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Enhanced *in vivo* absorption and biodistribution of curcumin loaded into emulsions with high medium-chain triglyceride content

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Abstract

The health benefits of curcumin have been demonstrated by clinical studies, but its low bioavailability compromises its functionality. In this regard, emulsions have proven to be effective encapsulation systems for curcumin. Nevertheless, emulsions with a high oil content (50%) may offer some advantages due to the large amount of compound they can incorporate. Therefore, the aim of this work was to study the pharmacokinetics and biodistribution of curcumin when carried in optimized emulsions containing 50% MCT oil and a plant-based emulsifier (soybean lecithin) at 2 h or 4 h post-oral administration to rats. The most stable emulsion was obtained using 50% of oil and a surfactant-oil-ratio 0.1, through a microfluidization process. After the oral administration of the systems (150 mg curcumin/kg body weight), curcumin glucuronide was the main compound present in plasma (AUC_{0-t} = 1556.3 $ng \cdot h \cdot ml^{-1}$), especially at 2-4 h post-administration. The total curcuminoid bioavailability was increased by 10.6-fold when rats were fed with the

curcumin emulsion rather than with a control suspension. Moreover, rats fed with the emulsion showed the highest accumulation of free curcuminoids, which present the highest biological activity, in the liver (129 ng curcumin/g tissue) and brown adipose tissue (193 ng curcumin/g tissue). The obtained results are of relevant interest since the presence of curcumin in the brown adipose tissue has been shown to play a relevant role in the prevention of obesity and its related metabolic disorders.

Keywords: Curcumin, MCT oil, emulsions, bioavailability, biodistribution

1. Introduction

Curcumin, a polyphenol extracted from the rhizomes of *Curcuma longa*, have been shown to be effective against several chronic diseases and targets diverse molecular pathways without any associated toxicity or resistance (Prasad et al., 2014). Specifically, this lipophilic compound presents antioxidant and anti-inflammatory properties that provide multiple health benefits (Hewlings & Kalman, 2017; Pulido-Moran et al., 2016). Despite its numerous applications, curcumin has molecular instability, poor solubility in water, rapid conjugation to hydrophilic molecules (like glucuronic acid and sulphate) in the liver with biliary excretion and poor enteral absorption, which limit its utility as a health-promoting agent (Anand et al., 2007). To overcome these challenges, delivery systems such as nanoparticles, liposomes, micelles, and phospholipid complexes have been developed, showing to prevent the degradation in the gastrointestinal tract and to increase the permeation in the small intestine, increasing curcumin bioavailability (Hu et al., 2017; Stohs et al., 2020). The potential of emulsion-based delivery systems to increase the bioavailability of curcumin has been widely investigated. As an example, by using an emulsion stabilized with a protein-polysaccharide conjugate (bovine serum albumin-dextran), the bioavailability of curcumin increased by 4.8-fold compared to a curcumin suspension (Wang et al., 2016). Other authors observed a 3.95-fold higher curcumin bioavailability using a self-nanoemulsifying drug delivery system containing synthetic emulsifiers than when using a curcumin suspension (Nazari-Vanani et al., 2017). The composition of these systems has been reported to affect their properties and functionality. Regarding the lipid type, long-chain triglycerides (LCT) have been the most used due to the high *in vitro* bioaccessibility they provide (Ozturk et al., 2015; Qian et al., 2012). However, recent *in vivo* studies reported no differences on lipophilic compounds bioavailability when comparing them with medium-chain triglycerides (MCT) (Lin et al., 2022; Salvia-Trujillo et al., 2015). Furthermore, MCT have been seen to present relevant health-related properties interesting for the treatment of obesity, cancer or malabsorption diseases (Nimbkar et al., 2022). Regarding the emulsifier type, it has been also seen to affect the properties of the emulsions (Gasa-Falcon et al., 2019), with synthetic ones being the most widely used in the last decades. However, due to the general concern about synthetic ingredients and the claims for more plant-based foods, there is a need to find natural plant-based options. Moreover, in previous works, we observed reduced particle size and polydispersity when using a plant-based emulsifier such as soybean lecithin rather than an animal-based like whey protein (Teixé-Roig et al., 2022).

To the best of our knowledge, there are no studies investigating the use plant-based emulsions with high MCT content (50%) to increase the *in vivo* bioavailability of curcumin. In this regard, this type of emulsions, present some advantages over more diluted emulsions. On the one hand, due to their high oil content, they can incorporate high concentrations of lipophilic bioactive compounds, reducing the dose of emulsion needed to achieve the beneficial effects. On the other hand, they can reduce the storage and transport costs, as well as increase the shelf-life of chemically labile products to a better extent than emulsions with lower oil droplet concentrations due to a reduced lipid oxidation rate (Luo et al., 2017).

Most *in vivo* studies investigating the use of emulsion-based delivery systems to increase the absorption and bioavailability of curcumin are based on pharmacokinetic studies. Curcumin delivered orally undergoes extensive metabolism reduction and conjugation, which results in the presence of so many different metabolites. Recent studies have revealed that some reductive metabolites such us tetrahydrocurcumin may present therapeutic properties, although any of them have shown more biological effect than curcumin in its native form (Pandey et al., 2020). In contrast, metabolites resulting from conjugation like curcumin glucuronide, have shown very reduced effects (Choudhury et al., 2015; Kunihiro et al., 2019; Shoji et al., 2014). Nevertheless, most *in vivo* pharmacokinetic studies have used enzymatic hydrolysis of plasma prior to analysis, reporting the results as total curcumin, and not determining the part of the compound present as curcumin glucuronide (Stohs et al., 2020). Furthermore, few studies have investigated the effect of curcumin incorporation into emulsion-based delivery systems on the biodistribution of the compound. In that sense, there is a need to perform studies investigating the target organs of curcumin and the form in which this compound is present when administered orally enclosed in emulsions. This is of great importance since it provides more information on which organs this compound can act and exert beneficial effects, providing insights into the different diseases in which these curcumin systems can improve treatment or prevention.

Therefore, the aim of this work was, firstly, to obtain a plant-based MCT emulsion containing a high dose of curcuminoids presenting high stability for 15 days at 4 ºC. Secondly, to study the effect of the encapsulation of curcuminoids in this emulsion on the *in vivo* absorption of the different curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), as well as their metabolization into curcumin glucuronide and the biodistribution of the different forms (free or conjugated) in the organs.

2. Materials and methods

2.1. Materials

Powdered curcumin extract (mixture of ≥ 78% curcumin, 15% demethoxycurcumin, and 1% bisdemethoxycurcumin) with a purity of ≥ 98% was purchased from Acros Organics. As a lipid phase MCT oil (Miglyol, Oxi-med expres) (99.9% of purity) was used. Soybean lecithin was acquired from Alfa Aesar (Thermo Fisher Scientific, Massachusetts, USA). Curcumin, demethoxycurcumin, and bisdemethoxycurcumin standards were obtained from Sigma Aldrich (St. Louis, MO). Curcumin glucuronide was acquired from Toronto Research Chemicals (Toronto, Canada). Ultrapure water obtained from a milli-Q filtration system was used to the preparation of all solutions.

2.2. Methods

2.2.1. Preparation of emulsions

To obtain the lipid phase, curcumin was solubilized in MCT oil (30 $mg \cdot g^{-1}$) by stirring for 20 min at 60 ºC and sonicating for 20 min. The concentration of curcumin was established considering the solubility of the compound in the MCT oil used. Different concentrations of oil were tested during the optimization process (10-50% w/w). To obtain the aqueous phase, soybean lecithin (which was used as emulsifier) was added in ultrapure water and stirred during 4 h. Different surfactant-oil-ratio (SOR) were tested (0.05, 0.1, and 0.2), thereby, varying the concentration of soybean lecithin in the emulsions.

Two different methodologies were tested to formulate the emulsions. In the first methodology only a high-shear homogenization (HSH) was used. The lipid phase (10 or 50% w/w) and the aqueous phase were mixed and homogenized at 11000 rpm for 2 min using an Ultra-Turrax (Janke & Kundel, Staufen, Germany). The second methodology included a HSH followed by a microfluidization process (MF). For the MF process, emulsions were passed through a microfluidizer (M-110P, Microfluidics, USA) at 100 MPa for 5 cycles.

2.2.2. Characterization of highly-concentrated emulsions

Particle size. The particle size of emulsions was measured using a Mastersizer 3000 (Malvern Instruments Ltd, Worcestershire, UK). Samples were diluted in ultrapure water and stirred in the dispersion unit with a constant speed of 1800 rpm. The mean particle size was expressed as surface area mean diameter (d_{32}) in nanometers (nm), fixing a refractive index of the MCT oil of 1.45 and 1.333 for water. The Span index was calculated using the following equation:

$$Span\ index = \frac{(d_{90} - d_{10})}{d_{50}}$$

ζ-potential. The ζ-potential was measured by phase-analysis light scattering (PALS) using Zetasizer laser diffractometer (NanoZS Malvern Instruments Ltd Worcestershire, UK). Prior to the analysis, emulsions were diluted (1:150) in ultrapure water. Then, the diluted samples were placed in a capillary cell equipped with two electrodes to assess the electrophoretic mobility of the particles. The results were reported in millivolts (mV).

Fluorescence microscopy. Emulsions were dyed with Nile Red, previously dissolved at 0.1% (w/v) in ethanol. Then, micrographs of emulsions were obtained using fluorescence with an optical microscope (Olympus BX41, Olympus, Göttingen, Germany) with a 100x objective lens. The images were obtained using a digital camera (Olympus DP74) and processed with the software CellSens (Olympus Göttingen, Germany).

Viscosity. Viscosity measurements were performed by using a vibro-viscometer (SV-10, A&D Company, Tokyo, Japan) vibrating at 30 Hz, with constant amplitude and working at room temperature. The results were expressed in mPa·s.

Stability. The stability of emulsions was studied using an optical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France), which is a non-destructive method that can

measure the static stability of samples and detect the cause of instability (flocculation, coalescence, sedimentation, or creaming) by the multiple light scattering technique. A sample of 7 mL was introduced into a glass cylindrical cell and analyzed by a light beam emitted in near infrared wavelength, which scanned vertically from the bottom to the top of the sample cell. Two synchronous optical sensors received light backscattered by the sample (45º from the incident radiation). In this study, the backscattering was measured during 15 days at 4 ºC to assess the stability of emulsions over time. The backscattering was analyzed at three different zones of the test tube (top, middle, and bottom) in order to study different instability phenomena throughout the tube such as creaming at the top, flocculation, or coalescence in the middle and sedimentation at the bottom.

2.2.3. *In vivo* studies

2.2.3.1. Animal experiments

Female Sprague Dawley rats weighing 0.20-0.25 kg were used for the in vivo studies. The animal procedures were conducted in accordance with EU Directive 2010/63/EU for animal experiments and approved by the Animal Ethics Committee of Universitat de Lleida (CEEA 01-04/18).

Pharmacokinetic study. For the pharmacokinetic study the animals were randomly divided into two groups (fed with an emulsion or suspension) containing five rats each group. After an acclimatation period, rats were fasted for 12 h before the experiment with free access to water. The animals were administered with curcumin emulsion or curcumin suspension, both containing a dose of 150 mg of curcumin/kg body weight. Blood samples were taken at 0, 30, 60, 120, 240, and 480 min after administration from the tail vein. Plasma was immediately separated by centrifuging the blood samples at 4000 rpm for 10 min at 4 ºC and stored at -80 ºC. Then, curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcumin glucuronide were extracted and quantified in plasma samples as described in 2.2.3.2. The pharmacokinetic analysis was performed for formulations using a non-compartmental design. The area under the drug concentration versus time curve from zero to 8 hours ($AUC_{0-8\ h}$) was calculated using the trapezoidal rule. The maximum plasma concentration of curcumin (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma analyses.

Biodistribution study. Rats were randomly divided in 2 groups; one group was fed with a curcumin emulsion (n=8) and the other group was fed with a curcumin suspension (n=8). Rats were fasted for 12 h before the experiment with free access to water. The animals were administered a dose of 150 mg of curcumin/kg body weight, irrespective of the curcumin vehicle used. Rats were sacrificed at 2 h or 4 h post-administration and tissue samples were collected (kidney, liver, duodenum, jejunum, ileum, colon, brown adipose tissue and white adipose tissue). Tissue samples were washed with phosphate buffered saline (PBS), dried on a filter paper, weighted, and stored at -80 ºC. Curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcumin glucuronide were extracted and quantified in tissue samples as described in 2.2.3.2.

2.2.3.2. Curcuminoid extraction and quantification in plasma and tissue samples

Extraction from plasma. Curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcumin glucuronide extraction in plasma samples was performed according to a previously reported method with some modifications (Li et al., 2011). Aliquots of 150 µL

of plasma were mixed with the internal standard (Honokiol) and 600 µL of acetonitrile and vortexed at 1800 rpm for 1 min. Then, the mixture was submerged into an ultrasonic bath for 30 sec, centrifuged at 9000 rpm for 10 min at 4 ºC, and the organic fraction was collected. Afterwards, the collected upper organic layers were evaporated under N_2 and stored at -80 ºC until quantification.

Extraction from tissues. Curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcumin glucuronide extraction in tissue samples was performed according to a previously reported method with some modifications (Chirio et al., 2019). Initially, tissue samples were mixed with milli-Q water and homogenized with an Ultra-Turrax at 9000 rpm for 1 min to obtain the tissue homogenates. Then internal standard (Honokiol) and 1 mL of acetonitrile were added to tissue homogenates and the mixture was vortexed at 2000 rpm for 1 min. Then, the mixture was centrifuged at 9000 rpm for 5 min at 4 ºC, and the organic fraction was collected. Afterwards, the collected upper organic layers evaporated under N_2 and stored at -80 ºC until quantification

Curcuminoids quantification. The quantification of curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcumin glucuronide was performed by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) using the method described by Liu et al. (2018), with some modifications. To perform the analyses an ACQUITY UPLC binary (Waters, Milford, MA, USA) coupled to a Xevo TQS (triple quadrupole) (Waters, Milford, MA, USA) was used. Chromatographic separation was performed on a 150 mm × 2.1 mm i.d., 1.6 µm CORTECS Phenyl Column (Waters, Milford, MA, USA) with mobile phase A [acetonitrile containing 0.1% (v/v) formic acid] and mobile phase B [98% water and 2% acetonitrile containing 0.1% (v/v) formic acid]. The injection volume was 2.5 µL and the flow rate was 0.4 mL/min. Separation was carried out in 5.5 min under the following conditions: 0 min, 60% B; 4 min, 25% B; 4.1 min, 60%B; 5.5 min, 06% B. The column was equilibrated for 10 min prior to each analysis. The column temperature was maintained at 40 ºC. The MS operated in electrospray ionization (ESI) in negative mode and nitrogen was used as the source gas in all cases.

2.2.4. Statistical analysis

All experiments were assayed in duplicate and three repetitions of each analysis were carried out on each parameter in order to obtain mean values. Analysis of the variance (ANOVA) was performed to compare treatments. Least significant difference (LSD) test was employed to determine differences between means. The confidence interval was set at 0.95 and all results were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md).

3. Results and discussion

3.1. Emulsion optimization

3.1.1. Effect of emulsification method and oil concentration

Particle size. Among emulsions obtained using HSH, those containing 50% oil presented a lower particle size than those with 10% oil, being ≈ 4.9 µm and ≈ 3.5 µm, respectively (Table 1). Moreover, emulsions with 50% oil presented a lower span index (Table 1), indicating less particle size polydispersity. Those facts could be attributed to the increased

packing state of droplets caused by the increased oil concentration, which helps to prevent droplet re-coalescence, resulting in a smaller and less polydisperse particle size (Artiga-Artigas et al., 2019). Additionally, other authors have also reported that by increasing the oil concentration, the stress forces during the emulsification process were higher, thereby reducing the particle size of droplets (Briceño et al., 2001). When a MF process was applied to emulsions, those with 50% oil showed a reduced particle size, lower span index and narrower particle size distribution than those with 10% of oil (Table 1 and Figure 1).

The particle size and span index of emulsions obtained with HSH+MF were lower than those obtained just with HSH (Table 1). Such a reduction can be attributed to the inertial forces in turbulent flow along with cavitation that promotes droplet disruption during the microfluidization process. The flow stream by high pressure through microchannels toward an impingement area creates a shearing action, which can provide fine emulsions (Maa & Hsu, 1999; Schultz et al., 2004). Furthermore, the particle size distribution became less polydisperse after the MF process (Figure 1), which has been also observed by other authors studying emulsions with similar oil concentrations (Artiga-Artigas et al., 2019).

Viscosity. By increasing the oil concentration, the viscosity of emulsions was greatly increased, regardless of the treatment applied (Table 1). Such an increase may be a consequence of the reduced particle size that increased the number of dispersed phase particles in the emulsion causing a high packing state of the droplets as well as the interactions between them (Brinkman, 1952; Krieger & Dougherty, 1959). Indeed, this was confirmed by the microscope images (Figure 1), in which more spherical and packed oil droplets can be observed in the emulsions formulated with 50% rather than 10% oil. Emulsions obtained by HSH+MF presented a higher viscosity than those obtained using HSH (Table 1) which can be attributed to the reduced particle size of the formers.

ζ-potential. The electrical charge of all emulsions was negative and values ranged from -61 mV to -68 mV (Table 1). The presence of lecithin molecules at the interface could be the reason of these values, since the majority of their phospholipids were negatively charged at pH 7 (Zhang et al., 2012). However, emulsions with 50% oil presented a more negative value than emulsions with 10% oil (Table 1). The smaller particle size of 50% oil compared to 10% oil, provided a higher surface area in the emulsions. Therefore, emulsions containing 50% oil may present more negatively charged molecules in the interface than those with 10%, as observed using other surfactants such as Tween 80 (Artiga-Artigas et al., 2019).

3.1.2. Effect of surfactant-oil-ratio (SOR)

In view of the results presented in the previous section, for the following study of the effect of SOR (0.05, 0.1 and 0.2) on the stabilisation of curcumin MCT-emulsions, those obtained by HSH+MF containing 50% oil were used.

Particle size. The smallest particle sizes were observed when using 0.1 SOR (≈ 0.53 μm), followed by emulsions containing 0.05 SOR (≈ 0.84 μm) and 0.2 SOR (≈ 3.73 μm) (Table 2). Moreover, the particle size distribution and span index increased in the same order: 0.1 SOR < 0.05 SOR < 0.2 SOR (Table 1 and Figure 2). This indicates that 0.1 SOR, which is a 5% of lecithin in an emulsion containing 50% of oil, was the most suitable concentration of surfactant to obtain small particle sizes and reduced polydispersity. It is well known that a higher concentration of surfactant can cover a greater surface area (Luo

et al., 2017). However, it is also known that an excess of surfactant can cause the aggregation of oil droplets (An et al., 2014; Dalvi & Dave, 2009). Therefore, it seems that, in the studied emulsions, 0.05 SOR (2.5% soybean lecithin) was not enough to completely cover the total oil surface. Conversely, at 0.2 SOR (10% soybean lecithin), an excess of surfactant that was promoting flocculation of lipid droplets was confirmed in the microscope images (Figure 2).

Viscosity. The viscosity of emulsions was increased when the SOR was augmented, being the emulsion with 0.2 SOR those with the highest viscosity (52.10 ± 1.22 mPa·s) (Table 2). It has been previously stated that the particle size governs the viscosity of emulsions with high oil content (Artiga-Artigas et al., 2019). In our study, this trend was observed in emulsions with 0.05 and 0.1 SOR, being the later the one with the smallest particle size (0.53 ± 0.06 μm) and the highest viscosity (27.87 ± 0.32 mPa·s). Nevertheless, this was not observed in the emulsion with a 0.2 SOR, which presented the highest viscosity although containing the droplets with the highest size and most polydisperse particle size distribution. In this case, the high viscosity may be a consequence of the aggregation of the droplets rather than the particle size.

ζ-potential. As observed in Table 2, the 0.2 SOR-emulsion presented the most negative ζ-potential (-68.50 ± 2.51 mV), followed by those with 0.1 SOR (-62.96 ± 2.03 mV) and 0.05 SOR (-52.83 ± 1.27 mV). When increasing the SOR, the number of lecithin molecules was also increased for the same oil content, so there were more negative charged molecules that could be positioned in the interface contributing to the negative ζ-potential.

Stability. Emulsions with 0.1 SOR showed the highest stability over the 15 days of storage at 4 ºC, presenting no instability phenomena (Figure 3). In this emulsion, it seems that the repulsive interactions generated by lecithin were strong enough to overcome any attractive interactions (van der Waals) between the droplets (Hunter, 2001; Luo et al., 2017). In contrast, both 0.05 and 0.2 SOR-emulsions were unstable, being the emulsion with 0.2 SOR the less stable. Indeed, the latter was immediately destabilized after its preparation, which could be attributed to an excess of surfactant that promoted the aggregation of droplets in the emulsion facilitating the destabilization of the system (Figure 1). In that sense, the rates of Ostwald ripening and coalescence in emulsions have been reported to be higher in the presence of excess surfactant due to micelle-mediated surfactant transport of oil between droplets (Klang & Valenta, 2011; Tadros et al., 2004). In contrast, 0.05 SOR-emulsion was stable for two days, and started to present sedimentation from day 5 (Figure 3). In this case, the instability phenomena may be caused by the lack of surfactant to completely cover the surface area of droplets. Therefore, since 0.1 was the most suitable SOR for emulsions containing 50% MCT oil, the following *in vivo* studies were performed using this emulsion.

3.2. In vivo studies

3.2.1. Pharmacokinetic study

Rats were administered with an emulsion containing 50% MCT oil and lecithin at a 0.1 SOR prepared using HSH+MF or a control suspension. After the oral administration of the curcuminoid carriers, the concentration in plasma of all studied compounds was higher in emulsion-fed rats than in suspension-fed rats (Figure 4 and Table 3). Specifically, the overall curcuminoid bioavailability was improved by 10.6-fold when the

compound was enclosed in the emulsion rather than in suspension. Values similar to those obtained in our work have been reported by other authors using other curcumin emulsion-based delivery systems. For instance, rice bran protein nanoparticles or organogel-based emulsions showed to improve the oral bioavailability of curcumin by approximately 9.2 or 9.5-fold compared to free curcumin (Liu et al., 2018; Yu & Huang, 2012). Such an increased bioavailability may be related to the capacity of emulsions increase the bioaccessibility of lipophilic compounds (Yi et al., 2021). In contrast, in the control group no mixed micelles were present after digestion to incorporate curcumin and facilitate its absorption.

Among the studied compounds, curcumin glucuronide was the main compound present in the plasma of rats, and especially in emulsion-fed rats. Indeed, the $AUC_{0\text{-}8h}$ for curcumin glucuronide was 1556.32 ± 210.44 $ng \cdot h \cdot ml^{-1}$ in rats fed with the emulsion, while it was 121.52 ± 137.39 $ng \cdot h \cdot ml^{-1}$ in those fed with the control suspension. In that sense, it is suggested that when curcumin is orally administered to rodents, it undergoes intestinal metabolism, as well as rapid first-pass metabolism and excretion in bile (Sharma et al., 2005). In fact, previous studies in rats have reported that curcumin undergoes O-conjugation to curcumin glucuronide and curcumin sulphate, as well as reduction to tetrahydrocurcumin, hexahydrocurcumin and others (Asai & Miyazawa, 2000; Ireson et al., 2001). However, according to previous works, among the metabolites formed, curcumin glucuronide is the major one found in the plasma after oral administration of curcumin to rats, while the free forms are normally negligible (Ozawa et al., 2017; Peng et al., 2018). Unlike curcumin, curcumin conjugates are water soluble, which may explain why a substantial proportion of curcumin in the general circulation exists as conjugates (Toden & Goel, 2017). Conjugation with glucuronic acid is considered a fundamental mechanism in nature for detoxifying and eliminating lipophilic chemicals from the organism, being the resultant glucuronides less biologically or chemically reactive than their corresponding aglycones (Ritter, 2000).

In our work, the $AUC_{0\text{-}8h}$ of curcumin glucuronide was > 100-fold higher than the $AUC_{0\text{-}8h}$ of the free forms (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) in the case of the emulsion, and up to 10-fold higher in the case of the suspension (Table 3). Moreover, among the free form compounds, curcumin exhibited a higher concentration in plasma (≈ 12-15 $ng \cdot h \cdot ml^{-1}$) compared to demethoxycurcumin and bisdemethoxycurcumin (≈ 7-9 $ng \cdot h \cdot ml^{-1}$), irrespective of the carrier used. This fact can be attributed to the composition of the administered curcuminoid extract, which contained a higher amount of curcumin (≥ 78%) than demethoxycurcumin (15%) or bisdemethoxycurcumin (1%).

The T_{max} of curcumin was lower using the suspension (0.5 h) than the emulsion (1 h) (Table 3). The presence of high quantities of oil (50%) of our emulsion could have retarded the gastric emptying and, therefore, the curcumin absorption. In contrast, the T_{max} of demethoxycurcumin and bisdemethoxycurcumin was 0.5 h for both curcumin vehicles (Table 3), although the concentration in plasma of these compounds was mostly constant along the studied time (Figure 4). The T_{max} of curcumin glucuronide was 4 h, irrespective of the carrier used (Table 4). This value is higher than that observed for the free forms and may be a consequence of curcumin metabolization. Indeed, a recent *in vivo* study with Caco-2 cell reported that the percentage of phase II metabolites (which includes curcumin glucuronide) increased from ≈ 10% at 0.5 h to > 60% at 4 h post-administration of different emulsions (Luo et al., 2022).

3.2.2. Biodistribution study

The concentration of curcuminoids in the different tissues of rats was quantified at 2 h and 4 h after the oral administration of the curcumin carriers (emulsion or suspension).

In general, the highest concentration of curcuminoids was observed in the intestinal tissues (Table 4), especially curcumin, which presented concentrations up to ≈ 841 ng/g tissue. In contrast, the concentration of curcuminoids in non-intestinal tissues (liver, kidney, white and brown adipose tissue) was lower (up to ≈193 ng/g tissue). These observations are in accordance with previous works that have also found the major concentration of curcumin in the intestine after an oral administration of curcumin in combination with a treated soybean lecithin in rats, followed by liver and kidney (Marczylo et al., 2009).

The concentration of all curcuminoids was higher in the intestine of rats fed with - emulsion rather than in those fed with the suspension, especially in the duodenum and jejunum (Table 4). In that sense, such an increased absorption when using the emulsion can be related to the capacity of emulsion-based delivery systems to increase the bioaccessibility of the enclosed compounds, as previously mentioned. So, emulsion-fed rats may present a higher amount of potentially absorbable curcumin in the intestinal digesta than those fed with the suspension, resulting in higher curcumin concentration in the intestinal cells. Moreover, the presence of lecithin could have increased the curcumin absorption by reducing the interfacial surface tension and increasing cell membrane fluidity and the penetration of curcumin through the epithelial cells (Pan-On et al., 2022). In contrast, the low concentration observed using the suspension may be a consequence of the inadequate absorption and avid metabolism of free curcumin that rodents present (Ireson et al., 2002).

Among the administered curcuminoids, curcumin showed the highest concentration in the intestine (up to ≈ 841 ng/g), followed by demethoxycurcumin (up to ≈ 233 ng/g), bisdemethoxycurcumin (up to ≈ 48 ng/g) (Table 4), which was expected due to the curcuminoid composition (≥ 78% curcumin, 12% demethoxycurcumin, and 1% bisdemethoxycurcumin). Curcumin glucuronide was also present in the intestine of rats, especially in the ileum of emulsion-fed rats at 4h post-administration (≈ 121 ng/g), which may indicate that some metabolization has occurred there. Previous works have reported the presence UDP-glucuronosyltransferases in the intestinal mucosa of rats, which is the enzyme responsible for curcumin glucuronidation (Asai & Miyazawa, 2000). Nevertheless, although curcumin glucuronide was the major metabolite observed in plasma (section 3.2.1), the concentration of this metabolite in the intestinal tissues of rats was low. Indeed, in our work, curcumin glucuronide has been quantified in plasma, intestinal mucosa and kidney (Table 4). These results are in accordance with previous works, which also detected this metabolite in plasma and intestinal mucosa of rats after the oral administration of curcumin with phosphatidylcholine (Marczylo et al., 2007) and can be a consequence of the fast metabolism of curcumin.

The concentration of curcumin, demethoxycurcumin and bisdemethoxycurcumin concentrations in the intestinal tissues was higher at 2 h rather than at 4 h post-administration (Table 4). This indicates that the absorption of the free forms was higher at early times (2 h) and decreased at longer times (4 h). In the same way, previous works reported the highest curcumin concentration at 1 h post-administration, which decreased at 3 h (Suresh & Srinivasan, 2010). Moreover, at 4 h post-administration, suspension-fed

rats presented a higher curcumin concentration in the colon than emulsion-fed rats. This may indicate that, by using the suspension, more compound was non-adsorbed and arrived at the colon to be excreted. The concentration of curcumin glucuronide in the duodenum and ileum of rats was the same at 2 h and 4 h post-administration. However, a higher concentration was observed in the ileum of emulsion-fed rats at 4 h than at 2 h, and no compound was detected in the colon.

Free form curcuminoids, which present the highest biological activity (Pandey et al., 2020), were detected in non-intestinal tissues. Among them, kidney and white adipose exhibited the smallest curcuminoid concentrations (up to 23.03 ± 10.29 ng/g), irrespective of the carrier used. In contrast, the liver and brown adipose tissue were those that presented the highest curcuminoid concentrations (up to 129.35 ± 52.07 and 192.94 ± 11.69, respectively). Furthermore, by using the emulsion, the concentration of curcumin in the liver and brown adipose tissue of rats was relatively higher than when using the suspension, at 2 h post-administration (Table 4). On the one hand, the liver has been seen to be an important accumulation site of curcumin even after being orally or intravenously administered to rodents (Arozal et al., 2019; Ryu et al., 2006; Wei et al., 2014). However, although the liver is an important glucuronidation site in humans, we could not detect this compound in the liver of rats. Previous works have postulated that most of the orally administered curcuminoids are conjugated to glucuronides in the intestinal mucosa of rats (Asai & Miyazawa, 2000). In fact, previous authors have investigated the capacity of rat hepatocytes to generate different curcumin metabolites, reporting that glucuronides are generated only in small amounts in the liver, whereas they are abundant in plasma after oral administration (Ireson et al., 2001). These authors reported that rat liver reduces curcumin to hexahydrocurcumin and to hexahydrocurcuminol, whereas conjugation of curcumin is only a minor hepatic biotransformation route. On the other hand, the enhanced curcumin concentration found in the brown adipose tissue when feeding rats with the emulsion is of high interest since previous authors have stated that the presence this compound in brown and white adipose tissues can play a relevant role in the prevention of obesity and its related metabolic disorders. It has been seen that curcumin can increase the expression of uncoupling protein 1 (UPC1) in the brown adipose tissue, which has been related to an increased energy expenditure. Moreover, it can also decrease inflammation in white adipose tissue (Santos et al., 2023; Song et al., 2018; Zhu et al., 2021).

4. Conclusions

Stable emulsions with high oil content were formulated using 50% MCT oil and lecithin (SOR 0.1) through homogenization and a further microfluidization process. After orally administered to rats, the absorption of curcuminoids was higher in the intestine of rats fed with the emulsion rather than with the suspension. As a consequence, the curcuminoid bioavailability was enhanced by 10.6-fold when using the emulsion compared to the control suspension. The increased absorption by using the emulsion may be a consequence of (1) the protection that emulsions provide against degradation during its pass through the gastrointestinal tract and enhanced bioaccessibility, and (2) the capacity of emulsifiers such as lecithin to improve the membrane fluidity and the penetration of curcumin through the epithelial cells. Furthermore, the concentration of curcumin, which presents the most biological activity, in the liver and brown adipose tissue was greatly enhanced when enclosing the curcuminoids in the emulsion. This work highlights the potential of plant-based emulsions with high MCT oil content to increase the

bioavailability of curcumin and increase its accumulation in organs where the compound can play a relevant role in the prevention of metabolic disorders.

Declarations of interest

The authors report no declarations of interest.

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References

An, Y., Yan, X., Li, B., & Li, Y. (2014). Microencapsulation of capsanthin by self-emulsifying nanoemulsions and stability evaluation. *European Food Research and Technology*, *239*(6), 1077–1085. https://doi.org/10.1007/s00217-014-2328-3

Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics*, *4*(6), 807–818. https://doi.org/10.1021/mp700113r

Arozal, W., Ramadanty, W. T., Louisa, M., Satyana, R. P. U., Hartono, G., Fatrin, S., Purbadi, S., Estuningtyas, A., & Instiaty, I. (2019). Pharmacokinetic Profile of Curcumin and Nanocurcumin in Plasma, Ovary, and Other Tissues. *Drug Research*, *69*(10), 559–564. https://doi.org/10.1055/a-0863-4355

Artiga-Artigas, M., Montoliu-Boneu, J., Salvia-Trujillo, L., & Martín-Belloso, O. (2019). Factors affecting the formation of highly concentrated emulsions and nanoemulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *578*(June), 123577. https://doi.org/10.1016/j.colsurfa.2019.123577

Asai, A., & Miyazawa, T. (2000). Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. *Life Sciences*, *67*(23), 2785–2793. https://doi.org/10.1016/S0024-3205(00)00868-7

Briceño, M., Salager, J. L., & Bertrand, J. (2001). Influence of dispersed phase content and viscosity on the mixing of concentrated oil-in-water emulsions in the transition flow regime. *Chemical Engineering Research and Design*, *79*(8), 943–948. https://doi.org/10.1205/02638760152721794

Brinkman, H. C. (1952). The viscosity of concentrated suspensions and solutions. *The Journal of Chemical Physics*, *20*(4), 571. https://doi.org/10.1063/1.1700493

Chirio, D., Peira, E., Dianzani, C., Muntoni, E., Gigliotti, C. L., Ferrara, B., Sapino, S., Chindamo, G., & Gallarate, M. (2019). Development of solid lipid nanoparticles by cold dilution of microemulsions: Curcumin loading, preliminary in vitro studies, and biodistribution. *Nanomaterials*, *9*(2). https://doi.org/10.3390/nano9020230

Choudhury, A. K., Raja, S., Mahapatra, S., Nagabhushanam, K., & Majeed, M. (2015). Synthesis and evaluation of the anti-oxidant capacity of curcumin glucuronides, the

major curcumin metabolites. *Antioxidants*, *4*(4), 750–767. https://doi.org/10.3390/antiox4040750

Dalvi, S. V., & Dave, R. N. (2009). Controlling particle size of a poorly water-soluble drug using ultrasound and stabilizers in antisolvent precipitation. *Industrial and Engineering Chemistry Research*, *48*(16), 7581–7593. https://doi.org/10.1021/ie900248f

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 and Function*, *10*(2), 713–722. https://doi.org/10.1039/c8fo02069h

Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its effects on human health. In *Foods* (Vol. 6, Issue 10, p. 92). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/foods6100092

Hu, B., Liu, X., Zhang, C., & Zeng, X. (2017). Food macromolecule based nanodelivery systems for enhancing the bioavailability of polyphenols. In *Journal of Food and Drug Analysis* (Vol. 25, Issue 1, pp. 3–15). No longer published by Elsevier. https://doi.org/10.1016/j.jfda.2016.11.004

Hunter, R. J. (2001). *Foundations of colloid science*. Oxford university press.

Ireson, C., Orr, S., Jones, D. J. L., Verschoyle, R., Lim, C. K., Luo, J. L., Howells, L., Plummer, S., Jukes, R., Williams, M., Steward, W. P., & Gescher, A. (2001). Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2production. *Cancer Research*, *61*(3), 1058–1064. https://cancerres.aacrjournals.org/content/61/3/1058.short

Ireson, C. R., Jones, D. J. L., Boocock, D. J., Farmer, P. B., Gescher, A. J., Orr, S., Coughtrie, M. W. H., Williams, M. L., & Steward, W. P. (2002). Metabolism of the cancer chemopreventive agent curcumin in human and rat intestine. *Cancer Epidemiology Biomarkers and Prevention*, *11*(1), 105–111.

Klang, V., & Valenta, C. (2011). Lecithin-based nanoemulsions. In *Journal of Drug Delivery Science and Technology* (Vol. 21, Issue 1, pp. 55–76). Editions de Sante. https://doi.org/10.1016/S1773-2247(11)50006-1

Krieger, I. M., & Dougherty, T. J. (1959). A Mechanism for Non-Newtonian Flow in Suspensions of Rigid Spheres. *Transactions of the Society of Rheology*, *3*(1), 137–152. https://doi.org/10.1122/1.548848

Kunihiro, A. G., Brickey, J. A., Frye, J. B., Luis, P. B., Schneider, C., & Funk, J. L. (2019). Curcumin, but not curcumin-glucuronide, inhibits Smad signaling in TGFβ-dependent bone metastatic breast cancer cells and is enriched in bone compared to other tissues. *Journal of Nutritional Biochemistry*, *63*, 150–156. https://doi.org/10.1016/j.jnutbio.2018.09.021

Li, R., Qiao, X., Li, Q., He, R., Ye, M., Xiang, C., Lin, X., & Guo, D. (2011). Metabolic and pharmacokinetic studies of curcumin, demethoxycurcumin and

bisdemethoxycurcumin in mice tumor after intragastric administration of nanoparticle formulations by liquid chromatography coupled with tandem mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *879*(26), 2751–2758. https://doi.org/10.1016/j.jchromb.2011.07.042

Lin, Y., Mcclements, D. J., Xiao, J., Cao, Y., & Liu, X. (2022). In Vitro-In Vivo Study of the Impact of Excipient Emulsions on the Bioavailability and Antioxidant Activity of Flavonoids: Influence of the Carrier Oil Type. *Journal of Agricultural and Food Chemistry*, *71*, 1488–1498. https://doi.org/10.1021/acs.jafc.2c05836

Liu, C., Yang, X., Wu, W., Long, Z., Xiao, H., Luo, F., Shen, Y., & Lin, Q. (2018). Elaboration of curcumin-loaded rice bran albumin nanoparticles formulation with increased in vitro bioactivity and in vivo bioavailability. *Food Hydrocolloids*, *77*, 834–842. https://doi.org/10.1016/J.FOODHYD.2017.11.027

Liu, Y., Siard, M., Adams, A., Keowen, M. L., Miller, T. K., Garza, F., Andrews, F. M., & Seeram, N. P. (2018). Simultaneous quantification of free curcuminoids and their metabolites in equine plasma by LC-ESI–MS/MS. *Journal of Pharmaceutical and Biomedical Analysis*, *154*, 31–39. https://doi.org/10.1016/j.jpba.2018.03.014

Luo, H., Li, Z., Yao, M., McClements, D. J., & Xiao, H. (2022). Impact of excipient emulsions made from different types of oils on the bioavailability and metabolism of curcumin in gastrointestinal tract. *Food Chemistry*, *370*, 130980. https://doi.org/10.1016/j.foodchem.2021.130980

Luo, X., Zhou, Y., Bai, L., Liu, F., Zhang, R., Zhang, Z., Zheng, B., Deng, Y., & McClements, D. J. (2017). Production of highly concentrated oil-in-water emulsions using dual-channel microfluidization: Use of individual and mixed natural emulsifiers (saponin and lecithin). *Food Research International*, *96*, 103–112. https://doi.org/10.1016/j.foodres.2017.03.013

Maa, Y. F., & Hsu, C. C. (1999). Performance of sonication and microfluidization for liquid-liquid emulsification. *Pharmaceutical Development and Technology*, *4*(2), 233–240. https://doi.org/10.1081/PDT-100101357

Marczylo, T. H., Steward, W. P., & Gescher, A. J. (2009). Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. *Journal of Agricultural and Food Chemistry*, *57*(3), 797–803. https://doi.org/10.1021/jf803038f

Marczylo, T. H., Verschoyle, R. D., Cooke, D. N., Morazzoni, P., Steward, W. P., & Gescher, A. J. (2007). Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. *Cancer Chemotherapy and Pharmacology*, *60*(2), 171–177. https://doi.org/10.1007/s00280-006-0355-x

Nazari-Vanani, R., Moezi, L., & Heli, H. (2017). In vivo evaluation of a self-nanoemulsifying drug delivery system for curcumin. *Biomedicine and Pharmacotherapy*, *88*, 715–720. https://doi.org/10.1016/j.biopha.2017.01.102

Nimbkar, S., Leena, M. M., Moses, J. A., & Anandharamakrishnan, C. (2022). Medium chain triglycerides (MCT): State-of-the-art on chemistry, synthesis, health benefits

and applications in food industry. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 21, Issue 2, pp. 843–867). John Wiley and Sons Inc. https://doi.org/10.1111/1541-4337.12926

Ozawa, H., Imaizumi, A., Sumi, Y., Hashimoto, T., Kanai, M., Makino, Y., Tsuda, T., Takahashi, N., & Kakeya, H. (2017). Curcumin β-D-glucuronide plays an important role to keep high levels of free-form curcumin in the blood. *Biological and Pharmaceutical Bulletin*, *40*(9), 1515–1524. https://doi.org/10.1248/bpb.b17-00339

Ozturk, B., Argin, S., Ozilgen, M., & McClements, D. J. (2015). Nanoemulsion delivery systems for oil-soluble vitamins: Influence of carrier oil type on lipid digestion and vitamin D3 bioaccessibility. *Food Chemistry*, *187*, 499–506. https://doi.org/10.1016/j.foodchem.2015.04.065

Pan-On, S., Dilokthornsakul, P., & Tiyaboonchai, W. (2022). Trends in advanced oral drug delivery system for curcumin: A systematic review. *Journal of Controlled Release*, *348*, 335–345. https://doi.org/10.1016/J.JCONREL.2022.05.048

Pandey, A., Chaturvedi, M., Mishra, S., Kumar, P., Somvanshi, P., & Chaturvedi, R. (2020). Reductive metabolites of curcumin and their therapeutic effects. In *Heliyon* (Vol. 6, Issue 11, p. e05469). Elsevier. https://doi.org/10.1016/j.heliyon.2020.e05469

Peng, S., Li, Z., Zou, L., Liu, W., Liu, C., & McClements, D. J. (2018). Enhancement of Curcumin Bioavailability by Encapsulation in Sophorolipid-Coated Nanoparticles: An in Vitro and in Vivo Study. *Journal of Agricultural and Food Chemistry*, *66*(6), 1488–1497. https://doi.org/10.1021/acs.jafc.7b05478

Prasad, S., Tyagi, A. K., & Aggarwal, B. B. (2014). Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. *Cancer Research and Treatment*, *46*(1), 2–18. https://doi.org/10.4143/crt.2014.46.1.2

Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C., & Ramirez-Tortosa, M. C. (2016). Curcumin and health. *Molecules*, *21*(3), 1–22. https://doi.org/10.3390/molecules21030264

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Nanoemulsion delivery systems: Influence of carrier oil on β-carotene bioaccessibility. *Food Chemistry*, *135*(3), 1440–1447. https://doi.org/10.1016/j.foodchem.2012.06.047

Ritter, J. K. (2000). Roles of glucuronidation and UDP-glucuronosyltransferases in xenobiotic bioactivation reactions. *Chemico-Biological Interactions*, *129*(1–2), 171–193. https://doi.org/10.1016/S0009-2797(00)00198-8

Ryu, E. K., Choe, Y. S., Lee, K. H., Choi, Y., & Kim, B. T. (2006). Curcumin and dehydrozingerone derivatives: Synthesis, radiolabeling, and evaluation for β-amyloid plaque imaging. *Journal of Medicinal Chemistry*, *49*(20), 6111–6119. https://doi.org/10.1021/jm0607193

Salvia-Trujillo, L., Sun, Q., Um, B. H., Park, Y., & McClements, D. J. (2015). In vitro and in vivo study of fucoxanthin bioavailability from nanoemulsion-based delivery

systems: Impact of lipid carrier type. *Journal of Functional Foods*, *17*, 293–304. https://doi.org/10.1016/j.jff.2015.05.035

Santos, A. C. C., Amaro, L. B. R., Jorge, A. H. B., Lelis, S. de F., Lelis, D. de F., Guimarães, A. L. S., Santos, S. H. S., & Andrade, J. M. O. (2023). Curcumin improves metabolic response and increases expression of thermogenesis-associated markers in adipose tissue of male offspring from obese dams. *Molecular and Cellular Endocrinology*, *563*, 111840. https://doi.org/10.1016/j.mce.2022.111840

Schultz, S., Wagner, G., Urban, K., & Ulrich, J. (2004). High-pressure homogenization as a process for emulsion formation. *Chemical Engineering and Technology*, *27*(4), 361–368. https://doi.org/10.1002/ceat.200406111

Sharma, R. A., Gescher, A. J., & Steward, W. P. (2005). Curcumin: The story so far. *European Journal of Cancer*, *41*(13), 1955–1968. https://doi.org/10.1016/j.ejca.2005.05.009

Shoji, M., Nakagawa, K., Watanabe, A., Tsuduki, T., Yamada, T., Kuwahara, S., Kimura, F., & Miyazawa, T. (2014). Comparison of the effects of curcumin and curcumin glucuronide in human hepatocellular carcinoma HepG2 cells. *Food Chemistry*, *151*, 126–132. https://doi.org/10.1016/j.foodchem.2013.11.021

Song, Z., Revelo, X., Shao, W., Tian, L., Zeng, K., Lei, H., Sun, H. S., Woo, M., Winer, D., & Jin, T. (2018). Dietary Curcumin Intervention Targets Mouse White Adipose Tissue Inflammation and Brown Adipose Tissue UCP1 Expression. *Obesity*, *26*(3), 547–558. https://doi.org/10.1002/oby.22110

Stohs, S. J., Chen, O., Ray, S. D., Ji, J., Bucci, L. R., & Preuss, H. G. (2020). Highly bioavailable forms of curcumin and promising avenues for curcumin-based research and application: A review. In *Molecules* (Vol. 25, Issue 6, p. 1397). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/molecules25061397

Suresh, D., & Srinivasan, K. (2010). Tissue distribution & elimination of capsaicin, piperine & curcumin following oral intake in rats. *Indian Journal of Medical Research*, *131*(5), 682–691.

Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, *108–109*, 303–318. https://doi.org/10.1016/j.cis.2003.10.023

Teixé-Roig, J., Oms-Oliu, G., Odriozola-Serrano, I., & Martín-Belloso, O. (2022). Enhancing the Gastrointestinal Stability of Curcumin by Using Sodium Alginate-Based Nanoemulsions Containing Natural Emulsifiers. *International Journal of Molecular Sciences 2023, Vol. 24, Page 498*, *24*(1), 498. https://doi.org/10.3390/IJMS24010498

Toden, S., & Goel, A. (2017). The Holy Grail of Curcumin and its Efficacy in Various Diseases: Is Bioavailability Truly a Big Concern? *Journal of Restorative Medicine*, *6*(1), 27–36. https://doi.org/10.14200/jrm.2017.6.0101

Wang, C., Liu, Z., Xu, G., Yin, B., & Yao, P. (2016). BSA-dextran emulsion for

protection and oral delivery of curcumin. *Food Hydrocolloids*, *61*, 11–19. https://doi.org/10.1016/j.foodhyd.2016.04.037

Wei, X., Senanayake, T. H., Bohling, A., & Vinogradov, S. V. (2014). Targeted nanogel conjugate for improved stability and cellular permeability of curcumin: Synthesis, pharmacokinetics, and tumor growth inhibition. *Molecular Pharmaceutics*, *11*(9), 3112–3122. https://doi.org/10.1021/mp500290f

Yi, J., Gan, C., Wen, Z., Fan, Y., & Wu, X. (2021). Development of pea protein and high methoxyl pectin colloidal particles stabilized high internal phase pickering emulsions for β-carotene protection and delivery. *Food Hydrocolloids*, *113*, 106497. https://doi.org/10.1016/j.foodhyd.2020.106497

Yu, H., & Huang, Q. (2012). Improving the oral bioavailability of curcumin using novel organogel-based nanoemulsions. *Journal of Agricultural and Food Chemistry*, *60*(21), 5373–5379. https://doi.org/10.1021/jf300609p

Zhang, H. Y., Arab Tehrany, E., Kahn, C. J. F., Ponot, M., Linder, M., & Cleymand, F. (2012). Effects of nanoliposomes based on soya, rapeseed and fish lecithins on chitosan thin films designed for tissue engineering. *Carbohydrate Polymers*, *88*(2), 618–627. https://doi.org/10.1016/j.carbpol.2012.01.007

Zhu, X., Du, S., Yan, Q., Min, C., Zhou, N., Zhou, W., & Li, X. (2021). Dietary curcumin supplementation promotes browning and energy expenditure in postnatal overfed rats. *Nutrition and Metabolism*, *18*(1), 1–14. https://doi.org/10.1186/s12986-021-00625-5

Table 1. Particle size, span index, ζ-potential and viscosity of curcumin emulsions containing 10% or 50% of oil phase formulated using high-shear homogenization (HSH) or high-shear homogenization and microfluidization (HSH+MF).

Emulsion	Particle size (μm)	Span index	ζ-potential (mV)	Viscosity (mPa·s)
10% HSH	4.87 ± 0.18^{Bb}	1.74 ± 0.01^{Bb}	-61.32 ± 1.04^{Ab}	1.50 ± 0.05^{Aa}
50% HSH	3.52 ± 0.06^{Ba}	1.66 ± 0.01^{Ba}	-68.47 ± 2.97^{Aa}	26.4 ± 0.32^{Ab}
10% HSH+MF	0.72 ± 0.01^{Ab}	1.45 ± 0.01^{Ab}	-60.73 ± 0.93^{Ab}	5.04 ± 0.20^{Ba}
50% HSH+MF	0.52 ± 0.01^{Aa}	1.24 ± 0.02^{Aa}	-62.96 ± 2.03^{Ba}	27.87 ± 0.32^{Bb}

Values are expressed as mean ± standard deviation. Different capital letters indicate significant differences ($p < 0.05$) between emulsions with different formulation methodology and same oil concentration. Different lowercase letters indicate significant differences ($p < 0.05$) between emulsions with different oil concentration and same formulation methodology.

Table 2. Mean particle size, span index, ζ-potential and viscosity of curcumin emulsions containing 50% of oil phase and different surfactant-oil-ratio (SOR).

SOR	Particle size (μm)	Span index	ζ-potential (mV)	Viscosity (mPa·s)
0.05	1.04 ± 0.01 ^{b}	1.79 ± 0.01 ^{b}	-52.83 ± 1.27 ^{c}	7.89 ± 0.11 ^{a}
0.1	0.52 ± 0.01 ^{a}	1.24 ± 0.02 ^{a}	-62.96 ± 2.03 ^{b}	27.87 ± 0.32 ^{b}
0.2	3.73 ± 0.19 ^{c}	2.78 ± 0.14 ^{c}	-68.50 ± 2.51 ^{a}	52.10 ± 1.22 ^{c}

Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences ($p < 0.05$) between emulsions with different oil concentration and same formulation methodology.

Table 3. Pharmacokinetic parameters of curcumin glucuronide (CUR-GLUC), curcumin (CUR), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) after oral administration of a single-dose curcumin emulsion with high oil content (HOE) or curcumin suspension (SP).

Carrier	Parameters	CUR-GLUC	CUR	DMC	BDMC
HOE	C_{max} (ng·ml^{-1})	256.66 ± 68.91 ^{b}	2.43 ± 0.65 ^{a}	1.15 ± 0.14 ^{a}	1.22 ± 0.12 ^{a}
	T_{max} (h)	4	1	0.5	0.5
	AUC_{0-t} (ng·h·ml^{-1})	1556.32 ± 210.44 ^{b}	14.47 ± 0.78 ^{b}	7.88 ± 0.47 ^{b}	9.19 ± 0.39 ^{b}
SP	C_{max} (ng·ml^{-1})	21.76 ± 34.69 ^{a}	1.61 ± 0.18 ^{a}	0.91 ± 0.08 ^{a}	1.05 ± 0.05 ^{a}
	T_{max} (h)	4	0.5	0.5	0.5
	AUC_{0-t} (ng·h·ml^{-1})	121.52 ± 137.93 ^{a}	11.52 ± 0.14 ^{a}	6.91 ± 0.24 ^{a}	7.92 ± 0.16 ^{a}

Data of C_{max} and AUC_{0-t} are expressed as mean ± standard deviation. AUC_{0-t} = area under the plasma concentration–time curve; C_{max} = peak concentration; T_{max} = time to reach peak concentration. Different letters (a, b) indicate significant differences ($p < 0.05$) among carriers.

Table 4. Curcumin (CUR), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) and curcumin glucuronide (CUR-GLUC) concentration in different rat tissues at 2 h or 4 h after the oral administration of curcumin suspension (SP) or curcumin emulsions with high oil content (HOE).

	Vehicle	Time (h)	Concentration in tissue (ng/g tissue)							
			Duodenum	*Jejunum*	*Ileum*	*Colon*	*Liver*	*Kidney*	*Brown*	*White*
CUR	*HOE*	2	562.36 ± 168.27	587.61 ±	840.69 ±	132.15 ±	139.35 ± 52.07 Bb	13.87 ± 5.77 Aa	192.94 ± 11.69 Bb	9.43 ± 4.49 Aa
		4	229.19 ± 128.9	212.79 ±	545.48 ±	444.58 ±	18.61 ± 23.41 Aa	10.75 ± 2.46 Aa	44.70 ± 24.75 Aa	17.91 ± 6.87 Ba
	SP	2	27.66 ± 11.79 Aa	68.83 ± 49.11	481.27 ±	12.00 ± 9.57 Aa	21.86 ± 16.27 Aa	23.03 ± 10.29 Aa	39.42 ± 31.34 Aa	10.66 ± 3.98 Aa
		4	20.15 ± 15.34 Aa	29.34 ± 19.20	200.92 ± 89.47	482.16 ±	10.62 ± 10.56 Aa	16.16 ± 10.04 Aa	30.22 ± 6.74 Aa	6.22 ± 2.35 Aa
DMC	*HOE*	2	136.02 ± 37.35	190.63 ± 67.90	232.85 ± 89.39	36.52 ± 29.19	20.54 ± 9.01 Aa	3.32 ± 2.06 Aa	103.79 ± 51.37 Bb	4.91 ± 0.98 Aa
		4	39.57 ± 23.70 Ba	46.37 ± 32.39	174.90 ± 47.83	123.20 ± 57.76	10.24 ± 4.96 Aa	1.99 ± 1.25 Aa	28.32 ± 21.77 Aa	5.77 ± 1.13 Aa
	SP	2	7.11 ± 3.29 Aa	17.43 ± 11.13	158.55 ± 51.99	10.01 ± 3.79 Aa	10.45 ± 2.49 Aa	5.22 ± 4.54 Aa	14.70 ± 4.78 Aa	6.17 ± 1.31 Aa
		4	7.74 ± 4.74 Aa	12.01 ± 6.28 Aa	90.74 ± 50.87	188.48 ± 39.25	8.49 ± 1.05 Aa	3.76 ± 1.38 Aa	14.45 ± 9.33 Aa	5.64 ± 1.11 Aa
BDMC	*HOE*	2	32.66 ± 11.29 Ba	47.10 ± 17.24	47.79 ± 18.46	10.19 ± 6.98 Aa	7.10 ± 2.15 Ab	2.57 ± 1.14 Aa	12.47 ± 2.07 Bb	3.33 ± 0.42 Aa
		4	12.28 ± 0.70 Ba	4.37 ± 8.61 Ab	10.61 ± 10.70	22.01 ± 8.85 Aa	3.87 ± 0.61 Aa	1.87 ± 1.25 Aa	3.88 ± 1.27 Aa	3.18 ± 1.62 Aa
	SP	2	0.47 ± 1.42 Aa	0.71 ± 1.39 Aa	51.63 ± 14.37	3.80 ± 0.56 Aa	4.28 ± 0.78 Aa	6.93 ± 1.91 Ba	4.09 ± 2.12 Aa	3.94 ± 0.85 Aa
		4	0.35 ± 0.75 Aa	0.15 ± 0.31 Aa	41.91 ± 18.15	26.36 ± 9.61 Ab	4.29 ± 0.99 Aa	3.91 ± 1.85 Aa	5.78 ± 1.40 Aa	4.64 ± 1.67 Aa
CUR-GLUC	*HOE*	2	7.45 ± 1.36 Aa	14.21 ± 5.79 Aa	60.83 ± 24.65	ND	ND	11.79 ± 2.40 Ab	ND	ND
		4	7.02 ± 0.64 Ba	13.33 ± 4.18 Aa	120.93 ± 48.28	ND	ND	ND Aa	ND	ND
	SP	2	6.06 ± 2.89 Ab	8.91 ± 0.80 Aa	44.52 ± 20.40	ND	ND	11.00 ± 1.81 Ab	ND	ND
		4	ND Aa	8.92 ± 1.35 Aa	33.82 ± 13.93	ND	ND	ND Aa	ND	ND

Values are expressed as mean ± standard deviation. Different capital letters (A, B) indicate significant differences ($p < 0.05$) among curcumin carriers at a same time. Different lowercase letters (a, b) indicate significant differences ($p < 0.05$) among same carrier and different time.

Figure captions

Figure 1. Particle size distribution and microscope images of curcumin emulsions containing 10% or 50% of oil phase formulated using high-shear homogenization (HSH) or high-shear homogenization and microfluidization (HSH+MF). (a) Microscope image of emulsion with 50% oil and SOR 0.1 obtained by HSH+MF; (b) Microscope image of emulsion with 10% oil and SOR 0.1 obtained by HSH+MF; (c) Microscope image of emulsion with 50% oil and SOR 0.1 obtained by HSH; (d) Microscope image of emulsion with 10% oil and SOR 0.1 obtained by HSH. Scale bars are 10 μm long.

Figure 2. Particle size distribution and microscope images of curcumin emulsions containing 50% of oil phase and different surfactant-oil-ratio (SOR). (a) Microscope image of emulsion with SOR 0.05; (b) Microscope image of emulsion with SOR 0.1; (c) Microscope image of emulsion with SOR 0.2. Scale bars are 10 μm long.

Figure 3. Backscattering profile of curcumin emulsions containing 50% of lipid phase and a different surfactant-oil-ratio (SOR 0.05, SOR 0.1, and SOR 0.2) during 15 days at 4 ºC.

Figure 4. Plasma-concentration times profile of (a) curcumin glucuronide (CUR-GLUC), (b) curcumin (CUR), (c) demethoxycurcumin (DMC) and (d) bisdemethoxycurcumin (BDMC) after oral administration of a single-dose curcumin emulsion with high oil content (HOE) or curcumin suspension (SP).

CRediT authorship contribution statement:

Júlia Teixé-Roig: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original Draft, Visualization. Gemma Oms-Oliu: Visualization, Conceptualization, Methodology, Writing-Review & Editing, Visualization, Supervision. María Artiga-Artigas: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original Draft, Visualization. Isabel Odriozola-Serrano: Visualization, Conceptualization, Methodology, Writing-Review & Editing, Supervision. Olga Martín-Belloso: Visualization, Conceptualization, Methodology, Writing-Review & Editing, Supervision, Project administration, Funding acquisition.

Highlights:

- Stable high content MCT-emulsions containing lecithin were obtained using microfluidization.
- Curcumin enclosed in MCT-emulsion was better absorbed than in suspension.
- The curcuminoid bioavailability was increased by 10.6-fold when using the MCT-emulsion.

- MCT-emulsion increased free-form curcumin concentration in rat liver and brown adipose tissue.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure 1

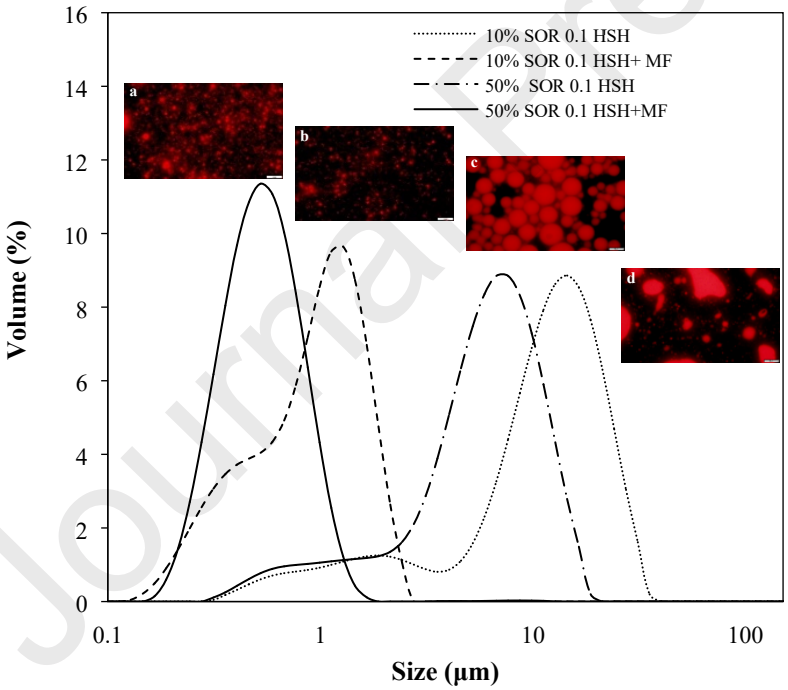


Figure 2

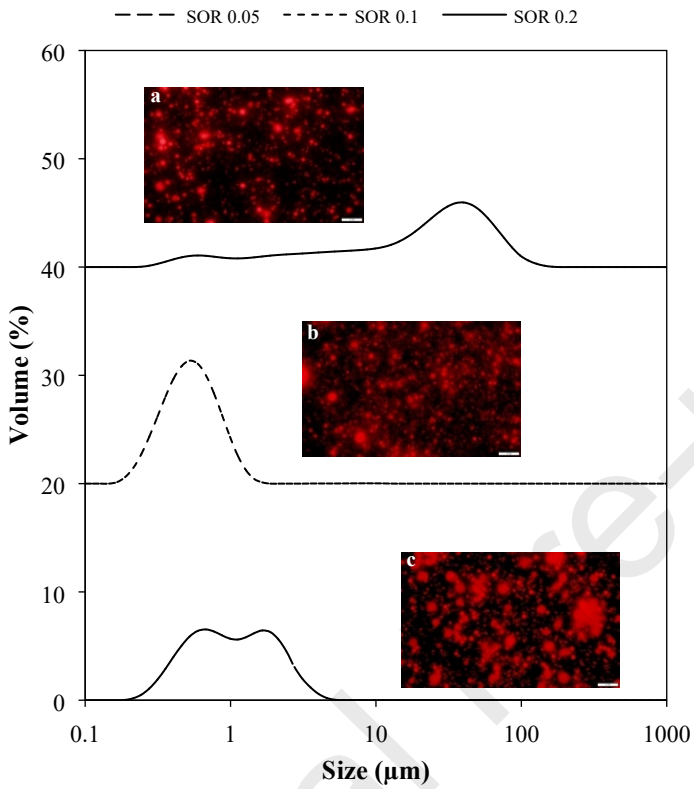


Figure 3

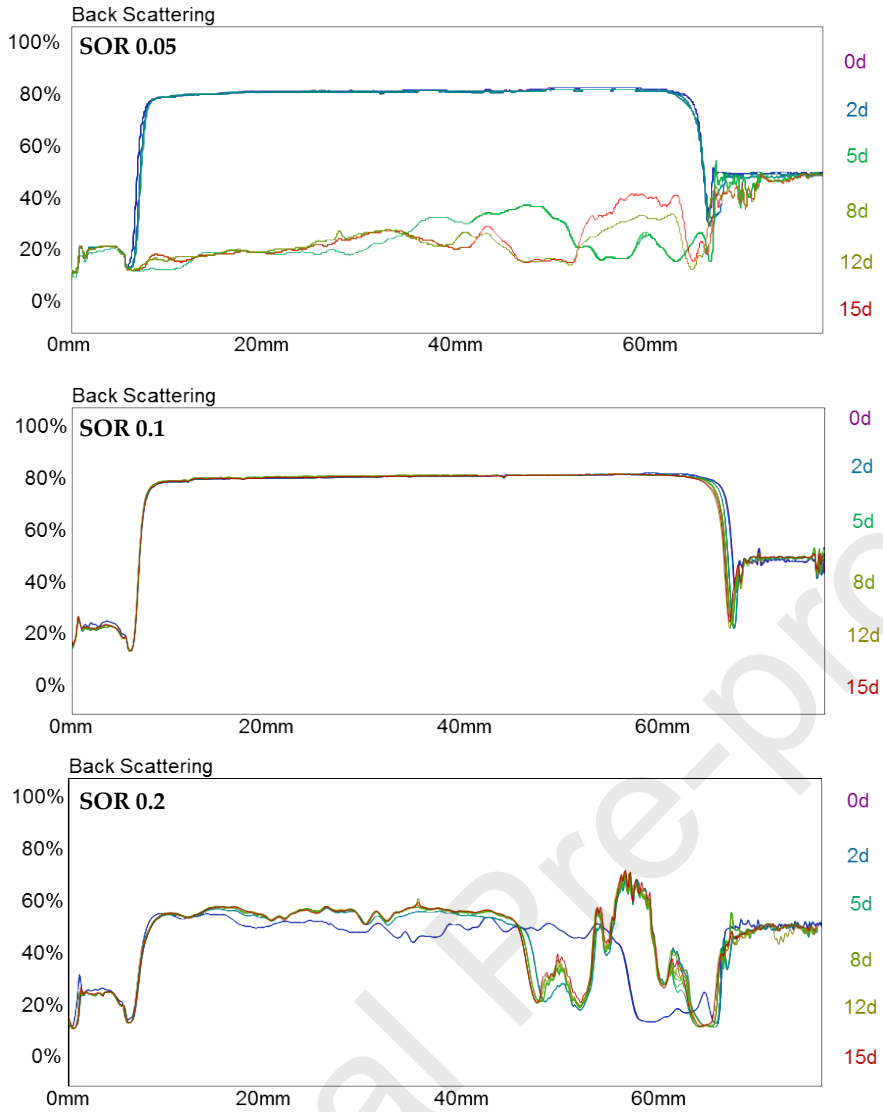
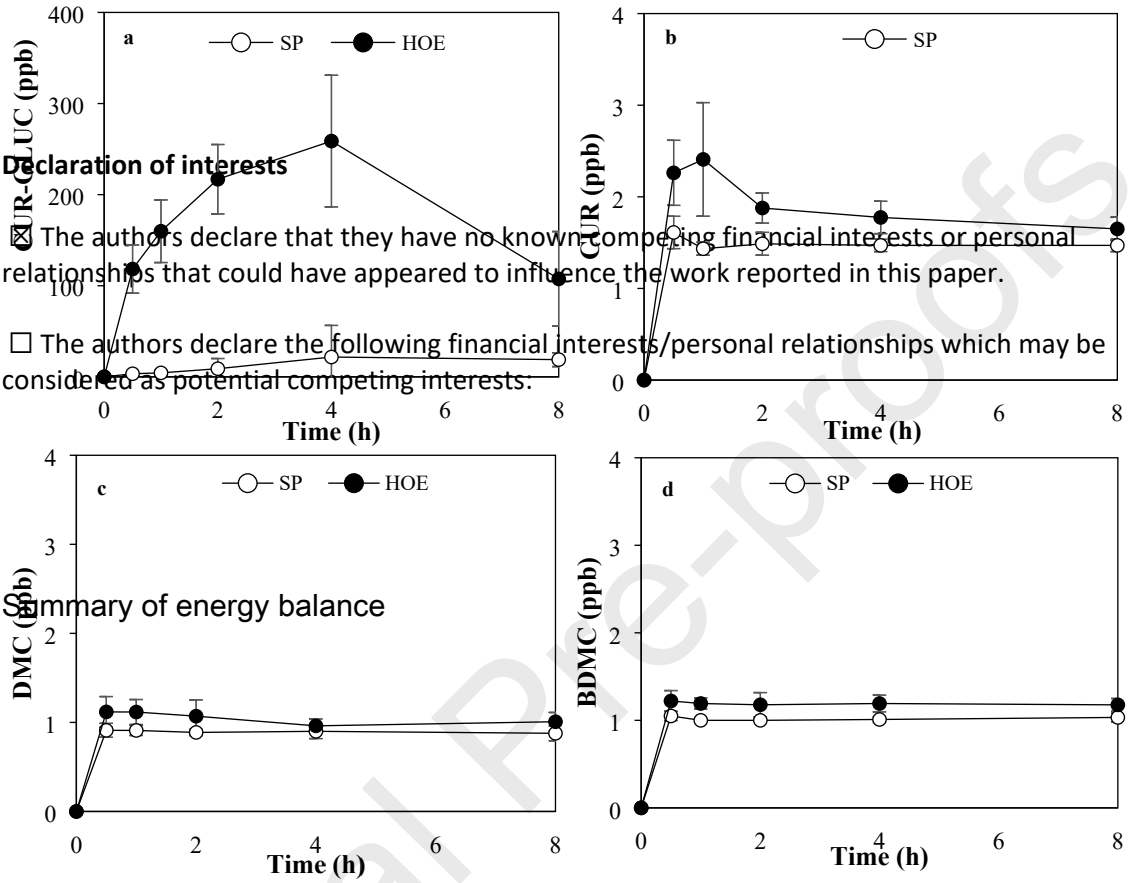


Figure 4



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Summary of energy balance