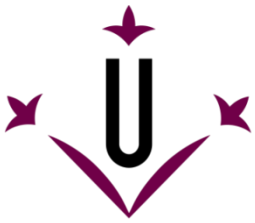

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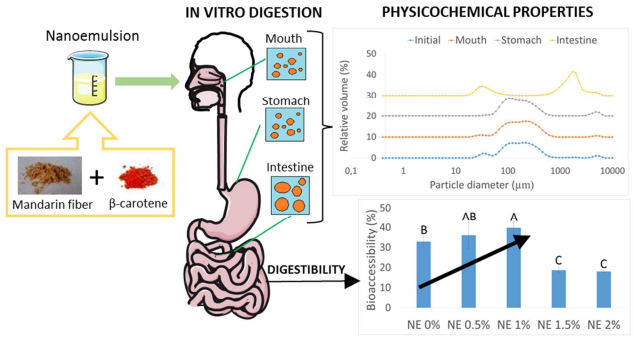

Title: Influence of mandarin fiber addition on physico-chemical properties of nanoemulsions containing β-carotene under simulated gastrointestinal digestion conditions

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Abstract

There is a lack of knowledge about how soluble fiber used as stabilizer may influence physicochemical properties of nanoemulsions during the lipid digestion process. In this study, different concentrations of mandarin fiber (0.5, 1.0, 1.5, 2.0 g/100 g) were added to nanoemulsions containing oil enriched with β-carotene (4 g/100 g emulsion) and tween 20 (1.5 g/100 g emulsion). As nanoemulsions were subjected to the different phases of an *in vitro* simulated gastrointestinal tract (GIT), its particle size was gradually increased. Furthermore, the higher the mandarin fiber content in the nanoemulsion, the greater the particle size. Nanoemulsions containing concentrations of mandarin fiber over 1.5 g/100 g showed the lowest ζ-potential, meaning that the droplets may become unstable and were likely to aggregate. Besides, adding until 1 g of mandarin fiber/100 g was effective to enhance bioaccessibility of the β-carotene incorporated in nanoemulsions. The present study provides valuable information on the phenomenology of incorporating mandarin fiber within β-carotene enriched nanoemulsions.

KEY WORDS: mandarin fiber, nanoemulsion, β-carotene, *in vitro* digestion, bioaccessibility.

Abbreviations

GIT, gastrointestinal tract; DF, dietary fibers; FFAs, free fatty acids

1. Introduction

Carotenoids are natural lipophilic pigments found in many natural sources, like fruits and vegetables, with β-carotene being one of the most important for its pro-vitamin A activity (Ferreira & Rodriguez-Amaya, 2008; Yonekura & Nagao, 2007). Protecting cells from free radicals (Kiokias & Gordon, 2004; Maiani et al., 2009), and preventing several chronic diseases, such as cancer, heart disease and aging (Wang, Liu, Mei, Nakajima, & Yin, 2012), are some of the attributed features to β-carotene owing to its strong antioxidant activity. However, incorporating β-carotene in foods can be challenging due to its low water-solubility but also poor stability (Boon, McClements, Weiss, & Decker, 2010). An outstanding technology based on nanoemulsion delivery systems as a way to encapsulate and protect lipophilic compounds dispersed in aqueous media could be a possible method to design healthier foodstuffs. The small droplets of nanoemulsions present advantages such as physical stability, improvement of optical quality and increased bioaccessibility of lipophilic compounds (Nik, Langmaid, & Wright, 2012). Preventing nanoemulsions from structure breakdown can be possible by incorporating stabilizers (Odriozola-Serrano, Oms-Oliu, & Martín-Belloso, 2014). Dietary fibers (DF) are important components of the human diet that are being applied in emulsion-based foods thanks to their stabilizing, texturizing properties, as well as health-promoting ingredients (Dikeman & Fahey, 2006; Eastwood, Martin A. & Morris, 1992; Elleuch et al., 2011). On the one hand, chronic illnesses like diabetes or obesity besides coronary heart and gastrointestinal diseases are linked to a low intake of DF (Anderson et al., 2009). On the other hand, its consumption in certain amounts may inhibit lipid absorption (Jenkins, Kendall, & Ransom, 1998; Lairon,

1996; Torcello-Gómez & Foster, 2016; Yokoyama et al., 2011), causing a decrease on the bioaccessibility of health-related compounds easily absorbed when lipids are present (Chawla & Patil, 2010). DF can be found not only in fruits and vegetables, but can also be obtained from fruit by-products, and mandarin fiber is especially rich in DF soluble fraction and thus, a feasible option to be used as a emulsion stabilizer. Hence, the purpose of this work was to analyze the effect of mandarin fiber on lipid digestibility of oil-in-water nanoemulsions containing β-carotene by subjecting them through an *in vitro* simulated GIT. Changes in the physicochemical properties of nanoemulsions during digestion were also characterized.

2. Material and methods

2.1. Materials

β-carotene, Tween 20, mucin (from porcine stomach), pepsin (from porcine gastric mucosa), lipase (from porcine pancreas), bile extract (porcine) and all the solvents were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Corn oil was from a local supermarket. Mandarin fiber was kindly donated by Indulleida S.A. (Alguaire, Spain). Its proximate analysis was provided by the manufacturer and is presented in Table 1. Milli-Q water was used to prepare emulsions and reagents of the experiment.

2.2. Methods

2.2.1. Nanoemulsions preparation

Firstly, β-carotene was dispersed in corn oil (0.5 g/100 g) by sonicating (1 min) and heating (<50 ºC, 5 min) to obtain the lipid phase. Then, mandarin fiber at

different concentrations (0.5, 1.0, 1.5 or 2.0 g/100 g) was solubilized in milli-Q water using a homogenizer (Ultra-Turrax, Janke & Kunkel, Staufen, Germany) at 9500 rpm for 5 min, obtaining the aqueous phase. Once both phases were ready, a coarse emulsion was prepared by putting together 4.0 g lipid phase, 1.5 g Tween 20 and 94.5 g aqueous phase per 100 g of emulsion, and mixing with a homogenizer (9500 rpm, 2 min). Lastly, a microfluidizer (M-110P, Microfluidics, Newton, MA, USA), equipped with a 75μm ceramic interaction chamber (F20Y) at an operational pressure of 30,000 psi, was used to form the nanoemulsions by passing the coarse emulsions for 5 cycles.

The main goal of the study was to study the mandarin addition effect on the physicochemical properties along an *in vitro* GIT, as well as, lipid digestibility and β-carotene bioaccessibility. Finally, it should be noted that the specific effect of the microfluiditzation process was studied by preparing the coarse emulsion and nanoemulsion without mandarin fiber, so as to determine the influence of particle size on β-carotene bioaccessibility. The term “emulsions” has been used through the text to involve all nanoemulsions and coarse emulsions, irrespective of their composition.

2.2.2. *In vitro* digestions

An *in vitro* gastrointestinal tract (GIT) was used to mimic the digestion process, adapting the proceeding proposed by Salvia-Trujillo, Qian, Martín-Belloso, & McClements (2013a).

For mouth stage, 50 mL of simulated saliva fluid, which contained mucin (0.03 g/mL) and various salts (Sarkar, Goh, & Singh, 2009), was mixed with 50 mL of

119 emulsion, adjusting its pH to 6.8. Finally, it was incubated in an orbital shaker
120 for 10 min at 100 rpm and 37 ºC. Following**,** the liquid proceeding from the
121 mouth phase was mixed with gastric fluids (Sarkar, Goh, Singh, & Singh, 2009)
122 at the same volume ratio simulate the gastric stage. The pH of the mixture was
123 adjusted to 2.5 and placed inside an orbital shaker at 37 ºC for 2 h with a
124 constant agitation (100 rpm). The gastric fluids were prepared by adding 2 g of
125 NaCl, 7 mL of HCl and 0.0032 mg/mL of pepsin in 1 L of milli-Q water. Finally,
126 to simulate intestinal stage a pH-stat (Metrohm USA Inc., Riverview, FL, USA)
127 was used (McClements & Li, 2010). The sample proceeding from the gastric
128 phase was placed in a water bath at 37 ºC. Solutions prepared using phosphate
129 buffer (0.005 mol/L, pH 7) consisting of 9.3 mL of bile extract (46.87 mg/mL)
130 and 1.0 mL of calcium chloride (110 mg/mL), were added to the sample
131 followed by an adjustment of pH to 7.0. Afterwards, 2.5 mL of lipase (24 mg/mL)
132 dissolved in phosphate buffer was also added. To compensate the free fatty
133 acids (FFAs) that were released during the lipid digestion, the pH was
134 constantly maintained at 7.0 by adding dropwise a NaOH solution. The volume
135 of NaOH was recorded after 2 h, employed to calculate the lipid digestibility
136 defined by the production of FFA (%) in the intestinal phase, using equation 1.

$$\text{FFA (\%)} = \frac{V_{NaOH} \times C_{NaOH} \times M_{oil}}{2 \times m_{oil}} \times 100 \qquad \text{Eq. (1)}$$

137

138 where V_{NaOH} is NaOH volume (mL) used to compensate the FFAs during the
139 digestion, C_{NaOH} is NaOH molarity (0.25 mol/L), M_{oil} is corn oil molecular weight
140 (800 g/mol), m_{oil} is corn oil total weight present in the emulsions (g).

141

2.2.3. Physicochemical and rheological properties

The physicochemical properties of emulsions before and during the different stages of the *in vitro* GIT (mouth, stomach, intestine) were determined with respect to droplet size, electrical charge and viscosity.

To determine nanoemulsions droplet size, a Zetasizer NanoZS (Malvern Instruments Ltd, Worcerstershire, UK) was used, working at 633 nm and 25 ºC, equipped with a backscatter detector. This device performs size measurements using a process called Dynamic Light Scattering (DLS), which measures Brownian motion and related it to the size of the particles. Prior to analysis, emulsions were diluted (1/10) in the appropriate solutions: saliva fluids for the mouth phase, gastric fluids for the stomach phase and buffer phosphate (pH 7) for the intestinal phase. Meanwhile, the droplet size of the coarse emulsion without mandarin fiber was determined using a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK), reporting the particle size as the surface area mean diameter (d_{32}). The Mie theory is employed for calculate the size of emulsions, fixing a refractive index of the corn oil of 1.473 and 1.333 for the water.

The emulsions electrical charge (ζ-potential) was determined using a Zetasizer NanoZS (Malvern Instruments Ltd, Worcerstershire, UK). Emulsions were previously diluted (1/10) as in the droplet size tests.

The viscosity was studied by a SV-10 vibro-viscometer (A&D Company, Tokyo, Japan).

2.2.4. Carotenoid extraction and quantification

To extract and quantify the carotenoids present in the emulsions, the method described by Morales-De La Peña, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso (2011) was used with some changes. Fifteen grams of emulsions were put together with magnesium hydroxide carbonate, butylated hydroxytoluene (BHT) and ethanol-hexane solution (4:3 v/v) under N_2 atmosphere. After agitation, the liquid was filtered under vacuum, washed with ethanol-hexane solution (4:3 v/v) and filtered with solvents. Then, all filtrates were washed with 100 g/L sodium chloride solution and water. The organic part was evaporated in a rotary evaporator and the residue was put under N_2 atmosphere, adding diethyl ether (DE) and methanolic KOH (0.5 mol/L) with BHT (1 g/L). Then, DE was added again and the solution was evaporated until dryness. The residue was dissolved by adding DE, evaporated it under N_2 and stored at -18 ºC until the chromatographic analysis. Before injecting into the HPLC, the carotenoid extract was reconstituted with 1 mL of methanol:tert-butyl methyl ether solution (70:30 v/v). β-carotene was identified using a reverse-phase C18 Spherisorb ODS2 (5 µm) stainless steel column (4.6 mm 250 mm), with the need of a gradient elution in order to separate β-carotene. The mobile phase consists of: methanol/ammonium acetate 0.1 mol/L, milli-Q water, methyl tert-butyl ether and methanol. The flow rate was established at 1 mL/min during 60 min and the column was fixed at 30 ºC. UV-vis spectral data and their retention times were used to determine the β-carotene present in the vials (Cortés, Esteve, Frígola, & Torregrosa, 2004; Mouly, Gaydou, & Corsetti, 1999) being quantified by comparing them with external β-carotene standards.

2.2.5. Bioaccessibility determination

A fraction resulting from the intestinal phase was centrifuged (AVANTI J-25, Beckman Instruments Inc., Fullerton, CA, USA) at 4000 rpm for 40 min at 4 ºC (Qian, Decker, Xiao, & McClements, 2012). The upper part of the resulted liquid was collected and considered being the micelle fraction in which the β-carotene was present. In some samples, a layer of non-digested oil could be observed in the top, not considering it. Lastly, the β-carotene bioaccessibility was calculated using the equation 2.

$$\text{Bioaccessibility} = 100 \times \frac{C_{micelle}}{C_{initial}} \qquad \text{Eq. (2)}$$

where $C_{micelle}$ is the carotenoid concentration of the micelle fraction and $C_{initial}$ the initial carotenoid concentration of emulsion.

The carotenoid concentration present in the micelle fraction was determined following the procedure explained above (2.2.4).

3. Statistical analysis

All tests were evaluated in duplicate while three replicate determinations were measured for each parameter. Interpretation of the variance (ANOVA) was examined using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md, USA).

4. Results and discussion

4.1. Particle Size

In the absence of mandarin fiber, the particle size of the coarse emulsion was 19200.0 ± 1686.4 nm, and it was efficiently lowered to 166.7 ± 8.1 nm, after

214 microfluidization (Table 2, Fig. 1). Microfluidization treatment consists of
215 pressurising the emulsions by passing them through an interaction chamber
216 which provokes impact forces and cavitation phenomena to reduce the
217 emulsions droplet size (Maa & Hsu, 1999). As can be seen in Fig. 1, the mean
218 particle size of initial nanoemulsions when mandarin fiber was added did not
219 appreciably change, but statistically significant differences existed when they
220 contained more than 1.5 g of mandarin fiber/100 g of nanoemulsion compared
221 to those with less mandarin fiber concentration. It is known that fiber is not
222 attracted to the surfaces of non-ionic surfactants due to electrostatic and steric
223 repulsion forces. Therefore, the increase of particle size in initial nanoemulsions
224 containing high concentration of mandarin fiber can be attributed to the creation
225 of an osmotic attraction between droplets (Espinal-Ruiz, Parada-Alfonso,
226 Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014) due to the
227 exclusion of non-absorbed mandarin fiber from the droplets surface.
228 During mouth and stomach phases, nanoemulsions droplets were quite
229 resistant to coalescence and breakdown because they presented similar size to
230 initial nanoemulsions (Fig. 1). In the present work, nanoemulsions were
231 stabilized using Tween 20, a non-ionic surfactant defined to be highly surface-
232 active because of the hydrophilic head group that form the molecule. Surface-
233 active substances were present in the oral and gastric fluids, and so the
234 surfactant incorporated seemed to rest at the droplets surface (Qian et al.,
235 2012). After the intestinal phase, all the nanoemulsions increased of particle
236 size up to 305.8 nm, indicating that flocculation or coalescence phenomena
237 occurred. In agreement with our results, other authors concluded that
238 nanoemulsions droplets were more susceptible to coalescence during the

intestinal phase, the stage where the lipid digestion takes place (Salvia Trujillo et al., 2013a). Morevover, other surface-active substances such as phospholipids, bile salts and lipase could have displaced Tween 20 molecules initially present in the droplets surface, bringing out a reduce on droplet stability. However, nanoemulsions with 0.5 and 1.0 g of mandarin fiber/100 g presented lower mean particle size in the intestinal phase compared to those nanoemulsions with a higher concentration of fiber. High pectin concentration from the mandarin fiber may have formed hydrogel structures when calcium ions are present in the intestine.

The mean particle size of the coarse emulsion without mandarin fiber changed significantly after stomach and intestinal phases, reaching values of 22860 and 28400 nm, respectively (Table 2). It might be expected that lipid digestion reduces droplets size, but droplet aggregation can occur as a consequence of changes in interfacial characteristics (Ozturk, Argin, Ozilgen, & McClements, 2015). Indeed, this droplet aggregation could lead to an increase of particle size, explained by the formation of micelles, undigested lipids droplets, insoluble calcium soaps, among other particles (Yao, Xiao, & McClements, 2014; Zou et al., 2016).

4.2. Electrical charge

Emulsions had negative surface charges with values ranging between -16.8 and -25.8 mV (Fig. 2). The surfactant utilized (Tween 20) would not be supposed to confer any charge to the droplets. However, some studies have concluded that non-ionic surfactants tended to have a considerable negative charge thereby

the presence of free fatty acids not only from the oil but also from the surfactant, as well as the adsorption of hydroxyl ions (OH-) present in the aqueous phase of emulsions (Chang & McClements, 2016; Mun, Decker, & McClements, 2005) . The addition of high amounts of negatively charged mandarin fiber in the emulsions (≥1.5 g/100 g), results in a decrease in negative repulsive forces between oil droplets. Competitive adsorption of mandarin fiber molecules and other species (hydroxyl ions, anionic impurities or free fatty acids) combined with compounds present in the emulsions, could have induced a neutralization of the electrical charge values.

The electrical charge along the GIT was similar in all emulsions. Generally, the emulsions ζ-potential after the exposure to the mouth had similar values to the initial emulsions (Fig. 2). Other authors have suggested that an appreciable increase of the negative charge could be caused by the absorption of mucin molecules negatively charged present in the saliva fluids to the droplet surfaces (Sarkar, Goh, Singh, et al., 2009). The particles' electrical charges after the stomach phase were less negative (between -4.3 and -7.3 mV) than those obtained after mouth phase. The salts present in the water of emulsions when they are in the stomach phase are known to screen electrostatic interactions and consequently increase the ζ-potential (Mun, Decker, Park, Weiss, & McClements, 2006). Meanwhile, the ζ-potential of all emulsions after the intestinal phase was significantly more negative, reaching electrical charges like those of the mouth and initial phases. This tendency could be attributed to the presence of surface-active anionic substances at droplets surfaces like bile salts and phospholipids from intestinal fluids or free fatty acids formed during lipid digestion (Pouton & Porter, 2008; Reis, Holmberg, Watzke, Leser, & Miller,

2009; Singh, Ye, & Horne, 2009). In addition, nanoemulsions with ≥1 g of mandarin fiber/100 g exhibited values of ζ-potential less negative in gastric and intestinal phases than those nanoemulsions with fewer mandarin fiber concentration. These results suggest that mandarin fiber, which is rich in pectin, might associate with the oil droplets by forming electrostatic interactions under a gastric environment (Espinal-Ruiz et al., 2014), which can be due to the less negative charge of the droplets and mandarin fiber at pH 2.5.

4.3. Viscosity

Regarding viscosity, no significant differences were noted between the initial coarse emulsion (1.04 mPa.s) and its nanoemulsion (1.08 mPa.s), both without mandarin fiber. Adding to nanoemulsions 1 and 2 g of mandarin fiber/100 g increased significantly its viscosity. The higher the concentration of mandarin fiber, the greater the viscosity of the emulsions was, reaching values of 9.11 mPa.s in nanoemulsions containing 2 g of mandarin fiber/100 g (Fig. 3). Mandarin fiber is rich in soluble fibers and has been applied in food and beverage industry during many years as thickening and gelling agent, as well as colloidal stabilizer. Actually, this type of components have several unique properties that capacitate them to be used as a matrix for the entrapment and/or delivery of different substances (Sáenz, Estévez Ana, & Sanhuenza, 2007).

Emulsions presented similar viscosity throughout the GIT, regardless of the mandarin fiber concentration (Fig. 3). Nanoemulsions with lower amount of 0.5 g of mandarin fiber/100 g and coarse emulsions without mandarin fiber presented the highest viscosity after the mouth phase because of the mucin addition. Otherwise, nanoemulsions containing over 0.5 g of mandarin fiber/100

313 g presented the lowest viscosity under simulated intestinal conditions.
314 Generally, viscosity tended to decrease as nanoemulsions passed through the
315 GIT, which can be due to the progressive dilution that occurs when in every
316 phase of the GIT the relevant fluids are added.
317
318 **4.4. Oil Digestibility**
319 In general, it was observed an initially rapid increase in the total free fatty acids
320 (FFA) released from emulsions throughout the first minutes of intestinal phase
321 digestion, followed by a progressive increment until the end. Accordingly, other
322 authors reported that the major amount of the corn oil in emulsions with pectin
323 was digested within the first ten minutes of intestinal phase (Zhang, Zhang,
324 Zhang, Decker, & McClements, 2015).
325 In Fig. 4 it can be appreciated that the initial rate along the first fifteen minutes
326 of intestinal phase was higher in nanoemulsions than in coarse emulsions,
327 irrespective of their composition. However, statistically significant differences
328 only existed during the first five minutes of the process. Surface area of oil
329 droplets exposed to lipase changed according to particle size of emulsions.
330 Hence, nanoemulsions would favor the contact between the oil droplets and
331 lipase, promoting the digestibility of the oil droplets. Nevertheless, the total
332 number of FFAs released after 2 h of intestinal digestion was similar for coarse
333 emulsion and nanoemulsions (74.7 and 74.6%, respectively), both without
334 mandarin fiber.
335 Adding mandarin fiber to nanoemulsions produced a decrease of FFAs
336 realesed during the intestinal digestion, with values ranging from 59.6% to
337 62.2% (Fig. 5). Espinal-Ruiz et al., (2014) reported a depletion in the rate and

338 extent of lipid digestion when high concentrations of hydrocolloids such us
339 pectin are present, resulting in a decrease in the total FFAs. In the present
340 study, nanoemulsions were prepared with mandarin fiber, which contains
341 soluble pectin, as it is shown in Table 1. The presence of pectin in
342 nanoemulsions could possibly affect lipid digestion: (i) binding free calcium ions
343 thereby reducing the amount available to remove long chain FFAs from droplet
344 surfaces; (ii) forming a coating round the droplets, by which could inhibit the
345 access of lipase to the lipid droplets; (iii) aqueous phase viscosity may increase
346 in consequence of mixing and/or diffusion processes; (iv) influence the way of
347 lipase to the oil droplets as a result of modified floc formation and structure
348 (Zhang et al., 2015).
349

350 **4.5. β-carotene bioaccessibility**

351 The bioaccessibility of β-carotene was found to significantly increase when
352 decreasing the droplet size of emulsions. In this sense, β-carotene
353 bioaccessibility increased from 24% in coarse emulsion up to 33% in
354 nanoemulsion, both without mandarin fiber (Fig. 6). In the same way, Salvia
355 Trujillo et al., (2013a) observed that the β-carotene bioaccessibility of coarse
356 emulsion was lower than in nanoemulsions, presenting values of 34% and 59%,
357 respectively. For the latter, spectrophotometer was used in the β-carotene
358 quantification which could be the main cause of the different bioaccessibility
359 results.
360 Particle size as well as digestibility would be supposed to have influence over
361 bioavailability of encapsulated lipophilic nutraceuticals (McClements & Xiao,
362 2012). Besides, bioactive components present in coarse emulsions may be

363 captured within the non-digested lipid fraction and consequently not be
364 released. Furthermore, substances produced as a result of the digestion such
365 as free fatty acids and monoacylglycerols, can form mixed micelles that can
366 solubilize and transport lipophilic components to the enterocytes where they are
367 absorbed (Cho et al., 2014). In our study, coarse emulsions and nanoemulsions
368 without mandarin fiber showed similar digestibility, thus the number of mixed
369 micelles present to solubilize the β-carotene should be similar. The low β-
370 carotene bioaccessibility of coarse emulsions could be associated to the
371 changes produced in the particles structure after lipid digestion, changing their
372 solubilizing capacity (Salvia Trujillo, Qian, Martín-Belloso, & McClements,
373 2013b). As can be seen in Fig. 6, bioaccessibility of β-carotene tended to
374 increase with the addition until 1 g of mandarin fiber/100 g nanoemulsion, but
375 higher concentrations led to lower bioaccessibility levels, being reduced until
376 18.7% and 18.0% for nanoemulsions containing 1.5 and 2 g of mandarin
377 fiber/100 g respectively, without statistical difference between them. These
378 results can be explained by rheological changes in the nanoemulsions when
379 adding high concentrations of mandarin fiber (≥1 g mandarin fiber/100 g
380 nanoemulsion). Actually, the presence of a certain amount of fiber could affect
381 the absorption of carotenoids (Riedl, Linseisen, Hoffmann, & Wolfram, 1999). In
382 this way, dietary fiber is supposed to decrease bioaccessibility by (i) hiding the
383 contact with micelles and small intestine, (ii) interacting with bile salts and lipase
384 (iii) and slowing down the transport of digestive enzymes because of the
385 viscosity increase (Verrijssen et al., 2014).

386

387 **5. Conclusions**

Particle size and ζ-potential of nanoemulsions during the GIT were similar irrespective of the mandarin fiber content. Conversely, viscosity of nanoemulsions appreciably increased when mandarin fiber was present at high concentrations. Based on the results observed, emulsion droplet initial size has an important role within lipid digestion. In fact, the initial digestion rate during the intestinal phase of nanoemulsions was faster than coarse emulsion. However, adding mandarin fiber to nanoemulsions produced a decrease of FFAs released. Finally, β-carotene bioaccessibility of emulsions increased appreciably as the initial droplet size decreased and until 1 g of mandarin fiber/100 g emulsion was added. The present work provides important phenomenological information of nanoemulsions containing mandarin fiber when they are subjected to different gastrointestinal phases using an *in vitro* method. However, more studies are required to define whether the presence of mandarin fiber in nanoemulsions could influence the β-carotene absorption, by using *in vivo* models.

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557

FIGURE CAPTIONS

Fig. 1.

The impact of the *in vitro* digestion phases (initial ■ , mouth ▨ , gastric ▩ , intestinal □) on the droplet size of nanoemulsions with β-carotene containing different concentrations of mandarin fiber (g/100 g). Equal capital letters indicate no significant differences ($p < 0.05$) of nanoemulsions droplet size during digestion phases. Equal lowercase letters indicate no significant differences ($p < 0.05$) of the droplet size among nanoemulsions within the same digestion phase. The values are the mean values of 2 experiments with standard deviation error bars.

Fig. 2.

Influence of *in vitro* digestion phases (initia ■ , mout ▧ , gastri ▩ , intesti □ l) on the ζ-potential of coarse emulsion without mandarin fiber (CE 0) and nanoemulsions (NE) containing different concentrations of mandarin fiber (g/100 g). Equal capital letters indicate no significant differences ($p < 0.05$) of the ζ-potential of an emulsion during digestion phases. Equal lowercase letters indicate no significant differences ($p < 0.05$) of the ζ-potential among emulsion types within the same digestion phase. The values are the mean values of 2 experiments with standard deviation error bars.

Fig. 3.

Influence of *in vitro* digestion phases (initial ■ , mouth ▨ , gastric ▩, intestinal □) of coarse emulsion without mandarin fiber (CE 0) and nanoemulsions (NE) containing different concentrations of mandarin fiber (g/100 g). Equal capital letters indicate no significant differences ($p < 0.05$) of the viscosity of an emulsion during digestion phases Equal lowercase letters indicate no significant differences ($p < 0.05$) of the viscosity among emulsion types within the same digestion phase. The values are the mean values of 2 experiments with standard deviation error bars.

Fig. 4.

Impact of emulsion droplet size (coarse emulsion and nanoemulsion) and mandarin fiber concentration (g/100 g) on the volume (mL) of NaOH during the intestinal phase to maintain the pH to 7. Equal capital letters indicate no significant differences ($p < 0.05$) of NaOH volume used to maintain the pH 7 among emulsion types. (: coarse emulsion without mandarin fiber; : nanoemulsion without mandarin fiber; : nanoemulsion with 0.5 g mandarin fiber/100 g; : nanoemulsion with 1 g mandarin fiber/100 g; : nanoemulsion with 1.5 g mandarin fiber/100 g; : nanoemulsion with 2 g mandarin fiber/100 g). Error bars are based on the standard deviation of two experiments.

Fig. 5.

Total free fatty acids (FFA) produced in the intestinal phase of the coarse emulsion without mandarin fiber (CE 0) and nanoemulsions (NE) containing different concentrations of mandarin fiber (g/100 g). Equal capital letters indicate no significant differences ($p < 0.05$) of the oil digestibility among emulsion types. Error bars are based on the standard deviation of two experiments.

Fig. 6.

β-carotene bioaccessibility (%) of coarse emulsion without mandarin fiber (CE 0) and nanoemulsions (NE) containing different concentrations of mandarin fiber (g/100 g). Equal capital letters indicate no significant differences ($p < 0.05$) of β-carotene bioaccessibility between emulsion types. Error bars are based on the standard deviation of two experiments.

Table 1. Proximate analysis of mandarin fiber.

Composition	(g/kg)
Soluble fiber (pectin)	289.10
Total carbohydrate	531.28
Protein	101.87
Total fat	9.70
Ashes	37.12

Table 2. Mean particle size of initial coarse emulsion during the different stages of the *in vitro* gastrointestinal tract (GIT).

Phase digestion	Mean particle size (nm)
Initial	19200.0 ± 1686.4a
Mouth	19850.0 ± 1456.7a
Stomach	22860.0 ± 7895.5b
Intestine	28400.0 ± 7185.5b

Different letters mean significant differences of particle size along digestion phases ($p < 0.05$).

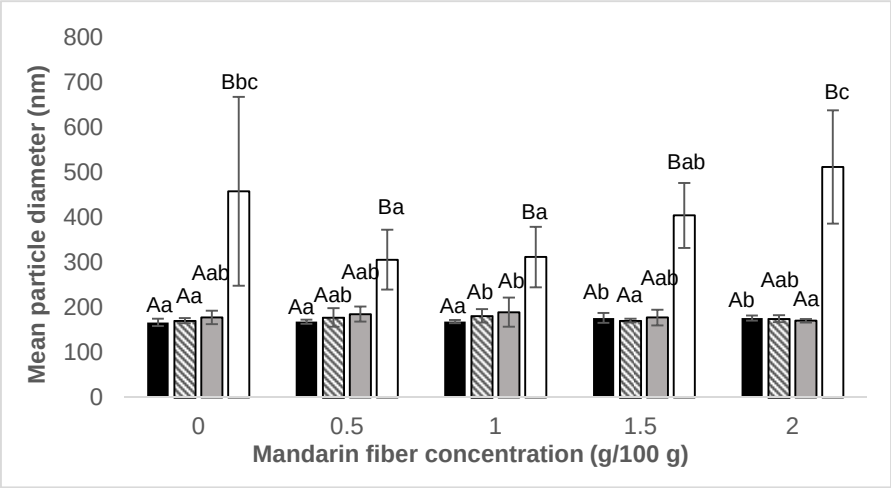

Fig. 1.

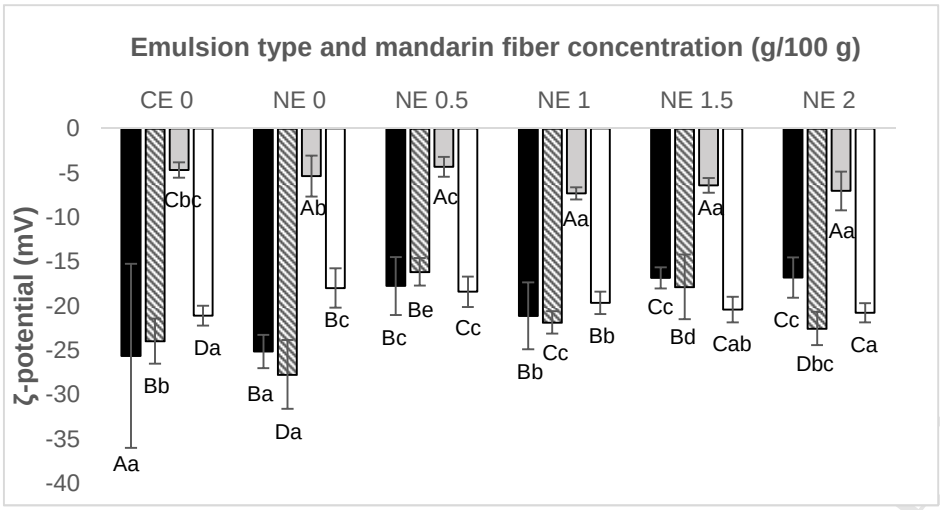

Fig. 2.

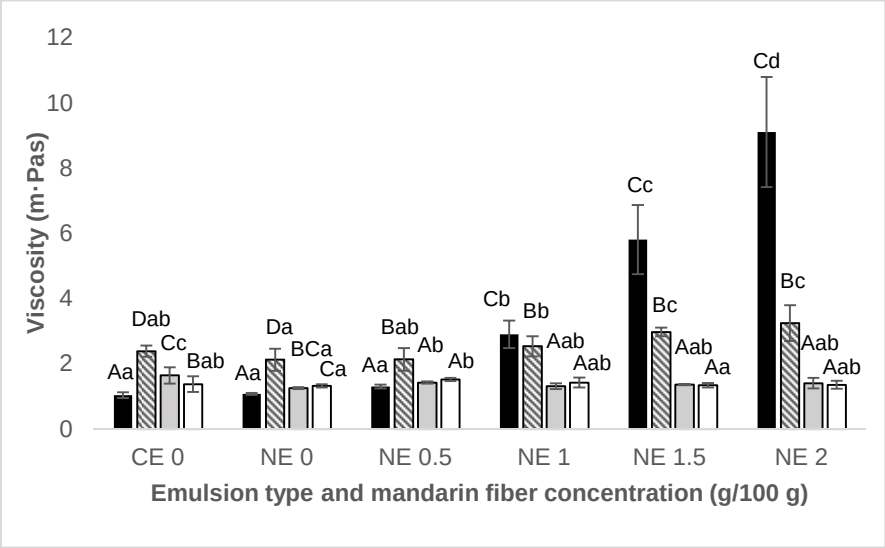

Fig. 3.

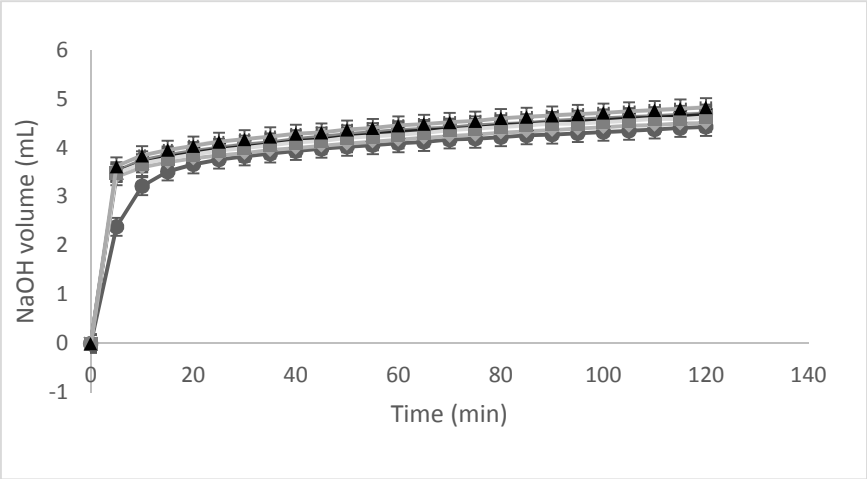

Fig. 4.

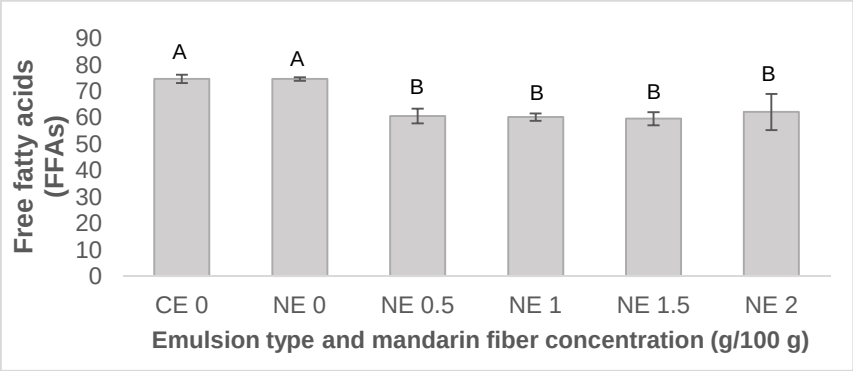

Fig. 5.

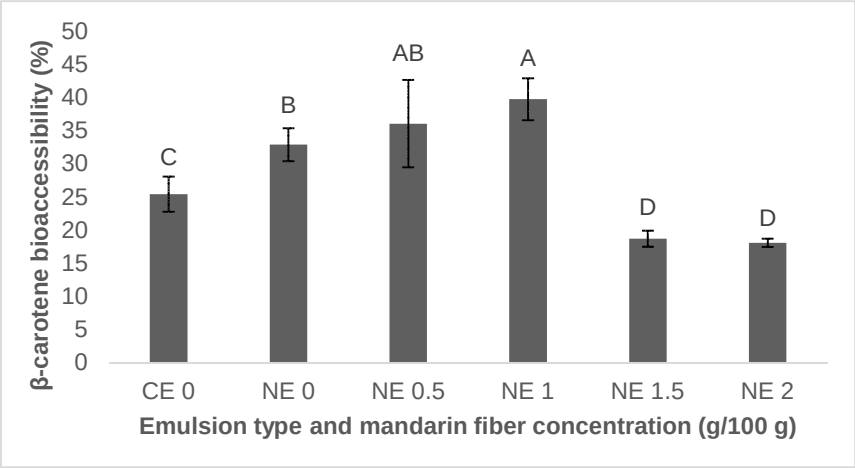

Fig. 6.

Highlights

1. Mandarin fiber was used to stabilize the nanoemulsions.
2. Physicochemical properties during the *in vitro* digestion were also determined.
3. Impact of mandarin fiber on lipid digestibility was determined.
4. β-carotene bioaccessibility increased as mandarin fiber was added (≤1 g/100 g).