Research Article

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Effect of operating parameters on the physical and chemical stability of an oil gelled-in-water emulsified curcumin delivery system

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Abstract

BACKGROUND: Curcumin is a natural antioxidant with important beneficial properties for health, although its low bioavailability and sensitivity to many environmental agents limits its use in the food industry. Furthermore, some studies mention a potential synergistic effect with omega-3 polyunsaturated fatty acids, comprising other bioactive compounds extremely unstable and susceptible to oxidation. A relatively novel strategy to avoid oxidation processes is to transform liquid oils into three-dimensional structures by adding a gelling agent and forming a self-assembled network that can later be vectorized by incorporating it into other systems. The present study aimed to design and optimize an oil gelled-in-water curcumin-loaded emulsion to maximize curcumin stability and minimize lipid oxidation in terms of some critical operating parameters, such as dispersed phase, emulsifier and stabilizer concentrations, and homogenization rate.

RESULTS: The operating conditions that had a significant effect on the formulation were the dispersed phase weight fraction affecting droplet size and total lipid oxidation, homogenization conditions affecting droplet size and primary lipid oxidation, and emulsifier concentration affecting droplet size (significance level = 95%). The optimal formulation for maximizing curcumin load and minimizing lipid oxidation in the oleogelified matrix was 140.4 g kg^{-1} dispersed phase, 50.0 g kg^{-1} emulsifier, 4.9 g kg^{-1} stabilizer and homogenization speed 1016 × *g*.

CONCLUSION: The results obtained in the present study provide a valuable tool for the rational design and development of oil gelled-in-water emulsions that stabilize and transport bioactive compounds such as curcumin.

Keywords: curcumin; oleogel-based delivery systems; emulsion-based delivery systems; oil gelled-in-water emulsions; ω-3 PUFAs

INTRODUCTION

Curcumin is a natural low-molecular-weight polyphenolic compound[1] mainly present in the rhizome of *Curcuma longa* L. or turmeric. Over the past decade, curcumin has been the subject of numerous investigations aiming to verify its therapeutic potential.[2-4] Recently, numerous modern scientific studies have supported a wide range of physiological effects and therapeutic properties, such as anti-inflammatory, antimicrobial, antiproliferative, anticarcinogenic, antioxidant, hypoglycemic, hepatoprotective, thrombosuppressive, hypocholesterolemic, cardiovascular, antiarthritic factors, etc.[5-9] Therefore, curcumin is considered as a 'polypharmacological agent' as a result of a large number of *in vitro* cellular effects, with review articles listing more than 100 different targets.[10-12] After promising *in vitro* results and animal studies, as well as its safe use as a dietary agent for centuries, curcumin is currently being tested in hundreds of clinical trials.[13]

However, we must not lose sight of the fact that the curcumin molecule is predominantly lipophilic (*n*-octanol–water partition coefficient equal to 3.29[14]) and quite sensitive to heat,[15] light[16] and physiological pH conditions,[17] or the presence of metal ions.[18] Hence, as a consequence of its poor water solubility and high rate of degradation during storage, the use of curcumin in food manufacturing is very limited. In addition to the physicochemical instability, curcumin presents a very low bioavailability[19] because of its poor intestinal absorption, rapid metabolism into inactive end-products (especially by glucuronidation conjugation) within the gastrointestinal tract and swift elimination from

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the body.[20] All of these factors seriously condition the use of curcumin in nutritional or clinical applications.[21]

To overcome these important limitations, the scientific community has investigated the incorporation of curcumin on other delivery vehicles that improve its bioavailability, absorption and stability. Good examples of this are the studies of Baglole *et al*.,[22] who investigated the incorporation of curcumin in complexes composed of α-, β- and γ-cyclodextrin and their hydroxypropylated derivatives. The inclusion complexes formed showed an important enhancement with respect to the solubility of curcumin in water. Nevertheless, major research efforts have been devoted to proposing and developing distinct types of aqueous colloidal delivery vehicles and non-aqueous systems for the improvement of encapsulation, preservation, delivery and bioavailability of hydrophobic bioactive compounds, including many lipid-based systems. This will facilitate their application in the food and pharmaceutical industries, amongst others. Some of the delivery systems investigated were hydrogels,[23] oleogels,[24] nanoparticles,[25] micelles,[26] liposomes[27] and emulsions.[28] Each of them has certain advantages and disadvantages depending on their structure, composition and functional performance.

A relatively novel strategy is to transform liquid oils into three-dimensional structures by adding a gelling agent to form a self-assembled network. In recent years, interest in gelled oils has increased dramatically as a result of their great potential for improving the food fat profile and their effectiveness in modulating lipid digestion and delivering nutrients and bioactive compounds in food matrices.[29-31] For example, Li *et al*.[32] investigated β-sitosterol/lecithin-structured curcumin-loaded oleogels as potential systems to release nutrients and drugs. The oleogel exhibited a lower oxidation rate than bulk oil and a very significant improvement in the bioaccessibility of curcumin was observed, making these delivery systems interesting alternatives, including as transporters and protectors of sensitive lipophilic bioactive compounds. Indeed, we have conducted previous studies in which we developed and optimized a physicochemically stable curcumin-loaded oleogel.[24] The oil phase chosen was a fish oil-enriched in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and fully hydrogenated rapeseed oil as the gelling agent. EPA and DHA are two very interesting omega-3 polyunsaturated fatty acids (ω-3 PUFAs), for which the bioactivity has been verified in countless epidemiological studies. They are involved in visual and neurological/brain development and maternal and child health. Moreover, they can promote human health and prevent the development of certain diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease, dementia, depression and other mental illnesses.[33] Furthermore, it has been found that, when combined with curcumin, they show synergistic anti-inflammatory and antioxidative stress effects.[34] However, these highly unsaturated bioactive lipids are quite unstable and susceptible to chemical degradation, producing volatile compounds with unpleasant smells and tastes, lower nutritional value and relative toxicity.[35,36] As far as we are aware, there are no previous studies available in the scientific literature on the transport of curcumin together with ω-3 PUFAs by emulsions containing oil gelled.

By contrast, food colloid researchers have highlighted the enormous potential of emulsions to encapsulate, protect and transport interesting compounds, such as curcumin, and enhance its stability and bioavailability by manipulating the compositions and structures of these colloidal delivery systems, and, consequenty, they are already widely utilized in the food industry.[37,38] Fundamentally, two main emulsion-based approaches have been considered to deliver curcumin: excipient emulsion systems and emulsion-based delivery systems, of which we focus on the latter.[21] In emulsion-based delivery systems, the curcumin isolated from its original environment is added to the oil phase as yet another ingredient in the emulsion. In this case, curcumin dissolves in the oil phase before the formation of the emulsion. Preliminary evidence suggests that emulsion-based delivery systems can be used to improve the intestinal bioavailability, permeability and resistance to metabolic processes of curcumin and other hydrophobic bioactive compounds by altering their bioaccessibility, absorption or transformation in the gastrointestinal tract.[39,21] Furthermore, these colloidal delivery systems are usually inexpensive, robust and suitable for introduction into a food product without adversely altering their physicochemical or sensory properties.[40] In this context, emulsion-based delivery systems are postulated as promising technology to meet this challenge.

In the present study, we have combined both delivery systems previously described to encapsulate, protect and transport curcumin and ω-3 PUFAs, aiming to enhance its stability and retention capacity and reduce oxidation processes. Accordingly, we have prepared oil-in-water emulsions in which the dispersed phase is not a liquid oil but an oleogel. We term these systems 'oil gelled-in-water emulsions' or 'Og/W emulsions'. The present study aims to investigate the influence of four different variables, namely dispersed phase concentrations, emulsifier concentrations, stabilizer concentrations and homogenization rate, on the physical and chemical stabilities of curcumin-loaded oil gelled-in-water emulsions. Finally, using multi-response surface methodology (M-RSM), the emulsion formulation was optimized to maximize the supplied curcumin load and minimize lipid oxidation of the oleogelified matrix.

MATERIALS AND METHODS

Materials

The dispersed phases were composed of (i) fish oil-enriched in ω-3 PUFAs (PronovaPure oil containing 36.0% EPA and 24.0% DHA TG Deodorized; BASF, Ludwigshafen am Rhein, Germany) as oil phase for the preparation of all oleogels and Og/W emulsions in this study; (ii) fully hydrogenated rapeseed oil (Palsgaard® 6111 powder; Palsgaard, Juelsminde, Denmark) as gelling agent; and (iii) curcumin powder (85% purity) supplied by Solutex (Zaragoza, Spain).

The continuous phases were composed of (i) deionized water; (ii) potassium sorbate and sodium benzoate as preservative agents to prevent the microbiological growth in the Og/W emulsions; (iii) Tween 20 as a hydrophilic emulsifier to stabilize Og/W emulsions (Merck, Kenilworth, NJ, USA); and (iv) xanthan gum (XG) as a thickening agent (HELM Iberica, SA, Madrid, Spain).

Other reagents used in the present study for lipid oxidation and curcumin concentration measurements were: ferrous sulphate heptahydrate, barium chloride dihydrate, hydrochloric acid and ammonium thiocyanate, and these were purchased from PanReac AppliChem (Chicago, IL, USA); cumene hydroperoxide was from Alfa Aesar (Haverhill, MA, USA); *p*-anisidine was from Acros Organics (Fair Lawn, NJ, USA); and *trans,trans*-2,4-decadienal was acquired from Sigma-Aldrich (St Louis, MO, USA). All of these were used as reagents for measuring lipid oxidation rates. Isooctane, 2-propanol, 1-butanol and methanol were all bought from PanReac AppliChem and acetic acid glacial was from J.T. Baker (Phillipsburg, NJ, USA). All of these were used as solvents during

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the measurements of the lipid oxidation rate. Ethanol was purchased from Guinama (Valenica, Spain) and was used as the solvent during the measurements of the curcumin content. All the materials used were of analytical grade without any purification or modification of their properties.

Methods

Preparation of oleogel

The procedures for oleogel preparation and optimization have been previously described by Vellido-Pérez *et al.*[24] In brief, previously weighed gelling agent and curcumin were premixed for 2 min using a magnetic stirrer at 168 rad s^{-1}. Later, the mixture was heated to 66.5 °C as quickly as possible to minimize curcumin and fish oil exposure to heat. Heating was performed in a bain-marie and under mild agitation (42 rad s^{-1}). Dispersion of curcumin was then immediately cooled to 23 °C when being stirred using the magnetic stirrer at 168 rad s^{-1} for 3 min, resulting in the formation of oleogel. The oleogel composition used for the preparation of all Og/W emulsions was optimal to minimize oxidation: gelling agent concentration equal to 46.483 g kg^{-1}, curcumin concentration equal to 1.500 g kg^{-1} and manufacturing temperature equal to 66.5 °C.

Preparation of oil gelled-in-water emulsions

All the Og/W emulsions in the study were prepared at room temperature (25 °C) and were formulated with different oleogel (dispersed phase) concentrations (50 g kg^{-1}, 100 g kg^{-1} and 150 g kg^{-1}), emulsifier concentrations (10 g kg^{-1}, 30 g kg^{-1} and 50 g kg^{-1}), stabilizer concentrations (2.0 g kg^{-1}, 3.5 g kg^{-1} and 5.0 g kg^{-1}) and homogenization speed (254, 572 and 1016 × *g*) according to the experimental design shown in Table 1. Accurately weighed emulsifier was added to deionized water, containing 0.6 g kg^{-1} sodium benzoate and 0.6 g kg^{-1} potassium sorbate to prevent the microbiological contamination of the Og/W emulsion, and the aqueous solution was blended using a vortex for 30 s until completely dissolved. The oleogel described above was then added to the previous aqueous solution and the Og/W emulsion was formed using a rotor-stator homogenizer (Ultra-Turrax® T 25; IKA, Staufen, Germany) for 5 min at the corresponding speed. Finally, the thickening agent was added to the previous Og/W emulsion, being dispersed by soft homogenization for 3 min at 64 × *g* to form the final Og/W emulsion. Lastly, Og/W emulsions (50 g) were placed immediately after manufacture in sealed screw cap plastic tubes and stored in an oven at 25 °C for 30 days to monitor their stabilities.

Characterization of the different delivery systems

Droplet size measurements. 4

$$d_{[4,3]} = \sum_i \frac{n_i d_i^4}{n_i d_i^3} \quad (1)$$

where n_i is the frequency of occurrence of particles in size class *i*, having a mean d_i diameter.

Lipid oxidation measurements. Oleogel and emulsion chemical stabilities were determined by measuring lipid oxidation after its preparation and at day 30 after its manufacture. Accordingly, both primary and secondary lipid oxidation products were measured.

The first ones were quantified by the peroxide value method (PV) using the colorimetric ferric-thiocyanate method, adapted from Shantha and Decker.[41] First, 0.1 mL of the emulsion (or 10 μL in the case of the oleogel) was taken and added to 1 mL of isooctane/2-propanol (3:1, v/v) solution, vortexed (three times for 10 s) and the resulting mixture was centrifuged at 12521 × *g* for 10 min. Afterwards, 0.2 mL of the clear upper solvent layer were taken and mixed with a 2.8 mL of methanol/1-butanol (2:1, v/v) solution and 30 μL of ferrous thiocyanate solution and vortexed for a few seconds. The ferrous thiocyanate solution was obtained by mixing 15 μL of 0.072 mol L^{-1} ferrous chloride solution with 15 μL of 3.94 mol L^{-1} ammonium thiocyanate solution. The ferrous chloride solution was prepared from the supernatant of a mixture of 25 mL of 0.144 mol L^{-1} ferrous sulphate solution and 25 mL of barium chloride solution (0.132 mol L^{-1} $BaCl_2$ in 0.4 mol L^{-1} HCl). The reaction tubes were kept in a dark room for 20 min at 25 °C. During this time, the lipid hydroperoxides in the sample would react with the ferrous ion, oxidizing it to ferric ion. Ferric thiocyanate is a red–violet complex that absorbs at a wavelength of 500–510 nm. Therefore, after 20 min, the absorbance values of samples were measured at a wavelength of 510 nm using a spectrophotometer (Spectronic Helios Gamma UV-Visible Spectrophotometer; Thermo Fisher Scientific, Waltham, MA, USA). To quantify the lipid hydroperoxides, a calibration curve was made with cumene hydroperoxide. The slope of the calibration curve was used for calculating hydroperoxide concentration in each sample. The final result was expressed as the molar ratio between the increase in lipid hydroperoxides 30 days after their manufacture and the quantity initially present, according to Eqn (2):

$$\%PV = \frac{[Lipid\ Hydroperoxides]_{Day\ 30} - [Lipid\ Hydroperoxides]_{Day\ 0}}{[Lipid\ Hydroperoxides]_{Day\ 0}} \times 100 \quad (2)$$

The secondary lipid oxidation products were quantified by using the *p*-anisidine value method (*p*-AnV), described in the American Oil Chemical Society (AOCS) CD 18-90 (1998).[42] This technique is based on the reaction of the unsaturated aldehydes with the *p*-anisidine, resulting in the formation of a yellowish product that absorbs at 350 nm. For this, 1 mL of the emulsion (or 100 μL in the case of the oleogel) was taken and mixed with 10 mL isooctane as the solvent and vortexed three times for 10 s. This mixture was centrifuged at 6662 × *g* for 20 min. Afterwards, 5 mL of the clear upper solvent layer were taken and added to 1 mL of 0.020 M *p*-anisidine solution (made by mixing *p*-anisidine in acetic acid). The reaction tubes were kept in a dark room for 10 min at 25 °C. After 10 min, the absorbance of samples was measured at a wavelength of 350 nm. To quantify the secondary lipid oxidation products, a calibration curve was made with *trans,trans*-2,4-decadienal. This pattern was used because it is a major decomposition product from sunflower oil oxidation. The slope of the calibration curve was used for calculating secondary lipid oxidation product concentration in each sample. The final result was expressed as the molar ratio between the increase in secondary lipid oxidation products 30 days after their manufacture and the quantity initially present, according to Eqn (3):

$$\%p\text{–}AnV = \frac{[Unsaturated\ Aldehydes]_{Day\ 30} - [Unsaturated\ Aldehydes]_{Day\ 0}}{[Unsaturated\ Aldehydes]_{Day\ 0}} \times 100 \quad (3)$$

Curcumin concentration measurements. First, an amount of 2 μL of the curcumin-loaded Og/W emulsion was added to 1 mL of

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Table 1. Box–Behnken design matrix for the optimization of Og/W emulsion formulation and measured values of the response variables

Experiment	Dispersed phase concentration (g kg^{-1})	Emulsifier concentration (g kg^{-1})	Stabilizer concentration (g kg^{-1})	Homogenization speed (× *g*)	$d_{[4,3]}$ (μm)	% Increase in lipid hydroperoxydes	% Increase in secondary products	% Curcumin transported
1	150.0	30.0	5.0	572	51.01 ± 9.42	217.701 ± 6.800	285.246 ± 10.172	100.000 ± 19.963
2	100.0	30.0	3.5	572	24.64 ± 3.10	92.228 ± 3.111	287.778 ± 3.880	79.235 ± 9.584
3	100.0	30.0	3.5	572	24.75 ± 4.75	87.982 ± 5.711	315.686 ± 3.806	86.500 ± 9.011
4	100.0	30.0	2.0	1016	66.25 ± 2.69	51.566 ± 2.290	431.818 ± 7.363	92.531 ± 7.961
5	100.0	30.0	5.0	254	67.24 ± 5.71	181.638 ± 1.228	393.388 ± 9.396	61.603 ± 8.047
6	100.0	30.0	3.5	572	23.93 ± 1.08	92.299 ± 2.139	311.494 ± 3.200	58.929 ± 7.247
7	150.0	50.0	3.5	572	37.42 ± 2.60	143.665 ± 2.915	80.233 ± 2.813	86.464 ± 5.549
8	100.0	10.0	2.0	572	22.41 ± 5.08	129.598 ± 5.056	526.984 ± 5.511	43.027 ± 6.187
9	100.0	30.0	2.0	254	62.57 ± 6.95	158.938 ± 3.338	413.265 ± 6.290	53.261 ± 7.311
10	50.0	10.0	3.5	572	11.01 ± 1.55	8.388 ± 5.072	458.621 ± 6.109	83.108 ± 13.790
11	50.0	30.0	2.0	572	7.01 ± 0.28	50.064 ± 7.972	493.548 ± 5.650	66.332 ± 9.042
12	50.0	30.0	5.0	572	29.12 ± 1.67	28.077 ± 6.150	451.515 ± 8.633	62.857 ± 8.717
13	100.0	50.0	3.5	1016	7.68 ± 3.55	60.597 ± 8.726	346.667 ± 7.412	99.588 ± 7.919
14	100.0	10.0	3.5	1016	23.04 ± 6.03	54.532 ± 6.423	381.034 ± 7.353	76.301 ± 5.947
15	50.0	30.0	3.5	1016	39.19 ± 16.96	70.207 ± 7.224	690.476 ± 10.760	81.287 ± 10.058
16	100.0	10.0	5.0	572	49.49 ± 3.62	73.213 ± 6.507	258.929 ± 4.914	77.170 ± 6.808
17	150.0	10.0	3.5	572	55.26 ± 8.72	277.352 ± 8.901	275.287 ± 5.354	54.978 ± 1.441
18	100.0	50.0	5.0	572	24.14 ± 4.17	76.605 ± 6.091	220.238 ± 6.522	85.348 ± 7.361
19	100.0	50.0	2.0	572	17.66 ± 0.96	133.440 ± 5.622	344.737 ± 6.078	60.081 ± 3.226
20	150.0	30.0	3.5	1016	3.31 ± 3.70	75.369 ± 4.519	206.186 ± 4.888	94.218 ± 3.615
21	100.0	30.0	5.0	1016	5.71 ± 3.10	67.276 ± 2.163	347.541 ± 9.099	85.862 ± 7.095
22	150.0	30.0	2.0	572	39.97 ± 3.41	288.126 ± 2.054	482.022 ± 11.879	73.462 ± 2.936
23	100.0	10.0	3.5	254	97.61 ± 5.96	243.436 ± 1.549	281.529 ± 6.446	70.288 ± 6.783
24	50.0	50.0	3.5	572	19.16 ± 8.94	60.778 ± 2.107	653.333 ± 11.881	72.892 ± 10.275
25	100.0	50.0	3.5	254	47.74 ± 3.16	254.708 ± 1.354	141.549 ± 5.362	77.544 ± 7.170
26	50.0	30.0	3.5	254	38.53 ± 2.88	121.938 ± 2.158	640.385 ± 16.376	53.927 ± 9.302
27	150.0	30.0	3.5	254	82.81 ± 3.71	328.268 ± 2.282	125.000 ± 3.247	89.474 ± 3.251
28	100.0	30.0	3.5	572	24.79 ± 4.51	87.083 ± 2.371	371.429 ± 6.640	60.880 ± 4.561
29	100.0	30.0	3.5	572	29.60 ± 4.25	63.143 ± 6.170	171.605 ± 39.109	20.216 ± 5.340

ethanol and vortexed for 30 s. The incorporated curcumin concentration was determined by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) technique. The UPLC-MS/MS analysis was carried out in positive ion ESI mode on a Xevo TQ-S Triple Quadrupole Mass Spectrometer connected to an Acquity UPLC H-Class System (Waters Corporation, Milford, MA, USA). The analysis was conducted on an Acquity UPLC BEH C18 130 Å column (50 mm × 2.1 mm, 1.7 µm) with a target column temperature of 40.0 °C. The mobile phase consisted of 13 mM ammonium fluoride solution and 1.0 g kg^{-1} ammonia (A) and acetonitrile (B) pumped at a constant flow rate of 0.300 mL min^{-1}. The gradient elution program was: 0–0.50 min, 95.0% A; 0.50–1.50 min, 95.0–5.0% A; 1.50–5.00 min, 5.0% A; 5.00–5.10 min, 5.0–95.0% A; and 5.10–7.00 min, 95.0% A. Other MS conditions were also optimized and set as: nebulizer gas flow, 7.00 bar; desolvation gas flow, 800 L h^{-1} (nitrogen was used as the drying gas); capillary voltage, 2.50 kV; source temperature, 150 °C; and desolvation temperature, 500 °C. The analyte was quantified using selected ion reaction monitoring. The ion transitions *m/z* 369.29 → 116.95, *m/z* 369.29 → 144.98, *m/z* 369.29 → 176.99 and *m/z* 369.29 → 285.22 were used for the determination of curcumin. The injection volume set on an Acquity FTN AutoSampler (Waters Corporation) was 5.00 µL.

The maintenance of drug content in the curcumin-loaded Og/W emulsion was determined as the molar ratio between the amount of curcumin present in the emulsion 30 days after their manufacture and the quantity initially incorporated into the emulsion, according to Eqn (4):

$$\%DC = \frac{[Curcumin]_{Day\ 30}}{[Curcumin]_{Day\ 0}} \times 100 \quad (4)$$

Statistical analysis, experimental design and optimization

Both the Og/W emulsion formulation and the manufacturing conditions were optimized by a statistical experimental design in combination with an analysis of the multi-response surface. In the present study, the dispersed phase percentage, the emulsifier and stabilizer concentrations, and the homogenization speed were chosen as independent variables. On the other hand, the droplet size, the oxidation degree of the Og/W emulsion and the maintenance of curcumin content were selected as response variables. The ranges tested for the selected variables were as indicated earlier above. Analysis of variance (ANOVA) was carried out to determine any significant differences among the operating parameters studied. $P < 0.05$ was considered statistically significant.

A Box–Behnken design was applied to simultaneously calculate the effect of the change in each of these variables and also their possible interactions. Five repetitions of the central point were included to verify the reproducibility of the model, resulting in a total of 29 experiments (which can be seen in Table 1, together with the obtained values of the response variables). Finally, the optimization was carried out using Statgraphics Centurion XV (https://www.statgraphics.com) and the results were interpreted using M-RSM.

RESULTS AND DISCUSSION

Physical stability of Og/W emulsions

Figure 1 represents the average droplet size of Og/W emulsions 30 days after their manufacture as a function of the different investigated variables. Each of the bars represents a certain value of two of these variables as indicated in the graph. The other two variables, for which the values are not indicated, take the tested medium values: 100 g kg^{-1} dispersed phase, 30 g kg^{-1} emulsifier, 3.5 g kg^{-1} stabilizer and 572 × *g* homogenization speed. According to ANOVA testing, the amount of dispersed phase (*P*-value = 0.0208), emulsifier concentration (*P*-value = 0.0473) and homogenization rate (*P*-value = 0.0001) were the variables that significantly influenced droplet size. Indeed, regardless of the concentration of emulsifier or stabilizer used (Fig. 1a), the average droplet sizes when 50 g kg^{-1} dispersed phase was used were between 7.0 ± 0.3 and 29.1 ± 11.1 µm in the worst case; however, when the dispersed phase was increased to 150 g kg^{-1}, the sizes increased to values of 37.4 ± 2.9 µm or greater. Therefore, these data suggest that the smaller the amount of dispersed phase, the smaller the final droplet size of the emulsion, which has influence on the surface exposed to oxidation and thus on chemical stability (investigated in more detail in the Results).

Regarding the emulsifier concentration, Fig. 1(b) shows how higher concentrations of emulsifier (in our case, 50 g kg^{-1}) lead to smaller average droplet sizes, which in some cases represent 78.8% but can reach 33.3% of the droplet size of the equivalent emulsion when smaller emulsifier concentrations (10 g kg^{-1}) were used. This translates into a greater physical stability of the Og/W emulsions over time at higher emulsifier concentrations. This result agrees with previous studies in which the physical stability of emulsions depends largely on the thickness of the interfacial area and electrostatic charge, which in turn is determined by the type and concentration of the emulsifier used: the emulsifier must be present in sufficient quantities to completely cover the droplet surfaces.[43,44] In our case, the emulsifier used is a non-ionic emulsifier that stabilizes oleogel droplets by exerting a steric hindrance as a result of the polyoxyethylene chains. Therefore, a higher emulsifier concentration would be expected to produce a greater coating of the oleogel droplets and a greater interfacial thickness, preventing the droplets from approaching each other and, consequently, reducing physical destabilization mechanisms such as flocculation, coalescence or Ostwald ripening.[43,44] These results are in line with the study developed by Kowalska *et al.*[45] in which the long-term stability of six rose oil emulsions with different amounts of emulsifier was studied. They concluded that higher emulsifier amounts lead to stable particle size distributions. However, this was not verified at the 50 g kg^{-1} dispersed phase where droplet size increased from 11.0 ± 1.7 to 19.2 ± 5.8 µm when we increased the emulsifier concentration from 10 g kg^{-1} to 50 g kg^{-1} because the droplets could be covered with smaller emulsifier concentrations. In this case, adding a higher amount of emulsifier than necessary, will produce an excess that will dissolve in the aqueous phase, providing slightly bigger sizes. Ma *et al.*[46] investigated the impact of the emulsifier type and surfactant-to-oil ratios on curcumin nanoemulsions and they concluded that reduced surfactant-to-oil ratio values were found to appropriately improve the physical stability of nanoemulsions.

On the other hand, the addition of XG as a stabilizer is essential for obtaining physically stable emulsions over time. The purpose of these types of polysaccharides is to increase the viscosity of the continuous phase, reduce the Brownian motion between the dispersed droplets and thus maintain stable emulsified components for longer periods.[47,48] However, despite being a key ingredient in the emulsion's physical stability, the concentration at which it is found may or may not favor its stability. Many studies

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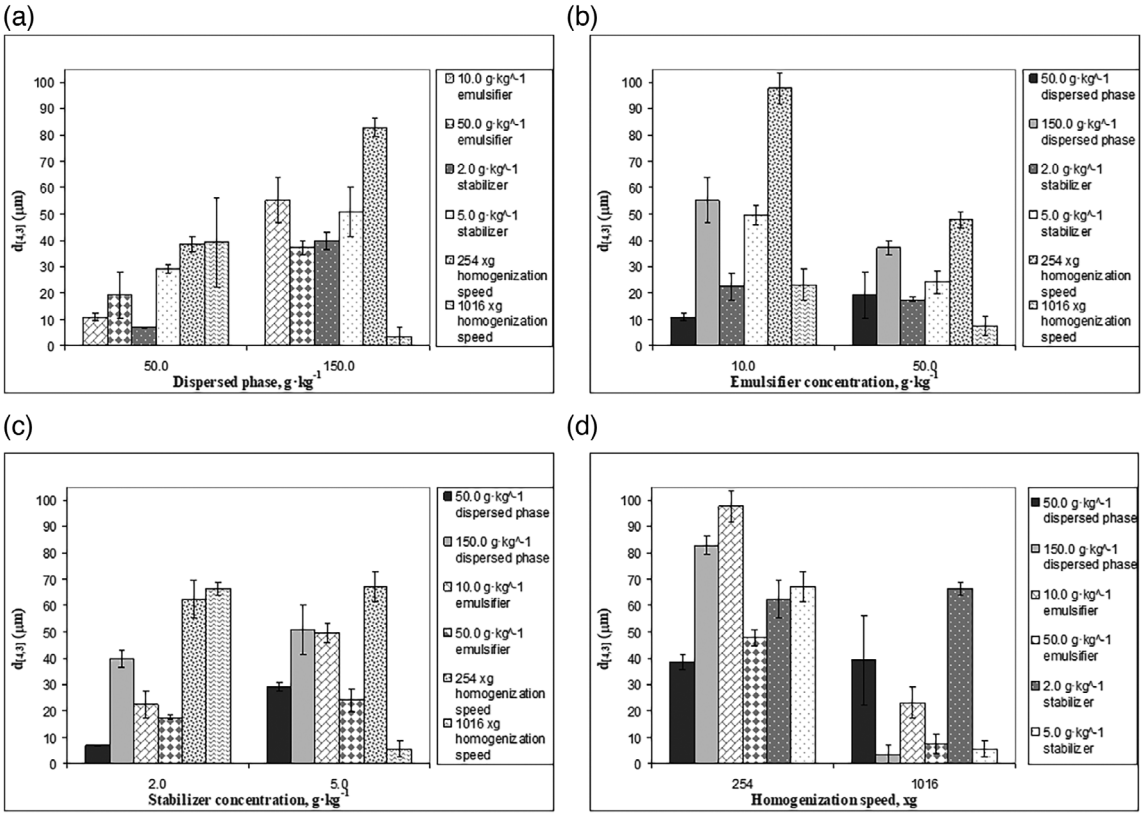

Figure 1. Average droplet size of Og/W emulsions 30 days after their manufacture as a function of: (a) dispersed phase percentage, (b) emulsifier concentration, (c) stabilizer concentration and (d) homogenization speed.

have already reported that, above a certain XG concentration, the creaming of O/W emulsions as a result of flocculation depletion has shown an increase.[47,48] This is what we can see in Fig. 1(a,b) where, by increasing the concentration of the thickener from 2.0 g kg^{-1} to 5.0 g kg^{-1}, we obtained Og/W emulsions with droplet sizes up to 3.15 times larger. The explanation for this is that, at low XG concentrations, and as a result of the stabilizing, thickening and gelling properties, they physically stabilize emulsions by increasing viscosity and yielding stress. However, at sufficiently high concentrations, aggregates can be formed. This is agreement with that observed by Viana *et al.*,[49] who evaluated the stability of vitamin D-enriched pecan oil emulsions. They found that smaller XG concentrations (0.3–3.0 g kg^{-1}) significantly decreased the droplet size of omega-3 vegetable oil emulsions, using this polysaccharide as the sole emulsifier. However, cream formation was observed probably because of the very low concentrations.

Figure 1(d) also shows the huge impact that homogenization speed has on droplet size: it shows that an increase in the homogenization rate from 254 to 1016 × *g* led to a drastic reduction in droplet size from 97.6 ± 6.6 to 23.0 ± 6.5 μm at 10 g kg^{-1} emulsifier or from 47.7 ± 3.5 to 7.7 ± 3.9 μm at 50 g kg^{-1}. The reason for this is that the oleogel droplets decrease in size as a consequence of the hydrodynamic shear stress generated by turbulent flow, which increases with the cube of the homogenization speed.[50] Furthermore, any contribution of energy made to a system leads to dissipation, in this case through heat. As a result of the increased temperature, the oleogel viscosity decreases significantly and this greatly facilitates their size reduction. These results are in line with those obtained by Scholz & Keck,[50] who investigated the droplet size and size distribution of nanoemulsions obtained by a high-speed stirrer as a function of stirring speed and production time.

Oxidative stability of Og/W emulsions

Lipid oxidation in Og/W emulsions is a complex process, depending on many factors including the type and concentration of the dispersed phase, as well as the emulsifier and stabilizer, the presence of compounds with antioxidant or pro-oxidant properties together with non-adsorbed ingredients in either of the phases, and the homogenization method, surface area, charge of the droplets and pH.[51] To better understand the role of some of these factors, different values for the dispersed phase percentage, emulsifier and stabilizer concentrations, and homogenization speeds were tested. Of them, the dispersed phase percentage was the only parameter that turned out to be statistically significant in both primary and secondary lipid oxidation ($P < 0.05$); the homogenization rate was only statistically significant in primary lipid oxidation ($P < 0.05$) and the rest of the factors were not statistically significant with a significance level of 95%. The results can be seen in Figs 2 and 3. Each of the bars represents the percentage of PV or p-AnV, respectively, at a certain value of two of these variables indicated in the graph. The other two variables (with no stated values) take the previously indicated tested medium values.

According to the scientific literature, one of the most important factors to affect oxidative stability of emulsions is oil concentration.[52] Indeed, Fig. 2(a) demonstrates that, by reducing dispersed phase quantities from 150 g kg^{-1} to 50 g kg^{-1}, primary lipid

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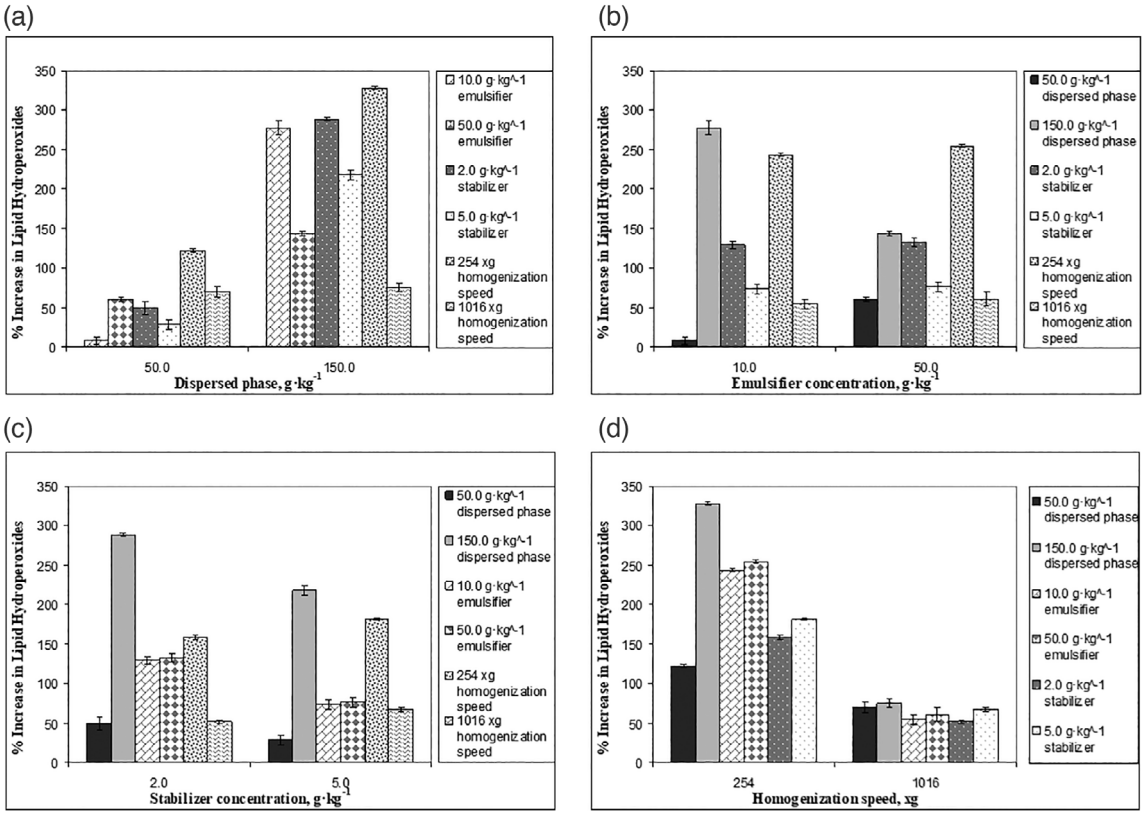

Figure 2. Primary lipid oxidation of Og/W emulsions 30 days after their manufacture as a function of: (a) dispersed phase percentage, (b) emulsifier concentration, (c) stabilizer concentration and (d) homogenization speed.

oxidation can be reduced from 75.4 ± 4.5 to 70.2 ± 7.2% of increase in lipid hydroperoxides (at 1016 × *g*, where the reduction of primary lipid oxidation products was lower) to reductions from 277.4 ± 8.9 to 8.4 ± 5.1% in the case of the largest reduction (at 10 g kg^{-1} emulsifier). This shows that, among the different ingredients making up the emulsion, the dispersed phase is the only one susceptible to oxidative processes leading to product degradation. Therefore, we must especially focus on this dispersed phase to achieve a chemically stable emulsion. It should be noted that the dispersed phase used was an oleogel composed of fish oil enriched in ω-3 PUFAs. This corroborates the tendency of these types of polyunsaturated compounds to undergo oxidative processes as a consequence of the molecule unsaturations, thus making them extremely reactive compounds. However, observing the data corresponding to secondary oxidation collected in Fig. 3, we found that the emulsions that showed less primary oxidation (those corresponding to a lesser dispersed phase amount) are those now exhibiting a greater secondary oxidation percentage. The one that showed an increase 2.77 times greater than its initial quantity in primary products now shows 2.75 times more secondary products than the initial one, whereas the one that showed 8.4 ± 5.1%, now produces 4.59 times more in secondary products. According to scientific literature, it is lipid hydroperoxides that give rise to secondary oxidation, and so this transformation could also explain the lower percentage of one *versus* the greater percentage of the other.[53] From the previous explanation and bearing in mind that oxidative processes are chain reactions in which a series of products are formed from others that previously occurred, it is evidently impossible to analyze one or the other separately. They should be analyzed together.

Another important variable to consider in the investigation of the oxidative stability of emulsions is the thickener concentration. Figs 2 and 3 show a distinct decrease of peroxide and *p*-anisidine values for emulsions with 5.0 g kg^{-1} XG, in line with those observed previously.[54] The ability of XG to delay lipid oxidation could be mainly attributed to the fact that XG increases the viscosity of the continuous phase, which could result in hindering oxygen diffusion and oxidizing species to the oil droplet surface area, such that the lipid oxidation rate could be reduced. Also, these results agree with those of Chen *et al.*[55] who reported that, although XG are highly hydrophilic molecules with little inherent surface activity, they can be partially adsorbed to the surfaces of the oil droplets through interactions with the emulsifier. As a result, the packing of the emulsifier molecules at the emulsion interface could be tighter, so that the membrane could serve as a valid barrier to restrict the lipid oxidation initiators into oil droplets to some extent. Finally, Shimada *et al.*[56] reported that XG can be used as an emulsion stabilizer to strongly inhibit the peroxidation of soybean oil in the emulsion system.

Lipid oxidation has been carefully investigated over the last decades.[35,57-60] Most published studies agree that oxidative processes take place at the interface of the system; namely, on the contact surface between the oil and aqueous phases.[61,62] This appears to contradict the results obtained in Fig. 2, where the higher the primary oxidation observed, the lower the homogenization rate. Lower homogenization speeds imply larger droplet sizes and consequently less average surface area; for example, in our case, we attained 1839 μm^2/droplet at 1016 × *g* to 13 718 μm^2/droplet at 254 × *g*. Morover, during the homogenization process, as a result of friction and shear, there is momentary

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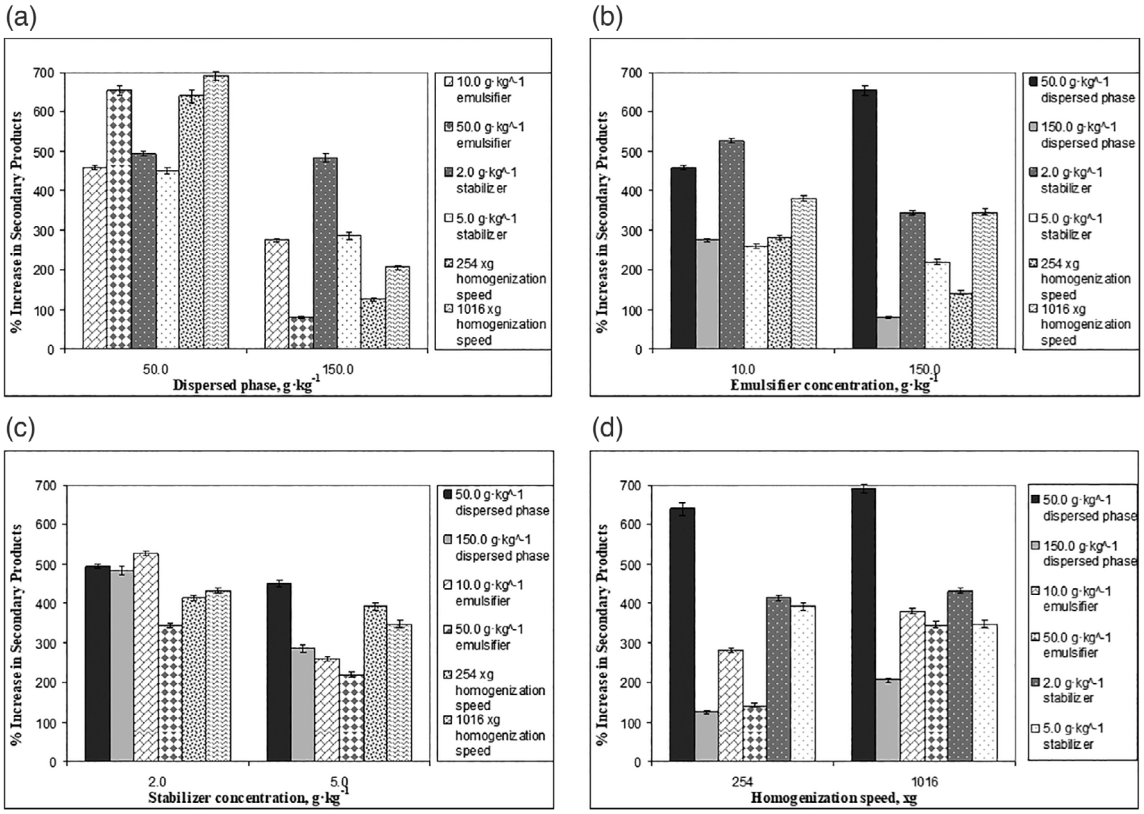

Figure 3. Secondary lipid oxidation of Og/W emulsions 30 days after their manufacture as a function of: (a) dispersed phase percentage, (b) emulsifier concentration, (c) stabilizer concentration and (d) homogenization speed.

heating of the product being emulsified, and this temperature increase should accelerate oxidation reactions. Such heat generation will be greater the higher the homogenization speed. However, if we look at the corresponding bars in Fig. 3, we observe that secondary oxidation was generally greater at higher oxidation rates.

Finally, the type and concentration of the emulsifier used have been shown to influence both the physical and chemical stability of Og/W emulsions.[44] Although the emulsifier concentration does not appear to influence the primary oxidation rate too much (there is a slight difference of 11% when different emulsifier concentrations were used, as indicated in Fig. 2b), it did show a much more significant effect on the secondary oxidation rate. Figure 3 (b,c) shows how the lower emulsifier and thickener concentrations produced an increase more than five times greater in secondary products compared to a less than a 3.5-fold increase observed when incrementing emulsifier concentration to 50 g kg^{-1}. We have already noted that the emulsifier concentration had quite an important impact on droplet size. A decrease in droplet size leads to a significant increase in droplet surface area, which could be the main reason for the decreased oxidative stability of emulsions. There are, however, conflicting studies supporting and disagreeing this theory, as extensively reviewed by Berton-Carabin *et al.*[51] On the other hand, it is important to note that the amount of emulsifier is related to steric hindrance and the thickness of the interfacial layer, and so it appears logical to deduce that the greater the amount of emulsifier, the greater the interface that oxygen will have to pass through to produce oxidation.

Maintenance of curcumin content in Og/W emulsions

In curcumin-loaded emulsions, the total curcumin content that can be incorporated into the delivery system is determined by its oil solubility and the concentration of oil droplets.[63] The dispersion of curcumin into the oil phase has been extensively investigated in many studies. It basically depends on the type of carrier oil and dissolution conditions (temperature, time, etc.). In our case, the type of carrier phase and dissolution conditions used were always the same: increasing the curcumin loading capacity by replacing the liquid oil with an oleogel, which has a greater ability to trap bioactive compounds. Therefore, oil droplet concentration directly related to the amount of dispersed phase is another of the fundamental factors in determining the curcumin incorporation efficiency. As shown in Fig. 4(a), generally, a higher percentage of dispersed phase implies a higher curcumin loading percentage 1 month after its manufacture. However, it should be noted that this is not always true, the opposite being the case at low emulsifier concentrations (Fig. 4b), where the curcumin loading percentage went from 83.1% with 50 g kg^{-1} of the dispersed phase to 55.0% when increasing the percentage of dispersed phase to 150 g kg^{-1}. As reported in the scientific literature[21], curcumin is usually located within the hydrophobic core of the oleogel droplets. Within the core, curcumin is safe from possible hydrophilic species (such as hydroxyl ions) present in the aqueous phase that could cause its chemical degradation). However, as a consequence of the polar groups and homogenization process itself on the curcumin, some of them may also be found close to the oleogel-water interface where the emulsifier is present.[63] Artiga-Artigas *et al.*[64] assessed the impact of emulsifier

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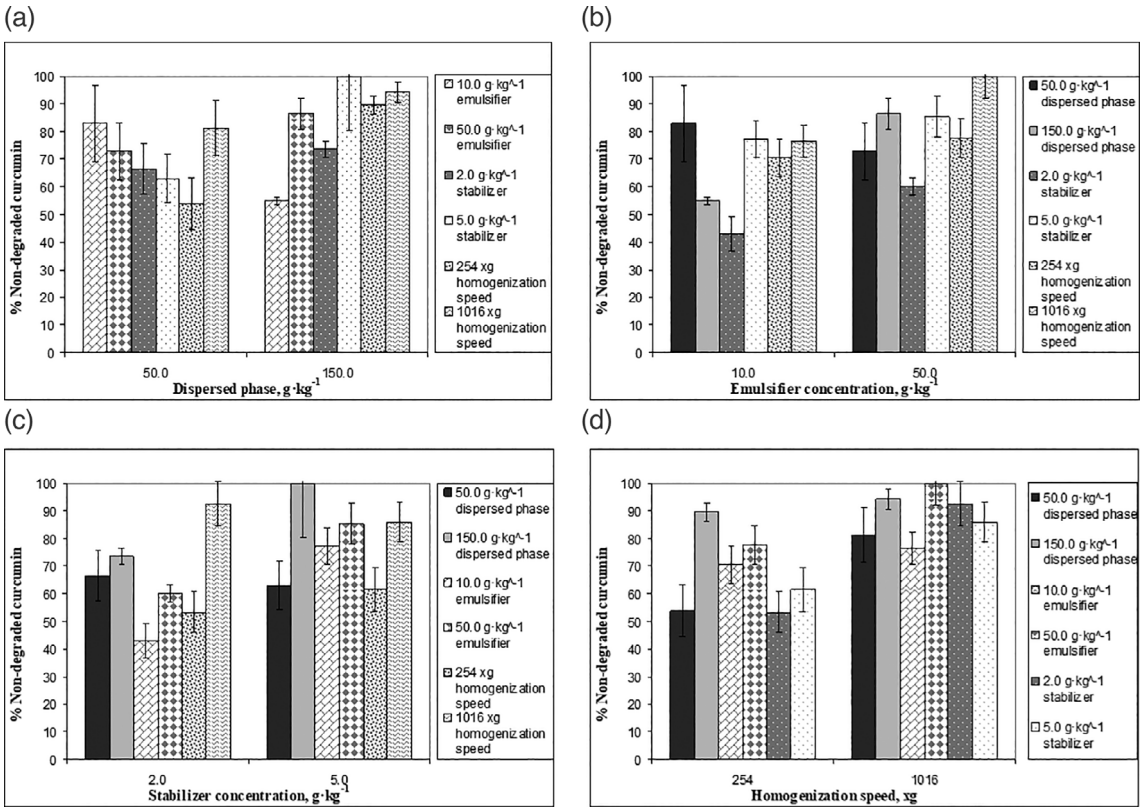

Figure 4. Maintenance of drug content in Og/W emulsions 30 days after their manufacture as a function of: (a) dispersed phase percentage, (b) emulsifier concentration, (c) stabilizer concentration and (d) homogenization speed.

nature and concentration on curcumin-loaded nanoemulsion stability and evaluated the encapsulation efficiency. They found the highest initial time encapsulation efficiency values in nanoemulsions containing Tween 20, with the results being even more effective at low emulsifier concentrations. This is consistent with the results obtained in the present study and as reported in Fig. 4(a) using a 50 g kg^{-1} dispersed phase: in this case, the curcumin retained percentage was 83.1% for 10 g kg^{-1} emulsifier compared to 72.9% retained using 50 g kg^{-1} emulsifier. However, at higher percentages of the dispersed phase, this trend is probably not verified as a result of an insufficient amount of emulsifier to completely coat the oleogel droplets, causing the system to destabilize over time. In that case, curcumin may be exposed to light and oxidation reactions, thus leading to degradation.

The homogenization speed is another parameter to consider in the investigation of curcumin incorporation efficiency. As shown in Fig. 4(d), a higher homogenization rate implies a higher amount of curcumin retained in the emulsion over time, reaching percentages of up to 99.6% at 1016 × *g* compared to a maximum of 89.5% reached when the homogenization speed was 254 × *g*. The homogenization rate is directly related to droplet size and this in turn to the emulsion's viscosity. Wang *et al.*[65] noted that the emulsion viscosity increases slightly as the average droplet diameter decreases due to the greater number and the interfacial area of emulsion droplets when the droplet size decreases. This slight increase in viscosity contributes to the physical stability of the emulsion, keeping curcumin encapsulated and protecting it from any the action of external agents leading to degradation. This can also be seen in Fig. 4(c), which verifies how an increase in the stabilizing concentration from 2.0 g kg^{-1} to 5.0 g kg^{-1} translated into a greater amount of curcumin present over time, increasing by 34.2% at 10 g kg^{-1} emulsifier or 25.2% at 50 g kg^{-1} emulsifier. Moreover, one of the effects produced by adding stabilizer is viscosity modification, as well as modification of the rheological properties of the emulsion. However, the statistical analysis revealed that none of these investigated variables had a significant influence on maintaining curcumin content to a 95% significance level.

Optimization of Og/W emulsion formulation

Finally, M-RSM was carried out to obtain the optimal formulation that, in this case, would maximize the amount of curcumin provided and, in turn, minimize both droplet size and lipid oxidation in the Og/W emulsion. The results are shown in Fig. 5, which represents the estimated response surface for the effect of dispersed phase percentage and stabilizer concentration on the overall desirability for a constant emulsifier concentration and homogenization speed (equal to the optimum reached).

In multivariate optimization, if all response variables are of equal relative importance, it appears reasonable to weigh all of them equally. However, when they have different variabilities and distinct deviations from the target value, the unit weight is not the most appropriate. In these cases, different weights can be assigned to each of the response variables, depending on the importance or influence that these have variables on the pursued objective. In the present study, we aimed to develop a new stable carrier capable of encapsulating, protecting and transporting curcumin; therefore, the variables related to the physicochemical

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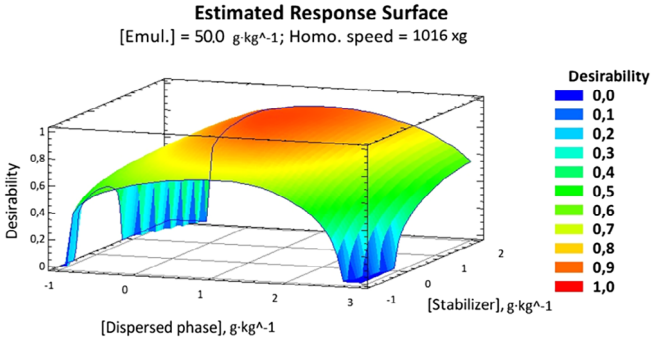

Figure 5. Estimated multi-response surface for the effect of dispersed phase percentage and stabilizer concentration on the overall desirability for a constant emulsifier concentration and homogenization speed.

Table 2. Optimized input parameters for Og/W emulsion formulation

Factor	Value
Dispersed phase (g kg^{-1})	140.4
Emulsifier concentration (g kg^{-1})	50.0
Stabilizer concentration (g kg^{-1})	4.9
Homogenization speed (× *g*)	1016

stability of our system have been weighted with a higher value, prioritizing them over the curcumin's load capacity. Following this criterion, droplet size has been given a weight of 4.0, primary oxidation a value of 5.0, secondary oxidation a weight of 2.0, and curcumin content a value of 3.0. The reason why primary oxidation weighs more than secondary is because of something already mentioned: secondary oxidation occurs from the products generated in primary oxidation; therefore, if we manage to reduce the extension of the first, it will be necessary to reduce the second as well. With all of these previous considerations, multivariate optimization has yielded the optimal values for the variables studied (Table 2).

For these values of the variables studied, the desirability function reached its optimum (maximum) of 0.932. Based on these data, we consider the obtained desirability value to be adequate, which reveals that the designed emulsion meets the global expectations we had set for ourselves. According to these results, we can conclude that high concentrations of emulsifier and thickener contribute to the stability of Og/W emulsions, both physically and chemically. In effect, the stabilizing action of the emulsifier, as widely reported in the scientific literature, and the change in viscosity caused by the thickener, hinders the mobility of both droplets and the oxidizing species and diffusion processes, favoring the physicochemical stability of the emulsion. We also observe that maximum values of homogenization speed contribute to stabilizing the system. Finally, employing an intermediate value for dispersed phase concentration, this balance is achieved between obtaining a stable emulsion and maximizing the content of curcumin supplied.

CONCLUSIONS

In the present study, an oil gelled-in-water emulsion was designed for maximizing the stability, encapsulation, protection and transportation of curcumin-enriched with ω-3 PUFAs. The influence of dispersed phase percentage, emulsifier and stabilizer concentrations, and homogenization speed on the physical and chemical stabilities over time of curcumin-loaded emulsions were evaluated.

In general, homogenization speed was the variable that most influenced both reduction of the average droplet size and encapsulated curcumin maintenance. In the first case, sizes up to 25 times smaller were reached when we increased the homogenization speed from 254 to 1016 × *g*. Regarding curcumin content, we reached percentages higher than 75% of retained curcumin even reaching 99.6% at speeds of 1016 × *g*; with some exceptions, the percentages of preserved curcumin ranged from 50% to 75% at 254 × *g*. Regarding lipid oxidation, higher stabilizer concentrations achieved lower oxidation rates, reaching reductions of approximately 56.7% in primary lipid oxidation products and 57.4% in secondary lipid oxidation products. Finally, by using the multi-response surface methodology, the optimal operating parameters were found to be: 140.4 g kg^{-1} dispersed phase, 50.0 g kg^{-1} emulsifier, 4.9 g kg^{-1} stabilizer and 1016 × *g*. Accordingly, oil gelled-in-water emulsion technology was confirmed to be a potential tool for transporting and stabilizing hydrophobic bioactive compounds such as ω-3 PUFAs and curcumin.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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