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ORIGINAL ARTICLE

# Increasing the Bioaccessibility of Antioxidants in Tomato Pomace Using Excipient Emulsions

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## Abstract

Plant-based foods contain numerous bioactive constituents ("nutraceuticals") that have beneficial effects on human health. However, their oral bioavailability is often relatively low, which limits their potential efficacy. The bioavailability of nutraceuticals can be increased through the utilization of excipient foods whose compositions and structures are specifically designed to increase the amount of nutraceuticals absorbed in an active form. In this study, olive oil excipient emulsions were designed to increase the bioaccessibility of lycopene and other natural antioxidants in tomato pomace. These emulsions consisted of 8 wt% olive oil and 1 wt% Tween 20 or Tween 80 and were prepared using a microfluidizer operated under different processing conditions (12,000 or 20,000 psi; 3 or 5 passes). Changes in particle size, charge, and bioaccessibility were assessed when tomato pomace-emulsion mixtures were exposed to simulated gastrointestinal digestion. The mean particle diameter of the particles in the excipient emulsions increased after digestion (416 to 446 nm) compared to the values before digestion (200 to 220 nm). The presence of excipient emulsions significantly increased the bioaccessibility of lycopene in tomato pomace compared to oil-free control samples. For instance, lycopene bioaccessibility was > 82% when the tomato pomace was mixed with excipient emulsions but only 29% when it was mixed with oil-free buffer solutions. The presence of excipient emulsions also increased the total phenolic content of the tomato pomace. For instance, the phenolic content was considerably higher in the presence of excipient emulsions (1489 to 2055 mg GAE /100 g FW) than in their absence (939 mg GAE /100 g FW). However, the excipient emulsions did not increase naringenin bioaccessibility, which was attributed to the fact that it was not strongly hydrophobic. The efficacy of the excipient emulsions was only modestly dependent on emulsifier type and homogenization conditions. In conclusion, excipient emulsions can be designed to enhance the bioaccessibility of strongly hydrophobic nutraceuticals in tomato-based products, which may boost their healthiness.

**Keywords** In vitro digestion · Nanoemulsions · Naringenin · Lycopene · Antioxidant

**Highlights**

- Tomato pomace (waste) was processed with an excipient emulsion.
- An in vitro digestion model was used to measure carotenoid bioaccessibility
- The excipient emulsion increased lycopene bioaccessibility.
- Excipient emulsions may improve health benefits of nutraceuticals in plant foods.

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## Introduction

Tomatoes (*Solanum lycopersicum*) are natural sources of several kinds of health-promoting substances, including vitamin C, carotenoids, chlorophyll, phenolics, folate, and flavonoids [1]. Epidemiological studies suggest that consumption of fresh or processed tomato products lowers the risk of heart disease and some cancers, which is mainly attributed to the antioxidant activity of tomato-based products since increased oxidative stress is one of the main reasons for these diseases [2]. Lycopene is a carotenoid that is associated with the deep red color of tomatoes [3]. More than 85% of lycopene in the human diet has been estimated to come from either fresh tomatoes or tomato-based products [4]. Consequently, tomato consumption can provide almost all of the lycopene requirements in the human diet [3].

The overall bioavailability of carotenoids from many natural sources is often extremely low because of their limited release from the food matrix, their poor resistance to chemical degradation, their low solubility within intestinal fluids, and their poor absorption by the epithelium cells [1, 5–7]. Many of these problems can be overcome by controlling the nature of the foods consumed with fruits and vegetables. For instance, the co-ingestion of fatty foods with carotenoid-rich fruits and vegetables can increase carotenoid bioavailability. However, the extent of this effect depends on the structure and composition of the co-ingested fats. Typically, emulsified fats are more effective at boosting bioavailability than bulk fats because they are digested more rapidly in the gastrointestinal tract (GIT) due to their higher surface areas. Oil type also has a pronounced effect on carotenoid bioavailability. Olive, soybean, and sunflower oils have been shown to be more effective at increasing carotenoid bioavailability than peanut and coconut oils [8]. Notably, the first three oils contain more polyunsaturated fatty acids than the last two. Other studies have shown that digestible lipids comprised of long chain fatty acids (such as corn oil) are more effective at increasing carotenoid bioavailability than those comprised of short or medium chain fatty acids (such as coconut oil) [5]. This effect was attributed to the greater solubilization capacity of mixed micelles formed from long chain FFAs, since they contain larger hydrophobic domains in their interiors. Emulsified fats have been shown to increase the bioaccessibility of carotenoids in various kinds of fruits and vegetables, including yellow peppers [9], carrots [5] and mangos [7].

"Excipient foods" are specifically designed to increase the bioavailability of nutraceuticals in other foods that they are consumed with [7]. For example, excipient creams increase carotenoid bioavailability in fruits, whereas excipient sauces improve carotenoid bioavailability in cooked vegetables [1]. Oil-in-water emulsions are especially suitable for the design of excipient foods because their compositions and structures can be carefully controlled, and they form the basis of a wide range of food products, including creams, sauces, dips, dressings, and desserts [10]. These multiphase systems consist of small fat droplets dispersed within a watery liquid [7]. Some regular foods also increase the bioavailability of nutraceuticals in the foods they are normally consumed with *e.g.,* salad dressings may increase the bioaccessibility of carotenoids in salads. However, the compositions and structures of excipient emulsions are specifically designed for this purpose based on knowledge of the main factors limiting nutraceutical bioaccessibility. Emulsions are particularly suitable as excipient foods because their compositions and structures can easily be manipulated using different ingredients and processes [11, 12]. An important advantage of using excipient emulsions arises from their small oil droplet size and high specific surface area, which leads to rapid lipid digestion and mixed micelle formation within the gastrointestinal tract (GIT), thereby facilitating the release and solubilization of hydrophobic nutraceuticals [3, 13]. Excipient emulsions are rapidly digested within the GIT, which leads to the rapid formation of mixed micelles that can solubilize any hydrophobic nutraceuticals released from the fruits and vegetables [8, 14, 15]. In vitro studies have shown that the micelle solubilization of carotenoids from carrot and tomato suspensions is enhanced when they are mixed with olive oil emulsions [1, 16]. They have also shown that the bioaccessibility of lycopene from tomato juice is increased when it is mixed with corn oil emulsions [3].

The industrial processing of tomatoes leads to an appreciable quantity (10 to 40%) of by-products, such as seeds and peels, which are traditionally used as animal feed or fertilizer, despite the fact they are rich in bioactive compounds [17]. It has been reported that the total carotenoid content of dried tomato by-products was around 793 and 158 μg $g^{-1}$ for peel and seeds, respectively. Lycopene (414 μg $g^{-1}$) and β-carotene (150 μg $g^{-1}$) were reported to be the main carotenoid compounds in the dried tomato waste [18]. The lycopene concentration in the skin is considerably higher than that in the seeds because lycopene accumulates in the skin during ripening [2]. Tomato pomace, which is composed of tomato peel and seeds, represents about 3–5% of the total weight of processed tomatoes, and should therefore be a good source of carotenoids.

The main goal of this research was to investigate the potential of olive oil excipient emulsions to increase the in vitro bioaccessibility of lycopene and other natural antioxidants in tomato pomace. The impact of emulsifier type (Tween 20 and Tween 80) and homogenization conditions (operating pressure and number of passes) on the efficacy of the excipient emulsions was also examined. These two non-ionic surfactants were selected because they are food-grade substances that are widely used to prepare oil-in-water emulsions. Moreover, they can protect the oil droplets from aggregation in the stomach, which can increase their digestibility in the small intestine (REF). This study may lead to the design of new tomato-based foods, such as sauces or condiments, that have increased carotenoid bioavailability.

## Materials and methods

### Emulsion Preparation

Olive oil-in-water emulsions were prepared by blending 8% olive oil (w/w), 1% (w/w) surfactant (Tween 20 or Tween 80), and 91% buffer solution (10 mM potassium

phosphate, pH 7.0) together. Eight excipient emulsions were prepared using different emulsifier types and homogenization conditions (Table 1). Homogenization was carried out using a two-step procedure. First, a coarse emulsion was prepared using a high-shear mixer operating at 10,000 rpm, 2 min (IKA A11 basic, IKA-Werke GmbH & Co., Staufen, Germany). Second, this coarse emulsion was passed through a microfluidizer at two different operating pressures (12,000 or 20,000 psi) using two different number of passes (3 or 5 passes) (LM10, Microfluidics, Newton, MA, USA).

## Tomato Pomace Preparation

The tomato pomace samples (seed and skin) were collected from a tomato paste factory (Döhler Co., Balıkesir, Turkey) and collected during the processing of fresh tomato (a commercial variety) into paste. Three different batches were used. The samples were stored at -80 °C until they were dried in a freeze dryer at -35 °C for 36 h. The dried tomato pomace was then ground into a powder and stored at -20 °C prior to use.

## Tomato-Emulsion and Control Sample Preparation

Dried and ground tomato pomace samples were mixed with buffer solution (10 mM potassium phosphate, pH 7.0) in a mass ratio of 1:5 w/w. These samples were then blended for 2 min using a high-shear mixer (IKA A11 basic, IKA-Werke GmbH & Co., Staufen, Germany) to breakdown the plant cell structure in the tomato peel and seeds. The resulting samples were then mixed with an equal mass (1:1 w/w) of 8 different excipient emulsions to acquire tomato-emulsion samples.

Control sample was prepared as PB (tomato pomace and buffer) to compare to the test samples. For the control, buffer solution (10 mM potassium phosphate, pH 7.0) was added to dried and ground tomato pomace in a mass ratio of 5:1 w/w and then the mixture was blended for 2 min using a high-shear mixer. The control group samples were stored at -20 °C before use. All samples were prepared in three batch replicates and then each batch was in vitro digested in triplicate.

## Extraction of Polyphenols

The polyphenols were extracted from the samples using a solvent extraction method described previously [19]. Briefly, 5 mL of 75% aqueous methanol solution containing 0.1% formic acid was mixed with 2 g of sample in a cooled ultrasonic bath for 15 min. The resulting mixtures were then centrifuged at 4000 rpm for 10 min at 40 °C and the supernatants were collected (Himac CR22N, Hitachi Koki, Japan). After removal of the supernatants, the same procedure was repeated using the remainder of the sample. For each sample, all the supernatants were then combined and then stored at -20 °C until analyses.

## In vitro Digestion Model

A simulated gastrointestinal tract (GIT) containing mouth, stomach, and small intestine phases was applied to all test and control samples. The in vitro GIT method used was based on the harmonized INFOGEST method described in detail elsewhere [20]. All solutions used were heated to 37 °C before the experiment, and the GIT simulation was performed at that temperature. For the mouth phase, each sample was mixed with simulated saliva fluid containing human salivary α-amylase solution (1500 U/mL), $CaCl_2$, and Milli-Q water. The mixture was then adjusted to pH 7 and incubated for 2 min at 37 °C with continuous agitation (100 rpm). The sample from the oral phase was then placed in a water bath (37 °C) to simulate the stomach phase that was mixed with simulated gastric fluid containing porcine pepsin solution (25,000 U/mL) and $CaCl_2$. The mixture was then adjusted to pH 3 with HCl and incubated at 37 °C in a shaker at 100 rpm for 2 h. For the small intestinal phase, the stomach phase sample was then mixed with the simulated intestinal fluid containing pancreatin (800 U/mL), and bile salts. The pH of the mixture was adjusted to 7.0 using NaOH. Then, the mixture was incubated at 37 ˚C in a shaker at 100 rpm for 2 h. Batches were in vitro digested in triplicate. In addition, a blank without the added samples, was also incubated under the same conditions and used for the correction of interferences from the digestive fluids. Afterwards, all samples were centrifuged at 23,000 g for 10 min, and the supernatants were stored at − 20 °C until further analysis. In vitro digestion experiments were carried out in triplicate.

## Total Phenolic Content and Antioxidant Capacity

All extracted samples and the fractions collected from the in vitro GIT procedure were passed through a PET filter before spectrophotometric analysis to remove any particulate matter that would scatter light. The total phenolic content (TPC) was measured using a spectrophotometric method described previously [21]. The results are reported in terms of gallic acid equivalent (GAE). The total antioxidant capacity (TACs) of the samples was ascertained using the CUPRAC [copper(II) reducing antioxidant capacity] method [22] and expressed as Trolox equivalent (TE)/100 g fresh weight.

### Particle Size and Charge Measurements

The size and charge of the small particles in the samples were determined after the large tomato tissue fragments were removed by filtration. Static light scattering was used for the determination of the particle size distribution (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK). Before analysis, the samples were diluted with buffer solution (10 mM potassium phosphate, pH 7.0) and then vigorously shaken to ensure homogeneity. The samples were diluted to obtain an appropriate light scattering intensity and to avoid multiple scattering effects. The average particle size was expressed as the surface-weighted mean diameter ($d_{32}$). The surface potential (ζ-potential) of the particles was measured using an electrophoresis instrument (Nano ZS, Malvern Instruments, Worcestershire, UK). Again, the samples were diluted with buffer solution (10 mM potassium phosphate, pH 7.0) before analysis.

### Lycopene Bioaccessibility

Lycopene was extracted from the samples and analyzed using a method described previously [23].

#### Lycopene Extraction

Samples (1 g) were mixed with an organic solvent (1 mL) that contained hexane/acetone/ethanol (50:25:25 v/v/v). The resulting mixtures were then vigorously shaken to facilitate transfer of the carotenoids from the tomato tissue into the organic solvent. Afterward, the mixtures were centrifuged at 4000 rpm for 1 min at 40 °C (Himac CR22N, Hitachi Koki, Japan), and then the supernatants were collected. This procedure was repeated twice. The collected supernatants were evaporated under $N_2$ gas flow until they were dry. The dry extracts obtained were then dissolved by adding 2 ml of a THFmethanol (50:50v/v) mixture. The resulting solutions were then passed through 0.45 µm PTFE filters to remove any large particles and prepare them for injection into the HPLC instrument.

#### Lycopene Analysis

The lycopene concentration in the samples was determined using high performance liquid chromatography (HPLC). A reversed phase column (Zorbax C8, 5 µm, 4.6 × 250 mm, PN 880,952–706) and UV–visible detector were used to separate and quantify the carotenoids during the HPLC analysis. Methanol/ACN (90:10 v/v) was used as the mobile phase. The flow rate of the mobile phase was 1 ml/min and the injection volume was 10 µl. The concentration of lycopene in the test and control samples was determined by measuring the absorbance at 475 nm using a calibration curve prepared using solutions with known lycopene contents. The bioaccessibility of lycopene in the samples was calculated using the following equation:

$$\text{Bioaccessibility} = 100 \times C_M/C_D.$$

Here, $C_M$ and $C_D$ are the concentrations of lycopene in the mixed micelle phase and the overall digesta collected after the completion of the small intestine phase, respectively.

### Phenolics Analysis

The quantification of selected phenolics in the samples was performed according to a HPLC method described previously [19]. The instrument used consisted of a W600 Waters HPLC coupled to a Waters 996 photodiode array detector (Waters, Milford, MA, USA). Phenolics were separated using a Luna C18 column (150 × 4.6 mm, 3 µm; Phenomenex, Torrance, CA, USA). Dose–response curves of quercetin, naringenin and chlorogenic acid (0–50 µg/mL) were used as a reference.

### Statistical Analysis

All analyses were performed using three replicates, and the data was confirmed to be normally distributed. The results are expressed as means ± standard deviations. Data were subjected to analysis using statistical software (SPSS 20.0, Town, State). ANOVA followed by a Tukey post hoc test was used to compare differences between treatments. $p < 0.05$ was considered statistically significant.

## Results and Discussion

### Initial Characteristics of the Excipient Emulsions

#### Particle Size

The size of the lipid droplets in excipient emulsions influences the rate and degree of lipid digestion under gastrointestinal conditions, which impacts the kinetics and extent of mixed micelle formation in the small intestine [5]. In general, the smaller the droplet size, the greater the surface area, and the faster the digestion rate. A faster digestion rate would be expected to lead to a higher bioaccessibility for hydrophobic nutraceuticals like carotenoids. For this reason, the mean particle diameter of the various excipient emulsion formulations was measured before and after digestion (Fig. 1).

The mean particle diameters of all the initial excipient emulsions were relatively small ($d_{32} < 230$ nm). This phenomenon can be attributed to the fact that the homogenization method used (microfluidization) is highly efficient at creating small droplets and the non-ionic surfactants used

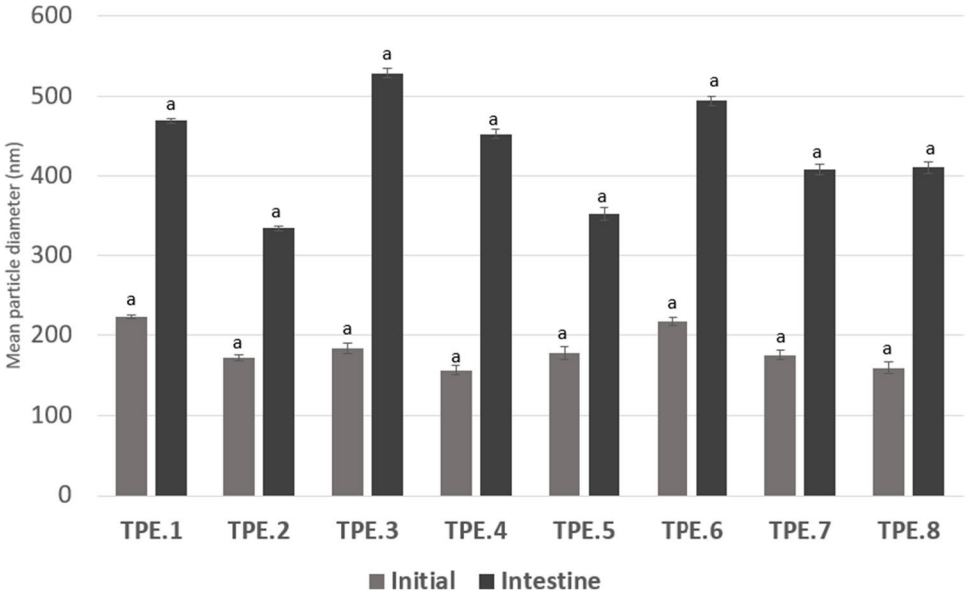


**Fig. 1** Mean particle diameters of olive oil excipient emulsions in different formulations before and after digestion. Different letters above bars represent statistically significant differences for each fraction, individually ($p < 0.05$)

(Tween 20 and Tween 80) rapidly adsorb to the oil droplet surfaces and inhibit droplet coalescence during homogenization. In general, the mean droplet diameter did not depend strongly on homogenization pressure and number of passes (Fig. 1, Table 1), which suggests that even the mildest microfluidization conditions used were sufficient to prepare fine emulsions. The ability to form excipient emulsions containing small lipid droplets by microfluidization has also been reported by other researchers [1, 7]. Overall, these results show that the homogenizer and emulsifier used were effective at finely emulsifying olive oil.

The mean particle diameter of all the excipient emulsions was higher after digestion (416 to 446 nm) than before digestion (200 to 220 nm). This effect is mainly because the nature of the particles within the samples changed substantially after digestion. Specifically, there will be mixed micelles (micelles and vesicles), colloidal calcium soaps, and protein aggregates present in the samples after digestion, and few (if any) of the original lipid droplets [1, 10]. An increase in mean particle diameter after exposure to simulated small intestine conditions has previously been reported for excipient emulsions containing corn oil droplets stabilized by Tween 80, caseinate, whey protein, or modified starch [6, 10]. It has also been reported for excipient emulsions containing olive oil droplets stabilized by whey protein [1].

**Table 1** Olive oil excipient emulsion formulations and sample codes

| Sample Code | Formulation | | | |
|---|---|---|---|---|
| | Olive oil (%8) | Emulsifier (%1) | Pressure (psi) | Number of Passess |
| TPE.1 | Olive Oil | Tween 20 | 12,000 | 3 |
| TPE.2 | Olive Oil | Tween 20 | 12,000 | 5 |
| TPE.3 | Olive Oil | Tween 20 | 20,000 | 3 |
| TPE.4 | Olive Oil | Tween 20 | 20,000 | 5 |
| TPE.5 | Olive Oil | Tween 80 | 12,000 | 3 |
| TPE.6 | Olive Oil | Tween 80 | 12,000 | 5 |
| TPE.7 | Olive Oil | Tween 80 | 20,000 | 3 |
| TPE.8 | Olive Oil | Tween 80 | 20,000 | 5 |

## Particle Charge

The electrical characteritics of the particles within foods and gastrointestinal fluids determine their aggregation stability, as well as their interactions with other charged components [5]. Moreover, they provide insights into the surface composition of the particles. Therefore, the ζ-potential of the colloidal particles in the samples was measured before and after digestion (Fig. 2). The surface potentials of all the samples were negative, both before and after digestion, indicating that anionic species were located at the particle surfaces. The initial excipient emulsions had a modest negative charge (-3 to -16 mV), even though they were stabilized by non-ionic surfactants, which can be attributed to anionic impurities in the oil or surfactant ingredients, such as free fatty acids. After digestion, the negative charge in most of the samples became stronger, which can be attributed to the presence of relatively high levels of anionic free fatty acids and bile acids in the mixed micelles. Other researchers have also reported a negative charge in the colloidal dispersions collected after the digestion of emulsions [1, 7, 10].

Overall, these results suggest that there were only slight differences in the properties of the excipient emulsions prepared using different homogenization conditions and surfactants.

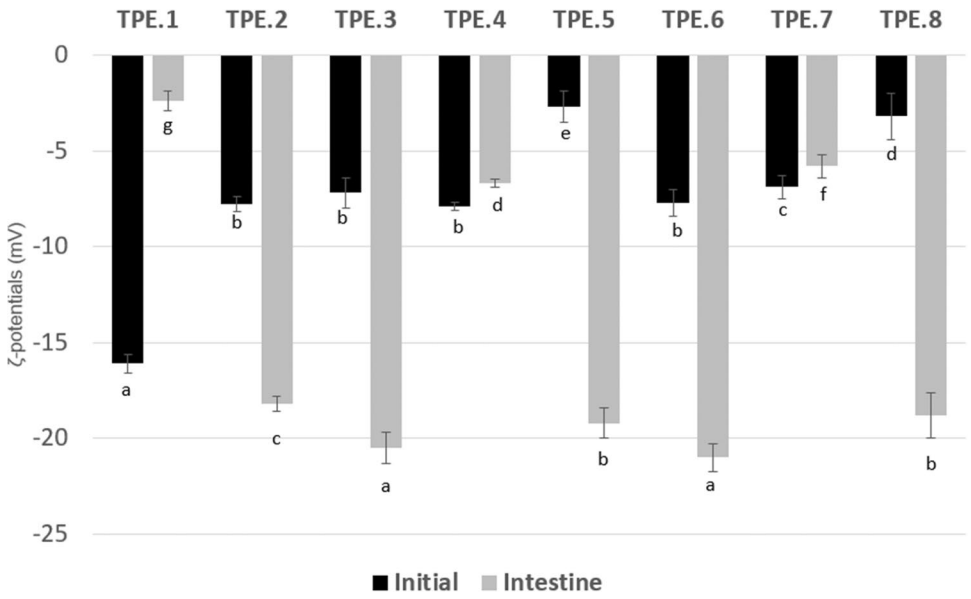


**Fig. 2** ζ-potentials of olive oil excipient emulsions. Different letters below bars for each fraction represent statistically significant differences ($p < 0.05$)

## Total Phenolic Content

Phenolic compounds are believed to have health benefits when consumed regularly as part of the human diet [24]. For this reason, the total phenolics content (TPC) of the different samples was measured before and after digestion (Table 2). The TPC of all the samples increased after being exposed to the in vitro digestion process, suggesting that the disruption of the tomato plant tissue by mechanical, chemical, and enzymatic processes occurring within the simulated GIT facilitated the release of phenolic compounds [25]. The TPC measurements in the digesta and mixed micelle phase suggest that the majority of phenolic compounds were solubilized in the mixed micelles and/or the aqueous phase, leading to a high bioaccessibility (Table 2). As expected, after digestion, the TPC was substantially higher for the samples containing excipient emulsions than for the control samples. For instance, the TPC values of the tomato pomace plus excipient emulsions in the mixed micelle phase (1489 to 2055 mg GAE/100 g FW) were considerably higher than those of the tomato pomace plus buffer (939 mg GAE /100 g FW). In general, the TPC values tended to increase as the homogenization pressure increased (Table 2), which can be attributed to more release of the phenolic compounds due to greater disruption of the tomato cell walls.

Other researchers have also found that the bioaccessibility of the phenolic compounds in fruits and vegetables depends on food composition and processing. For example, TPC in purple plums and blueberries increased after exposure to in vitro digestion [26] Similarly, an increase in TPC after digestion was reported for sweet potatoes [27] and apples [25]. This effect has been attributed to the conversion of polymerized polyphenolic components into monomers and aglycons by hydrolysis when exposed to GIT conditions, thereby increasing the total amount of free phenolic compounds present [27, 28]. Moreover, the concentrations of hydrophobic phenolic compounds released from the tomato pomace, such as quercetin, are increased due to the formation of mixed micelles that can solubilize them in the intestinal fluids. Indeed, other researchers have also reported that the bioaccessibility of quercetin is greatly increased when it is co-ingested with excipient emulsions [29].

**Table 2** Total phenolic content (TPC) of olive oil excipient emulsion and control samples

| Sample Code | TPC (mg GAE/100 g fresh weight) | | | |
|---|---|---|---|---|
| | Initial | After in vitro digestion process | | |
| | | Digesta | Micelle | Bioaccessibility (%) |
| TPE.1 | 288 ± 5 c | 1489 ± 70 b | 1592 ± 28 b | 106.9 |
| TPE.2 | 306 ± 32 c | 1508 ± 37 b | 1561 ± 12 b | 103.5 |
| TPE.3 | 303 ± 68 c | 1976 ± 9 a | 1930 ± 11 a | 97.7 |
| TPE.4 | 254 ± 16 c | 1893 ± 28 a | 1836 ± 33 a | 97.0 |
| TPE.5 | 698 ± 99 a | 1617 ± 33 b | 1558 ± 7 b | 96.4 |
| TPE.6 | 590 ± 48 b | 1669 ± 21 b | 1608 ± 45 b | 96.3 |
| TPE.7 | 362 ± 67 c | 2055 ± 21 a | 1808 ± 37 a | 88.0 |
| TPE.8 | 400 ± 32 c | 1904 ± 12 a | 1856 ± 43 a | 97.5 |
| Control (PB) | 346 ± 16 c | 1055 ± 17 c | 939 ± 57 c | 89.0 |

Data represent average quantities ± standard deviation (n = 3).

Different letters in the columns represent statistically significant differences ($p < 0.05$)

## Total Antioxidant Capacity

The health benefits of nutraceuticals in fruits and vegetables are often attributed to their antioxidant activity. For this reason, we measured the total antioxidant capacity (CUPRAC values) of the test and control samples before and after digestion (Table 3). Before digestion, all excipient emulsion samples had higher CUPRAC values than control samples, which may have been because of antioxidant components within the olive oil or because the presence of the emulsions facilitated the release of antioxidant components from the tomato tissue. After digestion, there was a pronounced increase in the antioxidant activity of all the samples containing excipient emulsions or buffer solution, which can be attributed to disruption of the tomato tissue and release of the antioxidants. In the case of the emulsions, there may also have been an increase in the amount of strongly hydrophobic antioxidants solubilized within the mixed micelles. Other researchers have reported an increase in antioxidant activity for black grapes, purple plums, and cranberries after in vitro digestion process, but a decrease for blueberries [26]. These results suggest that the impact of digestion on antioxidant activity depends on the nature of the fruits and vegetables tested.

## Naringenin Content and Bioaccessibility

Naringenin is a phenolic compound found in tomatoes that has been reported to exhibit beneficial health effects [30]. For this reason, the concentration of naringenin was determined in the test and control samples before and after digestion by HPLC analysis (Table 4).

**Table 3** Total Antioxidant Capacity of Olive Oil Excipient Emulsion and Control Samples

| Sample Code | CUPRAC (mg TE/100 g fresh weight) | | |
|---|---|---|---|
| | Initial | After in vitro digestion process | |
| | | Digesta | Micelle |
| TPE.1 | 1225 ± 17c | 2793 ± 32c | 3282 ± 39a |
| TPE.2 | 1136 ± 9c | 2940 ± 30c | 3027 ± 16a |
| TPE.3 | 1072 ± 20c | 3838 ± 21ba | 3027 ± 94a |
| TPE.4 | 1184 ± 1b | 4159 ± 85a | 3241 ± 70a |
| TPE.5 | 2106 ± 4a | 3233 ± 8bc | 3403 ± 5a |
| TPE.6 | 1773 ± 14b | 3418 ± 24abc | 3653 ± 86a |
| TPE.7 | 1978 ± 11b | 3997 ± 74ba | 3632 ± 43a |
| TPE.8 | 1916 ± 18b | 4003 ± 82ba | 3653 ± 39a |
| Control (PB) | 846 ± 9d | 1485 ± 96d | 1479 ± 6b |

Data represent average quantities ± standard deviation (n = 3).

Different letters in the columns represent statistically significant differences ($p < 0.05$)

**Table 4** Naringenin content in samples before and after digestion

| Sample Code | Naringenin (mg/100 g FW) | | | |
|---|---|---|---|---|
| | Initial | After in vitro digestion process | | |
| | | Digesta | Micelle | Bioaccessibility (%) |
| TPE.1 | 33.6 ± 3.1 a | 13.87 ± 0.27 a | 14.54 ± 0.06 a | 104.3 |
| TPE.2 | 35.09 ± 0.06 a | 14.1 ± 1.2 a | 14.47 ± 0.58 a | 102.8 |
| TPE.3 | 33.3 ± 1.5 a | 12.20 ± 0.00 a | 9.89 ± 0.15 b | 81.1 |
| TPE.4 | 34.85 ± 0.84 a | 13.02 ± 0.72 a | 10.20 ± 0.23 b | 78.3 |
| TPE.5 | 33.98 ± 0.56 a | 13.75 ± 0.54 a | 14.74 ± 0.31 a | 77.8 |
| TPE.6 | 34.3 ± 1.8 a | 14.13 ± 0.63 a | 14.45 ± 0.33 a | 102.5 |
| TPE.7 | 34.16 ± 0.20 a | 13.22 ± 0.22 a | 9.98 ± 0.03 b | 75.6 |
| TPE.8 | 34.95 ± 0.82 a | 13.57 ± 0.23 a | 9.07 ± 0.46 b | 66.7 |
| Control (PB) | 33.82 ± 0.61 a | 13.3 ± 2.8 a | 14.35 ± 0.55 a | 107.9 |

Data represent average quantities ± standard deviation (n = 3).

Different letters in the columns represent statistically significant differences ($p < 0.05$)

Initially, the naringenin concentration was similar in all samples (33 to 35 mg/100 g FW). After digestion, there was an appreciable decrease in the naringenin concentration in the samples, which suggests that there may have been some chemical degradation of this phenolic compound in the simulated GIT. The total amount of naringenin in the mixed micelle phase was fairly similar for the tomato pomace mixed with emulsion as when it was mixed with buffer solution, which may have been because this molecule is not strongly hydrophobic (like carotenoids). Indeed, the logD and water-solubility of naringenin are 1.74 and 1.9 mg $mL^{-1}$ (≈190 mg/100 g) at pH 7 (www.chemicalize.com), respectively, which means that it is only mildly hydrophobic. Consequently, the majority of the naringenin would be expected to be dissolved in the aqueous phase and so not strongly effected by the presence of the lipid droplets or their digestion products. Nevertheless, other studies have suggested that the bioavailability of naringenin can be increased when it is delivered in surfactant- or phospholipid-based colloidal dispersions [31–33]. This effect may have been due to the ability of these formulations to increase the dissolution and/or intestinal permeation of naringenin.

Excipient emulsions are specifically designed to increase the bioavailability of hydrophobic nutraceuticals in foods. For example, the bioaccessibility of carotenoids in boiled tomatoes [34], yellow pepper [9], carrots [5], and spinach [8] have been reported to be increased, as well as the bioaccessibility of quercetin [13] and curcumin [10] in powders. Overall, our results suggest that excipient emulsions are not as effective at increasing the bioaccessibility of less hydrophobic nutraceuticals, like naringenin, which is probably because they already have an appreciable water-solubility.

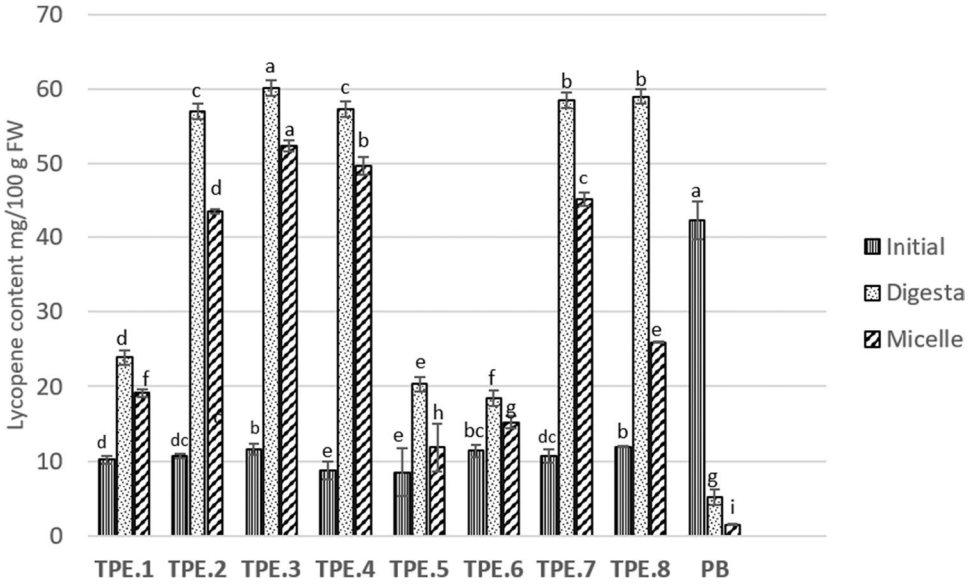


**Fig. 3** Lycopene content (mg/100 g) of olive oil excipient emulsions and control sample (PB). Different letters above bars for each fraction represent statistically significant differences ($p < 0.05$)

## Lycopene Content and Bioaccessibility

### Lycopene Content

Lycopene is a carotenoid found in tomatoes that is reported to have various health benefits [35]. Consequently, we measured the amount of lycopene released from the tomato pomace before and after digestion for the test and control samples (Fig. 3). Before digestion, the excipient emulsion samples had lower lycopene contents than the control samples. This result suggests that it may have been easier to extract the lycopene from the tomato pomace in the absence of lipid droplets. In contrast, after digestion, the concentration of lycopene in the excipient emulsions was higher than that in the control samples ($p < 0.05$). This effect can be attributed to the digestion of the lipid droplets and the formation of mixed micelles that could solubilize the carotenoids in their hydrophobic interiors. The concentrations of lycopene in the digesta were higher than those in the mixed micelle phase, which suggests that some of the carotenoids were not fully solubilized. Interestingly, there appeared to be quite large variations between different samples, despite the fact that the initial droplet sizes and charges in the excipient emulsions were fairly similar. This result suggests that the breakdown of the tomato tissue, the release of the carotenoids, and the solubilization of the carotenoids in the mixed micelles varied considerably between samples, which may have been because different parts of the tomato tissue were present in the original samples.

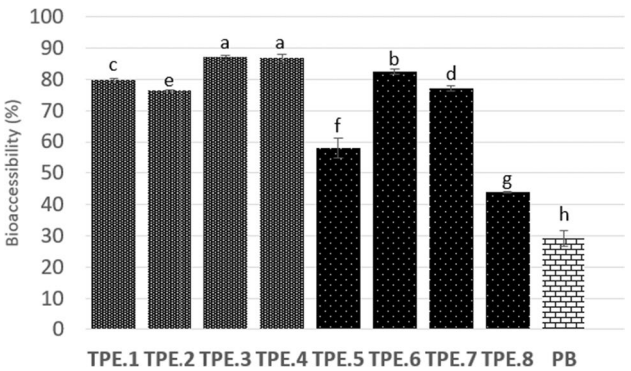


**Fig. 4** Bioaccessibility of lycopene in olive oil excipient emulsion stabilized with Tween 20 and Tween 80 and control sample (PB). Different letters above bars represent statistically significant differences ($p < 0.05$)

### Lycopene Bioaccessibility

The bioaccessibility was calculated from the ratio of lycopene in the mixed micelle phase and total digesta (Fig. 4). The bioaccessibility of lycopene in the oil-free control samples was relatively low (< 30%), which can be attributed to the fact that there were no mixed micelles formed to solubilize the carotenoids. Conversely, the bioaccessibility was relatively high in most of the samples containing excipient emulsions, being greater than 85% for some samples. This effect can be attributed to digestion of the lipid droplets and the formation of mixed micelles that could incorporate the hydrophobic carotenoids inside [1, 3, 34, 36].

Surprisingly, there were large variations in the bioaccessibility of lycopene for excipient emulsions prepared using different emulsifiers and homogenization conditions, with values ranging from around 44% to 87% depending on the formulation (Fig. 4). The origin of this effect is currently unknown. Emulsifier type and homogenization conditions would be

expected to impact the breakdown of the tomato tissue and the release of the lycopene, but there was not a clear trend of bioaccessibility with homogenization pressure, number of passes, or surfactant type. This result suggests that the release and solubilization of the carotenoids may have been highly sensitive to digestion conditions.

## Conclusions

The bioavailability of carotenoids in plant-based foods is relatively low due to their limited release from the food matrix, poor chemical stability, and low solubility within gastrointestinal fluids. This study showed that excipient emulsions significantly increased the bioaccessibility of lycopene in co-ingested tomato pomace, which was attributed to their ability to form mixed micelles that could solubilize the highly hydrophobic carotenoids released from the plant tissue. In contrast, there was little improvement in the bioaccessibility of naringenin in the presence of excipient emulsions, which was attributed to the fact that it is a much less hydrophobic molecule. Our results suggest that excipient foods can be designed to boost the bioavailability of hydrophobic nutraceuticals in natural fruits and vegetables, which may lead to an improvement in their health benefits.

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