Formulating orange oil-in-water beverage emulsions for effective delivery of bioactives: Improvements in chemical stability, antioxidant activity and gastrointestinal fate of lycopene using carrier oils

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Abstract

The influence of carrier oil type on the chemical stability, antioxidant properties and bioaccessibility of lycopene in orange oil-in-water beverage emulsions was investigated. The emulsions were formulated with orange oil (A), which was partially (50%) replaced with tributyrin (B) or corn oil (C) because of their distinctively different fatty acid composition. The addition of corn oil enhanced the physical stability of the beverage during chilled storage by inhibiting Ostwald ripening. The formation of oxidation products was insignificant after storage for 28 days at 4 °C, regardless the type of added oil. Lycopene was more susceptible to chemical degradation in the presence of unsaturated, long chain triglycerides and the retention followed the order: A (87.94%), B (64.41%) and C (57.39%). Interestingly, bioaccessibility of lycopene was significantly lower for emulsions formulated with 50% corn oil as opposed to 100% orange oil as indicated by the simulated *in vitro* gastric digestion model.

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Keywords: Beverage emulsion; Lycopene; Orange oil; In vitro digestion; Bioaccessibility

1. Introduction

Lycopene is one of over 600 naturally occurring carotenoids, which are pigments synthesized by plants and microorganisms, responsible for the colours of various fruits and vegetables (Britton, 1995; Paiva & Russell, 1999). The major contributors of lycopene in the western diet are tomato fruits and tomato-based products, such as tomato juice, ketchup, soup, pizza and sauces and account for over 85% of our dietary lycopene (Bramley, 2000; Mangels, Holden, Beecher, Forman & Lanza, 1993). Lycopene exhibits a plethora of biological properties associated with its

unique structure. It is the most efficient singlet oxygen quencher of the natural carotenoids (Conn, Schalch & Truscott, 1991; Di Mascio, Kaiser & Sies, 1989). The ability of lycopene to act as a potent antioxidant is thought to be responsible for protecting cells against oxidative damage and thereby decreasing the risk of chronic diseases (Tapiero, Townsend & Tew, 2004). In addition to its antioxidant properties, lycopene can induce cell to cell communication and modulate hormonal, immune systems, and other metabolic pathways (Aust et al., 2003; Stahl, von Laar, Martin, Emmerich & Sies, 2000). Both epidemiological and experimental studies have shown that lycopene is protective against different types of cancer and cardiovascular diseases (Krinsky & Johnson, 2005; Kun, Lule & Xiao-Lin, 2006, Rao & Agarwal, 2000). With increasing awareness of the health benefits of this carotenoid, the food and drink industry is interested to develop new products with formulations containing lycopene in order to meet consumers demand for products with improved nutritional value. However, lycopene is insoluble in water and a highly unsaturated molecule, which renders this compound very susceptible to thermal and oxidative degradation. Moreover, its bioavailability is low and depends on several factors such as co-ingestion with oil, isomerization and degradation during thermal processing (Ax, Mayer-Miebach, Link, Schuchmann & Schubert, 2003; Xianquan, Shi, Kakuda & Yueming, 2005).

Previous studies have investigated different aspects affecting the bioavailability of carotenoids and they have shown that one of the most important factors is the type and total amount of fat present during digestion. Recently it was demonstrated that lycopene bioaccessibility from raw tomato pulp can be significantly improved by the addition of 5% lipid, with variations for different kinds of fats (Colle, Van Buggenhout, Lemmens, Van Loey & Hendrickx, 2012). Moreover, the incorporation of lycopene into micelles is affected by the length of fatty acyl

chains: the amount of carotenoid available for absorption is higher when long chain triglycerides (LCTs) are predominantly present in the oil phase. This is due to the fact that long chain fatty acids are more efficient compared to short chain ones to form mixed micelles with a large hydrophobic core in which carotenoid molecules are accommodated and solubilised (Huo, Feruzzi, Schwartz & Failla, 2007; Salvia-Trujillo, Qian, Martín-Belloso & McClements, 2013). Lycopene being highly hydrophobic, may be dissolved within the oil phase of oil-in-water emulsions in order to improve its stability and bioavailability (McClements, Decker, Park & Weiss, 2009). Emulsions are particularly suitable matrices for encapsulation, protection and delivery of lipophilic components provided that they are properly formulated (Raikos & Ranawana, 2017). The food structure, processing methods and storage conditions are critical factors that need to be considered for interpreting bioaccessibility of lycopene, since they largely determine chemical stability, release from the matrix and incorporation into mixed micelles during digestion of the bioactive ingredient.

In this context, the purpose of the present work is to develop an edible orange oil-in-water beverage emulsion containing lycopene and investigate the influence of carrier lipid composition on 1) the physicochemical stability of the emulsions under four weeks of chilled storage; 2) the antioxidant properties of the beverage and 3) the bioaccessibility of β-carotene by using an *in vitro* gastro-intestinal digestion model. A limited number of studies are available on the processing factors affecting the properties of flavour emulsions containing lycopene. Furthermore, all data available regarding lycopene bioaccessibility is derived from studies using tomato juice or pulp, rather than lycopene in purified form. The results of this study have important implications for developing effective, emulsion-based delivery systems especially

designed to enhance the bioavailability of lycopene in food and beverage products which contain orange oil as a major ingredient.

2. Experimental

2.1. Materials

Lycopene (redivivo®) was purchased from DSM Nutritional Products Ltd (Heanor, UK). Tocopherol-stripped corn oil was used as an example of a long chain triglyceride (LCT) and was purchased from Sigma–Aldrich, Co Ltd (Dorset, UK). Tributyrin and orange oil were used as examples of short chain triglyceride (SCT) and non-digestible flavor oil respectively and were purchased from Sigma Aldrich (St. Louis, MO, USA). Citric acid, amylase (type VI-B), pepsin, pancreatin, bile extract were purchased from Sigma Aldrich (St. Louis, MO, USA). Pure Whey IsolateTM 97 powder (WPI) was used as emulsifier and was purchased from Bulk Powders (Colchester, UK). All reagents used were of analytical grade.

2.2. Fatty acid composition analysis

The fatty acid composition was determined by analyzing their methyl ester derivatives with gas-liquid chromatography (Liu, 1994). Analysis of the fatty acid methyl esters (FAMEs) was carried out using a gas chromatograph (HP6890, Hewlett Packard, Avondale, PA) using 50 m × 20 mm Chrompac CP7488 CP Sil-88 capillary column (film thickness 0.20 μm). Helium was used as carrier gas at a rate of 0.5 ml/min, and the split/splitless injector was used at a split ratio of 20:1. The injector and detector temperatures were 250°C. The column oven temperature was maintained at 80°C for 1 min after sample injection and was programmed to increase then at 25°C/min to 160°C where it was maintained for 3 min. Temperature was then increased to 190°C

115 at 1°C/min and then to 230°C at 10°C/min. The temperature was maintained at 230°C for 30
116 min. Separation was recorded with HP GC Chemstation software (Hewlett Packard, Avondale,
117 PA). The FAMEs were identified by comparison to previously essayed standards. Measurements
118 were taken in duplicate. Results are expressed as % of total fatty acids identified and includes
119 fatty acids ≥ 6 carbon atoms.
120
121 2.3. Preparation and storage of oil-in-water (o/w) beverage emulsions
122 Emulsion beverages were prepared using a standard weight-to-weight (w/w) recipe: 92% water,
123 3% WPI, 4% oil, 0.7% citric acid, 0.05% lycopene. To evaluate the effect of different oil carrier
124 type, the oil-phases were adjusted as follows: A. 4% orange oil, B. 2% orange oil + 2% tributyrin
125 (SCF) and C. 2% orange oil + 2% corn oil (LCT). In brief, WPI was initially reconstituted in
126 cold water to allow hydration for 20 min with mild agitation and then the pH was adjusted to 3.2
127 with citric acid. Lycopene was dispersed to the above chilled aqueous phase and the mixture was
128 agitated at room temperature for 5 minutes, according to the supplier's recommendations. A
129 coarse emulsion was initially formed by adding oil at a steady rate and mixing the rest of the
130 ingredients using a high speed blender (Morphy Richards, Argos, UK) for 2 min at room
131 temperature. Samples were then processed in a single stage valve homogenizer (APV-1000, SPX
132 Flow Technology, West Sussex, UK). Each sample was passed twice through the homogenizer to
133 ensure complete emulsification. Homogenization pressure was set at 40 MPa for both the
134 passages through the homogenizing valve. Three different batches for every oil type emulsion
135 beverages (500 gr) were prepared, which were stored at 4 ºC for a period of 4 weeks until further
136 analysis (sampling was performed on a weekly basis).
137

2.4. Emulsion physical stability

The physical stability of beverage emulsions was monitored using a Turbiscan MA2000 (Formulaction, Ramonville St. Agne, France). The apparatus comprises of a detection head equipped with a near-infrared light source (880 nm) which scans the length of the sample, acquiring transmission and backscattering data every 40 μm. The light source scanned the sample at 5 min intervals from top to bottom and measured the percentage of light backscattered or transmitted during 1 h period at 37°C. The refractive indices used for particle size calculation were 1.47 for the dispersed phase and 1.33 for the continuous phase. The Turbiscan stability index (TSI) was calculated according to backscattering changes that indicate the particles aggregation and dynamic migration by Turbisoft 2.0.

2.5. Emulsion microstructure

Light micrographs of the emulsion samples were taken using a Leica DM IL LED inverted laboratory microscope equipped with a Leica DFC295 digital colour camera (Leica microsystems Ltd, Milton Keynes, UK). The samples were observed with a 40 x dry objective lens. Pictures were taken using the in-built 3 MP digital camera and picture analysis was performed by Leica application suite software (V.3.6.0).

2.6. Quantification of lycopene

A reverse phase HPLC method was used to quantify lycopene in beverage emulsions using fluorescence and visible detection. In brief, lycopene was extracted from the oil phase as follows: 20 mg of oil was mixed with 280 μL H_2O and 400 μL ethanol. Each tube was vortexed for 10 seconds and 700 μL of hexane (containing BHT) and 100 μL of echinone were added and the

161 samples were shaken for 10 min in the vortex genie before centrifugation for 5 min. The
162 supernatant hexane layer (600 μL) was removed and dried down on the speed vacuum for 10
163 min. Each sample was then dissolved in 200 μL of DEA (20 % (v/v) 1.4 dioxan, 20 % (v/v)
164 ethanol, 60 % (v/v) acetonitrile) and was shaken for 5–10 min before injected for HPLC analysis.
165 HPLC analysis was performed using a Waters 717 plus Autosampler Module (Waters
166 Corporation, Milford, USA) equipped with a Waters 2475 scanning fluorescence detector, a
167 2487 UV/VIS absorbance detector and a C-18 silica (Beckman Ultrasphere ODS) analytical
168 column (250×4.6 mm ID 5 μm particle size). Elution flow rate was 1.1 ml/min, sample run was
169 30 min and injection volume was 150 μL. Measurements were determined with mixed standards
170 containing carotenoids and tocopherols at appropriate concentrations and results were expressed
171 in μg/g of oil. Echinone was used as an internal standard.

172

173 2.7. Antioxidant properties

174 2.7.1. Ferric Reducing Power

175 The reducing power of lycopene beverage emulsions was measured as described by Duan et al.
176 (2006) with slight modifications. One milliliter of samples was dissolved in 2.5 ml PBS buffer
177 (0.2 M, pH 7.0), mixed with 2.5 ml potassium ferricyanide (1% w/v) and incubated at 50°C for
178 20 min. At the end of the incubation period, 2.5 ml of trichloroacetic acid (10% w/v) were added,
179 and the mixtures were centrifuged at 1800 x g for 10 min. Two milliliters of distilled water and
180 0.5 ml of ferric chloride (0.1% w/v) were added to the supernatant. The absorbance of the
181 reaction mixture was determined at 700 nm after 10 min, using a UV-1600 (UV-VIS)
182 spectrophotometer (Spectronic Camspec Ltd., Leeds, UK). Blank and control samples were

prepared using water instead of potassium ferricyanide and emulsion without β-carotene respectively.

2.7.2. Thiobarbituric acid reactive substances (TBARs)

The lipid peroxidation inhibition capacity was determined by TBARS. Thiobarbituric acid (1ml of 0.34% w/v in 50% acetic acid) was added to the reaction mixture (150 µl of emulsion and 4 mL dH2O) before heating the samples for 30 min in a boiling water bath (VWR International Ltd, Leicestershire, UK). Samples were allowed to cool and were centrifuged at 2465 x g for 15 min (Eppendorf 5810R). The supernatant (100µl) was transferred to a black 96 well plate (Thermo Scientific) and fluorescence measured at excitation 515nm and emission 546nm on a plate fluorometer (SpectraMax GEMINI XS, Molecular Devices Ltd, Wokingham, UK), while concentrations of TBARs were calculated from a standard curve prepared with malonaldehyde in the range of 0 to 8 nmoles per tube.

2.7.3. Conjugated Dienes (CD)

The oxidative stability of the emulsions was determined by monitoring the formation of conjugated dienes (Kiokias & Oreopoulou, 2006). Emulsion samples (0.1 ml) were diluted to 10 ml in ethanol; this solution was diluted as necessary (1:1) to achieve spectrophotometric readings in the target absorbance range of 0.2-0.8 at 233 nm. Ethanol was used as the blank. The absorbance at 233 nm was determined with a UV–Vis Spectrophotometer (Spectronic Camspec Ltd., Leeds, UK) as an oxidative indicator. CD levels are expressed in units of raw absorbance.

2.8. *In vitro* gastro-intestinal digestion method

206 Each emulsion was passed through a three-step in vitro digestion model that simulates mouth,
207 gastric and intestine digestion. This method was based on the standardized static in vitro
208 digestion model suitable for food, proposed in a consensus paper (Minekus et al., 2014) with
209 several modifications to assess carotenoid bioaccessibility. Simulated digestion fluids were used
210 as follows: for the oral phase, a simulated salivary fluid (SSF) (pH 7); in the gastric phase, a
211 simulated gastric fluid (SGF) (pH 3); and in the duodenal phase, a simulated intestinal fluid (SIF)
212 (pH 7). Enzyme solutions were prepared using these fluids as solvents. Briefly, the protocol was
213 as follows: to initiating the oral phase 5 mL of SSF with α-amylase (final effective concentration:
214 75 U/mL) and $CaCl_2$ solution (150 mM) were added to 5 mL of emulsion sample, the mixture
215 was then shaken in an incubator (37 °C, 100 rpm) for 2 min (Shaking Incubator, Stuart, UK).
216 Upon completion of that phase, 10 mL of SGF, including porcine pepsin (final effective
217 concentration: 2000U/mL) and $CaCl_2$ (0.15 mM), were added to the sample (10 mL) from the
218 mouth phase. The pH was adjusted to 3.5 using HCl, then the mixture was again incubated for 2
219 hr (37 °C, 100 rpm) under agitation. After 2 hr, the intestinal phase digestion process was
220 initiated by adding 20 mL of SIF with the appropriate amount of enzyme stock: pancreatin (0.25
221 ml per ml of digesta) and bile (0.125 ml per ml of digesta). The amount of pancreatin added was
222 based on the trypsin activity (100U/mL). After 30 min the pH was monitored until it reached 7.
223 The samples were incubated at 37°C for 3 hr, whilst mixing. At the end of the 3 hours, the
224 mixtures obtained were placed into a fresh tube and used to determine the bioaccessibility of
225 lycopene.
226
227 2.9. Bioaccessibility determination

After *in vitro* digestion, raw digesta were collected and centrifuged (2647 x g) at 25 °C for 40 min using a MiniSpin® plus centrifuge (Fisher Scientific UK, Loughborough, UK). The middle phase was assumed to consist of mixed micelles that solubilized lycopene (Rao et al., 2013). The micelle phases were collected and prepared for HPLC analysis. Bioaccessibility was calculated using the following equation:

Bioaccessibility (%) = $(C_{micella}/C_{raw\ digesta}) *100$

where $C_{micelle}$ and $C_{raw\ digesta}$ are the β-carotene concentrations in the micelle phase and in the total digesta after the in vitro digestion respectively.

2.10. Statistical analysis

Results are expressed as mean±standard deviation (SD) of three replicates (each replicate corresponds to a different batch). Statistical analysis of the data was performed using the statistical software SPSS Statistics 22 (IBM). Data were analyzed by analysis of variance (ANOVA) and significant differences ($p<0.05$) were detected by the *Scheffé's* post hoc test.

3. Results and discussion

3.1. Physicochemical stability of beverage emulsions during storage

The susceptibility of beverage emulsions to physical and chemical degradation after their production and during storage is the most important factor determiming consumer acceptance and commercial viability (Piorkowski & McClements, 2014). In the present study, the influence of carrier oil type on the physicochemical stability of lycopene enriched emulsions during chilled storage was investigated by partially replacing orange oil with a SCT or a LCT oil phase. The fatty acid compostion of each oil phase is presented in Table 1. Fatty acids with 6 or more carbon

atoms were identified with this method and thus butyric acid (normally present in tributyrin) is not included. Turbiscan analysis was carried out every week for a total period of 28 days of storage at 4°C to investigate any effect due to the carrier oil on the physical stability of the beverages. Figure 1A presents the changes in Turbiscan stability Index (TSI), which is a parameter negatively correlated to the stability and takes into account all destabilization phenomena, such as gravitational separation, flocculation, coalescence and Ostwald ripening. Results clearly indicate that the type of carrier oil has a significant impact on the physical stability of the beverage emulsions and stability increases in the order C (50% LCT+50% Orange)> A (100% Orange) ≥ B (50% SCT+50% Orange). Particle size measurements (Fig. 1B) also confirm that the inclusion of LCT in the beverage formulation is important for maintaining stability during storage. On the day of preparation, all beverages were similar in terms of appearance and no indication of any form of instability was observed macro- or microscopically (Fig. 2). However, the increment in particle sizes was significant ($p<0.05$) during storage for beverages A and B (160.4% and 142.9% respectively), whereas it remained relatively stable for beverage C (11.6%). The increased instability of the beverages A and B during storage is likely to be attributed mainly to Ostwald Ripening (OR) phenomena. OR is the process whereby the oil droplet size increases over time due to diffusion of oil molecules from small to large droplets through the continuous aqueous phase (Kabalnov & Shchulkin, 1992). Flavor oils emulsions are susceptible to OR phenomena due to the high water solubility of the dispersed phase (Lim et al., 2011). In addition, emulsions containing non polar molecules of low molar volume and high water solubility such as tributyrin are highly prone to OR. (McClements, Henson, Popplewell, Decker & Choi, 2012; Wooster, Golding & Sanguansri, 2008). The partial replacement of orange oil with an oil phase of a nonpolar nature (i.e. corn oil), inhibits OR by generating an entropy of

mixing which is more thermodynamically favorable (Piorkowski & McClements, 2014). This thermodynamic driving force operates in opposition to the OR effect due to differences in curvature and results in having the two lipids evenly distributed in the droplets of the dispersed phase (Kabalnov, Pertzov & Shchukin, 1987). Previous studies suggest that incorporating relatively small amounts of poorly water soluble triglycerides (i.e.≥10% corn oil), commonly known as ripening inhibitors, is sufficient to inhibit OR (Li, Le Maux, Xiao & McClements, 2009).

Lycopene is particularly prone to degradation during emulsification and storage due to its highly unsaturated structure (Henry, Catignani & Schwartz, 1998). The data from the determination of lycopene concentration recovered in each beverage emulsion are presented in Table 2. The determined lycopene concentration for all samples at day 1 was lower than the theoretical calculated value (~500μg/ml) based on the recipe. This means that a significant proportion of lycopene is degraded during the preparation stage. According to Tan and Nakajima (2005), high pressure homogenisation, may lead to significant destruction of lycopene molecules due to the generation of heat. Furthermore, an additional factor contributing to the reduced stability of lycopene at the early stage of emulsification may be the presence of singlet oxygen (Ribeiro, Ax & Schubert, 2003). In the presence of oxygen, approximately 30% of lycopene is decomposed in an oil-in-water emulsion stored at 5 °C for 30 hr (Ax, Mayer-Miebach, Link, Schuchmann & Schubert, 2003). The results obtained in this study indicate that storage at 4 °C for 28 days in the dark resulted in noticeable but not significant losses of lycopene ($P<0.05$) for all beverages (A, B and C). Previous studies reported a decrease of approximately 35% in lycopene content of tomato pulp at storage times longer than 15 days at 5 °C (Anese, Bot, Panozzo, Mirolo & Lippe, 2015). The degradation of lycopene in this case was attributed to isomerisation and oxidation

297 reactions, mainly due to the oxidative deterioration of unsaturated lipids in close vicinity. An
298 additional factor which should be considered and may explain lycopene degradation is the acidic
299 nature of the beverage emulsions (pH ~ 3.2). Shi et al. (2015) reported that lycopene was highly
300 unstable in microemulsons when subjected to low pH conditions (2.21-3.03). Similar findings
301 were documented by Boon, McClements, Weiss and Decker (2009), suggesting that rapid
302 lycopene degradation occurs in emulsion-based systems at pH 4 and below. Transition metal
303 induced oxidation of lycopene was the predominant mechanism of degradation proposed.
304 Electron transfer is known to occur between transition metals like iron and carotenoids, resulting
305 in the formation of free radical species. Thus, in a low pH environment, acid-catalysed reactions
306 may lead to carotenoid destruction and increased iron solubility which, in turn can affect
307 oxidation rates (He & Kispert, 2001; Mei, Decker & McClements, 1998). Differences between
308 samples A, B and C were also detected when comparing lycopene levels of beverages stored for
309 28 days, but these were not statistically significant either ($P<0.05$). The beverage formulated
310 with orange oil (A) showed the highest retention of lycopene (87.94%), followed by the sample
311 B with 50% SCT (64.41%) and sample C with 50% LCT (57.39%). The small particle size of
312 beverages formulated with LCT may have contributed to the higher degradation rates of
313 lycopene. However, the effect of droplet size on the oxidative stability of emulsions is rather
314 ambiguous and difficult to interpret (Berton, Ropers & Genot, 2014).
315
316 3.2. Antioxidant properties of lycopene beverage emulsions
317 The reducing power of the emulsions containing lycopene was determined and data are presented
318 in Figure 3. The method used for measuring the antioxidant properties of the carotenoid
319 containing emulsions has been used and validated previously (Tan et al., 2014). It offers the

advantage that measurements are taken at 700nm, which is fairly distant from the maximum absorption intensity of lycopene (443-502 nm) and thus reduces the risk of spectral interference. The method was validated by determining the ferric reducing power of a control emulsion (D), which was formulated without lycopene. Our results show that lycopene addition in beverage emulsions significantly increases the antioxidant capacity. Furthermore, the type of carrier oil impacts on the reducing power of the beverage emulsions which followed the order A (100% Orange) > B (50% SCT+50% Orange) > C (50% LCT+50% Orange). These findings may be easily interpreted if the decomposition of lycopene in each type of beverage formulation is considered (Table 2). There seems to be a clear quantitative effect, which suggests that the higher the retention of lycopene the better the antioxidant capacity of the beverage. As briefly mentioned in 3.1, the major cause of lycopene degradation is attributed to transition metal induced oxidative deterioration due to the acidic environment of the emulsion. Previous research suggests that carotenoid oxidation is favoured by co-oxidation with lipid hydroperoxides generated by unsaturated lipids (Rodriguez-Amaya, 2001). The rate and extent of oxidative reactions occurring in turn depends on the fatty acid composition of the oil phase, which determines its susceptibility to rancidification. Long-chain polyunsaturated fatty acids in corn oil are more susceptible to oxidation, since they contain more bis-allylic C-H bonds in their hydrocarbon chain (Berton, Ropers & Genot, 2014). On the contrary, saturated lipids or short chain triglycerides with a higher degree of saturation are more stable, indicating that lycopene is better protected from degradation when dispersed in this type of oil phase. This hypothesis is further supported by the fatty acid composition analysis of the oil phases, which shows that corn oil contains high levels of unsaturated fatty acids (predominantly linoleic acid and oleic acid), as shown in Table 1. On the other hand, orange oil and tribyturin are mainly composed of caproic

343 and butyric acid respectively which are less susceptible to oxidation. All oil types used in the
344 present study were devoid of any natural antioxidants which would impact on the oxidative
345 stability of the beverages. Similar findings with respect to lycopene stability in emulsions
346 formulated with different oil types were previously reported by Boon et al. (Boon et al., 2008).
347 Lycopene was more resistant to degradation when dispersed in a fully saturated oil such as
348 hexadecane compared to stripped corn oil. Other studies suggest that particle size is also a major
349 determinant of the antioxidant activity of lycopene-enriched nanoemulsions (Ha et al., 2015),
350 which is not supported by the results of the present study.
351 Lipid oxidation is one of the most critical cause of quality deterioration in food and beverages.
352 The lipid peroxidation inhibition capacity of lycopene-enriched emulsions was assessed by
353 measuring oxidation products (TBARS and CD) in beverage emulsions on the day of formation
354 and after 28 days of storage at 4°C. The aim was to investigate whether the formation of lipid
355 peroxidation products during storage is inhibited, which is a critical factor affecting the quality
356 of the beverage and consumer acceptability. Comparing the oxidative rates of the samples
357 formulated with different oil types, both primary (CD) and secondary (TBARS) oxidation
358 products showed that the emulsions remained stable for the period of study (Fig. 4). The
359 presence of lycopene might protect the lipid fraction from oxidative reactions by virtue of its
360 strong antioxidant activity (Anese, Falcone, Fogliano, Nicoli & Massini, 2002). WPI may have
361 also contributed to the oxidative stability of the beverages by forming an interfacial physical and
362 electrostatic barrier to iron and other pro-oxidants that are common to the aqueous phase.
363 Previous research has indicated that hydroperoxides' formation may be inhibited in structured
364 oil-in-water emulsions by the antioxidant effect of protein emulsifiers (Osborn-Barnes & Akoh,
365 2003). No significant differences were detected for CD's for any of the beverages irrespective of

366 the type of carrier oil. TBARS results followed the same trend, with the values being slightly
367 higher ($P<0.05$) for beverage C (50% LCT+50% Orange) after 28 days of storage, possibly due
368 to the higher content of unsaturated fatty acids in the composition of corn oil. The results
369 obtained clearly suggest that the formulation used for emulsion fabrication and the processing
370 and storage conditions of the beverages are adequate to inhibit lipid peroxidation and ensure
371 oxidative stability after a 28 days period of storage at 4°C.
372
373 3.3. Effect of oil carrier type on lycopene bioaccessibility
374 Emulsion samples were digested using an *in vitro* gastro-intestinal digestion model, which
375 included an oral, stomach and intestinal phase. After the intestine stage, the digesta obtained was
376 centrifuged to separate the micelle phase (middle layer), which contained the solubilized fraction
377 of lycopene. Bioaccessibility was determined by measuring the lycopene concentration in the
378 micelle phase and the total digesta (Rao, Decker, Xiao, & McClements, 2013). Bioaccessibility
379 was unexpectedly high ranging from 35% to 50%, depending on the type of oil used for the
380 beverage formulation (Fig. 5). Most studies have reported lower bioaccessibility values for
381 lycopene (Colle, Van Buggenhout, Lemmens, Van Loey & Hendrickx, 2010; Colle, Van
382 Buggenhout, Lemmens, Van Loey & Hendrickx, 2012; Salvia-Trujillo & McClements, 2016);
383 however these studies used tomato pulp or juice and as a result lycopene's entrapment in the
384 chromoplasts may have hindered its release and subsequent availability for solubilisation into
385 mixed micelles. Furthermore, in the present study bioaccessibility is determined as the fraction
386 of lycopene in the raw digesta which is solubilized, rather than the fraction made available for
387 absorption in relation to the amount originally present in the beverage. The results also indicate
388 that lycopene bioaccessibility was highly dependent on the type of carrier oil present in the

beverage emulsions. Lycopene bioaccessibility was significantly higher from the beverage emulsions formulated with 50% SCT (B) than from those containing 50% LCT (C). This contradicts previous findings which suggest that mixed micelles (micelles and vesicles) formed by long chain fatty acids have higher solubilisation capacities than those formed by medium or short chain fatty acids due to the better ability of the former to accommodate large lipophilic bioactives in their non-polar regime (Qian, Decker, Xiao & McClements, 2012; Salvia-Trujillo, Sun, Um, Park & McClements, 2015). This statement is valid provided that lipid hydrolysis is complete and the micelle core which accommodates carotenoids is made up of the total monoglycerides and free fatty acids generated. An incomplete hydrolysis of LCT's in relation to the complete hydrolysis of SCT's may explain the enhanced solubilisation capacity of lycopene for beverage formulations with tributyrin as opposed to corn oil (Huo, Feruzzi, Schwartz & Failla, 2007). However, this hypothesis is more likely to apply for food systems with a relatively high lipid content (Porter et al., 2004). The present findings on bioaccessibility most probably reflect the decreased lycopene stability in formulations with unsaturated fatty acids (corn oil) which are more susceptible to oxidative deterioration. This hypothesis is supported by the difference in lycopene levels for each type of beverage (Table 2). The bioaccessibility of the beverage formulated with 100% orange oil was also higher than expected. Indigestible oils such as flavor oils are not hydrolyzed by lipases and as such do not form free fatty acids, which is an essential step in the formation of mixed micelles that can solubilize hydrophobic molecules. Similar findings have been reported in the past with the bioaccessibility of vitamin D_3 being higher in orange oil nanoemulsions than for emulsions containing medium chain triglycerides, despite the fact that the later were fully digestible (Ozturk, Argin, Ozilgen & McClements, 2015). Our findings also support the hypothesis that non-digested lipid droplets which contain

lycopene are not removed by centrifugation due to their small size and are therefore located in the micellar phase. This is an artefact for the method used to determine bioaccessibility and further work is needed to establish whether these droplets can be adsorbed by the human epithelial cells in the small intestine.

4. Conclusions

This study investigated the efficacy of orange oil beverage emulsions as delivery systems of lycopene. The type of carrier oil used for beverage formulation had a significant effect on the shelf-life and antioxidant properties of the emulsion and affected lycopene bioaccessibility. The partial replacement of orange oil with LCT's enhanced the physical stability of the beverage during chilled storage by inhibiting OR. The formation of primary and secondary oxidation products was insignificant during storage, regardless the type of carrier oil. On the other hand, beverage emulsions formulated with corn oil were more susceptible to chemical degradation. The lenght of fatty acyl chains in triglycerides and the degree of unsaturation might promote lycopene oxidation, which in turn results in reduced antioxidant capacity of the formulation. The bioccessibility data indicates that beverage emulsions containing orange oil may be used for the effective delivery of lycopene in its purified form Surprisingly, lycopene bioaccessibility was higher in beverage emulsions containing indigestible oil and SCT's. This finding contradicts our current knowledge which suggests that long chain fatty acids result in effective swelling of the mixed micellar species and enhance their solubilization capacity. Current results indicate that the chemical stability of lycopene is affected by oxidation phenomena in the presence of unsaturated fatty acids, which in turn results in lower bioaccessibility. This hypothesis requires further investigation using both *in vitro* and *in vivo* gastrointestinal models.

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Conflicts of interest

All authors declare that there are no conflicts of interest.

Figure Captions

Fig. 1 Changes in TSI (1A) and particle diameter (1B) values of beverage emulsions during 28 days of chilled storage formulated with: [A) 100% orange oil; B) 50% orange oil + 50% SCT; C) 50% orange oil + 100% LCT]. Small letters denote significant differences ($P < 0.05$) for each sample (A, B and C) due to storage effects. Data are mean ± S.D. (n=3).

Fig. 2 Bright field optical microscopy images and photographs of emulsion beverages formulated with A) 100% orange oil; B) 50% orange oil + 50% SCT; C) 50% orange oil + 100% LCT. Scale bar equals to 100 μm.

Fig. 3 Reducing power as an indicator of antioxidant capacity of lycopene beverage emulsions formulated with: A) 100% orange oil; B) 50% orange oil + 50% SCT; C) 50% orange oil + 50% LCT; D) emulsion without lycopene; E) water (blank). Different small letters denote significant differences ($P < 0.05$) between samples. Data are mean ± S.D. (n=3)

Fig. 4 Influence of oil carrier type on lipid peroxidation inhibition capacity of lycopene beverage emulsions. Changes in CD (3A) and TBARS values (3B) during storage at at 4 ºC . Different small letters denote significant differences ($P < 0.05$) between samples. Data are mean ± S.D. (n=3).

Fig. 5 Bioaccessibility (%) of lycopene beverage emulsions formulated with different carrier oil: A) 100% orange oil; B) 50% orange oil + 50% SCT; C) 50% orange oil + 50% LCT. Different small letters denote significant differences ($P < 0.05$) between samples. Data are mean ± S.D. (n=3).

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