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Emulsions vs excipient emulsions as α-tocopherol delivery systems: Formulation optimization and behaviour under *in vitro* digestion

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ABSTRACT

Oil-in-water emulsions (EM) have been extensively used for the encapsulation of lipophilic bioactive compounds and posterior incorporation into food matrices to obtain functional foods. Conversely, novel excipient oil-in-water emulsions (EXC) present identical composition and structure as EM, albeit are not bioactive by themselves since no bioactive compound is encapsulated. Instead, EXC aims at improving the bioavailability of foods' natural bioactive compounds upon co-ingestion with nutrient-rich foods. In this work, EM and EXC were produced and their stability and functionality as delivery systems for α-tocopherol compared. Emulsions were formulated with corn oil and lecithin, and their composition was optimized using experimental designs. Formulations produced with 3 % lecithin and 5 % oil attained smallest particles sizes with the lowest polydispersity index of all tested formulations and remained stable up to 60 days. Encapsulation of α-tocopherol did not have a significative impact on the structural properties of the particles produced with the same composition. α-tocopherol stability during *in vitro* digestion was superior in EM regardless the processing methodology (EM stability < 50 %, EXC stability < 29 %), indicating that EM offered greater protection against the digestive environment. α-tocopherol's bioaccessibility was significantly increased when encapsulated or when digested with added excipient emulsions (82–92 % and 87–90 % for EM and EXC, respectively). In conclusion, EM were more efficient vehicles for the selected bioactive compound, however, the good results obtained with EXC imply that excipient emulsions have a great potential for applications on foods to improve their natural bioactive compounds' bioavailability without the need of further processing.

1. Introduction

The application of bio-based delivery systems for the development and fortification of functional food products has been regarded as an excellent approach to improve foods' functional and sensorial characteristics, whilst enhancing the stability, bioaccessibility and bioavailability of bioactive compounds. Bio-based delivery systems have been extensively applied during recent years to overcome challenges of food fortification, that are linked to the low solubility of certain bioactive compounds, to the low stability of formulated foods and to the high degradation occurring during digestion. As such, several approaches using various matrices (e.g., lipids, proteins and polysaccharides) have been applied to produce delivery systems that grant protection to the incorporated bioactive compounds and modulate their release profile (Madalena et al., 2022).

Lipid-based systems, such as oil-in-water emulsions (EM) have been extensively used for the encapsulation of lipophilic bioactive compounds and posterior incorporation into food matrices to produce functional foods (Gonçalves et al., 2022). EM are obtained by processing two immiscible liquids with a surfactant, which reduces interfacial tension between the liquids and allows the formation of a colloidal dispersion (Liu et al., 2019). EM dispersions are characterized by the small oil droplets that are distributed through the aqueous phase, however, depending on the formulation and applied processing technology, the particles formed will vary in size, dispersity and stability, ultimately modulating their properties (Safaya & Rotliwala, 2020). EM are extremely advantageous for applications as delivery systems of lipophilic bioactive compounds. Lipophilic bioactive compounds

Abbreviations: EM, oil-in-water emulsions; EXC, excipient oil-in-water emulsions; UT, Ultra-Turrax; HPH, High pressure homogenizer; CCD, central composite design; Z-Ave, particle size; PDI, polydispersity index; SSF, simulated salivary fluid; SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

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generally present low water solubility and are chemically unstable in certain environmental conditions (Liu et al., 2019). Also, because of their sensitivity, their stability is greatly affected by humans' harsh physicochemical digestive conditions, which may result in lower absorption and reduced functionality (Bourbon et al., 2018).

One of the most important lipophilic bioactive compounds is vitamin E (i.e., α-tocopherol), which has a significant impact on healthy ageing (Fernandes et al., 2021). Vitamin E is an important antioxidant in the preservation of lipidic membranes and essential fatty acids, presenting high quenching capacity and removal of reactive oxygen species. As such, vitamin E has important physiological properties in the human body, that includes the regulation of genetic expression, modulation of cellular functions, inhibition of lipoxygenases and anti-inflammatory action (Boccardi et al., 2016; Csapó et al., 2017). Vitamin E is widely available in various sources (i.e., plant oils, seeds, nuts, fruits and vegetables), however, older adults often present vitamin E levels below the recommended daily dose (Millen et al., 2016). Moreover, this deficit has been linked to the occurrence of cancers, vision loss, arteriosclerosis, cardiovascular diseases, sarcopenia, and was suggested to contribute to immunosenescence associated with the loss of cognitive functions (Casas et al., 2018; Lorenzo-López et al., 2017; Rémond et al., 2015). Vitamin E fortified foods have been commercially available, however, due to the above-mentioned characteristics of this bioactive compound, the effectiveness of vitamin E delivery from these food products is often reduced (Lv et al., 2019).

Several studies regarding the encapsulation of vitamin E in bio-based delivery systems proved the efficacy of this strategy on increasing the stability, bioaccessibility and bioavailability of this bioactive compound in the gastrointestinal tract (Ribeiro et al., 2022; Yang & McClements, 2013). That said, novel and simpler delivery systems such as excipients emulsions (EXC), have been recently studied for their capacity to boost the biological activity of naturally available bioactive compounds (McClements et al., 2016). By definition, EXC do not contain bioactive functions but are designed to increase the functionality of bioactive compounds and modulate the digestive fate of the co-ingested compounds (Gonçalves et al., 2018). Conversely to emulsions encapsulating bioactive compounds, EXC aim at improving the bioavailability of foods' natural bioactive compounds upon their co-ingestion with such nutrient rich foods. After ingestion, EXC should interact with the available compounds during the digestion process, preventing undesired biotransformation and ultimately conferring them higher stability, increased bioaccessibility and intestinal absorption (McClements & Xiao, 2014). Studies using EXC demonstrated their capacity to improve the solubility and bioaccessibility of various liposoluble bioactive compounds. For instance, the application of oil-in-water excipient emulsions granted a faster solubilization rate of quercetin compared to the bulk oil or bulk water, which consequently enhanced quercetin *in vitro* bioaccessibility and *in vivo* antioxidant activity (Chen et al., 2016). Similarly, multiple studies demonstrated that the application of EXC increased the bioaccessibility of curcumin powder (Zou et al., 2016), omega-3 powder (Hwang et al., 2019), lycopene present in tomatoes (Salvia-Trujillo & McClements, 2016; Wang et al., 2023), carotenoids present in spinach (X. Liu et al., 2023; Tan et al., 2021), among others. Recently, authors tested the influence of various oil-in-water excipient formulations on curcumin, and concluded that the EXC application increased curcumin bioaccessibility by increasing its transportation rate across cellular models while reducing cellular metabolism (Luo et al., 2022).

The results obtained with the application of EM and EXC are dependent of their composition, production methodology and resultant characteristics (Shah et al., 2015). Various studies demonstrate the influence of lipid composition (Zou et al., 2016), emulsifier (Zou et al., 2015) and processing type (McClements, 2021) on the structure and functionality of emulsions. Also, despite the several studies of encapsulation of lipophilic vitamins to improve their bioavailability, there is little understanding of how EXC contribute to the stability and absorption of said compounds. In this sense, the aim of this work was to optimize the production of EM and EXC, using the same ingredients and applying different high-energy processing techniques. The optimized structures were then studied and compared for their stability and functionality as delivery systems for vitamin E. This work contributes to understand the impact of bioactive compounds encapsulation in the production of emulsions and how these delivery strategies (i.e., encapsulated or co-ingested) would modulate the bioactive compounds release and absorption efficacy.

2. Materials and methodology

2.1. Emulsions and excipient emulsions production

EM and EXC were produced with LIPOID S 75 lecithin (composed by fat-free soybean phospholipids with 70 % phosphatidylcholine, kindly provided by Lipoid GmbH, Germany), corn oil (Puro Milho, Fula) purchased in a local market (Braga, Portugal) and distilled water. To produce the EM, α-tocopherol (Sigma-Aldrich T3251, St. Louis, MO, USA) was solubilized in the lipid phase to achieve a final concentration of 0.75 mg/mL (i.e., 15 mg per 20 mL formulation). This step was disregard for EXC production. Lecithin was solubilized in distilled water at 80 °C for 1 h to obtain the aqueous phase. The emulsions were obtained by combining the two phases and processing the mixture via two different mechanical methods at room temperature: a) using an Ultra-Turrax (UT) homogenizer (T18, Ika-Werke, Staufen, Germany) at 15,000 rpm; b) premixed in the UT for 2 min at 8,200 rpm, and subsequently processed in a high-pressure homogenizer (HPH) (EmulsiFlex-C3, AVESTIN, Canada) at 50 psi (3.5 bar) of air pressure. The concentration of lecithin, oil:water ratio and processing time/cycles were optimized with experimental designs detailed in section 2.2.

After their production, EM and EXC were kept in the dark at 4 °C until further analysis and application.

2.2. Optimization of emulsion and excipient emulsion production

A Central Composite Design (CCD) was used to optimize the production of EM and EXC. The CCD was composed by a factorial 2^k with three independent variables plus a central point with three replicates, for a total of 11 experiments per design. The independent variables selected were: i) surfactant concentration (X_1); ii) oi:water (v/v) ratio (X_2); and iii) processing time on either HPH or UT ($X_{3\ HPH}$ and $X_{3\ UT}$, respectively). The range for the different levels (−1, 0, 1) was established based on previous works (data not shown) as follows: X_1 (1.0, 2.0, 3.0 % within the aqueous phase); X_2 (1:19, 1:9, 3:17 (*v/v*)); and $X_{3\ HPH}$ (10, 15, 20 HPH cycles) or $X_{3\ UT}$ (5, 10, 15 min). The particle size (Z-Ave) and polydispersity index (PDI) were the two dependent variables evaluated (to be minimized). Four CCDs were performed to optimize EM and EXC production using the HPH or the UT. For each CCD, three additional confirmation experiments were conducted to validate the statistical model.

2.3. Particle size and polydispersity index

Z-Ave and PDI of the EM and EXC produced were measured by dynamic light scattering, DLS (Zetasizer Nano SZ, Malvern, Worcestershire, UK). Measurements were performed after their production, after 30 and 60 days. All samples were diluted 1:10 with distilled water. Each individual measurement was determined from the average of three readings.

2.4. In vitro gastrointestinal digestion

Optimized EM and EXC functionality as delivery system for α-tocopherol was evaluated using the harmonized INFOGEST static *in vitro* gastrointestinal digestion protocol. *In vitro* digestion was performed

to evaluate emulsions influence on α-tocopherol stability, bioaccessibility and estimated bioavailability throughout the gastrointestinal tract (Brodkorb et al., 2019). EM were submitted to the digestion protocol as produced, whereas EXC were mixed with α-tocopherol prior to digestion to attain an equal α-tocopherol concentration. EM and EXC were produced in the preceding day and stored in the dark at 4 °C until the experiment. Control samples void of surfactant and without HPH/UT processing were also evaluated.

During the digestion protocol, EM and EXC were exposed to electrolytic and enzymatic conditions that mimic digestive parameters from the mouth, stomach and small intestinal. Briefly, to perform the oral phase, simulated salivary fluid (SSF, constituted by KCl 15.1 mmol/L, KH_2PO_4 3.7 mmol/L, $NaHCO_3$ 13.6 mmol/L, $MgCl_2.(H_2O)_6$ 0.15 mmol/L, $(NH_4)_2.CO_3$ 0.06 mmol/L and HCl 1.1 mmol/L), $CaCl_2.(H_2O)_2$ 0.3 mol/L (to achieve 0.75 mmol/L at the final mixture) and distilled water to attain a sample:SSF ratio of 1:1 (v/v) were added. Samples were incubated in a horizontal water shaking bath at 120 rpm at and 37 °C (B. BRAUN BIOTECH model CERTOMAT WR, Melsungen, Germany) for 2 min to complete the oral phase.

For the gastric phase, simulated gastric fluid (SGF, constituted by KCl 6.9 mmol/L, KH_2PO_4 0.9 mmol/L, $NaHCO_3$ 25 mmol/L, NaCl 47.2 mmol/L, $MgCl_2.(H_2O)_6$ 0.12 mmol/L, $(NH_4)_2.CO_3$ 0.5 mmol/L and HCl 15.6 mmol/L), 0.15 mmol/L $CaCl_2.(H_2O)_2$, porcine pepsin (Sigma-Aldrich P7012, to achieve a final concentration 2000 U/mL), HCl 1 mol/L (to adjust to pH 3.0) and distilled water to attain a bolus:SGF mixture ratio of 1:1 (v/v) were added. Samples were incubated in a horizontal water shaking bath at 120 rpm and 37 °C during 120 min.

The intestinal phase simulation consisted in the addition of simulated intestinal fluid (SIF, constituted by KCl 6.8 mmol/L, KH_2PO_4 0.8 mmol/L, $NaHCO_3$ 85 mmol/L, NaCl 38.4 mmol/L, $MgCl_2.(H_2O)_6$ 0.33 mmol/L and HCl 8.4 mmol/L), 0.015 mmol/L $CaCl_2.(H_2O)_2$, bile extract porcine (Sigma-Aldrich B8631, to achieve a final concentration 10 mmol/L), pancreatin from porcine pancreas (Sigma-Aldrich P7545, to achieve a final concentration 100 (TAME) U/mL)), NaOH 1 mol/L (to adjust to pH 7.0) and distilled water to attain a chyme:SIF mixture ratio of 1:1 (v/v). Samples were incubated in a horizontal water shaking bath at 120 rpm and 37 °C during 120 min.

Samples were collected at the end of each digestive phase to evaluate the morphological changes throughout *in vitro* digestion. Collected samples were placed in ice to decrease enzymatic activity until analysis. To the samples obtained at the end of intestinal phase (onwards described as digesta) Pefabloc® SC (Sigma-Aldrich) 1 mmol/L (10 μL per 1 mL of sample) was added to the samples obtained at the end of intestinal phase (onwards described as digesta) to stop the digestion reactions. Digesta samples were used to assess digestion stability and bioaccessibility of α-tocopherol. *In vitro* digestion experiments were performed in triplicate.

2.5. Emulsions morphological characterization during in vitro digestion

EM and EXC size and stability throughout the *in vitro* digestion were studied using dynamic light scattering (i.e., Z-Ave, PDI and ζ-Potential) as described in topic 2.3.

In addition to this analysis, fluorescence microscopy (BX51, Olympus Europe, Hamburg, Germany) was used to understand morphological changes on EM and EXC during the *in vitro* digestion. Briefly, 100 μL of sample was stained with 10 μL of Nile Red solution (0.1 % (w/v) dissolved in methanol). Samples were visualized with a wavelength filter (i.e., λ_{ex} = 488 nm; λ_{em} = 500 to 525 nm) and 100x magnification. Acquired images format type was set to 8-bit (to convert colours into greyscale) and their brightness was adjusted using ImageJ 1.53 t software.

2.6. α-tocopherol quantification and determination of bioaccessibility, stability and bioavailability

α-tocopherol released during *in vitro* digestion was quantified by UHPLC-DAD Nexera X2, Shimadzu, Japan) according to the protocol described by Ribeiro et al. (2022). Digesta samples were centrifuged at 18,000 *g* for 30 min at 4 °C (Multifuge X3R, Heraeus, Germany) to precipitate unsolvable matter and obtain the micellar phase. α-tocopherol was extracted from 3 mL of digesta or micellar samples, by mixing with 3 mL of hexane:ethanol (1:1, *v/v*), followed by centrifugation at 1,540 *g* for 2 min (EBA 20, Hettich, Germany) to collect the supernatant (i.e., hexane phase). The extraction procedure was repeated 3 times and hexane phases combined and dried under nitrogen. Samples were then resuspended in 1.5 mL of methanol and filtered through 0.22 μm PES syringe filter before UHPLC analysis. A Kinetex® core–shell 1.7 μm C18 column (Phenomenex, California, USA) was used. The parameters used in UHPLC were a isocratic elution mode at 0.4 mL/min of methanol: water mobile phase (95:5, v/v), column temperature at 50 °C and 10 μL injection volume. DAD detection wavelength was set to 295 nm.

After UHPLC α-tocopherol quantification, the bioaccessibility, digestion stability and estimated bioavailability were calculated. α-tocopherol bioaccessibility estimates the percentage of the bioactive compound that was released and formed mixed micelles, allowing it to be absorbed into the blood stream. Bioaccessibility was calculated as the percentage of α-tocopherol concentration in mixed micelles ($\alpha toc_{micelle}$) in regard to α-tocopherol concentration in the digesta ($\alpha toc_{digesta}$), according to Equation (1).

$$\boldsymbol{Bioaccessibility} = (\boldsymbol{\alpha toc_{micelle}}/\boldsymbol{\alpha toc_{digesta}}) \times 100 \quad (1)$$

Stability was calculated as the percentage of α-tocopherol concentration in the digesta that remained untransformed regarding the initial α-tocopherol concentration ([αtoc] = 0.75 mg/mL), according to Equation (2).

$$Stability = (\alpha toc_{digesta}/\alpha toc) \times 100 \quad (2)$$

Bioavailability estimates the α-tocopherol absorption, by considering the product between the stable and the bioaccessible bioactive compound, according to Equation (3).

$$Bioavailability = (Bioaccessibility \times Stability) \times 100 \quad (3)$$

2.7. Statistical analyses

Experimental designs' data, namely, variables and methodology outline, statistical analysis and charts were obtained using Protimiza Experimental Design web-based software. Independent variables statistical significance was determined by ANOVA ($p < 0.05$). Statistically significant independent variables were considered for parametrization of regression models and respective charts.

The remaining data obtained in this work was analysed using Prism version 8.0.2 (GraphPad Software Inc., California USA). The statistical significance was determined by two-way ANOVA followed by post-hoc Tukey's honestly significant difference (HSD) test. Statistically significant differences were considered when p *value* < 0.05. Results are presented as mean values of experiments performed in triplicate ± standard deviation (SD).

3. Results and discussion

3.1. Emulsions and excipient emulsions formulation optimization

The formulation of EM and EXC was optimized by performing different CCDs. The preferable ratio of lipid and aqueous phase, surfactant concentration and type or duration of processing were evaluated by granulometric analysis of the produced particles up to 60 days. All experimental data can be found in **Appendix A**. Generally, all CCDs for

either the production of EM or EXC using UT or HPH obtained similar results for the different formulations experiments. The smallest particles produced at each CCD presented average size of 253.5, 242.5, 260.5 and 241 nm for EM-HPH, EM-UT, EXC-HPH and EXC-UT, respectively. The regression coefficients for the different responses (i.e., Z-Ave (Y_1), PDI (Y_2), Z-Ave 30 (Y_3), PDI (Y_4), Z-Ave 60 (Y_5) and PDI 60 (Y_6)) were calculated and considered for reparameterization of regression models if they were statistically significant ($p < 0.05$ or 0.1 if there were not statistically significant variables at $p < 0.05$).

The obtained mathematical models for the various responses of all CCDs are presented on Table 1. As observed, the models differ substantially between the different CCDs conducted. Such outcome can be due to the variability inherit of such experiments and due to experimental errors (e.g., performed on different days, calibration errors, sample preparation errors, etc.). The variability found when comparing the models obtained for the same response at different time points can also be attributed to such errors (e.g., Z-Ave, Z-Ave 30 and Z-Ave 60 of EXC-HPH). Despite such variability, the independent variables that were more often statistically significant were the oil:water ratio (X_2) and processing (X_3), indicating that these parameters had higher influence on EM and EXC production. As expected, models fitted with more variables presented better correlation coefficients compared to those fitted with only one variable (e.g., PDI, PDI 30 and PDI 60 of EM-UT). Low correlation coefficients found may be related to the low variability between assays and, in the case of PDI responses, because the values are depicted in decimal scale.

Table 1
Mathematical models obtained for the Z-Ave and PDI on the production day, after 30 and 60 days for Central Composite Designs of emulsions (EM) and excipient emulsions (EXC) processed in high pressure homogenizer (HPH) and Ultraturrax (UT).

Models obtained for EM-HPH		
Z-Ave	$Y_1 = 291.34 + 29.11x_2$	$R^2 = 62.97$ %
Z-Ave 30	$Y_3 = 293.70 + 35.72x_2$	$R^2 = 77.61$ %
Z-Ave 60	$Y_5 = 279.26 + 38.13x_2$	$R^2 = 64.41$ %
PDI	$Y_2 = 0.21 - 0.02x_3$	$R^2 = 41.33$ %
PDI 30	$Y_4 = 0.21 + 0.01x_2 - 0.01x_3 - 0.01x_1x_2 + 0.01x_2x_3$	$R^2 = 85.19$ %
PDI 60	$Y_6 = 0.23 - 0.03x_3$	$R^2 = 43.77$ %
Models obtained for EM-UT		
Z-Ave	$Y_1 = 282.18 + 28.90x_3 + 17.17x_1x_2$	$R^2 = 80.43$ %
Z-Ave 30	$Y_3 = 276.34 - 24.43x_3 + 16.27x_1x_2$	$R^2 = 89.06$ %
Z-Ave 60	$Y_5 = 264.55 + 24.04x_3$	$R^2 = 50.40$ %
PDI	$Y_2 = 0.43 - 0.05x_1 + 0.04x_2 - 0.03x_3$	$R^2 = 83.77$ %
PDI 30	$Y_4 = 0.42 - 0.02x_1 + 0.03x_2 - 0.03x_3 + 0.03x_1x_2 - 0.01x_1x_3$	$R^2 = 98.54$ %
PDI 60	$Y_6 = 0.41 - 0.03x_1 - 0.03x_3$	$R^2 = 66.32$ %
Models obtained for EXC-HPH		
Z-Ave	$Y_1 = 336.66 - 35.37x_1 + 60x_2$	$R^2 = 59.70$ %
Z-Ave 30	$Y_3 = 311.02 - 47.15x_1x_2$	$R^2 = 31.39$ %
Z-Ave 60	$Y_5 = 342.63 - 58.75x_1 + 44.30x_2 - 52.47x_1x_2$	$R^2 = 83.30$ %
PDI	$Y_2 = 0.28 + 0.05x_2 - 0.05x_3 - 0.03x_2x_3$	$R^2 = 87.70$ %
PDI 60	$Y_6 = 0.31 - 0.06x_1 - 0.05x_3 - 0.05x_1x_2$	$R^2 = 82.77$ %
Models obtained for EXC-UT		
Z-Ave	$Y_1 = 299.99 + 28.25x_2 - 25.37x_3 + 20.10x_1x_2$	$R^2 = 94.24$ %
Z-Ave 30	$Y_3 = 306.38 - 41.14x_3 + 26.19x_1x_2$	$R^2 = 83.82$ %
Z-Ave 60	$Y_5 = 256.03 + 13.96x_2 - 19.64x_3 + 14.49x_1x_2$	$R^2 = 80.58$ %
PDI 30	$Y_4 = 0.21 - 0.06x_3$	$R^2 = 39.29$ %
PDI 60	$Y_6 = 0.39 + 0.04x_2 - 0.03x_3$	$R^2 = 71.20$ %

It can be concluded that the encapsulation of α-tocopherol did not cause a great influence on the produced EM and EXC, since their characteristics were similar. Conversely, EM and EXC composition and processing had a significant impact on the size and homogeneity of the produced samples, as observed in the mathematical modelling. These results can be linked to a basic principle of thermodynamics applied to emulsions formation: the surface free energy of the emulsion is correlated with the interfacial tension and total interfacial area (McClements, 2021). Therefore, changing the surfactant concentration, oil:water ratio and processing duration and type will have direct impact on the above-mentioned parameters by inducing different interactions between droplets, their flow conditions and surface elasticity (Schroën et al., 2020).

In Fig. 1 some examples of response surfaces for the Z-Ave and PDI (PDI 60 on the case of EXC-UT, because PDI results did not correlate statistically significant variables at $p < 0.1$) for the different CCDs are presented. The remaining obtained charts can be found on **Appendix B**. Fig. 1 shows that smaller particles were obtained when using higher concentrations of lecithin ($X_1 = 1$) and a lower oil:water ratio ($X_2 = -1$) (i.e., 3 % of lecithin on the aqueous phase and 5 % of oil) regardless of the emulsion type and processing methodology. Particles with better homogeneity were also obtained using similar conditions (data found on **Appendix B**). This outcome is in agreement with the literature, as increasing the surfactant-to-oil ratio and decreasing the oil-to-water ratio promotes formation of smaller particles (Zhang et al., 2020).

Longer processing times originated particles with better dispersity in either methodology (i.e., nanoparticles presented PDI < 0.23 and PDI < 0.29, when processed in HPH and UT, respectively), which was expected since the particles were submitted to disruptive forces for longer periods (Mendes et al., 2020). Also, homogeneity of samples was higher when processed in HPH compared to the samples processed in UT, possibly due to the higher relative energy efficiency of the HPH (Schroën et al., 2020).

Concerning particles' stability, particles produced with higher concentrations of lecithin, lower oil:water ratio and longer processing times remained stable up to 60 days with modest Z-Ave and PDI variations (i.e., EM Z-Ave changed < 6 % and PDI changed < 17 %, whereas EXC Z-Ave changed < 10 % and PDI changed < 13 %).

To validate the obtained models and the preferable emulsion formulation, three additional experiments were conducted applying the discussed conditions (i.e., $X_1 = 1$; $X_2 = -1$; $X_3 = 1$). Table 2 presents the theoretical values (determined using the mathematical models from Table 1) and experimental values (average value and standard deviation obtained from the experiments) for each formulation. Once again, particles with identical droplet dimensions (ca. 230 nm) were obtained for all formulations. Moreover, these results prove that the selected conditions were optimal to attain smaller particles, as particles exhibited the smallest size throughout all experiments. Validation experiments also confirmed that HPH provided more homogeneous samples (lower PDI). It can be also observed that some of the obtained models presented high percentage errors (i.e., EM-HPH Z-Ave (Y_1), EM-UT Z-Ave (Y_1) and EXC-HPH PDI (Y_2)), which could be attributed to low correlation of such models and to higher standard deviation obtained on the validation experiments.

Overall, encapsulation of the bioactive compound did not cause great

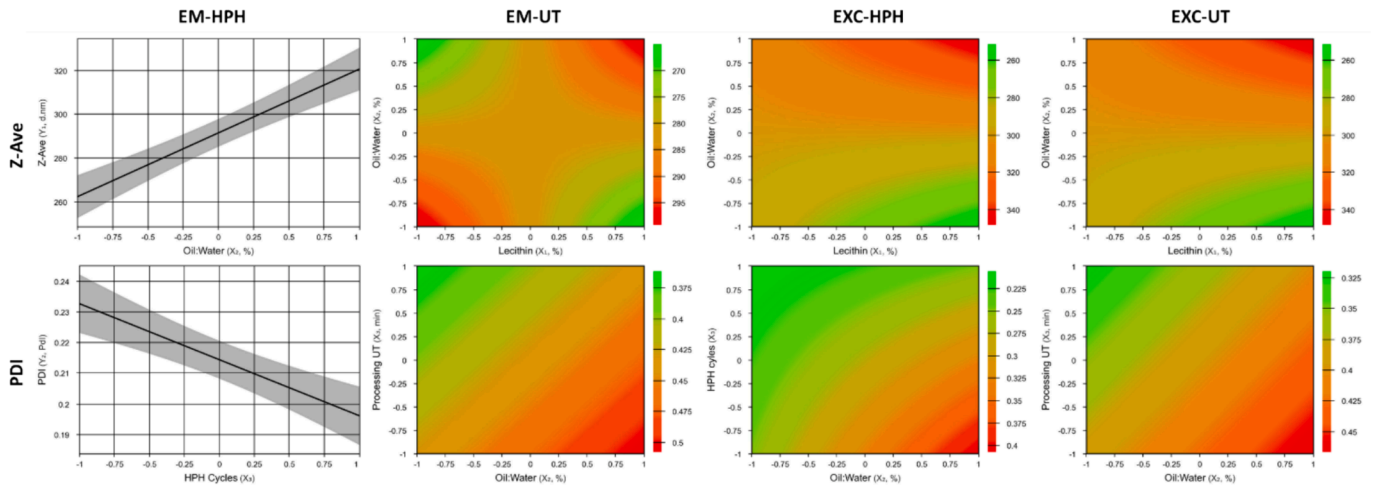

Fig. 1. Response surfaces of Z-Ave and PDI (PDI 60 on the case of EXC-UT) obtained for the Central Composite Designs of emulsions (EM) and excipient emulsions (EXC) processed in high pressure homogenizer (HPH) and Ultraturrax (UT).

Table 2
Theorical values and experimental values obtained for the validation of Central Composite Designs mathematical models of Z-Ave and PDI.

		Theoretical	Obtained	% Error
EM-HPH	Z-Ave (nm)	262.23	233.06 ± 14.08	11.13
	PDI	0.19	0.18 ± 0.02	3.16
EM-UT	Z-Ave (nm)	293.91	237.28 ± 4.48	19.27
	PDI	0.31	0.31 ± 0.04	−0.32
EXC-HPH	Z-Ave (nm)	241.29	225.22 ± 4.93	6.66
	PDI	0.26	0.21 ± 0.05	17.69
EXC-UT	Z-Ave (nm)	226.27	233.81 ± 5.43	−3.33

impact in emulsion formation. Conversely, the formulation recipe, duration and efficiency of processing did. Finally, it was validated that by increasing the surfactant concentration, reducing oil:water ratio and increasing processing duration and efficiency, smaller and more homogenous particles are obtained.

3.2. Emulsions and excipient emulsions behaviour during in vitro gastrointestinal digestion

In this study, the development of EM and EXC targeted its application as delivery systems, aiming at enhancing the functionality of α-tocopherol. To understand how these particles could contribute to the preservation and targeted release of α-tocopherol during digestion on the human gastrointestinal tract, the optimized formulations were submitted to INFOGEST *in vitro* static digestion protocol (Brodkorb et al., 2019). *In vitro* digestion protocols have been proposed as an efficient and simple methodology to simulate the parameters of the gastrointestinal tract, by mimicking conditions found in the mouth, stomach and small intestine. Prior the digestion, EXC formulations were mixed with α-tocopherol to achieve equal α-tocopherol concentration of EM formulations. Fig. 2 shows the results obtained for the particle size, polydispersity index and ζ-potential of the initial samples, as well as throughout the different digestion stages, for EM and EXC processed by either HPH and UT.

As above-mentioned, initial samples presented similar Z-Ave (ca. 230 nm) and ζ-potential (ca. − 60 mV) without statistically significant differences. Homogeneity of samples was higher when processed in HPH and presented statistically significant differences compared to samples processed in UT (PDI 0.184 ± 0.015, 0.311 ± 0.044, 0.214 ± 0.049 and 0.254 ± 0.073, for EM-HPH, EM-UT, EXC-HPH and EXC-UT, respectively). Fluorescence microscopy images (Fig. 3) indicate that the initial samples presented uniformly distributed particles, without the presence of aggregates. The characteristics of the obtained particles were in agreement with results described by others authors when producing O/W nanoemulsions using lecithin as surfactant (Nash & Erk, 2017). These properties are due to lecithin characteristics, a zwitterionic surfactant

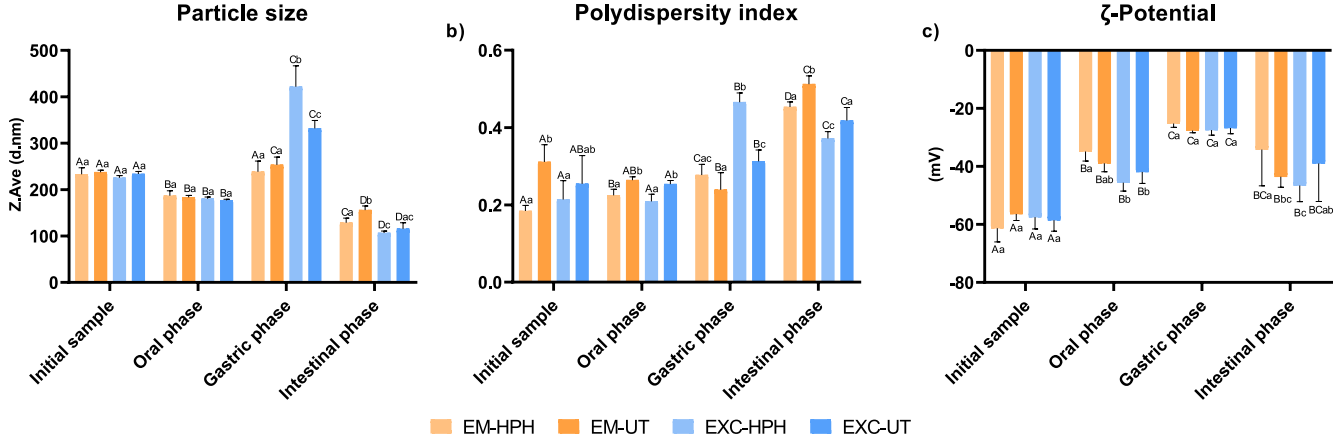

Fig. 2. Behaviour of emulsions and excipient emulsions during different stages of the simulated *in vitro* digestion, assessed by particle size (a), polydispersity index (b) and ζ-potential (c). Different capital letters represent statistically significant differences ($p < 0.05$) between the same sample in different digestion phases, whereas lowercase letters represent statistically significant differences ($p < 0.05$) between samples on the same digestion phase.

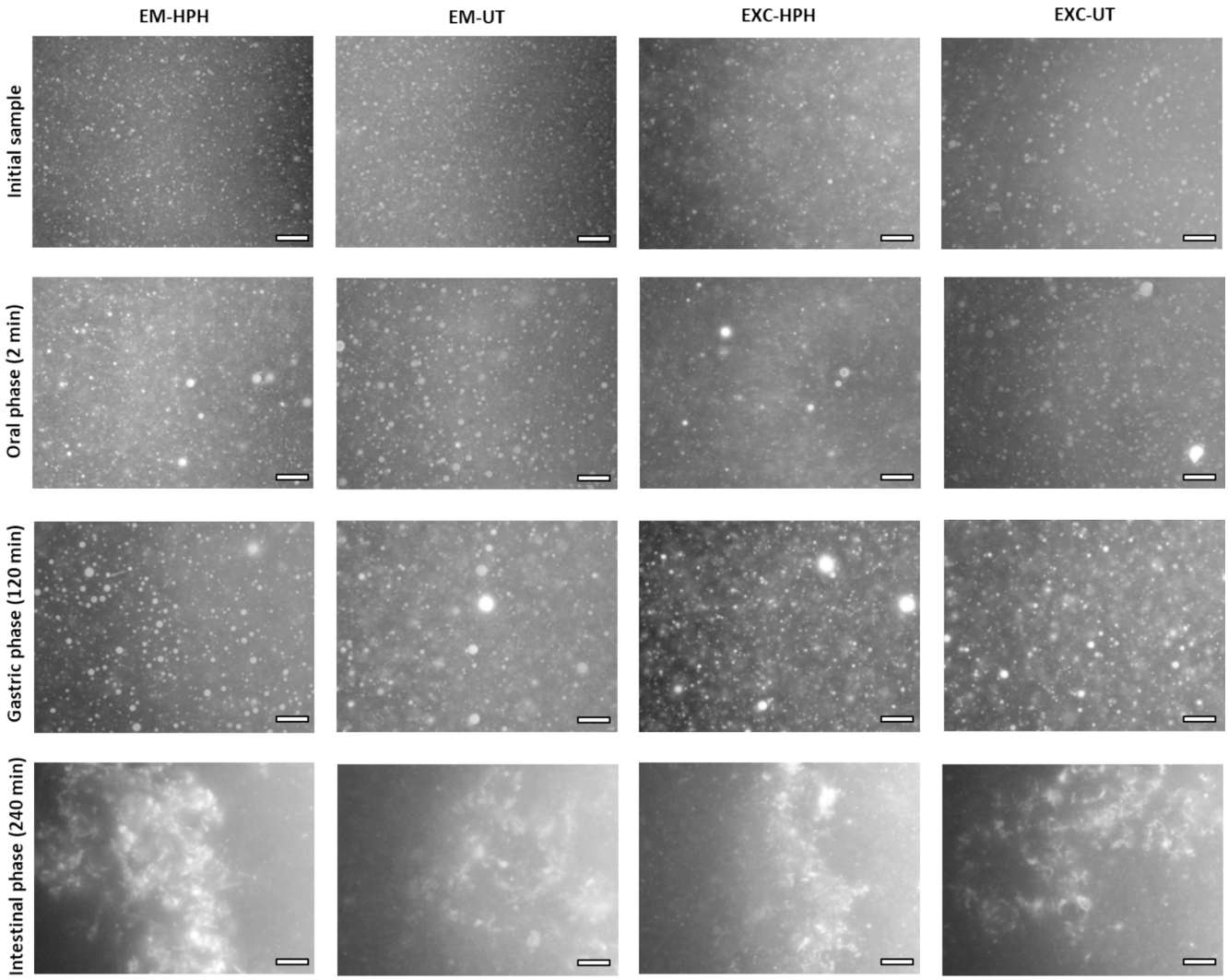

Fig. 3. Fluorescence microscopy of emulsions and excipient emulsions stained with Nile Red during different *in vitro* digestion stages. Scale corresponds to 10 μm. Images were obtained with 100x magnification and were processed using ImageJ software.

that self-assembles by means of hydrogen bonds at the oil–water interface, originating larger particles with lower ζ-potential values and generally higher stability compared to emulsions produced with other surfactants (Fuentes et al., 2021).

After the oral phase, particles from all samples presented a size decrease (Fig. 2a) and an ζ-potential increase (Fig. 2c) with statistically significant differences. Concerning PDI, only EM-HPH had statistically significant differences (Fig. 2b). Particle distribution remained unimodal (data not shown) however, larger particles were observed through fluorescence microscopy, suggesting the occurrence of coalescence to some extent (Fig. 3). Although some studies demonstrated that nanoemulsions remain stable at the oral phase (Gonçalves et al., 2021; Lv et al., 2019), the type of interactions established by lecithin (through sell-assembly) may contribute to higher probability of coalescence. The increase of ζ-potential on the oral phase has been suggested as electrostatic screening effects, caused by the interaction of lecithin with ionic molecules present in SSF, which may be adsorbed to the droplet interface (Gonçalves et al., 2021; Zhang et al., 2015).

EM and EXC were significantly destabilized after the gastric phase with increase in particles' size, PDI and ζ-potential (Fig. 2). Samples presented a bimodal distribution (data not shown), as well as coalescence and flocculation effects (Fig. 3). This outcome could be associated to the harsher enzymatic and environmental conditions specific to the gastric phase, characterized by acidic pH medium with high ionic strength. It has been described that these conditions induce aggregation on emulsion droplets by different mechanisms: i) reduced electrostatic repulsion; ii) electrostatic screening of available salts/ionic molecules to the droplets interface; iii) pepsin induced exposure of lecithin hydrophobic domain, among others (Lv et al., 2019; Yang et al., 2021). Consequently, these conditions could also be responsible for the increase in ζ-potential at this phase, triggering the adsorption of positive charged molecules (Gonçalves et al., 2021). Also, this increase in ζ-potential could be linked to the pKa of lecithin (ca. 1.5), because as the acidity of environment increases, more lecithin molecules will transition into a non-ionic state (Surh et al., 2008).

Comparing emulsion type, EXC presented statistically significant differences regarding particle size and PDI when compared to EM (Fig. 2a and 2b). This result could imply that the void core of excipient particles made them more susceptible to the gastric digestion. Moreover, it could be hinted that the incorporation of bioactive compound onto emulsions may present a symbiotic effect, contributing to the stability of the emulsion during digestion while being protected against the harsh digestive conditions.

After the intestinal phase, samples presented the smallest dimensions with similar sizes across samples (Fig. 2a). That said, these results should also report other structures formed with digesta products, since the formation of various colloidal structures is expected at this stage. At the end of digestion, a mixture with components from lipid digestion (e.g.,

free fatty acids, mono-, di- and triacylglycerides), surfactant digestion (e.g., phospholipids), biliary salts, enzymes, peptides and others, that can interact to form vesicles, emulsions, micelles, among others is obtained (Lv et al., 2019; Zhang et al., 2015). On the other hand, since there was a significant degradation of the particles, several aggregates were formed. These aggregates were observed in fluorescence microscopy (Fig. 3) and were also identified by the higher PDI values obtained (Fig. 2b). The reduction of ζ-potential could also be linked to the adsorption of anionic components present in the intestinal juices (e.g., bile salts) or the presence of free fatty acids (anionic), which are surface active products of lipolysis (Pinheiro et al., 2013).

Generally, particle destabilization throughout the digestive protocol was in agreement with reported emulsion/nanoemulsion digestion. The gastric phase had the highest impact on the degradation of particles, being more pronounced in EXC samples. The encapsulation of bioactive compounds may contribute to the preservation and resistance of EM to gastric degradation.

3.3. α-Tocopherol bioaccessibility, digestion stability and bioavailability comparison on emulsions vs excipient emulsions

To evaluate if the EM and EXC are effective for the delivery of α-tocopherol, their digestion stability, bioaccessibility and estimated bioavailability were determined after *in vitro* digestion. As described in Eqs. (1) to (3), these parameters estimate the digestive outcome of the compounds of interest, taking advantage of *in vitro* methodologies. Nonetheless, these cannot represent with certainty an outcome caused by the different physiological phenomena occurring during the digestive process. Fig. 4 presents results for the digestion stability (a), bioaccessibility (b) and estimated bioavailability (c) of α-tocopherol when digested with EM and EXC. A control digestion was performed using the same α-tocopherol concentration and oil:water ratio as EM and EXC formulations without surfactant or high energy processing.

The stability of α-tocopherol was higher in the control sample, followed by the EM and finally EXC (Fig. 4a). In control samples, α-tocopherol was dispersed in a free form without the presence of lecithin. As such, during the digestion experiment its ability to establish interactions with other components and form mixed micelles was lower compared to when it was digested in the presence of an emulsion. These phenomena may be responsible for the higher concentration found in the digesta of control samples. However, layering effects were observed on control samples at the final stages of the digestion protocol. This effect could have contributed to the greater variability between experiments, and may also have led to a separation α-tocopherol from the aqueous phase protecting it from enzymatic degradation.

Interestingly, bioaccessibility of control samples was 23.55 ± 9.83 % (Fig. 4b), whereas in all emulsion formulations the bioaccessibility was higher than 80 % (i.e., 92.05 ± 4.43, 82.99 ± 1.62, 90.43 ± 1.33 and 86.97 ± 1.31 % for EM-HPH, EM-UT, EXC-HPH and EXC-UT, respectively), representing a 3.5 to 3.9-fold increase. This significant increase in α-tocopherol bioaccessibility could be linked to several factors that impact α-tocopherol digestive fate. Firstly, the bioactive compound must be released either from the emulsion (in the case of this study) or from the food structures in which it was stored, followed by its incorporation into mixed micelles and consequent absorption via enterocyte cells (Nagao et al., 2013; Reboul, 2017). There are reports that foods with higher oil content may improve bioaccessibility of hydrophobic compounds by triggering an increase in phosphatidylcholine secretion and biliary salts, thus increasing micellization of these compounds (Yang et al., 2015; Yang & McClements, 2013). Since the oil proportion on the formulations was the same, the presence of lecithin (composed by ca. 70 % of phosphatidylcholine) should be the main responsible for the higher α-tocopherol bioaccessibility found in EM and EXC compared to the control samples. Literature suggests that the interaction of biliary salts with emulsifiers and dietary fibres have a direct effect on degradation, transport and absorption of nutrients, by contributing to colloidal structures assembly, affecting lipase adsorption, lipid solubilization and passage of lipids through the mucosal layer (Macierzanka et al., 2019). The interaction between the available lecithin and supplied biliary salts could have contributed to the rearrangement of the particles to form mixed micelles in higher proportions, granting higher solubilization to α-tocopherol thus higher estimated bioaccessibility. These findings are corroborated by other authors, that found that O/W nanoemulsions prepared with lecithin granted enhanced lipid digestion and bioaccessibility of encapsulated β-carotene compared to emulsions prepared with other emulsifiers (i.e., Tween® 20, sodium caseinate, sucrose palmitate) (Gasa-Falcon et al., 2019). Similarly, other authors found that lecithin-stabilized oil-in-water emulsions granted curcumin a 1700-fold water solubility increase and ca. 11-fold bioaccessibility increase (Yang et al., 2021).

Bioavailability was determined as the product between stable α-tocopherol in the digesta that was bioaccessible (Equation (3), and estimates how much of the initial compound of interest would be available at the site of action. It must be noted that relevant physiological phenomena, such as absorption and metabolism were not considered for this calculation (Zou et al., 2016). As observed in Fig. 4c, bioavailability was significantly higher in EM samples compared to the control samples, followed by EXC samples (i.e., 2.79 to 3.15-fold and 1.61 to 1.70-fold increase for EM and EXC, respectively). This result suggests that the encapsulation of bioactive compounds could extend their functionality, by protecting them against physicochemical alterations during gastrointestinal transit and by improving their absorption mechanisms. That said, the EXC tested also greatly extended α-tocopherol bioavailability, albeit following different action mechanisms compared to those of EM. The purpose of EM formulations was to preserve their structure until the intestinal phase, where α-tocopherol could be released and incorporated into micelles. Conversely, EXC needed to be destabilized in order to interact with free α-tocopherol molecules

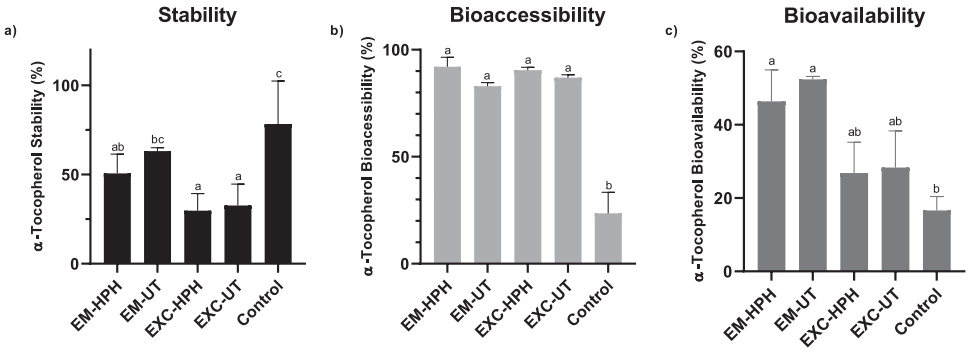

Fig. 4. α-tocopherol digestion stability (a), bioaccessibility (b) estimated bioavailability (c) when digested with different emulsions. Different letters represent statistically significant differences ($p < 0.05$) between samples.

throughout the digestion (as described in topic 3.2). As such, free α-tocopherol was exposed to digestive conditions for longer periods, which explains the lower bioavailability of EXC samples. Similar performance of excipient emulsions was reported when applying them to tomato sauce, where authors have reported that these contributed to a 2.65, 1.58 and 1.52-fold increase in the bioaccessibility of lycopene, lutein and β-carotene present in tomato sauce (Nemli et al., 2023).

Summarizing, lecithin-stabilized emulsions (both EM and EXC) increased the bioaccessibility and bioavailability of α-tocopherol. EM action mechanism may be responsible for higher protection of α-tocopherol and consequently higher bioaccessibility compared to EXC.

4. Conclusions

The optimization of emulsions' and excipient emulsions' formulations demonstrated that the composition and processing methodology applied significantly modified the developed structure and its characteristics, while incorporation of α-tocopherol did not. Also, modulating emulsion composition to reduce interfacial tension by increasing emulsifier and reducing the oil–water ratio, greatly reduced the obtained particle size and polydispersity index.

Comparing emulsions' and excipient emulsions' behaviour as α-tocopherol delivery systems using INFOGEST *in vitro* digestion protocol showed that emulsions had higher resistance to the gastric environment compared to excipient emulsions. These findings may be linked to the action mechanism of these structures: whereas emulsions with encapsulated compounds are designed to maintain their structure until the targeted release site, excipient emulsions must first be destabilized to interact with said compounds.

Even if biotransformation and degradation of α-tocopherol was relatively low during the *in vitro* digestion, all developed emulsions increased α-tocopherol bioaccessibility between 3.5 and 3.9-fold and its bioavailability between 1.6 and 3.2-fold. Despite their direct comparison in this study, application of emulsions and excipient emulsions should be case-dependent.

The results obtained indicate that emulsions greatly contribute to improve the functionality of hydrophobic bioactive compounds and that novel excipient emulsions present a viable and simpler methodology for improving the absorption of nutraceuticals available in foods.

CRediT authorship contribution statement

J.M. Fernandes: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J.F. Araújo:** Writing – review & editing, Methodology, Investigation. **R.F.S. Gonçalves:** Writing – review & editing, Methodology. **A.A. Vicente:** Writing – review & editing, Resources, Project administration, Funding acquisition. **A.C. Pinheiro:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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.foodres.2024.114743.

References

Boccardi, V., Baroni, M., Mangialasche, F., & Mecocci, P. (2016). Vitamin E family: role in the pathogenesis and treatment of Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions, 2*(3), 182–191. https://doi.org/10.1016/j.trci.2016.08.002

Bourbon, A. I., Gonçalves, R. F. S., Vicente, A. A., & Pinheiro, A. C. (2018). Characterization of particle properties in nanoemulsions. *Nanoemulsions: Formulation, Applications, and Characterization*, 519–546. https://doi.org/10.1016/B978-0-12-811838-2.00016-3

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Casas, R., Castro-Barquero, S., Estruch, R., & Sacanella, E. (2018). Nutrition and cardiovascular health. *In International Journal of Molecular Sciences., 19*(12). https://doi.org/10.3390/ijms19123988 (MDPI AG).

Chen, X., Zou, L., Liu, W., & McClements, D. J. (2016). Potential of excipient emulsions for improving quercetin bioaccessibility and antioxidant activity: an in vitro study. *Journal of Agricultural and Food Chemistry, 64*(18), 3653–3660. https://doi.org/10.1021/acs.jafc.6b01056

Csapó, J., Albert, C., & Prokisch, J. (2017). The role of vitamins in the diet of the elderly I. Fat-soluble vitamins. *Acta Universitatis Sapientiae Alimentaria, 10*(1), 127–145. https://doi.org/10.1515/ausal-2017-0009

Fernandes, J.-M., Araújo, J. F., Vieira, J. M., Pinheiro, A. C., & Vicente, A. A. (2021). Tackling older adults' malnutrition through the development of tailored food products. *Trends in Food Science & Technology., 115*(2020), 55–73. https://doi.org/10.1016/j.tifs.2021.06.028

Fuentes, K., Matamala, C., Martínez, N., Zúñiga, R. N., & Troncoso, E. (2021). Comparative study of physicochemical properties of nanoemulsions fabricated with natural and synthetic surfactants. *Processes, 9*(11), 2002. https://doi.org/10.3390/pr9112002

Gasa-Falcon, A., Odriozola-Serrano, I., Oms-Oliu, G., & Martín-Belloso, O. (2019). Impact of emulsifier nature and concentration on the stability of β-carotene enriched nanoemulsions during in vitro digestion. *Food & Function, 10*(2), 713–722. https://doi.org/10.1039/C8FO02069H

Gonçalves, R. F. S., Madalena, D. A., Fernandes, J. M., Marques, M., Vicente, A. A., & Pinheiro, A. C. (2022). Application of nanostructured delivery systems in food: from incorporation to detection and characterization. *Trends in Food Science & Technology, 129*(May), 111–125. https://doi.org/10.1016/j.tifs.2022.09.016

Gonçalves, R. F. S., Martins, J. T., Abrunhosa, L., Vicente, A. A., & Pinheiro, A. C. (2021). Nanoemulsions for enhancement of curcumin bioavailability and their safety evaluation: effect of emulsifier type. *Nanomaterials, 11*(3). https://doi.org/10.3390/nano11030815

Gonçalves, R. F. S., Martins, J. T., Duarte, C. M. M., Vicente, A. A., & Pinheiro, A. C. (2018). Advances in nutraceutical delivery systems: from formulation design for bioavailability enhancement to efficacy and safety evaluation. *Trends in Food Science and Technology, 78*(June), 270–291. https://doi.org/10.1016/j.tifs.2018.06.011

Hwang, C. J., Na, Y. G., Huh, H. W., Kim, M. K., Lee, H. K., & Cho, C. W. (2019). The effect of pharmaceutical excipients for applying to spray-dried omega-3 powder. *Applied Sciences (Switzerland), 9*(6), 1–11. https://doi.org/10.3390/app9061177

Liu, Q., Huang, H., Chen, H., Lin, J., & Wang, Q. (2019). Food-grade nanoemulsions: preparation, stability and application in encapsulation of bioactive compounds. *Molecules, 24*(23), 1–37. https://doi.org/10.3390/molecules24234242

Liu, X., Lin, Y., Yao, K., Xie, J., Xiao, J., & Cao, Y. (2023). Increasing β-carotene bioavailability and bioactivity in spinach demonstrated using excipient nanoemulsions—especially those of long-chain triglycerides. *Food Chemistry, 404*(PB), Article 134194. https://doi.org/10.1016/j.foodchem.2022.134194

Lorenzo-López, L., Maseda, A., De Labra, C., Regueiro-Folgueira, L., Rodríguez-Villamil, J. L., & Millán-Calenti, J. C. (2017). Nutritional determinants of frailty in older adults: a systematic review. *BMC Geriatrics, 17*(1), 1–13. https://doi.org/10.1186/s12877-017-0496-2

Luo, H., Li, Z., Yao, M., McClements, D. J., & Xiao, H. (2021). Impact of excipient emulsions made from different types of oils on the bioavailability and metabolism of

curcumin in gastrointestinal tract. *Food Chemistry, 370*, Article 130980. https://doi.org/10.1016/j.foodchem.2021.130980

Lv, S., Zhang, Y., Tan, H., Zhang, R., & McClements, D. J. (2019). Vitamin E encapsulation within oil-in-water emulsions: impact of emulsifier type on physicochemical stability and bioaccessibility. *Journal of Agricultural and Food Chemistry, 67*(5), 1521–1529. https://doi.org/10.1021/acs.jafc.8b06347

Macierzanka, A., Torcello-Gómez, A., Jungnickel, C., & Maldonado-Valderrama, J. (2019). Bile salts in digestion and transport of lipids. *Advances in Colloid and Interface Science, 274*, Article 102045. https://doi.org/10.1016/j.cis.2019.102045

Madalena, D., Fernandes, J.-M., Avelar, Z., Gonçalves, R. F. S. S., Ramos, Ó. L., Vicente, A. A., & Pinheiro, A. C. (2022). Emerging challenges in assessing bio-based nanosystems' behaviour under in vitro digestion focused on food applications – a critical view and future perspectives. *Food Research International, 157*(February), Article 111417. https://doi.org/10.1016/j.foodres.2022.111417

McClements, D. J. (2021). Advances in edible nanoemulsions: digestion, bioavailability, and potential toxicity. *Progress in Lipid Research, 81*, Article 101081. https://doi.org/10.1016/j.plipres.2020.101081

McClements, D. J., Saliva-Trujillo, L., Zhang, R., Zhang, Z., Zou, L., Yao, M., Xiao, H. (2016). Boosting the bioavailability of hydrophobic nutrients, vitamins, and nutraceuticals in natural products using excipient emulsions. In *Food Research International* (Vol. 88, pp. 140–152). Elsevier Ltd. doi: 10.1016/j.foodres.2015.11.017.

McClements, D. J., & Xiao, H. (2014). Excipient foods: designing food matrices that improve the oral bioavailability of pharmaceuticals and nutraceuticals. *Food and Function, 5*(7), 1320–1333. https://doi.org/10.1039/C4FO00100A

Mendes, M., Basso, J., Sousa, J., Pais, A., & Vitorino, C. (2020). Designing Ultra-Small Nanostructured Lipid Carriers: Critical Process Parameters. In *The 1st International Electronic Conference on Pharmaceutics* (p. 50). https://doi.org/10.3390/IECP2020-08691

Millen, B. E., Abrams, S., Adams-Campbell, L., Anderson, C. A. M., Brenna, J. T., Campbell, W. W., Clinton, S., Hu, F., Nelson, M., Neuhouser, M. L., Perez-Escamilla, R., Siega-Riz, A. M., Story, M., & Lichtenstein, A. H. (2016). *The 2015 dietary guidelines advisory committee scientific report: Development and major conclusions. In Advances in Nutrition* (Vol. 7(3,, 438–444.

Nagao, A., Kotake-Nara, E., & Hase, M. (2013). Effects of fats and oils on the bioaccessibility of carotenoids and vitamin E in vegetables. *Bioscience, Biotechnology, and Biochemistry, 77*(5), 1055–1060. https://doi.org/10.1271/bbb.130025

Nash, J. J., & Erk, K. A. (2017). Stability and interfacial viscoelasticity of oil-water nanoemulsions stabilized by soy lecithin and Tween 20 for the encapsulation of bioactive carvacrol. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 517*, 1–11. https://doi.org/10.1016/j.colsurfa.2016.12.056

Nemli, E., Capanoglu, E., McClements, D. J., & Tomas, M. (2023). Use of excipient emulsions for improving the bioaccessibility of antioxidants in tomato sauce. *Food Chemistry, 424*(May), Article 136395. https://doi.org/10.1016/j.foodchem.2023.136395

Pinheiro, A. C., Lad, M., Silva, H. D., Coimbra, M. A., Boland, M., & Vicente, A. A. (2013). Unravelling the behaviour of curcumin nanoemulsions during in vitro digestion: effect of the surface charge. *Soft Matter, 9*(11), 3147. https://doi.org/10.1039/c3sm27527b

Reboul, E. (2017). Vitamin E bioavailability: mechanisms of intestinal absorption in the spotlight. *Antioxidants, 6*(4), 95. https://doi.org/10.3390/antiox6040095

Rémond, D., Shahar, D. R., Gille, D., Pinto, P., Kachal, J., Peyron, M.-A.-A., Dos Santos, C. N., Walther, B., Bordoni, A., Dupont, D., Tomás-Cobos, L., & Vergères, G. (2015). Understanding the gastrointestinal tract of the elderly to develop dietary solutions that prevent malnutrition. *Oncotarget, 6*(16), 13858–13898. https://doi.org/10.18632/oncotarget.4030

Ribeiro, A., Gonçalves, R. F. S., Pinheiro, A. C., Manrique, Y. A., Barreiro, M. F., Lopes, J. C. B., & Dias, M. M. (2022). In vitro digestion and bioaccessibility studies of vitamin E-loaded nanohydroxyapatite Pickering emulsions and derived fortified foods. *LWT, 154*, Article 112706. https://doi.org/10.1016/j.lwt.2021.112706

Safaya, M., & Rotliwala, Y. C. (2020). Nanoemulsions: a review on low energy formulation methods, characterization, applications and optimization technique. *Materials Today: Proceedings, 27*, 454–459. https://doi.org/10.1016/j.matpr.2019.11.267

Salvia-Trujillo, L., & McClements, D. J. (2016). Enhancement of lycopene bioaccessibility from tomato juice using excipient emulsions: influence of lipid droplet size. *Food Chemistry, 210*, 295–304. https://doi.org/10.1016/j.foodchem.2016.04.125

Schroën, K., de Ruiter, J., & Berton-Carabin, C. (2020). The importance of interfacial tension in emulsification: connecting scaling relations used in large scale preparation with microfluidic measurement methods. *ChemEngineering, 4*(4), 63. https://doi.org/10.3390/chemengineering4040063

Shah, R., Eldridge, D., Palombo, E., & Harding, I. (2015). Lipid Nanoparticles: Production, Characterization and Stability. In *Springer Briefs in Pharmaceutical Science & Drug Development* (pp. 1–9). Springer International Publishing. https://doi.org/10.1007/978-3-319-10711-0_1.

Surh, J., Jeong, Y. G., & Vladisavljević, G. T. (2008). On the preparation of lecithin-stabilized oil-in-water emulsions by multi-stage premix membrane emulsification. *Journal of Food Engineering, 89*(2), 164–170. https://doi.org/10.1016/j.jfoodeng.2008.04.023

Tan, Z., Chen, Q., Yin, Y., Liu, Y., Lin, Y., Bai, Q., Wu, M., Yao, W., Xu, S., & Liu, J. (2021). Tracking the dissolution behavior of zinc oxide nanoparticles in skimmed milk powder solutions. *Food Chemistry, 365*. https://doi.org/10.1016/j.foodchem.2021.130520

Wang, C., Fu, Y., Cao, Y., Huang, J., Lin, H., Shen, P., Julian McClements, D., Han, L., Zhao, T., Yan, X., & Li, Q. (2023). Enhancement of lycopene bioaccessibility in tomatoes using excipient emulsions: Effect of dark tea polysaccharides. *Food Research International, 163*(November 2022), Article 112123. https://doi.org/10.1016/j.foodres.2022.112123

Yang, Q.-Q., Sui, Z., Lu, W., & Corke, H. (2021). Soybean lecithin-stabilized oil-in-water (O/W) emulsions increase the stability and in vitro bioaccessibility of bioactive nutrients. *Food Chemistry, 338*(August 2020), Article 128071. https://doi.org/10.1016/j.foodchem.2020.128071

Yang, Y., Decker, E. A., Xiao, H., & McClements, D. J. (2015). Enhancing vitamin E bioaccessibility: factors impacting solubilization and hydrolysis of α-tocopherol acetate encapsulated in emulsion-based delivery systems. *Food & Function, 6*(1), 83–96. https://doi.org/10.1039/C4FO00725E

Yang, Y., & McClements, D. J. (2013). Vitamin E bioaccessibility: Influence of carrier oil type on digestion and release of emulsified α-tocopherol acetate. *Food Chemistry, 141*(1), 473–481. https://doi.org/10.1016/j.foodchem.2013.03.033

Zhang, R., Zhang, Z., & McClements, D. J. (2020). Nanoemulsions: an emerging platform for increasing the efficacy of nutraceuticals in foods. *Colloids and Surfaces B: Biointerfaces, 194*(April), Article 111202. https://doi.org/10.1016/j.colsurfb.2020.111202

Zhang, R., Zhang, Z., Zhang, H., Decker, E. A., & McClements, D. J. (2015). Influence of emulsifier type on gastrointestinal fate of oil-in-water emulsions containing anionic dietary fiber (pectin). *Food Hydrocolloids, 45*, 175–185. https://doi.org/10.1016/j.foodhyd.2014.11.020

Zou, L., Liu, W., Liu, C., Xiao, H., & McClements, D. J. (2015). Designing excipient emulsions to increase nutraceutical bioavailability: emulsifier type influences curcumin stability and bioaccessibility by altering gastrointestinal fate. *Food & Function, 6*(8), 2475–2486. https://doi.org/10.1039/C5FO00606F

Zou, L., Zheng, B., Zhang, R., Zhang, Z., Liu, W., Liu, C., Zhang, G., Xiao, H., & McClements, D. J. (2016). Influence of lipid phase composition of excipient emulsions on curcumin solubility, stability, and bioaccessibility. *Food Biophysics, 11*(3), 213–225. https://doi.org/10.1007/s11483-016-9432-9