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

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ABSTRACT

Tomato fractions were subjected to pulsed electric fields treatment combined or not with heating. Results showed that pulsed electric fields and heating applied in combination or individually induced permeabilization of cell membranes in the tomato fractions. However, no changes in β-carotene and lycopene bioaccessibility were found upon combined and individual pulsed electric fields and heating, except in the following cases: (i) in tissue, a significant decrease in lycopene bioaccessibility upon combined pulsed electric fields and heating and heating only was observed; (ii) in chromoplasts, both β-carotene and lycopene bioaccessibility significantly decreased upon combined pulsed electric fields and heating and pulsed electric fields only. The reduction in carotenoids bioaccessibility was attributed to modification in chromoplasts membrane and carotenoids-protein complexes. Differences in the effects of pulsed electric fields on bioaccessibility among different tomato fractions were related to tomato structure complexity.

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, carotenoids bioavailability is strongly related to their bioaccessibility, that is the fraction released from the food 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 membranes as well as cell walls are the limiting factors for both β-carotene and lycopene bioaccessibility in tomatoes and carrots (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, 2010; Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010). However, the structural complexity of the tomato matrix did not allow to disentangle the effect of the various processes on the different cell barriers and to identify the key factors governing carotenoids bioaccessibility. To tackle this issue, Palmero and co-authors investigated *in vitro* carotenoids bioaccessibility in tomato fractions with different physical barriers (i.e. chromoplasts and cells clusters) and applied thermal or high pressure homogenization treatments (Palmero et al., 2013, 2016; Palmero, Lemmens, Hendrickx, & Van Loey, 2014). Thermal treatments (up to 125 °C for 25 min) caused the reduction in carotenoids bioaccessibility in tomato chromoplasts and cells clusters. According to Palmero et al. (2014), this was attributable to the formation of a network consisting of cell wall material that hinders the carotenoids incorporation into biliary micelles. On the other hand, high pressure homogenization (150 MPa) increased carotenoids bioaccessibility due to the disruption of the cell structure present in tomato 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

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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 pores formation and loss of cell membrane semi-permeability. 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 (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 et al., 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). Depending on the process parameters and origin of the matrix (tomato or carrot), both an increase or no effect in carotenoids concentration were found (Luengo et al., 2014; Jayathunge et al., 2017). With regards to carotenoids bioaccessibility, the effect of PEF on whole tomato fruit has been recently investigated (Jayathunge et al., 2017). According to the authors, PEF did not modify carotenoids bioaccessibility in tomato fruit, while only the blanching followed by PEF treatment allowed to increase the amount of carotenoids incorporated into biliary micelles during *in vitro* digestion.

On the other hand, to the best of our knowledge, the effect of PEF applied in combination with heating on tomato fractions characterized by different cellular barriers has not been previously investigated. Therefore, the aim of this research was to study whether PEF treatment applied in combination with heating could induce structural modifications in isolated tomato fractions namely tissue, cells clusters, isolated cells and chromoplasts, and lead to changes in carotenoids bioaccessibilty. Individual PEF and heating treatments were also applied to isolate the effect of electroporation from heat. Contextually, microstructure, conductivity and carotenoids concentration of untreated and treated tomato samples were determined.

2. Materials and methods

2.1. Materials

A 30 kg batch of red tomatoes at fully ripe stage (*Lycopersicum esculentum*, cv. Kommeet), harvested in The Netherlands, was purchased in April 2016 in a local store in The Netherlands and stored at 7 °C until their use for the experiments. 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 tomato fractions with different level of structural barriers corresponding to tissue, cells clusters, single cells and chromoplasts were subjected to combined PEF and heating as well as to individual PEF and heat treatments. The selected treatments were chosen in order to maximize the difference between PEF and heat as well as disentangle possible electrical effects by PEF from heat. Therefore, treatments have been compared at the similar temperature-time conditions. 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.

2.3. Preparation of tomato fractions

2.3.1. Tissue

Tomato tissue was prepared by removing skin and placental tissue and cutting the mesocarp into cubes of 0.5 cm length. The sample was immediately subjected to the treatments.

2.3.2. Cells clusters

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

2.3.3. Single cells

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

2.3.4. Chromoplasts

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

2.4. Treatments

2.4.1. Pulsed electric fields treatments

Pulsed electric fields (PEF) treatment was carried out using a NP110-60 system (IXL Netherlands B.V.) with an output voltage of 3.8 kV. The system provided monopolar, rectangular shaped pulses of average 350 μs width. The pulse duration was selected to lead to irreversible electroporation (Donsì et al., 2010). The treatment chamber consisted of a batch chamber with two circular 316 stainless steel electrodes with a surface area of 28.3 cm^2, resulting in a 56.5 cm^3 total volume. The distance between the electrodes was 2.0 cm. Aliquots of 57 g of tomato fractions were put into the chamber subjected to PEF treatments characterized by a total energy input (Q) (MJ/kg) of 7.6 MJ/kg. The latter was calculated according to Zhang, Barbosa-Cánovas and Swanson (1995) (Eq. (1)), by using the following equation:

$$Q = \frac{V^2 t}{Rm} \quad (1)$$

where V is the voltage (kV), t is the total treatment time (s), R is the resistance (Ohm) and m is the sample mass (kg). Two PEF treatments were performed: (i) 90 pulses at 1 Hz repetition rate in order to reach an initial temperature of 90 °C. Afterwards, 210 pulses where delivered at 0.167 Hz s within 30 min at an equilibrium temperature in the range of 85–90 °C. The temperature range was selected in order to inactivate the enzymes responsible for loss in tomato consistency (i.e. pectin methylesterase and polygalacturonase). This treatment was indicated as PEF + HEAT; (ii) 600 pulses where delivered at 0.33 Hz at an equilibrium temperature in the range of 40–45 °C. This treatment was indicated as PEF.

The frequency applied for both PEF + HEAT and PEF was selected

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