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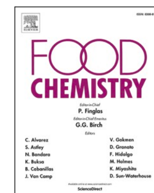

Olive and echium oil gelled emulsions: Simulated effect of processing temperature, gelling agent and *in vitro* gastrointestinal digestion on oxidation and bioactive compounds

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ABSTRACT

The impact and relative relevance of *in vitro* gastrointestinal digestion, processing temperature (room temperature or 180 °C), and gelling agent (GA) (carrageenan and alginate) on the bioactive compounds and oxidation status of olive and echium oils gelled formulations with 40% lipid incorporation was assessed. *In vitro* digestion was not affected by the GA, with >90% lipolysis in all formulations, but was the most relevant variable, promoting oxidation (MDA) regardless of the oil type, GA or temperature applied. Tocopherols and phenolic decreased with digestion, which could be interpreted as a protective response to pro-oxidative conditions during digestion. Temperature decreased olive oil phenolics. Gelification of echium oil using alginate reduced secondary oxidation products formation in comparison with carrageenan, with oxidation degrees after digestion equivalent to those shown with olive oil. The use of alginate with olive oil resulted in the most stable formulations, although not protecting its minor bioactive compounds from thermal degradation.

1. Introduction

Fat replacers are being developed using different technological approaches such as microemulsions, gelled emulsions, oleogels, hydrogels, nanoparticles, among others, aiming not just to mimic fat technological properties and decrease their caloric impact on food products, but also to deliver bioactive compounds (Gayoso et al., 2017; Muñoz-González, Ruiz-Capillas, Salvador, & Herrero, 2021). Vegetable oils are one of the main ingredients to these delivery systems, as they bring a wide range of possibilities referring to fatty acid composition, phytosterols, tocopherols and phenolic compounds (Dubois et al., 2007; Zarrouk et al., 2019; Prasad et al., 2021). Evidence suggests that delivering vegetable oils and bioactive compounds by structured systems can not only preserve their function but also improve their stability and bioavailability (Dong, Wei, & Xue, 2021; Gayoso, Ansorena, & Astiasarán, 2019). Additionally, recent studies showed that the application of these vehiculation systems in foodstuff has a promising future in the food industry due to their technological versatility and positive consumer response (Gutiérrez-Luna, Ansorena, & Astiasarán, 2022b). Therefore, there is an increased interest on the stability and fate of the lipid bioactives present in fat replacers or alternative fat sources during processing and after being ingested has increased (Van Hecke & De Smet, 2021). However, only limited information is available about the changes that these processes might inflict on bioactive compounds and their potential benefits (Salvia-Trujillo et al., 2019; Comunian et al., 2021; Mella et al., 2021).

Gastrointestinal lipid digestion complies a sequence of physico-chemical and enzymatic events that allows the body to absorb dietary lipids, fat-soluble vitamins and other minor compounds that can be present in the food matrix (Bauer, Jakob, & Mosenthin, 2008). However, this process also leads to secondary reactions that might negatively affect these compounds, namely lipid oxidation (Alberdi-Cedeño, Ibargoitia, & Guillén, 2020), due to the complexity of the pro-oxidative environment that includes mechanical (chewing, tongue movement and muscle relaxation and contraction) and chemical contributors (pH, ionic strength, enzymes, proteins) (Gayoso et al., 2019; Mao & Miao, 2015). Among the most common lipid oxidation markers, presence of 4-

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hydroxy-2-hexenal, 4-hydroxy-2-nonenal and malondialdehyde (MDA) can be highlighted (Hecke, Goethals, Vossen, & Smet, 2019) being the later one of the most abundant aldehydes generated during secondary lipid oxidation (Barriuso, Astiasarán, & Ansorena, 2013). Therefore, it is of great interest to assess the products derived from these reactions, especially when they might interfere in the bioavailability of the ingested compounds.

As different structures and formulations are used in emulsified lipids, their behavior during heating and digestion will differ as well. In this sense, studying different fatty acid arrangements, gelling agents and heat treatments could give a glimpse of the benefits or disadvantages of their use and combination for the delivery of bioactive lipidic compounds (Comunian et al., 2021). Extra virgin olive oil (*Olea europea* L.) (O), a well-known monounsaturated vegetable oil and a key ingredient of the Mediterranean diet and Echium oil (*Echium plantagineum* L.) (E), an excellent source of omega-3 fatty acids, have great potential to be incorporated into fat replacers due to their health-related properties (Minkowski et al., 2010; Comunian et al., 2016; Gutiérrez-Luna et al., 2022; Alongi, Lucci, Clodoveo, Schena, & Calligaris, 2022). However, gelling agents (GAs) can strongly interfere in lipid digestion, as they can affect oil droplet size and stability of the system or perform differently with pH changes in each phase of digestion (Tan, Zhang, Muriel Mundo, & McClements, 2020). In this sense, alginate (A) and carrageenan (C) are two algae polysaccharides often used for the development of structured food ingredients (Alejandre, Ansorena, Calvo, Cavero, & Astiasarán, 2019; Gutiérrez-Luna et al., 2022b) and their use in combination with vegetable oils offers the alternative to study the possible advantages of algae-based delivery systems. In fact, a previous paper optimized gelled emulsions using different hydrocolloids in combination with O or E, aiming to behave as butter replacers (Gutiérrez-Luna et al., 2022b), with promising results for alginate and carrageenan. However, the *in vitro* digestibility of bioactive compounds in all vegetable/algae origin delivery systems has not been explored enough yet (Comunian et al., 2021). Emulsion breakdown and lipolysis during digestion depends, among other factors, on the structuring agent, surfactants of choice and the effect of the matrix (Guo, Ye, Bellissimo, Singh, & Rousseau, 2017; Mat, Le Feunteun, Michon, & Souchon, 2016). Additionally, considering that the food matrices where these ingredients would be incorporated might be subjected to different cooking processes (baking, microwaving), the effect of temperature exposure is also an interesting variable to explore.

The goal of this study was to assess the effect of *in vitro* gastrointestinal digestion of four formulations of gelled emulsions using O or E, and alginate or carrageenan, subjected or not to previous heating (180 °C) on the presence of bioactive compounds and oxidation status of the lipids.

2. Material and methods

2.1. Materials

Echium oil (E) (*Echium plantagineum* L.) was kindly donated by NEWmega™ Echium Oil De Wit Specialty Oils (De Waal, Tescel, The Netherlands) and was enriched with a mix of tocopherols by the manufacturer. Extra virgin olive oil (O) (*Olea europaea* L.) (Urzante. Variety Hojiblanca, Spain) was purchased in a local supermarket. k-Carrageenan (C) was obtained from Grama aliment (San Sebastián, Spain) and Alginate (A) as Binder 1.0 (alginate and calcium sulphate) from BDF Natural Ingredients (Girona, Spain). Polysorbate 80 (PS80) was obtained from Sigma-Aldrich Chemical Co. (MO, USA). α-Amylase from human saliva (A1031; 852 U/ mg protein), pepsin from porcine gastric mucosa (P7000; 674 U/mg protein), pancreatin from porcine pancreas (P1750; 4 × United States Pharmacopeia specifications), lipase from porcine pancreas (L3226; 419 U/mg protein, activity using olive oil substrate) and bile extract (B8631) were obtained from Sigma-Aldrich Chemical Co. (MO, USA). The standards used for phenolics quantification (tyrosol, hydroxytirosol and syringic acid) were from Sigma-Aldrich, while those for fatty acid methyl esters analysis were from Supelco Inc (USA). Methanol and acetonitrile were of high-performance liquid chromatography (HPLC) grade (Riedel-de Häen, Germany). Heptane, isopropanol, and cyclohexane (all > 99% purity), were purchased from Carl Roth (Germany). 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), and sodium chloride (99.5% purity) were purchased from Panreac (Barcelona, Spain), and the 1,1,3,3-tetramethoxypropane (TEP, >95%) and anhydrous sodium sulphate (Na_2SO_4, analytical grade) from Merck (Germany).

2.2. Design

The effect of *in vitro* digestion over the bioactive compounds present in 4 types of gelled emulsion formulations, previously exposed or not to heat treatments simulating standard food processing practices was investigated (Fig. A1). The formulations used were previously optimized by our group (Gutiérrez-Luna et al., 2022b) and consisted oil-in-water emulsions prepared with 40% oil (olive oil or echium oil), 1.20% alginate or 0.75% carrageenan, 0.12% PS80, as detailed below. Samples were produced in three independent replicates, for each oil / gelling agent / thermal treatment combination / digestion with a total of 48 samples (2 oils × 2 gelling agents × 2 temperatures × digestion/non-digestion × triplicate).

2.3. Gel preparation

Gelled emulsions containing alginate were prepared as follows: the oil phase (40% for both formulations) containing the hydrophobic surfactant (0.12% Polysorbate 80) was added to the aqueous phase (water) and homogenized during 1–2 min (16000 rpm, Ultra-Turrax® T25basic). Once the two phases were unified, alginate was added (1.20%) and blended until a homogeneous mixture was achieved (2 min approx.). For those formulated with carrageenan, the method described by Poyato, Ansorena, Berasategi, Navarro-Blasco, & Astiasarán (2014) was followed. In this case, the oil phase containing the hydrophobic surfactant was added to the aqueous phase that included 0.75% of C and homogenized. Both phases were previously heated separately to 70 °C. After homogenization (3 min), the emulsions were cooled to room temperature, allowing the carrageenan to polymerize. All the gels were kept overnight under refrigeration (4 °C) before heat treatment.

2.4. Heat treatments

The stability of samples was evaluated at two different temperatures: room temperature (RT) (24 ± 1 °C) or 180 °C (common temperature for baking processes). The time of exposition to these temperatures was 28 min, according to the baking conditions reported by Gutiérrez-Luna et al., (2020). After heating treatments, samples were kept frozen (-20 °C) until analysis.

2.5. In vitro digestion

The *in vitro* digestion model for each type of gelled emulsion included three steps (oral, gastric and intestinal digestion) and it was based on the procedure described by Gayoso et al., (2016) including modifications following the Infogest method (Brodkorb et al., 2019). Briefly, 2.5 g of gelled emulsion were mixed with 20 mL of distilled water in a Falcon tube and homogenized for 5 s with an Ultra-Turrax® (T25 basic). Tubes were then warmed at 37 °C (water bath) to initiate the simulated oral digestion. Then, 625 μL of α-amylase (1.3 mg/mL solution in 1 mM $CaCl_2$) was added. The pH was adjusted to 6.5 with 1 M $NaHCO_3$ and the samples were incubated in a water bath at 37 °C for 2 min with magnetic stirring to complete the oral step. For the gastric digestion, on the same tubes, 825 μL of pepsin (160 mg/mL solution in 0.1 M HCl) was added, pH was adjusted to 2.5 with 3 M HCl and the incubation time was 2 h at

37 °C. In the simulated intestinal phase, 5 mL of pancreatin-bile-lipase extract (4 mg of pancreatin + 25 mg of bile extract mL/solution in 0.1 M $NaHCO_3$) were added to the gastric mixture. The digestion continued for another 2 h at 37 °C after adjusting the pH to 7.5 with 1 M or 0.1 M $NaHCO_3$. After intestinal digestion, samples were immediately frozen and kept at −20 °C until analysis. Samples were defrosted under refrigeration and subsequently centrifuged (10000*g*, 4 °C for 5 min, Eppendorf centrifuge 5810R) to separate the micellar fraction (upper phase of digesta that represents the bioaccessible fraction (Gayoso et al., 2019)) and the residual fractions for analysis.

Blanks with added enzymes and reagents in the absence of sample were run in parallel to ascertain the background contributions caused by the chemical environment in the assay.

All samples analyzed correspond to the intestinal phase.

2.6. Tyrosol and hydroxytyrosol analysis

2.6.1. Extraction

Polyphenol extraction protocols for non-digested and digested samples were based on Romero & Brenes (2012) and Bellumori et al., (2019) fitted to the characteristics of the samples and are described below. Chromatographic conditions were the same for both types of samples.

For non-digested samples, one gram of gelled emulsion or 400 mg of the oils was weighed into a 10 mL Falcon, followed by 40 μL of internal standard (IS) [0.15 mg/mL Syringic acid in MeOH/H_2O] and 2.5 mL HCl 2 M (MeOH:H_2O, 80:20) for direct aglycon hydrolysis. Tubes were vortexed for 30 s and kept under continuous agitation at 25 °C for 6 h. After this period, 2.5 mL ACN:H_2O (50:50 v/v) were added followed by 30 s vortexing. A 2 mL portion of the mixture was transferred to a micro tube and centrifuged for 10 min at 16,000 g. The upper phase was transferred to a 10 mL Falcon tube and deffated with 2 mL of hexane. Tubes were further vortexed and centrifuged for 3 min at 2000 g, the upper phase was rejected and the remaining lower hydroalcoholic phase was filtered (disposable filter PVDF 0.22 μm), and concentrated under N_2 flow to 200 μL prior to injection.

When processing digested samples, two milliliters of digested sample were measured in a 10 mL Falcon tube plus 250 μL of internal standard (0.15 mg/mL Syringic acid in MeOH/H_2O) and 5 mL MeOH/H_20 (80:20). Tubes were vortexed for 1 min and sonicated (ultrasound bath) for 15 min at room temperature. Afterwards, samples were centrifuged at 2800 g for 25 min. Supernatant was collected and transferred to a new tube for a second centrifugation step at 16,000 g for 5 min. In a 4 mL vial, 300 μL of hydroalcoholic extract (supernatant) was mixed with 300 μL of H_2SO_4 1 M and left for 2 h at 80ºC. Then, 400 μL H_2O was added and the mixture was transferred to a 1.5 mL microtube for centrifugation at 16,000 g for 5 min. The clear upper phase was transferred to a vial for analysis.

2.6.2. Chromatographic conditions

Hydroxytyrosol (HTyr) and tyrosol (Tyr) were analyzed by HPLC-PDA (Jasco, Japan), in a Jasco system equipped with two integrated pumps (PU–4180), an auto-sampler (AS–4150), a column oven (ECOM Eco2000, Czech Republic), and a photodiode array detector (Jasco MD—4010, Japan). Separation was accomplished on a C18 column (Gemini® 5 μm NX-C18 110 Å, 150 × 4.6 mm, Phenomenex, USA) using an eluent gradient of water and acetonitrile, both with 0.1% of formic acid at a flow rate of 1 mL/min. Quantification was performed at 280 nm based on individual calibration curves of HTyr and Tyr standards and the results expressed as mg/kg of oil.

2.7. Lipid extraction for further chromatographic analyses

Lipid extraction followed the Bligh & Dyer (1959) method, with modifications. Samples (oil, gelled formulations and digested samples, with an estimated fat content of approx. 45 mg) were weighted/measured in a 10 mL tube. A solution of NaCl 1% (H_2O) was added up to 1.5 mL, followed by 1.5 mL of chloroform, 2 mg TriC11:0 [triundecanoin, from a 10 mg/mL solution in Chloroform] (IS for Fatty acids), 15 μg Tocol [from a 1 mg/mL solution in Hexane] (IS for Vit E), 50 μL butylhydroxytoluene (BHT) (1% in MeOH) and “a spatulatip” of ascorbic acid. Then, 3 mL of methanol (MeOH) was added and vortexed. Finally, 1.5 mL of chloroform and 1.5 mL of NaCl 1% (H_2O) were added and the mixture was vortexed and centrifuged for 10 min at 3000 g. The aqueous phase was transferred to a new tube, while the organic phase (lower phase) was reserved. The aqueous phase was treated by lowering the pH (<1.5) with HCl 3 M (450 μL) and further extracted with 3 mL of chloroform, vortexed and centrifuged under the same conditions. The aqueous phase was removed and both organic phases were combined. Anhydrous Na_2SO_4 was added to remove any remaining water in the solution. The organic phase containing the extracted lipids was divided into three portions for the analysis of vitamin E, glycerides and fatty acids. They were all dried under gentle N_2 flow and reconstituted in adequate solvents as detailed below.

2.8. Tocopherol analysis

Tocopherols measurements were based on the ISO 9936:2016 standard with the addition of tocol as internal standard. Briefly, dried lipid extracts were reconstituted in 1 mL of hexane and transferred to a 1.5 mL micro tube, vortex and centrifuged for 3 min at 16,000 g. Samples were then transferred to vials for HPLC analysis, using the same Jasco equipment described above, with separation on a Luna Silica column (3 μm, 100 × 3.0 mm from Phenomenex, USA), with equivalent pre-column, operating at constant room temperature (23 °C). The eluent was a mixture of *n*-hexane and 1,4-dioxane (97:3) (v/v) at a flow rate of 0.7 mL/min. A fluorescence detector (Jasco FP-2020 Plus) was used, with excitation wavelength at 290 nm and emission wavelength at 330 nm. The concentrations were expressed as mg/kg of oil using calibration curves of alpha-tocopherol.

2.9. Glycerides analysis

Dried lipid extracts were diluted in THF (Tetrahydrofuran) to be analyzed by high-performance size exclusion chromatography (HPSEC) on a Jasco (Japan) HPLC system, equipped with a styrene–divinylbenzene copolymer R column (pore size 10 nm; 60 cm × 7 mm) (Phenomenex, Spain) and refractive index (RI) detection (Gilson, USA), using tetrahydrofuran as eluent (ISO 18395:2005). Results were expressed as % of total area.

2.10. Fatty acids analysis

Fatty acid profile was determined by gas chromatography (GC-FID) after acid transesterification. Lipid extracts were reconstituted in 0.5 mL of toluene and 1 mL of sulfuric acid 2% (MeOH) was added, vortex and placed (sealed) overnight (15 h) at 50 °C. Samples were cooled to room temperature and 1 mL of neutralizing solution ($NaHCO_3$ and K_2CO_3 in H_2O) and 1 mL of hexane were added. Vial was homogenized and centrifuged for 5 min at 100 g. Upper phase (hexane) was transferred to a 2 mL vial for analysis. Results are expressed as ratios of areas of individual fatty acids to the area of IS in g/100 g of fatty acid methyl ester, calculated by internal normalization of the chromatographic peak areas. A mixture of fatty acid methyl esters (Supelco 37 FAME Mix) was used for identification purposes and TriC11:0 (triundecanoin) for quantification (Sigma, Spain).

2.11. Malondialdehyde (MDA) analysis

TBARs (thiobarbituric acid reactive substances) measurement in all samples, quantified as free MDA equivalents, was performed following the method described by Sobral, Casal, Faria, Cunha, & Ferreira (2020) with modifications. 150 mg of gelled emulsions or 400 μL of digested,

standard or blank were measured and completed to 1 mL with trichloroacetic acid (TCA) at 7.5% for protein precipitation. 40 μL of BHT (4.5%); a lipophilic antioxidant, were added to avoid further oxidation during the experiment. Samples were centrifuged at 2800 g for 5 min. 500 μL of supernatant were transferred to a new micro tube and 500 μL of thiobarbituric acid (TBA) (40 mM) were added. Samples were stored overnight (24 h) in darkness at room temperature to help the reaction. Absorbance was read at 532 nm. MDA quantification was made using a standard curve with triethyl phosphate (TEP) (0.1–12.8 μmol dissolved in TCA 7.5%) and results were expressed as nmol/g of emulsion. Experiments were performed in duplicate and readings in triplicate.

2.12. Statistical analysis

Analysis was done using STATA/IC 12.1 program (StataCorp LP, TX, USA). One-way analysis of variance (ANOVA) was performed to evaluate statistical significance ($p \leq 0.05$) among formulations or treatments. Multiple comparisons of means were done by the Bonferroni post-hoc procedure to evaluate significance ($p \leq 0.05$) among formulations and treatments. Student *t*-test was used to compare the formulations before and after digestion. Values reported are the mean and standard deviations of all replicates. The influence of each variable (oil type, gelling agent, temperature and digestion) on MDA and tocopherols was studied by multiple regression analysis.

3. Results and discussion

3.1. Lipolysis and lipid profile

As shown in Fig. 1, a high degree of hydrolysis was achieved after *in vitro* digestion on all formulations tested, so it can be assumed that the polymers used did not represent a barrier for gastric lipase activity. Free fatty acids (FFAs) were the main products of lipolysis after the digestion process, accounting for 62 to 83% of the total glyceride profile, being the highest completeness verified in E gelled samples. Also, independently from the oil phase used and heat treatment (RT or 180 °C), those gelled emulsions with carrageenan had higher content of FFAs than their counterparts formulated with alginate (on average 73%, 68%, 82% and 79% for OC, OA, EC and EA respectively). Triglycerides percentage dropped from 91 to 94% to ~0% in E gelled emulsions and OC gelled emulsions. Formulations with OA presented a lower rate of lipolysis as ~10% of triglycerides were still quantified after the digestion process. Di- and monoglycerides were <9% and <17% respectively, while the unknown fraction was no higher than 3.5%.

Alginate and carrageenan are considered indigestible as they are not significantly degraded by low gastric pH or microflora in the gastrointestinal tract, therefore not being substantially absorbed or metabolized (Corstens, Berton-Carabin, Schroën, Viau, & Meynier, 2018). Various studies using alginate had reported that this polymer had little influence on the rate and extent of lipid digestion when present at relatively low levels (0.1 and 0.2 wt%), but greatly retarded lipid digestion when the concentration slightly increased (0.4 wt%) (Qin, Yang, Gao, Yao, & McClements, 2016). This effect had been associated with the ability of the polymer to bind calcium strongly (from enzymes and salts) and to be resistant to digestion in the stomach and small intestine (Hu, Li, Decker, & McClements, 2010). In this sense, the polymer might trap the lipid droplets in a highly viscous calcium-alginate gel that slows down mixing and reduces enzyme access to lipid droplet surfaces during intestinal digestion, resulting in lipase activity inhibition (Li, Hu, Du, & McClements, 2011). However, in our results, the use of higher concentrations of alginate (1.20%) did not seem to influence the lipolysis rate when used with Echium oil, and only to a very reduced extent with olive oil (~90%). Since the same reduction in the lipolysis was not observed with alginate, the GA might be the main responsible for this effect, and therefore the gel structure formed with alginate managed to retard the action of enzymes, probably by hindering their accessibility. The chemical nature of triacylglycerols, with a prevalence of polyunsaturated fatty acids in echium oil against monounsaturated in olive oil, together with the action of the GA might impose a higher degree of triglycerides structural fitting in olive oil, making them less available to enzymes. The importance of the positional isomers in triacylglycerols for the lipolysis rate is also referenced in the literature (Ji, Shin, Hong, & Lee, 2019).

Certain variability in the degree of lipolysis after *in vitro* gastrointestinal digestion of emulsion gels have also been reported in the literature. Mella et al. (2021) showed between 15 and 47.9% of FFAs released in emulsion gels stabilized by whey protein isolate, prepared under different pH conditions and pressures of homogenization, pointing out that this great variability was highly influenced by the digestion method, rather than pH or pressure. Verkempinck et al., (2018) reported only 4.6% of digested triacylglycerol (TAG) in olive oil enriched emulsion gels stabilized by pectin and attributed this low lipolysis degree to a possible interaction of the fiber with digestive components, slowing down the TAG hydrolysis. Interestingly, a higher hydrolysis degree (72–77%) was obtained when pectin was used in combination with other emulsifiers like Tween 80 (PS 80) (Verkempinck et al., 2018) as in our study. These results portray the important role of structuring agents on the efficiency of lipid digestion and whether they are fitted to the

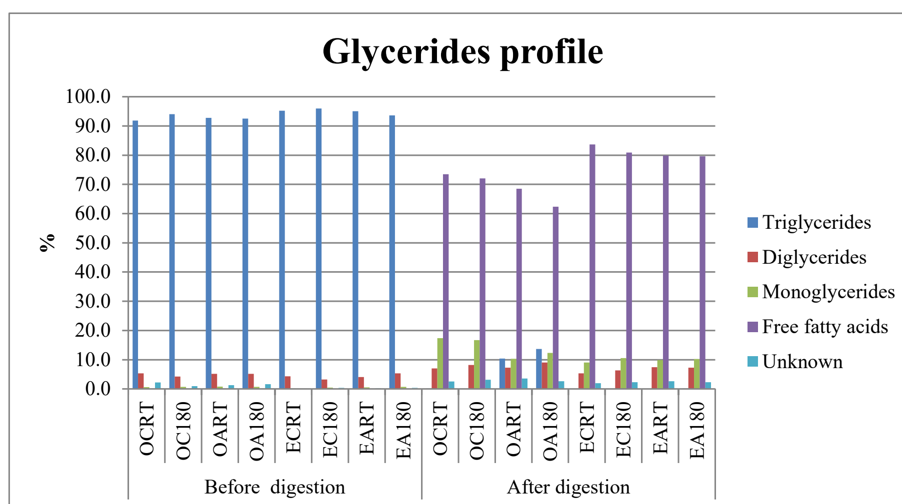

Fig. 1. Glyceride distribution (%) before and after digestion. OC: Olive oil + Carrageenan. OA: Olive oil + Alginate. EC: Echium oil + Carrageenan. EA: Echium oil + Alginate. RT: Room temperature. 180: Heated to 180 °C.

intended purpose (delivering a certain amount of components or retarding lipid digestion as a method to battle obesity) (Mella et al., 2021).

It can be hypothesized that lipase action and gastrointestinal environmental conditions can induce oxidative and detrimental effects that could be appreciated in the changes observed in the lipid fraction before and after digestion. Tables 1 and 2 show the most relevant individual fatty acids (g/100 g fat) and fatty acids sums together with w3/w6 ratio, respectively, for each analyzed sample.

Both studied oils are mostly unsaturated (>70%), being the major fatty acids those of the C18 series: oleic (18:1) in olive oil and echium oil, and γ- and α-linolenic (18:3), linoleic (18:2) and stearidonic (18:4) acid for echium oil (Carlini et al., 2021; Gutiérrez-Luna, Ansorena, & Astiasarán, 2022a).

The undigested samples of gelled emulsions showed reduced quantitative changes among formulations, regardless of the type of GA used or the heat treatment applied. The effect of digestion however, seemed to be much more relevant in terms of quantitative fatty acid profile modifications. A significant decrease in every fraction (SFA, MUFA and PUFA) was observed after the digestion process. It can be hypothesized that part of the fat might have been affected by oxidative processes and also it could occur that it would have remained in the pellet after the digestion affected by separation conditions, meaning that it would pass to the colon, where it could be metabolized by microbiota or excreted in feces. The reduction was, in every case, higher for SFA than for PUFA, although this last fraction was expected to be the most affected one by the potential oxidation process occurring during digestion. Other authors have also reported a decrease in the PUFA fraction when digesting bulk oils (Gayoso et al., 2019) or some food matrices (chicken meat burgers (Sobral et al., 2020), mushrooms (Liu et al., 2021), muscle food from mammals, poultry and fish (Hecke et al., 2019)). Regarding the SFA decrease during digestion, this fact has also been reported in algae oil delivered by an oil-in-water emulsion or by a gelled emulsion (Gayoso et al., 2019) and in digested pork liver pate (Lucas-González, Pérez-Álvarez, Viuda-Martos, & Fernández-López, 2021). Iriondo-Dehond et al. (2019) analyzing the bioaccessibility of lipids in digested spent coffee grounds found that half of the total fat was bioaccessible and half was excreted with the insoluble fraction. These authors also found a significant reduction in the saturated fatty acids of the bioaccessible fraction as compared to the non-digested sample.

3.2. Oxidation

Oxidation status of the emulsion gels was assessed and quantified as MDA equivalents, in order to determine the influence of all studied factors (digestion, oil, GA and temperature) in this process (Table 3). Regression analysis applied to these data (Table A1) and the resulting equation led to conclude that the *in vitro* digestion process was the most determinant factor in MDA formation, followed by the type of oil and GA used, whereas the temperature applied was the less relevant factor. Thus, the *in vitro* digestion process significantly increased MDA formation between 2.8 and 5.7-fold for every type of sample. This high impact on oxidation could be expected since the digestion environment complies with all the factors leading to oxidation. The *in vitro* model aims to mimic the conditions undergone during the natural gastrointestinal digestion, including the enzymatic activity and pH changes of the three phases: oral, gastric and intestinal. In the oral phase, mechanical (simulated mastication) and enzymatic activity takes place, as food is mixed with saliva. Once in the gastric phase, proteins are significantly hydrolyzed, carbohydrates experiment little breakdown and lipids are

Table 1
Fatty acids (g/100 g of fat) in gelled emulsion samples before and after *in vitro* digestion.

Echium oil gelled emulsions	ECRT		EC180		EART		EA180	
Fatty acids	Not digested	Digested	Not digested	Digested	Not digested	Digested	Not digested	Digested
C16:0	6.78 (0.08)b	2.36 (0.25)a	6.82 (0.07)b	2.99 (0.47)a	6.77 (0.05)b	2.59 (0.21)a	6.73 (0.07)b	2.19 (0.05)a
C16:1	0.15 (0.02)b	0.08 (0.02)a	0.14 (0.01)b	0.08 (0.02)a	0.13 (0.01)b	0.07 (0.02)a	0.12 (0.01)b	0.05 (0.02)a
C17:0	0.11 (0.00)b	0.04 (0.01)a	0.11 (0.00)b	0.06 (0.01)a	0.13 (0.03)b	0.05 (0.00)a	0.14 (0.04)b	0.04 (0.00)a
C17:1	0.05 (0.00)c	0.04 (0.00)b	0.05 (0.00)c	0.04 (0.00)b	0.05 (0.00)c	0.03 (0.00)a	0.05 (0.00)c	0.03 (0.00)a
C18:0	3.81 (0.01)b	1.01 (0.25)a	3.85 (0.04)b	1.58 (0.29)a	3.81 (0.05)b	1.24 (0.10)a	3.80 (0.02)b	1.02 (0.04)a
C18:1c	15.55 (0.27)c	12.77 (0.63)b	15.54 (0.32)c	12.30 (0.15)b	15.56 (0.33)c	10.60 (0.48)a	15.50 (0.31)c	9.76 (0.24)a
C18:2c	15.55 (0.34)c	13.07 (0.58)b	15.52 (0.32)c	13.26 (0.41)b	15.57 (0.32)c	11.42 (0.32)a	15.53 (0.33)c	10.71 (0.24)a
C18:3n6	10.32 (0.20)c	9.00 (0.38)b	10.32 (0.15)c	9.23 (0.23)b	10.33 (0.24)c	8.04 (0.20)a	10.31 (0.15)c	7.56 (0.10)a
C18:3n3	31.51 (0.23)d	26.44 (0.92)c	31.46 (0.15)d	27.11 (0.31)c	31.55 (0.12)d	23.68 (0.36)b	31.51 (0.11)d	22.42 (0.13)a
C18:4	13.79 (0.23)c	10.90 (0.32)b	13.56 (0.20)c	11.19 (0.24)b	13.64 (0.26)c	9.80 (0.23)a	13.68 (0.22)c	9.22 (0.17)a
C20:0	0.16 (0.00)c	0.07 (0.01)b	0.16 (0.00)c	0.08 (0.01)b	0.17 (0.03)c	0.06 (0.00)ab	0.16 (0.01)c	0.05 (0.00)a
C20:1	nd	nd	nd	nd	nd	nd	nd	nd
Olive oil gelled emulsions	**OCRT**		**OC180**		**OART**		**OA180**	
	Not digested	Digested	Not digested	Digested	Not digested	Digested	Not digested	Digested
C16:0	10.30 (0.38)c	5.06 (2.14)ab	10.60 (0.39)c	3.49 (0.21)a	12.49 (0.18)d	6.36 (0.26)b	12.69 (0.26)d	6.36 (0.30)b
C16:1	1.22 (0.06)c	0.93 (0.10)b	1.23 (0.10)c	0.94 (0.06)b	1.19 (0.10)bc	0.72 (0.07)a	1.15 (0.09)bc	0.79 (0.05)a
C17:0	0.12 (0.01)b	0.04 (0.03)a	0.10 (0.03)b	0.02 (0.01)a	0.10 (0.03)b	0.05 (0.02)a	0.12 (0.02)b	0.05 (0.02)a
C17:1	0.18 (0.06)b	0.13 (0.04)ab	0.18 (0.05)b	0.13 (0.04)ab	0.17 (0.05)b	0.10 (0.03)a	0.17 (0.05)b	0.11 (0.03)a
C18:0	3.08 (0.31)c	1.25 (0.65)ab	3.11 (0.34)c	0.76 (0.07)a	3.25 (0.28)c	1.44 (0.20)b	3.28 (0.30)c	1.42 (0.16)b
C18:1c	72.06 (0.79)c	55.98 (1.70)b	71.68 (0.93)c	56.22 (0.56)b	70.18 (0.51)c	43.87 (0.44)a	70.16 (0.44)c	45.24 (0.83)a
C18:2c	8.89 (0.72)c	6.76 (1.03)b	8.86 (0.79)c	6.86 (0.60)b	8.46 (0.82)c	5.24 (0.48)a	8.43 (0.80)c	5.40 (0.50)ab
C18:3n6	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a	0.00 (0.00)a	0.01 (0.00)a	0.01 (0.00)a	0.01 (0.00)a	0.00 (0.00)a
C18:3n3	0.75 (0.08)b	0.60 (0.07)b	0.75 (0.07)b	0.59 (0.06)ab	0.73 (0.08)b	0.47 (0.04)a	0.73 (0.07)b	0.47 (0.05)a
C18:4	nd	nd	nd	nd	nd	nd	nd	nd
C20:0	0.46 (0.03)d	0.21 (0.06)b	0.46 (0.03)d	0.16 (0.01)a	0.46 (0.02)d	0.22 (0.01)b	0.32 (0.23)c	0.22 (0.01)b
C20:1	0.21 (0.14)b	0.21 (0.00)b	0.30 (0.01)c	0.20 (0.01)b	0.27 (0.01)c	0.16 (0.01)a	0.27 (0.00)c	0.15 (0.01)a

Data correspond to mean value. Standard deviations appear in parentheses for each type of oil. For each type of oil, values with different letters within rows are significantly different ($p < 0.05$) based on post hoc Bonferroni test. OC: Olive oil + Carrageenan.. OA: Olive oil + Alginate.. EC: Echium oil + Carrageenan.. EA: Echium oil + Alginate.. RT: Room temperature.. 180: Heated to 180 °C. nd: Not detected.

Table 2
Sum of fatty acid fractions (g/100 g of fat) and W3/W6 in gelled emulsion samples before and after *in vitro* digestion.

Echium oil gelled emulsions								
	ECRT		EC180		EART		EA180	
	Not digested	Digested	Not digested	Digested	Not digested	Digested	Not digested	Digested
SFA	10.90 (0.09)c	3.60 (0.56)a	10.98 (0.07)c	4.84 (0.80)b	10.89 (0.04)c	4.03 (0.33)ab	10.86 (0.09)c	3.38 (0.09)a
MUFA	16.53 (0.26)c	13.45 (0.68)b	16.52 (0.30)c	12.93 (0.21)b	16.53 (0.34)c	11.12 (0.50)a	16.42 (0.31)c	10.25 (0.24)a
PUFA	71.83 (0.37)d	59.62 (2.10)c	71.57 (0.43)d	60.99 (1.03)c	71.77 (0.49)d	53.10 (0.91)b	71.75 (0.44)d	50.07 (0.17)a
W6	25.87 (0.54)c	22.07 (0.95)b	25.85 (0.45)c	22.50 (0.64)b	25.90 (0.53)c	19.47 (0.51)a	25.84 (0.47)c	18.29 (0.31)a
W3	31.51 (0.23)a	37.34 (1.23)c	31.46 (0.15)a	38.30 (0.52)c	31.55 (0.12)a	33.48 (0.56)b	31.51 (0.11)a	31.64 (0.28)a
W3/W6	1.22 (0.03)a	1.69 (0.03)b	1.22 (0.02)a	1.70 (0.04)b	1.22 (0.03)a	1.72 (0.04)b	1.22 (0.02)a	1.73 (0.04)b
Trans	0.33 (0.03)c	0.19 (0.02)b	0.35 (0.03)c	0.18 (0.02)b	0.33 (0.04)c	0.13 (0.02)a	0.36 (0.01)c	0.12 (0.02)a
Olive oil gelled emulsions								
	OCRT		OC180		OART		OA180	
	Not digested	Digested	Not digested	Digested	Not digested	Digested	Not digested	Digested
SFA	14.26 (0.53)c	6.68 (2.89)ab	14.56 (0.67)c	4.55 (0.28)a	16.65 (0.50)d	8.20 (0.49)b	16.72 (0.77)d	8.18 (0.43)b
MUFA	75.77 (0.92)d	59.11 (1.86)b	75.50 (0.91)d	59.38 (0.51)b	73.80 (0.53)c	46.30 (0.45)a	73.67 (0.38)c	47.80 (0.81)a
PUFA	9.86 (0.69)c	7.42 (1.03)b	9.83 (0.76)c	7.50 (0.57)b	9.42 (0.80)c	5.75 (0.45)a	9.38 (0.74)c	5.91 (0.48)a
W6	8.90 (0.72)c	6.78 (1.03)ab	8.87 (0.79)c	6.86 (0.59)b	8.47 (0.82)c	5.26 (0.47)a	8.45 (0.79)c	5.41 (0.49)a
W3	0.75 (0.08)c	0.60 (0.07)b	0.75 (0.07)c	0.59 (0.06)b	0.73 (0.08)c	0.47 (0.04)a	0.73 (0.07)c	0.47 (0.05)a
W3/W6	0.09 (0.01)a	0.09 (0.02)a	0.09 (0.01)a	0.09 (0.01)a	0.09 (0.01)a	0.09 (0.01)a	0.09 (0.01)a	0.09 (0.01)a
Trans	0.05 (0.01)b	0.04 (0.00)b	0.05 (0.01)b	0.05 (0.02)b	0.05 (0.01)b	0.02 (0.01)a	0.05 (0.01)b	0.04 (0.02)ab

Data correspond to mean value. Standard deviations appear in parentheses for each type of oil. For each type of oil, values with different letters within rows are significantly different ($p < 0.05$) based on post hoc Bonferroni test. OC: Olive oil + Carrageenan. OA: Olive oil + Alginate. EC: Echium oil + Carrageenan. EA: Echium oil + Alginate. RT: Room temperature. 180: Heated to 180 °C.

Table 3
TBARs values (nmol MDA/g of emulsion) for each sample, before and after *in vitro* digestion process.

		Before digestion	After digestion	p value
Echium Oil	EC RT	27.7 (2.2)b	133.7 (7.0)b	<0.05
	EC 180	32.8 (2.6)c	152.5 (6.5)b	<0.05
	EA RT	22.5 (1.8)a	62.0 (4.6)a	<0.05
	EA 180	26.4 (2.2)b	74.0 (1.9)a	<0.05
Olive oil	OC RT	13.2 (1.6)b	62.9 (5.5)b	<0.05
	OC 180	17.7 (0.7)c	70.7 (3.8)c	<0.05
	OA RT	11.2 (0.1)a	55.6 (4.5)a	<0.05
	OA 180	11.3 (0.6)a	64.2 (4.5)b	<0.05

Data correspond to mean value. Standard deviations appear in parentheses. For each type of oil, values with different letters within columns are significantly different ($p < 0.05$) based on post hoc Bonferroni test. P values lower than 0.05 indicate significant differences between columns, according to student *t*-test. OC: Olive oil + Carrageenan. OA: Olive oil + Alginate., EC: Echium oil + Carrageenan. EA: Echium oil + Alginate. RT: Room temperature. 180: Heated to 180 °C.

only partially digested. It is in this phase where further dietary lipid peroxidation and destabilization of oil in water emulsions occurs (Gorelik, Ligumsky, Kohen, & Kanner, 2008; Guo et al., 2017). However, as oil droplets are often dispersed within complex structures, most of them may not be released at all during gastric digestion. Then, in the intestinal phase most food macronutrients (both pre-existing emulsified oils or emulsions formed *in-situ* in the duodenum or the stomach) are physically and chemically broken down with the aid of a number of enzymes (trypsin, chymotrypsin, pancreatic lipase, colipase, and α-amylase), which facilitates their absorption but also contributes to further oxidation. (Guo et al., 2017).

Of all samples, olive oil gelled emulsions presented the lowest MDA values before digestion being significantly higher in those formulated with carrageenan. The higher oxidation in echium oil formulations can be naturally attributed to the instability of its polyunsaturated fatty acids (70–75%), particularly those with three and four double bonds. The initial heating (70 °C) required for the preparation of carrageenan gels could also have contributed to early oxidative reactions (Mohanan et al., 2018; Rincón-Cervera et al., 2020), being this increase particularly visible in the olive oil formulations when comparing A and C emulsions. Previous assessment of long-term oxidative stability in olive and echium oil gelled emulsions revealed the same tendency (Gutiérrez-Luna et al., 2022b), where formulations with olive oil and carrageenan showed a statistically significant increase in MDA values after Schaal oven test in comparison with alginate, while echium oil gels were at least 5–8 folds higher than their olive oil counterparts at 0 h and after 24 h, respectively. When assessing oxidative status of gelled olive oil, Alongi et al. (2022) reported that processing conditions, in particular the maintenance at relatively high temperatures during oleogels preparation, had a negative impact on the initial oxidative status of olive oil.

An interesting approach to oxidation of the samples under heating and digestion is that it can be attenuated by the action of antioxidant bioactive compounds such as tocopherols and polyphenols. Thus, the presence of these components was assessed in order to conclude about their potential reduction while exerting their protective effect over other compounds such as fatty acids.

3.3. Minor bioactive compounds

Tocopherols are the major natural antioxidants in vegetable oils, being susceptible to losses during processing (temperatures, storage, air exposition) (Cao et al., 2015). Detailed profile of tocopherols in gelled samples is presented in Table 4. Tocopherol profile in O undigested gel samples ranged between 200 and 290 mg/kg of oil depending on the type of samples, and included α-, β- and γ-tocopherol, with α-tocopherol as the main type. On the other hand, E gels tocopherol profile ranged between 1600 and 2000 mg/kg of oil, and was composed mainly by γ-tocopherol, followed by δ-, α- and β-tocopherol. It should be mentioned that echium oil was enriched by the manufacturer with a mix of tocopherols and our results are considerably higher than those reported by other authors. Nevertheless, the proportions could still be compared to those found in the literature. Nogala-Kalucka, Rudzinska, Zadernowski, Siger, & Krzyzostaniak (2010) and Minkowski et al. (2010) reported γ-tocopherol to be between 77 and 94% of the total amount of tocopherols in fresh echium oil, which is similar to the % observed in our samples before digestion (~70%).

As resembled in the regression analysis (Table A1), *in vitro*

Table 4
Tocopherol content in gelled emulsion samples before and after *in vitro* digestion.

mg/kg of oil	Before digestion				After digestion			
	EC RT	EC 180	EA RT	EA 180	EC RT	EC 180	EA RT	EA 180
α-Tocopherol	157(47)ab	108(38)a	175 (33)b	145 (44)ab	86 (23)a	82 (16)a	103 (16)a	92 (15)a
β-Tocopherol	17(5)a	13 (4)a	16 (4)a	13 (5)a	10 (3)a	10 (3)a	9 (3)a	9 (3)a
γ-Tocopherol	1376 (225)b	1132 (129)ab	1422 (157)b	1168 (222)ab	854 (110)a	865 (73)a	812 (70)a	782 (83)a
δ-Tocopherol	391 (104)a	325 (85)a	403 (87)a	336 (119)a	270 (79)a	274 (67)a	233 (60)a	231 (65)a
Total	1940 (364)b	1578 (254)ab	2017 (259)b	1661 (390)ab	1219 (214)a	1231 (158)a	1157 (145)a	1113 (165)a
	OC RT	OC 180	OA RT	OA 180	OC RT	OC 180	OA RT	OA 180
α-Tocopherol	273 (43)b	225 (29)ab	199 (35)ab	187 (54)ab	172 (27)a	168(41)a	166 (23)a	146 (18)a
β-Tocopherol	3 (1)b	2 (0)ab	2 (0)a	2 (1)a	2 (0)a	2 (0)a	2 (0)a	2 (0)a
γ-Tocopherol	18 (2)b	15 (6)b	13 (3)ab	10 (1)a	12 (3)ab	12 (3)ab	10 (3)a	9 (3)a
Total	294(2)b	242 (35)b	214(34)b	199 (55)ab	186 (27)a	182 (42)a	178 (25)a	157(20)a

Data correspond to mean value. Standard deviations appear in parentheses. Values with different letters within rows are significantly different ($p < 0.05$) based on post hoc Bonferroni test. OC: Olive oil + Carrageenan., OA: Olive oil + Alginate., EC: Echium oil + Carrageenan. EA: Echium oil + Alginate.,. RT: Room temperature., 180: Heated to 180 °C.

gastrointestinal digestion represented the leading cause for tocopherol decrease. After digestion, tocopherol losses for O gelled emulsions varied between 16 and 36% and for E gelled emulsions were 22–42%. For E gels, the reduction was similar for the two GA, confirmed with the regression analysis where GA did not represent a relevant factor in the reduction of tocopherol content. However, OA formulations had lower tocopherol contents before digestion than OC ones, with the impact of the GA being apparently higher than the one from heating. Since these formulations do not require heating as the carrageenan ones, other processing step might induce tocopherol degradation, as homogenization, totalizing 4 to 5 min of air incorporation, a technological step that might worth further studies. However, after digestion, all olive oil formulations presented equivalent contents of tocopherol, reducing the significance of the apparent lower tocopherol content in alginate formulas.

Even though the temperature effect was not statistically significant, results showed a reducing effect over tocopherols content in all samples, being higher for α-T and γ-T (major tocopherols for each type of oil) in O and E gelled emulsions respectively. Alongi et al. (2022) observed decreases of up to ~36% of α-tocopherol in O oleogels with heating requirement of 80 to 140 °C, structured using monoglycerides, rice wax, γ-oryzanol, β-sitosterol or ethylcellulose. This loss is significantly higher than our results, where α-tocopherol losses after heating at 180 °C were of 6.5% for OA and 17.9% in OC on average. This difference could be attributed to the proportions of oil in the formulations (oleogels are mainly composed by oil ~90%) and its higher exposure to temperatures during the gelation process (Pehlivanoğlu et al., 2018; Alongi et al., 2022).

Numerous health benefits are attributed to HTyr and Tyr; two of the most studied olive oil phenols, for their biological effects on physiological processes acting as antioxidants, antiatherogenic, cardioprotective, anticancer, neuroprotective, antidiabetic (EFSA, 2011; Marković et al., 2019; Alberdi-Cedeño et al., 2020). Therefore, polyphenol hydrolysis and further extraction was applied to O and E samples. Whereas HTyr and Tyr were found in O samples (Table 5), those containing E did not show any polyphenolic compounds.

Due to the extent of oxidation undergone by samples after heat exposure and digestion, a certain loss of both phenolic compounds was expected. In fact, data showed a high degradation of both HTyr and Tyr. HTyr content was between 15 and 17 mg/kg of oil in OC and OA gels at room temperature, and a highly significant loss was observed in OC 180 gels, with a loss of 87%. After digestion, no HTyr could still be detected in the micellar fraction and the Tyr contents were residual with both GA, representing less than 2% in all samples. The very low amounts of phenolic compounds found in the micellar fractions could be attributed to their degradation under digestion conditions (Alberdi-Cedeño et al., 2020). Moreover, the possibility of the polyphenols being entrapped in the residual phase, and therefore pass to the large intestine were gut microbiota might obtain through their metabolism other compounds with potential beneficial health effects (Mithul-Aravind, Wichienchot, Tsao, Ramakrishnan, & Chakkaravarthi, 2021), deserved to be explored.

Table 5
Total hydroxytyrosol (HTyr) and tyrosol (Tyr) content in EVOO gelled emulsions before and after digestion (mg/kg of oil).

		Before digestion	After digestion
HTyr	OC RT	18.0 (0.5)c	nd
	OC 180	2.3 (0.8)a	nd
	OA RT	15.5 (1.4)b	nd
	OA 180	11.4 (3.2)b	nd
Tyr	OC RT	103.3 (17.6)c	2.1 (0.2)a
	OC 180	40.4 (6.8)b	2.6 (0.2)a
	OA RT	120.5 (14.4)d	2.0 (0.2)a
	OA 180	19.0 (7.6)a	2.3 (0.3)a

Data correspond to mean value. Standard deviations appear in parentheses. Values with different letters within rows are significantly different ($p < 0.05$) based on post hoc Bonferroni test. OC: Olive oil + Carrageenan., OA: Olive oil + Alginate., EC: Echium oil + Carrageenan. EA: Echium oil + Alginate.,. RT: Room temperature., 180: Heated to 180 °C. nd: Not detected.

4. Conclusions

In vitro digestion showed to be the main contributor to O and E gelled emulsions oxidation, with higher impact than a short exposure to high temperature. Gelification using C, in comparison to A, seemed to have favored the formation of secondary oxidation products, especially when C was used in combination with highly polyunsaturated samples. The fatty acid profile and amounts (in both, O and E gelled emulsions) were modified by digestion, generally decreasing during the process. Tocopherols and phenolic content were also highly impacted by the digestion process and by heating, and their reduction could be interpreted as a protective response to pro-oxidative conditions. Nevertheless, olive and echium oil gelled emulsions formulated with A (1.20%) and C (0.75%) seemed to have great potential as delivery systems for unsaturated fatty acids, but further research is needed to understand better the behavior of structured oils under different conditions and improve their oxidative stability to avoid compromising their health benefits.

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CRediT authorship contribution statement

Katherine Gutiérrez-Luna: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Diana Ansorena:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Rebeca Cruz:** Conceptualization, Formal analysis, Methodology, Supervision, Visualization. **Iciar Astiasarán:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. **Susana Casal:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134416.

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