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Double emulsions as potential fat replacers with gallic acid and quercetin nanoemulsions in the aqueous phases

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ABSTRACT

The development of fat replacers to obtain healthier/functional foods is a constant challenge. With this aim, double emulsions (DE) with a blend of olive, linseed and fish oils as oil phase were developed. To prevent the oxidation of these oils, gallic acid and quercetin were incorporated in the internal and the external aqueous phase (W_2), respectively, according to a factorial design. Considering the low solubility of quercetin in water, it was included in O/W nanoemulsions (QN), thus being freely dispersible in W_2. The antioxidant activity in DE was attributed to QN, which significantly improved the oxidative stability of DE/QN. Furthermore, DE/QN showed good physical stability with a limited coalescence during storage at 4 °C for 28 days, significantly longer than time usually required for food ingredients. Therefore, DE/QN could be used as potential fat replacer in a variety of food formulations, providing blends of fatty acids consistent with dietary recommendations.

1. Introduction

Water-in-oil-in-water ($W_1/O/W_2$) double emulsions (DEs) are multi-compartmentalized systems where a water-in-oil (W_1/O) emulsion is dispersed as droplets within an external aqueous phase (W_2) (McClements, Decker, Park, & Weiss, 2009). Although encapsulation and controlled release of bioactive compounds is one of the main potential applications of $W_1/O/W_2$ DEs in the food industry, they may have other promising applications in foods. Thus, DEs offer the opportunity to design delivery systems for fatty acids to meet dietary recommendations for prevention of diet-related chronic diseases, namely reducing saturated fatty acids (SFA) intake and promoting the consumption of monounsaturated (MUFA) and polyunsaturated (PUFA) ω3 and ω6 fatty acids (FAO/WHO, 2010; Tressou et al., 2016). Therefore, by suitable formulation of the oil phase, DEs could be used as fat replacers in the development of healthier/functional foods with lower content of SFA and higher content of MUFA and PUFA. Several studies have been focused on the development of DEs enriched in unsaturated fatty acids to be used as functional ingredients. Vegetable oils such as olive, canola, chia, sunflower, linseed, soybean or perilla have been used as oil phase in the formulation of DEs (Jiménez-Colmenero, 2013), but blends of oils closer to health recommendations have not been yet studied.

Double emulsions designed with blends of unsaturated fatty acids may be susceptible to lipid oxidation, leading to the development of off-flavors and off-odors, together with the formation of potentially toxic compounds. Although there are many studies focused on the oxidative stability of conventional emulsions, especially oil-in-water (O/W) emulsions (Waraho, Cardenia, Decker, & McClements, 2010), research on oxidative stability of more complex emulsions such as multiple emulsions is scarce (Flaiz et al., 2016; Kiokias & Varzakas, 2017; O'Dwyer et al., 2013; O'Dwyer, O'Beirne, Ní Eidhin, & O'Kennedy, 2012; Poyato et al., 2013).

Oxidation of PUFA is a complex chemical reaction, and multiple antioxidant hurdle technologies are frequently required for their stabilization (McClements et al., 2009). Thus, the use of antioxidants is a common strategy for preventing lipid oxidation in food emulsions (Mcclements & Decker, 2000). Phenolic compounds have been widely used due to their ability to donate electrons and/or hydrogen atoms to free radicals, as well as to their capacity of chelating metal ions (Craft, Kerrihard, Amarowicz, & Pegg, 2012). The efficiency of phenolic compounds in O/W emulsions depends on several factors such as concentration, physical location, chemical structure, steric issues, nature of lipids, interactions with other components and relative polarity to the

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type of lipids present in the emulsion (Espinosa, Inchingolo, Alencar, Rodriguez-Estrada, & Castro, 2015; Jayasinghe, Gotoh, & Wada, 2013; Shahidi & Zhong, 2011). The antioxidant activity of gallic acid (GA) and quercetin (Q) has not been evaluated in multiple emulsions, but they have been incorporated in O/W emulsions (Di Mattia, Sacchetti, Mastrocola, Sarker, & Pittia, 2010; Espinosa et al., 2015; Gomes, Costa, de Assis Perrechil, & da Cunha, 2016). Quercetin has structural features that impart high antioxidant activity, such as the ortho 3′,4′-dihydroxy moiety in the B-ring, and the 2,3-double bond in combination with both a 4-keto group and a 3-hydroxyl group in the C ring (Heim, Tagliaferro, & Bobilya, 2002). However, one of the main disadvantages of using Q in functional foods is its low solubility in aqueous and oil media. Regarding this, several strategies have been used to increase the solubility/dispersability and bioavailability of Q, such as nanoemulsification, complexation with cyclodextrins or liposome encapsulation (Karadag, Yang, Ozcelik, & Huang, 2013).

The multi-compartmentalized structure of DEs offers the opportunity to incorporate antioxidants in different locations, thus potentially obtaining synergic effect due to the presence of antioxidants with different solubility and antioxidant mechanism. The aim of this study was to design DEs as delivery systems for blends of healthy fatty acids, with Q incorporated in the W_2 and GA in the internal aqueous phase (W_1). To overcome the disadvantage of the low solubility of quercetin in water, quercetin O/W nanoemulsions (QN) were developed as nanocarriers for Q in DE and they were dispersed in W_2. In contrast with most of the works addressing oxidative stability in emulsions, where effective antioxidant concentrations are determined through assay and error studies, we propose a factorial design to optimize the concentrations of GA and Q in DE. DEs obtained under optimal conditions were evaluated for droplet size, morphology and stability. Furthermore, their oxidative stability was evaluated under accelerated conditions.

2. Materials and methods

2.1. Materials

Double emulsions: Olive oil (Team Foods S.A., Chile), linseed oil (Nutra Andes Ltda., Chile) and fish oil (SPESS S.A., Chile). Polyglycerol ester of polyricinoleic acid (PGPR; Dimerco S.A., Chile), sodium caseinate (Prinal S.A., Chile), gallic acid (Sigma-Aldrich, Chile). Nanoemulsions: Miglyol 812 (neutral oil formed by esters of caprylic and capric fatty acids and glycerol, Sasol GmbH, Germany), Epikuron 145 V (phosphatidylcholine-enriched fraction of soybean lecithin, Cargill, Spain), ethanol (Merck, Chile). Quercetin was acquired from Sigma-Aldrich (Chile).

2.2. Fatty acid profile of the oil blend

A blend of olive, linseed and fish oils (70:20:10) was designed in previous studies (data not shown) and used as lipid phase in the formulation of DE. Fatty acid methyl esters (FAMEs) were prepared according to UNE-EN ISO 5509:2001. FAMEs were analyzed using a gas chromatograph (7890B, Agilent, Chile) fitted with a fused-silica capillary column (HP-88, 100 m × 0.25 mm i.d. × 0.20 μm film thickness) and a flame ionization detector. The injector and detector temperatures were set at 250 °C, and the hydrogen flow at 1 mL/min. The initial oven temperature was 180 °C, held from 20 min, and increased to 215 °C at a rate of 2 °C/min, held for 15 min. The injection volume was 0.5 μL. FAME standards (GLC 569, Nu-Chek Prep. Inc., USA) were used to identify the fatty acids, and tricosanoic acid was used as internal standard. The results were expressed as percentage of methyl esters. The determination was performed in triplicate.

2.3. Preparation of quercetin nanoemulsions

A quercetin O/W nanoemulsion (QN) was prepared by the solvent evaporation method according to Lozano et al. (2008), with some modifications. Briefly, Epikuron 145 V (600 mg) and quercetin (281 mg) were dissolved in ethanol (10 mL). Then, Miglyol 812 (2.5 mL) was added to the above solution, which was then verted into 190 mL of ethanol. Finally, this ethanolic phase was added to an aqueous phase (400 mL) and the final mixture was rotaevaporated to a final volume of 50 mL.

2.3.1. Characterization of quercetin nanoemulsions

The size and zeta potential of the QN was determined by photon correlation spectroscopy and laser Doppler anemometry, with a Zetasizer Nano-ZS (Malvern Instruments, UK). The concentration of Q in the O/W nanoemulsion was quantified by dissolving an aliquot of the nanosuspension in acetone (1:500) and the absorbance was measured in a UV–Vis spectrophotometer (Aquamate 8000, Orion, USA) at 370 nm. The standard calibration curve of quercetin was linear (0–10 μg/mL; $R^2 = 0.99$) in the range of tested concentrations (molar extinction coefficient 0.102 M^{-1} cm^{-1}). Scanning transmission electron microscopy (STEM) images were obtained to analyze the morphology of the carriers. The samples for STEM were prepared by depositing one droplet (10 μL) of the nanoemulsion, one droplet of MilliQ-water and one droplet of phosphotungstic acid (1%) on a Parafilm™ surface. Then, a copper grid (200 mesh, covered with Formvar) was incubated with each droplet for 2 min, and allowed to dry for 4 h. The association efficiency of Q in the nanocarriers was determined by calculating the difference between the total content of Q in the O/W nanoemulsion (obtained after the rotaevaporation) and that obtained in the continuous aqueous phase of the O/W nanoemulsion. Thus, the nanoemulsion was centrifuged (6000*g*, 10 min) to separate the continuous aqueous phase using VIVASPIN® tubes (100 kDa MWCO). The concentration of Q in the continuous phase was measured spectrophotometrically as described above. All the determinations were performed on three different nanoformulations.

2.4. Preparation of double emulsions

Double emulsions were formulated following a two-step emulsification process (Cofrades, Antoniou, Solas, Herrero, & Jiménez-Colmenero, 2013). GA was dissolved in W_1 (2–225 mg GA/kg DE) and QN was dispersed in W_2 (2–225 mg Q/kg DE). Furthermore, a control DE without GA and QN was prepared for comparative purposes (DE/C). In all the emulsions, the osmolarity of W_1 and W_2 was determined using an osmometer (3320, Advanced Instruments, USA) and equilibrated using NaCl to prevent diffusion phenomena. PRPG (lipophilic emulsifier) was added to the oil blend used as lipid phase (6% w/w). A W_1/O coarse emulsion was prepared by drop-wise addition of W_1 (20%) to the oil phase (80%) using a blender (Thermomix, Vorwek, Germany; 3250 rpm, 5 min, 50 °C). The coarse emulsion was homogenized twice with a two-stage high pressure homogenizer (Panda Plus 2000, GEA Niro Soavi, Italy) at 7977 psi (first stage) and 1015 psi (second stage). The W_1/O/W_2 coarse emulsion was prepared by gradually addition of the primary W_1/O emulsion (40%) to W_2 (60%) with sodium caseinate as hydrophilic emulsifier (0.5% w/w), followed by mixing (700 rpm, 5 min, room temperature). This coarse emulsion was homogenized twice at 2175 psi (first stage) and 435 psi (second stage). The main steps of the preparation of DE with GA and QN are shown as Supplementary material.

A three-level factorial design with 11 runs and two blocks was performed to optimize the GA content in the internal aqueous phase (W_1) and QN content in the external one (W_2). The concentrations of GA (2–225 mg GA/kg DE) and Q (2–225 mg Q/kg DE) were evaluated as independent variables. The dependent variable was lipid oxidation, evaluated as thiobarbituric acid reactive substances (TBARS) and expressed as induction time (IT_{TBARS}, time elapsed until TBARS curves showed an inflection point). Data were fitted to a polynomial function (time/MDA ratio vs. time) and the maximum point of polynomial