**Potential physicochemical basis of Mediterranean diet effect:
Ability of emulsified olive oil to increase carotenoid
bioaccessibility in raw and cooked tomatoes**

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Abstract

Excipient emulsions have compositions and structures specifically designed to increase the bioavailability of nutraceuticals in foods that they are co-ingested with. In this study, olive oil excipient emulsions were shown to significantly increase the bioaccessibility of carotenoids from tomatoes using a simulated gastrointestinal tract (GIT) model ($p < 0.05$): being $8.18 \pm 0.93\%$, $11.85 \pm 1.76\%$ for raw tomato-buffer mixture, and cooked tomato-buffer mixture; While being $32.3 \pm 2.60\%$, $55.7 \pm 3.28\%$ and $42.6 \pm 3.75\%$ for raw tomato-emulsion mixture, cooked tomato-emulsion mixture (boiled together) and cooked tomato- emulsion mixture (tomato boiled alone). These effects were attributed to: (i) the ability of digested olive oil droplets to form mixed micelles in the small intestine that solubilized the carotenoids; and, (ii) the ability of natural antioxidants (phenolics) in the olive oil to protect the carotenoids from oxidation. Thermal treatment (boiling) significantly increased carotenoid bioaccessibility from the tomatoes digested with emulsion, which was attributed to plant tissue disruption at elevated temperatures facilitating carotenoid release ($p < 0.05$). Carotenoid bioaccessibility was higher when the tomatoes were boiled with emulsions ($55.7 \pm 3.28\%$) than when they were boiled alone and then added to emulsions ($42.6 \pm 3.75\%$). In conclusion, this study indicates that olive oil emulsions boost the bioaccessibility and chemical stability of lipophilic nutraceuticals in tomatoes, which may make an important contribution to the potential health benefits of the Mediterranean diet.

Keywords: olive oil excipient emulsion; carotenoids bioaccessibility in diet; tomato carotenoids; gastrointestinal carotenoids bioaccessibility; carotenoids digestion; olive oil emulsions; Mediterranean diet

1. Introduction

Tomatoes (*Solanum lycopersicum*) are an important component of the human diet in many parts of the world, and they are a rich source of various micronutrients and nutraceuticals (carotenoids, chlorophyll, phenolics, folate, flavonoids, and Vitamin C) that may promote human health and wellbeing (Alshatwi et al., 2010; Li, Deng, Liu, Loewen, & Tsao, 2012). Carotenoids are a class of highly hydrophobic bioactive molecules found in tomatoes (Kopsell & Kopsell, 2006), with the most common types being lycopene, β -carotene, and lutein (Li et al., 2012). Epidemiological studies suggest a lowered risk of developing certain types of cancers, chronic degenerative diseases, cardiovascular diseases, and age-related macular degeneration following a diet rich in carotenoid-containing foods, which has been attributed to various *in vivo* biological actions, including antioxidant activity (Hornero-Méndez & Mínguez-Mosquera, 2007; Stahl & Sies, 2003, 2005; Tapiero, Townsend, & Tew, 2004). However, the oral bioavailability of carotenoids from plants is often rather low, which has been attributed to various causes, including limited release from plant tissues, chemical or biochemical instability, and low solubilization in gastrointestinal fluids (Biehler & Bohn, 2010; Zhang et al., 2015a).

Recently, a potential strategy for increasing the bioavailability of carotenoids from fruits and vegetables has been proposed based on the principle of excipient foods (Liu, Bi, Xiao, & McClements, 2015; Zhang et al., 2015b). An excipient food is designed to contain ingredients and structures that increase the bioavailability of nutraceuticals in foods that it is co-ingested with (McClements & Xiao, 2014). For example, carotenoid bioavailability in fruits could be improved by consuming them with excipient creams, or carotenoid bioavailability in vegetables could be improved by consuming them with excipient sauces. Indeed, previous studies have already demonstrated the potential of excipient foods for enhancing the bioavailability of hydrophobic nutraceuticals in fruits, vegetables, and spices. For example, excipient emulsions have been shown to increase the bioaccessibility of curcumin from spices (Zou, Liu, Liu, Xiao, & McClements, 2015b), and carotenoids from carrots (Zhang et al., 2015a), mangoes (Liu, Bi, Xiao, & McClements, 2016), and peppers (Liu et al., 2015).

The Mediterranean diet has been associated with a lower incidence of chronic disease and a higher life expectancy than the typical Western diet consumed in many developed countries (Cicerale, Lucas, & Keast, 2012; Dedoussis et al., 2004). The Mediterranean diet is characterized by high levels of certain plant-based foods (such as vegetables, legumes, fruits and nuts), as well as by the fact that olive oil is the main source of dietary fat (Ortega, 2006). It was therefore hypothesized that one of the reasons for the potential health benefits of the Mediterranean diet is that olive oil acts

as an excipient food that enhances the bioavailability of lipophilic nutraceuticals from plant-based foods. The major objective of this study was therefore to provide evidence that olive oil emulsions could increase the bioavailability of carotenoids in a major component of the Mediterranean diet. Tomato is one of the most common plant-based components of the Mediterranean diet, and so the ability of olive oil emulsions to increase the bioaccessibility and stability of carotenoids from tomato using was investigated using a simulated gastrointestinal tract (GIT) model. Cooking has been shown to increase carotenoid bioaccessibility from plants by disrupting their microstructure, thereby facilitating the release of the carotenoids from their natural environment inside the plant cells (Hedren, Diaz, & Svanberg, 2002; Hornero-Méndez & Mínguez-Mosquera, 2007). For this reason, the influence of thermal processing (boiling) on the ability of excipient emulsions to impact carotenoid bioaccessibility and stability was also examined.

This study provides some interesting information about how olive oil emulsions enhance the bioavailability of carotenoids from plant-based foods, which may help understand the origin of the health benefits of the Mediterranean diet. In addition, this information may be useful for designing olive oil excipient emulsions to improve the efficacy of lipophilic nutraceuticals in other natural foods. Nevertheless, it should be stressed that the potential contribution of this effect to the health benefits of the Mediterranean diet is currently only a conjecture, and that further animal and human **nutrition** studies would be needed to provide evidence for or against this hypothesis.

2. Materials and Methods

2.1 Materials

Raw tomato was purchased from a local supermarket. Whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). As stated by the manufacturer, the protein content was 97.6% (dry basis). Olive oil was purchased from a commercial food supplier (Bertolli, Mizkan America Inc., USA). The manufacturer reported that the saturated, monounsaturated, and polyunsaturated fat contents of this product were approximately 14.3, 71.4, and 14.3%, respectively. Mucin from porcine stomach, pepsin from porcine gastric mucosa (250 units/mg), porcine lipase (100-400 units/mg), and porcine bile extract were purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA). β -Carotene was purchased from Sigma-Aldrich (Sigma Chemical Co.). All other chemicals used in this paper were purchased from either Sigma-Aldrich or Fisher Scientific. All solvents and reagents were of analytical grade. Double distilled water from a water purification system (Nanopure Infinity, Barnstaeas International, Dubuque, IA, USA) was used for the preparation of all solutions.

2.2 Emulsion Preparation

A stock olive oil-in-water emulsion was prepared by homogenizing 8 wt% olive oil with 92 wt% aqueous phase (0.87 wt% WPI, pH 7.0, 10 mM phosphate buffer) using a high-speed blender (7000 rpm) for 2 min (M133/1281-0, Biospec Products,

Inc., ESGC, Basel, Switzerland) followed by passing through a microfluidizer (M110Y, Microfluidics, Newton, MA, USA) with a 75 µm interaction chamber (F20Y) at 12000 psi for three passes. Prior to use, emulsions containing 4 wt % oil content were prepared by diluting the stock emulsion with buffer solution.

2.3 Tomato Preparation

Tomatoes were cut into small pieces (approximately 10 mm high and 10 mm wide) that were used directly (“raw”) or were subjected to a thermal treatment (“boiled”) by heating (100 °C, 10 min).

Raw tomato - emulsion mixture (Raw-TE): Raw tomato disks were mixed with an equal mass of excipient emulsion (4 wt % oil) and then placed in a household blender for 2 min to break down the tomato structure.

Boiled tomato - emulsion mixture: The boiled tomatoes were prepared using two protocols: (1) Raw tomato disks were mixed with a half mass of excipient emulsion (8 wt % oil contents) and then placed in a household blender for 2 min to break down the tomato structure. Afterwards, the mixtures were heated for 10 min at 100°C. After heating, the selected samples were immediately placed in an ice-water bath and then diluted with phosphate buffer solution to make the final oil content of the mixture to be 4 wt % (Boiled-TE); (2) Raw tomato disks were mixed with a half mass of phosphate buffer solution and then placed in a household blender for 2 min to break down the tomato structure. Afterwards, the mixtures were heated for 10 min at 100 °C.

After heating, the selected samples were immediately placed in an ice-water bath and then diluted with excipient emulsion (8 wt % oil contents) to make the final oil content of mixture to be 4 wt % (Boiled-TE*).

These two treatments were used to take into account that tomato may be consumed raw (*e.g.*, in salads) or they may be cooked (*e.g.*, in soups or in sauces).

2.4 In Vitro Digestion Model

Mixtures of tomato with emulsion or phosphate buffer were passed through a GIT model designed to mimic the mouth, stomach, and small intestine phases, which was a slight modification of that described in previous studies (Qian, Decker, Xiao, & McClements, 2012; Zou et al., 2015b).

Initial System. An aliquot (20 mL) of tomatoes/emulsion or phosphate buffer mixture was placed into a glass beaker in an incubated shaker (Innova Incubator Shaker, model 4080, New Brunswick Scientific, New Brunswick, NJ, USA) at 37°C.

Mouth Phase. Twenty milliliters of simulated saliva fluid (SSF) containing 30 mg/mL mucin was preheated to 37°C and then mixed with the initial systems. After being adjusted to pH 6.8, the mixture was incubated in the incubator shaker for 2 min at 37°C to mimic agitation in the mouth.

Stomach Phase. Twenty milliliters of the “bolus” sample resulting from the mouth phase was mixed with 20 mL of simulated gastric fluid (containing 3.2 mg/mL pepsin) that had been preheated to 37°C, and then the pH was adjusted to 2.5 with

sodium hydroxide solution(0.1N and 1.0N). This mixture was incubated in the incubator shaker for 2 h at 37 °C to mimic stomach conditions.

Small Intestine Phase. Thirty grams of “chyme” sample from the stomach phase were poured into a 100 mL glass beaker, placed into a water bath at 37 °C, and then adjusted to pH 7.00. One and a half milliliters of simulated intestinal fluid were added to the reaction vessel, followed by 3.5 mL of bile salt solution with constant stirring. The pH of the reaction system was adjusted back to 7.00. Two and a half milliliters of lipase solution were then added to the sample, and an automatic titration unit (Metrohm, USA, Inc.) was used to monitor the pH and maintain it at pH 7.00 by titrating 0.25 M NaOH solution into the reaction vessel for 2 h at 37 °C. The percentage of FFA released was calculated using the following equation:

$$\text{FFA}(t)= 100 \times \frac{V_{\text{NaOH}}(t) \cdot C_{\text{NaOH}} \cdot M_{\text{oil}}}{2 \cdot m_{\text{oil}}}$$

Here, $V_{\text{NaOH}}(t)$ is the volume of NaOH solution required to neutralize the FFAs produced at digestion time t (L), C_{NaOH} is the molarity of the NaOH solution used to titrate the sample (mol/L), M_{oil} is the molecular weight of the oil (g/mol), and m_{oil} is the total mass of lipid initially present in the incubation cell (g). This equation assumes that there are two FFAs released per triacylglycerol molecule.

2.5 Particle Size and Charge Measurements

The particle size distribution and ζ -potential of the particles in the samples were measured as they passed through the various stages of the GIT model. It was not

possible to obtain information about the lipid droplets in the presence of the homogenized tomato tissues because the large plant tissue fragments dominated the light-scattering signal. For this reason, the microstructure of the samples was measured using microscopy (next section). The particle size distribution of the samples was determined using static light scattering (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). Samples were diluted in aqueous solutions and stirred in the dispersion unit with a speed of 1200 rpm to ensure homogeneity. Phosphate buffer (10 mM, pH 7.0) was used to dilute initial, mouth, and small intestine samples, whereas pH 2.5-adjusted distilled water was used to dilute stomach samples. Average particle sizes are reported as the surface-weighted mean diameter (d_{32}).

The ζ -potential of emulsions was measured using an electrophoresis instrument (Zetasizer Nano ZS series, Malvern Instruments Ltd.). Prior to analysis, initial, mouth, and small intestine samples were diluted with 10 mM phosphate buffer (pH 7.0), whereas stomach samples were diluted with pH 2.5-adjusted distilled water.

2.6 Microstructure Measurements

The microstructures of samples were measured after exposure to the various stages of the GIT model using either optical or confocal scanning laser microscopy with a 20 $\times$ objective lens or a 60 $\times$ oil immersion objective lens (Nikon D-Eclipse C1 80i, Nikon, Melville, NY, USA). Before analysis, 2 mL samples were mixed with 0.1

mL of Nile Red solution (1 mg/mL ethanol) to dye the oil phase. The excitation and emission spectra for Nile Red were 543 and 605 nm, respectively. An aliquot of sample was placed on a microscope slide, covered by a coverslip, and then microstructure images were acquired using image analysis software (NIS-Elements, Nikon).

2.7 Bioaccessibility and Stability

In the present study, the bioaccessibility and chemical stability of carotenoids were determined after each sample had been subjected to the full *in vitro* digestion process using a method described previously (Zhang et al., 2015b). The bioaccessibility was taken to be the fraction of carotenoids present within the small intestine digesta that were solubilized within the mixed micelle phase, while the chemical stability was taken to be the fraction of carotenoids present within the digesta compared to the initial amount. After the small intestinal stage, digesta samples were collected and centrifuged at 12000 rpm and 4 °C for 60 min, which resulted in samples that contained sediment at the bottom and a clear supernatant above. The supernatant was collected and assumed to be the “micelle” fraction, in which the carotenoids were solubilized. Aliquots of 5 mL of digesta or micelle fraction were extracted with 5 mL hexane/acetone (1:1, v/v) mixture, vortexed and centrifuged at 2000 rpm for 6 min at room temperature. The supernatant layer was collected in a second tube. The extraction process was repeated two times. The

combined organic fractions were mixed with saturated sodium chloride solution, and the mixture was shaken vigorously. After the supernatant hexane layer was collected, the lower phase was extracted again with hexane. Then the combined supernatant hexane phases were measured at 450 nm using a spectrophotometer (Ultrospec 3000 pro, GE Health Sciences, USA). A cuvette containing pure hexane was used as a reference cell.

The concentration of carotenoids extracted from a sample was determined from a calibration curve of absorbance versus β -carotene concentration in hexane. The bioaccessibility of carotenoids was then calculated the following equation:

$$\text{Bioaccessibility} = 100 \times \frac{C_{micelle}}{C_{digesta}}$$

Where, $C_{micelle}$ and $C_{digesta}$ are the concentrations of carotenoids in the micelle fraction and in the overall sample (raw digesta) after the simulated intestinal digestion experiment, respectively. The chemical stability of the carotenoids was calculated using the following expression:

$$\text{Stability} = 100 \times \frac{C_{digesta}}{C_{initial}}$$

Here, $C_{initial}$ is the initial concentration of carotenoids in the samples prior to passage through the simulated gastrointestinal tract.

It should be noted that the relatively simple spectroscopic method used in the current study is unable to discriminate between different kinds of carotenoids, or to provide information about the nature of the reaction products formed. In future studies it would therefore be useful to use more sophisticated methods (such as

chromatography) to provide more detailed information about the gastrointestinal fate of the different carotenoids.

2.8. Statistical Analysis

Experiments were carried out using three freshly prepared samples. The results are reported as averages and standard deviations, and the differences among treatments were calculated on the basis of an analysis of variance (ANOVA) and a post hoc Duncan test with a confidence level of 95%. These analyses were carried out using statistical analysis software (SPSS, IBM Corp., Armonk, NY, USA).

3. Results and Discussion

The potential impact of olive oil excipient emulsions on the bioaccessibility and chemical stability of the carotenoids in raw and cooked tomatoes was assessed using a static simulated GIT that included mouth, stomach, and small intestine phases.

3.1. Initial excipient emulsion properties

The properties of the olive oil excipient emulsions were characterized before they were mixed with tomato and exposed to the GIT model. The mean droplet diameter (d_{32}) of the excipient emulsions was 0.146 μm , the particle size distribution was monomodal, and no visible phase separation was observed throughout 4 weeks storage (data not shown). Optical and confocal microscopy images of the initial excipient emulsions indicated that they contained relatively small droplets that were

uniformly distributed throughout the samples (data not shown). Thus, the emulsifier (WPI) and homogenizer (microfluidizer) used to fabricate the excipient emulsions were effective at generating small stable olive oil droplets. The ζ -potential of the olive oil droplets was highly negative (-45.6 ± 3.0 mV), which can be attributed to the fact that the pH of the solution (pH 7.0) was well above the isoelectric point (pI 5.1) of the adsorbed proteins. Previous studies have reported that the electrical potential of excipient emulsions depends on emulsifier type (Liu et al., 2015; Zhang et al., 2015a; Zou, Liu, Liu, Xiao, & McClements, 2015a).

3.2. Impact of mixing tomatoes excipient emulsions

After the tomato samples were blended with excipient emulsions or buffer solutions (control), the initial mean particle diameters (d_{32}) were measured: 138 μm for Raw-Buffer; and, 186 μm for Boiled-Buffer; 23.7 μm for Raw-TE; 153 μm for Boiled-TE; and, 118 μm for Boiled-TE* (**Figure 1**).

Droplet-free systems: In the absence of lipid droplets (control), the mean particle diameter and particle size distribution results of both the raw and boiled tomato samples indicated that the mixtures contained relatively large particles (**Figures 1 and 2**). These large particles were presumably the fragments of tomato formed during the blending process designed to simulate mastication. The optical microscopy images also indicated the presence of relatively large particles within these samples (**Figure 4a**). Interestingly, there were some bright regions within the confocal fluorescence

microscopy images for the Raw-Buffer and Boiled-Buffer samples, which suggested that the Nile red dye may have been localized within some hydrophobic regions in the plant tissue or the carotenoids exhibited auto-fluorescence (**Figures 3 and 4**). Similar results were reported in recent studies using other types of plant-based foods, such as mangoes and carrots (Liu et al., 2015; Zhang et al., 2015b). Both the raw and boiled tomato control samples had high negative charges ($\zeta \approx -29$ and -28 mV, respectively), which may be due to the presence of anionic biopolymers (such as pectin) at the surfaces of the tomato fragments. The mean particle diameter of the boiled tomato samples was significantly higher than that of the raw samples ($p < 0.05$), which may have been because heating promoted swelling of the plant tissue. On the other hand, boiling had no significant effect on the electrical properties of the tomato fragments ($p > 0.05$).

Droplet-containing systems: The properties of the samples were appreciably different when the tomato pieces were mixed with excipient emulsions rather than with buffer solutions. For the raw tomato samples (Raw-TE), the mean particle diameter determined by light scattering was much smaller than that of the tomato tissue alone (**Figure 1**). The particle size distributions of these samples indicated that they had a minor peak of small particles around $5\ \mu\text{m}$ and a major peak of large particles around $100\text{-}1000\ \mu\text{m}$ (**Figure 2b**). In addition, the optical and confocal microscopy images showed that there were some small particles and some large particles in these samples (**Figures 3 and 4**). The relatively small particles were likely

to be individual or aggregated lipid droplet, while the large particles were likely to be tomato tissue fragments. The microscopy images suggested that some of the lipid droplets were trapped in or adhered to the tomato tissue fragments. Droplet aggregation may have occurred because some of the components released from the tomato tissue promoted droplet flocculation (such as acids, biopolymers, or minerals). As observed in the droplet-free control samples, the mean particle diameters of the boiled samples were significant larger than those of the raw samples ($p < 0.05$), which again suggests some swelling of the tomato tissue during boiling.

The mean particle diameter of the samples containing tomatoes and emulsions depended on whether they were boiled before or after mixing (**Figure 1**): the particle size was appreciably higher for Boiled-TE (mixed before heating) than for Boiled-TE* (mixed after heating). The optical and confocal microscopy images indicated that the Boiled-TE samples contained larger lipid-rich regions than the Boiled-TE* samples (**Figure 3**). These lipid-rich particles were probably olive oil droplets that had aggregated during the boiling process. The optical microscopy images indicated that the tomato tissues were still partly intact after boiling, whereas the confocal microscopy images showed that the tomato tissues had lipid-rich (bright) regions associated with them (**Figure 4**). This result suggests that some of the oil droplets were internalized by the tomato tissues or were associated with tomato tissue surfaces, with this effect being more pronounced for the samples where the oil droplets and tomato were boiled together. Previous studies have reported that the

internalization of the lipid droplets by the plant tissue may have due to capillary forces generated by the pores in the tomato tissue, or due to attractive interactions between the oil droplets and tomato tissue *e.g.*, electrostatic or hydrophobic attraction (Zhang et al., 2015a).

Compared to the original excipient emulsions, the magnitude of the negative charge on the particles in the system decreased after mixing with the tomato tissue: -31.6 ± 1.3 mV for Raw-TE; -29.6 ± 1.5 mV for Boiled-TE; and, -30.9 ± 0.3 mV for Boiled-TE* (**Figure 5**). These results suggest that there may have been some constituents released from the tomato tissues that altered the surface charge or ionic strength of the systems, such as acids, minerals, or biopolymers.

3.3. Gastrointestinal fate of tomato samples

The various types of samples were then passed through a simulated GIT that consisted of mouth, stomach, and small intestine phases, and changes in the properties of the particles were measured.

Mouth: In the absence of oil droplets (tomato plus buffer), there was a decrease in particle size after exposure to the mouth phase, but the particles remained relatively large, *i.e.*, for Raw-Buffer and Boiled-Buffer (**Figure 1**). Optical microscopy images indicated that these large particles were fragments of tomato tissue (**Figure 4**). The observed decrease in particle size may have been due to dissociation or shrinkage of the plant tissue due to sample dilution, a change in pH, or the presence of mucin. In

the presence of oil droplets (tomato plus emulsion), the mean particle diameter either increased or decreased after exposure to the mouth phase depending on the sample, *i.e.*, for Raw-TE, Boiled-TE, and Boiled-TE* (**Figure 1**). These effects were attributed to changes in the dimensions of both the tomato fragments (decreasing) and oil droplets (increasing). Indeed, confocal microscopy indicated that the oil droplets in the mouth samples were larger than those in the initial samples and that some droplet flocculation had occurred after exposure to oral conditions (**Figure 3**). Droplet flocculation under simulated mouth conditions may be due to bridging or depletion effects associated with the presence of mucin molecules from the saliva or components released from the tomatoes (Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez, Narváez-Cuenca, & McClements, 2014; Vingerhoeds, Blijdenstein, Zoet, & van Aken, 2005). Interestingly, the optical and confocal microscopy images of the tomato/emulsion mixtures showed that some of the oil droplets were adhered to or internalized within the tomato tissues (**Figure 4**), which again may be due to capillary forces or specific attractive interactions.

In the absence of oil droplets (tomato and buffer), the ζ -potential of the mouth samples was significantly less negative ($p < 0.05$) than the initial samples. This effect can be attributed to the presence of mineral ions and mucin in the saliva solution, which reduced the surface potential of tomato tissue fragments through electrostatic screening and ion binding effects. In the presence of oil droplets (tomato and emulsion), there a slight but not significant ($p > 0.05$) decrease in the magnitude of

the negative charge on the particles after exposure to the mouth conditions.

Furthermore, there was no significant difference in the electric properties of raw and

boiled samples ($p > 0.05$).

Stomach: The samples resulting from the oral phase were incubated in simulated gastric fluids, and then their size, microstructure, and charge were measured.

In the absence of oil droplets (tomato and buffer), the particle size of the stomach

samples was slightly less than that of the mouth samples, but they still remained

relatively large phase (**Figure 1**). Previous studies have also indicated that simulated

gastric fluids do not greatly breakdown plant tissue fragments (Liu et al., 2015). In

the presence of oil droplets (tomato and emulsion), the particle size of the stomach

samples was either smaller or larger than that of the mouth samples, depending on the

nature of the sample. Nevertheless, all of the samples still contained relatively large

particles after exposure to the stomach phase. These effects can be attributed to the

fact that there were changes in the size of both the tissue fragments (decrease) and the

oil droplets (increase). Indeed, the confocal microscopy images clearly showed that

there was extensive flocculation of the oil droplets in the samples in the stomach

phase (**Figure 3**). Interestingly, the particle size decreased for both the boiled

samples (Boiled-TE and Boiled-TE*) after exposure to the stomach phase, but it

increased for the raw samples (Raw-TE). The observed decrease in particle size for

the boiled samples may have occurred because heating made them softer and easier to

disrupt during the 2 h of stirring in the gastric fluids. The magnitude of the ζ -potential

of the samples significantly decreased after incubation in the gastric fluids ($p < 0.05$) (**Figure 5**), which can be attributed to the low pH and high ionic strength of the simulated stomach phase.

Small Intestine: After exposure to the small intestine phase, the particle size, microstructure, and charge of the samples were measured. In the absence of oil droplets, the mean particle size of the samples increased after incubation in the intestinal fluids ($p < 0.05$) (**Figure 1**). The optical microscopy images indicated that some of the large tomato tissue fragments remained in these samples (**Figure 4**). In addition, there may have been some contribution to the light scattering signal from the colloidal particles originating from the bile salts and lipase extract. The overall microstructures of the Boiled-Buffer samples were fairly similar to those of the Raw-Buffer samples (data not shown), indicating that tomato tissue fragments remained in both cases.

In the presence of lipid droplets, there was a significant increase in the particle size of both raw and boiled tomato samples after exposure to the intestinal phase ($p < 0.05$) (**Figure 1**). This change in particle dimensions may be due to the presence of tomato tissue fragments and the formation of a complex mixture of colloidal particles that scatter light, such as undigested lipid droplets, micelles, vesicles, insoluble calcium soaps, and liquid crystals (Liu et al., 2015). The optical microscopy images showed that there were intact tomato tissue fragments in the intestinal fluids (**Figure 4**) and the confocal microscopy images indicated that there were also various kinds of

lipid-rich colloidal structures present (**Figure 3**).

There was an appreciable increase in the magnitude of the negative charge on the particles in all the samples after exposure to the small intestine conditions. This relatively negative is due to the presence of various types of anionic species in the small intestinal fluids, such as bile salts, phospholipids, free fatty acids, peptides, and undigested lipid droplets. The ζ -potentials of the samples initially containing oil droplets were significantly higher than those containing no oil droplets, which can be attributed to the presence of more free fatty acids and undigested lipid droplets.

3.4. Lipid Digestion

In this section, an automatic titration method (pH stat) was used to determine the rate and extent of lipid digestion in the samples. The volume of alkaline solution added to the reaction vessel to neutralize the free fatty acids (and any other acidic species released) during the small intestine phase was measured. These data were then used to calculate the percentage of free fatty acids released from the lipid phase for the digestible olive oils. The volume of alkaline determined for the control sample (no oil droplets) was subtracted from the volume measured for the samples containing oil droplets prior to making these calculations to account for any changes in pH due to the reactants or tomatoes.

In the absence of lipids (controls), there was only a slight increase in the volume of alkaline solution added to the reaction chamber during the 2 h of digestion, which

may be due to the digestion of some components in the reagents (such as bile salts or lipase) or some acids released from the tomato tissues (**Figure 6a**). Interestingly, the volume of alkaline solution required to neutralize the Boiled-Buffer samples was significantly higher than for the Raw-Buffer samples. This may have been because the heat treatment promoted the release of acids from the tomato tissues, perhaps by partially disrupting the plant tissue structure and releasing some of the cell contents. The volume of alkaline solution titrated into the samples containing oil droplets was appreciably higher than that for the samples containing no oil droplets, which is due to the presence of the free fatty acids released digested from the oil droplets (**Figure 6a**).

For the tomato samples containing oil droplets, there was a rapid increase in free fatty acids released during the first few minutes, followed by a much slower increase at later times (**Figure 6b**). Interestingly, heat treatment and adding the oil droplets before or after boiling had no significant effect on the rate and extent of lipid digestion. This effect can be attributed to the fact that there was the same type and amount of oil droplets present in all of the samples. Consequently, by the end of the digestion period about 90 % of the olive oil had been digested in all samples.

3.5. Carotenoid Bioaccessibility and Chemical Stability

In this section, the bioaccessibility and chemical stability of the carotenoids released from the tomato tissue was measured after passage through the simulated

GIT. The bioaccessibility of the carotenoids in the droplet-free samples was relatively low ($\approx 10\%$) (**Figure 7a**). This phenomenon was attributed to a number of factors: (i) there was no fat phase present for the carotenoids to be transferred into; (ii) there were no free fatty acids generated in the small intestine that could increase the solubilization capacity of the mixed micelles. Consequently, the carotenoids released from the tomato tissue could only be solubilized within relatively small simple micelles formed by bile salts. Other studies have also reported that the bioaccessibility of carotenoids in vegetables is remarkably low, *e.g.*, only 1 to 3% of the β -carotene in raw carrots is available for absorption in simulated GIT systems (Hedren et al., 2002; Stahl et al., 2002). It is well established that digestible lipids are required to increase the intestinal absorption of carotenoids (Dimitrov et al., 1988; Huo, Ferruzzi, Schwartz, & Failla, 2007; Prince & Frisoli, 1993).

In the current study, there was a significant increase in the bioaccessibility of the carotenoids from the tomatoes (both raw and boiled) when they were mixed with the olive oil excipient emulsions ($p < 0.05$). This effect can be partly attributed to the fact that the fine oil droplets from the emulsions can penetrate into the pores in the plant tissue and help extract and solubilize the carotenoids (**Figure 4**), and then the free fatty acids produced by lipid digestion can be incorporated into mixed micelles and increase their solubilization capacity. It had been reported that the transfer of carotenoids from the food matrix to micelles is referred to as bioaccessibility, which represents the potential for their bioavailability (Failla, Chitchumroonchokchai, &

Ishida, 2008). Other studies have reported that the addition of 20% cooking oil to lyophilized raw carrots increased the bioaccessibility of β -carotene by 30% (Hedren et al., 2002). Previous work in our laboratory demonstrated that the presence of digestible lipids could significantly increase the bioaccessibility of carotenoids in carrots and peppers (Liu et al., 2015; Zhang et al., 2015a). The bioaccessibility of the carotenoids from the raw tomatoes (Raw-TE samples) was slightly lower than that from the boiled tomatoes (Boiled-TE and Boiled-TE* samples). This effect can be attributed to the ability of the heat treatment to soften, separate, and dissociate the cell walls thereby facilitating the release of the carotenoids. Indeed, a number of previous studies have reported that disrupting or weakening fibrous plant cell walls by heating substantially increases the release of carotenoids (Lemmens, Van Buggenhout, Oey, Van Loey, & Hendrickx, 2009; Rock et al., 1998; van het Hof et al., 2000). In addition, carotenoid bioaccessibility from tomatoes boiled in the presence of excipient emulsions (Boiled-TE) was significantly higher than from tomatoes to which the excipient emulsions were added after boiling (Boiled-TE*). This effect may be partly because boiling weakened the plant wall structure, thereby facilitating the transfer of carotenoids from the tomato tissue fragments into the olive oil droplets. Another recent study indicated that the point where the excipient emulsions were mixed with the produce may impact bioaccessibility, *i.e.*, before or after thermal processing (Zhang et al., 2016).

The chemical stability of the carotenoids was determined by measuring the

amount present at the end of the GIT model compared to the original amount present. The percentage of carotenoids remaining after chemical transformation were 75.6 ± 4.9 , 71.9 ± 6.1 , 89.9 ± 7.3 , 83.8 ± 6.7 and $84.6 \pm 8.2\%$ for Raw-Buffer, Boiled-Buffer, Raw-TE, Boiled-TE and Boiled-TE*, respectively (**Figure 7b**). The decrease in the total amount of carotenoids present in the samples may have occurred due to their chemical degradation when exposed to simulated GIT conditions, particularly within the highly acidic environment of the stomach. Interestingly, the chemical stability of the carotenoids was higher in the tomato samples containing oil droplets, (84-90%) than in the ones containing no oil droplets (72-76%). It has been reported that antioxidants can limit carotenoid oxidation in foods and in the GIT (Ferruzzi, Lumpkin, Schwartz, & Failla, 2006; Hedren et al., 2002), and olive oil contains a variety of phenolic compounds, which possesses potent antioxidant activity (Cicerale, Conlan, Sinclair, & Keast, 2008; Cicerale et al., 2012). Thus, it was speculated that the higher stability of the carotenoids in the samples containing oil droplets was due to two reasons: (i) the presence of antioxidant phenolic compounds in the olive oil inhibited carotenoid oxidation; (ii) the solubilization of carotenoids within the hydrophobic interior of the oil droplets prevented them from coming into close contact with acids in the aqueous phase that promote their degradation.

4. Conclusions

In summary, the present work has shown that olive oil excipient emulsions can be

512 used to increase the bioaccessibility of carotenoids from tomato. This effect may be
513 because the lipid droplets in the emulsion can penetrate into the pores in the plant
514 tissue and extract some of the carotenoids and/or because they form mixed micelles
515 that solubilize the carotenoids in the intestinal fluids. In addition, the olive oil droplets
516 may contain antioxidants that inhibit the chemical degradation of the carotenoids
517 within the GIT. Thermal processing (boiling) increased the bioaccessibility of the
518 carotenoids, which was attributed to the ability of high temperatures to soften the cell
519 wall structure and promote the release of carotenoids. Moreover, the point when the
520 excipient emulsions are mixed with the tomatoes may also be important (*e.g.* before
521 or after boiling). The bioaccessibility of carotenoids from tomatoes that were heated
522 with excipient emulsions was higher than that of tomatoes to which the excipient
523 emulsions were added after heating. This effect may be due to the increase mobility
524 of the carotenoid molecules at elevated temperatures, leading to faster mass transport
525 processes. Overall, these results highlight the potential of designing excipient oil
526 emulsions to boost the fraction of beneficial nutraceuticals obtained from natural
527 fruits and vegetables. For example, it may be possible to design an excipient dressing
528 or dip to consume with raw tomatoes in salads, or an excipient sauce to cook with
529 tomato soups. Finally, these results may help to partially explain the health benefits of
530 the Mediterranean diet: ingesting fruits and vegetables with olive oil may enhance the
531 chemical stability and bioaccessibility of health promoting hydrophobic bioactives,
532 such as carotenoids.

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Figure Captions

Figure 1. Surface-weighted mean particle diameter (d_{32}) of samples containing raw tomatoes and either buffer solution (control) or excipient emulsions exposure to simulated GIT conditions (a); Influence of passage through a simulated GIT on the mean particle diameter (d_{32}) of samples containing boiled tomato and either buffer solution (control) or excipient emulsions (b). Samples designated with different capital letters (a, b, c, d) were significantly different (Duncan, $p < 0.05$) when compared between different GIT regions (same sample). Samples designated with different upper case letters (A, B, C) were significantly different (Duncan, $p < 0.05$) when compared between different samples (same GIT region).

Figure 2. (a) Influence of gastrointestinal stage on the particle size distribution of Raw-Buffer samples. (b) Influence of gastrointestinal stage on the particle size distribution of Raw- TE samples; (c) Influence of gastrointestinal stage on the particle size distribution of Boiled-TE samples (d) Influence of gastrointestinal stage on the particle size distribution of Boiled-TE* samples.

Figure 3. Changes in the microstructure of Raw-Buffer mixtures and raw/boiled tomato-excipient emulsion mixtures as they passed through the simulated GIT as observed by confocal fluorescent microscopy using a lipid dye.

Figure 4. Microstructures of tomato/ buffer or emulsion mixtures after they were exposed to different regions of a simulated GIT by optical and confocal fluorescent microscopy. (a) The samples of raw tomatoes; (b) The samples of boiled tomatoes. Optical microscopy images show the microstructure of the tomato tissue, where confocal microscopy images show the location of the lipid phase. Similar results were obtained for the Boiled-buffer samples (data not shown).

Figure 5. (a) Influence of passage through a simulated GIT on the electrical characteristics of the particles (ζ -potential) of samples containing raw tomato and either buffer solution (control) or excipient emulsions. (b) Influence of passage through a simulated GIT on the electrical characteristics of the particles (ζ -potential) of samples containing boiled tomato and either buffer solution (control) or excipient emulsions. Samples designated with different letters (a, b, c, d) were significantly different (Duncan, $p < 0.05$) when compared between different GIT regions (same sample). Samples designated with different upper case letters (A, B, C) were significantly different (Duncan, $p < 0.05$) when compared between different samples (same GIT region).

Figure 6. Volume of NaOH (0.25 M) required to maintain a constant pH (7.00) in tomato/emulsion or phosphate buffer mixtures (a) and percentage of free fatty acids released in tomato/emulsion mixtures (b) under simulated small intestine conditions

704 as measured using a pH-stat method.

705

706 **Figure 7.** Bioaccessibility and stability of carotenoids released from samples

707 containing raw/boiled tomatoes and either phosphate buffer solutions or excipient

708 emulsions under simulated GIT conditions. Samples designated with different letters

709 (a, b, c, d) were significantly different (Duncan, $p < 0.05$) when compared between

710 different samples.

Figure1.

Figure2.

Figure 3.

Figure 4

Figure 5

Figure 6

Figure 7

Graphical Abstract

- Olive oil emulsions boost the bioaccessibility and chemical stability of carotenoids in tomato