapidly aliquoted (40 g) in tubes (50 mL capacity), which were screw-capped and kept for 24 h at 4 °C to form the final two GDEs: the control sample without Hyt (GDE-C) and the sample containing 300 mg Hyt/kg (GDE-Hyt). These GDEs were stored in darkness at 4 °C until analysis (0, 8, 15, 30 days). These systems were fully replicated.

2.2. Particle size and distribution of oil droplets

These parameters were determined (in triplicate) in the DEs with a Malvern Mastersizer S laser diffraction particle size analyzer (Malvern Instrument Ltd., Worcestershire, UK) equipped with a He-Ne laser (λ = 633 nm). The measurement range was 0.05–900 μm. Obscuration range was 8–15%. Particle size calculations were based on the Mie Scattering theory. Volume average diameter ($d_{4,3}$), (Tepsongkroh, Harnsilawat, Maisuthisakul, & Chantrapornchai, 2015) was measured immediately after 10-fold dilution with the same saline composition as the outer phase of the W/O/W, and immediately read after placing in the dispersing unit filled with deionized water. Particle size measurements were performed at room temperature. Particle size was not determined in the GDEs given that these are thermostable gels (Flaiz et al., 2016). The pH of the DEs was measured previously as reported in Section 2.5 and was not expected to change during measurement.

2.3. Dynamic rheological properties

Dynamic rheological analyses were performed using a controlled-stress Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK). The measurement system was a circular plate geometry PP20 (20 mm diameter) with a 1 mm gap for all samples. Emulsion gels were cut from graduated plastic tubes into disk-shaped slices 20-mm diameter and 1-mm thick with a stainless steel cell specially designed for this diameter (Canet, Fernández, & Alvarez, 2009). A film of Vaseline oil (Codex purissimun) was gently applied to the edge of each sample to prevent moisture losses Amplitude sweeps, to determine the linear viscoelastic (LVE) region, were conducted by varying the shear strain (γ) of the input signal from 0.001 to 1% at a frequency of 1 Hz. Samples were subjected to stress that varied harmonically with time at variable frequencies from 0.1 to 10 Hz. The strain amplitude was set at γ = 0.5% within the LVE range. The elastic modulus (G′; Pa) and the viscous modulus (G″; Pa) were plotted as functions of frequency. Samples were allowed to relax for 4 min before performing rheological tests such as equilibration time. Measurements were made at 25 °C since it is worth noting that lipids that are solid in the range of 20–25 °C are considered fats whereas those that are melted at these temperatures are typically referred to as oils. Since we aimed to mimic solid-fat characteristics, we studied rheological properties at room temperature.

Also, a temperature sweep was conducted from 20 to 80 °C at a heating rate of 1 °C with a frequency of 1 Hz at a constant strain of 0.5% (within the LVE range). Elastic modulus (G′), viscous modulus (G″) and phase angle (δ; °) were calculated with the analysis program software. Results were averages of three determinations.

Rheological analyses were conducted on GDE-C and GDE-Hyt at the beginning and at the end of storage (30 days at 4 °C).

2.4. Texture profile analysis

Texture profile analysis was performed with a TA.XTPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) as described by (Bourne, 1978). Six cores (20 mm height; 30 mm diameter) for each sample (GDE-C and GDE-Hyt) were axially compressed to 50% of their original height. Force–time deformation curves were obtained with a 5 kg load cell applied at a crosshead speed of 1 mm s^{-1}. Attributes were calculated as described by Freire, Bou, Cofrades, Solas, and Jiménez-Colmenero (2016).