The effect of pulsed electric fields on carotenoids bioaccessibility: the role of tomato matrix

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Abstract

Tomato fractions (tissue, cells clusters, single cells, chromoplasts) were subjected to PEF treatment combined or not with heating. Results show that PEF and heating applied in combination or individually induced permeabilization of cell membranes in the tomato fractions. However, in tissue no changes in β-carotene bioaccessibility were found upon combined and individual PEF and heating, while a decrease in lycopene bioaccessibility upon combined PEF and heating and heating only was observed. In cells clusters and single cells, carotenoids bioaccessibility did not change upon the treatments. In chromoplasts both β-carotene and lycopene bioaccessibility significantly decreased upon combined PEF and heating. Differences in the effects of PEF on carotenoids bioaccessibility were related to the structure complexity of the tomato fractions. In particular, for chromoplasts the reduction in bioaccessibility was attributed to the lower protection against PEF

treatment present in this fraction compared to multiple physical barriers present in the other fractions.

Keywords: pulsed electric fields, heating, tomato, food matrix, structural barriers, carotenoids bioaccessibility

1. Introduction

Several studies relate a high intake of bioactive compounds present in fruit and vegetables to human health benefits. Among the large spectrum of bioactive compounds, carotenoids are a widespread family of fat-soluble plant pigments giving yellow, orange and red colour to many plant foods. Lycopene and β-carotene, the major carotenoids present in tomato and derived products, play an important role in human health because of their powerful antioxidant properties and pro-vitamin A activity. Moreover, they are associated with a decreased risk of cardiovascular diseases and cancer (Giovannucci, 1999). To study the carotenoids health related functions, their bioavailability needs to be evaluated. However, the bioavailability is strongly related to their bioaccessibility, that is the fraction released from the matrix and available for the intestinal absorption (Parada & Aguilera, 2007). The specific localization of carotenoids into the chromoplasts as well as the structural barriers within the cell govern carotenoids bioaccessibility. In particular, chromoplasts and cell membrane as well as cell wall are the limiting factors for both β-carotene and lycopene bioaccessibility in tomato and carrot (Jeffery, Holzenburg, & King, 2012; Palmero et al., 2013).

Several studies investigated the effect of thermal treatment, high pressure homogenization or high power ultrasounds on carotenoids bioaccessibility in tomato juice (Anese, Mirolo, Beraldo, & Lippe 2013; Colle, Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010a; Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010b). However, the structural complexity of the tomato matrix did not allow to disentangle the effect of the various processes on the different cell barriers thus to understand which are the key factors governing the bioaccessibility. To tackle this issue, Palmero

and co-authors used tomato fractions posing different physical barriers to carotenoids bioaccessibility (i.e. chromoplasts and cells clusters) and applied thermal or high pressure homogenization treatments (Palmero et al., 2013; Palmero, Lemmens, Hendrickx, & Van Loey, 2014; Palmero, Panozzo, Colle, Chigwedere, Hendrickx, & Van Loey, 2016). Upon thermal treatment the carotenoids bioaccessibility decreased in cluster cells. It has been suggested that carotenoids became entrapped by a new formed network consisting of cell wall material (Palmero et al., 2014). On the other hand, high pressure homogenization treatment induced an increase of carotenoids bioaccessibility due to the disruption of the cell structure present in chromoplasts and cells clusters (Palmero et al., 2016).

Pulsed electric fields (PEF) is a widely explored technology for inducing the permeabilization of cell membranes. The exposure of a plant tissue to an electric field for short voltage pulses, typically in the range of μs, induces the formation of pores on the membrane (electroporation phenomena). More specifically, when the cells are exposed to an external electric field, the accumulation of oppositely charged ions on both sides of membrane causes membrane thickness reduction. Further increases of the electric field up to the critical values (0.5-5 kV/cm for plant cells) cause pore formation and loss of semi-permeability of the cell membrane. However, depending on electric field strength and treatment intensity, electroporation may be either reversible or irreversible (Zimmermann, 1986).

The effect of PEF at low electric fields applied individually or in combination with heating has been investigated in order to improve the extraction yield of intracellular compounds present in fruits and vegetables tissue (Donsì, Ferrari, & Pataro, 2010). Several studies found that PEF treatments at 0.1-10 kV/cm increased the extraction of hydrophilic compounds, such as sugar from sugar beet, betaine from red beet and anthocyanins from grapes, red cabbage or purple fleshed potatoes (Eshtiaghi & Knorr, 2002; Gachovska, Cassada, Subbiah, Hanna, Thippareddi, & Snow, 2010; López, Puértolas, Condón, Raso, & Alvarez, 2009; Puértolas, Cregenzán, Luengo, Álvarez, & Raso, 2013). By contrast, only a few studies investigated the effect of PEF on the extraction of

lipophilic compounds, such as carotenoids (Luengo, Álvarez, & Raso, 2014; Wiktor et al., 2015). Both an increase or no effect in carotenoids concentration was found, depending on the origin of the matrix (skin or tissue) (Luengo, Álvarez, & Raso, 2014; Jayathunge, Stratakos, Cregenzán-Albertia, Grant, Lyng, & Koidis, 2017). Recently, Jayathunge et al. (2017) investigated the effect of PEF as pre-treatment in on carotenoids bioaccessibility in whole tomato fruit during storage. These treatments, while causing an increase in carotenoids concentration, induced both an increase or a decrease of carotenoids bioaccessibility depending on the number of pulses and storage time. On the other hand, to the best of our knowledge, there is a lack of information on the effect of PEF on tomato fractions characterized by different structural barriers. Therefore, the aim of this study was to investigate the effect of PEF on carotenoids bioaccessibility in tomato fractions. To this purpose tomato tissue, cells clusters, single cells and chromoplasts were isolated and subjected to PEF and heating, in combination or individually. Contextually, microstructure, conductivity and carotenoids concentration of untreated and treated samples were determined.

2. Materials and methods

2.1. Materials

A 30 kg batch of red tomatoes (*Lycopersicum esculentum*) was purchased in a local store in the Netherlands and stored at 7 °C until their use for the experiment. Tomato samples were prepared fresh for every trial from the same batch of fruits to minimize the influence of the matrix.

2.2 Experimental set-up

Four fractions with different level of structural barriers corresponding to tissue, cells clusters, single cells and chromoplasts were isolated from tomato and subjected to PEF (PEF), heating (HEAT) or combined PEF and heating (PEF+HEAT). Microstructure, conductivity, carotenoids concentration and *in vitro* bioaccessibility analyses were performed to each fraction. Fig. 1 depicts schematically the experimental set-up performed in the present study.

105

106 *2.3.Preparation of tomato fractions*

107 *2.3.1 Tissue*

108 Tomato tissue was prepared by removing skin and placental tissue and cutting the mesocarp into
109 cubes of 5 cm length.

110

111 *2.3.2 Cells clusters*

112 Tomato cubes, obtained by previous discard of skin and placental tissue, were blended in a kitchen
113 blender (5 s for 3 times). The cells clusters were obtained by separating the juice with the use of wet
114 sieving equipment (Analysette 3 Spartan, Idar-Oberstein, Germany). The size of the fraction
115 considered in this study ranged between 71-350 µm.

116

117 *2.3.3. Single cells*

118 Single cells were obtained based on the procedure of McAtee, Hallett, Johnston, & Schaffer (2009)
119 with minor modifications. Tomato cubes, obtained by previous discard of skin and placental tissue,
120 were immersed in a 0.05 M Na_2CO_3 in 0.3 M mannitol solution. The solution was heated at 90 °C
121 for 30 minutes under continuous stirring, and filtered with a sieve (1 mm). The cells were isolated
122 by filtering the solution through a cheesecloth.

123

124 *2.3.4. Chromoplasts*

125 Chromoplasts were isolated based on the procedure of Hansen and Chiu (2005) as described by
126 Palmero et al. (2013). Tomato cubes, obtained by previous discard of skin and placental tissue, were
127 blended in a kitchen blender (5 s for 5 times) with 0.05 M EDTA solution (1:1 ratio). The obtained
128 juice was filtrated using a cheesecloth and the filtrate was centrifuged (Beckman Coulter Avanti J-
129 26XP centrifuge, Palo Alto, CA, USA) at 27250 g and 4 °C for 30 min. The pellet, consisting of the
130 chromoplasts, was re-dissolved in 5 mg/mL NaCl.

131

132 2.4. Treatments

133 *2.4.1.Pulsed electric fields treatments*

134 Pulsed electric fields (PEF) treatment was carried out using a NP110-60 system (IXL Netherlands
135 B.V.) with an output voltage of 3.8 kV. The system provided monopolar, rectangular shaped pulses
136 of average 350 μs width. The treatment chamber consisted of a batch chamber with two circular 316
137 stainless steel electrodes with a surface area of 28.3 cm^2, resulting in a 56.5 cm^3 total volume. The
138 distance between the electrodes was 2.0 cm. Aliquots of 57 g of tomato fractions were put into the
139 chamber subjected to PEF treatments characterized by a total energy input (*Q*) (MJ/kg) of 7.6
140 MJ/kg. The latter was calculated according to Zhang, Barbosa-Cánovas and Swanson (1995) (eq.
141 1), by using the following equation:

142 $$Q = \frac{V^2\, t}{R\, m} \qquad \textit{(eq. 1)}$$

143 where *V* is the voltage (kV), *t* is the total treatment time (s), *R* is the resistance (Ohm) and *m* is the
144 sample mass (kg). Two PEF treatments were performed: (i) 90 pulses at 1Hz repetition rate in order
145 to reach an initial temperature of 90 °C. Afterwards, 210 pulses where delivered at 0.167 Hz s
146 within 30 minutes at an equilibrium temperature in the range of 85-90 °C. This treatment was
147 indicated as PEF+HEAT; (ii) 600 pulses with an interval time of 3 s was applied. The equilibrium
148 temperature of this treatment was within the range of 40-45 °C. This treatment was indicated as
149 PEF.

150 The temperature was measured at the end of the treatment by using a copper-constantan
151 thermocouple probe connected to a data logger (YC 727UD, TMS Europe Ltd, Hope Valley, United
152 Kingdom). After the treatments, the samples were cooled in a water-ice bath.

153

154 *2.4.2. Heating*

155 The total temperature–time combination received by the samples during PEF+HEAT was applied to
156 the sample in absence of the electric field. To this purpose, aliquots of 57 g of tomato fractions were
157 heated in a thermostatic water bath (Ika Werke, MST BC, Staufen, Germany) under continuous
158 stirring. The sample reached 90 °C within 5 min and the temperature was maintained constant at 90
159 °C for 25 min. This treatment was indicated as HEAT. The temperature was recorded using a
160 copper-constantan thermocouple probe connected to a data logger (YC 727UD, TMS Europe Ltd,
161 Hope Valley, United Kingdom). After the treatment, the samples were cooled in a water-ice bath.

162

163 *2.5. Microscopy analysis*

164 Microstructure of tomato fractions was analysed using an optical microscope (Axioskop Zeiss,
165 Göttingen Germany). The pictures were taken by a digital camera (Axiocam HCR, Göttingen,
166 Germany). Tissue, cells clusters and single cells were analysed with 10x lens, while 100x objective
167 lens was used for chromoplasts.

168

169 *2.6. Impedance measurement*

170 Measurement of electrical complex impedance was used to characterized tissue permeabilization
171 after treatments (Donsì, Ferrari, & Pataro, 2010). The measurement was conducted by loading the
172 sample in a test vessel between two 0.3 mm platinum wires separated 1.0 cm and inserted 1.0 cm
173 deep into the sample. The platinum wires were connected to an impedance analyser which consisted
174 of a Rigol DG1022 function generator and a Rigol DS1054Z oscilloscope. The generator produced
175 a sinusoidal voltage of 2 V peak-peak with a frequency ranging between 1 kHz and 1 MHz. All the
176 measurement were carried out at 20 °C. The electrical conductivity is the ratio of the current
177 through the sample and the voltage drop across the sample, multiplied by the cell constant ($1 cm^{-1}$)
178 of the wire electrode set-up. Conductivities, given in the unit S/m, are corrected for evaporation in

179 the heated samples (leading to an increase in ion concentration) by normalizing to the sample
180 weight i.e. conductivity is expressed in units of S/m·g.
181
182 *2.7. Cell disintegration index*
183 Cell disintegration index (Z_p) was computed according to Angersbach, Heinz and Knorr (1999).
184 This index indicate the proportion of permeabilized cells based on the frequency dependence of
185 conductivity of intact and permeabilized plant tissue. The Z_p was calculated by using the following
186 equation (eq. 2):
187 $$Z_p = 1 - \left(\frac{K_h}{K_h'}\right) \cdot \frac{(K_h' - K_l')}{(K_h - K_l)} \qquad (eq.\ 2)$$
188 Where K_l, and K_l' are the conductivities of untreated and treated tomato fraction at 1 kHz,
189 respectively, and K_h and K_h' are the electrical conductivities of untreated and treated tomato fraction
190 at 1 MHz, respectively. The Z_p varies between 0 for intact tissue and 1 for a tissue with all the cells
191 permeabilized.
192
193 *2.9. Carotenoids concentration*
194 The extraction of carotenoids from tomato fractions or micelles was performed following the
195 procedure of Sadler, Davis and Dezman (1990) with minor modifications. The analysis was
196 carried out under subdued light to prevent carotenoid degradation and isomerisation. 0.5 g NaCl
197 and 50 mL extraction solution (hexane:acetone:ethanol, 2:1:1 v/v/v) were added to 2 g of tomato
198 sample or supernatant containing micelle fraction. After 20 min of stirring at room temperature,
199 15 mL of reagent grade water was added and stirring was continued for 10 min. The apolar
200 phase, containing carotenoids, was collected, filtered (Chromafil PET filters, Düren, Germany;
201 0.20 µm pore size, 25 mm diameter) and transferred to an amber HPLC vial. The HPLC analyses
202 were performed on a Ultimate 3000 Rapid Separation LC System (Thermo Fisher Scientific,
203 Sunnyvale, CA, USA) equipped with a Ultimate 300 RS photodiode Diode array detector

(DAD-3000RS, Thermo Fisher Scientific, Sunnyvale, CA, USA). β-carotene and all-*trans* lycopene were separated at 25 °C on a reversed phase C_{30} column (250 x 4.6 mm, particle size 5 µm, YMC Europe, Dinslaken, Germany) with a gradient of two methanol:methyl tert-butyl ether eluents (eluent A 90:10, eluent B 10:90) containing 0.1% BHT. The gradient was as follow: 0 min: 88% A, 12% B; 2-4 min: 73% A, 26% B; 4-6 min: 57% A, 43% B; 6-8 min: 20% A, 80% B; 9-14 min: 0% A and 100% B; 15-25 min: 88% A, 12% B. The flow rate was 1.05 mL/min with an injection volume of 20 µL. β-carotene and all-*trans* lycopene were identified based on retention times and spectral characteristics compared to the standards (data not shown). To quantify the carotenoids, HPLC-DAD responses were measured at 450 nm for β-carotene and at 470 nm for all-*trans* lycopene. The carotenoids content was calculated based on their calibration curves and expressed as µg/g tomato fraction dry weight (µg/g dw).

2.10. Carotenoids in vitro *bioaccessibility*

The carotenoids *in vitro* bioaccessibility was measured the day after the processing following the procedure described by Minekus et al. (2014) with minor modifications. Five g of sample was weighed into a 50 mL capacity falcon tube. The sample was diluted with 4 mL of Simulated Salivary Fluid (SSF: 15.1 mL of 0.5 M KCl, 3.7 mL of 0.5 M KH_2PO_4, 6.8 mL of 1 M $NaHCO_3$, 0.5 mL of 0.15 M $MgCl_2(H_20)_6$, 0.06 mL of 0.5 M $(NH_4)_2CO_3$), 975 µL miliQ water, 7.24 mL of Simulated Gastric Fluid (SGF: 6.9 mL of 0.5 M KCl, 0.9 mL of 0.5 M KH_2PO_4, 12.5 mL of 1 M $NaHCO_3$, 11.8 mL of 2 M NaCl, 0.4 mL of 0.15 M $MgCl_2(H_20)_6$, 0.5 mL of 0.5 M of $(NH_4)_2CO_3$), 50 µL of 0.3 M $CaCl_2$ solution, 260 µL of freshly prepared L-α-phosphatidylcholine solution (10 mg/mL in SGF). The latter was obtained by preparing 50 mg/mL L-α-phosphatidylcholine (Sigma-Aldrich) solution in chloroform:methanol (1:1 v:v). To simulate the gastric digestion, the pH of the mixture was adjusted to 3 ± 0.05 with 1 M HCl or 1 M NaOH and 1.6 mL of porcine pepsin solution (25000 U/mL) was added. After flushing sample headspace with nitrogen for 10 s, the mixture was incubated at 37 °C for 2 hours while shaking end-over-end. Afterwards, to mimic the

passage through the small intestine, 11 mL of Simulated Intestinal Fluid (SIF: 6.8 mL of 0.5 M KCl, 0.8 mL of 0.5 M KH_2PO_4, 42.5 mL of 1 M $NaHCO_3$, 9.6 mL of 2 M NaCl, 1.1 mL of 0.15 M $MgCl_2(H_20)_6$), 2.5 mL of fresh bile (0.089 g/ml in SIF), 40 μL of 0.3 M $CaCl_2$ and 5.0 mL of pancreatin (0.33 g/ml in SIF) were added. The amount of pancreatin added to SIF was calculated based on a trypsin activity measured in the pancreatin according to the assay described in the harmonized protocol (Minekus et al., 2014).The pH of the solution was adjusted to 7 ± 0.05 with 1 M HCl or 1 M NaOH. Finally, the sample headspace was flushed with nitrogen for 10 seconds and the solution was incubated at 37 °C for 2 hours while shaking end-over-end. The digest was centrifuged (Beckman L-60 Ultracentrifuge, Palo Alto, CA, USA) at 162000 g for 67 min at 4 °C to separate the micelles containing the carotenoids. The supernatant was collected and carotenoids quantified according to the method described above. After the extraction procedure, the carotenoids extract was up-concentrated under vacuum using a rotary evaporator at 35 °C and re-dissolved in hexane:dichlorometane (4:1 v:v). The concentration factor was calculated by adding a specific amount of β-apo-8'-carotenale prior to evaporation. The carotenoid *in vitro* bioaccessibility (B/C) in each fraction and for each treatment was defined as the percentage ratio between the carotenoids concentration in the micelles (B) and the carotenoid concentration (C) of the corresponding fraction and treatment before digestion.

2.11. Total solids content

The total solids content was measured by gravimetric method (AOAC, 1995).

2.12. Data analysis

The results are the average of at least two measurements carried out on two replicated experiments ($n \geq 4$). Data are reported as mean value ± standard error. Statistical analysis was performed using R v.2.15.0 (The R foundation for Statistical Computing). Bartlett's test was used to check the

homogeneity of variance, one way ANOVA was carried out and Tukey test was used to determine statistically significant differences among means ($p<0.05$).

3. Results and discussion

3.1 Effect of PEF on microstructure and conductivity of tomato fractions

Fig. 2 shows the microstructure of untreated and PEF+HEAT treated tissue, cells clusters, single cells and chromoplasts. In tomato tissue, carotenoids were dispersed within the cells glued together through the middle lamella (Moelants, Cardinaels, Van Buggenhout, Van Loey, Moldenaers, & Hendrickx, 2014). The isolation procedure allowed to obtain in the cells clusters fraction a mixture of intact and broken cells and debris with carotenoids homogenously dispersed, while in single cells intact membranes can be observed. In chromoplasts fraction, a single thin membrane layer enveloped carotenoid crystals (Jeffery, Holzemburg, & King, 2012). PEF+HEAT caused cell detachment in tomato tissue, while in single cells, a large number of cells were damaged. In cells clusters and chromoplasts, no visual differences were found among untreated and PEF+HEAT treated samples. No microstructural differences were found among the samples subjected to PEF+HEAT, PEF and HEAT (data not shown).

To understand the effect of PEF+HEAT, PEF and HEAT on modification of cell membranes, conductivity in a frequency range between 1 kHz and 1 MHz was measured. Fig. 3 shows the conductivity spectra of untreated and PEF+HEAT treated tomato tissue as well as those relevant to samples subjected to PEF and HEAT treatments. In untreated tomato tissue low conductivity values were found at low frequency because the cell membrane acts as capacitor, preventing the current electric flow into the medium. As expected, by increasing the frequency, higher conductivity values were found because the cell membrane became less resistant to the current flow applied during the conductivity measurement. Upon PEF+HEAT, PEF and HEAT treatments, high conductivity values were found in the whole frequency range, indicating a modification of the membrane permeability. In particular, at low frequency the higher conductivity level indicated the irreversible membrane

electroporation (Donsì et al., 2010). However, no significant differences ($p>0.05$) in conductivity were found among the treated samples. In order to obtain an indication of the proportion of permeabilized cells due to treatments, the disintegration index (Z_p) was calculated (Angersbach, Heinz, & Knorr, 1999). Upon PEF+HEAT, Z_p reached the value of 0.8, indicating that most of the cells membrane were damaged. Similar Z_p values were found for PEF and HEAT (data not shown). Thus, although these results give an indication that cell membrane modification actually occurred as a consequence of processing, they do not allow to discriminate among the different cell damages, which have been described in the literature. For instance, it has been reported that PEF treatments cause membrane pores formation, while thermal treatments induce pectin depolymerisation in the cell wall and disruption of cell membrane (Moelants et al., 2014; Zimmermann, 1986).

In cells clusters, single cells and chromoplasts, the conductivity did not increase upon the treatments as compared to the untreated counterparts (data not shown). It is likely that samples preparation steps (e.g. blending and heating at 90 °C for 30 min) have already damaged cell membranes, causing an increase in conductivity to a maximum value. Therefore, no further conductivity increase was found for the treated tomato fractions, although membrane damage can be hypothesised as occurring under our process conditions. In fact, according to Zhang, Chang and Barbosa-Cánovas (1994), membrane electroporation depends not only on process parameters but also on cell size. Moreover, Janositz and Knorr (2010) found that PEF treatments at 0.25 to 7.5 kV/cm caused electroporation of tobacco cells, which are characterized by cell size similar to chromoplasts (~ 10µm).

3.2 Effect of PEF on carotenoids concentration and bioaccessibility

In order to understand the effect of PEF+HEAT treatment on bioactive compounds, carotenoids concentration and bioaccessibility were determined. Table 1 shows β-carotene and all-*trans* lycopene concentrations of untreated and treated tomato fractions. It can be observed that the application of PEF+HEAT did not change β-carotene concentration in tomato tissue, cells clusters

307 and isolated cells. However, a decrease of β-carotene concentration was found upon PEF or HEAT
308 in cells clusters and single cells, likely due to oxidation and isomerization phenomena favoured by
309 electrochemical reactions and metal release (Morren, Roodenburg, & de Haan, 2003; Pataro,
310 Falcone, Donsì, & Ferrari, 2014; Roodenburg, Morren, Berg, & de Haan, 2005). By contrast, no
311 significant differences ($p>0.05$) in all-*trans* lycopene concentration were found in tissue, cells
312 clusters and single cells upon the application of the above technologies, probably due to the higher
313 stability to oxidation and isomerization phenomena of lycopene compared to β-carotene (Lemmens,
314 Tchuenche, Van Loey, & Hendrickx, 2013; Seybold, Fröchlich, Bitsch, Otto, & Böhm, 2004).
315 These results are in agreement with the literature in the framework of PEF and heating. In
316 particular, PEF treatment at 3-7 kV/cm did not affect the lycopene concentration in tomato tissue
317 (Luengo et al., 2014). Similarly, temperature below 100 °C did not induce lycopene isomerization
318 and degradation in tomato derivatives (Nguyen & Schwartz, 1998; Colle et al., 2010a). It is
319 noteworthy that under our experimental conditions, temperature never exceeded 90 °C. In
320 chromoplasts fraction, HEAT caused a decrease of about 21% in β-carotene concentration, while no
321 changes were found in all-*trans* lycopene concentration. By contrast, both β-carotene and all-*trans*
322 lycopene concentrations decreased by 47% and 36%, respectively, upon the application of
323 PEF+HEAT or PEF. Such a decrease that is attributable to carotenoids oxidation and/or
324 isomerization could be favoured by the higher susceptibility of carotenoids, enveloped in a thin
325 membrane layer, towards oxygen permeability, metal release by the electrodes and electrochemical
326 reaction occurring during PEF treatment (Jeffery et al., 2012; Morren et al., 2003; Pataro et al.,
327 2014; Roodenburg et al., 2005). In order to understand the effect of PEF+HEAT, PEF and HEAT on
328 the functional properties of the tomato fractions, carotenoids bioaccessibility was investigated.
329 Carotenoids bioaccessibility indicates the carotenoids fraction released from the matrix and
330 incorporated into micelles during the *in vitro* digestion. In our study, bioaccessibility was calculated
331 as the ratio of the carotenoids incorporated into micelles after the digestion to the initial carotenoid
332 concentration in the same processed sample. Fig. 4 shows β-carotene and all-*trans* lycopene

bioaccessibility in tomato fractions upon PEF+HEAT, PEF and HEAT. In untreated tissue, cells clusters and single cells the β-carotene and all-*trans* lycopene bioaccessibility ranged between 2% and 5%, in agreement with the literature (Palafox-Carlos et al., 2011; Palmero et al., 2013). As expected, lower β-carotene and all-*trans* lycopene bioaccessibility values were found in untreated tissue, cells clusters and single cells than in chromoplasts. It can be inferred that intact cell wall polysaccharides may prevent the digestive enzymes, bile salts and surfactants (i.e. phospholipids) reaching the bioactive compounds within the chromoplasts and hampered micelle formation during intestinal step (Palafox-Carlos et al., 2011). Moreover, the higher carotenoids bioaccessibility in tissue compared to cells clusters and single cells could be attributed to the presence of chromoplast leaked from the broken cells along the surface during the preparation step (i.e. cutting). Moreover, from Fig. 4A it can be also observed that in tissue β-carotene bioaccessibility did not change upon PEF+HEAT, PEF and HEAT. It is likely that, although the treatments caused the modification of cell permeability (Fig. 1), the presence of multiple encapsulation barriers is enough to prevent the carotenoids release (Palafox-Carlos et al., 2011). All-*trans* lycopene bioaccessibility in tomato tissue were not affected by PEF, in agreement with Jayathunge et al. (2017), while a significant decrease was found in PEF+HEAT and HEAT treated samples. It can be inferred that heating induced the formation of a barrier consisting of cell wall and cell membrane that hinders the lycopene release (Palmero et al., 2014). Differences in β-carotene and all-*trans* lycopene bioaccessibility upon the treatments can be attributable to their different molecular structure. In fact, the linear isoprenoid chain confers to lycopene lower solubility capacity compared to β-carotene and thus lower bioaccessibility (Tyssandier, Lyan, & Borel, 2001; Van het Hof, West, Weststrate, & Hautvast, 2000).

In cells clusters and single cells (Fig. 4B and 4C), β-carotene and all-*trans* lycopene bioaccessibility did not change upon PEF+HEAT, PEF and HEAT. So we concluded that these treatments did not affect the carotenoids bioaccessibility due to the presence of cell wall polysaccharides in cells clusters and cell barriers induced by thermal treatment during samples

359 preparation in isolated cells (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011; Palmero et
360 al., 2014). To understand the role of membranes, carotenoids bioaccessibility was studied in the
361 chromoplasts fraction (Fig. 4D), which has a single membrane layer. In untreated chromoplasts
362 fraction, β-carotene and all-*trans* lycopene bioaccessibility ranged between 16% and 20%. The
363 application of PEF+HEAT caused a significant reduction of carotenoids bioaccessibility to 5%.
364 Similar results were obtained for the PEF treated chromoplasts. On the contrary, HEAT did not
365 cause significant modifications ($p>0.05$) of carotenoids *in vitro* bioaccessibility, in agreement with
366 Colle et al. (2010a).
367 The reduction in carotenoids bioaccessibility at chromoplasts level is attributable to the effect of
368 PEF rather than to HEAT. This decrease in the bioaccessibility might suggest that PEF induced
369 modifications not only in the membrane but also in the carotenoids-protein complexes. In fact, it is
370 well known that carotenoids are tightly bound to subcellular lipids and binding proteins within the
371 chromoplast structure (Faulks & Southon, 2005) and that complexes constitute a further structural
372 barrier for carotenoids bioaccessibility. Moreover, Perez and Pilosof (2004) found that PEF
373 treatment can induce modification in protein conformation. Although further research is needed,
374 these results clearly show a role of PEF on carotenoids bioaccessibility.
375

376 **4. Conclusions**

377 The results of this study show that PEF treatment applied individually or in combination with
378 heating induced changes of the microstructure and membrane permeability of tomato fractions.
379 These treatments caused slight or no changes in β-carotene concentration and bioaccessibility in
380 tissue. Similarly, all-*trans* lycopene concentration and bioaccessibility did not change upon PEF. A
381 decrease of all-*trans* lycopene bioaccessibility upon PEF+HEAT and HEAT was found, suggesting
382 the formation of a barrier that hinders carotenoids release. In cells clusters and single cells, slight or
383 no changes were found in carotenoids concentration and bioaccessibility. On the contrary, in
384 chromoplasts both β-carotene and all-*trans* lycopene concentration and bioaccessibility significantly

decreased upon PEF with or without heating due to PEF related phenomena. This effect was attributed to the comparatively higher susceptibility of the chromoplasts fraction towards PEF treatment compared to tomato fractions which possess further structural barriers. It can be concluded that the effect of PEF on carotenoids bioaccessibility strongly depends on the vegetable structure complexity and presence of physical barriers naturally present or induced by the process that impede the carotenoids release. The results of this study clearly indicate individual PEF can be applied to tomato based products, mainly constituted by tissue, cells clusters and single cells, without impairing carotenoids functionality.

Conflict of interest

Hennie Mastwijk has been involved as a consultant in the high voltage engineering of the NP110-60 pulsed electrical field system.

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538 **Figure captions**

539 **Fig. 1.** Experimental set-up.

540

541 **Fig. 2.** Micrographs of untreated and PEF+HEAT treated tissue, cells clusters, single cells and
542 chromoplasts fractions obtained from tomatoes.

543

544 **Fig. 3.** Conductivity (S/m·g) spectra of tomato tissue subjected to combined pulsed electric fields
545 and heating (PEF+HEAT), pulsed electric fields (PEF) and heating (HEAT) only. Data relevant to
546 untreated samples are also shown.

547

548 **Fig. 4**. β-carotene (■) and all-*trans* lycopene (□) bioaccessibility (B/C %) in tissue (A), cells
549 clusters (B), single cells (C) and chromoplasts (D) subjected to combined pulsed electric fields and
550 heating (PEF+HEAT), pulsed electric fields (PEF) or heating (HEAT) only. Data relevant to
551 untreated samples are also shown.

552 $^{a, b}$: Means with different letters within each tomato fraction indicate significant differences
553 ($p<0.05$) for β-carotene.

554 $^{a', b'}$: Means with different letters within each tomato fraction indicate significant differences
555 ($p<0.05$) for all-*trans* lycopene.

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564 **Table 1.** β-carotene and all-*trans* lycopene concentrations (μg/g dw) in tomato tissue, cluster cells,
565 single cells and chromoplasts subjected to combined pulsed electric fields and heating
566 (PEF+HEAT), pulsed electric fields (PEF) or heating (HEAT) only. Data relevant to untreated
567 samples are also shown.
568

		Concentration (μg/g dw)	
Sample	**Treatment**	β-carotene	all-*trans* lycopene
Tissue	Untreated	32.2 ± 5.2 [a]	647.6 ± 65.9 [a']
	PEF+HEAT	40.6 ± 3.1 [a]	557.1 ± 58.1 [a']
	PEF	26.5 ± 2.3 [a]	463.8 ± 53.2 [a']
	HEAT	25.9 ± 2.2 [a]	588.2 ± 51.0 [a']
Cells cluster	Untreated	32.2 ± 1.3 [a]	346.8 ± 47.8 [a']
	PEF+HEAT	30.4 ± 0.9 [a]	345.5 ± 5.6 [a']
	PEF	16.9 ± 0.5 [c]	395.3 ± 2.8 [a']
	HEAT	24.2 ± 0.4 [b]	304.3 ± 1.0 [a']
Single cells	Untreated	75.7 ± 4.2 [a]	839.4 ± 119.4 [a'b']
	PEF+HEAT	68.4 ± 6.4 [ab]	825.9 ± 134.2 [a'b']
	PEF	52.6 ± 0.7 [bc]	1096.8 ± 31.7 [a']
	HEAT	45.5 ± 3.1 [c]	569.8 ± 69.1 [b']
Chromoplasts	Untreated	341.6 ± 5.9[a]	2970.1 ± 44.0 [a']
	PEF+HEAT	187.3 ± 7.7 [c]	1881.7± 43.9 [b']
	PEF	255.8 ± 18.4 [b]	2191.7 ± 227.2 [b']
	HEAT	283.6 ± 1.3 [b]	2794.1 ± 46.3 [a']

569

570 [a, b] : means with different letters within each tomato fraction indicate significant differences
571 ($p<0.05$) for β-carotene.

572 [a', b'] : means with different letters within each tomato fraction indicate significant differences

573 ($p<0.05$) for all-*trans* lycopene.