In Vitro Bioaccessibility Of Colored Carotenoids In Tomato Derivatives As Affected By Ripeness Stage And The Addition Of Different Types Of Oil

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ABSTRACT

The simultaneous effect of tomato ripeness stage (mature green, pink and red-ripe), mechanical processing (dicing and grinding) and oil addition (coconut, sunflower and olive oils) on the amount and bioaccessible fraction of carotenoids were evaluated. Tomato products obtained from fruits at the most advanced ripeness stage exhibited the greatest values of both concentration and bioaccessible fraction of total carotenoids and lycopene. The type of processing also exerted an important influence on carotenoids content, as well as on its bioaccessibility. Thus, despite the concentration of carotenoids in tomato puree significantly decreased (36-59%), their bioaccessibility was greater (up to 2.54-fold increase) than in tomato cubes. Moreover, the addition of oil significantly improved the carotenoid bioaccessibility, especially when olive oil was added, reaching up to 21-fold increase with respect to samples without oil. The results obtained clearly indicate that carotenoids bioaccessibility of tomato derivatives was strongly influenced by the ripeness stage of the fruit, processing and the addition of oil.

PRACTICAL APPLICATION

Bioaccessibility of carotenoids is known to be affected by different factors. This study provides useful information about the synergic effect of different factors affecting the amount and the bioaccessible fraction of carotenoids, especially lycopene, in two common tomato derivatives. The findings of this work may contribute to develop tomato derivatives with high content of bioaccessible carotenoids, leading to the enhancement of their health-promoting properties.

KEYWORDS

Lycopene, tomato products, oil, bioaccessibility, ripening
1. INTRODUCTION

The consumption of raw tomatoes and tomato derivatives has increased worldwide over the last years, thus becoming one of the most important sources of carotenoids in the human diet (Kotíková and others 2011). Carotenoids have received special attention because of their relation with a decreased risk in the incidence of some types of cancer, atherosclerosis and cardiovascular diseases (Schweiggert and Carle 2017).

Several researchers have reported that the amount of carotenoids in tomatoes are influenced by many factors, such as type of cultivar/variety, climate, agronomic aspects, harvesting and ripening (Ilahy and others 2011; Hdider and others 2013). Tomato fruits are typically harvested at different ripeness stages depending on the consumer and market preferences, ranging from breaker (pink or red colour shows no more than 10% of tomato surface) to red (fully ripe) (USDA 1991). Nevertheless, the amount of bioactive compounds, particularly carotenoids, is also variable over tomato ripening. Hence, both nutritional value and health-promoting properties change during tomato fruit development. The ripening of tomato fruit implies morphological, physiological, biochemical and molecular changes including chlorophyll degradation and synthesis of carotenoids, especially lycopene (Ilahy and others 2011). In this sense, several authors have shown that the concentration of total carotenoids and lycopene in tomato significantly increases during ripening (Ilahy and others 2011; Cano and others 2003). However, there is a lack of information about the influence of tomato ripeness stage on the bioaccessibility of carotenoids.

Carotenoid bioaccessibility may be influenced by a number of food properties and dietary factors, namely the type of carotenoid, molecular linkage, amount of carotenoids consumed in a meal and matrix in which carotenoids are contained, among others (Priyadarshani 2017). In addition, food processing, including mechanical operations,
has been shown to affect both the amount of carotenoids and their bioaccessible fraction. In this sense, processing operations could produce a significant reduction in the carotenoids content of tomato products (Martínez-Hernández and others 2015). However, processing appears to have a positive effect in the bioaccessibility of carotenoids since it favours the disruption of the food matrix and facilitates the release, transformation and absorption of these health-related compounds during digestion (Barba and others 2017).

Moreover, it has been noticed that carotenoids bioaccessibility is enhanced when lipids are added during processing and/or digestion due to their lipophilic behaviour (Lemmens and others 2014). Colle and others (2012) reported that lycopene bioaccessibility significantly increased after adding smaller amounts of sunflower oil, olive oil and cocoa butter. Similarly, Failla and others (2014) found that the micellarization of β-carotene and lycopene of mixed salad vegetables increased by adding dietary lipids. To ensure carotenoids absorption in the human body, they must be released from the food matrix, dispersed into the lipid phase and incorporated into mixed micelles (Desmarchelier and Borel 2016). The ability of micelles to incorporate carotenoids depends on their structural features and the dietary fatty acid characteristics, such as its chain length and degree of unsaturation. In this regard, it has been suggested that long-chain-triglycerides increase carotenoid bioaccessibility more than short/medium-chain molecules (Colle and others 2012; Nagao and others 2013). Moreover, controversial results have been reported regarding the effect of the degree of unsaturation of dietary fatty acids on the carotenoid bioaccessibility (Colle and others 2012; Mashurabad and others 2017).

As far we are concerned there are no previous studies dealing with the effect of the ripening stage on the carotenoids bioaccessibility of different tomato-based products.
Therefore, the objective of this study was to evaluate the content and bioaccessible fraction of both total carotenoids and lycopene of two tomato derivatives (cubes and puree) as affected by the fruit ripening stage (mature-green, pink or red-ripe) as well as by the addition of different types of oil characterized by their different fatty acid composition (coconut, sunflower and olive).

2. MATERIALS AND METHODS

2.1. REAGENTS

All digestive enzymes (α-amylase from porcine pancreas, pepsin from hog stomach, pancreatin from porcine pancreas, bile extract porcine) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Calcium chloride dehydrate, magnesium chloride hexahydrate (99%), magnesium sulphate hexahydrate, sodium chloride, sodium bicarbonate and sodium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium chloride was obtained from Panreac (Barcelona, Spain). Monopotassium phosphate was purchased from Acros Organics (New Jersey, U.S.A.). Butyl hydroxytoluene (BHT), hydrochloric acid and sodium hydroxide were acquired from Scharlau Chemie S.A. (Barcelona, Spain).

2.2. MATERIALS

Tomatoes (Lycopersicum esculentum cv. Raf) were purchased in a local market (Lleida, Spain) at mature-green stage. They were stored at 12 ºC until they reached the desired degree of ripeness corresponding to mature-green (fruit surface completely green, varying from light to dark green), pink (partially ripe – approximately 50% red) and red (fully ripe – over 90% red) fruit colour, according to the US colour standard for classifying tomato ripeness (USDA 1991).
A number of oils with different fatty acid composition were purchased in a local market:

- Coconut oil (88% of saturated fatty acids, 9% of oleic acid and 3% of linoleic acid).
- Olive oil (15% of saturated fatty acids, 75% of oleic acid, 8% of linoleic acid and 2% of linolenic acid).
- Sunflower oil (9% of saturated fatty acids, 25% of oleic acid and 66% of linolenic acid).

### 2.3. PHYSICOCHEMICAL CHARACTERIZATION OF TOMATO

Colour, soluble solids, pH and titratable acidity of tomato were determined at each ripeness stage according to Soliva-Fortuny and others (2005). Tomato surface colour was directly measured with a CR-400 Minolta colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan). Colour was measured using the CIE L*, a*, b* coordinates (lightness, L*; green-red chromaticity, a*; and blue-yellow chromaticity, b*). The equipment was set up for a D65 illuminant and 10° observer angle. A white standard plate (Y = 94.00, x = 0.3158, y = 0.3322) was used for calibration. The a*/b* ratio on the skin of tomato was calculated in order to observe the colour development during tomato ripening. Each sample was homogenised with a blender (Solac Professional Mixter BV5722, Spain). Afterwards, soluble solids content was determined by refractometry (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan) and expressed as ºBrix. pH measurements were carried out on the homogenized tomatoes using a Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain). Titratable acidity was estimated after titration at pH 8.1 with 0.1 N NaOH and results were expressed as grams of citric acid kg⁻¹.
2.4. TOMATO PROCESSING

Tomatoes at each ripeness stage (mature-green, pink or red) were washed with tap water and the excess of water was carefully removed from the surface with paper cloth. Unpeeled tomatoes were then diced or ground in order to obtain tomato cubes and puree, respectively. The choice of these tomato derivatives was based on the traditional products used in homes. On the one hand, tomato cubes were obtained by cutting the fruits approximately into 1-cm³ pieces. Afterwards, they were mixed with 5% of coconut, olive or sunflower oils. On the other hand, puree was obtained by crushing tomatoes for 90 seconds in a blender (Solac Professional Mixter BV5722, Spain). Then, 5% of coconut oil, olive oil or sunflower oil was added and mixed for 10 seconds in a grinder (Moulinex DP700G-BP, France) in order to obtain a homogeneous puree. The selection of the amount of oil added was in accordance with the common amount used in the Spanish commercial tomato-based products. Tomato derivatives without oil were also prepared as control.

Each tomato product was divided in two sets of samples. The first one, aimed at determining total carotenoids and lycopene contents in the undigested products, was directly freeze-dried (Cryodos, Telstar, Terrasa, Spain) and stored at -40 ºC until analysis. The second set of samples was subjected to in vitro gastrointestinal conditions in order to determine the total carotenoids and lycopene contents after digestion.

2.5. IN VITRO DIGESTION

A static in vitro gastrointestinal digestion model consisting of oral, gastric and small intestinal phases was simulated based on the procedures reported by Tagliazucchi and others (2012) and Rodríguez-Roque and others (2013) with slight modifications.
Oral phase: 75 g of each tomato derivative were mixed with 75 mL of simulated salivary fluid (SSF) which contains 150 - 200 uds mL⁻¹ of α-amylase. The composition of SSF was 0.1854 g L⁻¹ of CaCl₂·2H₂O, 0.4 g L⁻¹ of KCl, 0.06 g L⁻¹ of KH₂PO₄, 0.1 g L⁻¹ of MgCl₂·6H₂O, 0.049 g L⁻¹ of MgSO₄·7H₂O, 8 g L⁻¹ of NaCl, 0.35 g L⁻¹ of NaHCO₃ and 0.048 g L⁻¹ of Na₂HPO₄ (pH 6.8). The mixture was homogenized in a stomacher laboratory blender (IUL Instruments, Barcelona, Spain) for 1 min to simulate mastication. Then it was incubated using an orbital shaker (Ovan, Badalona, Spain) at 37 ºC for 10 min with continuous agitation at 95 rpm.

Gastric phase: the pH of the digesta was adjusted in two steps to mimic the gradual drop of the gastric pH after the intake of a meal. First, the pH was adjusted to 4 with 1 M HCl. Subsequently, a porcine pepsin solution from hog stomach (40 g L⁻¹ in 0.1 M HCl) was added to assure a final concentration of 1.8 g L⁻¹ in the gastric digesta. Finally, pH was adjusted to 2 with 5 M HCl. The mixture was incubated for 120 min at 37 ºC in an orbital shaker at 95 rpm.

Small intestinal phase: to simulate duodenal conditions, the pH of the digesta was set to 5.3 with 2 M NaOH. Then, for the preparation of the pancreatin/bile extract solution, 4 g L⁻¹ of pancreatin from porcine pancreas and 25 g L⁻¹ of bile extract from porcine were dissolved in 0.1 M NaHCO₃. It was added into the small intestinal digesta to provide final concentrations of 0.4 g L⁻¹ and 2.5 g L⁻¹, respectively. Afterwards, the pH was adjusted to 7.5 with 2 M NaOH. The mixture was incubated at 37 ºC for 120 min with agitation at 95 rpm.

The digested fraction was centrifuged at 33,768 x g for 20 min at 4 ºC (Beckman Coulter, Avanti J-26 XP, California, USA) to separate the micellar phase from the undigested oils droplets and from the undigested tomato pulp. The micellar fraction was collected and filtered across a Whatman 1 filter paper and then, across a cellulose filter
(1-3 µm pore size, 70 mm diameter, Filtros Anoia S.A., Barcelona, Spain) to remove any crystalline carotenoid or lipid. Finally, the micellar fraction was freeze-dried and stored at -40 °C until analysis.

2.6. DETERMINATION OF CAROTENOIDS

2.6.1. Extraction

The lipophilic fraction was extracted according to the procedure described by Rodriguez-Roque and others (2013) with slight modifications. First, 1 g of lyophilized non-digested or digested samples was mixed with 0.01 g of magnesium hydroxide carbonate, 0.01 g of butylhydroxytolune (BHT) and 15 mL of ethanol:hexane (4:3 v/v) in an Ultraturrax (T-25 Basic, IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. Then, the mixture was filtered once under reduced pressure using a Whatman no.1 filter paper. The residue was re-extracted with a second volume of 10 mL of ethanol:hexane (4:3 v/v) and again filtered. The pellet was washed twice with 5 mL of ethanol and once with 5 mL of hexane, until the residue was colourless. All the extracts were combined and washed twice with 10 mL of sodium chloride (100 g L⁻¹) and thrice with 10 mL of distilled water to remove unwanted water-soluble substances. The aqueous layer was discarded and the organic phase was collected. All the procedures were carried out under dim lighting using amber glassware in order to prevent carotenoid oxidation and isomerization.

2.6.2. Analysis of total carotenoids

Total carotenoids content (TCC) was measured spectrophotometrically following the methodology described by Ilahy and others (2011) with slight modifications.
The absorbance was measured at 470 nm versus a blank of hexane solvent, using a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK). TCC were calculated following the Equation 1, according to Li and others (2013):

\[
\text{Total carotenoids (mg kg}^{-1}\text{)} = \frac{A_{470} \times V \times 10^4}{A_{1\%}^{1\%} \times G} \quad (1)
\]

where \(A_{470}\) is the absorbance at 470 nm, \(V\) is the total volume of extract (mL), \(A_{1\%}^{1\%}\) is extinction coefficient (2500 \(100 \text{ ml} \div g \text{ cm}\)), and \(G\) is sample weight (g). Total carotenoids results were expressed in mg kg\(^{-1}\) of fresh weight (fw).

2.6.3. Analysis of lycopene

Lycopene content (LC) was measured spectrophotometrically following the method proposed by Odriozola-Serrano and others (2007). The absorbance of the extract was measured in a 1-cm path length quartz cuvette at 503 nm to avoid interference with other carotenoids. LC was calculated according to Equation 2.

\[
\text{Lycopene (mg kg}^{-1}\text{)} = \frac{A_{503} \times MW \times DF \times 10^6}{\varepsilon \times L} \quad (2)
\]

where \(A_{503}\) is the absorbance at 503 nm, \(MW\) is the molecular weight of lycopene (536.9 g mol\(^{-1}\)), \(DF\) is the dilution factor, \(\varepsilon\) is the molar extinction coefficient of lycopene (17.2 \(\cdot 10^4\) L mol\(^{-1}\) cm\(^{-1}\)) and \(L\) is the pathlength (1 cm). Results of lycopene content were expressed in mg kg\(^{-1}\) (fw).

2.7. BIOACCESSIBILITY

Total carotenoid bioaccessibility (TCB) and lycopene bioaccessibility (LB) were calculated using Equation 3. Results were expressed as the percentage of carotenoids transferred from tomato matrix to the micellar fraction after the \textit{in vitro} digestion.

\[
\text{Bioaccessibility (\%) = } \frac{BC_{\text{digested}}}{BC_{\text{undigested}}} \times 100 \quad (3)
\]
where $BC_{\text{digested}}$ corresponded to the overall concentration of bioactive compound in the micellar fraction and $BC_{\text{undigested}}$ was the concentration in the non-digested samples.

### 2.8. STATISTICAL ANALYSIS

Each treatment replicate was obtained from five fruits. Four different replicates for each assayed condition were subjected to an *in vitro* gastrointestinal digestion. Each analysis was conducted twice ($n = 8$). A multifactor analysis of variance (ANOVA) was performed at $p < 0.05$ in order to determine significant differences in concentration and bioaccessibility of carotenoids from the tomato derivatives in relation to the factors studied in this research (tomato ripening, type of processing and addition of different types of oil). In addition, a correlation analysis based on Pearson’s test was carried out in order to determine the relationship between each assayed parameter. All statistical analyses were performed with the program JMP Pro v.12.0.1 software (SAS Institute, Cary, NC, USA).

### 3. RESULTS AND DISCUSSION

#### 3.1. PHYSICOCHEMICAL CHARACTERIZATION

A physicochemical characterization of tomato fruits at selected ripeness stages is shown in Table 1. Significant ($p < 0.05$) differences in surface colour of the fruits were observed as tomatoes ripened. The $a^*/b^*$ ratio, indicative of redness, significantly increased during ripening as a consequence of the increase of $a^*$ values, which ranged from $-13.8 \pm 1.5$ at mature-green stage and $15.0 \pm 2.9$ at red stage. Regarding soluble solids, pH and titratable acidity, no significant ($p > 0.05$) differences were observed between tomato fruits differing in ripeness stage.
3.2. CAROTENOID CONTENT

Changes in both total carotenoids and lycopene concentration as affected by tomato ripeness, type of processing and the addition of oil can be observed in Table 2. Pooled data indicate that the total carotenoids and lycopene content was influenced by the ripening stage and the type of processing, as well as by their interaction. However, the addition of different types of oil did not lead to significant (p > 0.05) changes in carotenoids content in the derived tomato products. These changes in carotenoids concentration in tomato products were accompanied by several changes in the main physicochemical properties of the tomato fruits (Table 1).

Total carotenoid content (TCC) in tomato-based products markedly increased as fruits ripened, ranging from 0.53 ± 0.11 mg kg⁻¹ at mature-green stage to 14.82 ± 1.62 mg kg⁻¹ when tomatoes were processed at the most advanced stage of ripeness (Table 2). Changes in LC during tomato ripening showed a similar pattern to that followed by TCC. LC in tomato derivatives processed at green-mature stage was very low and continuously increased by 40-fold during ripening, reaching values of 8.07 ± 0.87 mg kg⁻¹ at red-ripe stage (Table 2). These values were consistent with published data (Maiani and others 2009). It is important to consider that the spectrophotometric method used in this study could only allow the detection of the colored carotenoids. Therefore, colourless carotenoids, such as phytoene and phytofluene, which are also found in tomatoes (Engelman and others 2011) were not assessed. Further HPLC analysis should be carried out in order to precisely quantify the specific concentration of each individual compound during tomato ripening.

The accumulation of lycopene was simultaneous with the reddening of tomato fruits (Table 1). In this regard, a significant (p < 0.001) correlation between a*/b* ratio and
LC ($r = 0.991 - 0.998$) was found, which is consistent with the well-established relationship between the reddening of tomato and the accumulation of lycopene (Arias and others 2000). The results obtained in this work were in accordance with those found by Ilahy and others (2011) who also reported a continuous increase in TCC and LC during tomato ripening. A number of physiological, morphological and biochemical changes during tomato ripening has been described, including chlorophylls degradation and biosynthesis and accumulation of carotenoids, especially lycopene, during chloroplast to chromoplast transition (Ilahy and others 2011; Hdider and others 2013).

The degree of tissue disruption of tomato led to changes in TCC and LC (Table 2). Thus, significant decreases ($p < 0.05$) in TCC and LC contents, ranging between 4 - 59% and 9 – 46% respectively, were found when tomatoes were ground into puree with respect to tomato cubes. The principal causes of tomato carotenoids degradation during processing are isomerization, oxidation and co-oxidation reactions produced by lipoxygenases and peroxidases, which could be activated during tomato puree processing (Martínez-Hernández and others 2015). The molecular configuration of carotenoids, rich in conjugated double bonds, makes them susceptible to oxidation and isomerization (Takeoka and others 2001). Thus, all operations that disrupt food matrices, such as cutting or grinding, expose carotenoids to pro-oxidative conditions (light, heat, oxygen and/or acids), favouring the reduction of carotenoids content of tomato products, as outlined previously (Martínez-Hernández and others 2015).

The losses of TCC and LC during tomato puree production in presence of oil were lower than in absence of oil, in all the conditions (Table 2). Thus, TCC and LC losses ranged between 4 – 25% and 8 – 27%, respectively, after the addition of oil into samples, while these losses reached values of 36 – 59% for TCC, and 40 – 46% for LC.
in absence of oil. These data are in accordance with those results reported by Chen and others (2009), who found that the oxidative degradation of lycopene was greater in water-based tomato products than in oil-based samples. As oxygen is more soluble in oil than in water (Cuvelier and others, 2017), the reduction of the extent of oxidative phenomena affecting carotenoids could be related to the protecting action of oils against photo-oxidation as well as to the quenching of molecular oxygen.

Moreover, the type of oil had also an impact on carotenoids degradation. Thus, carotenoids degradation in tomato products after adding olive oil and sunflower oil, which are characterized to be rich in unsaturated fatty acids, ranged from 24 – 27%, while samples mixed with coconut oil, which is mainly composed by saturated fatty acids, exhibited losses ranging between 11 – 17%. This fact could be partially explained by the oxidative stability of the fatty acids composition (Liu and others 2015). Thus, the higher degree of unsaturation, the lower the oil stability. This may explain the greater degradation of carotenoids during processing when olive and sunflower oils were incorporated. Besides, other factors including the role of the oxidative stability of the oils, the carotenoid location inside the crystal network as well as the physical state of the lipid have been reported to affect the chemical stability of carotenoids (Calligaris and others 2014). According to Cornacchia and Roos (2011), a partial solid lipid (coconut oil) may entrap the carotenoids in isolated domains and keep them apart from oxidative species in a better way than liquid oils (sunflower or olive oil), thus leading to a lower carotenoid oxidation.

3.3. BIOACCESSIBILITY OF CAROTENOIDS

The influence of the addition of different types of oil on the bioaccessibility of carotenoids (TCB) and lycopene (LB) in two tomato derivatives (cubes and puree) at
three ripeness stages (mature-green, pink and red) is presented in Figures 1 and 2, respectively. Statistical analysis indicate that the total carotenoids and lycopene bioaccessibility was influenced by the ripening stage and the type of processing, as well as by the interaction of these factors with the type of added-oil (p < 0.001).

In spite of the fact that, to the best our knowledge, no data are available regarding the influence of the stage of ripeness of tomato on the bioaccessibility of carotenoids, our results seem to point out that the stage of ripeness at processing is an important variable affecting the bioaccessibility of carotenoids in tomato products (p < 0.05). Thus, a markedly increase in TCB and LB values were found throughout tomato ripening. In this sense, the amount of colored carotenoids released from tomato matrix during the simulated digestion of samples obtained from mature-green tomatoes could not be determined, because the carotenoids concentration in digested samples was negligible. Nevertheless, TCB and LB in tomato derivatives obtained from pink fruits exhibited a sharp increase, and reached the maximum values when tomatoes were processed at the most advanced ripeness stage. This trend was especially evident after the incorporation of different types of oil, leading to TCB and LB values ranging from 5.4 ± 1.2% to 29.3 ± 6.1% and from 4.6 ± 0.6% to 27.2 ± 5.2%, respectively. In addition, a good correlation between TCC of tomato and the amount of carotenoids released from the matrix after the in vitro digestion was found (r = 0.8; p < 0.0001). Thus, the accumulation of TCC as tomato ripened, led to an increase in the amount of released carotenoids during digestion and in turn, in their bioaccessibility. These findings are in accordance with those reported by Ornelas-Paz and others (2008), who found that the quantity of carotenoids of mango transferred into the micellar fraction during the simulated digestion significantly increased as the fruit ripened. Moreover, several studies have reported that the intake of pectin and other fibres decrease the
bioaccessibility of carotenoids (Rodríguez-Roque and others 2014). These food constituents increase the viscosity of duodenal medium and affect the emulsification and lipolysis of fat, necessary for carotenoids micellarization (Ornelas-Paz and others 2008). Moreover, it is well known that during ripening, a series of pectic enzymes, especially pectin methylesterase (PME) and polygalacturonase (PG), breakdown the pectin of cell walls, thus leading to a decrease in the methyl-esterification degree (DM) (Paniagua and others 2014; Manrique & Lajolo 2002). Recent studies have demonstrated that the DM of pectin plays an important role in β-carotene bioaccessibility in emulsions (Verrijssen and others 2014; Verrijssen and other 2016). In this regard, the higher DM of pectin in unripe tomatoes could hinder the incorporation of carotenoids into micelles resulting in lower bioaccessibility. Similar results were found by Verrijssen and others (2015) who reported an increase of the incorporation of β-carotene into the micelles by decreasing the pectin DM of the emulsions. In addition, the depolymerisation process could also facilitate the disruption of cell walls during digestion, allowing the release of carotenoids from tomato matrix and promoting their micellar solubilisation (Ornelas-Paz and others 2008).

Changes in tomato tissue structure, as a consequence of processing operations, exerted a significant influence (p < 0.05) on TCB and LB (Figure 1 and Figure 2). When tomatoes were ground into puree, TCB and LB values were greater than those observed in tomato cubes in all of the studied conditions. Thus, after the in vitro digestion of tomato puree, TCB and LB values were 55 – 209% and 46 – 251% greater than in tomato cubes, respectively. These results could be explained by the effect of processing operations in both the food matrix and the molecular structure of the carotenoids. On the one hand, several studies have reported that the physical state and location of carotenoids in food strongly affects their release from the matrix (Ryan and others 2008). Processing
operations involve changes in the microstructure of tomato, reducing the particle size
and breaking down cell walls, thus facilitating the liberation and solubilization of
carotenoids (Maiani and others 2009). According to Parada and Aguilera (2007), this
mechanical disruption enlarges the surface area available to the access of digestive
enzymes, thus facilitating the release of carotenoids from the food matrix (Ryan and
others 2008). As a consequence, the incorporation of carotenoids into micelles could be
promoted through processing, thus increasing their bioaccessibility. Tomato purees are
generally subjected to different thermal treatments. It has been confirmed that these
thermal processes would also increase the extractability of carotenoids from food matrix
and, therefore, their bioaccessibility (Tibäck and others 2009).

On the other hand, being highly unsaturated, carotenoids are thought to be isomerized
from all-trans form, which are the native form in fresh fruits, to cis-isomers during
processing (Martínez-Hernández and others 2015). It has been reported that cis-isomer
carotenoids may be easily incorporated in bile acid micelles because the bends in cis-
configurations decrease the space occupied by the molecule in comparison to the linear
all-trans structure (Failla and others 2008) and consequently increase its
bioaccessibility. However, further investigation would be interesting in order to clarify
the influence of the isomerization of carotenoids through mechanical processing on the
bioaccessibility of these health-related compounds. Furthermore, in vivo studies support
the hypothesis that cis-isomers are more efficiently absorbed (Unlu et al. 2007; Richelle
et al. 2012).

The addition of 5% of oil to tomato derivatives led to an increase in TCB and LB
values, regardless the studied conditions (Figure 1 and Figure 2). In samples without oil,
the amount of carotenoids released from tomato matrix was very low, ranging from
undetectable values to 2.9 ± 0.4% for TCB and 1.8 ± 0.2% for LB. After the addition of
different types of oil, TCB and LB were significantly (p < 0.05) enhanced, reaching values of 29.3% for TCB and 27.2% for LB. These maximum values corresponded to the puree obtained from red tomatoes with added olive oil. Previous studies have already revealed that the presence of oil enhances the bioaccessibility of carotenoids because dietary fats and oils may promote the dispersion of carotenoids in mixed micelles necessary to be taken up by intestinal enterocytes (Mashurabad and others 2017). Regarding the type of oil, the largest enhancement on TCB was noticed after the addition of olive oil, which can lead to a 21-fold increase in relation to samples without oil. In contrast, 11- and 7-fold increase in TCB values was observed when sunflower and coconut oils were added, respectively. Changes in LB exhibited similar trend than TCB. Thus the maximum values of LB were reached after the addition of olive oil (15-fold increase), followed by sunflower oil and coconut oil (11- and 7-fold increase, respectively). This trend was especially evident when tomatoes were ground into puree at fully ripe stage. The differences between the distinct added oils may be related to the chain length of fatty acids as well as their degree of unsaturation. Thus, the TCB and LB values in tomato products containing olive and sunflower oils, rich in long-chain fatty acids, were 32 – 68% higher than in products with addition of coconut oil, which is rich in medium-chain fatty acids. This is due to the fact that oils rich in medium-chain fatty acids have shown less effective swelling of the micelles compared to oils containing long-chain free fatty acids (Colle and others 2012). As the chain length of fatty acids increased, the hydrophobicity of the digested product increased and carotenoids incorporation from the food matrix into micellar phase was facilitated (Huo and others 2007). Additionally, transfer of carotenoids from tomato matrix to mixed micelles was significantly greater when the added oil was rich in unsaturated fatty acids (i.e., olive and sunflower oils) compared to saturated fatty acids (i.e., coconut oil). This
is similar to recent studies which observed an increment in carotenoids bioaccessibility after the *in vitro* digestion of different products with oils containing unsaturated long chain fatty acids (Colle and others 2012; Failla and others 2014). However, there are controversial conclusions about the influence of the degree of unsaturation of fatty acids on the bioaccessibility of carotenoids (Colle et al. 2012; Mashurabad et al. 2017).

Results obtained in this study suggest that the influence of the degree of unsaturation of added oils on the amount of bioaccessible carotenoids of tomato depends on the degree of tissue disruption during processing. Nevertheless, further investigations are necessary to clarify the influence of the fatty acid composition of added oils on the physicochemical characteristics of generated mixed micelles in order to elucidate the observed differences in carotenoids bioaccessibility.

**CONCLUSION**

Ripening-induced changes in tomato matrix influenced the amount and bioaccessible fraction of carotenoids, especially lycopene, in tomato-based products. Marked increases in TCC and LC were observed during tomato ripening, which were maxima when fruits were processed at red-ripe stage. These increments were accompanied by an improvement of TCB and LB. In addition, the type of processing also influenced the concentration of carotenoids before and after the *in vitro* digestion. Thus, in spite of TCC and LC in tomato puree significantly decreased, TCB and LB were greater than in tomato cubes. The addition of oil may play a protective role against carotenoids degradation in tomato-based products. Moreover, TCB and LB showed a significant improvement after the addition of different types of oil, especially when olive oil was added, following by sunflower and coconut oil. Differences could be explained by the fatty acids composition of the added oils. This study provides useful information about
the synergic effect of different factors affecting the amount and the bioaccessible
fraction of carotenoids, especially lycopene, in two common tomato derivatives.

However, further investigations are needed in order to assess the individual carotenoid
compounds, as well as their isomers, before and after the simulated digestion, with the
purpose of confirming the hypotheses reported in this work.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

BHT, butyl hydroxytoluene; $L^*$, lightness; $a^*$, green-red chromacity; $b^*$, blue-yellow
chromacity; SSF, simulated salivary fluid; TCC, total carotenoids content; LC, lycopene
content; TCB, total carotenoid bioaccessibility; LB, lycopene bioaccessibility; ANOVA,
analysis of variance; PME, pectin methylesterase; PG, polygalacturonase; DM, methyl
esterification degree.
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Figure 1. Total carotenoid bioaccessibility (%) in tomato cubes (A) and tomato puree (B) processed at three ripeness stages (mature-green, pink and red-ripe) after the addition of 5% of different types of oil (coconut, olive and sunflower oil). Results were expressed as mean ± standard deviation. Different lower case and capital letters represent statistically significant differences between different oils added at each stage of ripening (pink and red stage, respectively) (p < 0.05). ND: no detected.
Figure 2. Lycopene bioaccessibility (%) in tomato cubes (A) and tomato puree (B) processed at three ripeness stages (mature-green, pink and red-ripe) after the addition of 5% of different types of oil (coconut, olive and sunflower oil). Data represents average values ± standard deviation. Different lower case and capital letters represent statistically significant differences between different oils added at each stage of ripening (pink and red stage, respectively) (p < 0.05). ND: no detected.
Table 1. Physicochemical characterization of tomato at different ripeness stages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ripeness stage</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mature-green</td>
<td>Pink</td>
<td>Red</td>
<td></td>
</tr>
<tr>
<td>Chromaticity of fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$L^*$</td>
<td>45.9 ± 2.8 ab</td>
<td>46.9 ± 1.8 a</td>
<td>44.9 ± 2.8 b</td>
<td></td>
</tr>
<tr>
<td>$a^*$</td>
<td>-13.8 ± 1.5 c</td>
<td>1.7 ± 3.8 b</td>
<td>15.0 ± 2.9 a</td>
<td></td>
</tr>
<tr>
<td>$b^*$</td>
<td>25.2 ± 1.9 a</td>
<td>24.9 ± 2.4 a</td>
<td>23.8 ± 2.8 a</td>
<td></td>
</tr>
<tr>
<td>$a^<em>/b^</em>$</td>
<td>-0.6 ± 0.1 c</td>
<td>0.1 ± 0.2 b</td>
<td>0.6 ± 0.2 a</td>
<td></td>
</tr>
<tr>
<td>Soluble solids (°Brix)</td>
<td>4.77 ± 0.15 a</td>
<td>4.85 ± 0.14 a</td>
<td>5.05 ± 0.07 a</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>4.09 ± 0.13 a</td>
<td>4.02 ± 0.02 a</td>
<td>4.07 ± 0.03 a</td>
<td></td>
</tr>
<tr>
<td>Titratable acidity (g citric acid · kg⁻¹)</td>
<td>0.45 ± 0.06 a</td>
<td>0.46 ± 0.05 a</td>
<td>0.45 ± 0 a</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (n = 8).
**Table 2.** Changes of total carotenoids and lycopene contents (mg kg\(^{-1}\)) of two tomato derivatives (cubes and puree) at different ripening stages added or not with coconut oil, olive oil or sunflower oil.

<table>
<thead>
<tr>
<th>Ripeness stage</th>
<th>Oil type</th>
<th>Tomato cubes</th>
<th>Tomato puree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total carotenoids</td>
<td>Lycopene</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mg kg(^{-1}))</td>
<td>(mg kg(^{-1}))</td>
</tr>
<tr>
<td>Mature-green</td>
<td>No oil</td>
<td>1.27 ± 0.24 (^a)</td>
<td>0.21 ± 0.04 (^b)</td>
</tr>
<tr>
<td></td>
<td>Coconut oil</td>
<td>2.02 ± 0.12 (^A)</td>
<td>0.43 ± 0.01 (^D)</td>
</tr>
<tr>
<td></td>
<td>Olive oil</td>
<td>2.02 ± 0.25 (^A)</td>
<td>0.37 ± 0.08 (^B)</td>
</tr>
<tr>
<td></td>
<td>Sunflower oil</td>
<td>1.92 ± 0.14 (^A)</td>
<td>0.33 ± 0.02 (^B)</td>
</tr>
<tr>
<td>Pink</td>
<td>No oil</td>
<td>7.52 ± 0.40 (^A)</td>
<td>3.49 ± 0.43 (^A)</td>
</tr>
<tr>
<td></td>
<td>Coconut oil</td>
<td>6.67 ± 0.96 (^A)</td>
<td>3.19 ± 0.54 (^A)</td>
</tr>
<tr>
<td></td>
<td>Olive oil</td>
<td>5.31 ± 0.17 (^A)</td>
<td>2.63 ± 0.12 (^A)</td>
</tr>
<tr>
<td></td>
<td>Sunflower oil</td>
<td>6.45 ± 1.28 (^A)</td>
<td>2.95 ± 0.62 (^A)</td>
</tr>
<tr>
<td>Red</td>
<td>No oil</td>
<td>14.82 ± 1.62 (^A)</td>
<td>8.07 ± 0.87 (^A)</td>
</tr>
<tr>
<td></td>
<td>Coconut oil</td>
<td>11.44 ± 0.24 (^A)</td>
<td>6.33 ± 0.12 (^A)</td>
</tr>
<tr>
<td></td>
<td>Olive oil</td>
<td>11.53 ± 0.55 (^A)</td>
<td>6.39 ± 0.29 (^A)</td>
</tr>
<tr>
<td></td>
<td>Sunflower oil</td>
<td>11.39 ± 0.94 (^A)</td>
<td>6.46 ± 1.28 (^A)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (\(n = 8\)). Different lower case letters within a same column denote statistically significant differences. Different capital letters within the same ripeness stage indicate statistically significant differences in total carotenoids or lycopene contents (\(p < 0.05\)).