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Research article

Preparation and characterization of nanoemulsions of curcumin and echium oil

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HIGHLIGHTS

- Curcumin addition increased antioxidant activities of EO nanoemulsions.
- Curcumin incorporated nanoemulsions had significantly higher SDA content after *in vitro* digestion.
- In nanoemulsion form, *in vitro* curcumin bioaccessibility was 35.5%.

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ABSTRACT

The search for the plant origin bioactive compounds is increasing over animal origin compounds. Echium oil (EO) contains high amounts of plant based omega-3 fatty acids. Moreover, curcumin addition may increase the release of these omega-3 fatty acids during digestion. The study's objective is to determine the bioaccessibility of curcumin in simulated intestinal digestion conditions and the release behavior of fatty acids of echium oil from nanoemulsions. We prepared curcumin and EO nanoemulsions with a microfluidizer using two different concentrations of surfactant, Tween 80 (5% and 10%). Emulsion stability tests, antioxidant analysis, in vitro oil release and fatty acid composition assays were conducted. Results showed that curcumin-containing nanoemulsions provide higher radical scavenging activity than the EO nanoemulsions. In addition, *in vitro* bioaccessibility of curcumin after *in vitro* simulated intestinal digestion was calculated as 35.5%. Gas chromatography results of the digested nanoemulsions revealed that curcumin addition decreases oleic acid release while increasing stearidonic acid (SDA) release.

1. Introduction

Long-chain polyunsaturated fatty acids (PUFAs) are crucial for human health. Among these, omega-3 fatty acids are substantially effective for the treatment of diseases, such as cardiovascular diseases, hypertension, various types of cancer, depression, and Alzheimer's disease and strengthening the immune system. Also, they include essential fatty acids (EFA), which the human body cannot synthesize, and for this reason, regular consumption is critical (Us-Medina et al., 2018). Of the omega-3 family, each fatty acid has a unique characteristic on preventing diseases (Botelho et al., 2015).

Plant seed oils, generally, contain high amounts of omega-6 fatty acids; however, they do not contain the omega-3 family, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (James et al. 2003). In the human body, EPA and DHA can be synthesized from α-linolenic acid (ALA) by a series of enzymatic reactions. Delta-6 desaturase (D6D) enzyme is responsible for the conversion of ALA to stearidonic acid (SDA) and linoleic acid (LA) to γ-linolenic acid (GLA) as the first steps of n-3 and n-6 production pathway (Brown et al., 2019; Lee et al., 2019). Nonetheless, both ALA and LA are the substrates of D6D; thus, constituent metabolite production is not sufficient. SDA is more efficient in increasing EPA levels than ALA (Guil-Guerrero, 2007). However, plant-based edible oils have lower amounts of SDA than animal oils (Lenihan-Geels et al., 2013). *Echium plantagineum* oil contains SDA, which can be metabolized to EPA in humans. Furthermore, the functionality of SDA in the human body is similar to EPA and DHA obtained from animal sources, according to recent studies (Gray et al., 2010).

Curcumin, extracted from *Curcuma longa* rhizome, is a significant bioactive compound widely used in food, medicine, and cosmetics. The vast utilization of curcumin is justified by antimicrobial, antioxidant or

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disease-preventive properties (Artiga-Artigas et al., 2018). Moreover, it affects omega-3 fatty acid production from n-3 precursors. Curcumin promotes D6D (FADS2) and elongase 2 enzymes in the brain and liver, which significantly increases the conversion of ALA to DHA according to *in vivo* studies (Wu et al., 2015). On the other hand, curcumin has low bioavailability and water solubility; moreover, it is expeditiously metabolized to less active products in the human body. Poor absorption and rapid elimination of curcumin from the body reduce its bioavailability, thereby preventing its prevalent usage (Anand et al., 2007). Curcumin bioaccessibility is also another critical property that other components of the food products can influence. For instance, longer chain fatty acids are more likely to form micelles, which leads to increased solubilization capacity, providing higher curcumin bioaccessibility (Shah et al., 2016). Nanoemulsion applications are solutions to overcome low bioavailability, low bioaccessibility and low water solubility of curcumin (Joung et al., 2016).

To the best of our knowledge, there are no reports on the biological activity of EO nanoemulsions incorporated with curcumin. In this context, this work evaluated the effects of curcumin addition on the composition and the *in vitro* digestibility of EO nanoemulsions.

2. Materials and methods

2.1. Materials

Echium oil (*Echium plantagineum*) was purchased from De Wit Speciality Oils (Texel, Netherlands); curcumin (95% purity) from Herbaturk Co. (Istanbul, Turkey) and Tween 80 was purchased from Merck (Whitehouse, NJ). The lipase from porcine pancreas, and sodium chloride were provided from Sigma-Aldrich (St. Louis, MO). All other reagents and solvents were purchased from Sigma Chemical Co. and Merck (Whitehouse, NJ), and were of analytical or chromatographic grade.

2.2. Methods

2.2.1. Preparation of echium oil nanoemulsions

Two different ratios of Tween 80 (5% and 10%) were used for the preparation of curcumin incorporated echium oil nanoemulsions (CEONE) and echium oil nanoemulsions (EONE) as a control. As seen from Table 1, EO content of nanoemulsions was kept at 10% and curcumin content of CEONE was 40 mg curcumin/100 mL emulsion. Nanoemulsion preparation was conducted triplicate.

First of all, aqueous phase containing distilled water and surface active agent, Tween 80, and EO phase were mixed by using a high speed homogenizer (Ultra-Turrax T18, IKA, Germany) at 12,500 rpm for 10 min. After that, homogenous samples were exposed to 15,000 psi pressure for 5 times by employing LM 10 Microfluidizer (Microfluidics, Newton, Mass., USA). Obtained samples were stored at refrigerated temperatures (4 °C) during analysis period.

Table 1. Compositions, zeta potential, droplet size and polydispersity index values of nanoemulsions.†,‡

Sample	Surfactant (%) (v)	Zeta Potential Value* (mV)	Droplet Size* (d.nm)	Polidispersity Index*
EONE1	10	-39.6 ± 2.1^{a}	127 ± 2^{a}	0.28 ± 0.02^{a}
CEONE1	10	-27.6 ± 1.3^{b}	155 ± 2^{ab}	0.23 ± 0.02^{a}
EONE2	5	-27.6 ± 1.9^{b}	161 ± 1^{ab}	0.23 ± 0.01^{a}
CEONE2	5	-28.9 ± 2^{b}	171 ± 1^{b}	0.25 ± 0.01^{a}

†Abbreviations: EONE = Echium oil nanoemulsion, CEONE = Curcumin added echium oil nanoemulsion.

‡Results are given as the mean value and standard deviation of 9 different measurements of each sample for zeta potential, droplet size and polydispersity index values.

* Different letters for each column indicates statistical significance ($p < 0.05$).

2.2.2. Nanoemulsion stability tests

CEONE and EONE samples were put in a 20 mL graduated cylinder and waited for 21 days at refrigerated temperature to observe whether phase separation occurred or not (Arancibia2016). For oil-in-water emulsions, continuous phase is denser than dispersed phase. Thus, creaming occurs as a result of phase separation (Karakus, 2018). In order to determine the emulsion stability, creaming index values were calculated according to phase separation. Creaming index (CI) was determined according to Eq. (1) (Arancibia et al., 2016).

$$CI\ (\%) = (H_S/H_E) \times 100 \quad (1)$$

H_S is the volume of cream layer (mL) and H_E is the volume of the emulsion (mL).

2.2.3. Zeta potential and droplet size measurements

Nanoemulsions were diluted 1000 times by adding distilled water at room temperature and zeta potential assay was performed according to Lei et al. (2019). Zeta potential and size of the particles were measured at 25 °C and at a voltage of 3.9 V by a dynamic light scattering instrument, Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK).

2.2.4. Curcumin solubility in echium oil

Curcumin solubility assay was performed according to Cunico et al. (2017). Curcumin at an amount of 10 mg was added to 10 mL of EO and the dilutions were done based on a ratio curcumin to EO of 1:200 (v/v). Moreover, known amounts of curcumin-EO solutions (0.02, 0.04, 0.08 and 0.1 mg curcumin/mL EO) were prepared and homogenized using vortex. For the precipitation of insoluble curcumin, samples were centrifuged at 400 rpm for 30 min (Nüve, CN180, Turkey). Curcumin solubility in EO was determined by measuring the absorbances at 428 nm (LR45227, Thermo Fischer Scientific, USA). EO was run as a control. In order to assess curcumin solubility, calibration curve (R^2: 0.9587) was drawn and solubility was calculated accordingly.

2.2.5. Antioxidant potential

In order to explore the effects of curcumin addition and nanoemulsification on the antioxidant property of EO, ABTS and DPPH antioxidant capacity tests were applied on curcumin and nanoemulsions before and after *in vitro* digestion. These assays were performed according to the method of Ak and Gülçin (2008). For DPPH assay, 0.1 mM 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical in ethanol solution was produced. ABTS solution was prepared with 2 mM 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) in H_2O and 2.45 mM potassium persulfate. The solution was stored in a dark place at room temperature for 12 h. Afterwards, in order to have an absorbance of 0.750 at 734 nm, prepared solution was diluted. The solutions were added to nanoemulsions with a ratio of 3:1 (v/v). For the radical reactions to occur, samples both having and not having radicals were stored in the dark for 30 min. At the end of this period, sample absorbances were read at 734 nm and 517 nm for DPPH and ABTS tests, respectively. Calibration curves were drawn according to known concentrations of Trolox in ethanol (0.01, 0.02, 0.04, 0.08 and 0.1 M) for both DPPH (R^2: 0.9975) and ABTS (R^2: 0.9659) assays. Antioxidant analysis results are given as M Trolox/g sample.

Since curcumin has a high antioxidant potential and it is not water soluble, using the organic phases of the digested solutions results in more accurate antioxidant results. For this reason, after *in vitro* digestion assay, digested solutions were treated with 5 mL of n-hexane and then centrifuged at 1000 rpm for 3 min. Oil phases were obtained after the removal of hexane at 40 °C by rotary evaporator (IKA, HB10 basic, Germany) (Yüksel and Şahin-Yeşilçubuk, 2018).

2.2.6. In vitro simulated digestion of nanoemulsions

Gastrointestinal environment was imitated to observe *in vitro* bioavailability of nanoemulsions. Bioavailability assay was carried out

pursuant to the method of Yüksel-Bilsel and Şahin-Yeşilçubuk (2019) with a slight difference. Since enzymatic lipid digestion occurs in intestinal stage, gastric phase was not performed in the study. Intestinal fluid was composed of 0.3% bile salts, 0.9% sodium chloride, 1% pancreatin and distilled water. Intestinal fluid (20 mL) and pancreatic lipase (4.8 mg/mL) addition into samples having 0.5 g of EO took place. In order to provide intestinal digestion environment, pH of the solutions were adjusted to 7 by using 1 M NaOH. IKA brand KS4000i orbital shaker (Germany) was operated at 120 rpm for 2h to mimic the churning of digestion system. At the end of every 30 min period, obtained sample solutions (10 mL) were titrated with 0.1 N NaOH after the addition of 1% (w/v) phenolphthalein. Free fatty acid (FFA) release was calculated by using Eq. (2).

$$FFA\ (\%) = 100 \times (V \times N \times M_{oil}/m_{oil} \times 2) \quad (2)$$

V, the volume of NaOH used for titration; N, normality of NaOH; M_{oil}, molecular weight of EO (calculated as 871,332 g/mol regarding to its fatty acid content); and m_{oil}, total mass of EO (0.5 g for this experiment).

2.2.7. Determination of the fatty acid composition of echium oil and nanoemulsions after in vitro digestion

For the removal of enzymes from the digested solutions, FFAs were extracted according to the method of Yüksel and Şahin-Yeşilçubuk (2018), which was mentioned in antioxidant potential section (Section 2.2.5). After the recovery of oil phase, fatty acid methylation took place by the addition of 6% methanolic HCl acid (3 mL) onto fatty acids and these solutions were incubated at 75 °C for 2 h. At the end of the incubation period, n-hexane (2 mL) and 0.1 M KCl were included into solutions and centrifuged at 1000 rpm for 3 min. In order to remove the moisture of fatty acid methyl esters, they were filtered through anhydrous Na_2SO_4 column (Yüksel and Şahin-Yeşilçubuk, 2018).

With the aim of determining the fatty acid composition of EO, 1 mL hexane was added to 15 mg EO and mixed by vortex for 30 s. Afterwards, 1 mL of sodium methoxide (0.4 mol) was added to mixture and mixing was recurred for 30 s. The mixture was waited for 15 min and upper hexane layer was collected for further analysis (Zahran and Tawfeuk, 2019).

Fatty acid methyl esters were analyzed in GC-FID (gas chromatography coupled with a flame ionization detector) (Hewlett Packard, ABD) implemented in HP 6890 Gas Chromatography equipment by the use of Supelco ™ SP-2380 (60 m × 0.25 mm × 0.20 μm) (Sigma-Aldrich, ABD) capillary column. Detector and injector temperatures were adjusted at 250 °C. Column temperature was held at 140 °C for 5 min, then increased to 240 °C by 4 °C/min acceleration and held for 10 min. Helium was used as carrier gas and the flow rate was set as 1.2 mL/min. A 1 μL sample was injected into the GC. Peaks obtained after the analysis were compared to the peaks of standard solutions, and fatty acids were determined by the retention time similarity (Zahran and Tawfeuk, 2019).

2.2.8. Curcumin bioaccessibility by in vitro simulated intestinal digestion

Curcumin bioaccessibility assay was done according to Ahmed et al. (2012). Digested sample, at an amount of 5 mL was mixed with 5 mL chloroform. This mixture was centrifuged at 1750 rpm for 10 min. Lower phase was collected while upper phase was treated with the same procedure. The first and second phases were blended and their absorbances were determined at 419 nm using spectrophotometer. Calibration curve was drawn according to the absorbances of different concentrations of curcumin - chloroform solutions.

2.2.9. Statistical analysis

The results were given as means ± standard deviation of three independent repetitions and two experimental repetitions. One factor analysis of variance (ANOVA) test was used for statistical analysis of the results, at the 95% level ($p < 0.05$) of significance. Also, Tukey test was applied to determine significant differences. In order to perform statistical analysis tests, MINITAB software (version 16.1.0) program was used.

3. Results and discussion

3.1. Nanoemulsion stability tests

Nanoemulsions were stored for 21 days at 4 °C. At the end of the time period, there were no phase separations observed for both EONE and CEONE samples (CI % = 0). Tween 80 was used as surface active agent for the nanoemulsion samples. It can be interpreted that Tween 80 was a suitable agent for EO – water nanoemulsion systems as phase separation did not take place. Furthermore, microfluidization technique was applied for nanoemulsion preparation, which could be an appropriate method for obtaining stable nanoemulsions. Since there were no differences between EONE and CEONE samples in terms of stability, it could be concluded that curcumin incorporation had no impact on nanoemulsion stability.

Aranciaba et al. (2016) studied olive oil nanoemulsions composed of Tween 80 as emulsifying agent and carboxymethyl cellulose (CMC) as thickening agent. Physical stability tests were applied to nanoemulsions having different CMC:olive oil ratio and nanoemulsions stored at different temperatures (5 °C and 20 °C). Phase separation took place at 15th and 21st days of storage. It was interpreted that the lower the storage temperature, the higher physical stability was observed. Moreover, as CMC:olive oil ratio increased, more stable nanoemulsions were obtained.

In another study, Ahmed et al. (2012) investigated oil-in-water emulsions and nanoemulsions incorporated with curcumin. Lipids differing in triacylglycerol content (short, medium and long chain) were emulsified using β-lactoglobulin. The effects of dissimilar emulsion types and lipid contents on physical stability were examined for 24 h storage at ambient temperature. It was concluded that emulsions consisting of short chain triacylglycerols were the least stable when lipid types were compared. Furthermore, there were no phase separations observed for nanoemulsions. Thus; conventional emulsions were less stable than nanoemulsions, which were obtained by microfluidization.

Emulsification process, stabilizer type and amount, and lipid content affect the physical stability of emulsions as previously discussed. It can be interpreted that EO, which has high amount of long chain fatty acids, is therefore a suitable potential oil to obtain stable oil-in-water nanoemulsions. Moreover, microfluidization can be an appropriate emulsification process providing stable nanoemulsions.

3.2. Zeta potential and droplet size measurements

Zeta potential and droplet size measurements were done for all nanoemulsions. Results are given in Table 1.

For the estimation of nanoemulsion stability, zeta potential is one of the widely used methods measuring the attractions and repulsions between the particles. Zeta potential values of the prepared emulsions were varied between -27.6 mV and -39.6 mV. The magnitude of zeta potential value determines emulsions stability and emulsions having low magnitude tend to be decompose. Roughly, emulsions having zeta potential values more than +30 or less than -30 values are said to be stable (Larsson et al., 2012). In addition, it is shown that nanoemulsions having 10% surface active agent are more stable than the others.

Zeta sizes differed between 127 nm and 171 nm. Nanoemulsions having more surface active agent (10%) were smaller in size than the others, which complies with the studies of Joung et al. (2016) and Chang and McClements (2014). Sugasini and Lokesh (2012) studied sunflower oil, flaxseed oil, and coconut oil nanoemulsions with curcumin addition. Zeta sizes were determined as 115 ± 12 nm, 103 ± 11 nm, and 685 ± 14 nm, respectively. Curcumin added nanoemulsions having PUFAs were indicated to be more efficient than curcumin added nanoemulsions having saturated fatty acids.

Polydispersity index value was calculated as 0.25 ± 0.01 PdI. For dynamic light scattering assay, nanoemulsions having a value of nearly 0.2 PdI demonstrate monodisperse distribution (Matos et al., 2018).

Ganta and Amiji (2009) studied flaxseed oil and curcumin nanoemulsion, and the results were -42.89 mV for zeta potential, 132 ± 1.7 nm for zeta size, and 0.3 PdI for polydispersity index. Matos et al. (2018) compared the results for both curcumin included and not included medium chain triglyceride (MCT) oil nanoemulsions and results were not significantly different from each other. When the zeta analysis results of nanoemulsion having 10% surfactant and having 5% surfactant were compared no statistically significant difference was observed ($p < 0.05$). It can be seen from Table 1 that curcumin addition into nanoemulsions had no effect on correlative effects on either zeta values or polydispersity index. According to zeta potential assay, the most appropriate samples were found to be EONE1 and CEONE1; thus, further analysis were conducted with these samples.

3.3. Curcumin solubility in echium oil

Absorbance of 10 mg curcumin/10 mL EO solution was measured as 0.66 ± 0.12 and the maximum solubility of curcumin in EO was calculated as 0.07 mg curcumin/mL EO based on the calibration curve.

Takenaka et al. (2013) investigated the solubility of curcumin in different edible oils, such as, MCT, rapeseed, soybean, olive and corn oil. MCT oil was found to have the highest solubility of curcumin. Other edible oils had a rather less solubility compared to MCT oil. Joung et al. (2016) compared the solubility of curcumin in MCT, coconut, olive and corn oil, and corn oil was found to have the same solubility as EO. The most suitable solvent for curcumin among these oils was found as MCT. Also, Ma et al. (2018) studied curcumin solubility of MCT, canola, sunflower, linseed and corn oil. Although solubility results for the studies mentioned above differed from each other, the common ground between the studies was on the highest solubility of curcumin in MCT oil. Takenaka et al. (2013) commented that MCT oil has a relatively more polar nature resulting from its triacylglycerol content. Thus, it can provide a better interaction between oil and curcumin molecules.

Due to the merely water soluble nature of curcumin, its incorporation into emulsion systems is determined by the solubility in the lipophilic phase of the emulsion (Ahmed et al., 2012). Hence, measuring the maximum solubility of curcumin in EO helped to determine how much curcumin is needed for the EO nanoemulsion.

3.4. Antioxidant potential

Curcumin and nanoemulsions before and after *in vitro* digestion were analyzed in terms of antioxidant capacity. Antioxidant analysis results were given as M Trolox equivalent. DPPH and ABTS results of samples are given in Figure 1. It is clearly shown that curcumin addition increased the antioxidant capacity of nanoemulsions, significantly ($p < 0.05$). Following that, antioxidant potentials of digested and not digested nanoemulsions differ from each other, significantly ($p < 0.05$). There was an increase in the digested samples in terms of antioxidants capacities. This can be explained by the chemical change of antioxidant components' functionalities (Lima et al., 2019; Bhatt and Patel, 2013; Pavez-Guajardo et al., 2020). ABTS results were higher than DPPH results for all nanoemulsion samples. Different transfer mechanisms occur in these assays. Moreover, DPPH radicals are less reactive than ABTS radicals (Ak and Gülçin, 2008). It can be interpreted that due to the differences in radical activities and radical scavenging mechanisms, antioxidant activity results differed from each other.

Sari et al. (2015) compared the antioxidant activity of curcumin and its nanoemulsion form which were 3.53 ± 0.11 mM Trolox/mg and 3.33 ± 0.02 mM Trolox/mg, respectively. It was specified that curcumin incorporated nanoemulsions had a slightly lower activity. Artiga-Artigas et al. (2018) determined antioxidant capacities of curcumin loaded nanoemulsions having different emulsifiers with different concentrations. For Tween 20 added nanoemulsions, antioxidant activities of samples were found as 792, 1029 and 1279 μg Trolox/g sample for concentrations of 0.5%, 1.0% and 2.0% (w/w) surfactant, respectively.

In another study, antioxidant capacities of curcumin incorporated MCT oil nanoemulsions were investigated. It was found that the more surface active agent used, the more soluble curcumin in the oil phase; thus, antioxidant capacity increased due to the increased solubility (Joung et al., 2016). Also, Kiralan et al. (2014) remarked that surface active agent provided better dispersion of oil phase in aqueous phase with raising the antioxidant capacity. On the other hand, Richa and Choudhury (2019) specified that in nanoemulsions, if polysaccharides

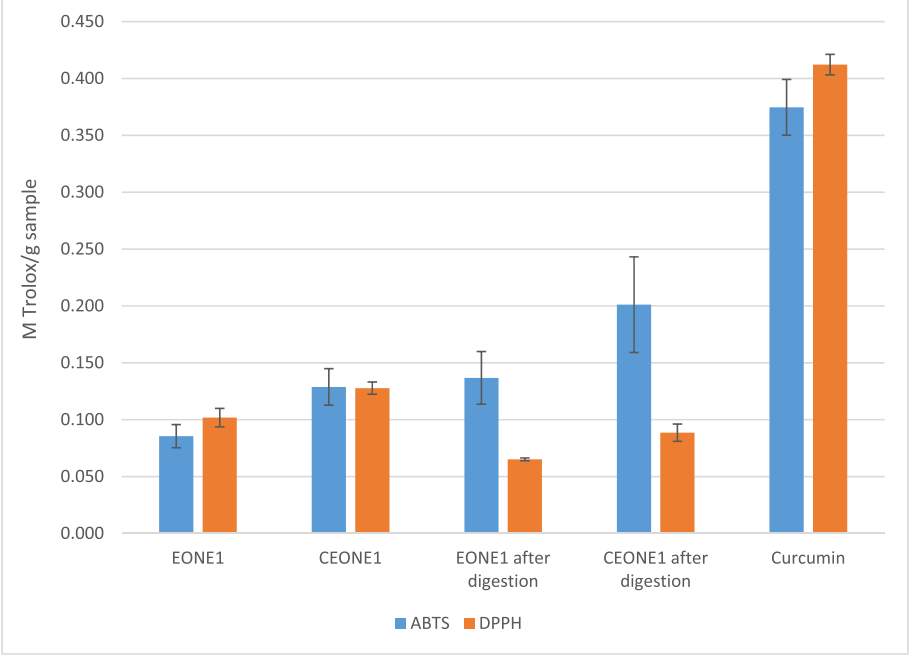

Figure 1. Antioxidant capacities of EONE1 and CEONE1 before and after *in vitro* digestion, and curcumin determined by DPPH and ABTS methods. †Abbrevations: EONE1 = Echium oil nanoemulsion, CEONE1 = Curcumin added echium oil nanoemulsion.

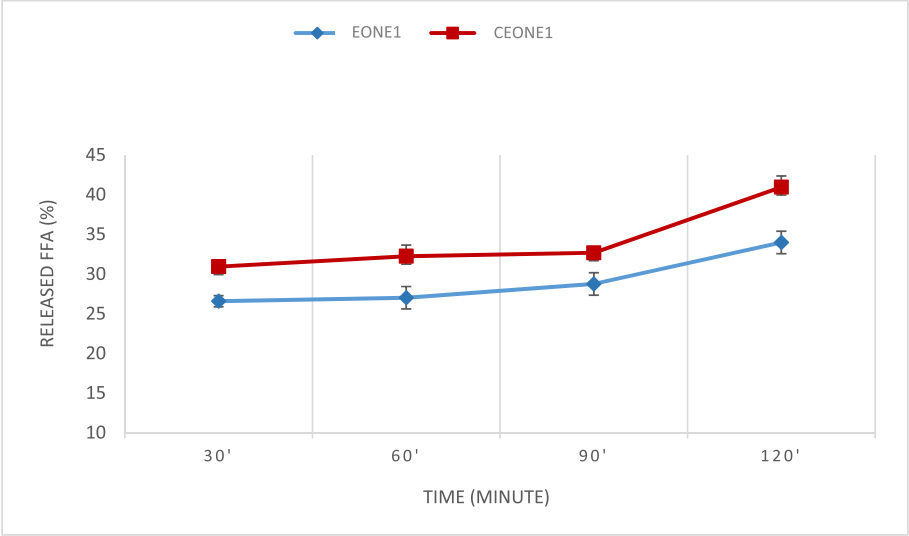

Figure 2. Released FFA% during *in vitro* intestinal digestion. †Abbrevations: EONE1 = Echium oil nanoemulsion, CEONE1 = Curcumin added echium oil nanoemulsion.

were used, they could increase scavenging activity of curcumin whereas if surfactants, such as Tween 20, were used, they could decrease the activity substantially. Furthermore, *in vitro* cell culture studies suggested that curcumin could have a pro-oxidant activity by increasing the production of reactive oxygen species (ROS) (Sandur et al., 2007; Atsumi et al., 2005; Syng-Ai et al., 2004). Therefore, in our study, the possibility of curcumin to show pro-oxidant effect with the interaction of Tween 80 was not negligible.

3.5. *In vitro simulated digestion of nanoemulsions*

Intestinal phase of digestion was mimicked for 2 h and samples were collected in each 30 min period. Figure 2 shows the fatty acids released during simulated digestion. At the end of the 120 min, fatty acid release for EONE1 was 33.98%, and for CEONE1 it was increased to 40.95%. Although FFA release was higher in CEONE1, there were no statistically significant differences between EONE1 and CEONE1 ($p < 0.05$) during *in vitro* digestion.

An incremental FFA release was observed for both EONE1 and CEONE1 between 90 and 120 min interval. Zero order kinetics are applied for lipolysis process. Therefore, enzyme binding increases with the increased product concentration due to the alteration of emulsion interface (Wickham et al., 1998). Incremental FFA release could be explained by increased lipase dispersion between the aqueous phase and the emulsion interface as lipase was activated when binding to an insoluble emulsified substrate.

Studies relevant to curcumin nanoemulsions show that the amount of fatty acid released is dependent on the type of the lipid. According to a

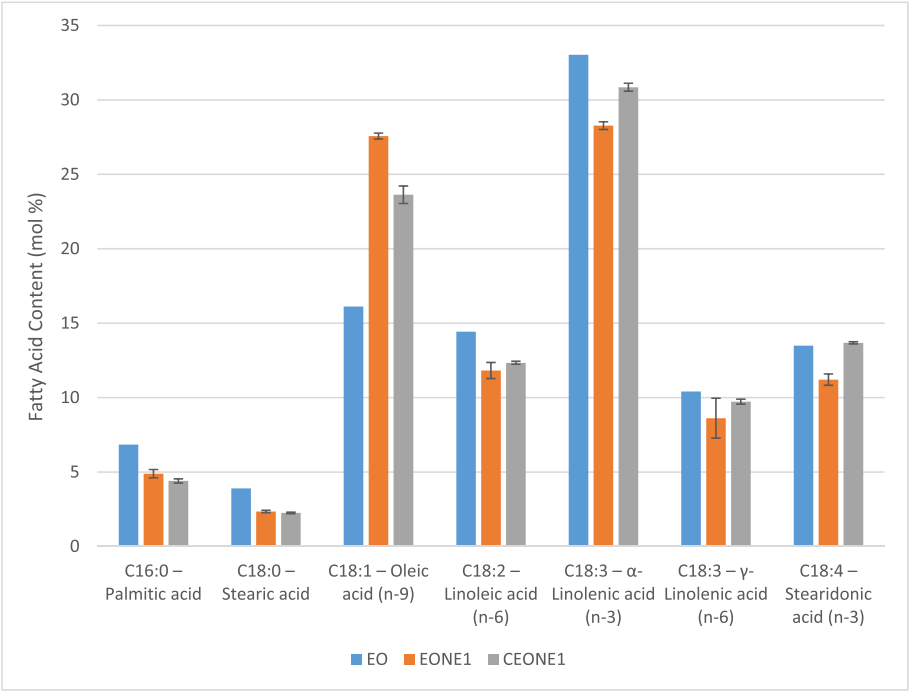

Figure 3. Fatty acid compositions of EO and *in vitro* digested EONE1 and CEONE1. †Abbrevations: EO = Echium oil, EONE1 = Echium oil nanoemulsion, CEONE1 = Curcumin added echium oil nanoemulsion.

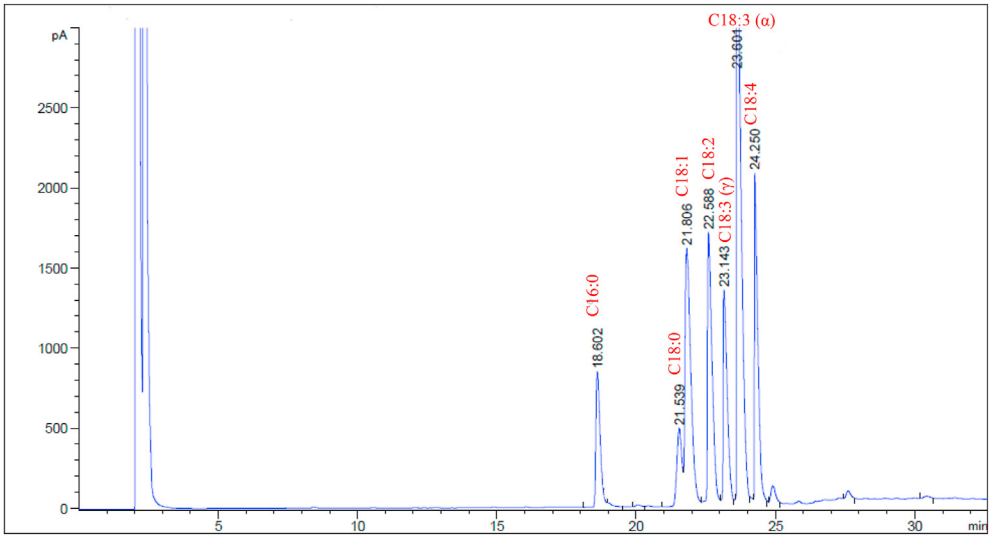

Figure 4. Chromatogram of EO fatty acid composition. †Abbrevations: EO = Echium oil, C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (ã-linolenic acid), C18:3 (á-linolenic acid), C18:4 (stearidonic acid).

research, released fatty acid amounts were highest for medium chain fatty acids (MCFA) with 113% FFA amount, and lowest for long chain fatty acids (LCFA) with 68% FFA amount. This issue can be explained by the physicochemical mechanisms of fatty acids (Ahmed et al. 2012).

Dey et al. (2019) compared *ex vivo* bioavailability of conventional fish oil emulsion and fish oil nanoemulsion. It was remarked that nano-emulsification process increased bioavailability of fish oil significantly, due to reduced droplet size.

Joung et al. (2016) investigated the effect of surfactant concentration on lipid release during *in vitro* digestion. In the study, Tween 20 was used as surfactant. It was found that lipid release decreased while surfactant concentration increased. This phenomenon was explained by the higher surface activity of surfactant than lipase, which resulted in a competition in the favor of surfactant.

3.6. Determination of the fatty acid composition of echium oil and nanoemulsions after in vitro digestion

By comparing the retention times of reference standards to samples, all fatty acids were identified. Fatty acid profiles of EO and nanoemulsions after *in vitro* digestion are given in Figure 3. Also, Figure 4 shows a chromatogram of EO fatty acids. For mono-unsaturated fatty acids, oleic acid release were seen to be decreased when EO and nanoemulsions were compared, and it can be seen from Figure 3 that curcumin addition also decreased the release of oleic acid amount from 27.57% to 23.63%. When PUFA contents were examined, it was found that EONE1 had a lower SDA content than EO; whereas, CEONE1 had a slightly higher content than the oil. Thus, it can be interpreted that curcumin addition had an positive impact of SDA release. Statistical analysis demonstrated that curcumin addition led to the decayed release of oleic acid, and elevated release of SDA, significantly ($p < 0.05$). For the other fatty acids, there were no statistically significant differences observed between EONE1 and CEONE1 samples ($p > 0.05$).

3.7. Curcumin bioaccessibility by in vitro simulated intestinal digestion

Digested CEONE1 sample after 120 min was subjected to curcumin bioaccessibility assay. Curcumin amount was calculated to be 0.71 ± 0.06 mg/mL. Taking initial amount into consideration, it can be concluded that 35.5 % curcumin in the nanoemulsions was bioaccessible meaning that, 35.5% is the remaining fraction after *in vitro* intestinal simulated digestion which is suitable for the absorption in the gut.

Pinheiro et al. (2016) studied curcumin added lactoferrin and alginate-stabilized nanoemulsions. For these nanoemulsions, curcumin bioaccessibility was found about 4%. The reason for low bioaccessibility was explained by lactoferrin interactions with other molecules. In another study, Ahmed et al. (2012) implied that curcumin bioaccessibility could be as high as 38% for nanoemulsions having long chain fatty acids.

Pinheiro et al. (2013) investigated curcumin incorporated corn oil nanoemulsions during *in vitro* digestion. Emulsifier type was found to be effective on curcumin bioaccessibility, which was explained by the highly lipophilic nature of curcumin. Bioaccessibility assays revealed that nanoemulsions having high amount of released FFA provided higher bioavailable curcumin. Noack, Oidtmann, Kutza and Mäder (2012) interpreted that the curcumin release during *in vitro* digestion was affected by the features of media and lipid carrier. When these studies were taken into consideration, curcumin behaviour during *in vitro* digestion was concluded to be influenced by characteristics of nano-emulsions, such as lipid type and emulsifier content.

4. Conclusions

Curcumin and EO nanoemulsions were obtained and analyzed in this study. The effects of curcumin on the fatty acid release, antioxidant capacity and omega-3 content of EO were investigated. Curcumin incorporation into nanoemulsions resulted in significantly higher antioxidant capacities than control samples, both before and after *in vitro* digestion. Curcumin was found as 35.5% bioaccessible in simulated gastrointestinal environment, in accordance with similar literature studies. Moreover, curcumin significantly increased SDA release of nanoemulsions, which is an intermediate precursor for EPA and DHA biosynthesis from ALA.

For further studies nanoemulsions having different curcumin:EO ratios can be investigate and alternate nanoemulsification methods could be applied in order to differentiate methods in terms of nanoemulsion stability. Furthermore, additional *in vitro*, *in vivo* and *ex vivo* digestion studies and fatty acid analysis should be performed to assess if EO and curcumin could increase DHA concentrations in serum, liver, heart and brain lipids which have implications for meeting the DHA requirements of vegan populations.

Declarations

Author contribution statement

Aslı İNAL: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Hande YENİPAZAR: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Neşe ŞAHİN-YEŞİLÇUBUK: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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