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Emulsion gels containing n-3 fatty acids and condensed tannins designed as functional fat replacers

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ABSTRACT

The purpose of this study was to ascertain the potential of several food-grade emulsion (O/W) gels (GEs) for use as healthier fat replacers. The emulsions, formulated with a lipid phase rich in n-3 fatty acids and different emulsifiers (sodium caseinate, SC; whey protein isolate, WPI and isolated soy protein, ISP), were cold gelled after adding a natural extract rich in condensed tannins (CT). The GEs were characterized and their oxidative stability evaluated during storage (4 °C). All GEs formulated presented a solid-like structure showing generally excellent emulsion stability, which improved in GEs with the addition of CT. Non-extractable proanthocyanidins (NEPA) were the main source of polyphenol in samples enriched with CTs. The antioxidant activity of the systems was not affected by the use of different proteins as emulsifiers, but it was improved in GEs containing CT. The oxidation values recorded in the GE systems can generally be regarded as low even considering their enrichment with unsaturated fatty acids, which thus assures their suitability for use as fat replacers.

1. Introduction

Reformulation is one of the most important approaches for improving the fat content of meat products and developing healthy and functional foods (Jimenez-Colmenero, 2007). In this regard, the consumption and development of food products enriched with n-3 fatty acids is of particular interest because of the reported beneficial health effects, mainly relating to cardiovascular and inflammatory diseases (Ruxton, Reed, Simpson, & Millington, 2004; Simopoulos, 2006). Healthier-lipid meat product reformulation generally entails replacement of the animal fat with liquid plant or marine oils. However, these unsaturated lipid materials have different physicochemical characteristics from the fats normally used in the food industry and hence their removal or substitution by liquid lipids (oils) may have a negative effect on the desired quality attributes in the reformulated product, as in the case of meat products.

Recently, novel proposals for oil stabilization and structuring have been reported for development of fat alternatives to improve the quality of the reformulated systems (Jiménez-Colmenero et al., 2015). These strategies aim to mimic a plastic fat and thus retain solid-like properties while possessing a healthier fatty acid profile. In this context, the formation of structured emulsions such as gelled emulsions (GEs; or emulsion hydrogels) offer interesting possibilities as fat replacers. Among different possibilities, the initial stage in emulsion gel formulation typically involves the production of a protein stabilized liquid oil-in-water emulsion (O/W). Using thermal, enzymatic or chemical means, a solid-like emulsion gel may be generated from a stable liquid-like emulsion by gelling the continuous aqueous phase. The gelation process depends to a great extent on the nature of the system (Jiménez-Colmenero et al., 2015). Gels formed by non-thermal treatments are referred to as cold set gels and have been shown to be particularly well suited to deliver heat-labile bioactives and nutraceuticals (Jones & McClements, 2010; Yang, Liu, & Tang, 2013; Zeeb et al., 2013).

The employment of enzymes such as microbial transglutaminase (MTG) to cross-link protein components and thus form thermally stable emulsion gels is of particular interest when dealing with labile ingredients such as n-3 fatty acids (Flaiz et al., 2016; Herrero, Carmona, Pintado, Jiménez-Colmenero, & Ruíz-Capillas, 2011; Yang et al., 2013). Transglutaminase has also been used to improve the processing properties of gluten-free cereals (Han et al., 2013). This enzyme induces

Abbreviations: GEs, gelled emulsions; CT, condensed tannins; O/W, oil-in-water emulsion; MTG, microbial transglutaminase; SC, sodium caseinate; WPI, whey protein isolate; ISP, isolated soy protein

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covalent cross-linking by acyl transfer between glutamine and lysine residues in proteins (Motoki & Seguro, 1998; Yang et al., 2013). In comparison with heat-induced gels, emulsion gels formed by means of MTG not only minimize lipid oxidation of n-3 fatty acids during preparation but may also offer additional protection in food products (Flaiz et al., 2016). In this connection, various biopolymers such as gelatine and carrageenans have also been used to make different types of cold-set emulsion-filled gels (Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasaran, 2014; Sala, van Vliet, Stuart, van de Velde, & van Aken, 2009).

Lipid oxidation not only causes nutritional loss and rancidity but also constitutes a potential hazard in that several of the compounds thus formed have been associated with neurodegenerative and cardiovascular diseases (Esterbauer, Wag, & Puhl, 1993; Perluigi, Coccia, & Butterfield, 2012). Antioxidant addition is a suitable strategy to tackle this problem, especially if it involves the use of natural compounds and extracts, as these are widely accepted by consumers (Ahn, Grun, & Fernando, 2002; Decker, Elias, & McClements, 2010). In this connection the use of denatured carob dietary fibre, very rich in condensed tannins (CT) in the form of oligomeric proanthocyanidin complexes, has demonstrated antioxidant efficacy in frying oils (Sanchez-Muniz et al., 2007) and meat systems (Bastida et al., 2009). Moreover, diets rich in dietary fibre have been associated with a reduced risk of colon cancer and cardiovascular disease (Bingham et al., 2003; Kaczmarczyk, Miller, & Freund, 2012; Ye, Chacko, Chou, Kugizaki, & Liu, 2012). In this connection, the insoluble dietary fibre from carob pod has shown hypocholesterolemic properties in animal studies (Perez-Olleros, Garcia-Cuevas, Ruiz-Roso, & Requejo, 1999; Wursch, 1979) and clinical trials (Ruiz-Roso, Quintela, de la Fuente, Haya, & Perez-Olleros, 2010; Zunft et al., 2003).

The purpose of this study is thus to examine the potential of several food-grade O/W gels (GEs) for use as healthier fat replacers. The different emulsions were prepared using a lipid phase rich in n-3 fatty acids and three commonly used emulsifiers, sodium caseinate (SC), whey protein isolate (WPI) and isolated soy protein (ISP) with and without the addition of CT. These emulsions were then induced to form cold-set gels, so as to improve the lipid content quantitatively and qualitatively for use in various food applications such as meat products. To that end, these GEs were characterized and their oxidative stability was evaluated during chilled storage at 4 °C.

2. Material and methods

2.1. Materials and reagents

Extra virgin olive oil (Carbonell Virgen Extra, SOS Cuétara SA; Madrid, Spain), linseed oil (Natursoy, Alimentos Ecológicos; Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold; Cognis GmbH; Illertissen, Germany) were used as lipid phases in emulsion preparations. Sodium caseinate (Excellion EM 6, *FrieslandCampina* DMV; Veghel, the Neteherlands), whey protein isolate (Provon 295, Glanbia Nutritionals; Kilkenny, Ireland) and isolated soy protein (Wilpro G-300, Wilmar Group; Qinhuangdao, China) were used as emulsifiers, and hereafter simply referred to as SC, WPI and ISP respectively. Condensed tannins (Exxenterol®; CT) extracted from carob fruit were obtained from Biosearch SA (Granada, Spain). Bovine gelatine (200–220 bloom) was from Manuel Riesgo, S.A. (Madrid, Spain), Texturalia k-carrageenan was from Trades S.A. (Barcelona, Spain) and Activa GS microbial transglutaminase (MTG) was from Ajinomoto (Tokyo, Japan). According to the supplier the enzymatic activity of MTG was approximately 100 U/g of powder.

Methanol, chloroform stabilized with ethanol, 1-butanol, trichloroacetic acid, ammonium thiocyanate, ammonium sulphate, sodium chloride, ferrous sulphate heptahydrate, barium chloride dihydrate, sodium carbonate, hydrochloric acid, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), sodium azide and hexane were from Panreac Quimica, SA (Barcelona, Spain). The 2-thiobarbituric acid reagent was from Merck KGaA (Madrid, Spain) and 1,1,3,3-tetraethoxypropane (TEP) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), butylated hydroxytoluene (BHT), gallic acid and Folin-Ciocalteau reagent were from Sigma-Aldrich (Madrid, Spain).

2.2. Formulation and preparation of O/W emulsion gels

2.2.1. Preparation of O/W emulsions

The emulsion composition and preparation processes were optimized in previous tests to obtain various gelled emulsions with the desired physicochemical characteristics. In this study, six different types of oil-in-water (O/W) emulsion gel samples were formulated, three of them containing CT and the others three without. Three different aqueous phases were prepared by dispersing 2 g/100 ml SC or 6 g/100 ml of WPI or 3 g/100 ml of ISP (as emulsifiers) in distilled water at room temperature until fully dissolved (at least 4 h). The emulsions were prepared with the same lipid phase, consisting of olive oil, linseed oil and fish oil added at 44.39, 37.87 and 17.74 g/100 g respectively. This oil combination was designed to produce a healthier lipid formulation (Delgado-Pando, Cofrades, Ruiz-Capillas, Solas, & Jimenez-Colmenero, 2010b) in line with health recommendations.

Coarse emulsions were prepared by dropwise addition of the lipid phase (50 g/100 g) in a food processor (Thermomix, Vorwerk, Germany) containing the different aqueous phases (50 g/100 g) and mixing at 3250 rpm at room temperature. The process took a total of 20 min. The coarse emulsions prepared with SC and WPI were passed once through a high-pressure homogenizer (GEA Niro Soavi MODEL Panda Plus 2000, Parma, Italy) at 55/7 MPa (first-stage pressure/second-stage pressure) to obtain fine emulsions. The emulsions prepared with SC and WPI are designated SC- and WPI-emulsions. The coarse emulsion prepared with ISP was not homogenized like its counterparts as this would have increased the viscosity, making any further processing difficult. This last emulsion is hereafter referred to as ISP-emulsion. These emulsions are hereafter designated SC-R, WPI-R and ISP-R. The same procedure was used to prepare the emulsions with CT, which were added after each emulsion preparation (3.9 g powder/100 g emulsion) and subsequently mixed. These emulsions are hereafter designated SC-CT, WPI-CT and ISP-CT.

2.2.2. Gelation process of emulsions

All emulsions were structured in a similar way as follows. 20 g of water per 100 g of O/W was heated to 80 °C in the food processor at 1625 rpm. Then, κ-carrageenan was added at 0.3 g/100 g of emulsion and stirred until it was completely dissolved, following which bovine gelatine was added at 0.5 g/100 g of emulsion. Once solubilized, this solution with hydrocolloids was mixed with the different O/W emulsions while stirring at 1625 rpm in the food processor at the 37 °C setting. Similarly, 1.5 g MTG/100 g of emulsion was added in 10 g of water per 100 g of O/W. Once solubilized, these mixtures were immediately mixed with their respective emulsions until completely homogenized. Subsequently, aliquots of 80 ml were rapidly poured into 100 ml tubes and stored at 4 °C. Under these conditions, two types of gelled emulsions were prepared (within 24 h) for each emulsifier, without (control, used as reference, R) and with condensed tannins (CT), denominated as follows: with SC (GE-SC-R and GE-SC-CT), with WPI (GE-WPI-R and GE-WPI-CT) and with ISP (GE-ISP-R and GE-ISP-CT).

2.3. Instrumental colour and pH

CIE-Lab tristimulus values were analysed using a 3500d spectrophotometer (Konica Minolta Business Technologies, Tokyo, Japan). The instrument was set for standard illuminant D-65 and a 2° observer angle and standardized using white and black standards. Ten determinations were performed from each formulation.

The pH was determined three times on 10 g of sample homogenate