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**Research article** 

# Thermal concentration effects of tomato pulp and carotenoids extraction from the residue

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### Introduction

Tomato (*Solanum lycopersicum* L.) and its derivatives are widely known for their nutritional and antioxidant properties due to the presence of minerals, vitamins, carotenoids, and phenolic compounds. Lycopene, the predominant carotenoid in tomatoes, is responsible for their red color and is known to have significant antioxidant potential. Studies have proved the benefits of lycopene and other bioactive compounds of tomatoes and tomato-based products with immunoprotective activity, as well as a reduction in the risk of cancer and cardiovascular diseases through reductions in the oxidative stress of cells (Yang et al., 2001; Xu et al., 2019).

Bioactive compounds in tomatoes are modified according to the variety and growing conditions of the fruit but they are also affected by time and the temperature of processing its derivatives. Ascorbic acid, for example, degrades easily in the presence of light, heat, and oxygen. However, depending on the temperature and time used, a higher concentration of lycopene can be achieved (Kelebek et al., 2017), since it is considered a very stable molecule (Manzo et al., 2018).

Lycopene is linked to the food matrix in *trans* form, which hinders its complete release and makes it susceptible to less digestion and absorption in the human digestive tract. *In vitro* digestion methods have been widely used to assess the impact that food processing has on the bioaccessibility of bioactive compounds, in addition to its being considered economical, quick, and easily reproducible when compared to *in vivo* methods.

**ABSTRACT:** The present study evaluated the effect of two thermal concentration systems on bioactive compounds, the sugar content of tomato (*Solanum lycopersicum* L.) pulp, and the carotenoid bioaccessibility of pulp concentrate. The closed processing system ensured a higher retention of phenolic and carotenoid compounds. The bioaccessibility of lycopene in tomato pulp concentrate was relatively low (0.54 %) but higher than in raw tomato pulp (0.15 %), corroborating other results that have reported the low availability of the compound in these matrices. Carotenoid extraction from tomato residue was also evaluated through both conventional (CE) and ultrasound (UAE) extractions together with the stability of extracts over 30 days. UAE promoted a superior release of lycopene and lutein than conventional extraction. Lycopene showed less stability with a reduction of 18 % in 30 days.

Keywords: phenolic compounds, bioaccessibility, ultrasound, conventional extraction

Tomato is considered a versatile fruit because it can be consumed *in natura* or processed in different ways. However, when processing tomatoes, peels and seeds are generally separated from the pulp, also referred to as tomato waste. It is known that certain bioactive compounds can be found in higher concentration in fruit peel in general, and that the nutritional importance of tomato residue has also been reported on account of a skin rich in dietary fiber and lycopene and seeds as significant sources of protein and fat (Lu et al., 2019).

There are several methods of carotenoid extraction, and the most conventional, the solid-liquid extraction, uses organic solvents and usually takes longer. Adaptations to this method aimed at reducing the cost and time of processing and increasing the purity of the compounds have already been evaluated (Naviglio et al., 2008a, b).

Thus, the aim of this work was to evaluate the impact on bioactive compounds, sugars and the carotenoid bioaccessibility of tomato pulp concentrate by two different thermal processes. It was also intended to verify the use of tomato residue, focusing on carotenoids through the evaluation of two extraction methods and the stability of extracts over 30 days under refrigeration conditions.

# **Materials and Methods**

#### Fruit material and sample preparation

Fresh ripe Italian-type tomatoes (*Solanum lycopersicum* L.) were purchased from a local market (Guaratiba, Rio de Janeiro, Brazil). They were washed, sanitized by immersion in a 200 mg  $L^{-1}$  chlorine solution for 20 min,

manually cut and depulped in a horizontal depulper equipped with a 0.8 mm diameter sieve that separated the whole pulp from the peels and seeds. The pulp obtained was placed in 5 L plastic containers. Tomato residue was dried in a convective dryer at 60 °C with an average air flow of 0.42 m s<sup>-1</sup> for 30 h, followed by grinding in a disk mill.

#### Thermal concentration

The concentrations were carried out through two processing systems: an open system to simulate a homemade concentration and a closed system to simulate an industrial concentration, both processes being carried out once. The concentrate in the open system was prepared in a jacketed pan equipped with a steam generation boiler, under constant agitation at 8.90 rad s<sup>-1</sup> with a temperature ranging between 80-90 °C, monitored by a thermocouple. The closed system was carried out using a rotary evaporator, at 8.90 rad s<sup>-1</sup>, 85 °C and a vacuum of 4 hPa. The soluble solids content was the parameter required to finish the experiments, stopping when the content had at least doubled as checked by a refractometer.

The two tomato concentrates were named TC1 (80-90 °C for 120 min) and TC2 (85 °C for 45 min), respectively. The Concentration Factor (CF) was determined by the ratio between the initial and the final content of the evaluated parameter.

#### Extraction of carotenoids from tomato waste

Absolute ethanol was used as the solvent. Two methods of extraction were evaluated: Conventional Extraction (CE) and Ultrasound Assisted Extraction (UAE), with time ( $x^1$ ) and solvent-residue ratio ( $x^2$ ) as variables. In both processes, a Central Composite Design  $2^2$  was formulated, resulting in 11 independent assays with triplicates of the central point and four axial points as follows:  $x^1$  was 30, 45 and 60 min for CE and 10, 15 and 20 min for UAE. The  $x^2$  variable was 1:10, 1:20 and 1:30 (v/w) for both experiments.

Conventional extraction was carried out in a thermostatic bath with orbital shaking at 30 °C and 85 rpm. Three consecutive extractions were performed in each assay, combining the aliquots in a volumetric flask. In the UAE, only one extraction was performed for each assay, using a 1000 W ultrasound unit equipped with a titanium sonotrode BS2d18 (18 mm in diameter) and a booster B4.18, magnifying the working amplitude. The power had been previously set at 80 W and the sonotrode immersed 2 cm into the extraction solution. The sample was immersed in an ice bath during processing to delay overheating of the system.

The extracts from both processes were filtered through qualitative paper and then stored at -18 °C until analysis. For total carotenoids, the ethanolic extract was washed with water in a separatory funnel containing

petroleum ether, filtered through qualitative paper containing anhydrous sodium sulfate and avolumated with petroleum ether. Next, for a chromatographic profile, samples were dried and resuspended in acetone for High-Performance Liquid Chromatography (HPLC) characterization. The assay from CE and UAE that achieved the highest recovery in total carotenoids was chosen to evaluate carotenoid stability. For CE, the best recovery was achieved when using the highest solvent residue ratio and the shortest extraction time. For the UAE, maximum recovery was also achieved with the highest solvent residue ratio but in a higher process time. The recovery was calculated using the initial concentration of each carotenoid in tomato waste according to Rodriguez-Amaya (2001) and Pacheco et al. (2014).

#### Extract storage stability

The chosen assay of both extractions was repeated to evaluate carotenoid stability for 30 days. Aliquots were separated for each period evaluated (0, 15 and 30 days) and were kept in glass containers with a screw cap under refrigeration conditions (0-10 °C). The results were expressed as the ratio between the carotenoid content in the extract of each period and the initial carotenoid content of tomato residue (C/C<sub>o</sub>).

# Analytical determinations, pH, moisture, soluble solids and total titrable acidity

The pH was analysed in a pHmeter by direct reading at 25 °C. Total acidity was determined using the potentiometric method in an automatic titrator. Soluble solids were ascertained with an Abbé-type refractometer. Moisture was analysed in an oven at 105 °C until constant weight. All analyses were performed according to AOAC (2000).

#### Sugar profile

Sugar compositions were determined according to Macrae (1998). Sugar extraction was conducted in a 20 mL volumetric flask, using 1 g of sample and 10 mL ultrapure water in an ultrasonic bath for 20 min with subsequent addition of 5 mL of acetonitrile. An aliquot of the extract was filtered directly into the autosampler vial. The samples were analyzed in a HPLC system equipped with a refractive index detector and an amino column  $30 \text{ cm} \times 4.6 \text{ mm}$  (High Performance Carbohydrate). The flow rate used was 1.4 mL min<sup>-1</sup>, the injection volume was 20 µL and an acetonitrile:water ratio (75:15 v/v) was applied in the mobile phase. The total run time was 20 min. The column and detector temperatures were set at 30 °C and 45 °C, respectively. Sugar was quantified by external standardization according to commercial analytical standards.

#### Vitamin C

The extraction was carried out in a 25 mL volumetric flask, using 2.5 g of sample and 10 mL of 50 mol m<sup>-3</sup> sulfuric acid in an ultrasound bath for 10 min (Rosa et al., 2007). An aliquot of the extract was filtered directly into the 1.5 mL autosampling vial. An HPLC system equipped with a photodiode array detector was used for the chromatographic analysis. The system was run at 0.7 mL min<sup>-1</sup>, using an ion exchange. A sulfuric acid solution 50 mol m<sup>-3</sup> was used as the mobile phase. The total run time was 12 min. The column and detector temperatures were set at 30 °C and 45 °C, respectively. An injection volume of 20 µL was used, which was quantified by external standardization through the construction of an analytical curve from dilutions of the standard solution prepared.

#### Total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteau reagent by a spectrophotometric method (Singleton and Rossi, 1965). Phenolic compounds were extracted in 25 mL volumetric flasks using a 10 g sample and a 70 % acetone solution, under constant magnetic stirring, for 30 min. The samples were filtered, and the acetone concentration was corrected to 7 % by dilution with distilled water. The extract was left to react with the 10 % Folin-Ciocalteau solution at room temperature for 2 min, followed by the addition of 7.5 % sodium carbonate solution, which reacted with the mixture at 50 °C for 15 min. A spectrophotometer took the reading at 760 nm. The results were expressed in mg of gallic acid equivalent per 100 g of sample (mg GAE  $100^{-1}$  g<sup>-1</sup>).

#### ABTS radical scavenging capacity

The antioxidant capacity was evaluated by the ABTS radical cation (ABTS<sup>+</sup>) assay according to Re et al. (1999). ABTS radical was prepared with the addition of 5 mL of an aqueous ABTS solution (7 mol m<sup>-3</sup>) and 0.088  $\mu$ L of K<sub>2</sub>SO<sub>5</sub> solution (140 mol m<sup>-3</sup>) in an amber flask for 16 h. The previously prepared ABTS radical cation stock solution was diluted in 95 % ethanol to prepare ABTS<sup>+</sup> working solution with initial absorbance of 0.7  $\pm$  0.02 at 734 nm. The reaction was generated by mixing 30  $\mu$ L of the extract with 3 mL of the radical solution and rested for 6 min in the dark at room temperature. Next, the absorbance was read at 734 nm in a spectrophotometer. A standard Trolox curve (0-0.0025 mol m<sup>-3</sup>) was used and the results expressed as  $\mu$ mol Trolox-Equivalent antioxidant capacity ( $\mu$ mol ET g<sup>-1</sup>).

#### Total carotenoids and carotenoid profile

The carotenoid extraction was carried out according to the method described by Rodriguez-Amaya (2001). The sample was weighed (1 g) for the extraction. Total carotenoids were determined by spectrophotometry at 470 nm. The carotenoid profile was determined in an acetone extract by HPLC (Pacheco et al., 2014) at 470 nm. Carotenoid separation was obtained in a C30 column (S-3 Carotenoid, 4.6 mm × 250 mm, YCM) by a gradient elution of methanol and methyl tert-butyl ether. The flow rate was 0.8 mL min<sup>-1</sup> and the running time 28 min. The column temperature was 33 °C and the injection volume of the samples 15 µL. Carotenoids were identified on the basis of their retention times and UV/V absorption spectra compared to the retention times of the carotenoid standards. Carotenoid content was calculated according to Eq. (1).

$$Carotenoids(\mu g \ 100^{-1} g^{-1}) = \frac{Absorvance \times flask \ volume \ (mL) \times 10^3 \times dilution \ (if \ have)}{A_{1cm}^{1\%} \times mass \ (g)}$$
(1)

where:  $A_{1cm}^{1\%}$  molar absorptivity coefficient of lycopene in petroleum ether = 3450 (major compound).

#### In vitro digestion assay

The oral, gastric, and intestinal phases were implemented according to Brodkorb et al. (2019). The compositions of Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF), and Simulated Intestinal Fluid (SIF) were reproduced as described by Minekus et al. (2014). At the end of each stage of immersion in a water bath at 37 °C with 10.47 rad s<sup>-1</sup> of agitation, the tubes were immersed in an ice bath to interrupt the action of the enzyme. A control tube was used for adjusting the pH.

Initially, 5 g of tomato pulp was weighed in a 50 mL centrifuge tube to which 4 mL of SSF stock solution (pH 7.0  $\pm$  0.1), 0.5 mL of amylase solution (7.19 units mg<sup>-1</sup> in ultrapure water), 25 µL of 300 mol m<sup>-3</sup> CaCl<sub>2</sub> and 475 µL of ultrapure water were added. The oral phase lasted for 2 min in the water bath. A volume of 8 mL of SGF stock solution (pH 3.0  $\pm$  0.1), 0.5 mL of pepsin solution (2227.3 units mg-1 in ultrapure water), 5 µL of 300 mol m<sup>-3</sup> CaCl<sub>21</sub> 995 µL of ultrapure water were added, and the pH of the mixture was adjusted to 3.0  $\pm$  0.2 with 1000 mol m<sup>-3</sup> HCl. The gastric phase lasted for 2 h in a water bath. The pH of the gastric phase was adjusted to seven with 1000 mol m<sup>-3</sup> NaOH to perform the intestinal digestion. A volume of 8.5 mL of SIF stock solution (pH 7.0  $\pm$  0.1), 5 mL of pancreatin solution (7.1 units mg<sup>-1</sup> in SIF), 2.5 mL of bile solution, and 40 µL of 300 mol m<sup>-3</sup> CaCl, were added. The intestinal phase lasted for 2 h in water bath. All enzyme activities were determined according to Minekus et al. (2014).

#### Micellar extraction

The control tube was discarded. Aliquots of 15 mL from each tube with digested sample were transferred to new tubes and centrifuged at 5000  $\times$  g for 45 min at 4 °C. The supernatant was filtered through a 0.22 µm cellulose acetate filter. The filtrate was transferred

to a separatory funnel containing 10 mL of petroleum ether, and 200 mL of aqueous NaCl 10 % (w/v) solution was added and agitated. The organic phase was washed using a 200 mL Na<sub>2</sub>SO<sub>4</sub> 2 % solution, and the extract was filtered into an amber volumetric flask containing a funnel with anhydrous Na<sub>2</sub>SO<sub>4</sub>. The funnel was rinsed with petroleum ether to avoid sample losses. The volume was completed with petroleum ether and read at 470 nm. The bioaccessibility of the total carotenoids was obtained and calculated according to equation Eq. (2).

To obtain the carotenoid profile after digestion, the ethereal fraction was dried with nitrogen for subsequent resuspension in acetone for determination by HPLC.

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% Bioaccessibility = \frac{\text{carotenoids concentration after digestion (µg 100<sup>-1</sup>g<sup>-1</sup>)×100}{\text{initial carotenoid concentration in the whole sample (µg 100<sup>-1</sup>g<sup>-1</sup>)}
(2)
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#### Statistical analysis

Data were subjected to Univariate Analysis of Variance (ANOVA) and Tukey test with a significance level of 5 % (p < 0.05), using the Statistica 10.0 software program. All measurements were carried out in triplicate.

### **Results and Discussion**

The main steps in the experiment taken in the present study are summarized in Figure 1A-I.

# Effect of thermal processing on the chemical composition of tomato pulp

Tomato concentrates did not show significant variations in pH (4.18 in both) compared to the raw pulp (4.28). As regards acidity, an increase of 41 % in both pulp concentrates was observed, resulting in 0.41 mg of citric acid  $100^{-1}~{\rm g}^{-1}.$ 

The final soluble solids content of TC1 and TC2 were very similar (Table 1), and it seemed that the effect of thermal treatment on bioactive compounds was related more to the extension of thermal processing rather than to the loss of water. The processing time in the open system was much higher than in the closed system, in addition to the better exposure to oxidative factors, which facilitate the degradation of nutrients and bioactive compounds.

Thermal treatment decreased (p < 0.05) the ascorbic acid content of the samples. Losses of 21 % and 5 % were observed for TC1 and TC2, respectively. Although the final pH was lower in the open system, which in fact could increase the stability of ascorbic acid, in this processing there was more exposure to light and oxygen, which probably favored more significant degradation of the compound (Boonpangrak et al., 2016).

On the other hand, the thermal processing increased (p < 0.05) the bioactive compound and sugar concentration. Glucose and fructose showed an increase of 32 % and 31 %, respectively, when the pulp was concentrated in an open system. Under the closed system, the increase was 52 % and 62 %, respectively. The closed system provides more energy to break the bonds between compounds and the molecules of the matrix, thereby facilitating the release of sugars since the closed system retains more heat than the open one. No sucrose was detected in the samples.

Total phenolic compounds and total carotenoid content increased 85 % and 91 % when the closed system was used, which was very close to the concentration



Figure 1 – Processing steps and experiments. (A) Tomato *in natura*; (B) Raw pulp; (C) Tomato residue; (D) Tomato pulp concentrated by open system; (E) Tomato pulp concentrated by closed system; (F) Dried tomato residue; (G) Conventional Extraction; (H) Ultrasound Assisted Extraction; (I) Carotenoids extract. Credits: Miranda, N.G.M.

factor achieved in the process (CF  $\sim$  2). In the open process, the levels of these compounds increased only by 25 % and 46 %, respectively, indicating degradation of these compounds probably due to the more prolonged exposure to heat, light, and oxygen.

As regards the carotenoid profile, lycopene was the major carotenoid found in raw pulp, TC1, and TC2, which accounted for 88 %, 82 %, and 77 %, respectively, of the total carotenoids in each sample evaluated and, in minor contents, betacarotene (3.7 % to 4.9 %), and lutein (0.8 % to 1.1 %) were also detected. However, lycopene concentration factors were minor compared to the CF of the process, which can be explained because of possible lycopene oxidation or isomerization reactions during the processing. The concentration factors of antioxidant activity and lycopene content were close in the two experiments, reinforcing the advantage of concentration processes in preserving the lycopene as a bioactive tomato compound since it's a relatively thermal stable compound.

#### **Bioaccessibility of carotenoids**

The bioaccessibility of total carotenoids in the raw (0.15 %) and concentrated tomato pulps was less than 1 %, regardless of the pulp processing system (Table 2). An increase was observed in the concentrated pulp, which corroborated studies that reported a higher release of carotenoids from the food matrix when subjected to heat (Colle et al., 2010; Svelander et al.,

2010). The isomeric form of lycopene directly influences the availability of the compound. The effects of thermic treatments are known to increase this availability since heat facilitates membrane rupture and provides the conversion of lycopene *trans* form into *cis* (Knockaert et al., 2012).

However, in the present study, neither heat treatment favored the transformation into *cis* lycopene, since the *trans* form remained in a higher amount even after thermic concentration. A lower ratio between the *cis/trans* isomers of lycopene was observed in the pulp concentrated by the open system (1:24) when compared to that obtained in the closed system (1:55). Open system processing promoted slightly higher bioaccessibility (0.54 %) than the closed system (0.41 %), which is also explained by the shorter processing time in the closed system being insufficient for a higher release of the compounds.

In both processes, a low concentration in all compounds was observed after digestion, indicating a small available fraction, reaching a maximum of 0.34 % for *trans* lycopene in the open system. A study found bioaccessibility of 2.9 % for total carotenoids in tomato puree and almost 30 % after adding 5 % olive oil, using 33,768 g of centrifugation for 20 min at 4 °C in micellar extraction (González-Casado et al., 2018). In the present study, oil was not added to samples since it was intended to maintain the integrity of the tomato pulps as much as possible, even knowing that the presence of oil would probably increase the bioaccessibility of

Table 1 – Characterization of the raw and concentrated tomato pulps obtained by open and closed systems.

	neter Raw Pulp Cor Open System CF <sub>open</sub>	Concentrated Pulp				
Parameter		CF <sub>open</sub>	Closed System	CF <sub>closed</sub>		
Total soluble solids	4.0	8.0	2.0	8.3	2.1	
Total phenolics	$57.04^{\circ} \pm 1.18$	71.2 <sup>b</sup> ± 0.25	1.2	106.3° ± 3.54	1.9	
Antioxidant capacity	$1.12^{a} \pm 0.03$	$1.60^{a} \pm 0.03$	1.4	2.01 <sup>b</sup> ± 0.09	1.8	
Total carotenoids	$4.47^{a} \pm 0.03$	$6.54^{b} \pm 0.05$	1.5	8.55° ± 0.03	1.9	
Lycopene	$3.91^{\circ} \pm 0.02$	$5.35^{b} \pm 0.11$	1.4	6.62° ± 0.05	1.7	
β-carotene	$0.22^{a} \pm 0.01$	$0.24^{a} \pm 0.02$	1.1	$0.37^{b} \pm 0.00$	1.7	
Lutein	$0.05^{a} \pm 0.01$	$0.05^{a} \pm 0.00$	1.0	$0.07^{a} \pm 0.02$	1.4	
Vitamin C	7.21°± 0.15	$5.71^{b} \pm 0.01$	0.8	6.83° ± 0.10	0.9	
Glucose	$1.49^{\circ} \pm 0.04$	$1.96^{b} \pm 0.01$	1.3	2.27 <sup>b</sup> ± 0.01	1.5	
Fructose	$1.44^{a} \pm 0.00$	$1.88^{b} \pm 0.03$	1.3	2.33° ± 0.04	1.6	

Total soluble solids expressed as "Brix; Total phenolics expressed as mg gallic acid  $100^{-1}$  g<sup>-1</sup>; Antioxidant capacity is expressed as µmol ET g<sup>-1</sup>; Carotenoids and Vitamin C are expressed as mg  $100^{-1}$  g<sup>-1</sup>; Sugars are expressed as g  $100^{-1}$  g<sup>-1</sup>; Results expressed on average ± standard deviation (n = 3); Different letters on the same line indicate difference (p < 0.05) between samples.

	Table 2 – Carote	noid profile of	concentrated tomato	pulps before	e and after	in vitro digestion
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Corretencid (up 100-1 g-1)	Open system		Closed System	
	Before digestion	After digestion	Before digestion	After digestion
Lutein	46.12 ± 1.3	nd	102.51 ± 0.71	nd
Beta-carotene	$505.05 \pm 5.24$	$0.74 \pm 0.01$	370.21 ± 7.07	$0.43 \pm 0.01$
Cis lycopene	751.14 ± 7.84	$1.03 \pm 0.02$	269.06 ± 21.04	$0.31 \pm 0.05$
Trans lycopene	7204.35 ± 10.24	24.43 ± 0.12	6615.07 ± 52.31	17.21 ± 0.06

nd = not detected; Results expressed as average  $\pm$  standard deviation (n = 3).

carotenoids. The maximum centrifugation available was 1047.2 rad s<sup>-1</sup>; therefore, it was decided to increase the centrifugation time to 1 h to guarantee an efficient separation of the phases. Centrifugation is essential in this determination since it separates the aqueous phase containing oil and micelles (supernatant) from the rest of the undigested material.

#### Carotenoid extraction from residue

A chromatogram of the carotenoids extracted from the tomato residue, with each compound identified in the respective retention time and UV/Vis spectra, is represented in Figure 2. Several forms of *cis* lycopene



Figure 2 – Chromatogram of carotenoid standards found in vegetables (black) and of carotenoids found in the tomato waste sample (red) at 450 nm, followed by the UV/Vis spectra of carotenoids (Lutein,  $\beta$ -carotene, Lycopene) identified in the sample.

were identified at lower concentrations, represented by the smallest peaks between the retention times of 19 and 24 min, with identical spectra to all *trans* lycopene, with a band in the UV region, identifying the presence of double bonds *cis*.

In both processes, lycopene was the most extracted compound (Table 3). The higher percentual retention in CE was 52 % for betacarotene, followed by 45 % and 37 % for lycopene and lutein, respectively. Betacarotene in CE remained stable for the 30 days of storage (Figure 3A). Lycopene and lutein showed a reduction in the concentration at 15 days of storage, remaining constant until 30 days had passed.

The extract obtained by UAE showed better retention for lycopene (Figure 3B) and lutein than CE, reaching 76 % and 47 %, respectively. Despite having presented the highest retention level, lycopene was also the less stable compound, representing





Table 3 – Physical and chemical characterization of tomato dried residue and the extracts from both extraction processes.

Parameter	Residue powder*	Conventional Extract	Ultrassound Assisted Extract
Moisture	$1.42 \pm 0.05$	na	na
Total carotenoids	$13601.66 \pm 101.5$	8336.72 ± 100.43	9962.82 ± 133.12
Lycopene	6159.30 ± 154.21	2773.53 ± 16.91	4686.67 ± 115.03
Beta carotene	3451.02 ± 143.16	$1636.48 \pm 16.18$	1458.15 ± 55.15
Lutein	703.50 ± 10.44	256.78 ± 4.27	333.08 ± 10.08

Carotenoids are expressed as  $\mu g \ 100^{-1} \ g^{-1}$  dry weight; Moisture is expressed as  $g \ 100^{-1} \ g^{-1}$ ; na = not applicable; \*Total carotenoid analysis according to Rodriguez-Amaya (2001) and Pacheco et al. (2014).

18 % of reduction over the period. Ultrasound assisted extraction did not favor betacarotene retention (42 %), but it was the single compound that remained stable throughout the time evaluated. Lutein decreased only after 30 days due to its greater stability in organic solvents (Becerra et al., 2020).

Comparing the two processes evaluated, 20 min of UAE were enough to extract more lycopene and lutein than the closed obtained in 90 min of CE, also considering that three consecutive extractions were carried out in each assay in CE, resulting in an extensive process time. The bubbles generated in the solvent on UAE grow until they break and produce shock waves that promote an increase in temperature and pressure, affecting cell walls and membranes that facilitate the release of compounds (Yilmaz et al., 2017). The solvent polarity directly affects its ability to dissolve certain groups of antioxidant compounds (Briones-Labarca et al., 2019). This study chose ethanol as the solvent for extraction due to its lower cost and toxicity than other commonly used solvents. However, it was considered that ethanol is an exceptionally polar solvent, while carotenoids, as fat-soluble compounds, are nonpolar, especially lycopene, one of the most nonpolar among them. Under the chosen experimental conditions, total recovery was not expected; however, it can be concluded that the results were satisfactory considering the circumstances.

# Conclusions

The closed system conditions favored a higher concentration of bioactive compounds and sugars on the tomato pulp, increasing 52 % and 62 % of glucose and fructose, respectively, 85 % on total phenolic compounds and 91 % and total carotenoids. Despite the expected reduction of ascorbic acid in both processes, the loss in the closed system was lower, falling only 5 %, against 21 % with the opened system. An increase in the bioaccessibility of carotenoids when the pulp was concentrated was also observed; however, it did not reach 1 %. Ultrasound assisted extraction has been shown to be a valiable tool for improving the release and extraction of potentially health-related compounds, in addition to being faster than conventional extraction. Betacarotene was the only compound that remained stable throughout the 30 days that was evaluated under refrigeration conditions.

# **Authors' Contributions**

Data curation: Miranda, N.G.M.; Gomes, F.S.; Jesus, M.S.C. Formalanalysis: Miranda, N.G.M.; Jesus, M.S.C. Funding acquisition: Cabral, L.M.C. Methodology: Miranda, N.G.M. Project administration: Gomes, F.S.; Cabral, L.M.C. Supervision: Gomes, F.S. Writingoriginal draft: Miranda, N.G.M. Writing-review & editing: Gomes, F.S.; Cabral, L.M.C.; Jesus, M.S.C.

# References

- Association of Official Analytical Chemists International [AOAC]. 2000. Official Methods of Analysis. 17ed. AOAC, Gaithersburg, MD, USA.
- Becerra, M.O.; Contreras, L.M.; Lo, M.H.; Díaz, J.M.; Herrera, G.C. 2020. Lutein as a functional food ingredient: Stability and bioavailability. Journal of Functional Foods 66: 103771. https:// doi.org/10.1016/j.jff.2019.103771
- Briones-Labarca, V.; Giovagnoli-Vicuña, C.; Cañas-Sarazúa, R. 2019. Optimization of extraction yield, flavonoids and lycopene from tomato pulp by high hydrostatic pressureassisted extraction. Food Chemistry 278: 751-759. https://doi. org/10.1016/j.foodchem.2018.11.106
- Brodkorb, A.; Egger, L.; Alminger, M.; Alvito, P.; Assunção, R.; Balance, S.; Bohn, T.; Bourlieu-Lacanal, C.; Boutrou, R.; Carrière, F.; Clemente, A.; Corredig, M.; Dupont, D.; Dufour, C.; Edwards, C.; Golding, M.; Karakaya, S.; Kirkhus, B.; Le Feunteun, S.; Lesmes, U.; Macierzanka, A.; Mackie, A.R.; Martins, C.; Marze, S.; McClements, D.J.; Ménard, O.; Minekus, M.; Portmann, R.; Santos, C.N.; Souchon, I.; Singh, R.P.; Vegarud, G.E.; Wickham, M.S.J.; Weitschies, W.; Recio, I. 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols 14: 991-1014. https://doi.org/10.1038/s41596-018-0119-1
- Boonpangrak, S.; Lalitmanat, S.; Suwanwong, Y.; Prachayasittikul, S.; Prachayasittikul, V. 2016. Analysis of ascorbic acid and isoascorbic acid in orange and guava fruit juices distributed in Thailand by LC-IT-MS/MS. Food Analytical Methods 9: 1616-1626. https://doi.org/10.1007/s12161-015-0337-x
- Colle, I.; Van Buggenhout, S.; Van Loey, A.; Hendrickx, M. 2010. High pressure homogenization followed by thermal processing of tomato pulp: influence on microstructure and lycopene in vitro bioaccessibility. Food Research International 43: 2193-2200. https://doi.org/10.1016/j.foodres.2010.07.029
- González-Casado, S.; Martín-Belloso, O.; Elez-Martínez, P.; Soliva-Fortuny, R. 2018. *In vitro* bioaccessibility of colored carotenoids in tomato derivatives as affected by ripeness stage and the addition of different types of oil. Journal of Food Science 83: 1404-1411. https://doi.org/10.1111/1750-3841.14140
- Kelebek, H.; Selli, S.; Kadiroglu, P.; Kola, O.; Kesen, S.; Uçar, B.; Çetiner, B. 2017. Bioactive compounds and antioxidant potential in tomato pastes as affected by hot and cold break process. Food Chemistry 220: 31-41. https://doi.org/10.1016/j. foodchem.2016.09.190
- Knockaert, G.; Pulissery, S.K.; Colle, I.; Van Buggenhout, S.; Hendrickx, M.; Loey, A.V. 2012. Lycopene degradation, isomerization and *in vitro* bioaccessibility in high pressure homogenized tomato puree containing oil: effect of additional thermal and high pressure processing. Food Chemistry 135: 1290-1297. https://doi.org/10.1016/j. foodchem.2012.05.065
- Lu, Z.; Wang, J.; Gao, R.; Ye, F.; Zhao, G. 2019. Sustainable valorization of tomato pomace: a comprehensive review. Trends in Food Science and Technology 86: 172-187. https:// doi.org/10.1016/j.tifs.2019.02.020
- Macrae, R. 1998. HPLC in Food Analysis (Food Science and Technology). 2ed. Academic Press, Cambridge, USA.

- Manzo, N.; Santini, A.; Pizzolongo, F.; Aiello, A.; Romano, R. 2018. Degradation kinetic (D<sub>100</sub>) of lycopene during the thermal treatment of concentrated tomato paste. Natural Product Research 33: 1835-1841. https://doi.org/10.1080/14786419.20 18.1477147
- Minekus, M.; Alminger, M.; Alvito, P.; Balance, S.; Bohn, T.; Bourlieu, C.; Carrière, F.; Boutrou, R.; Corredig, M.; Dupont, D.; Dufour, C.; Egger, L.; Golding, M.; Karakaya, S.; Kirkhus, B.; Le Feunteun, S.; Lesmes, U.; Macierzanka, A.; Mackie, A.; Marze, S.; McClements, D.J.; Menard, O.; Recio, I.; Santos, C.N.; Singh, R.P.; Vegarud, G.E.; Wickham, M.S.J.; Weitschies, W.; Brodkorb, A. 2014. A standardized static *in vitro* digestion method suitable for food: an international consensus. Food and Function 5: 1113-1124. https://doi.org/10.1039/C3FO60702J
- Naviglio, D.; Pizzolongo, F.; Ferrara, L.; Aragon, A.; Santini, A. 2008a. Extraction of pure lycopene from industrial tomato byproducts in water using a new high-pressure process. Journal of the Science of Food and Agriculture 88: 2414-2420. https:// doi.org/10.1002/jsfa.3334
- Naviglio, D.; Caruso, T.; Iannece, P.; Aragòn, A.; Santini, A. 2008b. Characterization of high purity lycopene from tomato wastes using a new pressurized extraction approach. Journal of Agricultural and Food Chemistry 56: 6227-6231. https://doi. org/10.1021/jf703788c
- Pacheco, S.; Peixoto, F.M.; Borguini, R.G.; Nascimento, L.S.M.; Bobeda, C.R.R.; Santiago, M.C.P.A.; Godoy, R.L.O. 2014. Microscale extraction method for HPLC carotenoid analysis in vegetable matrices. Scientia Agricola 71: 416-419. https://doi. org/10.1590/0103-9016-2013-0402
- Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

- Rodriguez-Amaya, D.B. 2001. A Guide to Carotenoid Analysis in Foods. OMNI Press, Washington, DC, USA.
- Rosa, J.S.; Godoy, R.L.O.; Oiano Neto, J.; Campos, R.S.; Matta, V.M.; Freire, C.A.; Silva, A.S.; Souza, R.S. 2007. Development of a method for vitamin C analysis in food using high performance liquid chromatography and ion exclusion. Food Science and Technology 27: 837-846 (in Portuguese, with abstract in English). https://doi.org/10.1590/S0101-20612007000400025
- Singleton, V.L.; Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acids reagents. American Journal of Enology and Viticulture 16: 144-158.
- Svelander, C.A.; Tibäck, E.A.; Ahrné, L.M.; Langton, M.I.; Svanberg, U.S.; Alminger, M.A. 2010. Processing of tomato: impact on *in vitro* bioaccessibility of lycopene and textural properties. Journal of the Science of Food and Agriculture 90: 1665-1672. https://doi.org/10.1002/jsfa.4000
- Xu, F.; Wang, P.; Yao, Q.; Shao, B.; Yu, H.; Yu, K.; Li, Y. 2019. Lycopene alleviates AFB 1-induced immunosuppression by inhibiting oxidative stress and apoptosis in the spleen of mice. Food and Function 10: 3868-3879. https://doi.org/10.1039/ C8FO02300J
- Yang, C.S.; Landau, J.M.; Huang, M.T.; Newmark, H.L. 2001. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual Review of Nutrition 21: 381-406. https:// doi.org/10.1146/annurev.nutr.21.1.381
- Yilmaz, T.; Kumcuoglu, S.; Tavman, S. 2017. Ultrasound assisted extraction of lycopene and β-carotene from tomato processing wastes. Italian Journal of Food Science 29: 186-194. https://doi. org/10.14674/1120-1770/ijfs.v481